

# Package: ORFik (via r-universe)

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**Type** Package

**Title** Open Reading Frames in Genomics

**Version** 1.32.0

**Encoding** UTF-8

**Description** R package for analysis of transcript and translation features through manipulation of sequence data and NGS data like Ribo-Seq, RNA-Seq, TCP-Seq and CAGE. It is generalized in the sense that any transcript region can be analysed, as the name hints to it was made with investigation of ribosomal patterns over Open Reading Frames (ORFs) as it's primary use case. ORFik is extremely fast through use of C++, data.table and GenomicRanges. Package allows to reassign starts of the transcripts with the use of CAGE-Seq data, automatic shifting of RiboSeq reads, finding of Open Reading Frames for whole genomes and much more.

**biocViews** ImmunoOncology, Software, Sequencing, RiboSeq, RNASeq, FunctionalGenomics, Coverage, Alignment, DataImport

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**LazyData** TRUE

**BugReports** <https://github.com/Roleren/ORFik/issues>

**URL** <https://github.com/Roleren/ORFik>

**Depends** R (>= 4.1.0), IRanges (>= 2.17.1), GenomicRanges (>= 1.35.1), GenomicAlignments (>= 1.19.0)

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## Description

Main goals:

1. Finding Open Reading Frames (very fast) in the genome of interest or on the set of transcripts/sequences.
2. Utilities for metaplots of RiboSeq coverage over gene START and STOP codons allowing to spot the shift.
3. Shifting functions for the RiboSeq data.
4. Finding new Transcription Start Sites with the use of CageSeq data.
5. Various measurements of gene identity e.g. FLOSS, coverage, ORFscore, entropy that are recreated based on many scientific publications.
6. Utility functions to extend GenomicRanges for faster grouping, splitting, tiling etc.

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## See Also

Useful links:

- <https://github.com/Roleren/ORFik>
- Report bugs at <https://github.com/Roleren/ORFik/issues>

---

```
add_pseudo_5utrs_txdb_if_needed
      add_pseudo_5utrs_txdb_if_needed
```

---

**Description**

add\_pseudo\_5utrs\_txdb\_if\_needed

**Usage**

```
add_pseudo_5utrs_txdb_if_needed(
  txdb,
  pseudo_5UTRS_if_needed = NULL,
  minimum_5UTR_percentage = 30
)
```

**Arguments**

txdb                    a TxDb object

pseudo\_5UTRS\_if\_needed  
                        integer, default NULL. If defined > 0, will add pseudo 5' UTRs of maximum this length if 'minimum\_5UTR\_percentage" (default 30 mRNAs (coding transcripts) do not have a leader. (NULL and 0 are both the ignore command)

minimum\_5UTR\_percentage  
                        numeric, default 30. What minimum percentage of mRNAs must have a 5' UTRs (leaders), to not do the pseudo\_UTR addition. If percentage is higher, addition is ignored, set to 101 to always do it.

**Value**

txdb (new txdb if it was done, old if not)

---

```
append_gene_symbols    Append gene symbols to a data.table with tx ids
```

---

**Description**

Main use case is to add gene symbols to data.table outputs from ORFik with tx ids only, like the DTEG.analysis etc.

**Usage**

```
append_gene_symbols(dt, symbols_dt, extend_id = TRUE, id_col = "id")
```

**Arguments**

dt	a data.table, must have a id_col with transcript ids
symbols_dt	the data.table with symbols, must have a column with tx, transcript or value in the name. And only 1 of those!
extend_id	logical, if TRUE, paste together old id from dt, with the symbol id like: tx_id(symbol_id)
id_col	character, default "id". The name of the id column in dt.

**Value**

a data.table

**Examples**

```
library(data.table)
df <- ORFik.template.experiment()

cds_names <- names(loadRegion(df, "cds"))
dt <- data.table(id = cds_names[-1], LFC = seq(5), p.value = 0.05)

symbols_dt <- data.table(ensembl_tx_name = cds_names,
  ensembl_gene_id = txNamesToGeneNames(cds_names, df),
  external_gene_name = c("ATF4", "AAT1", "ML4", "AST2", "RPL4", "RPL12"))
append_gene_symbols(dt, symbols_dt)
append_gene_symbols(dt, symbols_dt, extend_id = FALSE)
```

---

artificial.orfs

---

*Create small artificial orfs from cds*


---

**Description**

Usefull to see if short ORFs prediction is dependent on length.

Split cds first in two, a start part and stop part. Then say how large the two parts can be and merge them together. It will sample a value in range give.

Parts will be forced to not overlap and can not extend outside original cds

**Usage**

```
artificial.orfs(
  cds,
  start5 = 1,
  end5 = 4,
  start3 = -4,
  end3 = 0,
  bin.if.few = TRUE
)
```

**Arguments**

<code>cds</code>	a GRangesList of orfs, must have width <code>%% 3 == 0</code> and length <code>&gt;= 6</code>
<code>start5</code>	integer, default: 1 (start of orf)
<code>end5</code>	integer, default: 4 (max 4 codons from start codon)
<code>start3</code>	integer, default -4 (max 4 codons from stop codon)
<code>end3</code>	integer, default: 0 (end of orf)
<code>bin.if.few</code>	logical, default TRUE, instead of per codon, do per 2, 3, 4 codons if you have few samples compared to lengths wanted, If you have 4 cds' and you want 7 different lengths, which is the standard, it will give you possible nt length: 6-12-18-24 instead of original 6-9-12-15-18-21-24. If you have more than 30x cds than lengths wanted this is skipped. (for default arguments this is: $7*30 = 210$ cds)

**Details**

If artificial cds length is not divisible by 2, like 3 codons, the second codon will always be from the start region etc.

Also If there are many very short original cds, the distribution will be skewed towards more smaller artificial cds.

**Value**

GRangesList of new ORFs (sorted: + strand increasing start, - strand decreasing start)

**Examples**

```
txdb <- ORFik.template.experiment()
#cds <- loadRegion(txdb, "cds")
## To get enough CDSs, just replicate them
# cds <- rep(cds, 100)
#artificial.orfs(cds)
```

---

as.character,GRangesList-method

*Convert GRangesList to character vector*

---

**Description**

Single exon format:

"1:14598834-14598914:+"

Multi-exon format (exon separator: ';'):

"1:15210514-15210562:+;1:15214895-15215025:+"

**Usage**

```
## S4 method for signature 'GRangesList'
as.character(x, ...)
```

**Arguments**

x	A <a href="#">GRangesList</a>
...	Not used for now, to preserve generic requirement

**Value**

a character vector, 1 element per element in GRangesList

---

assignTSSByCage	<i>Input a txdb and add a 5' leader for each transcript, that does not have one.</i>
-----------------	--

---

**Description**

For all cds in txdb, that does not have a 5' leader: Start at 1 base upstream of cds and use CAGE, to assign leader start. All these leaders will be 1 exon based, if you really want exon splicings, you can use exon prediction tools, or run sequencing experiments.

**Usage**

```
assignTSSByCage(
  txdb,
  cage,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
  removeUnused = FALSE,
  preCleanup = TRUE,
  pseudoLength = 1
)
```

**Arguments**

txdb	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite) or an ORFik experiment
cage	Either a filePath for the CageSeq file as .bed .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: <code>convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE)</code> The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
extension	The maximum number of bases upstream of the TSS to search for CageSeq peak.
filterValue	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.

restrictUpstreamToTx	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
removeUnused	logical (FALSE), if False: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.
preCleanup	logical (TRUE), if TRUE, remove all reads in region (-5:-1, 1:5) of all original tss in leaders. This is to keep original TSS if it is only +/- 5 bases from the original.
pseudoLength	a numeric, default 1. Add a pseudo length for all the UTRs. Will not extend a leader if it would make it go outside the defined seqlengths of the chromosome (for non circular chromosomes), or extending closer than 50 nucleotides to upstream cds. So this length is not guaranteed for all!

### Details

Given a TxDb object, reassign the start site per transcript using max peaks from CageSeq data. A max peak is defined as new TSS if it is within boundary of 5' leader range, specified by 'extension' in bp. A max peak must also be higher than minimum CageSeq peak cutoff specified in 'filterValue'. The new TSS will then be positioned where the cage read (with highest read count in the interval). If no CAGE supports a leader, the width will be set to 1 base.

### Value

a TxDb object of reassigned transcripts

### See Also

Other CAGE: [reassignTSSbyCage\(\)](#), [reassignTxDbByCage\(\)](#)

### Examples

```
txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
  package = "GenomicFeatures")
cagePath <- system.file("extdata", "cage-seq-heart.bed.bgz",
  package = "ORFik")

## Not run:
assignTSSByCage(txdbFile, cagePath)
#Minimum 20 cage tags for new TSS
assignTSSByCage(txdbFile, cagePath, filterValue = 20)
# Create pseudo leaders for the ones without hits
assignTSSByCage(txdbFile, cagePath, pseudoLength = 100)
# Create only pseudo leaders (in example 2 leaders are added)
assignTSSByCage(txdbFile, cage = NULL, pseudoLength = 100)

## End(Not run)
```

asTX

*Map genomic to transcript coordinates by reference***Description**

Map range coordinates between features in the genome and transcriptome (reference) space.

**Usage**

```
asTX(
  grl,
  reference,
  ignore.strand = FALSE,
  x.is.sorted = TRUE,
  tx.is.sorted = TRUE
)
```

**Arguments**

<code>grl</code>	a <a href="#">GRangesList</a> of ranges within the reference, <code>grl</code> must either have names matching, or a meta column called 'names' that gives grouping names. i.e. <code>grl</code> named <code>uORF_1_ENST00001</code> , must then have a names meta column with <code>ENST00001</code> .
<code>reference</code>	a <a href="#">GRangesList</a> of ranges that include <code>grl</code> as a subset of ranges. Example: <code>cds</code> is <code>grl</code> and <code>mrna</code> can be <code>reference</code>
<code>ignore.strand</code>	When <code>ignore.strand</code> is <code>TRUE</code> , strand is ignored in overlaps operations (i.e., all strands are considered "+") and the strand in the output is '*'. When <code>ignore.strand</code> is <code>FALSE</code> (default) strand in the output is taken from the transcripts argument. When transcripts is a <a href="#">GRangesList</a> , all inner list elements of a common list element must have the same strand or an error is thrown. Mapped position is computed by counting from the transcription start site (TSS) and is not affected by the value of <code>ignore.strand</code> .
<code>x.is.sorted</code>	if <code>x</code> is a <a href="#">GRangesList</a> object, are "-" strand groups pre-sorted in decreasing order within group, default: <code>TRUE</code>
<code>tx.is.sorted</code>	if transcripts is a <a href="#">GRangesList</a> object, are "-" strand groups pre-sorted in decreasing order within group, default: <code>TRUE</code>

**Details**

Similar to `GenomicFeatures`' `pmapToTranscripts`, but in this version the `grl` ranges are compared to reference ranges with same name, not by index. This gives a large speedup, but also requires all objects must be named.

**Value**

a [GRangesList](#) in transcript coordinates

**See Also**

Other ExtendGenomicRanges: [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

**Examples**

```
seqname <- c("tx1", "tx2", "tx3")
seqs <- c("ATGGGTATTATA", "AAAAA", "ATGGGTAATA")
grIn1 <- GRanges(seqnames = "1",
                 ranges = IRanges(start = c(21, 10), end = c(23, 19)),
                 strand = "-")
grIn2 <- GRanges(seqnames = "1",
                 ranges = IRanges(start = c(1), end = c(5)),
                 strand = "-")
grIn3 <- GRanges(seqnames = "1",
                 ranges = IRanges(start = c(1010), end = c(1019)),
                 strand = "-")
grl <- GRangesList(grIn1, grIn2, grIn3)
names(grl) <- seqname
# Find ORFs
test_ranges <- findMapORFs(grl, seqs,
                          "ATG|TGG|GGG",
                          "TAA|AAT|ATA",
                          longestORF = FALSE,
                          minimumLength = 0)
# Genomic coordinates ORFs
test_ranges
# Transcript coordinate ORFs
asTX(test_ranges, reference = grl)
# seqnames will here be index of transcript it came from
```

---

bamVarName

*Get library variable names from ORFik [experiment](#)*


---

**Description**

What will each sample be called given the columns of the experiment? A column is included if more than 1 unique element value exist in that column.

**Usage**

```
bamVarName(
  df,
  skip.replicate = length(unique(df$rep)) == 1,
  skip.condition = length(unique(df$condition)) == 1,
  skip.stage = length(unique(df$stage)) == 1,
  skip.fraction = length(unique(df$fraction)) == 1,
  skip.experiment = !tryCatch(df@expInVarName, error = function(e) FALSE),
```

```

    skip.libtype = FALSE,
    fraction_prepend_f = TRUE
  )

```

### Arguments

```

df          an ORFik experiment
skip.replicate a logical (FALSE), don't include replicate in variable name.
skip.condition a logical (FALSE), don't include condition in variable name.
skip.stage    a logical (FALSE), don't include stage in variable name.
skip.fraction a logical (FALSE), don't include fraction
skip.experiment
              a logical (FALSE), don't include experiment
skip.libtype  a logical (FALSE), don't include libtype
fraction_prepend_f
              a logical (TRUE), include "f" in front of fraction, useful for knowing what frac-
              tion is.

```

### Value

variable names of libraries (character vector)

### See Also

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

### Examples

```

df <- ORFik.template.experiment()
bamVarName(df)

## without libtype
bamVarName(df, skip.libtype = TRUE)
## Without experiment name
bamVarName(df, skip.experiment = TRUE)

```

---

browseSRA

*Open SRA in browser for specific bioproject*

---

### Description

Open SRA in browser for specific bioproject

**Usage**

```
browseSRA(x, browser = getOption("browser"))
```

**Arguments**

**x** character, bioproject ID.

**browser** a non-empty character string giving the name of the program to be used as the HTML browser. It should be in the PATH, or a full path specified. Alternatively, an R function to be called to invoke the browser.

Under Windows NULL is also allowed (and is the default), and implies that the file association mechanism will be used.

**Value**

invisible(NULL), opens webpage only

**See Also**

Other sra: [download.SRA\(\)](#), [download.SRA.metadata\(\)](#), [download.ebi\(\)](#), [get\\_bioproject\\_candidates\(\)](#), [install.sratoolkit\(\)](#), [rename.SRA.files\(\)](#)

**Examples**

```
#browseSRA("PRJNA336542")

#' # For windows make sure a valid browser is defined:
browser <- getOption("browser")
#browseSRA("PRJNA336542", browser)
```

---

canonical\_isoforms      *Get canonical isoforms of organism*

---

**Description**

Search for a txt file at location: `file.path(refFolder(x), "canonical_isoforms.txt")`, where `x` is an ORFik experiment.

**Usage**

```
canonical_isoforms(x)
```

**Arguments**

**x** an ORFik [experiment](#)

**Value**

a character vector

**Examples**

```
df <- ORFik.template.experiment()
canonical_isoforms(df)
```

---

```
canonical_isoforms,experiment-method
Get canonical isoforms of organism
```

---

**Description**

Search for a txt file at location: `file.path(refFolder(x), "canonical_isoforms.txt")`, where `x` is an ORFik experiment.

**Usage**

```
## S4 method for signature 'experiment'
canonical_isoforms(x)
```

**Arguments**

`x` an ORFik [experiment](#)

**Value**

a character vector

**Examples**

```
df <- ORFik.template.experiment()
canonical_isoforms(df)
```

---

```
codon_usage Codon usage
```

---

**Description**

Per AA / codon, analyse the coverage, get a multitude of features. For both A sites and P-sites (Input reads must be P-sites for now) This function takes inspiration from the codonDT paper, and among others returns the negative binomial estimates, but in addition many other features.

**Usage**

```
codon_usage(
  reads,
  cds,
  mrna,
  faFile,
  filter_table,
  filter_cds_mod3 = TRUE,
  min_counts_cds_filter = max(min(quantile(filter_table, 0.5), 1000), 1000),
  with_A_sites = TRUE,
  aligned_position = "center",
  code = GENETIC_CODE
)
```

**Arguments**

reads	either a single library (GRanges, GAlignment, GAlignmentPairs), or a list of libraries returned from outputLibs(df) with p-sites. If list, the list must have names corresponding to the library names.
cds	a GRangesList
mrna	a GRangesList
faFile	a FaFile from genome
filter_table	a matrix / vector of length equal to cds
filter_cds_mod3	logical, default TRUE. Remove all ORFs that are not mod3, this speeds up the computation a lot, and usually removes malformed ORFs you would not want anyway.
min_counts_cds_filter	numeric, default: max(min(quantile(filter_table, 0.50), 100), 100). Minimum number of counts from the 'filter_table' argument.
with_A_sites	logical, default TRUE. Not used yet, will also return A site scores.
aligned_position	what positions should be taken to calculate per-codon coverage. By default: "center", meaning that positions -1,0,1 will be taken. Alternative: "left", then positions 0,1,2 are taken.
code	a named character vector of size 64. Default: GENETIC_CODE. Change if organism does not use the standard code.

**Details**

The primary column to use is "mean\_txNorm", this is the fair normalized score.

**Value**

a data.table of rows per AA:codon. All values are given per library, per site (A or P) per codon type (start, internal, stop), sorted by the mean\_txNorm\_percentage column of the first library in the set, the columns are:

- variable (character) : Library name
- seq (character) : Amino acid:codon , for start codons: Amino acid is #, and stop codons are "\*". So for human, there will be both #:ATG (the start sites), and M:ATG (internal ATGs)
- sum (integer) : total counts per seq
- sum\_txNorm (integer) : total counts per seq normalized per tx
- var (numeric) : variance of total counts per seq
- N (integer) : total number of genes with this codon, per type (start, stop, internal codon)
- N.total (integer) : total number of codons over all genes, per type (start, stop, internal codon)
- mean\_txNorm (numeric) : Default use output, the fair codon usage, normalized both for gene and genome level for codon and read counts
- mean\_txNorm\_percentage : Percentage transform of mean\_txNorm
- dispersion :  $(\text{mean}^2) / (\text{var} - \text{mean})$
- dispersion\_txNorm :  $(\text{mean\_txNorm}^2) / (\text{var\_txNorm} - \text{mean\_txNorm})$
- alpha (numeric) : dirichlet alpha MOM estimator (imagine mean and variance of probability in 1 value, the lower the value, the higher the variance, mean is decided by the relative value between samples)
- sum\_txNorm (integer) : total counts per seq normalized per tx
- relative\_to\_max\_score (integer) : Max scaled percentage of mean\_txNorm\_percentage, so percentage on the ratio of mean\_txNorm\_percentage / max(mean\_txNorm\_percentage)
- type (factor(character)) : "P" or "A"

## References

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196831/>

## See Also

Other codon: `codon_usage_exp()`, `codon_usage_plot()`

## Examples

```
df <- ORFik.template.experiment()[9:10,] # Subset to 2 Ribo-seq libs

## For single library
reads <- fimport(filepath(df[1,], "pshifted"))
cds <- loadRegion(df, "cds", filterTranscripts(df))
mrna <- loadRegion(df, "mrna", names(cds))
filter_table <- assay(countTable(df, type = "summarized")[names(cds)])
faFile <- findFa(df)
res <- codon_usage(reads, cds, mrna, faFile = faFile,
                  filter_table = filter_table, min_counts_cds_filter = 10)
```

codon\_usage\_exp

*Codon analysis for ORFik experiment***Description**

Per AA / codon, analyse the coverage, get a multitude of features. For both A sites and P-sites (Input reads must be P-sites for now) This function takes inspiration from the codonDT paper, and among others returns the negative binomial estimates, but in addition many other features.

**Usage**

```
codon_usage_exp(
  df,
  reads,
  cds = loadRegion(df, "cds", filterTranscripts(df)),
  mrna = loadRegion(df, "mrna", names(cds)),
  filter_cds_mod3 = TRUE,
  filter_table = assay(countTable(df, type = "summarized")[names(cds)]),
  faFile = df@fafile,
  min_counts_cds_filter = max(min(quantile(filter_table, 0.5), 1000), 1000),
  with_A_sites = TRUE,
  code = GENETIC_CODE,
  aligned_position = "center"
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
reads	either a single library (GRanges, GAlignment, GAlignmentPairs), or a list of libraries returned from outputLibs(df) with p-sites. If list, the list must have names corresponding to the library names.
cds	a GRangesList, the coding sequences, default: loadRegion(df, "cds", filterTranscripts(df)), longest isoform per gene.
mrna	a GRangesList, the full mRNA sequences (matching by names the cds sequences), default: loadRegion(df, "mrna", names(cds)).
filter_cds_mod3	logical, default TRUE. Remove all ORFs that are not mod3, this speeds up the computation a lot, and usually removes malformed ORFs you would not want anyway.
filter_table	an numeric(integer) matrix, where rownames are the names of the full set of mRNA transcripts. This will be subsetted to the cds subset you use. Then CDSs are filtered from this table by the 'min_counts_cds_filter' argument.
faFile	<a href="#">FaFile</a> , BSgenome, fasta/index file path or an ORFik <a href="#">experiment</a> . This file is usually used to find the transcript sequences from some GRangesList.

min_counts_cds_filter	numeric, default: $\max(\min(\text{quantile}(\text{filter\_table}, 0.50), 100), 100)$ . Minimum number of counts from the 'filter_table' argument.
with_A_sites	logical, default TRUE. Not used yet, will also return A site scores.
code	a named character vector of size 64. Default: GENETIC_CODE. Change if organism does not use the standard code.
aligned_position	what positions should be taken to calculate per-codon coverage. By default: "center", meaning that positions -1,0,1 will be taken. Alternative: "left", then positions 0,1,2 are taken.

### Details

The primary column to use is "mean\_txNorm", this is the fair normalized score.

### Value

a data.table of rows per AA:codon. All values are given per library, per site (A or P) per codon type (start, internal, stop), sorted by the mean\_txNorm\_percentage column of the first library in the set, the columns are:

- variable (character) : Library name
- seq (character) : Amino acid:codon , for start codons: Amino acid is #, and stop codons are "\*". So for human, there will be both #:ATG (the start sites), and M:ATG (internal ATGs)
- sum (integer) : total counts per seq
- sum\_txNorm (integer) : total counts per seq normalized per tx
- var (numeric) : variance of total counts per seq
- N (integer) : total number of genes with this codon, per type (start, stop, internal codon)
- N.total (integer) : total number of codons over all genes, per type (start, stop, internal codon)
- mean\_txNorm (numeric) : Default use output, the fair codon usage, normalized both for gene and genome level for codon and read counts
- mean\_txNorm\_percentage : Percentage transform of mean\_txNorm
- dispersion :  $(\text{mean}^2) / (\text{var} - \text{mean})$
- dispersion\_txNorm :  $(\text{mean\_txNorm}^2) / (\text{var\_txNorm} - \text{mean\_txNorm})$
- alpha (numeric) : dirichlet alpha MOM estimator (imagine mean and variance of probability in 1 value, the lower the value, the higher the variance, mean is decided by the relative value between samples)
- sum\_txNorm (integer) : total counts per seq normalized per tx
- relative\_to\_max\_score (integer) : Max scaled percentage of mean\_txNorm\_percentage, so percentage on the ratio of mean\_txNorm\_percentage /  $\max(\text{mean\_txNorm\_percentage})$
- type (factor(character)) : "P" or "A"

### References

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196831/>

**See Also**

Other codon: [codon\\_usage\(\)](#), [codon\\_usage\\_plot\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()[9:10,] # Subset to 2 Ribo-seq libs
## For single library
res <- codon_usage_exp(df, fimport(filepath(df[1,], "pshifted")),
  min_counts_cds_filter = 10)
# mean_txNorm is adviced scoring column
# codon_usage_plot(res, res$mean_txNorm)
# Default for plot function is the percentage scaled version of mean_txNorm
# codon_usage_plot(res) # This gives check error
## For multiple libs
res2 <- codon_usage_exp(df, outputLibs(df, type = "pshifted", output.mode = "list"),
  min_counts_cds_filter = 10)
# codon_usage_plot(res2)
```

---

codon_usage_plot	<i>Plot codon_usage</i>
------------------	-------------------------

---

**Description**

Plot codon\_usage

**Usage**

```
codon_usage_plot(
  res,
  score_column = res$relative_to_max_score,
  ylab = "Ribo-seq library",
  legend.position = "none",
  limit = c(0, max(score_column)),
  midpoint = max(limit/2),
  monospace_font = TRUE,
  ignore_start_stop_codons = FALSE
)
```

**Arguments**

<code>res</code>	a data.table of output from a <code>codon_usage</code> function
<code>score_column</code>	numeric, default: <code>res\$relative_to_max_score</code> . Which parameter to use as score column.
<code>ylab</code>	character vector, names for libraries to show on Y axis
<code>legend.position</code>	character, default "none", do not display legend.
<code>limit</code>	numeric, 2 values for plot color limits. Default: <code>c(0, max(score_column))</code>

midpoint            numeric, default:  $\max(\text{limit} / 2)$ . midpoint of color limit.

monospace\_font    logical, default TRUE. Use monospace font, this does not work on systems (require specific font packages), set to FALSE if it crashes for you.

ignore\_start\_stop\_codons  
                    logical, default FALSE. If TRUE, remove start (#) and stop (\*) codons.

**Value**

a ggplot object

**See Also**

Other codon: [codon\\_usage\(\)](#), [codon\\_usage\\_exp\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()[9:10,] # Subset to 2 Ribo-seq libs
## For multiple libs
res2 <- codon_usage_exp(df, outputLibs(df, type = "pshifted", output.mode = "list"),
                       min_counts_cds_filter = 10)
# codon_usage_plot(res2, monospace_font = TRUE) # This gives check error
codon_usage_plot(res2, monospace_font = FALSE) # monospace font looks better
```

---

collapse.fastq            *Very fast fastq/fasta collapser*

---

**Description**

For each unique read in the file, collapse into 1 and state in the fasta header how many reads existed of that type. This is done after trimming usually, works best for reads < 50 read length. Not so effective for 150 bp length mRNA-seq etc.

**Usage**

```
collapse.fastq(
  files,
  outdir = file.path(dirname(files[1]), "collapsed"),
  header.out.format = "ribotoolkit",
  compress = FALSE,
  prefix = "collapsed_"
)
```

**Arguments**

files	paths to fasta / fastq files to collapse. I tries to detect format per file, if file does not have .fastq, .fastq.gz, .fq or fq.gz extensions, it will be treated as a .fasta file format.
outdir	outdir to save files, default: file.path(dirname(files[1]), "collapsed"). Inside same folder as input files, then create subfolder "collapsed", and add a prefix of "collapsed_" to the output names in that folder.
header.out.format	character, default "ribotoolkit", else must be "fastx". How the read header of the output fasta should be formatted: ribotoolkit: ">seq1_x55", sequence 1 has 55 duplicated reads collapsed. fastx: ">1-55", sequence 1 has 55 duplicated reads collapsed
compress	logical, default FALSE
prefix	character, default "collapsed_" Prefix to name of output file.

**Value**

invisible(NULL), files saved to disc in fasta format.

**Examples**

```
seqs <- rep(c("AAAAAAAAAAAAAAAAAAAAAAAAAAAA", "GGGGGGGGGGGGGGGGGGGG"), times = c(2,3))
fastq.folder <- tempfile()
dir.create(fastq.folder)
fastq.file <- file.path(fastq.folder, "test.fasta")
writeXStringSet(DNAStringSet(seqs), filepath = fastq.file)
infiles <- dir(fastq.folder, "fasta|fastq", full.names = TRUE)
collapse.fastq(infiles)
readDNAStringSet(file.path(fastq.folder, "collapsed", "collapsed_test.fasta"))
# You see names says x3 of read 1 (GGGG...) and x2 of read 2 (AAAA...)
```

---

collapseDuplicatedReads

*Collapse duplicated reads*

---

**Description**

For every GRanges, GAlignments read, with the same: seqname, start, (cigar) / width and strand, collapse and give a new meta column called "score", which contains the number of duplicates of that read. If score column already exists, will return input object!

**Usage**

```
collapseDuplicatedReads(x, addScoreColumn = TRUE, ...)
```

**Arguments**

x a GRanges, GAlignments or GAlignmentPairs object

addScoreColumn logical, default: (TRUE), if FALSE, only collapse and not keep score column of counts for collapsed reads. Returns directly without collapsing if reuse.score.column is FALSE and score is already defined.

... alternative arguments for class instances. For example, see: ?'collapseDuplicatedReads,GRanges-method'

**Value**

a GRanges, GAlignments, GAlignmentPairs or data.table object, same as input

**Examples**

```
gr <- rep(GRanges("chr1", 1:10,"+"), 2)
collapseDuplicatedReads(gr)
```

---

collapseDuplicatedReads,data.table-method  
*Collapse duplicated reads*

---

**Description**

For every GRanges, GAlignments read, with the same: seqname, start, (cigar) / width and strand, collapse and give a new meta column called "score", which contains the number of duplicates of that read. If score column already exists, will return input object!

**Usage**

```
## S4 method for signature 'data.table'
collapseDuplicatedReads(
  x,
  addScoreColumn = TRUE,
  addSizeColumn = FALSE,
  reuse.score.column = TRUE,
  keepCigar = FALSE
)
```

**Arguments**

x a GRanges, GAlignments or GAlignmentPairs object

addScoreColumn logical, default: (TRUE), if FALSE, only collapse and not keep score column of counts for collapsed reads. Returns directly without collapsing if reuse.score.column is FALSE and score is already defined.

addSizeColumn logical (FALSE), if TRUE, add a size column that for each read, that gives original width of read. Useful if you need original read lengths. This takes care of soft clips etc. If collapsing reads, each unique range will be grouped also by size.

`reuse.score.column` logical (TRUE), if `addScoreColumn` is TRUE, and a score column exists, will sum up the scores to create a new score. If FALSE, will skip old score column and create new according to number of replicated reads after conversion. If `addScoreColumn` is FALSE, this argument is ignored.

`keepCigar` logical, default FALSE. Keep the cigar information

**Value**

a GRanges, GAlignments, GAlignmentPairs or data.table object, same as input

**Examples**

```
gr <- rep(GRanges("chr1", 1:10, "+"), 2)
collapseDuplicatedReads(gr)
```

---

*collapseDuplicatedReads, GAlignmentPairs-method*  
*Collapse duplicated reads*

---

**Description**

For every GRanges, GAlignments read, with the same: seqname, start, (cigar) / width and strand, collapse and give a new meta column called "score", which contains the number of duplicates of that read. If score column already exists, will return input object!

**Usage**

```
## S4 method for signature 'GAlignmentPairs'
collapseDuplicatedReads(x, addScoreColumn = TRUE)
```

**Arguments**

`x` a GRanges, GAlignments or GAlignmentPairs object

`addScoreColumn` logical, default: (TRUE), if FALSE, only collapse and not keep score column of counts for collapsed reads. Returns directly without collapsing if `reuse.score.column` is FALSE and score is already defined.

**Value**

a GRanges, GAlignments, GAlignmentPairs or data.table object, same as input

**Examples**

```
gr <- rep(GRanges("chr1", 1:10, "+"), 2)
collapseDuplicatedReads(gr)
```

---

collapseDuplicatedReads,GAlignments-method  
*Collapse duplicated reads*

---

### Description

For every GRanges, GAlignments read, with the same: seqname, start, (cigar) / width and strand, collapse and give a new meta column called "score", which contains the number of duplicates of that read. If score column already exists, will return input object!

### Usage

```
## S4 method for signature 'GAlignments'
collapseDuplicatedReads(x, addScoreColumn = TRUE, reuse.score.column = TRUE)
```

### Arguments

`x` a GRanges, GAlignments or GAlignmentPairs object

`addScoreColumn` logical, default: (TRUE), if FALSE, only collapse and not keep score column of counts for collapsed reads. Returns directly without collapsing if reuse.score.column is FALSE and score is already defined.

`reuse.score.column` logical (TRUE), if addScoreColumn is TRUE, and a score column exists, will sum up the scores to create a new score. If FALSE, will skip old score column and create new according to number of replicated reads after conversion. If addScoreColumn is FALSE, this argument is ignored.

### Value

a GRanges, GAlignments, GAlignmentPairs or data.table object, same as input

### Examples

```
gr <- rep(GRanges("chr1", 1:10,"+"), 2)
collapseDuplicatedReads(gr)
```

---

collapseDuplicatedReads,GRanges-method  
*Collapse duplicated reads*

---

### Description

For every GRanges, GAlignments read, with the same: seqname, start, (cigar) / width and strand, collapse and give a new meta column called "score", which contains the number of duplicates of that read. If score column already exists, will return input object!

**Usage**

```
## S4 method for signature 'GRanges'
collapseDuplicatedReads(
  x,
  addScoreColumn = TRUE,
  addSizeColumn = FALSE,
  reuse.score.column = TRUE
)
```

**Arguments**

`x` a GRanges, GAlignments or GAlignmentPairs object

`addScoreColumn` logical, default: (TRUE), if FALSE, only collapse and not keep score column of counts for collapsed reads. Returns directly without collapsing if `reuse.score.column` is FALSE and score is already defined.

`addSizeColumn` logical (FALSE), if TRUE, add a size column that for each read, that gives original width of read. Useful if you need original read lengths. This takes care of soft clips etc. If collapsing reads, each unique range will be grouped also by size.

`reuse.score.column` logical (TRUE), if `addScoreColumn` is TRUE, and a score column exists, will sum up the scores to create a new score. If FALSE, will skip old score column and create new according to number of replicated reads after conversion. If `addScoreColumn` is FALSE, this argument is ignored.

**Value**

a GRanges, GAlignments, GAlignmentPairs or data.table object, same as input

**Examples**

```
gr <- rep(GRanges("chr1", 1:10,"+"), 2)
collapseDuplicatedReads(gr)
```

---

combn.pairs

*Create all unique combinations pairs possible*

---

**Description**

Given a character vector, get all unique combinations of 2.

**Usage**

```
combn.pairs(x)
```

**Arguments**

`x` a character vector, will unique elements for you.

**Value**

a list of character vector pairs

**Examples**

```
df <- ORFik.template.experiment()
ORFik:::combn.pairs(df[, "libtype"])
```

---

computeFeatures	<i>Get all main features in ORFik</i>
-----------------	---------------------------------------

---

**Description**

If you want to get all the NGS and/or sequence features easily, you can use this function. Each feature have a link to an article describing its creation and idea behind it. Look at the functions in the feature family (in the "see also" section below) to see all of them. Example, if you want to know what the "te" column is, check out: [?translationalEff](#).

A short description of each feature is also shown here:

**\*\* NGS features \*\*** If not stated otherwise stated, the feature apply to Ribo-seq.

- countRFP : raw counts of Ribo-seq
- fpkmRFP : FPKM
- fpkmRNA : FPKM of RNA-seq
- te : Translation efficiency Ribo-seq / RNA-seq FPKM
- floss : Fragment length similarity score
- entropyRFP : Positional entropy
- disengagementScores : downstream coverage from ORF
- RRS : Ribosome release score
- RSS : Ribosome staling score
- ORFScores : Periodicity score, does frame 0 have more reads
- ioScore : inside outside score: coverage ORF / coverage rest of transcript
- startCodonCoverage : Coverage over start codon + 2nt before start codon
- startRegionCoverage : Coverage over codon 2 & 3
- startRegionRelative : Peakness of TIS, startCodonCoverage / startRegionCoverage, 0-n

**\*\* Sequence features \*\***

- kozak : Similarity to kozak sequence for organism score, 0-1
- gc : GC percentage, 0-1
- StartCodons : Start codon as a string, "ATG"
- StopCodons : stop codon as a string, "TAA"

- fractionLengths : ORF length compared to transcript, 0-1

**\*\* uORF features \*\***

- distORFCDS : Distance from ORF stop site to CDS, -n:n
- inFrameCDS : Is ORF in frame with downstream CDS, T/F
- isOverlappingCds : Is ORF overlapping with downstream CDS, T/F
- rankInTx : ORF with most upstream start codon is 1, 1-n

## Usage

```
computeFeatures(
  grl,
  RFP,
  RNA = NULL,
  Gtf,
  faFile = NULL,
  riboStart = 26,
  riboStop = 34,
  sequenceFeatures = TRUE,
  uorfFeatures = TRUE,
  grl.is.sorted = FALSE,
  weight.RFP = 1L,
  weight.RNA = 1L
)
```

## Arguments

grl	a <a href="#">GRangesList</a> object with usually ORFs, but can also be either leaders, cds', 3' utrs, etc. This is the regions you want to score.
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
RNA	RnaSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
Gtf	a TxDb object of a gtf file or path to gtf, gff .sqlite etc.
faFile	a path to fasta indexed genome, an open <a href="#">FaFile</a> , a BSgenome, or path to <a href="#">ORFik experiment</a> with valid genome.
riboStart	usually 26, the start of the floss interval, see ?floss
riboStop	usually 34, the end of the floss interval
sequenceFeatures	a logical, default TRUE, include all sequence features, that is: Kozak, fractionLengths, distORFCDS, isInFrame, isOverlapping and rankInTx. uorfFeatures = FALSE will remove the 4 last.
uorfFeatures	a logical, default TRUE, include all uORF sequence features, that is: distORFCDS, isInFrame, isOverlapping and rankInTx
grl.is.sorted	logical (F), a speed up if you know argument grl is sorted, set this to TRUE.
weight.RFP	a vector (default: 1L). Can also be character name of column in RFP. As in translationalEff(weight = "score") for: GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times.
weight.RNA	Same as weightRFP but for RNA weights. (default: 1L)

**Details**

If you used CageSeq to reannotate your leaders, your txDB object must contain the reassigned leaders. Use [reassignTxDbByCage()] to get the txdb.

As a note the library is reduced to only reads overlapping 'tx', so the library size in fpkm calculation is done on this subset. This will help remove rRNA and other contaminants.

Also if you have only unique reads with a weight column, explaining the number of duplicated reads, set weights to make calculations correct. See [getWeights](#)

**Value**

a data.table with scores, each column is one score type, name of columns are the names of the scores, i.g [floss()] or [fpkm()]

**See Also**

Other features: [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
# Here we make an example from scratch
# Usually the ORFs are found in orfik, which makes names for you etc.
gtf <- system.file("extdata/references/danio_rerio", "annotations.gtf",
  package = "ORFik") ## location of the gtf file

suppressWarnings(txdb <- loadTxdb(gtf))
# use cds' as ORFs for this example
ORFs <- loadRegion(txdb, "cds")
ORFs <- makeORFNames(ORFs) # need ORF names
# make Ribo-seq data,
RFP <- unlistGrl(firstExonPerGroup(ORFs))
computeFeatures(ORFs, RFP, Gtf = txdb)
# For more details see vignettes.
```

---

computeFeaturesCage    *Get all main features in ORFik*

---

**Description**

If you have a txdb with correctly reassigned transcripts, use: [computeFeatures()]

**Usage**

```
computeFeaturesCage(
  grl,
  RFP,
  RNA = NULL,
  Gtf = NULL,
  tx = NULL,
  fiveUTRs = NULL,
  cds = NULL,
  threeUTRs = NULL,
  faFile = NULL,
  riboStart = 26,
  riboStop = 34,
  sequenceFeatures = TRUE,
  uorfFeatures = TRUE,
  grl.is.sorted = FALSE,
  weight.RFP = 1L,
  weight.RNA = 1L
)
```

**Arguments**

grl	a <a href="#">GRangesList</a> object with usually ORFs, but can also be either leaders, cds', 3' utrs, etc. This is the regions you want to score.
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
RNA	RnaSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
Gtf	a TxDb object of a gtf file or path to gtf, gff .sqlite etc.
tx	a <a href="#">GRangesList</a> of transcripts, normally called from: exonsBy(Gtf, by = "tx", use.names = T) only add this if you are not including Gtf file If you are using CAGE, you do not need to reassign these to the cage peaks, it will do it for you.
fiveUTRs	fiveUTRs as <a href="#">GRangesList</a> , if you used cage-data to extend 5' utrs, remember to input CAGE assigned version and not original!
cds	a <a href="#">GRangesList</a> of coding sequences
threeUTRs	a <a href="#">GRangesList</a> of transcript 3' utrs, normally called from: threeUTRsByTranscript(Gtf, use.names = T)
faFile	a path to fasta indexed genome, an open <a href="#">FaFile</a> , a BSgenome, or path to <a href="#">ORFik experiment</a> with valid genome.
riboStart	usually 26, the start of the floss interval, see ?floss
riboStop	usually 34, the end of the floss interval
sequenceFeatures	a logical, default TRUE, include all sequence features, that is: Kozak, fractionLengths, distORFCDS, isInFrame, isOverlapping and rankInTx. uorfFeatures = FALSE will remove the 4 last.
uorfFeatures	a logical, default TRUE, include all uORF sequence features, that is: distORFCDS, isInFrame, isOverlapping and rankInTx

`grl.is.sorted` logical (F), a speed up if you know argument `grl` is sorted, set this to TRUE.

`weight.RFP` a vector (default: 1L). Can also be character name of column in RFP. As in `translationalEff(weight = "score")` for: `GRanges("chr1", 1, "+", score = 5)`, would mean score column tells that this alignment region was found 5 times.

`weight.RNA` Same as `weightRFP` but for RNA weights. (default: 1L)

### Details

A specialized version if you don't have a correct txdb, for example with CAGE reassigned leaders while txdb is not updated. It is 2x faster for tested data. The point of this function is to give you the ability to input transcript etc directly into the function, and not load them from txdb. Each feature have a link to an article describing feature, try `?floss`

### Value

a data.table with scores, each column is one score type, name of columns are the names of the scores, i.g `[floss()]` or `[fpkm()]`

### See Also

Other features: [computeFeatures\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
# a small example without cage-seq data:
# we will find ORFs in the 5' utrs
# and then calculate features on them

if (requireNamespace("BSgenome.Hsapiens.UCSC.hg19")) {
  library(GenomicFeatures)
  # Get the gtf txdb file
  txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
    package = "GenomicFeatures")
  txdb <- loadDb(txdbFile)

  # Extract sequences of fiveUTRs.
  fiveUTRs <- fiveUTRsByTranscript(txdb, use.names = TRUE)[1:10]
  faFile <- BSgenome.Hsapiens.UCSC.hg19::Hsapiens
  tx_seqs <- extractTranscriptSeqs(faFile, fiveUTRs)

  # Find all ORFs on those transcripts and get their genomic coordinates
  fiveUTR_ORFs <- findMapORFs(fiveUTRs, tx_seqs)
  unlistedORFs <- unlistGr1(fiveUTR_ORFs)
  # group GRanges by ORFs instead of Transcripts
  fiveUTR_ORFs <- groupGRangesBy(unlistedORFs, unlistedORFs$names)

  # make some toy ribo seq and rna seq data
```

```

starts <- unlistGrl(ORFik::firstExonPerGroup(fiveUTR_ORFs))
RFP <- promoters(starts, upstream = 0, downstream = 1)
score(RFP) <- rep(29, length(RFP)) # the original read widths

# set RNA seq to duplicate transcripts
RNA <- unlistGrl(exonsBy(txdb, by = "tx", use.names = TRUE))

#ORFik::computeFeaturesCage(grl = fiveUTR_ORFs, RFP = RFP,
# RNA = RNA, Gtf = txdb, faFile = faFile)

}
# See vignettes for more examples

```

---

config

*Read directory config for ORFik experiments*

---

## Description

Defines a folder for: 1. fastq files (raw data)  
 2. bam files (processed data)  
 3. references (organism annotation and STAR index)  
 4. experiments (Location to store and load all [experiment](#) .csv files) Update or use another config using `config.save()` function.

## Usage

```

config(
  file = config_file(old_config_location = old_config_location),
  old_config_location = "~/Bio_data/ORFik_config.csv"
)

```

## Arguments

`file` location of config csv, default: `config_file(old_config_location = old_config_location)`  
`old_config_location` path, old config location before BiocFileCache implementation. Will copy this to cache directory and delete old version. This is done to follow bioc rules on not writing to user home directory.

## Value

a named character vector of length 3

## Examples

```

## Make with default config path
#config()

```

---

config.exper	<i>Set directories for experiment</i>
--------------	---------------------------------------

---

### Description

- Defines a folder for: 1. fastq files (raw\_data)
- 2. bam files (processed data)
- 3. references (organism annotation and STAR index)
- 4. Experiment (name of experiment)

### Usage

```
config.exper(
  experiment,
  assembly,
  type,
  config = ORFik::config(),
  sub_dir_single = file.path(type, experiment, ""),
  name_with_type_suffix = TRUE
)
```

### Arguments

experiment	short name of experiment (must be valid as a folder name)
assembly	name of organism and assembly (must be valid as a folder name)
type	name of sequencing type, Ribo-seq, RNA-seq, CAGE.. Can be more than one.
config	a named character vector of length 3, default: ORFik::config()
sub_dir_single	character, path. Default: file.path(type, experiment, "") The subdirectory relative to config defined main locations. If defined location should be used directly without making subdirectories, set to "".
name_with_type_suffix	logical, default TRUE. Make fastq name like 'fastq RNA-seq', setting it to FALSE gives name 'fastq'. Only allowed when length(type) == 1

### Value

named character vector of paths for experiment

### Examples

```
# Where should files go in general?
ORFik::config()
# Paths for project: "Alexaki_Human" containing Ribo-seq and RNA-seq:
#config.exper("Alexaki_Human", "Homo_sapiens_GRCh38_101", c("Ribo-seq", "RNA-seq"))
```

---

 config.save

*Save/update directory config for ORFik experiments*


---

### Description

Defines a folder for fastq files (raw\_data), bam files (processed data) and references (organism annotation and STAR index)

### Usage

```
config.save(
  file = config_file(),
  fastq.dir = file.path(base.dir, "raw_data"),
  bam.dir = file.path(base.dir, "processed_data"),
  reference.dir = file.path(base.dir, "references"),
  exp.dir = file.path(base.dir, "ORFik_experiments/"),
  base.dir = "~/Bio_data",
  conf = data.frame(type = c("fastq", "bam", "ref", "exp"), directory = c(fastq.dir,
    bam.dir, reference.dir, exp.dir))
)
```

### Arguments

file	location of config csv, default: config_file(old_config_location = old_config_location)
fastq.dir	directory where ORFik puts fastq file directories, default: file.path(base.dir, "raw_data"), which is retrieved with: config()["fastq"]
bam.dir	directory where ORFik puts bam file directories, default: file.path(base.dir, "processed_data"), which is retrieved with: config()["bam"]
reference.dir	directory where ORFik puts reference file directories, default: file.path(base.dir, "references"), which is retrieved with: config()["ref"]
exp.dir	directory where ORFik puts experiment csv files, default: file.path(base.dir, "ORFik_experiments/"), which is retrieved with: config()["exp"]
base.dir	base directory for all output directories, default: "~/Bio_data"
conf	data.frame of complete conf object, default: data.frame(type = c("fastq", "bam", "ref", "exp"), directory = c(fastq.dir, bam.dir, reference.dir, exp.dir))

### Value

invisible(NULL), file saved to disc

### Examples

```
# Overwrite default config, with new base directory for files
#config.save(base.dir = "/media/Bio_data/") # Output files go here instead
# of ~/Bio_data
## Dont do this, but for understanding here is how to make a second config
```

```
#new_config_path <- config_file(query = "ORFik_config_2")
#config.save(new_config_path, "/media/Bio_data/raw_data/",
# "/media/Bio_data/processed_data", /media/Bio_data/references/)
```

---

config\_file                      *Get path for ORFik config in cache*

---

### Description

Get path for ORFik config in cache

### Usage

```
config_file(
  cache = BiocFileCache::getBFCOption("CACHE"),
  query = "ORFik_config",
  ask = interactive(),
  old_config_location = "~/Bio_data/ORFik_config.csv"
)
```

### Arguments

cache                      path to bioc cache directory with rname from query argument. Default is: BiocFileCache::getBFCOption("CACHE") For info, see: [BiocFileCache::BiocFileCache()]

query                      default: "ORFik\_config". Exact rname of the file in cache.

ask                        logical, default interactive().

old\_config\_location        path, old config location before BiocFileCache implementation. Will copy this to cache directory and delete old version. This is done to follow bioc rules on not writing to user home directory.

### Value

a file path in cache

### Examples

```
config_file()
# Another config path
config_file(query = "ORFik_config_2")
```

---

convert\_bam\_to\_ofst     *Convert libraries to ofst*

---

### Description

Saved by default in folder "ofst" relative to default libraries of experiment. Speeds up loading of full files compared to bam by large margins.

### Usage

```
convert_bam_to_ofst(
  df,
  in_files = filepath(df, "default"),
  out_dir = file.path(libFolder(df, unique_mappers = only_unique_mappers), "ofst"),
  verbose = TRUE,
  strandMode = rep(0, length(in_files)),
  only_unique_mappers = uniqueMappers(df)
)
```

### Arguments

df	an ORFik <a href="#">experiment</a> , or NULL is allowed if both in_files and out_dir is specified manually.
in_files	paths to input files, default: filepath(df, "default") with bam format files.
out_dir	paths to output files, default file.path(libFolder(df), "cov_RLE").
verbose	logical, default TRUE, message about library output status.
strandMode	numeric, default 0. Only used for paired end bam files. One of (0: strand = *, 1: first read of pair is +, 2: first read of pair is -). See ?strandMode. Note: Sets default to 0 instead of 1, as readGAlignmentPairs uses 1. This is to guarantee hits, but will also make mismatches of overlapping transcripts in opposite directions.
only_unique_mappers	logical, default uniqueMappers(df). Load file of only unique format type, located in './unique_mappers' relative to bam files / default files. See ?uniqueMappers for more information.

### Details

If you want to keep bam files loaded or faster conversion if you already have them loaded, use ORFik::convertLibs instead

### Value

invisible(NULL), files saved to disc

**See Also**

Other lib\_converters: [convertLibs\(\)](#), [convert\\_to\\_bigWig\(\)](#), [convert\\_to\\_covRle\(\)](#), [convert\\_to\\_covRleList\(\)](#)

**Examples**

```
df <- ORFik.template.experiment.zf()
## Usually do default folder, here we use tmpdir
folder_to_save <- file.path(tmpdir(), "ofst")
convert_bam_to_ofst(df, out_dir = folder_to_save)
fimport(file.path(folder_to_save, "ribo-seq.ofst"))
folder_to_save_unique <- file.path(tmpdir(), "unique_mappers", "ofst")
convert_bam_to_ofst(df, out_dir = folder_to_save_unique, only_unique_mappers = TRUE)
fimport(file.path(folder_to_save_unique, "ribo-seq.ofst"))
```

---

convert\_to\_bigWig      *Convert to BigWig*

---

**Description**

Convert to BigWig

**Usage**

```
convert_to_bigWig(
  df,
  in_files = filepath(df, "pshifted"),
  out_dir = file.path(libFolder(df), "bigwig"),
  split.by.strand = TRUE,
  split.by.readlength = FALSE,
  seq_info = seqinfo(df),
  weight = "score",
  is_pre_collapsed = FALSE,
  verbose = TRUE
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a> , or NULL is allowed if both in_files and out_dir is specified manually.
in_files	paths to input files, default pshifted files: <code>filepath(df, "pshifted")</code> in ofst format
out_dir	paths to output files, default <code>file.path(libFolder(df), "bigwig")</code> .
split.by.strand	logical, default TRUE, split into forward and reverse strand RleList inside covRle object.

split.by.readlength  
 logical, default FALSE, split into files for each readlength, defined by readWidths(x) for each file.

seq\_info  
 SeqInfo object, default seqinfo(findFa(df))

weight  
 integer, numeric or single length character. Default "score". Use score column in loaded in\_files.

is\_pre\_collapsed  
 logical, default FALSE. Have you already collapsed reads with collapse.by.scores, so each positions is only in 1 GRanges object with a score column per readlength? Set to TRUE, only if you are sure, will give a speedup.

verbose  
 logical, default TRUE, message about library output status.

**Value**

invisible(NULL), files saved to disc

**See Also**

Other lib\_converters: [convertLibs\(\)](#), [convert\\_bam\\_to\\_ofst\(\)](#), [convert\\_to\\_covRle\(\)](#), [convert\\_to\\_covRleList\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()[10,]
## Usually do default folder, here we use tmpdir
folder_to_save <- file.path(tmpdir(), "bigwig")
convert_to_bigWig(df, out_dir = folder_to_save)
fimport(file.path(folder_to_save, c("RFP_Mutant_rep2_forward.bigWig",
  "RFP_Mutant_rep2_reverse.bigWig")))
```

---

convert\_to\_covRle      *Convert libraries to covRle*

---

**Description**

Saved by default in folder "cov\_RLE" relative to default libraries of experiment

**Usage**

```
convert_to_covRle(
  df,
  in_files = filepath(df, "pshifted"),
  out_dir = file.path(libFolder(df), "cov_RLE"),
  split.by.strand = TRUE,
  split.by.readlength = FALSE,
  seq_info = seqinfo(df),
  weight = "score",
  format = "qs",
  verbose = TRUE
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a> , or NULL is allowed if both in_files and out_dir is specified manually.
in_files	paths to input files, default pshifted files: filepath(df, "pshifted") in ofst format
out_dir	paths to output files, default file.path(libFolder(df), "cov_RLE").
split.by.strand	logical, default TRUE, split into forward and reverse strand RleList inside covRle object.
split.by.readlength	logical, default FALSE, split into files for each readlength, defined by readWidths(x) for each file.
seq_info	SeqInfo object, default seqinfo(findFa(df))
weight	integer, numeric or single length character. Default "score". Use score column in loaded in_files.
format	character, default "qs", alternative "rds". File format to save R object.
verbose	logical, default TRUE, message about library output status.

**Value**

invisible(NULL), files saved to disc

**See Also**

Other lib\_converters: [convertLibs\(\)](#), [convert\\_bam\\_to\\_ofst\(\)](#), [convert\\_to\\_bigWig\(\)](#), [convert\\_to\\_covRleList\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()[10,]
## Usually do default folder, here we use tmpdir
folder_to_save <- file.path(tmpdir(), "cov_RLE")
convert_to_covRle(df, out_dir = folder_to_save)
fimport(file.path(folder_to_save, "RFP_Mutant_rep2.covqs"))
```

---

convert\_to\_covRleList *Convert libraries to covRleList objects*

---

**Description**

Useful to store reads separated by readlength, for much faster coverage calculation. Saved by default in folder "cov\_RLE\_List" relative to default libraries of experiment

**Usage**

```

convert_to_covRleList(
  df,
  in_files = filepath(df, "pshifted"),
  out_dir = file.path(libFolder(df), "cov_RLE_List"),
  out_dir_merged = file.path(libFolder(df), "cov_RLE"),
  split.by.strand = TRUE,
  seq_info = seqinfo(df),
  weight = "score",
  format = "qs",
  verbose = TRUE
)

```

**Arguments**

<code>df</code>	an ORFik <a href="#">experiment</a> , or NULL is allowed if both <code>in_files</code> and <code>out_dir</code> is specified manually.
<code>in_files</code>	paths to input files, default pshifted files: <code>filepath(df, "pshifted")</code> in ofst format
<code>out_dir</code>	paths to output files, default <code>file.path(libFolder(df), "cov_RLE_List")</code> .
<code>out_dir_merged</code>	character vector of paths, default: <code>file.path(libFolder(df), "cov_RLE")</code> . Paths to merged output files, Set to NULL to skip making merged covRle.
<code>split.by.strand</code>	logical, default TRUE, split into forward and reverse strand RleList inside covRle object.
<code>seq_info</code>	SeqInfo object, default <code>seqinfo(findFa(df))</code>
<code>weight</code>	integer, numeric or single length character. Default "score". Use score column in loaded <code>in_files</code> .
<code>format</code>	character, default "qs", alternative "rds". File format to save R object.
<code>verbose</code>	logical, default TRUE, message about library output status.

**Value**

invisible(NULL), files saved to disc

**See Also**

Other lib\_converters: [convertLibs\(\)](#), [convert\\_bam\\_to\\_ofst\(\)](#), [convert\\_to\\_bigWig\(\)](#), [convert\\_to\\_covRle\(\)](#)

**Examples**

```

df <- ORFik.template.experiment()[10,]
## Usually do default folder, here we use tmpdir
folder_to_save <- file.path(tempdir(), "cov_RLE_List")
folder_to_save_merged <- file.path(tempdir(), "cov_RLE")
ORFik:::convert_to_covRleList(df, out_dir = folder_to_save,
out_dir_merged = folder_to_save_merged)
fimport(file.path(folder_to_save, "RFP_Mutant_rep2.covqs"))

```

---

convert_to_fstWig	<i>Convert to fstwig</i>
-------------------	--------------------------

---

### Description

Will split files by chromosome for faster loading for now. This feature might change in the future!

### Usage

```
convert_to_fstWig(
  df,
  in_files = filepath(df, "pshifted"),
  out_dir = file.path(libFolder(df), "fstwig"),
  split.by.strand = TRUE,
  split.by.readlength = FALSE,
  seq_info = seqinfo(df),
  weight = "score",
  is_pre_collapsed = FALSE,
  verbose = TRUE
)
```

### Arguments

df	an ORFik <a href="#">experiment</a> , or NULL is allowed if both in_files and out_dir is specified manually.
in_files	paths to input files, default pshifted files: filepath(df, "pshifted") in ofst format
out_dir	paths to output files, default file.path(libFolder(df), "bigwig").
split.by.strand	logical, default TRUE, split into forward and reverse strand RleList inside covRle object.
split.by.readlength	logical, default FALSE, split into files for each readlength, defined by readWidths(x) for each file.
seq_info	SeqInfo object, default seqinfo(findFa(df))
weight	integer, numeric or single length character. Default "score". Use score column in loaded in_files.
is_pre_collapsed	logical, default FALSE. Have you already collapsed reads with collapse.by.scores, so each positions is only in 1 GRanges object with a score column per readlength? Set to TRUE, only if you are sure, will give a speedup.
verbose	logical, default TRUE, message about library output status.

### Value

invisible(NULL), files saved to disc

---

 convertLibs

*Converted format of NGS libraries*


---

### Description

Export as either .ofst, .wig, .bigWig, .bedo (legacy format) or .bedoc (legacy format) files:

Export files as .ofst for fastest load speed into R.

Export files as .wig / bigWig for use in IGV or other genome browsers.

The input files are checked if they exist from: envExp(df).

### Usage

```
convertLibs(
  df,
  out.dir = libFolder(df),
  addScoreColumn = TRUE,
  addSizeColumn = TRUE,
  must.overlap = NULL,
  method = "None",
  type = "ofst",
  input.type = "ofst",
  reassign.when.saving = FALSE,
  envir = envExp(df),
  force = TRUE,
  library.names = bamVarName(df),
  libs = outputLibs(df, type = input.type, chrStyle = must.overlap, library.names =
    library.names, output.mode = "list", force = force, BPPARAM = BPPARAM),
  BPPARAM = bpparam()
)
```

### Arguments

df	an ORFik <a href="#">experiment</a>
out.dir	optional output directory, default: libFolder(df), if it is NULL, it will just reassign R objects to simplified libraries. Will then create a final folder specified as: paste0(out.dir, "/", type, "/"). Here the files will be saved in format given by the type argument.
addScoreColumn	logical, default TRUE, if FALSE will not add replicate numbers as score column, see ORFik::convertToOneBasedRanges.
addSizeColumn	logical, default TRUE, if FALSE will not add size (width) as size column, see ORFik::convertToOneBasedRanges. Does not apply for (GAlignment version of ofst) or .bedoc. Since they contain the original cigar.
must.overlap	default (NULL), else a GRanges / GRangesList object, so only reads that overlap (must.overlap) are kept. This is useful when you only need the reads over transcript annotation or subset etc.

method	character, default "None", the method to reduce ranges, for more info see <a href="#">convertToOneBasedRanges</a>
type	character, output format, default "ofst". Alternatives: "ofst", "bigWig", "wig", "bedo" or "bedoc". Which format you want. Will make a folder within out.dir with this name containing the files.
input.type	character, input type "ofst". Remember this function uses the loaded libraries if existing, so this argument is usually ignored. Only used if files do not already exist.
reassign.when.saving	logical, default FALSE. If TRUE, will reassign library to converted form after saving. Ignored when out.dir = NULL.
envir	environment to save to, default envExp(df), which defaults to .GlobalEnv, but can be set with envExp(df) <- new.env() etc.
force	logical, default TRUE. If TRUE, reload library files even if matching named variables are found in environment used by experiment (see <a href="#">envExp</a> ) A simple way to make sure correct libraries are always loaded. FALSE is faster if data is loaded correctly already.
library.names	character vector, names of libraries, default: name_decider(df, naming)
libs	list, output of outputLibs as list of GRanges/GAlignments/GAlignmentPairs objects. Set input.type and force arguments to define parameters.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

## Details

We advice you to not use this directly, as other function are more safe for library type conversions. See family description below. This is mostly used internally in ORFik. It is only advised to use if large bam files are already loaded in R and conversions are wanted from those.

See [export.ofst](#), [export.wiggle](#), [export.bedo](#) and [export.bedoc](#) for information on file formats.

If libraries of the experiment are already loaded into environment (default: .globalEnv) is will export using those files as templates. If they are not in environment the .ofst files from the bam files are loaded (unless you are converting to .ofst then the .bam files are loaded).

## Value

invisible NULL (saves files to disc or R .GlobalEnv)

## See Also

Other lib\_converters: [convert\\_bam\\_to\\_ofst\(\)](#), [convert\\_to\\_bigWig\(\)](#), [convert\\_to\\_covRle\(\)](#), [convert\\_to\\_covRleList\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
#convertLibs(df, out.dir = NULL)
# Keep only 5' ends of reads
#convertLibs(df, out.dir = NULL, method = "5prime")
```

---

```
convertToOneBasedRanges
```

*Convert a GRanges Object to 1 width reads*

---

**Description**

There are 5 ways of doing this

1. Take 5' ends, reduce away rest (5prime)
2. Take 3' ends, reduce away rest (3prime)
3. Tile to 1-mers and include all (tileAll)
4. Take middle point per GRanges (middle)
5. Get original with metacolumns (None)

You can also do multiple at a time, then output is GRangesList, where each list group is the operation (5prime is [1], 3prime is [2] etc)

Many other ways to do this have their own functions, like startSites and stopSites etc. To retain information on original width, set addSizeColumn to TRUE. To compress data, 1 GRanges object per unique read, set addScoreColumn to TRUE. This will give you a score column with how many duplicated reads there were in the specified region.

**Usage**

```
convertToOneBasedRanges(
  gr,
  method = "5prime",
  addScoreColumn = FALSE,
  addSizeColumn = FALSE,
  after.softclips = TRUE,
  along.reference = FALSE,
  reuse.score.column = TRUE
)
```

**Arguments**

gr	GRanges, GAlignment or GAlignmentPairs object to reduce.
method	character, default "5prime", the method to reduce ranges, see NOTE for more info.
addScoreColumn	logical (FALSE), if TRUE, add a score column that sums up the hits per unique range. This will make each read unique, so that each read is 1 time, and score column gives the number of collapsed hits. A useful compression. If addSizeColumn is FALSE, it will not differentiate between reads with same start

	and stop, but different length. If addSizeColumn is FALSE, it will remove it. Collapses after conversion.
addSizeColumn	logical (FALSE), if TRUE, add a size column that for each read, that gives original width of read. Useful if you need original read lengths. This takes care of soft clips etc. If collapsing reads, each unique range will be grouped also by size.
after.softclips	logical (TRUE), include softclips in width. Does not apply if along.reference is TRUE.
along.reference	logical (FALSE), example: The cigar "26MI2" is by default width 28, but if along.reference is TRUE, it will be 26. The length of the read along the reference. Also "1D20M" will be 21 if by along.reference is TRUE. Intronic regions (cigar: N) will be removed. So: "1M200N19M" is 20, not 220.
reuse.score.column	logical (TRUE), if addScoreColumn is TRUE, and a score column exists, will sum up the scores to create a new score. If FALSE, will skip old score column and create new according to number of replicated reads after conversion. If addScoreColumn is FALSE, this argument is ignored.

## Details

NOTE: Note: For cigar based ranges (GAlignments), the 5' end is the first non clipped base (neither soft clipped or hard clipped from 5'). This is following the default of bioconductor. For special case of GAlignmentPairs, 5prime will only use left (first) 5' end and read and 3prime will use only right (last) 3' end of read in pair. tileAll and middle can possibly find point that are not in the reads since: lets say pair is 1-5 and 10-15, middle is 7, which is not in the read.

## Value

Converted GRanges object

## See Also

Other utils: [bedToGR\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

## Examples

```
gr <- GRanges("chr1", 1:10, "+")
# 5 prime ends
convertToOneBasedRanges(gr)
# is equal to convertToOneBasedRanges(gr, method = "5prime")
# 3 prime ends
convertToOneBasedRanges(gr, method = "3prime")
# With lengths
convertToOneBasedRanges(gr, addSizeColumn = TRUE)
# With score (# of replicates)
gr <- rep(gr, 2)
convertToOneBasedRanges(gr, addSizeColumn = TRUE, addScoreColumn = TRUE)
```

---

cor_plot	<i>Get correlation between columns</i>
----------	--

---

**Description**

Get correlation between columns

**Usage**

```
cor_plot(
  dt_cor,
  col = c(low = "blue", high = "red", mid = "white", na.value = "white"),
  limit = c(ifelse(min(dt_cor$Cor, na.rm = TRUE) < 0, -1, 0), 1),
  midpoint = mean(limit),
  label_name = "Pearson\nCorrelation",
  text_size = 4,
  legend.position = c(0.4, 0.7),
  legend.direction = "horizontal"
)
```

**Arguments**

dt_cor	a data.table, with column Cor
col	colors c(low = "blue", high = "red", mid = "white", na.value = "white")
limit	default (-1, 1), defined by: c(ifelse(min(dt_cor\$Cor, na.rm = TRUE) < 0, -1, 0), 1)
midpoint	midpoint of correlation values in label coloring.
label_name	name of correlation method, default "Pearson Correlation" with newline after Pearson.
text_size	size of correlation numbers
legend.position	default c(0.4, 0.7), other: "top", "right", ..
legend.direction	default "horizontal", or "vertical"

**Value**

a ggplot (heatmap)

---

cor_table	<i>Get correlation between columns</i>
-----------	--

---

**Description**

Get correlation between columns

**Usage**

```
cor_table(
  dt,
  method = c("pearson", "spearman")[1],
  upper_triangle = TRUE,
  decimals = 2,
  melt = TRUE,
  na.rm.melt = TRUE
)
```

**Arguments**

dt	a data.table
method	c("pearson", "spearman")[1]
upper_triangle	logical, default TRUE. Make lower triangle values NA.
decimals	numeric, default 2. How many decimals for correlation
melt	logical, default TRUE.
na.rm.melt	logical, default TRUE. Remove NA values from melted table.

**Value**

a data.table with 3 columns, Var1, Var2 and Cor

---

correlation.plots	<i>Correlation plots between all samples</i>
-------------------	--

---

**Description**

Get correlation plot of raw counts and/or  $\log_2(\text{count} + 1)$  over selected region in: c("mrna", "leaders", "cds", "trailers")

Note on correlation: Pearson correlation, using pairwise observations to fill in NA values for the covariance matrix.

**Usage**

```
correlation.plots(
  df,
  output.dir,
  region = "mrna",
  type = "fpkm",
  height = 400,
  width = 400,
  size = 0.15,
  plot.ext = ".pdf",
  complex.correlation.plots = TRUE,
  data_for_pairs = countTable(df, region, type = type),
  as_gg_list = FALSE,
  text_size = 4,
  method = c("pearson", "spearman")[1]
)
```

**Arguments**

<code>df</code>	an ORFik <a href="#">experiment</a>
<code>output.dir</code>	directory to save to, named : <code>cor_plot</code> , <code>cor_plot_log2</code> and/or <code>cor_plot_simple</code> with either <code>.pdf</code> or <code>.png</code>
<code>region</code>	a character (default: <code>mrna</code> ), make raw count matrices of whole mrnas or one of (leaders, cds, trailers)
<code>type</code>	which value to use, "fpkm", alternative "counts".
<code>height</code>	numeric, default 400 (in mm)
<code>width</code>	numeric, default 400 (in mm)
<code>size</code>	numeric, size of dots, default 0.15. Deprecated.
<code>plot.ext</code>	character, default: ".pdf". Alternatives: ".png" or ".jpg".
<code>complex.correlation.plots</code>	logical, default TRUE. Add in addition to simple correlation plot two computationally heavy dots + correlation plots. Useful for deeper analysis, but takes longer time to run, especially on low-quality gpu computers. Set to FALSE to skip these.
<code>data_for_pairs</code>	a data.table from <code>ORFik::countTable</code> of counts wanted. Default is fpkm of all mRNA counts over all libraries.
<code>as_gg_list</code>	logical, default FALSE. Return as a list of ggplot objects instead of as a grob. Gives you the ability to modify plots more directly.
<code>text_size</code>	size of correlation numbers
<code>method</code>	<code>c("pearson", "spearman")[1]</code>

**Value**

invisible(NULL) / if `as_gg_list` is TRUE, return a list of raw plots.

---

countOverlapsW	<i>CountOverlaps with weights</i>
----------------	-----------------------------------

---

**Description**

Similar to countOverlaps, but takes an optional weight column. This is usually the score column

**Usage**

```
countOverlapsW(query, subject, weight = NULL, ...)
```

**Arguments**

query	IRanges, IRangesList, GRanges, GRangesList object. Usually transcript a transcript region.
subject	GRanges, GRangesList, GAlignment or covRle, usually reads.
weight	(default: NULL), if defined either numeric or character name of valid meta column in subject. If weight is single numeric, it is used for all. A normal weight is the score column given as weight = "score". GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times. Ignored if subject is covRle.
...	additional arguments passed to countOverlaps/findOverlaps

**Value**

a named vector of number of overlaps to subject weighed by 'weight' column.

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
gr1 <- GRanges(seqnames="chr1",
               ranges=IRanges(start = c(4, 9, 10, 30),
                              end = c(4, 15, 20, 31)),
               strand="+")
gr2 <- GRanges(seqnames="chr1",
               ranges=IRanges(start = c(1, 4, 15, 25),
                              end = c(2, 4, 20, 26)),
               strand=c("+"),
               score=c(10, 20, 15, 5))
countOverlaps(gr1, gr2)
countOverlapsW(gr1, gr2, weight = "score")
```

---

countTable	<i>Extract count table directly from experiment</i>
------------	---

---

### Description

Used to quickly load pre-created read count tables to R.

If df is experiment: Extracts by getting /QC\_STATS directory, and searching for region Requires [ORFikQC](#) to have been run on experiment, to get default count tables!

### Usage

```
countTable(
  df,
  region = "mrna",
  type = "count",
  collapse = FALSE,
  count.folder = "default",
  full_path = countTablePath(df, region, count.folder)
)
```

### Arguments

df	an ORFik <a href="#">experiment</a> or path to folder with countTables, use path if not same folder as experiment libraries. Will subset to the count tables specified if df is experiment. If experiment has 4 rows and you subset it to only 2, then only those 2 count tables will be outputted.
region	a character vector (default: "mrna"), make raw count matrices of whole mrnas or one of (leaders, cds, trailers).
type	character, default: "count" (raw counts matrix). Which object type and normalization do you want ? "summarized" (SummarizedExperiment object), "deseq" (Deseq2 experiment, design will be all valid non-unique columns except replicates, change by using DESeq2::design, normalization alternatives are: "fpkm", "log2fpkm" or "log10fpkm").
collapse	a logical/character (default FALSE), if TRUE all samples within the group SAMPLE will be collapsed to one. If "all", all groups will be merged into 1 column called merged_all. Collapse is defined as rowSum(elements_per_group) / ncol(elements_per_group)
count.folder	character, default "auto" (Use count tables from original bam files stored in "QC_STATS", these are like HTseq count tables). To load your custome count tables from pshifted reads, set to "pshifted" (remember to create the pshifted tables first!). If you have custom ranges, like reads over uORFs stored in a folder called "/uORFs" relative to the bam files, set to "uORFs". Always create these custom count tables with <a href="#">makeSummarizedExperimentFromBam</a> . Always make the location of the folder directly inside the bam file directory!
full_path	Full path to countTable, default: countTablePath(df, region, count.folder)

## Details

If df is path to folder: Loads the the file in that directory with the regex region.rds, where region is what is defined by argument, if multiple exist, see if any start with "countTable\_", if so, subset. If loaded as SummarizedExperiment or deseq, the colData will be made from ORFik.experiment information.

## Value

a data.table/SummarizedExperiment/DESeq object of columns as counts / normalized counts per library, column name is name of library. Rownames must be unique for now. Might change.

## See Also

Other countTable: [countTable\\_regions\(\)](#)

## Examples

```
# Make experiment
df <- ORFik.template.experiment()
# Make QC report to get counts ++ (not needed for this template)
# ORFikQC(df)

# Get count Table of mrnas
# countTable(df, "mrna")
# Get count Table of cds
# countTable(df, "cds")
# Get count Table of mrnas as fpkm values
# countTable(df, "mrna", type = "count")
# Get count Table of mrnas with collapsed replicates
# countTable(df, "mrna", collapse = TRUE)
# Get count Table of mrnas as summarizedExperiment
# countTable(df, "mrna", type = "summarized")
# Get count Table of mrnas as DESeq2 object,
# for differential expression analysis
# countTable(df, "mrna", type = "deseq")
```

---

countTable\_regions      *Make a list of count matrices from experiment*

---

## Description

By default will make count tables over mRNA, leaders, cds and trailers for all libraries in experiment. Saved as "qs" or "rds" format files.

**Usage**

```

countTable_regions(
  df,
  out.dir = libFolder(df),
  longestPerGene = FALSE,
  geneOrTxNames = "tx",
  regions = c("mrna", "leaders", "cds", "trailers"),
  type = "count",
  lib.type = "ofst",
  weight = "score",
  rel.dir = "QC_STATS",
  forceRemake = FALSE,
  library.names = bamVarName(df),
  format = "qs",
  path_prefix = if (!is.null(out.dir)) {
    pasteDir(file.path(out.dir, rel.dir,
      "countTable_"))
  } else {
    NULL
  },
  libraries = outputLibs(df, chrStyle = seqinfo(df), paths = filepath(df, lib.type), type
    = lib.type, force = FALSE, library.names = library.names, BPPARAM =
    BiocParallel::SerialParam()),
  BPPARAM = bpparam()
)

```

**Arguments**

<code>df</code>	an ORFik <a href="#">experiment</a>
<code>out.dir</code>	character, output directory, default: <code>resFolder(df)</code> . Will make a folder within this called "QC_STATS" with all results in this directory. Warning: If you assign not default path, you will have a hazzle to load files later. Much easier to load count tables, statistics, ++ later with default. Update <code>resFolder</code> of <code>df</code> instead if needed.
<code>longestPerGene</code>	a logical (default FALSE), if FALSE all transcript isoforms per gene. Ignored if "region" is not a character of either: "mRNA", "tx", "cds", "leaders" or "trailers".
<code>geneOrTxNames</code>	a character vector (default "tx"), should row names keep trancript names ("tx") or change to gene names ("gene")
<code>regions</code>	a character vector, default: <code>c("mrna", "leaders", "cds", "trailers")</code> , make raw count matrices of whole regions specified. Can also be a custom <code>GRangesList</code> of for example uORFs or a subset of cds etc.
<code>type</code>	default: "count" (raw counts matrix), alternative is "fpkm", "log2fpkm" or "log10fpkm"
<code>lib.type</code>	a character(default: "default"), load files in experiment or some precomputed variant, either "ofst", "pshifted" or "cov" These are made with <code>ORFik:::convertLibs()</code> or <code>shiftFootprintsByExperiment()</code> . Can also be custom user made folders inside the experiments bam folder. Format "cov" (i.e. <code>covRle</code> format) is by far the fastest to use if existing.

weight	numeric or character, a column to score overlaps by. Default "score", will check for a metacolumn called "score" in libraries. If not found, will not use weights.
rel.dir	relative output directory for out.dir, default: "QC_STATS". For pshifted, write "pshifted".
forceRemake	logical, default FALSE. If TRUE, will not look for existing file count table files.
library.names	character, default: bamVarName(df). Names to load libraries as to environment and names to display in plots.
format	character, default "qs", alternative: "rds". Which format to save summarizedExperiment.
path_prefix	the prefix names of tables, default: if (!is.null(out.dir) {pasteDir(file.path(out.dir, rel.dir, "countTable_")}) else {NULL} i.e. directory + countTable_ or NULL if out.dir is NULL.
libraries	The call to output libraries, the input is not used! Default: outputLibs(df, chrStyle = seqinfo(df), paths = filepath(df, lib.type), type = lib.type, force = force, library.names = library.names, BPPARAM = BPPARAM)
BPPARAM	how many cores/threads to use? default: bpparam()

**Value**

a list of data.table, 1 data.table per region. The regions will be the names the list elements.

**See Also**

Other countTable: [countTable\(\)](#)

**Examples**

```
##Make experiment
df <- ORFik.template.experiment()
## Create count tables for all default regions
countTable_regions(df, NULL)
## Pshifted reads (first create pshiftead libs)
# countTable_regions(df, lib.type = "pshifted", rel.dir = "pshifted")
```

---

coverage\_to\_dt

*Convert coverage RleList to data.table*


---

**Description**

Convert coverage RleList to data.table

**Usage**

```
coverage_to_dt(
  coverage,
  keep.names = TRUE,
  withFrames = FALSE,
  weight = "score",
  drop.zero.dt = FALSE,
  fraction = NULL
)
```

**Arguments**

coverage	RleList with names
keep.names	logical (TRUE), keep names or not. If as.data.table is TRUE, names (genes column) will be a factor column, if FALSE it will be an integer column (index of gene), so first input grl element is 1. Dropping names gives ~ 20 % speedup. If drop.zero.dt is FALSE, data.table will not return names, will use index (to avoid memory explosion).
withFrames	a logical (FALSE), only available if as.data.table is TRUE, return the ORF frame, 1,2,3, where position 1 is 1, 2 is 2 and 4 is 1 etc.
weight	(default: 'score'), if defined a character name of valid meta column in subject. GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGEr CAGE files and many other package formats. You can also assign a score column manually.
drop.zero.dt	logical FALSE, if TRUE and as.data.table is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)
fraction	integer or character, a description column. Useful for grouping multiple outputs together. If returned as Rle, this is added as: metadata(coverage) <- list(fraction = fraction). If as.data.table it will be added as an additional column.

**Value**

a data.table with column names c("count" [numeric or integer], "genes" [integer], "position" [integer])

---

coverageByTranscriptC *coverageByTranscript with coverage input*

---

**Description**

Extends the function with direct genome coverage input, see [coverageByTranscript](#) for original function.

**Usage**

```
coverageByTranscriptC(x, transcripts, ignore.strand = !strandMode(x))
```

**Arguments**

x a covRle (one RleList for each strand in object), must have defined and correct seqlengths in its SeqInfo object.

transcripts [GRangesList](#)

ignore.strand a logical (default: length(x) == 1)

**Value**

Integer Rle of coverage, 1 per transcript

---

```
coverageByTranscriptFST
```

*Get coverage from fst large coverage format*

---

**Description**

Get coverage from fst large coverage format

**Usage**

```
coverageByTranscriptFST(grl, fst_index, columns = NULL)
```

**Arguments**

grl a GRangesList

fst\_index a path to an existing fst index file

columns NULL or character, default NULL. Else must be a subset of names in the fst files. Run ids etc.

**Value**

a list, each element is a data.table of coverage

**Examples**

```
library(data.table)
library(ORFik)
grl <- GRangesList("1:1-5:+")
tempdir <- tempdir()
fst_index <- file.path(tempdir, "coverage_index.fst")
mock_run_names <- c("SRR1010101", "SRR1010102", "SRR1010103")
coverage_file <- file.path(tempdir, paste0("coverage_1_part1_",
c("forward", "reverse"), ".fst"))
```

```

mock_coverage <- setnames(setDT(lapply(mock_run_names, function(x) {
  sample(seq(0, 100), 100, replace = TRUE, prob = c(0.95, rep(0.01, 100)))))),
  mock_run_names)
mock_index <- data.table(chr = "1", start = 1, end = nrow(mock_coverage),
  file_forward = coverage_file[1], file_reverse = coverage_file[2])

fst::write_fst(mock_index, fst_index)
fst::write_fst(mock_coverage, coverage_file[1])

coverageByTranscriptFST(gr1, fst_index)
coverageByTranscriptFST(gr1, fst_index, c("SRR1010101", "SRR1010102"))

```

---

coverageByTranscriptW *coverageByTranscript with weights*

---

## Description

Extends the function with weights, see [coverageByTranscript](#) for original function.

## Usage

```

coverageByTranscriptW(
  x,
  transcripts,
  ignore.strand = FALSE,
  weight = 1L,
  seqinfo.x.is.correct = FALSE
)

```

## Arguments

x	reads ( <a href="#">GRanges</a> , <a href="#">GAlignments</a> )
transcripts	<a href="#">GRangesList</a>
ignore.strand	a logical (default: FALSE)
weight	a vector (default: 1L), if single number applies for all, else it must be the string name of a defined meta column in "x", that gives number of times a read was found. <a href="#">GRanges</a> ("chr1", 1, "+", score = 5), would mean score column tells that this alignment was found 5 times.
seqinfo.x.is.correct	logical, default FALSE. If you know x, has correct seqinfo, then you can save some computation time by setting this to TRUE.

## Value

Integer Rle of coverage, 1 per transcript

---

coverageHeatMap	<i>Create a heatmap of coverage</i>
-----------------	-------------------------------------

---

### Description

Creates a ggplot representing a heatmap of coverage:

- Rows : Position in region
- Columns : Read length
- Index intensity : (color) coverage scoring per index.

Coverage rows in heat map is fraction, usually fractions is divided into unique read lengths (standard Illumina is 76 unique widths, with some minimum cutoff like 15.) Coverage column in heat map is score, default zscore of counts. These are the relative positions you are plotting to. Like +/- relative to TIS or TSS.

### Usage

```
coverageHeatMap(
  coverage,
  output = NULL,
  scoring = "zscore",
  legendPos = "right",
  addFracPlot = FALSE,
  xlab = "Position relative to start site",
  ylab = "Protected fragment length",
  colors = "default",
  title = NULL,
  increments.y = "auto",
  gradient.max = max(coverage$score)
)
```

### Arguments

coverage	a data.table, e.g. output of scaledWindowCoverage
output	character string (NULL), if set, saves the plot as pdf or png to path given. If no format is given, is save as pdf.
scoring	character vector, default "zscore", Which scoring did you use to create? either of zscore, transcriptNormalized, sum, mean, median, .. see ?coverageScorings for info and more alternatives.
legendPos	a character, Default "right". Where should the fill legend be ? ("top", "bottom", "right", "left")
addFracPlot	Add margin histogram plot on top of heatmap with fractions per positions
xlab	the x-axis label, default "Position relative to start site"

ylab	the y-axis label, default "Protected fragment length"
colors	character vector, default: "default", this gives you: c("white", "yellow2", "yellow3", "lightblue", "blue", "navy"), do "high" for more high contrasts, or specify your own colors.
title	a character, default NULL (no title), what is the top title of plot?
increments.y	increments of y axis, default "auto". Or a numeric value < max position & > min position.
gradient.max	numeric, default: max(coverage\$score). What data value should the top color be? Good to use if you want to compare 2 samples, with the same color intensity, in that case set this value to the max score of the 2 coverage tables.

### Details

Colors: Remember if you want to change anything like colors, just return the ggplot object, and reassign like: obj + scale\_color\_brewer() etc. Standard colors are:

- 0 reads in whole readlength : gray
- few reads in position : white
- medium reads in position : yellow
- many reads in position : dark blue

### Value

a ggplot object of the coverage plot, NULL if output is set, then the plot will only be saved to location.

### See Also

Other heatmaps: [heatMapL\(\)](#), [heatMapRegion\(\)](#), [heatMap\\_single\(\)](#)

Other coveragePlot: [pSitePlot\(\)](#), [savePlot\(\)](#), [windowCoveragePlot\(\)](#)

### Examples

```
# An ORF
gr1 <- GRangesList(tx1 = GRanges("1", IRanges(1, 6), "+"))
# Ribo-seq reads
range <- IRanges(c(rep(1, 3), 2, 3, rep(4, 2), 5, 6), width = 1 )
reads <- GRanges("1", range, "+")
reads$size <- c(rep(28, 5), rep(29, 4)) # read size
coverage <- windowPerReadLength(gr1, reads = reads, upstream = 0,
                                downstream = 5)

coverageHeatMap(coverage)

# With top sum bar
coverageHeatMap(coverage, addFracPlot = TRUE)
# See vignette for more examples
```

---

coveragePerTiling      *Get coverage per group*

---

## Description

It tiles each GRangesList group to width 1, and finds hits per position. A range from 1:5 will split into c(1,2,3,4,5) and count hits on each. This is a safer speedup of coverageByTranscript from GenomicFeatures. It also gives the possibility to return as data.table, for faster computations.

## Usage

```
coveragePerTiling(
  grl,
  reads,
  is.sorted = FALSE,
  keep.names = TRUE,
  as.data.table = FALSE,
  withFrames = FALSE,
  weight = "score",
  drop.zero.dt = FALSE,
  fraction = NULL
)
```

## Arguments

grl	a GRangesList of 5' utrs, CDS, transcripts, etc.
reads	a GAlignments, GRanges, or precomputed coverage as covRle (one for each strand) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better. File streaming is still in beta, so use with care!
is.sorted	logical (FALSE), is grl sorted. That is + strand groups in increasing ranges (1,2,3), and - strand groups in decreasing ranges (3,2,1)
keep.names	logical (TRUE), keep names or not. If as.data.table is TRUE, names (genes column) will be a factor column, if FALSE it will be an integer column (index of gene), so first input grl element is 1. Dropping names gives ~ 20 % speedup. If drop.zero.dt is FALSE, data.table will not return names, will use index (to avoid memory explosion).
as.data.table	a logical (FALSE), return as data.table with 2 columns, position and count.
withFrames	a logical (FALSE), only available if as.data.table is TRUE, return the ORF frame, 1,2,3, where position 1 is 1, 2 is 2 and 4 is 1 etc.

weight	(default: 'score'), if defined a character name of valid meta column in subject. GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGER CAGE files and many other package formats. You can also assign a score column manually.
drop.zero.dt	logical FALSE, if TRUE and as.data.table is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)
fraction	integer or character, a description column. Useful for grouping multiple outputs together. If returned as Rle, this is added as: metadata(coverage) <- list(fraction = fraction). If as.data.table it will be added as an additional column.

### Details

NOTE: If reads contains a \$score column, it will presume that this is the number of replicates per reads, weights for the coverage() function. So delete the score column or set weight to something else if this is not wanted.

### Value

a numeric RleList, one numeric-Rle per group with # of hits per position. Or data.table if as.data.table is TRUE, with column names c("count" [numeric or integer], "genes" [integer], "position" [integer])

### See Also

Other ExtendGenomicRanges: [asTX\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

### Examples

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20),
                                end = c(5, 15, 25)),
               strand = "+")
grl <- GRangesList(tx1_1 = ORF)
RFP <- GRanges("1", IRanges(25, 25), "+", seqlengths = c("1" = 25))
coveragePerTiling(grl, RFP, is.sorted = TRUE)
# now as data.table with frames
coveragePerTiling(grl, RFP, is.sorted = TRUE, as.data.table = TRUE,
                  withFrames = TRUE)
# With score column (usually replicated reads on that position)
RFP <- GRanges("1", IRanges(25, 25), "+", score = 5)
dt <- coveragePerTiling(grl, RFP, is.sorted = TRUE,
                       as.data.table = TRUE, withFrames = TRUE)
class(dt$count) # numeric
# With integer score column (faster and less space usage)
RFP <- GRanges("1", IRanges(25, 25), "+", score = 5L,
               seqlengths = c("1" = 50)) # We need to know size of the chromosome here
```

```
dt <- coveragePerTiling(gr1, RFP, is.sorted = TRUE,
                        as.data.table = TRUE, withFrames = TRUE)
class(dt$count) # integer
# Much faster coverage format for genomes (ORFik often stores these on disc
# with .qs format)
cov <- covRleFromGR(RFP)
dt <- coveragePerTiling(gr1, cov, is.sorted = TRUE)
```

---

coverageScorings      *Add a coverage scoring scheme*

---

### Description

Different scorings and groupings of a coverage representation.

### Usage

```
coverageScorings(coverage, scoring = "zscore", copy.dt = TRUE)
```

### Arguments

coverage	a data.table containing at least columns (count, position), it is possible to have additional: (genes, fraction, feature)
scoring	a character, one of (zscore, transcriptNormalized, mean, median, sum, log2sum, log10sum, sumLength, meanPos and frameSum, periodic, NULL). More info in details
copy.dt	logical TRUE, copy object, to avoid overwriting original object. Set to false to run function using reference to object, a speed up if original object is not needed.

### Details

Usually output of metaWindow or scaledWindowPositions is input in this function.

Content of coverage data.table: It must contain the count and position columns.

genes column: If you have multiple windows, the genes column must define which gene/transcript grouping the different counts belong to. If there is only a meta window or only 1 gene/transcript, then this column is not needed.

fraction column: If you have coverage of i.e RNA-seq and Ribo-seq, or TCP -seq of large and small subunit, divide into fractions. Like factor(RNA, RFP)

feature column: If gene group is subdivided into parts, like gene is transcripts, and feature column can be c(leader, cds, trailer) etc.

Given a data.table coverage of counts, add a scoring scheme. per: the grouping given, if genes is defined, group by per gene in default scoring.

Scorings:

- zscore (count-mean(window))/sd(window) per) # Outlier detection
- modzscore (count-median(window))/mad(window) per) # Outlier detection for signal with extreme values
- transcriptNormalized (sum(count / sum of counts per)) this is scaled per group
- mean (mean(count per))
- median (median(count per))
- sum (count per)
- log2sum (count per)
- log10sum (count per)
- sumLength (count per) / number of windows
- meanPos (mean per position per gene) used in scaledWindowPositions
- sumPos (sum per position per gene) used in scaledWindowPositions
- frameSum (sum per frame per gene) used in ORFScore
- frameSumPerL (sum per frame per read length)
- frameSumPerLG (sum per frame per read length per gene)
- fracPos (fraction of counts per position per gene) non scaled version of transcriptNormalized
- periodic (Fourier transform periodicity of meta coverage per fraction)
- NULL (no grouping, return input directly)

### Value

a data.table with new scores (size dependent on score used)

### See Also

Other coverage: [metaWindow\(\)](#), [regionPerReadLength\(\)](#), [scaledWindowPositions\(\)](#), [windowPerReadLength\(\)](#)

### Examples

```
dt <- data.table::data.table(count = c(4, 1, 1, 4, 2, 3),
                             position = c(1, 2, 3, 4, 5, 6))
coverageScorings(dt, scoring = "zscore")

# with grouping gene
dt$genes <- c(rep("tx1", 3), rep("tx2", 3))
coverageScorings(dt, scoring = "zscore")
```

---

covRle	<i>Coverage Rlelist for both strands</i>
--------	--

---

**Description**

Coverage Rlelist for both strands

**Usage**

```
covRle(forward = RleList(), reverse = RleList())
```

**Arguments**

forward	a RleList with defined seqinfo for forward strand counts
reverse	a RleList with defined seqinfo for reverse strand counts

**Value**

a covRle object

**See Also**

Other covRLE: [covRle-class](#), [covRleFromGR\(\)](#), [covRleList](#), [covRleList-class](#)

**Examples**

```
covRle()  
covRle(RleList(), RleList())  
chr_rle <- RleList(chr1 = Rle(c(1,2,3), c(1,2,3)))  
covRle(chr_rle, chr_rle)
```

---

covRle-class	<i>Coverage Rle for both strands or single</i>
--------------	--

---

**Description**

Given a run of coverage(x) where x are reads, this class combines the 2 strands into 1 object

**Value**

a covRLE object

**See Also**

Other covRLE: [covRle](#), [covRleFromGR\(\)](#), [covRleList](#), [covRleList-class](#)

---

covRleFromGR	<i>Convert GRanges to covRle</i>
--------------	----------------------------------

---

## Description

Convert GRanges to covRle

## Usage

```
covRleFromGR(x, weight = "AUTO", ignore.strand = FALSE)
```

## Arguments

x	a GRanges, GAlignment or GAlignmentPairs object. Note that coverage calculation for GAlignment is slower, so usually best to call <code>convertToOneBasedRanges</code> on GAlignment object to speed it up.
weight	default "AUTO", pick 'score' column if exist, else all are 1L. Can also be a manually assigned meta column like 'score2' etc.
ignore.strand	logical, default FALSE.

## Value

covRle object

## See Also

Other covRLE: [covRle](#), [covRle-class](#), [covRleList](#), [covRleList-class](#)

## Examples

```
seqlengths <- as.integer(c(200, 300))
names(seqlengths) <- c("chr1", "chr2")
gr <- GRanges(seqnames = c("chr1", "chr1", "chr2", "chr2"),
              ranges = IRanges(start = c(10, 50, 100, 150), end = c(40, 80, 129, 179)),
              strand = c("+", "+", "-", "-"), seqlengths = seqlengths)
cov_both_strands <- covRleFromGR(gr)
cov_both_strands
cov_ignore_strand <- covRleFromGR(gr, ignore.strand = TRUE)
cov_ignore_strand
strandMode(cov_both_strands)
strandMode(cov_ignore_strand)
```

---

covRleList	<i>Coverage Rlelist for both strands</i>
------------	--

---

**Description**

Coverage Rlelist for both strands

**Usage**

```
covRleList(list, fraction = names(list))
```

**Arguments**

list	a list or List of covRle objects of equal length and lengths
fraction	character, default names(list). Names to elements of list, can be integers, as readlengths etc.

**Value**

a covRleList object

**See Also**

Other covRLE: [covRle](#), [covRle-class](#), [covRleFromGR\(\)](#), [covRleList-class](#)

**Examples**

```
covRleList(List(covRle()))
```

---

covRleList-class	<i>List of covRle</i>
------------------	-----------------------

---

**Description**

Given a run of coverage(x) where x are reads, this covRle combines the 2 strands into 1 object This list can again combine these into 1 object, with accession functions and generalizations.

**Value**

a covRleList object

**See Also**

Other covRLE: [covRle](#), [covRle-class](#), [covRleFromGR\(\)](#), [covRleList](#)

---

create.experiment      *Create an ORFik experiment*

---

## Description

Create a single R object that stores and controls all results relevant to a specific Next generation sequencing experiment. Click the experiment link above in the title if you are not sure what an ORFik experiment is.

By using files in a folder / folders. It will make an experiment table with information per sample, this object allows you to use the extensive API in ORFik that works on experiments.

Information Auto-detection:

There will be several columns you can fill in, when creating the object, if the files have logical names like (RNA-seq\_WT\_rep1.bam) it will try to auto-detect the most likely values for the columns. Like if it is RNA-seq or Ribo-seq, Wild type or mutant, is this replicate 1 or 2 etc.

You will have to fill in the details that were not auto detected. Easiest way to fill in the blanks are in a csv editor like libre Office or excel. You can also remake the experiment and specify the specific column manually. Remember that each row (sample) must have a unique combination of values. An extra column called "reverse" is made if there are paired data, like +/- strand wig files.

## Usage

```
create.experiment(  
  dir,  
  exper,  
  saveDir = ORFik::config()["exp"],  
  txdb = "",  
  fa = "",  
  organism = "",  
  assembly = "",  
  pairedEndBam = FALSE,  
  viewTemplate = FALSE,  
  types = c("bam", "bed", "wig", "bigWig", "ofst"),  
  libtype = "auto",  
  stage = "auto",  
  rep = "auto",  
  condition = "auto",  
  fraction = "auto",  
  author = "",  
  files = findLibrariesInFolder(dir, types, pairedEndBam),  
  result_folder = NULL,  
  runIDs = extract_run_id(files)  
)
```

**Arguments**

dir	Which directory / directories to create experiment from, must be a directory with NGS data from your experiment. Will include all files of file type specified by "types" argument. So do not mix files from other experiments in the same folder!
exper	Short name of experiment. Will be name used to load experiment, and name shown when running <code>list.experiments</code>
saveDir	Directory to save experiment csv file, default: <code>ORFik::config()["exp"]</code> , which has default: <code>~/Bio_data/ORFik_experiments/</code> . Set to NULL if you don't want to save it to disc.
txdb	A path to TxDb (preferred) or gff/gtf (not advised, slower) file with transcriptome annotation for the organism.
fa	A path to fasta genome/sequences used for libraries, remember the file must have a fasta index too.
organism	character, default: "" (no organism set), scientific name of organism. Homo sapiens, Danio rerio, Rattus norvegicus etc. If you have a SRA metadata csv file, you can set this argument to <code>study\$ScientificName[1]</code> , where study is the SRA metadata for all files that was aligned.
assembly	character, default: "" (no assembly set). The genome assembly name, like GRCh38 etc. Useful to add if you want detailed metadata of experiment analysis.
pairedEndBam	logical FALSE, else TRUE, or a logical list of TRUE/FALSE per library you see will be included (run first without and check what order the files will come in) 1 paired end file, then two single will be c(T, F, F). If you have a SRA metadata csv file, you can set this argument to <code>study\$LibraryLayout == "PAIRED"</code> , where study is the SRA metadata for all files that was aligned.
viewTemplate	run View() on template when finished, default (FALSE). Usually gives you a better view of result than using print().
types	Default <code>c("bam", "bed", "wig", "bigWig", "ofst")</code> , which types of libraries to allow as NGS data.
libtype	character, default "auto". Library types, must be length 1 or equal length of number of libraries. "auto" means ORFik will try to guess from file names. Example: RFP (Ribo-seq), RNA (RNA-seq), CAGE, SSU (TCP-seq 40S), LSU (TCP-seq 80S).
stage	character, default "auto". Developmental stage, tissue or cell line, must be length 1 or equal length of number of libraries. "auto" means ORFik will try to guess from file names. Example: HEK293 (Cell line), Sphere (zebrafish stage), ovary (Tissue).
rep	character, default "auto". Replicate numbering, must be length 1 or equal length of number of libraries. "auto" means ORFik will try to guess from file names. Example: 1 (rep 1), 2 rep(2). Insert only numbers here!
condition	character, default "auto". Library conditions, must be length 1 or equal length of number of libraries. "auto" means ORFik will try to guess from file names. Example: WT (wild type), mutant, etc.

<code>fraction</code>	character, default "auto". Fractionation of library, must be length 1 or equal length of number of libraries. "auto" means ORFik will try to guess from file names. This column is used to make experiment unique, if the other columns are not sufficient. Example: cyto (cytosolic fraction), dms0 (dms0 treated fraction), etc.
<code>author</code>	character, default "". Main author of experiment, usually last name is enough. When printing will state "author et al" in info.
<code>files</code>	character vector or data.table of library paths in dir. Default: <code>findLibrariesInFolder(dir, types, pairedEndBam)</code> . Do not touch unless you want to do some subsetting, it will automatically remove files that are not of file format defined by 'type' argument. Note that sorting on number that: 10 is before 2, so 1, 2, 10, is sorted as: 1, 10, 2. If you want to fix this, you could update this argument with: <code>ORFik:::findLibrariesInFolder()[1,3,2]</code> to get order back to 1,2,10 etc.
<code>result_folder</code>	character, default NULL. The folder to output analysis results like QC, count tables etc. By default the <code>libFolder(df)</code> folder is used, the folder of first library in experiment. If you are making a new experiment which is a collection of other experiments, set this to a new folder, to not contaminate your other experiment directories.
<code>runIDs</code>	character ids, usually SRR, ERR, or DRR identifiers, default is to search for any of these 3 in the filename by: <code>extract_run_id(files)</code> . They are optional.

### Value

a data.frame, NOTE: this is not a ORFik experiment, only a template for it!

### See Also

Other ORFik\_experiment: `ORFik.template.experiment()`, `ORFik.template.experiment.zf()`, `bamVarName()`, `experiment-class`, `filepath()`, `libraryTypes()`, `organism`, `experiment-method`, `outputLibs()`, `read.experiment()`, `save.experiment()`, `validateExperiments()`

### Examples

```
# 1. Pick directory
dir <- system.file("extdata/Homo_sapiens_sample", "", package = "ORFik")
# 2. Pick an experiment name
exper <- "ORFik"
# 3. Pick .gff/.gtf location
txdb <- system.file("extdata/references/homo_sapiens",
                   "Homo_sapiens_dummy.gtf.db", package = "ORFik")
# 4. Pick fasta genome of organism
fa <- system.file("extdata/references/homo_sapiens",
                 "Homo_sapiens_dummy.fasta", package = "ORFik")
# 5. Set organism (optional)
org <- "Homo sapiens"

# Create temple not saved on disc yet:
template <- create.experiment(dir = dir, exper, txdb = txdb,
                             saveDir = NULL,
```

```

                                fa = fa, organism = org,
                                viewTemplate = FALSE)
## Now fix non-unique rows: either is libre office, microsoft excel, or in R
template$X5[6] <- "heart" # here a dummy example, even though data is correct
# read experiment (if you set correctly)
df <- read.experiment(template)

## Default location of experiments is ORFik::config()["exp"]
# default_experiments_path <- ORFik::config()["exp"]
# exp_path <- file.path(default_experiments_path, paste0("exper", ".csv"))
# Save with: save.experiment(df, file = exp_path)
# Then you can simply load with read.experiment(exper),
# since you saved in the default directory

## Custom location (If you work in a team, use a shared folder)
# Remember to update ORFik::config() to ripple the effect through whole
# of ORFik if you want to use this as default
new_dir <- tempdir() # Here we just use tempdir
create.experiment(dir = dir, exper, txdb = txdb,
                  saveDir = new_dir, fa = fa, organism = org)
template_loaded <- read.experiment(exper, new_dir)
# The csv template paths (from index 5) is equal to file paths of loaded exp
identical(template$X6[-seq(4)], filepath(template_loaded, "default"))

```

---

defineTrailer

*Defines trailers for ORF.*


---

## Description

Creates GRanges object as a trailer for ORFranges representing ORF, maintaining restrictions of transcriptRanges. Assumes that ORFranges is on the transcriptRanges, strands and seqlevels are in agreement. When lengthOftrailer is smaller than space left on the transcript than all available space is returned as trailer.

## Usage

```
defineTrailer(ORFranges, transcriptRanges, lengthOftrailer = 200)
```

## Arguments

ORFranges            GRanges object of your Open Reading Frame.  
transcriptRanges       GRanges object of transtript.  
lengthOftrailer       Numeric. Default is 10.

**Details**

It assumes that ORFranges and transcriptRanges are not sorted when on minus strand. Should be like: (200, 600) (50, 100)

**Value**

A GRanges object of trailer.

**See Also**

Other ORFHelpers: [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

**Examples**

```
ORFranges <- GRanges(seqnames = Rle(rep("1", 3)),
                    ranges = IRanges(start = c(1, 10, 20),
                                     end = c(5, 15, 25)),
                    strand = "+")
transcriptRanges <- GRanges(seqnames = Rle(rep("1", 5)),
                           ranges = IRanges(start = c(1, 10, 20, 30, 40),
                                             end = c(5, 15, 25, 35, 45)),
                           strand = "+")
defineTrailer(ORFranges, transcriptRanges)
```

---

 DEG.analysis

*Run differential TE analysis*


---

**Description**

Expression analysis of 1 dimension, usually between conditions of RNA-seq.

Using the standardized DESeq2 pipeline flow.

Creates a DESeq model (given x is the target.contrast argument) (usually 'condition' column)

1. RNA-seq model: design = ~ x (differences between the x groups in RNA-seq)

**Usage**

```
DEG.analysis(
  df,
  target.contrast = design[1],
  design = ORFik::design(df),
  p.value = 0.05,
  counts = countTable(df, "mrna", type = "summarized"),
  batch.effect = TRUE,
  pairs = combn.pairs(unlist(df[, target.contrast])),
  as.data.table = TRUE,
```

```

fitType = c("parametric", "local", "mean", "glmGamPoi"),
lfcShrinkType = "normal"
)

```

### Arguments

<code>df</code>	an <a href="#">experiment</a> of usually RNA-seq.
<code>target.contrast</code>	a character vector, default <code>design[1]</code> . The column in the ORFik experiment that represent the comparison contrasts. By default: the first design factor of the full experimental design. This is the factor you will do the comparison on. DESeq will normalize the counts based on the full design, but the log fold change values will be based on this contrast only. It is usually the 'condition' column.
<code>design</code>	a character vector, default <code>design(df.rfp)</code> . The full experiment design. Which factors have more than 1 level. Example: stage column are all HEK293, so it can not be a design factor. The condition column has 2 possible values, WT and mutant, so it is a factor of the experiment. Replicates column is not part of design, that is inserted later with setting <code>batch.effect = TRUE</code> . Library type 'libtype' column, can also no be part of initial design, it is always added inside the function, after initial setup.
<code>p.value</code>	a numeric, default 0.05 in interval (0,1). Defines adjusted p-value to be used as significance threshold for the result groups. I.e. for exclusive translation group significant subset for <code>p.value = 0.05</code> means: <code>TE\$padj &lt; 0.05 &amp; Ribo\$padj &lt; 0.05 &amp; RNA\$padj &gt; 0.05</code> .
<code>counts</code>	a <code>SummarizedExperiment</code> , default: <code>countTable(df, "mrna", type = "summarized")</code> , all transcripts. Assign a subset if you don't want to analyze all genes. It is recommended to not subset, to give DESeq2 data for variance analysis.
<code>batch.effect</code>	logical, default TRUE. Makes replicate column of the experiment part of the design. If you believe you might have batch effects, keep as TRUE. Batch effect usually means that you have a strong variance between biological replicates. Check out <a href="#">pcaExperiment</a> and see if replicates cluster together more than the design factor, to verify if you need to set it to TRUE.
<code>pairs</code>	list of character pairs, the experiment contrasts. Default: <code>combn.pairs(unlist(df.rfp[, target.contrast])</code>
<code>as.data.table</code>	logical, default TRUE. Return as <code>data.table</code> or list of DESeq result objects (FALSE).
<code>fitType</code>	either "parametric", "local", "mean", or "glmGamPoi" for the type of fitting of dispersions to the mean intensity. See <a href="#">estimateDispersions</a> for description.
<code>lfcShrinkType</code>	character or NULL. Default "normal", which shrinkage to apply to results for low count gene subset. This avoids the problem of extreme fold changes, when counts are low. See <a href="#">lfcShrink</a> . A note for DTEG.analysis function: The interaction term (TE), is not shrunked as this is not counts, but a ratio.

### Details

#' Analysis is done between each possible combination of levels in the target contrast If target contrast is the condition column, with factor levels: WT, mut1 and mut2 with 3 replicates each. You

get comparison of WT vs mut1, WT vs mut2 and mut1 vs mut2.  
 The respective result categories are defined as: (given a user defined p value, shown here as 0.05):  
 Significant - p-value adjusted < 0.05 (p-value cutoff decided by 'p.value' argument)

The LFC values are shrunken by `lfcShrink(type = "normal")`.

Remember that DESeq by default can not do global change analysis, it can only find subsets with changes in LFC!

### Value

a data.table with columns: (contrast variable, gene id, regulation status, log fold changes, p.adjust values, mean counts)

### References

<https://doi.org/10.1002/cpmb.108>

### See Also

Other DifferentialExpression: `DEG.plot.static()`, `DEG_model()`, `DTEG.plot()`, `te.table()`, `te_rna.plot()`

### Examples

```
## Simple example (use ORFik template, then use only RNA-seq)
df <- ORFik.template.experiment()
df.rna <- df[df$libtype == "RNA",]
design(df.rna) # The full experimental design
design(df.rna)[1] # Default target contrast
dt <- DEG.analysis(df.rna)
```

---

DEG.plot.static

*Plot DEG result*

---

### Description

Plot setup:

X-axis: mean counts Y-axis: Log2 fold changes For explanation of plot, see [DEG.analysis](#)

### Usage

```
DEG.plot.static(
  dt,
  output.dir = NULL,
  p.value.label = 0.05,
  plot.title = "",
  plot.ext = ".pdf",
```

```

width = 6,
height = 6,
dpi = 300,
dot.size = 0.4,
xlim = "auto",
ylim = "bidir.max",
relative.name = paste0("DEG_plot", plot.ext)
)

```

### Arguments

<code>dt</code>	a data.table with the results from <a href="#">DEG.analysis</a>
<code>output.dir</code>	a character path, default NULL(no save), or a directory to save to a file. Relative name of file, specified by 'relative.name' argument.
<code>p.value.label</code>	a numeric, default <code>ifelse(!is.null(attr(dt, "p.value")), attr(dt, "p.value"), 0.05)</code> Interval (0,1), use "" to not show. What p-value used for the analysis? Will be shown as a caption.
<code>plot.title</code>	title for plots, usually name of experiment etc
<code>plot.ext</code>	character, default: ".pdf". Alternatives: ".png" or ".jpg". Multiple values allowed, if so will save file in each format specified.
<code>width</code>	numeric, default 6 (in inches)
<code>height</code>	numeric, default 6 (in inches)
<code>dpi</code>	numeric, default 300.
<code>dot.size</code>	numeric, default 0.4, size of point dots in plot.
<code>xlim</code>	numeric vector or character preset, default: "bidir.max" (Equal in both + / - direction, using max value + 0.5 of meanCounts column in dt). If you want ggplot to decide limit, set to "auto". For numeric vector, specify min and max x limit: like <code>c(-5, 5)</code>
<code>ylim</code>	numeric vector or character preset, default: "bidir.max" (Equal in both + / - direction, using max value + 0.5 of LFC column in dt). If you want ggplot to decide limit, set to "auto". For numeric vector, specify min and max y limit: like <code>c(-10, 10)</code>
<code>relative.name</code>	character, Default: <code>paste0("DEG_plot", plot.ext)</code> Relative name of file to be saved in folder specified in <code>output.dir</code> . Change to .pdf if you want pdf file instead of png. Multiple values allowed, if so will save file in each format specified.

### Value

a ggplot object, will `facet_wrap` if `length(unique(dt$contrasts)) > 1`

### See Also

Other DifferentialExpression: [DEG\\_model\(\)](#), [DTEG.analysis\(\)](#), [DTEG.plot\(\)](#), [te.table\(\)](#), [te\\_rna.plot\(\)](#)

## Examples

```
df <- ORFik.template.experiment()
df.rna <- df[df$libtype == "RNA",]
#dt <- DEG.analysis(df.rna)
#Default scaling
#DEG.plot.static(dt)
#Manual scaling
#DEG.plot.static(dt, xlim = c(-2, 2), ylim = c(-2, 2))
```

---

DEG\_gorilla

*GO analysis with GOrilla*

---

## Description

Per contrast, split list into regulation groups and run GOrilla go analysis. If you want to change LFC threshold or p-value cutoff, you reassign Regulation column by LFC and/or p-value.

## Usage

```
DEG_gorilla(dt, output_dir, organism)
```

## Arguments

dt	a data.table of DEG or DTEG results, must also have appended gene symbols column called "external_gene_name"
output_dir	path to save results
organism	organism(df), example "Homo sapiens"

## Value

a data.table with 2 columns (id: name of contrast), urls: url to html online, this table is also saved in output\_dir.

## See Also

Other GOrilla: [DEG\\_gorilla\\_copy\\_to\\_local\(\)](#)

---

 DEG\_gorilla\_copy\_to\_local

*Copy GOrilla result htms to local*


---

### Description

Will retrieve full html, png and xls structure so analysis can be used even when results are deleted online (1 month after creation).

Files are saved to disc in directory `"/GORilla_local_html_outputs/"`, relative to input directory `'gorilla_output_dir'`. There is 1 subfolder per analysis url. Open the GOResults.html to view.

### Usage

```
DEG_gorilla_copy_to_local(
  gorilla_output_dir,
  local_html_dir = file.path(gorilla_output_dir, "GORilla_local_html_outputs")
)
```

### Arguments

`gorilla_output_dir`

character, directory path to existing results of a DEG\_gorilla call. Must contain a file with relative path `"/All_contrasts_GOrilla_urls.csv"`

`local_html_dir` character, output directory, default: `file.path(gorilla_output_dir, "GORilla_local_html_output"`

### Value

invisible(NULL), files are saved to disc.

### See Also

Other GOrilla: [DEG\\_gorilla\(\)](#)

---

 DEG\_gorilla\_local\_load\_data

*Load all GOrilla xls files from study*


---

### Description

Output as data.table with column `'analysis'` describing DEG contrast and column `'go_category'` describing COMPONENT, FUNCTION or PROCESS.

### Usage

```
DEG_gorilla_local_load_data(gorilla_local_dir)
```

**Arguments**

`gorilla_local_dir`  
path to directory of local GORilla html and xls data

**Value**

a data.table

---

<code>DEG_gorilla_plot</code>	<i>Plot GORilla xls results</i>
-------------------------------	---------------------------------

---

**Description**

Inspired by the enrichment plot from the package clusterProfiler.

**Usage**

```
DEG_gorilla_plot(dt, top_n = 20L, enrich_cutoff = 8)
```

**Arguments**

`dt` a data.table with results loaded using `DEG_gorilla_local_load_data`

`top_n` maximum number of GO terms per analysis / component split. The sorting is done as `-GeneRatio`, `-p.adjust`, so it extracts the top 20 highest GeneRatio terms by `p.adjust` values (decreasing sort).

`enrich_cutoff` numeric, default 8. Minimum enrichment, set it lower to get more generic groups, higher to get more specific groups.

**Value**

a ggplot, it uses `facet_wrap` to split analysis / components.

---

<code>DEG_model</code>	<i>Get DESeq2 model without running results</i>
------------------------	---

---

**Description**

This is the preparation step of DESeq2 analysis using `ORFik::DEG.analysis`. It is exported so that you can do this step in standalone, usually you want to use `DEG.analysis` directly.

**Usage**

```
DEG_model(
  df,
  target.contrast = design[1],
  design = ORFik::design(df),
  p.value = 0.05,
  counts = countTable(df, "mrna", type = "summarized"),
  batch.effect = TRUE,
  fitType = c("parametric", "local", "mean", "glmGamPoi")
)
```

**Arguments**

<code>df</code>	an <a href="#">experiment</a> of usually RNA-seq.
<code>target.contrast</code>	a character vector, default <code>design[1]</code> . The column in the ORFik experiment that represent the comparison contrasts. By default: the first design factor of the full experimental design. This is the factor you will do the comparison on. DESeq will normalize the counts based on the full design, but the log fold change values will be based on this contrast only. It is usually the 'condition' column.
<code>design</code>	a character vector, default <code>design(df, rfp)</code> . The full experiment design. Which factors have more than 1 level. Example: stage column are all HEK293, so it can not be a design factor. The condition column has 2 possible values, WT and mutant, so it is a factor of the experiment. Replicates column is not part of design, that is inserted later with setting <code>batch.effect = TRUE</code> . Library type 'libtype' column, can also no be part of initial design, it is always added inside the function, after initial setup.
<code>p.value</code>	a numeric, default 0.05 in interval (0,1). Defines adjusted p-value to be used as significance threshold for the result groups. I.e. for exclusive translation group significant subset for <code>p.value = 0.05</code> means: <code>TE\$padj &lt; 0.05 &amp; Ribo\$padj &lt; 0.05 &amp; RNA\$padj &gt; 0.05</code> .
<code>counts</code>	a SummarizedExperiment, default: <code>countTable(df, "mrna", type = "summarized")</code> , all transcripts. Assign a subset if you don't want to analyze all genes. It is recommended to not subset, to give DESeq2 data for variance analysis.
<code>batch.effect</code>	logical, default TRUE. Makes replicate column of the experiment part of the design. If you believe you might have batch effects, keep as TRUE. Batch effect usually means that you have a strong variance between biological replicates. Check out <a href="#">pcaExperiment</a> and see if replicates cluster together more than the design factor, to verify if you need to set it to TRUE.
<code>fitType</code>	either "parametric", "local", "mean", or "glmGamPoi" for the type of fitting of dispersions to the mean intensity. See <a href="#">estimateDispersions</a> for description.

**Value**

a DESeqDataSet object with results stored as metadata columns.

**See Also**

Other DifferentialExpression: `DEG.plot.static()`, `DTEG.analysis()`, `DTEG.plot()`, `te.table()`, `te_rna.plot()`

**Examples**

```
## Simple example (use ORFik template, then use only RNA-seq)
df <- ORFik.template.experiment()
df.rna <- df[df$libtype == "RNA",]
design(df.rna) # The full experimental design
target.contrast <- design(df.rna)[1] # Default target contrast
#ddsMat_rna <- DEG_model(df.rna, target.contrast)
```

---

DEG_model_results	<i>Get DESeq2 model results from DESeqDataSet</i>
-------------------	---

---

**Description**

Get DESeq2 model results from DESeqDataSet

**Usage**

```
DEG_model_results(
  ddsMat_rna,
  target.contrast,
  pairs,
  p.value = 0.05,
  lfcShrinkType = "normal",
  as.data.table = TRUE
)
```

**Arguments**

<code>ddsMat_rna</code>	a DESeqDataSet object with results stored as metadata columns.
<code>target.contrast</code>	a character vector, default <code>design[1]</code> . The column in the ORFik experiment that represent the comparison contrasts. By default: the first design factor of the full experimental design. This is the factor you will do the comparison on. DESeq will normalize the counts based on the full design, but the log fold change values will be based on this contrast only. It is usually the 'condition' column.
<code>pairs</code>	list of character pairs, the experiment contrasts. Default: <code>combn.pairs(unlist(df.rfp[, target.contrast])</code>
<code>p.value</code>	a numeric, default 0.05 in interval (0,1). Defines adjusted p-value to be used as significance threshold for the result groups. I.e. for exclusive translation group significant subset for <code>p.value = 0.05</code> means: <code>TE\$padj &lt; 0.05 &amp; Ribo\$padj &lt; 0.05 &amp; RNA\$padj &gt; 0.05</code> .

lfcShrinkType character or NULL. Default "normal", which shrinkage to apply to results for low count gene subset. This avoids the problem of extreme fold changes, when counts are low. See [lfcShrink](#). A note for DTEG.analysis function: The interaction term (TE), is not shrunked as this is not counts, but a ratio.

as.data.table logical, default TRUE. Return as data.table or list of DESeq result objects (FALSE).

### Value

a data.table or list

### Examples

```
## Simple example (use ORFik template, then use only RNA-seq)
df <- ORFik.template.experiment()
df.rna <- df[df$libtype == "RNA",]
design(df.rna) # The full experimental design
target.contrast <- design(df.rna)[1] # Default target contrast
#ddsMat_rna <- DEG_model(df.rna, target.contrast)
#pairs <- combn.pairs(unlist(df[, target.contrast]))
#dt <- DEG_model_results(ddsMat_rna, target.contrast, pairs)
```

---

DEG\_model\_simple      *Simple Fpkm ratio test DEG*

---

### Description

If you do not have a valid DESEQ2 experimental setup (contrast), you can use this simplified test

### Usage

```
DEG_model_simple(
  df,
  target.contrast = design[1],
  design = ORFik::design(df),
  p.value = 0.05,
  counts = countTable(df, "mrna", type = "summarized"),
  design_ids = as.character(df[, target.contrast]),
  batch.effect = FALSE
)
```

### Arguments

df an [experiment](#) of usually RNA-seq.

target.contrast a character vector, default design[1]. The column in the ORFik experiment that represent the comparison contrasts. By default: the first design factor of the full experimental design. This is the factor you will do the comparison on. DESeq will normalize the counts based on the full design, but the log fold change values will be based on this contrast only. It is usually the 'condition' column.

design	a character vector, default <code>design(df.rfp)</code> . The full experiment design. Which factors have more than 1 level. Example: stage column are all HEK293, so it can not be a design factor. The condition column has 2 possible values, WT and mutant, so it is a factor of the experiment. Replicates column is not part of design, that is inserted later with setting <code>batch.effect = TRUE</code> . Library type 'libtype' column, can also no be part of initial design, it is always added inside the function, after initial setup.
p.value	a numeric, default 0.05 in interval (0,1). Defines adjusted p-value to be used as significance threshold for the result groups. I.e. for exclusive translation group significant subset for <code>p.value = 0.05</code> means: <code>TE\$padj &lt; 0.05 &amp; Ribo\$padj &lt; 0.05 &amp; RNA\$padj &gt; 0.05</code> .
counts	a <code>SummarizedExperiment</code> , default: <code>countTable(df, "mrna", type = "summarized")</code> , all transcripts. Assign a subset if you don't want to analyze all genes. It is recommended to not subset, to give DESeq2 data for variance analysis.
design_ids	character vector of contrast group ids, e.g. <code>c("WT", "WT", "Mutant", "Mutant")</code>
batch.effect	logical, default TRUE. Makes replicate column of the experiment part of the design. If you believe you might have batch effects, keep as TRUE. Batch effect usually means that you have a strong variance between biological replicates. Check out <a href="#">pcaExperiment</a> and see if replicates cluster together more than the design factor, to verify if you need to set it to TRUE.

**Value**

a data.table of fpkm ratios

**Examples**

```
## Simple example (use ORFik template, then use only RNA-seq)
df <- ORFik.template.experiment()
df <- df[df$libtype == "RNA",]
#dt <- DEG_model_simple(df)
```

---

design,experiment-method

*Get experimental design Find the column/columns that create a separation between samples, by default skips replicate and choose first that is from either: libtype, condition, stage and fraction.*

---

**Description**

Get experimental design Find the column/columns that create a separation between samples, by default skips replicate and choose first that is from either: libtype, condition, stage and fraction.

**Usage**

```
## S4 method for signature 'experiment'
design(
  object,
  batch.correction.design = FALSE,
  as.formula = FALSE,
  multi.factor = TRUE
)
```

**Arguments**

`object` an ORFik [experiment](#)

`batch.correction.design` logical, default FALSE. If true, add replicate as a trailing design factor (only if  $\geq 2$  replicates exists).

`as.formula` logical, default FALSE. If TRUE, return as formula

`multi.factor` logical, default TRUE. If FALSE, return first factor only (+ rep, if `batch.correction.design` is true). Order of picking for `single.factor` is: does libtype have  $> 1$  level, if not then: stage, if not then: condition, if not then: fraction.

**Value**

a character (name of column) or a formula

**Examples**

```
df <- ORFik.template.experiment()
design(df) # The 2 columns that decides the design here
# If we subset it changes
design(df[df$libtype == "RFP",])
# Only single factor design, it picks first
design(df, multi.factor = FALSE)
```

---

detect_drive	<i>Detects the mounted drive based on a mounted path</i>
--------------	--

---

**Description**

Detects the mounted drive based on a mounted path

**Usage**

```
detect_drive(ref_path = path.expand(config()["ref"]))
```

**Arguments**

`ref_path` = `path.expand(config()["ref"])`

**Value**

character, name of FileSystem drive of mounted path, NA\_character\_ if not found

**Examples**

```
detect_drive(tempdir())
```

---

detect_ribo_orfs	<i>Detect ORFs by Ribosome profiling data</i>
------------------	---

---

**Description**

Finding all ORFs: 1. Find all ORFs in mRNA using ORFik findORFs, with defined parameters.  
To create the candidate ORFs (all ORFs returned):

Steps (candidate set):

Define a candidate search set by these 3 rules:

- 1.a Allowed ORF type: uORF, NTE, etc (only keep these in candidate list)
- 1.b Must have at least x reads over whole orf (default 10 reads)
- 1.c Must have at least x reads over start site (default 3 reads)

The total list is defined by these names, and saved according to allowed ORF type/types.

To create the prediction status (TRUE/FALSE) per candidate

Steps (prediction status)

(UP\_NT is a 20nt window upstream of ORF, that stops 2NT before ORF starts) :

1. ORF mean reads per NT > (UP\_NT mean reads per NT \* 1.3)
2. ORFScore > 2.5
3. TIS total reads + 3 > ORF median reads per NT
4. Given expression above, a TRUE prediction is defined with the AND operator: 1. & 2. & 3.

In code that is:

```
predicted <- (orfs_cov_stats$mean > upstream_cov_stats$mean*1.3) & orfs_cov_stats$ORFScores > 2.5 & ((reads_start[candidates] + 3) > orfs_cov_stats$median)
```

**Usage**

```
detect_ribo_orfs(
  df,
  out_folder,
  ORF_categories_to_keep,
  prefix_result = paste(c(ORF_categories_to_keep, gsub(" ", "_", organism(df))), collapse = "_"),
  mrna = loadRegion(df, "mrna"),
  cds = loadRegion(df, "cds"),
  libraries = outputLibs(df, type = "pshifted", output.mode = "envirlist"),
  orf_candidate_ranges = findORFs(seqs = txSeqsFromFa(mrna, df, TRUE), longestORF = longestORF, startCodon = startCodon, stopCodon = stopCodon, minimumLength = minimumLength),
  orfs_gr = categorize_and_filter_ORFs(orf_candidate_ranges, ORF_categories_to_keep, cds,
```

```

    mrna),
  export_metrics_table = TRUE,
  longestORF = FALSE,
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  minimumLength = 0,
  minimum_reads_ORF = 10,
  minimum_reads_start = 3
)

```

## Arguments

**df** an ORFik [experiment](#)

**out\_folder** Directory to save files

**ORF\_categories\_to\_keep** options, any subset of: c("uORF", "uoORF", "annotated", "NTE", "NTT", "internal", "doORF", "dORF", "ncORF", "a\_error", "all").

- uORF : Upstream ORFs (Starting in 5' UTR), not overlapping CDS
- uoORF : Upstream ORFs (Starting in 5' UTR), overlapping CDS
- annotated : The defined CDS for that transcript
- NTE : 5' Start codon extension of annotated CDS
- NTT : 5' Start codon truncation of annotated CDS
- CTE : 3' stop codon extension of annotated CDS, i.e. readthrough
- CTT : 5' Start codon truncation of annotated CDS, original cds was defined with readthrough
- internal : Starting inside CDS, ending before CDS ends
- doORF : Downstream ORFs (Ending in 3' UTR), overlapping CDS
- dORF : Downstream ORFs (Ending in 3' UTR), not overlapping CDS
- ncORF : Any ORF on a transcript without a defined CDS
- a\_error : Any ORF detect not in the above categories
- all : use all ORF types above

**prefix\_result** the prefix name of output files to out\_folder. Default: paste(c(ORF\_categories\_to\_keep, gsub(" ", "\_", organism(df))), collapse = "\_")

**mrna** = loadRegion(df, "mrna")

**cds** = loadRegion(df, "cds")

**libraries** the ribo-seq libraries loaded into R as list, default: outputLibs(df, type = "pshifted", output.mode = "envirlist")

**orf\_candidate\_ranges** IRangesList, = findORFs(seqs = txSeqsFromFa(mrna, df, TRUE), longestORF = longestORF, startCodon = startCodon, stopCodon = stopCodon, minimumLength = minimumLength)

**orfs\_gr** = categorize\_and\_filter\_ORFs(orf\_candidate\_ranges, ORF\_categories\_to\_keep, cds, mrna). The GRangesList set of ORFs to actually search.

export_metrics_table	logical, default TRUE. Export table of statistics to file with suffix: "_prediction_table.rds"
longestORF	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.
startCodon	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.
stopCodon	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.
minimumLength	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example minimumLength = 8 will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.
minimum_reads_ORF	numeric, default 10, orf removed if less reads overlap whole orf
minimum_reads_start	numeric, default 3, orf removed if less reads overlap start

### Value

invisible(NULL), all ORF results saved to disc

### Examples

```
# Pre requisites
# 1. Create ORFik experiment
# ORFik::create.experiment(...)
# 2. Create ORFik optimized annotation:
# makeTxdbFromGenome(gtfile = ORFik::getGtfPathFromTxdb(df), genome = df@fai,
#                   organism = organism(df), optimize = TRUE)
# 3. There must exist pshifted reads, either as default files, or in a relative folder called
# ".pshifted/". See ?shiftFootprintsByExperiment
# EXAMPLE:
df <- ORFik.template.experiment()
df <- df[df$libtype == "RFP",][c(1,2),]
result_folder <- riboORFsFolder(df, tempdir())
results <- detect_ribo_orfs(df, result_folder, c("uORF", "uoORF", "annotated", "NTE"))

# Load results of annotated ORFs
table <- riboORFs(df[1,], type = "table", result_folder)
table # See all statistics
sum(table$predicted) # How many were predicted as Ribo-seq ORFs
# Load 2 results
table <- riboORFs(df[1:2,], type = "table", result_folder)
table # See all statistics
sum(table$predicted) # How many were predicted as Ribo-seq ORFs
```

```

# Load GRangesList
candidates_gr <- riboORFs(df[1,], type = "ranges_candidates", result_folder)
prediction <- riboORFs(df[1,], type = "predictions", result_folder)

predicted_gr <- riboORFs(df[1:2,], type = "ranges_predictions", result_folder)
identical(predicted_gr[[1]], candidates_gr[[1]][prediction[[1]]])
## Inspect predictions in RiboCrypt
# library(RiboCrypt)
# Inspect Predicted
view <- predicted_gr[[1]][1]
#multiOmicsPlot_ORFikExp(view, df, view, leader_extension = 100, trailer_extension = 100)
# Inspect not predicted
view <- candidates_gr[[1]][!prediction[[1]]][1]
#multiOmicsPlot_ORFikExp(view, df, view, leader_extension = 100, trailer_extension = 100)

```

---

detectRibosomeShifts    *Detect ribosome shifts*

---

## Description

Utilizes periodicity measurement (Fourier transform), and change point analysis to detect ribosomal footprint shifts for each of the ribosomal read lengths. Returns subset of read lengths and their shifts for which top covered transcripts follow periodicity measure. Each shift value assumes 5' anchoring of the reads, so that output offsets values will shift 5' anchored footprints to be on the p-site of the ribosome. The E-site will be shift + 3 and A site will be shift - 3. So update to these, if you rather want those.

## Usage

```

detectRibosomeShifts(
  footprints,
  txdb,
  start = TRUE,
  stop = FALSE,
  top_tx = 10L,
  minFiveUTR = 30L,
  minCDS = 150L,
  minThreeUTR = if (stop) {
    30
  } else NULL,
  txNames = filterTranscripts(txdb, minFiveUTR, minCDS, minThreeUTR),
  firstN = 150L,
  tx = NULL,
  min_reads = 1000,
  min_reads_TIS = 50,
  accepted.lengths = 26:34,
  heatmap = FALSE,

```

```

    must.be.periodic = TRUE,
    strict.fft = TRUE,
    verbose = FALSE
)

```

## Arguments

footprints	<a href="#">GAlignments</a> object of RiboSeq reads - footprints, can also be path to the .bam / .ofst file. If GAlignment object has a meta column called "score", this will be used as replicate numbering for that read. So be careful if you have custom files with score columns, with another meaning.
txdb	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .db or .sqlite) or an ORFik experiment
start	(logical) Whether to include predictions based on the start codons. Default TRUE.
stop	(logical) Whether to include predictions based on the stop codons. Default FALSE. Only use if there exists 3' UTRs for the annotation. If periodicity around stop codon is stronger than at the start codon, use stop instead of start region for p-shifting.
top_tx	(integer), default 10. Specify which % of the top TIS coverage transcripts to use for estimation of the shifts. By default we take top 10 top covered transcripts as they represent less noisy data-set. This is only applicable when there are more than 1000 transcripts.
minFiveUTR	(integer) minimum bp for 5' UTR during filtering for the transcripts. Set to NULL if no 5' UTRs exists for annotation.
minCDS	(integer) minimum bp for CDS during filtering for the transcripts
minThreeUTR	(integer) minimum bp for 3' UTR during filtering for the transcripts. Set to NULL if no 3' UTRs exists for annotation.
txNames	a character vector of subset of CDS to use. Default: txNames = filterTranscripts(txdb, minFiveUTR, minCDS, minThreeUTR) Example: c("ENST1000005"), will use only that transcript (You should use at least 100!). Remember that top_tx argument, will by default specify to use top 10 % of those CDSs. Set that to 100, to use all these specified transcripts.
firstN	(integer) Represents how many bases of the transcripts downstream of start codons to use for initial estimation of the periodicity.
tx	a GRangesList, if you do not have 5' UTRs in annotation, send your own version. Example: extendLeaders(tx, 30) Where 30 bases will be new "leaders". Since each original transcript was either only CDS or non-coding (filtered out).
min_reads	default (1000), how many reads must a read-length have in total to be considered for periodicity.
min_reads_TIS	default (50), how many reads must a read-length have in the TIS region to be considered for periodicity.
accepted.lengths	accepted read lengths, default 26:34, usually ribo-seq is strongest between 27:32.

heatmap	a logical or character string, default FALSE. If TRUE, will plot heatmap of raw reads before p-shifting to console, to see if shifts given make sense. You can also set a filepath to save the file there.
must.be.periodic	logical TRUE, if FALSE will not filter on periodic read lengths. (The Fourier transform filter will be skipped). This is useful if you are not going to do periodicity analysis, that is: for you more coverage depth (more read lengths) is more important than only keeping the high quality periodic read lengths.
strict.fft	logical, TRUE. Use a FFT without noise filter. This means keep only reads lengths that are "periodic for the human eye". If you want more coverage, set to FALSE, to also get read lengths that are "messy", but the noise filter detects the periodicity of 3. This should only be done when you do not need high quality periodic reads! Example would be differential translation analysis by counts over each ORF.
verbose	logical, default FALSE. Report details of analysis/periodogram. Good if you are not sure if the analysis was correct.

### Details

Check out vignette for the examples of plotting RiboSeq metaplots over start and stop codons, so that you can verify visually whether this function detects correct shifts.

For how the Fourier transform works, see: [isPeriodic](#)

For how the changepoint analysis works, see: [changePointAnalysis](#)

NOTE: It will remove softclips from valid width, the CIGAR 3S30M is qwidth 33, but will remove 3S so final read width is 30 in ORFik. This is standard for ribo-seq.

### Value

a data.table with lengths of footprints and their predicted coresponding offsets

### References

<https://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-018-4912-6>

### See Also

Other pshifting: [changePointAnalysis\(\)](#), [shiftFootprints\(\)](#), [shiftFootprintsByExperiment\(\)](#), [shiftPlots\(\)](#), [shifts\\_load\(\)](#), [shifts\\_save\(\)](#)

### Examples

```
## Basic run
# Transcriptome annotation ->
gtf_file <- system.file("extdata/references/danio_rerio", "annotations.gtf", package = "ORFik")
# Ribo seq data ->
riboSeq_file <- system.file("extdata/Danio_rerio_sample", "ribo-seq.bam", package = "ORFik")
## Not run:
footprints <- readBam(riboSeq_file)
```

```

## Using CDS start site as reference point:
detectRibosomeShifts(footprints, gtf_file)
## Using CDS start site and stop site as 2 reference points:
#detectRibosomeShifts(footprints, gtf_file, stop = TRUE)
## Debug and detailed information for accepted reads lengths and p-site:
detectRibosomeShifts(footprints, gtf_file, heatmap = TRUE, verbose = TRUE)
## Debug why read length 31 was not accepted or wrong p-site:
#detectRibosomeShifts(footprints, gtf_file, must.be.periodic = FALSE,
#                      accepted.lengths = 31, heatmap = TRUE, verbose = TRUE)

## Subset bam file
param = ScanBamParam(flag = scanBamFlag(
                      isDuplicate = FALSE,
                      isSecondaryAlignment = FALSE))
footprints <- readBam(riboSeq_file, param = param)
detectRibosomeShifts(footprints, gtf_file, stop = TRUE)

## Without 5' Annotation
library(GenomicFeatures)

txdb <- loadTxdb(gtf_file)
tx <- exonsBy(txdb, by = "tx", use.names = TRUE)
tx <- extendLeaders(tx, 30)
## Now run function, without 5' and 3' UTRs
detectRibosomeShifts(footprints, txdb, start = TRUE, minFiveUTR = NULL,
                    minCDS = 150L, minThreeUTR = NULL, firstN = 150L,
                    tx = tx)

## End(Not run)

```

---

disengagementScore      *Disengagement score (DS)*

---

### Description

Disengagement score is defined as

$$(RPFs \text{ over ORF}) / (RPFs \text{ downstream to transcript end})$$

A pseudo-count of one is added to both the ORF and downstream sums.

### Usage

```

disengagementScore(
  grl,
  RFP,
  GtfOrTx,

```

```

RFP.sorted = FALSE,
weight = 1L,
overlapGr1 = NULL
)

```

### Arguments

gr1	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs.
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
GtfOrTx	If it is <a href="#">TxDb</a> object transcripts will be extracted using <code>exonsBy(Gtf, by = "tx", use.names = TRUE)</code> . Else it must be <a href="#">GRangesList</a>
RFP.sorted	logical (FALSE), an optimizer, have you ran this line: <code>RFP &lt;- sort(RFP[countOverlaps(RFP, tx, type = "within") &gt; 0])</code> Normally not touched, for internal optimization purposes.
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <code>countOverlaps()</code> presumes, if single number ( $\neq 1$ ), it repeats for all ranges, if vector with length > 1, it must be equal size of the reads object.
overlapGr1	an integer, (default: NULL), if defined must be <code>countOverlaps(gr1, RFP)</code> , added for speed if you already have it

### Value

a named vector of numeric values of scores

### References

doi: 10.1242/dev.098344

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```

ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
gr1 <- GRangesList(tx1_1 = ORF)
tx <- GRangesList(tx1 = GRanges("1", IRanges(1, 50), "+"))
RFP <- GRanges("1", IRanges(c(1,10,20,30,40), width = 3), "+")
disengagementScore(gr1, RFP, tx)

```

---

`distanceToFollowing`    *Distance to following range group*

---

**Description**

Follow means downstream GRangesList element, not including itself.

**Usage**

```
distanceToFollowing(gr1, gr12 = gr1, ignore.strand = FALSE)
```

**Arguments**

`gr1`                    a GRangesList  
`gr12`                    a GRangesList, default 'gr1'. The list that defines restrictions on extension. Can also be another set, which is used as 'roadblocks' for extension.  
`ignore.strand`    logical, default FALSE

**Value**

numeric vector of distance

---

`distanceToPreceding`    *Distance to preceding range group*

---

**Description**

Preceding means upstream GRangesList element, not including itself.

**Usage**

```
distanceToPreceding(gr1, gr12 = gr1, ignore.strand = FALSE)
```

**Arguments**

`gr1`                    a GRangesList  
`gr12`                    a GRangesList, default 'gr1'. The list that defines restrictions on extension. Can also be another set, which is used as 'roadblocks' for extension.  
`ignore.strand`    logical, default FALSE

**Value**

numeric vector of distance

---

distToCds	<i>Get distances between ORF ends and starts of their transcripts cds.</i>
-----------	--

---

### Description

Will calculate distance between each ORF end and beginning of the corresponding cds (main ORF). Matching is done by transcript names. This is applicable practically to the upstream (fiveUTRs) ORFs only. The cds start site, will be presumed to be on + 1 of end of fiveUTRs.

### Usage

```
distToCds(ORFs, fiveUTRs, cds = NULL)
```

### Arguments

ORFs	orfs as <a href="#">GRangesList</a> , names of orfs must be transcript names
fiveUTRs	fiveUTRs as <a href="#">GRangesList</a> , remember to use CAGE version of 5' if you did CAGE reassignment!
cds	cds' as <a href="#">GRangesList</a> , only add if you have ORFs going into CDS.

### Value

an integer vector, +1 means one base upstream of cds, -1 means 2nd base in cds, 0 means orf stops at cds start.

### References

doi: 10.1074/jbc.R116.733899

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
gr1 <- GRangesList(tx1_1 = GRanges("1", IRanges(1, 10), "+"))
fiveUTRs <- GRangesList(tx1 = GRanges("1", IRanges(1, 20), "+"))
distToCds(gr1, fiveUTRs)
```

---

distToTSS	<i>Get distances between ORF Start and TSS of its transcript</i>
-----------	--

---

### Description

Matching is done by transcript names. This is applicable practically to any region in Transcript If ORF is not within specified search space in tx, this function will crash.

### Usage

```
distToTSS(ORFs, tx)
```

### Arguments

ORFs	orfs as <a href="#">GRangesList</a> , names of orfs must be txname_[rank]
tx	transcripts as <a href="#">GRangesList</a> .

### Value

an integer vector, 1 means on TSS, 2 means second base of Tx.

### References

doi: 10.1074/jbc.R116.733899

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
gr1 <- GRangesList(tx1_1 = GRanges("1", IRanges(5, 10), "+"))
tx <- GRangesList(tx1 = GRanges("1", IRanges(2, 20), "+"))
distToTSS(gr1, tx)
```

---

download.SRA	<i>Download read libraries from SRA</i>
--------------	---

---

### Description

Multicore version download, see documentation for SRA toolkit for more information.

### Usage

```
download.SRA(
  info,
  outdir,
  rename = TRUE,
  fastq.dump.path = install.sratoolkit(),
  settings = paste("--skip-technical", "--split-files"),
  subset = NULL,
  compress = TRUE,
  use.ebi.ftp = is.null(subset),
  ebiDLMethod = "auto",
  timeout = 5000,
  BPPARAM = bpparam()
)
```

### Arguments

info	character vector of only SRR numbers or a data.frame with SRA metadata information including the SRR numbers in a column called "Run" or "SRR". Can be SRR, ERR or DRR numbers. If only SRR numbers can not rename, since no additional information is given.
outdir	directory to store runs, files are named by default (rename = TRUE) by information from SRA metadata table, if (rename = FALSE) named according to SRR numbers.
rename	logical or character, default TRUE (Auto guess new names). False: Skip renaming. A character vector of equal size as files wanted can also be given. Priority of renaming from the metadata is to check for unique names in the LibraryName column, then the sample_title column if no valid names in LibraryName. If new names found and still duplicates, will add "_rep1", "_rep2" to make them unique. If no valid names, will not rename, that is keep the SRR numbers, you then can manually rename files to something more meaningful.
fastq.dump.path	path to fastq-dump binary, default: path returned from install.sratoolkit()
settings	a string of arguments for fastq-dump, default: paste("-gzip", "-skip-technical", "-split-files")
subset	an integer or NULL, default NULL (no subset). If defined as a integer will download only the first n reads specified by subset. If subset is defined, will force to use fastq-dump which is slower than ebi download.

compress	logical, default TRUE. Download compressed files ".gz".
use.ebi.ftp	logical, default: is.null(subset). Use ORFiks much faster download function that only works when subset is null, if subset is defined, it uses fastqdump, it is slower but supports subsetting. Force it to use fastqdump by setting this to FALSE.
ebiDLMethod	character, default "auto". Which download protocol to use in download.file when using ebi ftp download. Sometimes "curl" is might not work (the default auto usually), in those cases use wget. See "method" argument of ?download.file, for more info.
timeout	5000, how many seconds before killing download if still active? Will overwrite global option until R session is closed. Increase value if you are on a very slow connection or downloading a large dataset.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers

### Value

a character vector of download files filepaths

### References

<https://ncbi.github.io/sra-tools/fastq-dump.html>

### See Also

Other sra: [browseSRA\(\)](#), [download.SRA.metadata\(\)](#), [download.ebi\(\)](#), [get\\_bioproject\\_candidates\(\)](#), [install.sratoolkit\(\)](#), [rename.SRA.files\(\)](#)

### Examples

```
SRR <- c("SRR453566") # Can be more than one

## Simple single SRR run of YEAST
outdir <- tempdir() # Specify output directory
# Download, get 5 first reads
#download.SRA(SRR, outdir, rename = FALSE, subset = 5)

## Using metadata column to get SRR numbers and to be able to rename samples
outdir <- tempdir() # Specify output directory
info <- download.SRA.metadata("SRP226389", outdir) # By study id
## Download, 5 first reads of each library and rename
#files <- download.SRA(info, outdir, subset = 5)
#Biostrings::readDNAStringSet(files[1], format = "fastq")

## Download full libraries of experiment
## (note, this will take some time to download!)
#download.SRA(info, outdir)
```

---

download.SRA.metadata *Downloads metadata from SRA*

---

### Description

Given an experiment identifier, query information from different locations of SRA to get a complete metadata table of the experiment. It first finds Runinfo for each library, then sample info, if pubmed id is not found searches for that and searches for author through pubmed.

### Usage

```
download.SRA.metadata(  
  SRP,  
  outdir = tempdir(),  
  remove.invalid = TRUE,  
  auto.detect = FALSE,  
  abstract = "printsave",  
  force = FALSE,  
  rich.format = FALSE,  
  fetch_GSE = FALSE  
)
```

### Arguments

SRP	character string, a study ID as either the PRJ, SRP, ERP, DRP, GSE or SRA of the study, examples would be "SRP226389" or "ERP116106". If GSE it will try to convert to the SRP to find the files. The call works as long the runs are registered on the efetch server, as their is a linked SRP link from bioproject or GSE. Example which fails is "PRJNA449388", which does not have a linking like this.
outdir	character string, directory to save file, default: tempdir(). The file will be called "SraRunInfo_SRP.csv", where SRP is the SRP argument. We advice to use bioproject IDs "PRJNA...". The directory will be created if not existing.
remove.invalid	logical, default TRUE. Remove Runs with 0 reads (spots)
auto.detect	logical, default FALSE. If TRUE, ORFik will add additional columns: LIBRARYTYPE: (is this Ribo-seq or mRNA-seq, CAGE etc), REPLICATE: (is this replicate 1, 2 etc), STAGE: (Which time point, cell line or tissue is this, HEK293, TCP-1, 24hpf etc), CONDITION: (is this Wild type control or a mutant etc). These values are only qualified guesses from the metadata, so always double check!
abstract	character, default "printsave". If abstract for project exists, print and save it (save the file to same directory as runinfo). Alternatives: "print", Only print first time downloaded, will not be able to print later. "save" save it, no print "no" skip download of abstract

force	logical, default FALSE. If TRUE, will redownload all files needed even though they exists. Useful if you wanted auto.detection, but already downloaded without it.
rich.format	logical, default FALSE. If TRUE, will fetch all Experiment and Sample attributes. It means, that different studies can have different set of columns if set to TRUE.
fetch_GSE	logical, default FALSE. Search for GSE, if exists, appends a column called GEO. Will be included even though this study is not from GEO, then it sets all to NA.

### Details

A common problem is that the project is not linked to an article, you will then not get a pubmed id.

The algorithm works like this:

If GEO identifier, find the SRP.

Then search Entrez for project and get sample identifier.

From that extract the run information and collect into a final table.

### Value

a data.table of the metadata, 1 row per sample, SRR run number defined in 'Run' column.

### References

doi: 10.1093/nar/gkq1019

### See Also

Other sra: [browseSRA\(\)](#), [download.SRA\(\)](#), [download.ebi\(\)](#), [get\\_bioproject\\_candidates\(\)](#), [install.sratoolkit\(\)](#), [rename.SRA.files\(\)](#)

### Examples

```
## Originally on SRA
download.SRA.metadata("SRP226389")
## Now try with auto detection (guessing additional library info)
## Need to specify output dir as tempfile() to re-download
#download.SRA.metadata("SRP226389", tempfile(), auto.detect = TRUE)
## Originally on ENA (RCP-seq data)
# download.SRA.metadata("ERP116106")
## Originally on GEO (GSE) (save to directory to keep info with fastq files)
# download.SRA.metadata("GSE61011")
## Bioproject ID
# download.SRA.metadata("PRJNA231536")
```

---

`download_gene_homologues`*Download homologue information of a gene*

---

**Description**

Uses ncbi gene database for vertebrates

**Usage**

```
download_gene_homologues(  
  gene_id = "ENSG00000110092",  
  organism = "Homo sapiens"  
)
```

**Arguments**

<code>gene_id</code>	character, gene name (ensembl gene id, not symbol!)
<code>organism</code>	default NULL. Scientific name (e.g. Homo sapiens)

**Value**

character, summary text for gene from the database.

---

`download_gene_info`*Download summary information of a gene*

---

**Description**

Uses ncbi gene database summary from RefSeq

**Usage**

```
download_gene_info(gene = "CCND1", organism = "Homo sapiens", by = "symbol")
```

**Arguments**

<code>gene</code>	character, gene name (symbol)
<code>organism</code>	default NULL. Scientific name (e.g. Homo sapiens)
<code>by</code>	character, default symbol (search by gene symbol name). If "ensembl id", it searches as it is ensembl gene id ENSG.. etc.

**Value**

character, summary text for gene from the database.

## Examples

```
# Wrap in 'try' to avoid wrong bioc test error
try(download_gene_info(gene = "CCND1"))
try(download_gene_info("ENSG00000110092", by = "ensembl_id")) # By ensembl id
try(download_gene_info(gene = "CCND1", organism = "Mus musculus"))
```

---

DTEG.analysis

*Run differential TE analysis*


---

## Description

Expression analysis of 2 dimensions, usually Ribo-seq vs RNA-seq.

Using an equal reimplementation of the deltaTE algorithm (see reference).

Creates a total of 3 DESeq models (given  $x$  is the target.contrast argument) (usually 'condition' column) and libraryType is RNA-seq and Ribo-seq):

### \*\* The 3 differential sub models \*\*

- 1. Ribo-seq model : design =  $\sim x$  (differences between the  $x$  groups in Ribo-seq)
- 2. RNA-seq model: design =  $\sim x$  (differences between the  $x$  groups in RNA-seq)
- 3. TE model: design =  $\sim x + \text{libraryType} + \text{libraryType}:x$  (differences between the  $x$  and libraryType groups and the interaction between them)

You need at least 2 groups and 2 replicates per group. By default, the Ribo-seq counts will be over CDS and RNA-seq counts over whole mRNAs, per transcript. See notes section below for more details.

## Usage

```
DTEG.analysis(
  df.rfp,
  df.rna,
  output.dir = QCfolder(df.rfp),
  target.contrast = design[1],
  design = ORFik::design(df.rfp),
  p.value = 0.05,
  RFP_counts = countTable(df.rfp, "cds", type = "summarized"),
  RNA_counts = countTable(df.rna, "mrna", type = "summarized"),
  batch.effect = FALSE,
  pairs = combn.pairs(unlist(df.rfp[, design])),
  plot.title = "",
  plot.ext = ".pdf",
  width = 6,
  height = 6,
  dot.size = 0.4,
  relative.name = paste0("DTEG_plot", plot.ext),
```

```

    complex.categories = FALSE,
    plot_to_console = TRUE,
    fitType = c("parametric", "local", "mean", "glmGamPoi"),
    lfcShrinkType = "normal"
  )

```

### Arguments

<code>df.rfp</code>	a <a href="#">experiment</a> of usually Ribo-seq or 80S from TCP-seq. (the numerator of the experiment, usually having a primary role)
<code>df.rna</code>	a <a href="#">experiment</a> of usually RNA-seq. (the denominator of the experiment, usually having a normalizing function)
<code>output.dir</code>	character, default <code>QCfolder(df.rfp)</code> . <code>output.dir</code> directory to save plots, plot will be named "TE_between". If NULL, will not save.
<code>target.contrast</code>	a character vector, default <code>design[1]</code> . The column in the ORFik experiment that represent the comparison contrasts. By default: the first design factor of the full experimental design. This is the factor you will do the comparison on. DESeq will normalize the counts based on the full design, but the log fold change values will be based on this contrast only. It is usually the 'condition' column.
<code>design</code>	a character vector, default <code>design(df.rfp)</code> . The full experiment design. Which factors have more than 1 level. Example: stage column are all HEK293, so it can not be a design factor. The condition column has 2 possible values, WT and mutant, so it is a factor of the experiment. Replicates column is not part of design, that is inserted later with setting <code>batch.effect = TRUE</code> . Library type 'libtype' column, can also no be part of initial design, it is always added inside the function, after initial setup.
<code>p.value</code>	a numeric, default 0.05 in interval (0,1). Defines adjusted p-value to be used as significance threshold for the result groups. I.e. for exclusive translation group significant subset for <code>p.value = 0.05</code> means: <code>TE\$padj &lt; 0.05 &amp; Ribo\$padj &lt; 0.05 &amp; RNA\$padj &gt; 0.05</code> .
<code>RFP_counts</code>	a <a href="#">SummarizedExperiment</a> , default: <code>countTable(df.rfp, "cds", type = "summarized")</code> , unshifted libraries, all transcript CDSs. If you have pshifted reads and <code>countTables</code> , do: <code>countTable(df.rfp, "cds", type = "summarized", count.folder = "pshifted")</code> Assign a subset if you don't want to analyze all genes. It is recommended to not subset, to give DESeq2 data for variance analysis.
<code>RNA_counts</code>	a <a href="#">SummarizedExperiment</a> , default: <code>countTable(df.rna, "mrna", type = "summarized")</code> , all transcripts. Assign a subset if you don't want to analyze all genes. It is recommended to not subset, to give DESeq2 data for variance analysis.
<code>batch.effect</code>	logical, default TRUE. Makes replicate column of the experiment part of the design. If you believe you might have batch effects, keep as TRUE. Batch effect usually means that you have a strong variance between biological replicates. Check out <a href="#">pcaExperiment</a> and see if replicates cluster together more than the design factor, to verify if you need to set it to TRUE.
<code>pairs</code>	list of character pairs, the experiment contrasts. Default: <code>combn.pairs(unlist(df.rfp[, target.contrast])</code>

<code>plot.title</code>	title for plots, usually name of experiment etc
<code>plot.ext</code>	character, default: ".pdf". Alternatives: ".png" or ".jpg". Multiple values allowed, if so will save file in each format specified.
<code>width</code>	numeric, default 6 (in inches)
<code>height</code>	numeric, default 6 (in inches)
<code>dot.size</code>	numeric, default 0.4, size of point dots in plot.
<code>relative.name</code>	character, Default: <code>paste0("DTEG_plot", plot.ext)</code> Relative name of file to be saved in folder specified in <code>output.dir</code> . Change to .pdf if you want pdf file instead of png. Multiple values allowed, if so will save file in each format specified.
<code>complex.categories</code>	logical, default FALSE. Separate into more groups, see above for details.
<code>plot_to_console</code>	logical, default TRUE. Plot to console before returning, set to FALSE to save some run time.
<code>fitType</code>	either "parametric", "local", "mean", or "glmGamPoi" for the type of fitting of dispersions to the mean intensity. See <a href="#">estimateDispersions</a> for description.
<code>lfcShrinkType</code>	character or NULL. Default "normal", which shrinkage to apply to results for low count gene subset. This avoids the problem of extreme fold changes, when counts are low. See <a href="#">lfcShrink</a> . A note for DTEG.analysis function: The interaction term (TE), is not shrunked as this is not counts, but a ratio.

## Details

Log fold changes and p-values are created from a Walds test on the comparison contrast described bellow. The RNA-seq and Ribo-seq LFC values are shrunken using `DESeq2::lfcShrink(type = "normal")`. Note that the TE LFC values are not shrunken, since these are ratios and not counts (as following specifications from deltaTE paper). The adjusted p-values are created using `DESeq2 pAdjustMethod = "BH"` (Benjamini-Hochberg correction). All other DESEQ2 arguments are default.

Analysis is done between each possible combination of levels in the target contrast If target contrast is condition column, with factor levels: WT, mut1 and mut2 with 3 replicates each. You get comparison of WT vs mut1, WT vs mut2 and mut1 vs mut2.

The respective result categories are defined through 4 main categories, first some intuition. The number of ribosomes (Ribo-seq) is significantly different between 2 contrast elements in the model if the relative counts is statistically higher/lower, for mRNA levels (RNA-seq) it is the same. So TE is then RFP / RNA which is basically how many ribosomes translated per mRNA in the sample, if contrast group 1 has TE of 2, it means 2 ribosomes per mrna fragment, while TE of 4 would be a doubling of 4 ribosomes per mRNA.

Mathematically the groups are defined by the p adjusted values as the following (`te.sign` means `na_safe(te.padj < p.value)`, `na_safe` is a function where NA values are FALSE for '<=' test and TRUE for '>' test), we also use a helper function: `te.sign & rfp.sign & rna.sign, all_models_sign :=`

TRUE.

**\*\* Significant DTEG Classifications \*\***

- No change : None of the below categories
- Translation (only RFP) : `te.sign & rfp.sign & !rna.sign`
- Expression (only RNA) : `!te.sign & !rfp.sign & rna.sign`
- mRNA abundance : `all_models_sign & na_safe(te.lfc * rna.lfc, ">", 0)`
- Inverse (inverse mRNA abundance) : `all_models_sign & te.lfc * rna.lfc, "<", 0)`
- Buffering (Stable protein output) : `te.sign & !rfp.sign & rna.sign`
- Forwarded (diagonal bottom left to top right) : `!te.sign & rfp.sign & rna.sign`

If `complex.categories` is FALSE, then Expression, Inverse and forwarded are defined 'Buffering'. mRNA abundance is called "Intensified" in original article For code, of classification, run: `View(ORFik:::DTEG_add_regulation)`. Feel free to redefine the categories as you want them.

See Figure 1 in the reference article for a clear definition of the groups!

If you do not need isoform variants, subset to longest isoform per gene either before or in the returned object (See examples). If you do not have RNA-seq controls, you can still use DESeq on Ribo-seq alone.

The LFC values are shrunken by `lfcShrink(type = "normal")`.

Remember that DESeq by default can not do global change analysis, it can only find subsets with changes in LFC!

## Value

a data.table with columns: (contrast variable, gene id, regulation status, log fold changes, p.adjust values, mean counts, significant (as logical))

## References

<https://doi.org/10.1002/cpmb.108>

## See Also

Other DifferentialExpression: `DEG.plot.static()`, `DEG_model()`, `DTEG.plot()`, `te.table()`, `te_rna.plot()`

## Examples

```
## Simple example (use ORFik template, then split on Ribo and RNA)
df <- ORFik.template.experiment()
df.rfp <- df[df$libtype == "RFP",]
df.rna <- df[df$libtype == "RNA",]
design(df.rfp) # The experimental design, per libtype
design(df.rfp)[1] # Default target contrast
#dt <- DTEG.analysis(df.rfp, df.rna)
#dt_with_gene_ids <- append_gene_symbols(dt, symbols(df))
## If you want to use the pshifted libs for analysis:
```

```

#dt <- DTEG.analysis(df.rfp, df.rna,
#                   RFP_counts = countTable(df.rfp, region = "cds",
#                                           type = "summarized", count.folder = "pshifted"))
## Restrict DTEGs by log fold change (LFC):
## subset to abs(LFC) < 1.5 for both rfp and rna
#dt[abs(rfp) < 1.5 & abs(rna) < 1.5, Regulation := "No change"]

## Only longest isoform per gene:
#tx_longest <- filterTranscripts(df.rfp, 0, 1, 0)
#dt <- dt[id %in% tx_longest,]
## Convert to gene id
#dt[, id := txNamesToGeneNames(id, df.rfp)]
## To get by gene symbol, use biomaRt conversion
## To flip directionality of contrast pair nr 2:
#design <- "condition"
#pairs <- combn.pairs(unlist(df.rfp[, design])
#pairs[[2]] <- rev(pairs[[2]])
#dt <- DTEG.analysis(df.rfp, df.rna,
#                   RFP_counts = countTable(df.rfp, region = "cds",
#                                           type = "summarized", count.folder = "pshifted"),
#                   pairs = pairs)

```

---

DTEG.plot

*Plot DTEG result*


---

## Description

For explanation of plot categories, see [DTEG.analysis](#)

## Usage

```

DTEG.plot(
  dt,
  output.dir = NULL,
  p.value.label = ifelse(!is.null(attr(dt, "p.value")), attr(dt, "p.value"), 0.05),
  plot.title = "",
  plot.ext = ".pdf",
  width = 6,
  height = 6,
  dpi = 300,
  dot.size = 0.4,
  xlim = "bidir.max",
  ylim = "bidir.max",
  relative.name = paste0("DTEG_plot", plot.ext),
  plot_to_console = TRUE
)

```

**Arguments**

<code>dt</code>	a data.table with the results from <a href="#">DTEG.analysis</a>
<code>output.dir</code>	a character path, default NULL(no save), or a directory to save to a file. Relative name of file, specified by 'relative.name' argument.
<code>p.value.label</code>	a numeric, default <code>ifelse(!is.null(attr(dt, "p.value")), attr(dt, "p.value"), 0.05)</code> Interval (0,1), use "" to not show. What p-value used for the analysis? Will be shown as a caption.
<code>plot.title</code>	title for plots, usually name of experiment etc
<code>plot.ext</code>	character, default: ".pdf". Alternatives: ".png" or ".jpg". Multiple values allowed, if so will save file in each format specified.
<code>width</code>	numeric, default 6 (in inches)
<code>height</code>	numeric, default 6 (in inches)
<code>dpi</code>	numeric, default 300.
<code>dot.size</code>	numeric, default 0.4, size of point dots in plot.
<code>xlim</code>	numeric vector or character preset, default: "bidir.max" (Equal in both + / - direction, using max value + 0.5 of rna column in dt). If you want ggplot to decide limit, set to "auto". For numeric vector, specify min and max x limit: like <code>c(-5, 5)</code>
<code>ylim</code>	numeric vector or character preset, default: "bidir.max" (Equal in both + / - direction, using max value + 0.5 of rfp column in dt). If you want ggplot to decide limit, set to "auto". For numeric vector, specify min and max y limit: like <code>c(-10, 10)</code>
<code>relative.name</code>	character, Default: <code>paste0("DTEG_plot", plot.ext)</code> Relative name of file to be saved in folder specified in <code>output.dir</code> . Change to .pdf if you want pdf file instead of png. Multiple values allowed, if so will save file in each format specified.
<code>plot_to_console</code>	logical, default TRUE. Plot to console before returning, set to FALSE to save some run time.

**Value**

a ggplot object, will `facet_wrap` if `length(unique(dt$contrasts)) > 1`

**See Also**

Other DifferentialExpression: [DEG.plot.static\(\)](#), [DEG\\_model\(\)](#), [DTEG.analysis\(\)](#), [te.table\(\)](#), [te\\_rna.plot\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
df.rfp <- df[df$libtype == "RFP",]
df.rna <- df[df$libtype == "RNA",]
#dt <- DTEG.analysis(df.rfp, df.rna)
#Default scaling
```

```
#DTEG.plot(dt)
#Manual scaling
#DTEG.plot(dt, xlim = c(-2, 2), ylim = c(-2, 2))
```

---

end,GRanges-method      *Get ends of GRanges*

---

### Description

Faster version than S4Vector generic caller

### Usage

```
## S4 method for signature 'GRanges'
end(x)
```

### Arguments

x                      a GRanges

### Value

an integer (length equal to x)

---

entropy                      *Percentage of maximum entropy*

---

### Description

Calculates percentage of maximum entropy of the ‘reads’ coverage over each ORF in ‘grl’ group. The entropy value per group is a real number in the interval (0:1), where 0 indicates no variance in reads over all codons of group For example c(0,0,0,0) has 0 entropy, since no reads overlap.

Interval: [0]: No reads or all reads in 1 place

Interval: [0.01-0.99]:  $\geq 2$  positions covered

Interval: [1]: all positions covered perfectly in frame

### Usage

```
entropy(grl, reads, weight = 1L, is.sorted = FALSE, overlapGr1 = NULL)
```

**Arguments**

<code>grl</code>	a <a href="#">GRangesList</a> object can be either transcripts, 5' utrs, cds', 3' utrs or ORFs as a special case (uORFs, potential new cds' etc). If regions are not spliced you can send a <a href="#">GRanges</a> object.
<code>reads</code>	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object, usually of RiboSeq, RnaSeq, CageSeq, etc.
<code>weight</code>	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If <code>weight</code> is name of defined meta column in reads object, it gives the number of times a read was found at that position. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <code>countOverlaps()</code> presumes, if single number ( $\neq 1$ ), it repeats for all ranges, if vector with length $> 1$ , it must be equal size of the reads object.
<code>is.sorted</code>	logical (FALSE), is <code>grl</code> sorted. That is + strand groups in increasing ranges (1,2,3), and - strand groups in decreasing ranges (3,2,1)
<code>overlapGr1</code>	an integer, (default: NULL), if defined must be <code>countOverlaps(grl, RFP)</code> , added for speed if you already have it.

**Value**

A numeric vector containing one entropy value per element in 'grl'

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
# a toy example with ribo-seq p-shifted reads
ORF <- GRangesList(tx1 = GRanges("1", IRanges(1, width = 9), "+"))
entropy(ORF, GRanges()) # 0
entropy(ORF, GRanges("1", IRanges(c(1)), "+")) # 0
entropy(ORF, GRanges("1", IRanges(c(1,4,6,7)), "+")) # 0.94
entropy(ORF, GRanges("1", IRanges(c(1,4,7)), "+", score = c(1,2,1)),
       weight = "score") # 0.94
entropy(ORF, GRanges("1", IRanges(c(1,4,7)), "+")) # Perfect = 1
```

envExp

*Get ORFik experiment environment*

---

**Description**

More correctly, get the pointer reference, default is .GlobalEnv

**Usage**

```
envExp(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

environment pointer, name of environment: pointer

---

envExp,experiment-method

*Get ORFik experiment environment*

---

**Description**

More correctly, get the pointer reference, default is .GlobalEnv

**Usage**

```
## S4 method for signature 'experiment'  
envExp(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

environment pointer, name of environment: pointer

---

envExp<-                    *Set ORFik experiment environment*

---

**Description**

More correctly, set the pointer reference, default is .GlobalEnv

**Usage**

```
envExp(x) <- value
```

**Arguments**

x                    an ORFik [experiment](#)  
value                environment pointer to assign to experiment

**Value**

an ORFik [experiment](#) with updated environment

---

envExp<- ,experiment-method  
                              *Set ORFik experiment environment*

---

**Description**

More correctly, set the pointer reference, default is .GlobalEnv

**Usage**

```
## S4 replacement method for signature 'experiment'  
envExp(x) <- value
```

**Arguments**

x                    an ORFik [experiment](#)  
value                environment pointer to assign to experiment

**Value**

an ORFik [experiment](#) with updated environment

---

exonsWithPseudoIntronsPerGroup

*Get exons with pseudo introns per Group*

---

### Description

If an intron is of length < 'width' \* 2, it will not be split into pseudo.

### Usage

```
exonsWithPseudoIntronsPerGroup(gr1, width = 100)
```

### Arguments

gr1                    a GRangesList of length 1  
width                  numeric, default 100. The size of pseudo flanks.

### Value

a GRangesList

### Examples

```
tx <- GRangesList(GRanges("1", IRanges(c(1, 150, 1e5, 1e6)), "+"))
exonsWithPseudoIntronsPerGroup(tx) # See intron 1 is not split
tx_2 <- rep(GRangesList(GRanges("1", IRanges(c(1, 150, 1e5, 1e6)), "+")), 2)
exonsWithPseudoIntronsPerGroup(tx_2)
tx_3 <- tx_2
names(tx_3) <- c("tx1", "tx2")
exonsWithPseudoIntronsPerGroup(tx_3, 1e6)
```

---

experiment-class

*experiment class definition*

---

### Description

It is an object that simplify and error correct your NGS workflow, creating a single R object that stores and controls all results relevant to a specific experiment.

It contains following important parts:

- **filepaths:** Information for each library in the experiment (for multiple file formats: bam, bed, wig, ofst, etc.)
- **genome:** Annotation files for the experiment (fasta genome, index, gtf, txdb)
- **organism:** Name (for automatic GO, sequence analysis, etc.)

- description: Author information and experiment details (use `'list.experiments()'` to show all experiments made with ORFik; this makes it easy to find and load them later)
- API: ORFik supports a rich API for using the experiment, e.g., `'outputLibs(experiment, type = "wig")'` to load all libraries in the wig format into R, `'loadTxdb(experiment)'` to load the txdb (gtf) of the experiment, `'transcriptWindow()'` to plot metacoverage for all libraries, and `'countTable(experiment)'` to load count tables, etc.
- Safety: Verifies that experiments contain no duplicate, empty, or non-accessible files.

Act as a way of extension of [SummarizedExperiment](#) by allowing more ease to find not only counts, but rather information about libraries, and annotation, so that more tasks are possible. Like coverage per position in some transcript etc.

## Constructor:

Simplest way to make is to call:

```
create.experiment(dir)
```

On some folder with NGS libraries (usually bam files) and see what you get. Some of the fields might be needed to fill in manually. Each resulting row must be unique (not including filepath, they are always unique), that means if it has replicates then that must be said explicit. And all filepaths must be unique and have files with size > 0.

Here all the columns in the experiment will be described: name (column info): examples

- filepaths: Information for each library in the experiment (for multiple file formats: bam, bed, wig, ofst, etc.)
- genome: Annotation files for the experiment (fasta genome, index, gtf, txdb)
- organism: Name (for automatic GO, sequence analysis, etc.)
- description: Author information and experiment details (use `'list.experiments()'` to show all experiments made with ORFik; this makes it easy to find and load them later)
- API: ORFik supports a rich API for using the experiment, e.g., `'outputLibs(experiment, type = "wig")'` to load all libraries in the wig format into R, `'loadTxdb(experiment)'` to load the txdb (gtf) of the experiment, `'transcriptWindow()'` to plot metacoverage for all libraries, and `'countTable(experiment)'` to load count tables, etc.
- Safety: Verifies that experiments contain no duplicate, empty, or non-accessible files.

## Details

Special rules:

Supported:

Single/paired end bam, bed, wig, ofst + compressions of these

The reverse column of the experiments says "paired-end" if bam file. If a pair of wig files, forward and reverse strand, reverse is filepath to '-' strand wig file. Paired forward / reverse wig files, must have same name except `_forward` / `_reverse` in name

Paired end bam, when creating experiment, set `pairedEndBam = c(T, T, T, F)`. For 3 paired end libraries, then one single end.

Naming: Will try to guess naming for tissues / stages, replicates etc. If it finds more than one hit for one file, it will not guess. Always check that it guessed correctly.

**Value**

a ORFik experiment

**See Also**

Other ORFik\_experiment: `ORFik.template.experiment()`, `ORFik.template.experiment.zf()`, `bamVarName()`, `create.experiment()`, `filepath()`, `libraryTypes()`, `organism`, `experiment-method`, `outputLibs()`, `read.experiment()`, `save.experiment()`, `validateExperiments()`

**Examples**

```
## To see an internal ORFik example
df <- ORFik.template.experiment()
## See libraries in experiment
df
## See organism of experiment
organism(df)
## See file paths in experiment
filepath(df, "default")
## Output NGS libraries in R, to .GlobalEnv
#outputLibs(df)
## Output cds of experiment annotation
#loadRegion(df, "cds")

## This is how to make it:
## Not run:
library(ORFik)

# 1. Update path to experiment data directory (bam, bed, wig files etc)
exp_dir = "/data/processed_data/RNA-seq/Lee_zebrafish_2013/aligned/"

# 2. Set a short character name for experiment, (Lee et al 2013 -> Lee13, etc)
exper_name = "Lee13"

# 3. Create a template experiment (gtf and fasta genome)
temp <- create.experiment(exp_dir, exper_name, saveDir = NULL,
  txdb = "/data/references/Zv9_zebrafish/Danio_rerio.Zv9.79.gtf",
  fa = "/data/references/Zv9_zebrafish/Danio_rerio.Zv9.fa",
  organism = "Homo sapiens")

# 4. Make sure each row(sample) is unique and correct
# You will get a view open now, check the data.frame that it is correct:
# library type (RNA-seq, Ribo-seq), stage, rep, condition, fraction.
# Let say it did not figure out it is RNA-seq, then we do:"

temp[5:6, 1] <- "RNA" # [row 5 and 6, col 1] are library types

# You can also do this in your spread sheet program (excel, libre office)
# Now save new version, if you did not use spread sheet.
saveName <- paste0("/data/processed_data/experiment_tables_for_R/",
  exper_name, ".csv")
save.experiment(temp, saveName)
```

```
# 5. Load experiment, this will validate that you actually made it correct
df <- read.experiment(saveName)

# Set experiment name not to be assigned in R variable names
df@expInVarName <- FALSE
df

## End(Not run)
```

---

experiment.colors      *Decide color for libraries by grouping*

---

### Description

Pick the grouping wanted for colors, by default only group by libtype. Like RNA-seq(skyblue4) and Ribo-seq(orange).

### Usage

```
experiment.colors(
  df,
  color_list = "default",
  skip.libtype = FALSE,
  skip.stage = TRUE,
  skip.replicate = TRUE,
  skip.fraction = TRUE,
  skip.condition = TRUE
)
```

### Arguments

df	an ORFik <a href="#">experiment</a>
color_list	a character vector of colors, default "default". That is the vector c("skyblue4", "orange", "green", "red", "gray", "yellow", "blue", "red2", "orange3"). Picks number of colors needed to make groupings have unique color
skip.libtype	a logical (FALSE), don't include libtype
skip.stage	a logical (FALSE), don't include stage in variable name.
skip.replicate	a logical (FALSE), don't include replicate in variable name.
skip.fraction	a logical (FALSE), don't include fraction
skip.condition	a logical (FALSE), don't include condition in variable name.

### Value

a character vector of colors

---

export.bed12	<i>Export as bed12 format</i>
--------------	-------------------------------

---

### Description

bed format for multiple exons per group, as transcripts. Can be use as alternative as a sparse .gff format for ORFs. Can be direct input for ucsc browser or IGV

### Usage

```
export.bed12(grl, file, rgb = 0)
```

### Arguments

grl	A GRangesList
file	a character path to valid output file name
rgb	integer vector, default (0), maximum (255), either single integer or vector of same size as grl to specify groups. It is adviced to not use more than 8 different groups. (In IGV / UCSC 0 is black and 255 is blue)

### Details

If grl has no names, groups will be named 1,2,3,4..

### Value

invisible(NULL) (File is saved only)

### References

<https://bedtools.readthedocs.io/en/latest/content/general-usage.html#bed-format>

### See Also

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

### Examples

```
grl <- GRangesList(GRanges("1", c(1,3,5), "+"))
```

---

export.bedo	<i>Store GRanges object as .bedo</i>
-------------	--------------------------------------

---

### Description

.bedo is .bed ORFik, an optimized bed format for coverage reads with read lengths .bedo is a text based format with columns (6 maximum):

1. chromosome
2. start
3. end
4. strand
5. ref width (cigar # M's, match/mismatch total)
6. duplicates of that read

### Usage

```
export.bedo(object, out)
```

### Arguments

object	a GRanges object
out	a character, location on disc (full path)

### Details

Positions are 1-based, not 0-based as .bed. End will be removed if all ends equals all starts. Import with import.bedo

### Value

NULL, object saved to disc

---

export.bedoc	<i>Store GAlignments object as .bedoc</i>
--------------	---

---

### Description

A fast way to store, load and use bam files. (we now recommend using `link{export.ofst}` instead!)

.bedoc is .bed ORFik, an optimized bed format for coverage reads with cigar and replicate number. .bedoc is a text based format with columns (5 maximum):

1. chromosome
2. cigar: (cigar # M's, match/mismatch total)
3. start (left most position)

4. strand (+, -, \*)
5. score: duplicates of that read

### Usage

```
export.bedoc(object, out)
```

### Arguments

object	a GAlignments object
out	a character, location on disc (full path)

### Details

Positions are 1-based, not 0-based as .bed. Import with import.bedoc

### Value

NULL, object saved to disc

---

export.bigWig	<i>Export as bigWig format</i>
---------------	--------------------------------

---

### Description

Will create 2 files, 1 for + strand (\*\_forward.bigWig) and 1 for - strand (\*\_reverse.bigWig). If all ranges are \* stranded, will output 1 file. Can be direct input for ucsc browser or IGV

### Usage

```
export.bigWig(
  x,
  file,
  split.by.strand = TRUE,
  is_pre_collapsed = FALSE,
  seq_info = seqinfo(x)
)
```

### Arguments

x	A GRangesList, GAlignment GAlignmentPairs with score column. Will be converted to 5' end position of original range. If score column does not exist, will group ranges and give replicates as score column. Since bigWig needs a score column to represent counts!
file	a character path to valid output file name

split.by.strand logical, default TRUE. Split bigWig into 2 files, one for each strand.

is\_pre\_collapsed logical, default FALSE. Have you already collapsed reads with collapse.by.scores, so each positions is only in 1 GRanges object with a score column per readlength? Set to TRUE, only if you are sure, will give a speedup.

seq\_info a Seqinfo object, default seqinfo(x). Must have non NA seqlengths defined!

**Value**

invisible(NULL) (File is saved as 2 .bigWig files)

**References**

<https://genome.ucsc.edu/goldenPath/help/bigWig.html>

**See Also**

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

**Examples**

```
x <- c(GRanges("1", c(1,3,5), "-"), GRanges("1", c(1,3,5), "+"))
seqlengths(x) <- 10
file <- file.path(tempdir(), "rna.bigWig")
# export.bigWig(x, file)
# export.bigWig(covRleFromGR(x), file)
```

---

export.fstwig

*Export as fstwig (fastwig) format*

---

**Description**

Will create 2 files, 1 for + strand (\*\_forward.fstwig) and 1 for - strand (\*\_reverse.fstwig). If all ranges are \* stranded, will output 1 file.

**Usage**

```
export.fstwig(
  x,
  file,
  by.readlength = TRUE,
  by.chromosome = TRUE,
  compress = 50
)
```

**Arguments**

x	A GRangesList, GAlignment GAlignmentPairs with score column or coverage RLElist Will be converted to 5' end position of original range. If score column does not exist, will group ranges and give replicates as score column.
file	a character path to valid output file name
by.readlength	logical, default TRUE
by.chromosome	logical, default TRUE
compress	value in the range 0 to 100, indicating the amount of compression to use. Lower values mean larger file sizes. The default compression is set to 50.

**Value**

invisible(NULL) (File is saved as 2 .fstwig files)

**References**

"TODO"

**See Also**

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

**Examples**

```
x <- c(GRanges("1", c(1,3,5), "-"), GRanges("1", c(1,3,5), "+"))
x$size <- rep(c(28, 29), length.out = length(x))
x$score <- c(5,1,2,5,1,6)
seqlengths(x) <- 5
# export.fstwig(x, "~/Desktop/ribo")
```

---

export.ofst

*Store GRanges / GAlignments object as .ofst*

---

**Description**

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)

4. width (not added if cigar exists)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)
5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from GAlignmentPairs, it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

## Usage

```
export.ofst(x, file, ...)
```

## Arguments

x	a GRanges, GAlignments or GAlignmentPairs object
file	a character, location on disc (full path)
...	additional arguments for write_fst

## Details

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

## Value

NULL, object saved to disc

## Examples

```
tempfile <- tempfile(fileext = ".ofst")

## GRanges
gr <- GRanges("1:1-3:-")
export.ofst(gr, file = tempfile)
identical(gr, fimport(tempfile))
# Save from data.frame of GAlignment columns
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
export.ofst(df, tempfile)
res <- fimport(tempfile) # Imports as GAlignments because you have cigar
res
# Save from GAlignments
ga <- ORFik:::getGAlignments(df)
export.ofst(ga, tempfile)
res2 <- fimport(tempfile)
identical(res, res2)
# Save from GAlignmentsPair
ga_rev <- ga
strand(ga_rev) <- "-"
ga2 <- GAlignmentPairs(ga, ga_rev)
export.ofst(ga2, tempfile)
res3 <- fimport(tempfile)
```

```
identical(ga2, res3)
```

---

```
export.ofst,data.frame-method
```

*Store GRanges / GAlignments object as .ofst*

---

## Description

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)
4. width (not added if cigar exists)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)
5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from GAlignmentPairs, it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

## Usage

```
## S4 method for signature 'data.frame'
export.ofst(x, file, NAMES = NULL, elementMetadata = DataFrame(), ...)
```

## Arguments

x	a GRanges, GAlignments or GAlignmentPairs object
file	a character, location on disc (full path)
NAMES	character, default NULL. Names of ranges.
elementMetadata	DataFrame, default DataFrame(). Metadata of ranges.
...	additional arguments for write_fst

## Details

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

**Value**

NULL, object saved to disc

**Examples**

```
tempfile <- tempfile(fileext = ".ofst")

## GRanges
gr <- GRanges("1:1-3:-")
export.ofst(gr, file = tempfile)
identical(gr, fimport(tempfile))
# Save from data.frame of GAlignment columns
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
export.ofst(df, tempfile)
res <- fimport(tempfile) # Imports as GAlignments because you have cigar
res
# Save from GAlignments
ga <- ORFik::getGAlignments(df)
export.ofst(ga, tempfile)
res2 <- fimport(tempfile)
identical(res, res2)
# Save from GAlignmentsPair
ga_rev <- ga
strand(ga_rev) <- "-"
ga2 <- GAlignmentPairs(ga, ga_rev)
export.ofst(ga2, tempfile)
res3 <- fimport(tempfile)
identical(ga2, res3)
```

---

export.ofst,GAlignmentPairs-method

*Store GRanges / GAlignments object as .ofst*

---

**Description**

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)
4. width (not added if cigar exists)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)

5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from GAlignmentPairs, it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

### Usage

```
## S4 method for signature 'GAlignmentPairs'
export.ofst(x, file, ...)
```

### Arguments

x	a GRanges, GAlignments or GAlignmentPairs object
file	a character, location on disc (full path)
...	additional arguments for write_fst

### Details

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

### Value

NULL, object saved to disc

### Examples

```
tempfile <- tempfile(fileext = ".ofst")

## GRanges
gr <- GRanges("1:1-3:-")
export.ofst(gr, file = tempfile)
identical(gr, fimport(tempfile))
# Save from data.frame of GAlignment columns
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
export.ofst(df, tempfile)
res <- fimport(tempfile) # Imports as GAlignments because you have cigar
res
# Save from GAlignments
ga <- ORFik:::getGAlignments(df)
export.ofst(ga, tempfile)
res2 <- fimport(tempfile)
identical(res, res2)
# Save from GAlignmentsPair
ga_rev <- ga
strand(ga_rev) <- "-"
ga2 <- GAlignmentPairs(ga, ga_rev)
export.ofst(ga2, tempfile)
res3 <- fimport(tempfile)
identical(ga2, res3)
```

---

`export.ofst,GAlignments-method`*Store GRanges / GAlignments object as .ofst*

---

## Description

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)
4. width (not added if cigar exists)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)
5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from GAlignmentPairs, it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

## Usage

```
## S4 method for signature 'GAlignments'
export.ofst(x, file, ...)
```

## Arguments

x	a GRanges, GAlignments or GAlignmentPairs object
file	a character, location on disc (full path)
...	additional arguments for write_fst

## Details

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

## Value

NULL, object saved to disc

**Examples**

```

tempfile <- tempfile(fileext = ".ofst")

## GRanges
gr <- GRanges("1:1-3:-")
export.ofst(gr, file = tempfile)
identical(gr, fimport(tempfile))
# Save from data.frame of GAlignment columns
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
export.ofst(df, tempfile)
res <- fimport(tempfile) # Imports as GAlignments because you have cigar
res
# Save from GAlignments
ga <- ORFik:::getGAlignments(df)
export.ofst(ga, tempfile)
res2 <- fimport(tempfile)
identical(res, res2)
# Save from GAlignmentsPair
ga_rev <- ga
strand(ga_rev) <- "-"
ga2 <- GAlignmentPairs(ga, ga_rev)
export.ofst(ga2, tempfile)
res3 <- fimport(tempfile)
identical(ga2, res3)

```

---

export.ofst,GRanges-method

*Store GRanges / GAlignments object as .ofst*

---

**Description**

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)
4. width (not added if cigar exists)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)
5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from GAlignmentPairs, it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

**Usage**

```
## S4 method for signature 'GRanges'
export.ofst(x, file, ...)
```

**Arguments**

x	a GRanges, GAlignments or GAlignmentPairs object
file	a character, location on disc (full path)
...	additional arguments for write_fst

**Details**

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

**Value**

NULL, object saved to disc

**Examples**

```
tempfile <- tempfile(fileext = ".ofst")

## GRanges
gr <- GRanges("1:1-3:-")
export.ofst(gr, file = tempfile)
identical(gr, fimport(tempfile))
# Save from data.frame of GAlignment columns
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
export.ofst(df, tempfile)
res <- fimport(tempfile) # Imports as GAlignments because you have cigar
res
# Save from GAlignments
ga <- ORFik::getGAlignments(df)
export.ofst(ga, tempfile)
res2 <- fimport(tempfile)
identical(res, res2)
# Save from GAlignmentsPair
ga_rev <- ga
strand(ga_rev) <- "-"
ga2 <- GAlignmentPairs(ga, ga_rev)
export.ofst(ga2, tempfile)
res3 <- fimport(tempfile)
identical(ga2, res3)
```

---

export.wiggle	<i>Export as wiggle format</i>
---------------	--------------------------------

---

## Description

Will create 2 files, 1 for + strand (\*\_forward.wig) and 1 for - strand (\*\_reverse.wig). If all ranges are \* stranded, will output 1 file. Can be direct input for ucsc browser or IGV

## Usage

```
export.wiggle(x, file)
```

## Arguments

x	A GRangesList, GAlignment GAlignmentPairs with score column. Will be converted to 5' end position of original range. If score column does not exist, will group ranges and give replicates as score column.
file	a character path to valid output file name

## Value

invisible(NULL) (File is saved as 2 .wig files)

## References

<https://genome.ucsc.edu/goldenPath/help/wiggle.html>

## See Also

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

## Examples

```
x <- c(GRanges("1", c(1,3,5), "-"), GRanges("1", c(1,3,5), "+"))
# export.wiggle(x, "output/path/rna.wig")
```

---

extendLeaders	<i>Extend the leaders transcription start sites.</i>
---------------	--

---

### Description

Will extend the leaders or transcripts upstream (5' end) by extension. The extension is general not relative, that means splicing will not be taken into account. Requires the grl to be sorted beforehand, use [sortPerGroup](#) to get sorted grl.

### Usage

```
extendLeaders(
  grl,
  extension = 1000L,
  cds = NULL,
  is.circular = all(isCircular(grl) %in% TRUE)
)
```

### Arguments

grl	usually a <a href="#">GRangesList</a> of 5' utrs or transcripts. Can be used for any extension of groups.
extension	an integer, how much to max extend upstream (5' end). Either single value that will apply for all, or same as length of grl which will give 1 update value per grl object. Or a <a href="#">GRangesList</a> where start / stops by strand are the positions to use as new starts. Will not cross the chromosome boundary for non circular chromosomes.
cds	a <a href="#">GRangesList</a> of coding sequences, If you want to extend 5' leaders downstream, to catch upstream ORFs going into cds, include it. It will add first cds exon to grl matched by names. Do not add for transcripts, as they are already included.
is.circular	logical, default FALSE if not any is: <code>all(isCircular(grl))</code> Where grl is the ranges checked. If TRUE, allow ranges to extend below position 1 on chromosome. Since circular genomes can have negative coordinates.

### Value

an extended [GRangesList](#)

### See Also

Other `ExtendGenomicRanges`: [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

**Examples**

```

library(GenomicFeatures)
samplefile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
                          package = "GenomicFeatures")
txdb <- loadDb(samplefile)
fiveUTRs <- fiveUTRsByTranscript(txdb, use.names = TRUE) # <- extract only 5' leaders
tx <- exonsBy(txdb, by = "tx", use.names = TRUE)
cds <- cdsBy(txdb, "tx", use.names = TRUE)
## extend leaders upstream 1000
extendLeaders(fiveUTRs, extension = 1000)
## now try(extend upstream 1000, add all cds exons):
extendLeaders(fiveUTRs, extension = 1000, cds)

## when extending transcripts, don't include cds' of course,
## since they are already there
extendLeaders(tx, extension = 1000)
## Circular genome (allow negative coordinates)
circular_fives <- fiveUTRs
isCircular(circular_fives) <- rep(TRUE, length(isCircular(circular_fives)))
extendLeaders(circular_fives, extension = 32672841L)

```

---

extendLeadersUntil      *Extend Leaders Until*

---

**Description**

Extend leaders until a restriction group / position. This makes you extend until you hit another gene boundary etc.

**Usage**

```

extendLeadersUntil(
  gr1,
  grl2 = gr1,
  extension = 500,
  until = 200,
  min_ext = 25,
  is.circular = all(isCircular(gr1) %in% TRUE),
  ...
)

```

**Arguments**

**gr1**                    a GRangesList

**grl2**                    a GRangesList, default 'gr1'. The list that defines restrictions on extension. Can also be another set, which is used as 'roadblocks' for extension.

extension	an integer, how much to max extend upstream (5' end). Either single value that will apply for all, or same as length of grl which will give 1 update value per grl object. Or a GRangesList where start / stops by strand are the positions to use as new starts. Will not cross the chromosome boundary for non circular chromosomes.
until	numeric, default 200. The nearest you can go to the neighbour boundaries of grl2 (the "other" genes). Defined as boundary hit + 1, so if hit other gene with distance 22, and 'until' argument is 2, will set final extension to 22-2-1 = 19. Usually if Leaders/trailers are not defined, this makes a good pseudo leader boundary around your other genes.
min_ext	numeric, default 25. What is the minimum extension, even though it crosses a boundary. Will not cross the chromosome boundary for non circular chromosomes.
is.circular	logical, default FALSE if not any is: all(isCircular(grl) Where grl is the ranges checked. If TRUE, allow ranges to extend below position 1 on chromosome. Since circular genomes can have negative coordinates.
...	Arguments sent to distanceToPreceding

**Value**

a GRangesList of extended grl input

**Examples**

```
grl <- GRangesList(tx1 = GRanges("1", IRanges(c(10, 15), c(13, 20)), "+"),
                  tx2 = GRanges("1", IRanges(30, 50), "+"))
extendLeadersUntil(grl, min_ext = 5)
extendLeadersUntil(grl, min_ext = 5, until = 1)
extendLeadersUntil(grl, min_ext = 5, until = 1)
extendLeadersUntil(grl, min_ext = 5, until = 1, extension = 4)
```

---

extendTrailers                      *Extend the Trailers transcription stop sites*

---

**Description**

Will extend the trailers or transcripts downstream (3' end) by extension. The extension is general not relative, that means splicing will not be taken into account. Requires the grl to be sorted beforehand, use [sortPerGroup](#) to get sorted grl.

**Usage**

```
extendTrailers(
  grl,
  extension = 1000L,
  is.circular = all(isCircular(grl) %in% TRUE)
)
```

**Arguments**

grl	usually a <a href="#">GRangesList</a> of 3' utrs or transcripts. Can be used for any extension of groups.
extension	an integer, how much to max extend downstream (3' end). Either single value that will apply for all, or same as length of grl which will give 1 update value per grl object. Or a GRangesList where start / stops sites by strand are the positions to use as new starts. Will not cross the chromosome boundary for non circular chromosomes.
is.circular	logical, default FALSE if not any is: all(isCircular(grl) Where grl is the ranges checked. If TRUE, allow ranges to extend below position 1 on chromosome. Since circular genomes can have negative coordinates.

**Value**

an extended GRangeslist

**See Also**

Other ExtendGenomicRanges: [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

**Examples**

```
library(GenomicFeatures)
samplefile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
                          package = "GenomicFeatures")
txdb <- loadDb(samplefile)
threeUTRs <- threeUTRsByTranscript(txdb) # <- extract only 5' leaders
tx <- exonsBy(txdb, by = "tx", use.names = TRUE)
## now try(extend downstream 1000):
extendTrailers(threeUTRs, extension = 1000)
## Or on transcripts
extendTrailers(tx, extension = 1000)
## Circular genome (allow negative coordinates)
circular_three <- threeUTRs
isCircular(circular_three) <- rep(TRUE, length(isCircular(circular_three)))
extendTrailers(circular_three, extension = 126200008L)[41] # <- negative stop coordinate
```

---

extendTrailersUntil     *Extend Trailers Until*

---

**Description**

Extend trailers until a restriction group / position. This makes you extend until you hit another gene boundary etc.

**Usage**

```

extendTrailersUntil(
  grl,
  grl2 = grl,
  extension = 500,
  until = 200,
  min_ext = 25,
  is.circular = all(isCircular(grl) %in% TRUE),
  ...
)

```

**Arguments**

grl	a GRangesList
grl2	a GRangesList, default 'grl'. The list that defines restrictions on extension. Can also be another set, which is used as 'roadblocks' for extension.
extension	an integer, how much to max extend downstream (3' end). Either single value that will apply for all, or same as length of grl which will give 1 update value per grl object. Or a GRangesList where start / stops sites by strand are the positions to use as new starts. Will not cross the chromosome boundary for non circular chromosomes.
until	numeric, default 200. The nearest you can go to the boundary. #' Defined as boundary hit + 1, so if hit on 22, and until is 2, will set to 22+2+1 = 25. Usually if Leaders/trailers are not defined, this makes a good pseudo leader boundary around your other genes.
min_ext	numeric, default 25. What is the minimum extension, even though it crosses a boundary. Will not cross the chromosome boundary for non circular chromosomes.
is.circular	logical, default FALSE if not any is: all(isCircular(grl) Where grl is the ranges checked. If TRUE, allow ranges to extend below position 1 on chromosome. Since circular genomes can have negative coordinates.
...	Arguments sent to distanceToFollowing

**Value**

a GRangesList of extended grl input

**Examples**

```

grl <- GRangesList(tx1 = GRanges("1", IRanges(c(10, 15), c(13, 20)), "+"),
  tx2 = GRanges("1", IRanges(30, 50), "+"))
extendTrailersUntil(grl, min_ext = 5)
extendTrailersUntil(grl, min_ext = 5, until = 1)
extendTrailersUntil(grl, min_ext = 5, until = 1)
extendTrailersUntil(grl, min_ext = 5, until = 1, extension = 4)

```

---

extract_run_id	<i>Extract SRR/ERR/DRR run IDs from string</i>
----------------	--

---

### Description

Extract SRR/ERR/DRR run IDs from string

### Usage

```
extract_run_id(
  x,
  search = "(SRR[0-9]+|DRR[0-9]+|ERR[0-9]+)",
  only_valid = FALSE
)
```

### Arguments

x	character vector to search through.
search	the regex search, default: "(SRR[0-9]+ DRR[0-9]+ ERR[0-9]+)"
only_valid	logical, default FALSE. If TRUE, return only the hits.

### Value

a character vector of run accepted run ids according to search, if only\_valid named character vector for which indices are returned

### Examples

```
search <- c("SRR1230123_absdb", "SRR1241204124_asdasd", "asd_ERR1231230213",
  "DRR12412412_asdqwe", "ASDASD_ASDASD", "SRRASDASD")
ORFik::extract_run_id(search)
ORFik::extract_run_id(search, only_valid = TRUE)
```

---

f	<i>strandMode covRle</i>
---	--------------------------

---

### Description

strandMode covRle

### Usage

```
f(x)
```

### Arguments

x	a covRle object
---	-----------------

**Value**

the forward RleList

---

f, covRle-method	<i>strandMode covRle</i>
------------------	--------------------------

---

**Description**

strandMode covRle

**Usage**

```
## S4 method for signature 'covRle'
f(x)
```

**Arguments**

x                    a covRle object

**Value**

the forward RleList

---

file_ext_without_compression	<i>Get file extension of files without compressions</i>
------------------------------	---

---

**Description**

Get file extension of files without compressions

**Usage**

```
file_ext_without_compression(x, compressions = c("gzip", "gz", "bgz", "zip"))
```

**Arguments**

x                    character paths  
 compressions       character vector, default: c("gzip", "gz", "bgz", "zip"). Expand if you have other formats

**Value**

character vector of file extensions of paths excluding suffix vector defined in compression.

**Examples**

```
file_ext_without_compression(c("abc.bam.gz", "def.bam.zip"))
```

---

 filepath
 

---

*Get filepaths to ORFik experiment*


---

## Description

If other type than "default" is given and that type is not found (and 'fallback' is TRUE), it will return you ofst files, if they do not exist, then default filepaths without warning.

## Usage

```
filepath(
  df,
  type,
  basename = FALSE,
  fallback = type %in% c("pshifted", "bed", "ofst", "bedoc", "bedo"),
  suffix_stem = "AUTO",
  base_folders = libFolder(df, unique_mappers = only_unique_mappers),
  only_unique_mappers = uniqueMappers(df) & type != "default"
)
```

## Arguments

df	an ORFik <a href="#">experiment</a>
type	<p>a character(default: "default"), load files in experiment or some precomputed variant, like "ofst" or "pshifted". These are made with <code>ORFik:::convertLibs()</code>, <code>shiftFootprintsByExperiment()</code>, etc. Can also be custom user made folders inside the experiments bam folder. It acts in a recursive manner with priority: If you state "pshifted", but it does not exist, it checks "ofst". If no .ofst files, it uses "default", which always must exist.</p> <p>Presets are (folder is relative to default lib folder, some types fall back to other formats if folder does not exist):</p> <ul style="list-style-type: none"> <li>- "default": load the original files for experiment, usually bam.</li> <li>- "ofst": loads ofst files from the ofst folder, relative to lib folder (falls back to default)</li> <li>- "pshifted": loads ofst, wig or bigwig from pshifted folder (falls back to ofst, then default)</li> <li>- "cov": Load covRle objects from cov_RLE folder (fail if not found)</li> <li>- "covl": Load covRleList objects, from cov_RLE_List folder (fail if not found)</li> <li>- "bed": Load bed files, from bed folder (falls back to default)</li> <li>- Other formats must be loaded directly with <code>fimport</code></li> </ul>
basename	logical, default (FALSE). Get relative paths instead of full. Only use for inspection!
fallback	logical, default: type If TRUE, will use type fallback, see above for info.

suffix_stem	character, default "AUTO". Which is "" for all except type = "pshifted". Then it is "_pshifted" appended to end of names before format. Can be vector, then it searches suffixes in priority: so if you insert c("_pshifted", ""), it will look for suffix _pshifted, then the empty suffix.
base_folders	character vector, default libFolder(df), path to base folder to search for library variant directories. If single path (length == 1), it will apply to all libraries in df. If df is a collection, an experiment where libraries are put in different folders and library variants like pshifted are put inside those respective folders, set base_folders = libFolder(df, mode = "all")
only_unique_mappers	logical, default uniqueMappers(df). Load file of only unique format type, located in './unique_mappers' relative to bam files / default files. See ?uniqueMappers for more information.

### Details

For pshifted libraries, if "pshifted" is specified as type: if multiple formats exist it will use a priority: ofst -> bigwig -> wig -> bed. For formats outside default, all files must be stored in the directory of the first file: base\_folder <- libFolder(df)

### Value

a character vector of paths, or a list of character with 2 paths per, if paired libraries exists

### See Also

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

### Examples

```
df <- ORFik.template.experiment()
filepath(df, "default")
# Subset
filepath(df[9,], "default")
# Other format path
filepath(df[9,], "ofst")
## If you have pshifted files, see shiftFootprintsByExperiment()
filepath(df[9,], "pshifted") # <- falls back to ofst
```

---

filterExtremePeakGenes

*Filter out transcript by a median filter*

---

### Description

For removing very extreme peaks in coverage plots, use high quantiles, like 99. Used to make your plots look better, by removing extreme peaks.

**Usage**

```

filterExtremePeakGenes(
  tx,
  reads,
  upstream = NULL,
  downstream = NULL,
  multiplier = "0.99",
  min_cutoff = "0.999",
  pre_filter_minimum = 0,
  average = "median"
)

```

**Arguments**

tx	a GRangesList
reads	a GAlignments, GRanges, or precomputed coverage as covRle (one for each strand) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better. File streaming is still in beta, so use with care!
upstream	numeric or NULL, default NULL. if you want window of tx, instead of whole, specify how much upstream from start of tx, 10 is include 10 bases before start
downstream	numeric or NULL, default NULL. if you want window of tx, instead of whole, specify how much downstream from start of tx, 10 is go 10 bases into tx from start.
multiplier	a character or numeric, default "0.99", either a quantile if input is string[0-1], like "0.99", or numeric value if input is numeric. How much bigger than median / mean counts per gene, must a value be to be defined as extreme ?
min_cutoff	a character or numeric, default "0.999", either a quantile if input is string[0-1], like "0.999", or numeric value if input is numeric. Lowest allowed value
pre_filter_minimum	numeric, default 0. If value is x, will remove all positions in all genes with coverage < x, before median filter is applied. Set to 1 to remove all 0 positions.
average	character, default "median". Alternative: "mean". How to scale the multiplier argument, from median or mean of gene coverage.

**Value**

GRangesList (filtered)

---

filterTranscripts      *Filter transcripts by lengths*

---

### Description

Filter transcripts to those who have leaders, CDS, trailers of some lengths, you can also pick the longest per gene.

### Usage

```
filterTranscripts(
  txdb,
  minFiveUTR = 30L,
  minCDS = 150L,
  minThreeUTR = 30L,
  longestPerGene = TRUE,
  stopOnEmpty = TRUE,
  by = "tx",
  create.fst.version = FALSE
)
```

### Arguments

txdb	a TxDb object, ORFik experiment object or a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite), Only in the loadRegion function: if it is a GRangesList, it will return it self.
minFiveUTR	(integer) minimum bp for 5' UTR during filtering for the transcripts. Set to NULL if no 5' UTRs exists for annotation.
minCDS	(integer) minimum bp for CDS during filtering for the transcripts
minThreeUTR	(integer) minimum bp for 3' UTR during filtering for the transcripts. Set to NULL if no 3' UTRs exists for annotation.
longestPerGene	logical (TRUE), return only longest valid transcript per gene. NOTE: This is by priority longest cds isoform, if equal then pick longest total transcript. So if transcript is shorter but cds is longer, it will still be the one returned.
stopOnEmpty	logical TRUE, stop if no valid transcripts are found ?
by	a character, default "tx" Either "tx" or "gene". What names to output region by, the transcript name "tx" or gene names "gene". NOTE: this is not the same as cdsBy(txdb, by = "gene"), cdsBy would then only give 1 cds per Gene, loadRegion gives all isoforms, but with gene names.
create.fst.version	logical, FALSE. If TRUE, creates a .fst version of the transcript length table (if it not already exists), reducing load time from ~ 15 seconds to ~ 0.01 second next time you run filterTranscripts with this txdb object. The file is stored in the same folder as the genome this txdb is created from, with the name: paste0(ORFik:::remove.file_ext(metadata(txdb)[3,2]), "_", gsub("\\(.*)"

```
|:", "", metadata(txdb)[metadata(txdb)[,1] == "Creation time",2]), "_txLengths.fst")
```

Some error checks are done to see this is a valid location, if the txdb data source is a repository like UCSC and not a local folder, it will not be made.

### Details

If a transcript does not have a trailer, then the length is 0, so they will be filtered out if you set `minThreeUTR` to 1. So only transcripts with leaders, cds and trailers will be returned. You can set the integer to 0, that will return all within that group.

If your annotation does not have leaders or trailers, set them to NULL, since 0 means there must exist a column called `utr3_len` etc. Genes with `gene_id = NA` will be removed.

### Value

a character vector of valid transcript names

### Examples

```
df <- ORFik.template.experiment.zf()
txdb <- loadTxdb(df)
txNames <- filterTranscripts(txdb, minFiveUTR = 1, minCDS = 30,
                             minThreeUTR = 1)
loadRegion(txdb, "mrna")[txNames]
loadRegion(txdb, "5utr")[txNames]
```

---

fimport

*Load any type of sequencing reads*

---

### Description

Wraps around ORFik file format loaders and `rtracklayer::import` and tries to speed up loading with the use of `data.table`. Supports `gzip`, `gz`, `bgz` compression formats. Also safer chromosome naming with the argument `chrStyle`

### Usage

```
fimport(
  path,
  chrStyle = NULL,
  param = NULL,
  strandMode = 0,
  only_unique_mappers = FALSE
)
```

**Arguments**

path	a character path to file (1 or 2 files), or data.table with 2 columns(forward&reverse) or a GRanges/Galignment/GAlignmentPairs object etc. If it is ranged object it will presume to be already loaded, so will return the object as it is, updating the seqlevelsStyle if given.
chrStyle	a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -> pick "NCBI"
param	NULL or a <a href="#">ScanBamParam</a> object. Like for <a href="#">scanBam</a> , this influences what fields and which records are imported. However, note that the fields specified thru this <a href="#">ScanBamParam</a> object will be loaded <i>in addition</i> to any field required for generating the returned object ( <a href="#">GAlignments</a> , <a href="#">GAlignmentPairs</a> , or <a href="#">GappedReads</a> object), but only the fields requested by the user will actually be kept as meta-data columns of the object.  By default (i.e. param=NULL or param=ScanBamParam()), no additional field is loaded. The flag used is scanBamFlag(isUnmappedQuery=FALSE) for readGAlignments, readGAlignmentsList, and readGappedReads. (i.e. only records corresponding to mapped reads are loaded), and scanBamFlag(isUnmappedQuery=FALSE, isPaired=TRUE, hasUnmappedMate=FALSE) for readGAlignmentPairs (i.e. only records corresponding to paired-end reads with both ends mapped are loaded).
strandMode	numeric, default 0. Only used for paired end bam files. One of (0: strand = *, 1: first read of pair is +, 2: first read of pair is -). See ?strandMode. Note: Sets default to 0 instead of 1, as readGAlignmentPairs uses 1. This is to guarantee hits, but will also make mismatches of overlapping transcripts in opposite directions.
only_unique_mappers	logical, default FALSE. Only load unique mappers. For bam files it extracts NH flag, for other formats, it presumes the presence of a directory './unique_mappers' relative to bam file directory.

**Details**

NOTE: For wig/bigWig files you can send in 2 files, so that it automatically merges forward and reverse stranded objects. You can also just send 1 wig/bigWig file, it will then have "\*" as strand.

**Value**

a [GAlignments/GRanges](#) object, depending on input.

**See Also**

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

**Examples**

```
bam_file <- system.file("extdata/Danio_rerio_sample", "ribo-seq.bam", package = "ORFik")
fimport(bam_file)
# Certain chromosome naming
fimport(bam_file, "NCBI")
# Paired end bam strandMode 1:
fimport(bam_file, strandMode = 1)
# (will have no effect in this case, since it is not paired end)
```

---

find\_url\_ebi

*Locates and check if fastq files exists in ebi*


---

**Description**

Look for files in ebi file servers, Paired end and single end fastq files.  
 Fastq ftp url: ftp://ftp.sra.ebi.ac.uk/vol1/fastq  
 SRA ftp url: ftp://ftp.sra.ebi.ac.uk/vol1/srr  
 Fastq ASCP url: era-fasp@fasp.sra.ebi.ac.uk:vol1/fastq  
 SRA ASCP url: era-fasp@fasp.sra.ebi.ac.uk:vol1/srr

**Usage**

```
find_url_ebi(
  SRR,
  stop.on.error = FALSE,
  study = NULL,
  ebi_file_format = c("fastq_ftp", "sra_ftp")[1],
  convert_to_ascp = FALSE
)
```

**Arguments**

SRR	character, SRR, ERR or DRR numbers.
stop.on.error	logical FALSE, if TRUE will stop if all files are not found. If FALSE returns empty character vector if error is caught.
study	default NULL, optional PRJ (study id) to speed up search for URLs.
ebi_file_format	character, format of run download, default is fastq (ftp): c("fastq_ftp", "sra_ftp")[1]
convert_to_ascp	logical, default FALSE. If TRUE use server: era-fasp@fasp.sra.ebi.ac.uk:

**Value**

full url to fastq files, same length as input (2 urls for paired end data). Returns empty character() if all files not found.

**Examples**

```

# Test the 3 ways to get fastq files from EBI
# Both single end and paired end data

# Most common: SRR(3 first)/0(2 last)/whole
# Single
ORFik::find_url_ebi("SRR10503056")
# Paired
ORFik::find_url_ebi("SRR10500056")

# less common: SRR(3 first)/00(1 last)/whole
# Single
#ORFik::find_url_ebi("SRR1562873")
# Paired
#ORFik::find_url_ebi("SRR1560083")
# least common SRR(3 first)/whole
# Single
#ORFik::find_url_ebi("SRR105687")
# Paired
#ORFik::find_url_ebi("SRR105788")

```

---

findFa

*Convenience wrapper for Rsamtools FaFile*


---

**Description**

Get fasta file object, to find sequences in file.  
Will load and import file if necessary.

**Usage**

```
findFa(faFile)
```

**Arguments**

faFile            [FaFile](#), BSgenome, fasta/index file path or an ORFik [experiment](#). This file is usually used to find the transcript sequences from some GRangesList.

**Value**

a [FaFile](#) or BSgenome

**See Also**

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

**Examples**

```
# Some fasta genome with existing fasta index in same folder
path <- system.file("extdata/references/danio_rerio", "genome_dummy.fasta", package = "ORFik")
findFa(path)
```

---

findMapORFs

*Find ORFs and immediately map them to their genomic positions.*


---

**Description**

This function can map spliced ORFs. It finds ORFs on the sequences of interest, but returns relative positions to the positions of 'grl' argument. For example, 'grl' can be exons of known transcripts (with genomic coordinates), and 'seq' sequences of those transcripts, in that case, this function will return genomic coordinates of ORFs found on transcript sequences.

**Usage**

```
findMapORFs(
  grl,
  seqs,
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  longestORF = TRUE,
  minimumLength = 0,
  groupByTx = FALSE,
  grl_is_sorted = FALSE
)
```

**Arguments**

grl	A <a href="#">GRangesList</a> of the original sequences that gave the orfs in Genomic coordinates. If <code>grl_is_sorted = TRUE</code> (default), negative exon ranges per grl object must be sorted in descending orders.
seqs	(DNAStrngSet or character vector) - DNA/RNA sequences to search for Open Reading Frames. Can be both uppercase or lowercase. Easiest call to get seqs if you want only regions from a fasta/fastq index pair is: <code>seqs = ORFik:::txSeqsFromFa(grl, faFile)</code> , where grl is a <a href="#">GRanges/List</a> of search regions and faFile is a <a href="#">FaFile</a> . Note: Remember that if you extracted through a <a href="#">GRanges</a> object, that must have been sorted with negative strand exons descending.
startCodon	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.
stopCodon	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.

longestORF	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.
minimumLength	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example minimumLength = 8 will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.
groupByTx	logical (default: FALSE), should output GRangesList be grouped by exons per ORF (TRUE) or by orfs per transcript (FALSE)?
grl_is_sorted	logical, default FALSE If FALSE will sort negative transcript in descending order for you. If you loaded ranges with default methods this is already the case, so you can set to TRUE to save some time.

### Details

This function assumes that 'seq' is in widths relative to 'grl', and that their orders match. 1st seq is 1st grl object, etc.

See vignette for real life example.

### Value

A GRangesList of ORFs.

### See Also

Other findORFs: [findORFs\(\)](#), [findORFsFasta\(\)](#), [findUORFs\(\)](#), [startDefinition\(\)](#), [stopDefinition\(\)](#)

### Examples

```
# First show simple example using findORFs
# This sequence has ORFs at 1-9 and 4-9
seqs <- DNASTringSet("ATGATGTAA") # the dna transcript sequence
findORFs(seqs)
# lets assume that this sequence comes from two exons as follows
# Then we need to use findMapORFs instead of findORFs,
# for splicing information
gr <- GRanges(seqnames = "1", # chromosome 1
              ranges = IRanges(start = c(21, 10), end = c(23, 15)),
              strand = "-", #
              names = "tx1") #From transcript 1 on chr 1
grl <- GRangesList(tx1 = gr) # 1 transcript with 2 exons
findMapORFs(grl, seqs) # ORFs are properly mapped to its genomic coordinates

grl <- c(grl, grl)
names(grl) <- c("tx1", "tx2")
findMapORFs(grl, c(seqs, seqs))
# More advanced example and how to save sequences found in vignette
```

findORFs

*Find Open Reading Frames.***Description**

Find all Open Reading Frames (ORFs) on the simple input sequences in ONLY 5' - 3' direction (+), but within all three possible reading frames. Do not use findORFs for mapping to full chromosomes, then use [findMapORFs](#)! For each sequence of the input vector [IRanges](#) with START and STOP positions (inclusive) will be returned as [IRangesList](#). Returned coordinates are relative to the input sequences.

**Usage**

```
findORFs(
  seqs,
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  longestORF = TRUE,
  minimumLength = 0
)
```

**Arguments**

seqs	(DNAStrngSet or character vector) - DNA/RNA sequences to search for Open Reading Frames. Can be both uppercase or lowercase. Easiest call to get seqs if you want only regions from a fasta/fastq index pair is: seqs = ORFik::txSeqsFromFa(grl, faFile), where grl is a GRanges/List of search regions and faFile is a <a href="#">FaFile</a> . Note: Remember that if you extracted through a GRanges object, that must have been sorted with negative strand exons descending.
startCodon	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.
stopCodon	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.
longestORF	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.
minimumLength	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example minimumLength = 8 will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.

**Details**

If you want antisense strand too, do: `#positive strands pos <- findORFs(seqs) #negative strands (DNAStrngSet only if character) neg <- findORFs(reverseComplement(DNAStrngSet(seqs))) relist(c(GRanges(pos, strand = "+"), GRanges(neg, strand = "-")), skeleton = merge(pos, neg))`

**Value**

(IRangesList) of ORFs locations by START and STOP sites grouped by input sequences. In a list of sequences, only the indices of the sequences that had ORFs will be returned, e.g. 3 sequences where only 1 and 3 has ORFs, will return size 2 IRangesList with names `c("1", "3")`. If there are a total of 0 ORFs, an empty IRangesList will be returned.

**See Also**

Other findORFs: [findMapORFs\(\)](#), [findORFsFasta\(\)](#), [findUORFs\(\)](#), [startDefinition\(\)](#), [stopDefinition\(\)](#)

**Examples**

```
## Simple examples
findORFs("ATGTAA")
findORFs("ATGTTAA") # not in frame anymore

findORFs("ATGATGTAA") # only longest of two above
findORFs("ATGATGTAA", longestORF = FALSE) # two ORFs

findORFs(c("ATGTAA", "ATGATGTAA")) # 1 ORF per transcript

## Get DNA sequences from ORFs
seq <- DNAStrngSet(c("ATGTAA", "AAA", "ATGATGTAA"))
names(seq) <- c("tx1", "tx2", "tx3")
orfs <- findORFs(seq, longestORF = FALSE)

# you can get sequences like this:
gr <- unlist(orfs, use.names = TRUE)
gr <- GRanges(seqnames = names(seq)[as.integer(names(gr))],
  ranges = gr, strand = "+")
# Give them some proper names:
names(gr) <- paste0("ORF_", seq.int(length(gr)), "_", seqnames(gr))
orf_seqs <- getSeq(seq, gr)
orf_seqs
# Save as .fasta (orf_seqs must be of type DNAStrngSet)
writeXStringSet(orf_seqs, "orfs.fasta")
## Reading from file and find ORFs
#findORFs(readDNAStrngSet("path/to/transcripts.fasta"))
```

---

 findORFsFasta

*Finds Open Reading Frames in fasta files.*


---

### Description

Should be used for prokaryote genomes or transcript sequences as fasta. Makes no sense for eukaryote whole genomes, since those contains splicing (use `findMapORFs` for spliced ranges). Searches through each fasta header and reports all ORFs found for BOTH sense (+) and antisense strand (-) in all frames. Name of the header will be used as seqnames of reported ORFs. Each fasta header is treated separately, and name of the sequence will be used as seqname in returned GRanges object. This supports circular genomes.

### Usage

```
findORFsFasta(
  filePath,
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  longestORF = TRUE,
  minimumLength = 0,
  is.circular = FALSE
)
```

### Arguments

<code>filePath</code>	(character) Path to the fasta file. Can be both uppercase or lowercase. Or a already loaded R object of either types: "BSgenome" or "DNAStringSet" with named sequences
<code>startCodon</code>	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.
<code>stopCodon</code>	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.
<code>longestORF</code>	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.
<code>minimumLength</code>	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example <code>minimumLength = 8</code> will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.
<code>is.circular</code>	(logical) Whether the genome in <code>filePath</code> is circular. Prokaryotic genomes are usually circular. Be carefull if you want to extract sequences, remember that <code>seqlengths</code> must be set, else it does not know what last base in sequence is before loop ends!

**Details**

Remember if you have a fasta file of transcripts (transcript coordinates), delete all negative stranded ORFs afterwards by: `orfs <- orfs[strandBool(orfs)]` # negative strand orfs make no sense then. Seqnames are created from header by format: `>name info`, so name must be first after "biggern than" and space between name and info. Also make sure your fasta file is valid (no hidden spaces etc), as this might break the coordinate system!

**Value**

(GRanges) object of ORFs mapped from fasta file. Positions are relative to the fasta file.

**See Also**

Other findORFs: [findMapORFs\(\)](#), [findORFs\(\)](#), [findUORFs\(\)](#), [startDefinition\(\)](#), [stopDefinition\(\)](#)

**Examples**

```
# location of the example fasta file
example_genome <- system.file("extdata/references/danio_rerio", "genome_dummy.fasta",
  package = "ORFik")
orfs <- findORFsFasta(example_genome)
# To store ORF sequences (you need indexed genome .fai file):
fa <- FaFile(example_genome)
names(orfs) <- paste0("ORF_", seq.int(length(orfs)), "_", seqnames(orfs))
orf_seqs <- getSeq(fa, orfs)
# You sequences (fa), needs to have isCircular(fa) == TRUE for it to work
# on circular wrapping ranges!

# writeXStringSet(DNAStringSet(orf_seqs), "orfs.fasta")
```

---

findPeaksPerGene

*Find peaks per gene*

---

**Description**

For finding the peaks (stall sites) per gene, with some default filters. A peak is basically a position of very high coverage compared to its surrounding area, as measured using zscore.

**Usage**

```
findPeaksPerGene(
  tx,
  reads,
  top_tx = 0.5,
  min_reads_per_tx = 20,
  min_reads_per_peak = 10,
  type = "max",
  gene_ids = names(tx),
```

```
coverage = coveragePerTiling(tx, reads, TRUE, as.data.table = TRUE)
)
```

### Arguments

tx	a GRangesList
reads	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> , or precomputed coverage as <a href="#">covRle</a> (one for each strand) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better. File streaming is still in beta, so use with care!
top_tx	numeric, default 0.50 (only use 50% top transcripts by read counts).
min_reads_per_tx	numeric, default 20. Gene must have at least 20 reads, applied before type filter.
min_reads_per_peak	numeric, default 10. Peak must have at least 10 reads.
type	character, default "max". Get only max peak per gene. Alternatives: "all", all peaks passing the input filter will be returned. "median", only peaks that is higher than the median of all peaks. "maxmedian": get first "max", then median of those.
gene_ids	character vector, names of genes, default names(tx)
coverage	a data.table of coverage, with columns position, score and genes

### Details

The 'reads' argument should be 1 width reads like p-shifts, or other reads that is single positioned. It will work with non 1 width bases, but you then get larger areas for peaks.

For more details see reference, which uses a slightly different method by zscore of a sliding window instead of over the whole tx.

### Value

a data.table of gene\_id, position, counts of the peak, zscore and standard deviation of the peak compared to rest of gene area.

### References

doi: 10.1261/rna.065235.117

### Examples

```
df <- ORFik.template.experiment()
cds <- loadRegion(df, "cds")
# Load ribo seq from ORFik
rfp <- fimport(df[3,]$filepath)
# All transcripts passing filter
findPeaksPerGene(cds, rfp, top_tx = 0)
```

```
# Top 50% of genes
findPeaksPerGene(cds, rfp)
```

---

findUORFs

*Find upstream ORFs from transcript annotation*


---

## Description

Procedure: 1. Create a new search space starting with the 5' UTRs. 2. Redefine TSS with CAGE if wanted. 3. Add the whole of CDS to search space to allow uORFs going into cds. 4. find ORFs on that search space. 5. Filter out wrongly found uORFs, if CDS is included. The CDS, alternative CDS, uORFs starting within the CDS etc.

## Usage

```
findUORFs(
  fiveUTRs,
  fa,
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  longestORF = TRUE,
  minimumLength = 0,
  cds = NULL,
  cage = NULL,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
  removeUnused = FALSE
)
```

## Arguments

fiveUTRs	(GRangesList) The 5' leaders or full transcript sequences
fa	a <a href="#">FaFile</a> . With fasta sequences corresponding to fiveUTR annotation. Usually loaded from the genome of an organism with <code>fa = ORFik::findFa("path/to/fast/genome")</code>
startCodon	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.
stopCodon	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.
longestORF	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.

minimumLength	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example minimumLength = 8 will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.
cds	(GRangesList) CDS of relative fiveUTRs, applicable only if you want to extend 5' leaders downstream of CDS's, to allow upstream ORFs that can overlap into CDS's.
cage	Either a filePath for the CageSeq file as .bed .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE) The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
extension	The maximum number of bases upstream of the TSS to search for CageSeq peak.
filterValue	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.
restrictUpstreamToTx	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
removeUnused	logical (FALSE), if False: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.

### Details

From default a filtering process is done to remove "fake" uORFs, but only if cds is included, since uORFs that stop on the stop codon on the CDS is not a uORF, but an alternative cds by definition, etc.

### Value

A GRangesList of uORFs, 1 granges list element per uORF.

### See Also

Other findORFs: [findMapORFs\(\)](#), [findORFs\(\)](#), [findORFsFasta\(\)](#), [startDefinition\(\)](#), [stopDefinition\(\)](#)

### Examples

```
# Load annotation
txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
                        package = "GenomicFeatures")

## Not run:
txdb <- loadTxdb(txdbFile)
fiveUTRs <- loadRegion(txdb, "leaders")
cds <- loadRegion(txdb, "cds")
if (requireNamespace("BSgenome.Hsapiens.UCSC.hg19")) {
  # Normally you would not use a BSgenome, but some custom fasta-
```

```

# annotation you have for your species
findUORFs(fiveUTRs, BSgenome.Hsapiens.UCSC.hg19::Hsapiens, "ATG",
          cds = cds)
}

## End(Not run)

```

---

findUORFs\_exp

*Find upstream ORFs from transcript annotation*


---

## Description

Procedure: 1. Create a new search space starting with the 5' UTRs. 2. Redefine TSS with CAGE if wanted. 3. Add the whole of CDS to search space to allow uORFs going into cds. 4. find ORFs on that search space. 5. Filter out wrongly found uORFs, if CDS is included. The CDS, alternative CDS, uORFs starting within the CDS etc.

## Usage

```

findUORFs_exp(
  df,
  faFile = findFa(df),
  leaders = loadRegion(txdb, "leaders"),
  startCodon = startDefinition(1),
  stopCodon = stopDefinition(1),
  longestORF = TRUE,
  minimumLength = 0,
  overlappingCDS = FALSE,
  cage = NULL,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
  removeUnused = FALSE,
  save_optimized = FALSE
)

```

## Arguments

df	a txdb or <a href="#">experiment</a>
faFile	FaFile of genome, default findFa(df). Default only works for ORFik experiments, if TxDb, input manually like: findFa(genome_path)
leaders	GRangesList, default: loadRegion(txdb, "leaders"). If you do not have any good leader annotation, a hack is to use ORFik:::groupGRangesBy(startSites(loadRegion(txdb, "cds")), asGR = TRUE, keep.names = TRUE, is.sorted = TRUE)
startCodon	(character vector) Possible START codons to search for. Check <a href="#">startDefinition</a> for helper function. Note that it is case sensitive, so "atg" would give 0 hits for a sequence with only capital "ATG" ORFs.

stopCodon	(character vector) Possible STOP codons to search for. Check <a href="#">stopDefinition</a> for helper function. Note that it is case sensitive, so "tga" would give 0 hits for a sequence with only capital "TGA" ORFs.
longestORF	(logical) Default TRUE. Keep only the longest ORF per unique stopcodon: (seqname, strand, stopcodon) combination, Note: Not longest per transcript! You can also use function <a href="#">longestORFs</a> after creation of ORFs for same result.
minimumLength	(integer) Default is 0. Which is START + STOP = 6 bp. Minimum length of ORF, without counting 3bps for START and STOP codons. For example minimumLength = 8 will result in size of ORFs to be at least START + 8*3 (bp) + STOP = 30 bases. Use this param to restrict search.
overlappingCDS	logical, default FALSE. Include uORFs that overlap CDS.
cage	Either a filePath for the CageSeq file as .bed .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: <code>convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE)</code> The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
extension	The maximum number of bases upstream of the TSS to search for CageSeq peak.
filterValue	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.
restrictUpstreamToTx	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
removeUnused	logical (FALSE), if False: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.
save_optimized	logical, default FALSE. If TRUE, save in the optimized folder for the experiment. You must have made this directory before running this function (call <code>makeTxdbFromGenome</code> first if not).

### Details

From default a filtering process is done to remove "fake" uORFs, but only if cds is included, since uORFs that stop on the stop codon on the CDS is not a uORF, but an alternative cds by definition, etc.

### Value

A GRangesList of uORFs, 1 granges list element per uORF.

### See Also

Other findORFs: [findMapORFs\(\)](#), [findORFs\(\)](#), [findORFsFasta\(\)](#), [startDefinition\(\)](#), [stopDefinition\(\)](#)

## Examples

```
df <- ORFik.template.experiment()
# Without cds overlapping, no 5' leader extension
findUORFs_exp(df, extension = 0)
# Without cds overlapping, extends 5' leaders by 1000 (good for yeast etc)
findUORFs_exp(df)
# Include cds overlapping uorfs
findUORFs_exp(df, overlappingCDS = TRUE)
```

---

firstEndPerGroup	<i>Get first end per granges group</i>
------------------	--

---

## Description

grl must be sorted, call `ORFik:::sortPerGroup` if needed

## Usage

```
firstEndPerGroup(grl, keep.names = TRUE)
```

## Arguments

grl                    a [GRangesList](#)  
keep.names            a boolean, keep names or not, default: (TRUE)

## Value

a `Rle(keep.names = T)`, or integer vector(F)

## Examples

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
firstEndPerGroup(grl)
```

---

firstExonPerGroup      *Get first exon per GRangesList group*

---

**Description**

grl must be sorted, call ORFik:::sortPerGroup if needed

**Usage**

```
firstExonPerGroup(grl)
```

**Arguments**

grl                    a [GRangesList](#)

**Value**

a GRangesList of the first exon per group

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
firstExonPerGroup(grl)
```

---

firstStartPerGroup      *Get first start per granges group*

---

**Description**

grl must be sorted, call ORFik:::sortPerGroup if needed

**Usage**

```
firstStartPerGroup(grl, keep.names = TRUE)
```

**Arguments**

grl                    a [GRangesList](#)  
 keep.names          a boolean, keep names or not, default: (TRUE)

**Value**

a Rle(keep.names = TRUE), or integer vector(FALSE)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(7, 14), width = 3),
  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
  ranges = IRanges(c(4, 1), c(9, 3)),
  strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
firstStartPerGroup(grl)
```

---

fix\_malformed\_gff      *Fix a malformed gff file*

---

**Description**

Basically removes all info lines with character length > 32768 and save that new file.

**Usage**

```
fix_malformed_gff(gff)
```

**Arguments**

gff                    character, path to gtf, can not be gzipped!

**Value**

path of fixed gtf

**Examples**

```
# fix_malformed_gff("my_bad_gff.gff")
```

---

flankPerGroup	<i>Get flanks per group</i>
---------------	-----------------------------

---

**Description**

For a GRangesList, get start and end site, return back as GRangesList.

**Usage**

```
flankPerGroup(gr1)
```

**Arguments**

gr1            a GRangesList

**Value**

a GRangesList, 1 GRanges per group with: start as minimum start of group and end as maximum per group.

**Examples**

```
gr1 <- GRangesList(tx1 = GRanges("1", IRanges(c(1,5), width = 2), "+"),
                  tx2 = GRanges("2", IRanges(c(10,15), width = 2), "+"))
flankPerGroup(gr1)
```

---

floss	<i>Fragment Length Organization Similarity Score</i>
-------	--

---

**Description**

This feature is usually calculated only for RiboSeq reads. For reads of width between ‘start’ and ‘end’, sum the fraction of RiboSeq reads (per read widths) that overlap ORFs and normalize by CDS read width fractions. So if all read length are width 34 in ORFs and CDS, value is 1. If width is 33 in ORFs and 34 in CDS, value is 0. If width is 33 in ORFs and 50/50 (33 and 34) in CDS, values will be 0.5 (for 33).

**Usage**

```
floss(gr1, RFP, cds, start = 26, end = 34, weight = 1L)
```

**Arguments**

gr1	a <a href="#">GRangesList</a> object can be either transcripts, 5' utrs, cds', 3' utrs or ORFs as a special case (uORFs, potential new cds' etc). If regions are not spliced you can send a <a href="#">GRanges</a> object.
RFP	ribosomal footprints, given as <a href="#">GAlignments</a> or <a href="#">GRanges</a> object, must be already shifted and resized to the p-site. Requires a \$size column with original read lengths.
cds	a <a href="#">GRangesList</a> of coding sequences, cds has to have names as gr1 so that they can be matched
start	usually 26, the start of the floss interval (inclusive)
end	usually 34, the end of the floss interval (inclusive)
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <code>countOverlaps()</code> presumes, if single number ( $\neq 1$ ), it repeats for all ranges, if vector with length $> 1$ , it must be equal size of the reads object.

**Details**

Pseudo explanation of the function:

$$\text{SUM}[\text{start to stop}]((\text{gr1}[\text{start:end}][\text{name}]/\text{gr1}) / (\text{cds}[\text{start:end}][\text{name}]/\text{cds}))$$

Where 'name' is transcript names.

Please read more in the article.

**Value**

a vector of FLOSS of length same as gr1, 0 means no RFP reads in range, 1 is perfect match.

**References**

doi: 10.1016/j.celrep.2014.07.045

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```

ORF1 <- GRanges(seqnames = "1",
                ranges = IRanges(start = c(1, 12, 22),
                                end = c(10, 20, 32)),
                strand = "+")
gr1 <- GRangesList(tx1_1 = ORF1)
# RFP is 1 width position based GRanges
RFP <- GRanges("1", IRanges(c(1, 25, 35, 38), width = 1), "+")
RFP$size <- c(28, 28, 28, 29) # original width in size col
cds <- GRangesList(tx1 = GRanges("1", IRanges(35, 44), "+"))
# gr1 must have same names as cds + _1 etc, so that they can be matched.
floss(gr1, RFP, cds)
# or change ribosome start/stop, more strict
floss(gr1, RFP, cds, 28, 28)

# With repeated alignments in score column
ORF2 <- GRanges(seqnames = "1",
                ranges = IRanges(start = c(12, 22, 36),
                                end = c(20, 32, 38)),
                strand = "+")
gr1 <- GRangesList(tx1_1 = ORF1, tx1_2 = ORF2)
score(RFP) <- c(5, 10, 5, 10)
floss(gr1, RFP, cds, weight = "score")

```

fpkm

*Create normalizations of overlapping read counts.***Description**

FPKM is short for "Fragments Per Kilobase of transcript per Million fragments in library". When calculating RiboSeq data FPKM over ORFs, use ORFs as 'grl'. When calculating RNASeq data FPKM, use full transcripts as 'grl'. It is equal to RPKM given that you do not have paired end reads.

**Usage**

```
fpkm(gr1, reads, pseudoCount = 0, librarySize = "full", weight = 1L)
```

**Arguments**

gr1	a <a href="#">GRangesList</a> object can be either transcripts, 5' utrs, cds', 3' utrs or ORFs as a special case (uORFs, potential new cds' etc). If regions are not spliced you can send a <a href="#">GRanges</a> object.
reads	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object, usually of RiboSeq, RnaSeq, CageSeq, etc.
pseudoCount	a numeric, default 0, set it to 1 if you want to avoid NA and inf values.

librarySize	either numeric value or character vector. Default ("full"), number of alignments in library (reads). If you just have a subset, you can give the value by librarySize = length(wholeLib) or sum(wholeLib\$score), if you want lib size to be only number of reads overlapping grl, do: librarySize = "overlapping" sum(countOverlaps(reads, grl) > 0), if reads[1] has 3 hits in grl, and reads[2] has 2 hits, librarySize will be 2, not 5. You can also get the inverse overlap, if you want lib size to be total number of overlaps, do: librarySize = "DESeq" This is standard fpkm way of DESeq2::fpkm(robust = FALSE) sum(countOverlaps(grl, reads)) if grl[1] has 3 reads and grl[2] has 2 reads, librarySize is 5, not 2.
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. GRanges("chr1", 1, "+", score = 5), would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others countOverlaps() presumes, if single number (!= 1), it repeats for all ranges, if vector with length > 1, it must be equal size of the reads object.

### Details

Note also that you must consider if you will use the whole read library or just the reads overlapping 'grl' for library size. A normal question here is, does it make sense to include rRNA in library size ? If you only want overlapping grl, do: librarySize = "overlapping"

### Value

a numeric vector with the fpkm values

### References

doi: 10.1038/nbt.1621

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20),
                               end = c(5, 15, 25)),
               strand = "+")
grl <- GRangesList(tx1_1 = ORF)
RFP <- GRanges("1", IRanges(25, 25), "+")
fpkm(grl, RFP)

# With weights (10 reads at position 25)
```

```
RFP <- GRanges("1", IRanges(25, 25), "+", score = 10)
fpkm(gr1, RFP, weight = "score")
```

---

fractionLength	<i>Fraction Length</i>
----------------	------------------------

---

### Description

Fraction Length is defined as

$$(\text{widths of gr1})/\text{tx\_len}$$

so that each group in the gr1 is divided by the corresponding transcript.

### Usage

```
fractionLength(gr1, tx_len = widthPerGroup(tx, TRUE), tx = NULL)
```

### Arguments

gr1	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs. ORFs are a special case, see argument tx_len
tx_len	the transcript lengths of the transcripts, a named (tx names) vector of integers. If you have the transcripts as GRangesList, call 'ORFik::widthPerGroup(tx, TRUE)'. If you used CageSeq to reannotate leaders, then the tss for the the leaders have changed, therefore the tx lengths have changed. To account for that call: 'tx_len <- widthPerGroup(extendLeaders(tx, cageFiveUTRs))' and calculate fraction length using 'fractionLength(gr1, tx_len)'.
tx	default NULL, a <a href="#">GRangesList</a> object of transcript to get lengths from. Pass in for wrapping to widths inside the function.

### Value

a numeric vector of ratios

### References

doi: 10.1242/dev.098343

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
grl <- GRangesList(tx1_1 = ORF)
# grl must have same names as cds + _1 etc, so that they can be matched.
tx <- GRangesList(tx1 = GRanges("1", IRanges(1, 50), "+"))
fractionLength(grl, tx = tx)
```

fread.bed

*Load bed file as GRanges***Description**

Wraps around [import.bed](#) and tries to speed up loading with the use of `data.table`. Supports gzip, gz, bgz and bed formats. Also safer chromosome naming with the argument `chrStyle`

**Usage**

```
fread.bed(filePath, chrStyle = NULL)
```

**Arguments**

<code>filePath</code>	The location of the bed file
<code>chrStyle</code>	a <code>GRanges</code> object, <code>TxDb</code> , <code>FaFile</code> , , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: <code>NULL</code> ) to get <code>seqlevelsStyle</code> from. In addition if it is a <code>Seqinfo</code> object, <code>seqinfo</code> will be updated. Example of <code>seqlevelsStyle</code> update: Is chromosome 1 called <code>chr1</code> or <code>1</code> , is mitochondrial chromosome called <code>MT</code> or <code>chrM</code> etc. Will use 1st <code>seqlevel-style</code> if more are present. Like: <code>c("NCBI", "UCSC")</code> -> pick "NCBI"

**Value**

a [GRanges](#) object

**See Also**

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

**Examples**

```
# path to example CageSeq data from hg19 heart sample
cageData <- system.file("extdata", "cage-seq-heart.bed.bgz",
                       package = "ORFik")
fread.bed(cageData)
```

---

gcContent	<i>Get GC content</i>
-----------	-----------------------

---

**Description**

0.5 means 50

**Usage**

```
gcContent(seqs, fa = NULL)
```

**Arguments**

seqs	a character vector of sequences, or ranges as GRangesList
fa	fasta index file .fai file, either path to it, or the loaded FaFile, default (NULL), only set if you give ranges as GRangesList

**Value**

a numeric vector of gc content scores

**Examples**

```
# Here we make an example from scratch
seqName <- "Chromosome"
ORF1 <- GRanges(seqnames = seqName,
                ranges = IRanges(c(1007, 1096), width = 60),
                strand = c("+", "+"))
ORF2 <- GRanges(seqnames = seqName,
                ranges = IRanges(c(400, 100), width = 30),
                strand = c("-", "-"))
ORFs <- GRangesList(tx1 = ORF1, tx2 = ORF2)
# get path to FaFile for sequences
faFile <- system.file("extdata/references/danio_rerio", "genome_dummy.fasta",
                      package = "ORFik")
gcContent(ORFs, faFile)
```

---

geneToSymbol	<i>Get gene symbols from Ensembl gene ids</i>
--------------	---

---

## Description

If your organism is not in this list of supported organisms, manually assign the input arguments. There are 2 main fetch modes:

By gene ids (Single accession per gene)

By tx ids (Multiple accessions per gene)

Run the mode you need depending on your required attributes.

Will check for already existing table of all genes, and use that instead of re-downloading every time (If you input valid experiment or txdb and have run [makeTxdbFromGenome](#) with symbols = TRUE, you have a file called gene\_symbol\_tx\_table.fst) will load instantly. If df = NULL, it can still search cache to load a bit slower.

## Usage

```
geneToSymbol(
  df,
  organism_name = organism(df),
  gene_ids = filterTranscripts(df, by = "gene", 0, 0, 0),
  org.dataset = paste0(tolower(substr(organism_name, 1, 1)), gsub(".*", replacement =
    "", organism_name), "_gene_ensembl"),
  ensembl = biomaRt::useEnsembl("ensembl", dataset = org.dataset),
  attribute = "external_gene_name",
  include_tx_ids = FALSE,
  uniprot_id = FALSE,
  force = FALSE,
  verbose = TRUE
)
```

## Arguments

df	an ORFik <a href="#">experiment</a> or TxDb object with defined organism slot. If set will look for file at path of txdb / experiment reference path named: 'gene_symbol_tx_table.fst' relative to the txdb/genome directory. Can be set to NULL if gene_ids and organism is defined manually.
organism_name	default, organism(df). Scientific name of organism, like ("Homo sapiens"), remember capital letter for first name only!
gene_ids	default, filterTranscripts(df, by = "gene", 0, 0, 0). Ensembl gene IDs to search for (default all transcripts coding and noncoding) To only get coding do: filterTranscripts(df, by = "gene", 0, 1, 0)
org.dataset	default, paste0(tolower(substr(organism_name, 1, 1)), gsub(".*", replacement = "", organism_name), "_gene_ensembl") the ensembl dataset to use. For Homo sapiens, this converts to default as: hsapiens_gene_ensembl
ensembl	default, useEnsembl("ensembl", dataset=org.dataset) .The mart connection.
attribute	default, "external_gene_name", the biomaRt column / columns default(primary gene symbol names). These are always from specific database, like hgnc symbol for human, and mgi symbol for mouse and rat, sgd for yeast etc.

include_tx_ids	logical, default FALSE, also match tx ids, which then returns as the 3rd column. Only allowed when 'df' is defined. If
uniprot_id	logical, default FALSE. Include uniprotspiremb and/or uniprotswissprot. If include_tx_ids you will get per isoform if available, else you get canonical uniprot id per gene. If both uniprotspiremb and uniprotswissprot exists, it will make a merged uniprot id column with rule: if id exists in uniprotswissprot, keep. If not, use uniprotspiremb column id.
force	logical FALSE, if TRUE will not look for existing file made through <a href="#">makeTxdbFromGenome</a> corresponding to this txdb / ORFik experiment stored with name "gene_symbol_tx_table.fst".
verbose	logical TRUE, if FALSE, do not output messages.

### Value

data.table with 2, 3 or 4 columns: gene\_id, gene\_symbol, tx\_id and uniprot\_id named after attribute, sorted in order of gene\_ids input. (example: returns 3 columns if include\_tx\_ids is TRUE), and more if additional columns are specified in 'attribute' argument.

### Examples

```
## Without ORFik experiment input
gene_id_ATF4 <- "ENSG00000128272"
#geneToSymbol(NULL, organism_name = "Homo sapiens", gene_ids = gene_id_ATF4)
# With uniprot canonical isoform id:
#geneToSymbol(NULL, organism_name = "Homo sapiens", gene_ids = gene_id_ATF4, uniprot_id = TRUE)

## All genes from Organism using ORFik experiment
# df <- read.experiment("some_experiment")
# geneToSymbol(df)

## Non vertebrate species (the ones not in ensembl, but in ensemblGenomes mart)
#txdb_ylipolytica <- loadTxdb("txdb_path")
#dt2 <- geneToSymbol(txdb_ylipolytica, include_tx_ids = TRUE,
# ensembl = useEnsemblGenomes(biomart = "fungi_mart", dataset = "ylipolytica_eg_gene"))
```

---

get\_bioproject\_candidates

*Query utils for bioproject IDs*

---

### Description

The default query of Ribosome Profiling human, will result in internal entrez search of: Ribosome[All Fields] AND Profiling[All Fields] AND ("Homo sapiens"[Organism] OR human[All Fields])

**Usage**

```
get_bioproject_candidates(  
  term = "Ribosome Profiling human",  
  as_accession = TRUE,  
  add_study_title = FALSE,  
  RetMax = 10000  
)
```

**Arguments**

term	character, default "Ribosome Profiling human". A space is translated into AND, that means "Ribosome AND Profiling AND human", will give same as above. To do OR operation, do: "Ribosome OR profiling OR human".
as_accession	logical, default TRUE. Get bioproject accessions: PRJNA, PRJEB, PRJDB values, or IDs (FALSE), numbers only. Accessions are usually the thing needed for most tools.
add_study_title	logical, default FALSE. If TRUE, return as data table with 2 columns: id: ID or accessions. title: The title of the study.
RetMax	integer, default 10000. How many IDs to return maximum

**Value**

character vector of Accessions or IDs. If add\_study\_title is TRUE, returns a data.table.

**References**

<https://www.ncbi.nlm.nih.gov/books/NBK25501/>

**See Also**

Other sra: [browseSRA\(\)](#), [download.SRA\(\)](#), [download.SRA.metadata\(\)](#), [download.ebi\(\)](#), [install.sratoolkit\(\)](#), [rename.SRA.files\(\)](#)

**Examples**

```
term <- "Ribosome Profiling Saccharomyces cerevisiae"  
# get_bioproject_candidates(term)
```

---

get\_silva\_rRNA

*Download Silva SSU & LSU sequences*

---

**Description**

Version downloaded is 138.1. NR99\_tax (non redundant)

**Usage**

```
get_silva_rRNA(output.dir)
```

**Arguments**

output.dir      directory to save downloaded data

**Details**

If it fails from timeout, set higher timeout: options(timeout = 200)

**Value**

filepath to downloaded file

**Examples**

```
output.dir <- tempdir()
# get_silva_rRNA(output.dir)
```

---

get\_system\_usage      *System usage for Linux (Auto-detects correct drive if not provided)*

---

**Description**

System usage for Linux (Auto-detects correct drive if not provided)

**Usage**

```
get_system_usage(drive = detect_drive(), one_liner = FALSE)
```

**Arguments**

drive              path, the Filesystem drive !(Not the mounted name), use drive = ORFik:::detect\_drive("My\_directory\_insi this mount\_name") to get custom drive

one\_liner          Logical, default FALSE. Instead return a length 1 character string with all the info.

**Value**

A list with system info, if one\_liner is TRUE, then a length 1 character string.

**Examples**

```
get_system_usage()
```

---

`getGenomeAndAnnotation`*Download genome (fasta), annotation (GTF) and contaminants*

---

### Description

This function automatically downloads (if files not already exists) genomes and contaminants specified for genome alignment. By default, it will use ensembl reference, upon completion, the function will store a file called `file.path(output.dir, "outputs.rds")` with the output paths of your completed genome/annotation downloads. For most non-model nonvertebrate organisms, you need my fork of biomartr for it to work: `remotes::install_github("Roleren/biomartr")` If you misspelled something or crashed, delete wrong files and run again. Do `remake = TRUE`, to do it all over again.

### Usage

```
getGenomeAndAnnotation(  
  organism,  
  output.dir,  
  db = "ensembl",  
  GTF = TRUE,  
  genome = TRUE,  
  merge_contaminants = TRUE,  
  phix = FALSE,  
  ncRNA = FALSE,  
  tRNA = FALSE,  
  rRNA = FALSE,  
  gunzip = TRUE,  
  remake = FALSE,  
  assembly_type = c("primary_assembly", "toplevel"),  
  optimize = FALSE,  
  gene_symbols = FALSE,  
  uniprot_id = FALSE,  
  pseudo_5UTRS_if_needed = NULL,  
  remove_annotation_outliers = TRUE,  
  notify_load_existing = TRUE,  
  assembly = organism,  
  refseq_genbank_format = c("gtf", "gff3")[1]  
)
```

### Arguments

<code>organism</code>	scientific name of organism, <i>Homo sapiens</i> , <i>Danio rerio</i> , <i>Mus musculus</i> , etc. See <code>biomartr::get.ensembl.info()</code> for full list of supported organisms.
<code>output.dir</code>	directory to save downloaded data

db	database to use for genome and GTF, default advised: "ensembl" (remember to set assembly_type to "primary_assembly", else it will contain haplotypes, very large file!). Alternatives: "refseq" (reference assemblies) and "genbank" (all assemblies)
GTF	logical, default: TRUE, download gtf of organism specified in "organism" argument. If FALSE, check if the downloaded file already exist. If you want to use a custom gtf from you hard drive, set GTF = FALSE, and assign: <pre>annotation &lt;- getGenomeAndAnnotation(gtf = FALSE) annotation["gtf"] = "path/to/gtf.gtf"</pre> If db is not "ensembl", you will instead get a gff file.
genome	logical, default: TRUE, download genome of organism specified in "organism" argument. If FALSE, check if the downloaded file already exist. If you want to use a custom gtf from you hard drive, set GTF = FALSE, and assign: <pre>annotation &lt;- getGenomeAndAnnotation(genome = FALSE) annotation["genome"] = "path/to/genome.fasta"</pre> Will download the primary assembly from Ensembl.
merge_contaminants	logical, default TRUE. Will merge the contaminants specified into one fasta file, this considerably saves space and is much quicker to align with STAR than each contaminant on it's own. If no contaminants are specified, this is ignored.
phix	logical, default FALSE, download phiX sequence to filter out Illumina control reads. ORFik defines Phix as a contaminant genome. Phix is used in Illumina sequencers for sequencing quality control. Genome is: refseq, Escherichia phage phiX174. If sequencing facility created fastq files with the command bcl2fastq, then there should be very few phix reads left in the fastq files received.
ncRNA	logical or character, default FALSE (not used, no download), if TRUE or defined path, ncRNA is used as a contaminant reference. If TRUE, will try to find ncRNA sequences from the gtf file, usually represented as lncRNA (long non-coding RNA's). Will let you know if no ncRNA sequences were found in gtf. If not found try character input: Alternatives; "auto": Will try to find ncRNA file on NONCODE from organism, Homo sapiens -> human etc. "auto" will not work for all, then you must specify the name used by NONCODE, go to the link below and find it. If not "auto" / "" it must be a character vector of species common name (not scientific name) Homo sapiens is human, Rattus norvegicus is rat etc, download ncRNA sequence to filter out with. From NONCODE online server, if you cant find common name see: <a href="http://www.noncode.org/download.php/">http://www.noncode.org/download.php/</a>
tRNA	logical or character, default FALSE (not used, no download), tRNA is used as a contaminant genome. If TRUE, will try to find tRNA sequences from the gtf file, usually represented as Mt_tRNA (mature tRNA's). Will let you know if no tRNA sequences were found in gtf. If not found try character input: if not "" it must be a character vector to valid path of mature tRNAs fasta file to remove as contaminants on your disc. Find and download your wanted mtRNA at: <a href="http://gtrnadb.ucsc.edu/">http://gtrnadb.ucsc.edu/</a> , or run trna-scan on you genome.
rRNA	logical or character, default FALSE (not used, no download), rRNA is used as a contaminant reference If TRUE, will try to find rRNA sequences from the gtf

file, usually represented as rRNA (ribosomal RNA's). Will let you know if no rRNA sequences were found in gtf. If not found you can try character input: If "silva" will download silva SSU & LSU sequences for all species (250MB file) and use that. If you want a smaller file go to <https://www.arb-silva.de/> If not "" or "silva" it must be a character vector to valid path of mature rRNA fasta file to remove as contaminants on your disc.

gunzip	logical, default TRUE, uncompress downloaded files that are zipped when downloaded, should be TRUE!
remake	logical, default: FALSE, if TRUE remake everything specified
assembly_type	character, default c("primary_assembly", "toplevel"). Used for ensembl only, specifies the genome assembly type. Searches for both primary and toplevel, and if both are found, uses the first by order (so primary is prioritized by default). The Primary assembly should usually be used if it exists. The "primary assembly" contains all the top-level sequence regions, excluding alternative haplotypes and patches. If the primary assembly file is not present for a species (only defined for standard model organisms), that indicates that there were no haplotype/patch regions, and in such cases, the 'toplevel file is used. For more details see: <a href="#">ensembl tutorial</a>
optimize	logical, default FALSE. Create a folder within the output folder (defined by txdb_file_out_path), that includes optimized objects to speed up loading of annotation regions from up to 15 seconds on human genome down to 0.1 second. ORFik will then load these optimized objects instead. Currently optimizes filterTranscript() function and loadRegion() function for 5' UTRs, 3' UTRs, CDS, mRNA (all transcript with CDS) and tx (all transcripts).
gene_symbols	logical default FALSE. If TRUE, will download and store all gene symbols for all transcripts (coding and noncoding)- In a file called: "gene_symbol_tx_table.fst" in same folder as txdb. hgc for human, mouse symbols for mouse and rat, more to be added.
uniprot_id	logical default FALSE. If TRUE, will download and store all uniprot id for all transcripts (coding and noncoding)- In a file called: "gene_symbol_tx_table.fst" in same folder as txdb.
pseudo_5UTRS_if_needed	integer, default NULL. If defined > 0, will add pseudo 5' UTRs of maximum this length if 'minimum_5UTR_percentage" (default 30 mRNAs (coding transcripts) do not have a leader. (NULL and 0 are both the ignore command)
remove_annotation_outliers	logical, default TRUE. Only for refseq. shall outlier lines be removed from the input annotation_file? If yes, then the initial annotation_file will be overwritten and the removed outlier lines will be stored at tempdir for further exploration. Among others Aridopsis refseq contains malformed lines, where this is needed
notify_load_existing	logical, default TRUE. If annotation exists (defined as: locally (a file called outputs.rds) exists in outputdir), print a small message notifying the user it is not redownloading. Set to FALSE, if this is not wanted
assembly	character, default is assembly = organism, which means getting the first assembly in list, otherwise the name of the assembly wanted, like "GCA_000005845"

will get ecoli substrain k12, which is the most used ones for references. Usually ignore this for non bacterial species.

```
refseq_genbank_format
= c("gtf", "gff3")[1] Gtf format files are usually more secure from bugs down-
stream, so we highly advice to use them. GFF3 files can sometimes include
information you might not find in the gtf, so sometimes it makes sense to use it.
```

## Details

Some files that are made after download:

- A fasta index for the genome
- A TxDb to speed up GTF/GFF reading
- Separat of merged contaminant files

Files that can be made:

- Gene symbols (hgnc, etc)
- Uniprot ids (For name of protein structures)

If you want custom genome or gtf from you hard drive, assign existing paths like this:

```
annotation <- getGenomeAndAnnotation(GTF = "path/to/gtf.gtf", genome = "path/to/genome.fasta")
```

## Value

a named character vector of path to genomes and gtf downloaded, and additional contaminants if used. If merge\_contaminants is TRUE, will not give individual fasta files to contaminants, but only the merged one.

## References

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4919035/>

## See Also

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [install.fastp\(\)](#)

## Examples

```
## Get Saccharomyces cerevisiae genome and gtf (create txdb for R)
#getGenomeAndAnnotation("Saccharomyces cerevisiae", tempdir(), assembly_type = "toplevel")
## Download and add pseudo 5' UTRs
#getGenomeAndAnnotation("Saccharomyces cerevisiae", tempdir(), assembly_type = "toplevel",
# pseudo_5UTRS_if_needed = 100)
## Get Danio rerio genome and gtf (create txdb for R)
#getGenomeAndAnnotation("Danio rerio", tempdir())

output.dir <- "/Bio_data/references/zebrafish"
## Get Danio rerio and Phix contamints to deplete during alignment
#getGenomeAndAnnotation("Danio rerio", output.dir, phix = TRUE)

## Optimize for ORFik (speed up for large annotations like human or zebrafish)
```

```

#getGenomeAndAnnotation("Danio rerio", tempdir(), optimize = TRUE)

# Drosophila melanogaster (toplevel exists only)
#getGenomeAndAnnotation("drosophila melanogaster", output.dir = file.path(config["ref"],
# "Drosophila_melanogaster_BDGP6"), assembly_type = "toplevel")
## How to save malformed refseq gffs:
## First run function and let it crash:
#annotation <- getGenomeAndAnnotation(organism = "Arabidopsis thaliana",
# output.dir = "~/Desktop/test_plant/",
# assembly_type = "primary_assembly", db = "refseq")
## Then apply a fix (example for linux, too long rows):
# fixed_gff <- fix_malformed_gff("~/Desktop/test_plant/Arabidopsis_thaliana_genomic_refseq.gff")
## Then updated arguments:
# annotation <- c(fixed_gff, "~/Desktop/test_plant/Arabidopsis_thaliana_genomic_refseq.fna")
# names(annotation) <- c("gtf", "genome")
# Then make the txdb (for faster R use)
# makeTxdbFromGenome(annotation["gtf"], annotation["genome"], organism = "Arabidopsis thaliana")

```

---

go\_analaysis\_gorilla *GO analysis with GOrilla*

---

## Description

Supports Gene symbols as default, and will produce the best results. You can also use ensembl gene ids, refseq gene ids and Entrez gene ids, but this will give weaker results.

## Usage

```

go_analaysis_gorilla(
  target_genes,
  background_genes,
  organism,
  analysis_name = paste0("Go_analysis_", organism),
  open_browser = TRUE,
  pvalue_thresh = 0.01,
  db = "all"
)

```

## Arguments

target_genes	a path to a txt file with the target Gene symbols, or presumed to be a character vector of genes (if length > 1). Minimum 10 genes, maximum 1 million.
background_genes	a path to a txt file with the background Gene symbols, or presumed to be a character vector of genes (if length > 1). Minimum 10 genes, maximum 2 million.
organism	organism(df), example "Homo sapiens"
analysis_name	character name, default "test". Used for saved file names and analysis name in GOrilla.

open\_browser = TRUE, open the URL  
 pvalue\_thresh fixed set numeric, default 0.001, Alternatives: 10e-3 to 10e-11  
 db character, default "all". Which GO ontology categories to use, all means process, function and component. Alternatives: "proc", "func" and "comp", if you only want that single category subset.

### Value

a url path to results, will also open your default web browser if open\_browser is TRUE.

### References

<https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-10-48>

### Examples

```
target_genes <- system.file("extdata/Homo_sapiens_sample/QC_STATS",
  "/DTEG_Comparison_Translation.txt", package = "ORFik")
background_genes <- system.file("extdata/Homo_sapiens_sample/QC_STATS",
  "/DTEG_Comparison_Background.txt", package = "ORFik")
#go_analysis_gorilla(target_genes, background_genes, "Homo sapiens",
#                    analysis_name = "Translation vs background")
```

---

groupGRangesBy	<i>Group GRanges</i>
----------------	----------------------

---

### Description

It will group / split the GRanges object by the argument 'other'. For example if you would like to group GRanges object by gene, set other to gene names.

If 'other' is not specified function will try to use the names of the GRanges object. It will then be similar to 'split(gr, names(gr))'.

### Usage

```
groupGRangesBy(gr, other = NULL)
```

### Arguments

gr a GRanges object  
 other a vector of unique names to group by (default: NULL)

### Details

It is important that all intended groups in 'other' are uniquely named, otherwise duplicated group names will be grouped together.

**Value**

a GRangesList named after names(GRanges) if other is NULL, else names are from unique(other)

**Examples**

```
ORFranges <- GRanges(seqnames = Rle(rep("1", 3)),
                    ranges = IRanges(start = c(1, 10, 20),
                                     end = c(5, 15, 25)),
                    strand = "+")
ORFranges2 <- GRanges("1",
                    ranges = IRanges(start = c(20, 30, 40),
                                     end = c(25, 35, 45)),
                    strand = "+")
names(ORFranges) = rep("tx1_1", 3)
names(ORFranges2) = rep("tx1_2", 3)
gr1 <- GRangesList(tx1_1 = ORFranges, tx1_2 = ORFranges2)
gr <- unlist(gr1, use.names = FALSE)
## now recreate the gr1
## group by orf
grltest <- groupGRangesBy(gr) # using the names to group
identical(gr1, grltest) ## they are identical

## group by transcript
names(gr) <- txNames(gr)
grltest <- groupGRangesBy(gr)
identical(gr1, grltest) ## they are not identical
```

---

groupings

*Get number of ranges per group as an iteration*


---

**Description**

Get number of ranges per group as an iteration

**Usage**

```
groupings(gr1)
```

**Arguments**

```
gr1          GRangesList
```

**Value**

an integer vector

**Examples**

```
grl <- GRangesList(GRanges("1", c(1, 3, 5), "+"),
                  GRanges("1", c(19, 21, 23), "+"))
ORFik::groupings(grl)
```

---

heatMap_single	<i>Coverage heatmap of single libraries</i>
----------------	---

---

**Description**

Coverage heatmap of single libraries

**Usage**

```
heatMap_single(
  region,
  tx,
  reads,
  outdir,
  scores = "sum",
  upstream,
  downstream,
  zeroPosition = upstream,
  returnCoverage = FALSE,
  acceptedLengths = NULL,
  legendPos = "right",
  colors = "default",
  addFracPlot = TRUE,
  location = "start site",
  shifting = NULL,
  skip.last = FALSE,
  title = NULL,
  gradient.max = "default"
)
```

**Arguments**

region	#' a <a href="#">GRangesList</a> object of region, usually either leaders, cds', 3' utrs or ORFs, start region, stop regions etc. This is the region that will be mapped in heatmap
tx	default NULL, a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all grl names. The names of grl can also be the ORFik orf names. that is "txName_id"
reads	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> , or precomputed coverage as <a href="#">covRleList</a> (where names of covRle objects are readlengths) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better.

outdir	a character path to save file as: not just directory, but full name.
scores	character vector, default "sum", either of zscore, transcriptNormalized, sum, mean, median, .. see ?coverageScorings for info and more alternatives.
upstream	an integer, relative region to get upstream from.
downstream	an integer, relative region to get downstream from
zeroPosition	an integer DEFAULT (upstream), what is the center point? Like leaders and cds combination, then 0 is the TIS and -1 is last base in leader. NOTE!: if windows have different widths, this will be ignored.
returnCoverage	logical, default: FALSE, return coverage, if FALSE returns plot instead.
acceptedLengths	an integer vector (NULL), the read lengths accepted. Default NULL, means all lengths accepted.
legendPos	a character, Default "right". Where should the fill legend be ? ("top", "bottom", "right", "left")
colors	character vector, default: "default", this gives you: c("white", "yellow2", "yellow3", "lightblue", "blue", "navy"), do "high" for more high contrasts, or specify your own colors.
addFracPlot	Add margin histogram plot on top of heatmap with fractions per positions
location	a character, default "start site", will make xlabel of heatmap be Position relative to "start site" or alternative given.
shifting	a character, default NULL (no shifting), can also be either of c("5prime", "3prime")
skip.last	skip top(highest) read length, default FALSE
title	a character, default NULL (no title), what is the top title of plot?
gradient.max	numeric or character, default: "default", which is: max(coverage\$score), the max coverage over all readlengths. If you want all plots to use same reference point for max scaling, then first detect this point, look at max in plot etc, and use that value, to get all plots to have same max point.

**Value**

ggplot2 grob (default), data.table (if returnCoverage is TRUE)

**See Also**

Other heatmaps: [coverageHeatMap\(\)](#), [heatMapL\(\)](#), [heatMapRegion\(\)](#)

---

heatMapRegion                      *Create coverage heatmaps of specified region*

---

### Description

Simplified input space for easier abstraction of coverage heatmaps  
 Pick your transcript region and plot directly  
 Input CAGE file if you use TSS and want improved 5' annotation.

### Usage

```
heatMapRegion(
  df,
  region = "TIS",
  outdir = "default",
  scores = c("transcriptNormalized", "sum"),
  type = "ofst",
  cage = NULL,
  plot.ext = ".pdf",
  acceptedLengths = 21:75,
  upstream = c(50, 30),
  downstream = c(29, 69),
  shifting = c("5prime", "3prime"),
  longestPerGene = TRUE,
  colors = "default",
  scale_x = 5.5,
  scale_y = 15.5,
  gradient.max = "default",
  BPPARAM = BiocParallel::SerialParam()
)
```

### Arguments

df	an ORFik <a href="#">experiment</a>
region	a character, default "TIS". The centering point for the heatmap (what is position 0, between -50 and 50 etc), can be any combination of the set: c("TSS", "TIS", "TTS", "TES"), which are: <ul style="list-style-type: none"> <li>- Transcription start site (5' end of mrna)</li> <li>- Translation initiation site (5' end of CDS)</li> <li>- Translation termination site (5' end of 3' UTRs)</li> <li>- Transcription end site (3' end of 3' UTRs)</li> </ul>
outdir	a character path, default "default", saves to: <code>file.path(QCfolder(df), "heatmaps/")</code> , a created folder within the ORFik experiment data folder for plots. Change if you want custom location.
scores	character vector, default <code>c("transcriptNormalized", "sum")</code> , either of <code>zscore</code> , <code>transcriptNormalized</code> , <code>sum</code> , <code>mean</code> , <code>median</code> , .. see <code>?coverageScorings</code> for info and more alternatives.

type	character, default: "ofst". Type of library: either "default", usually bam format (the one you gave to experiment), "pshifted" pshifted reads, "ofst", "bed", "bedo" optimized bed, or "wig"
cage	a character path to library file or a <a href="#">GRanges</a> , <a href="#">GAlignments</a> preloaded file of CAGE data. Only used if "TSS" is defined as region, to redefine 5' leaders.
plot.ext	a character, default ".pdf", alternative ".png"
acceptedLengths	an integer vector (NULL), the read lengths accepted. Default NULL, means all lengths accepted.
upstream	1 or 2 integers, default c(50, 30), how long upstream from 0 should window extend (first index is 5' end extension, second is 3' end extension). If only 1 shifting, only 1 value should be given, if two are given will use first.
downstream	1 or 2 integers, default c(29, 69), how long upstream from 0 should window extend (first index is 5' end extension, second is 3' end extension). If only 1 shifting, only 1 value should be given, if two are given will use first.
shifting	a character, default c("5prime", "3prime"), can also be NULL (no shifting of reads). If NULL, will use first index of 'upstream' and 'downstream' argument.
longestPerGene	logical, default TRUE. Use only longest transcript isoform per gene. This will speed up your computation.
colors	character vector, default: "default", this gives you: c("white", "yellow2", "yellow3", "lightblue", "blue", "navy"), do "high" for more high contrasts, or specify your own colors.
scale_x	numeric, how should the width of the single plots be scaled, bigger the number, the bigger the plot
scale_y	numeric, how should the height of the plots be scaled, bigger the number, the bigger the plot
gradient.max	numeric or character, default: "default", which is: max(coverage\$score), the max coverage over all readlengths. If you want all plots to use same reference point for max scaling, then first detect this point, look at max in plot etc, and use that value, to get all plots to have same max point.
BPPARAM	a core param, default: single thread: BiocParallel::SerialParam(). Set to BiocParallel::bpparam() to use multicore. Be aware, this uses a lot of extra ram (40GB+) for larger human samples!

**Value**

invisible(NULL), plots are saved

**See Also**

Other heatmaps: [coverageHeatMap\(\)](#), [heatMapL\(\)](#), [heatMap\\_single\(\)](#)

## Examples

```
# Toy example, will not give logical output, but shows how it works
df <- ORFik.template.experiment()[9:10,] # Subset to 2 Ribo-seq libs
#heatMapRegion(df, "TIS", outdir = "default")
#
# Do also TSS, add cage for specific TSS
# heatMapRegion(df, c("TSS", "TIS"), cage = "path/to/cage.bed")

# Do on pshifted reads instead of original files
remove.experiments(df) # Remove loaded experiment first
# heatMapRegion(df, "TIS", type = "pshifted")
```

---

import.bedo

*Load GRanges object from .bedo*

---

## Description

.bedo is .bed ORFik, an optimized bed format for coverage reads with read lengths .bedo is a text based format with columns (6 maximum):

1. chromosome
2. start
3. end
4. strand
5. ref width (cigar # M's, match/mismatch total)
6. duplicates of that read

## Usage

```
import.bedo(path)
```

## Arguments

path                    a character, location on disc (full path)

## Details

Positions are 1-based, not 0-based as .bed. export with export.bedo

## Value

GRanges object

---

import.bedoc	<i>Load GAlignments object from .bedoc</i>
--------------	--

---

### Description

A much faster way to store, load and use bam files.  
.bedoc is .bed ORFik, an optimized bed format for coverage reads with cigar and replicate number.  
.bedoc is a text based format with columns (5 maximum):

1. chromosome
2. cigar: (cigar # M's, match/mismatch total)
3. start (left most position)
4. strand (+, -, \*)
5. score: duplicates of that read

### Usage

```
import.bedoc(path)
```

### Arguments

path	a character, location on disc (full path)
------	---

### Details

Positions are 1-based, not 0-based as .bed. export with export.bedo

### Value

GAlignments object

---

import.fstwig	<i>Import region from fastwig</i>
---------------	-----------------------------------

---

### Description

Import region from fastwig

### Usage

```
import.fstwig(gr, dir, id = "", readlengths = "all")
```

**Arguments**

gr	a GRanges object of exons
dir	prefix to filepath for file strand and chromosome will be added
id	id to column type, not used currently!
readlengths	integer / character vector, default "all". Or a subset of readlengths.

**Value**

a data.table with columns specified by readlengths

---

import.ofst	<i>Load GRanges / GAlignments object from .ofst</i>
-------------	---

---

**Description**

A much faster way to store, load and use bam files.

.ofst is ORFik fast serialized object, an optimized format for coverage reads with cigar and replicate number. It uses the fst format as back-end: [fst-package](#).

A .ofst ribo seq file can compress the information in a bam file from 5GB down to a few MB. This new files has super fast reading time, only a few seconds, instead of minutes. It also has random index access possibility of the file.

.ofst is represented as a data.frame format with minimum 4 columns:

1. chromosome
2. start (left most position)
3. strand (+, -, \*)
4. width (not added if cigar exists, see below for more details)
5. cigar (not needed if width exists): (cigar # M's, match/mismatch total)
5. score: duplicates of that read
6. size: qwidth according to reference of read

If file is from [GAlignmentPairs](#), it will contain a cigar1, cigar2 instead of cigar and start1 and start2 instead of start

**Usage**

```
import.ofst(file, strandMode = 0, seqinfo = NULL)
```

**Arguments**

file	a path to a .ofst file
strandMode	numeric, default 0. Only used for paired end bam files. One of (0: strand = *, 1: first read of pair is +, 2: first read of pair is -). See ?strandMode. Note: Sets default to 0 instead of 1, as readGAlignmentPairs uses 1. This is to guarantee hits, but will also make mismatches of overlapping transcripts in opposite directions.
seqinfo	Seqinfo object, default NULL (created from ranges). Add to avoid warnings later on differences in seqinfo.

**Details**

If 'width' is not defined, it checks for column called 'end'. If neither width or end is defined, then all widths are presumed to be 1. I.e. single nucleotide points.

Other columns can be named whatever you want and added to meta columns. Positions are 1-based, not 0-based as .bed. Import with import.ofst

**Value**

a GAlignment, GAlignmentPairs or GRanges object, dependent of if cigar/cigar1 is defined in .ofst file.

**Examples**

```
## GRanges
gr <- GRanges("1:1-3:-")
tmp <- file.path(tempdir(), "path.ofst")
# export.ofst(gr, file = tmp)
# import.ofst(tmp)
## GAlignment
# Make input data.frame
df <- data.frame(seqnames = "1", cigar = "3M", start = 1L, strand = "+")
ga <- ORFik:::getGAlignments(df)
# export.ofst(ga, file = tmp)
# import.ofst(tmp)
```

---

importGtfFromTxdb	<i>Import the GTF / GFF that made the txdb</i>
-------------------	--

---

**Description**

Import the GTF / GFF that made the txdb

**Usage**

```
importGtfFromTxdb(txdb, stop.error = TRUE)
```

**Arguments**

txdb	a TxDb, path to txdb / gff or ORFik experiment object
stop.error	logical TRUE, stop if Txdb does not have a gtf. If FALSE, return NULL.

**Value**

data.frame, the gtf/gff object imported with rtracklayer::import. Or NULL, if stop.error is FALSE, and no GTF file found.

---

initiationScore      *Get initiation score for a GRangesList of ORFs*

---

### Description

initiationScore tries to check how much each TIS region resembles, the average of the CDS TIS regions.

### Usage

```
initiationScore(grl, cds, tx, reads, pShifted = TRUE, weight = "score")
```

### Arguments

grl	a <a href="#">GRangesList</a> object with ORFs
cds	a <a href="#">GRangesList</a> object with coding sequences
tx	a <a href="#">GRangesList</a> of transcripts covering grl.
reads	ribo seq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
pShifted	a logical (TRUE), are riboseq reads p-shifted?
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <a href="#">GRanges</a> ("chr1", 1, "+", score = 5), would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <a href="#">countOverlaps()</a> presumes, if single number (!= 1), it repeats for all ranges, if vector with length > 1, it must be equal size of the reads object.

### Details

Since this features uses a distance matrix for scoring, values are distributed like this:

As result there is one value per ORF:

0.000: means that ORF had no reads

-1.000: means that ORF is identical to average of CDS

1.000: means that orf is maximum different than average of CDS

If a score column is defined, it will use it as weights, see [getWeights](#)

### Value

an integer vector, 1 score per ORF, with names of grl

### References

doi: 10.1186/s12915-017-0416-0

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
# Good hitting ORF
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(21, 40),
               strand = "+")
names(ORF) <- c("tx1")
gr1 <- GRangesList(tx1 = ORF)
# 1 width p-shifted reads
reads <- GRanges("1", IRanges(c(21, 23, 50, 50, 50, 53, 53, 56, 59),
                              width = 1), "+")
score(reads) <- 28 # original width
cds <- GRanges(seqnames = "1",
               ranges = IRanges(50, 80),
               strand = "+")
cds <- GRangesList(tx1 = cds)
tx <- GRanges(seqnames = "1",
               ranges = IRanges(1, 85),
               strand = "+")
tx <- GRangesList(tx1 = tx)

initiationScore(gr1, cds, tx, reads, pShifted = TRUE)
```

---

insideOutsideORF	<i>Inside/Outside score (IO)</i>
------------------	----------------------------------

---

**Description**

Inside/Outside score is defined as

$$(\text{reads over ORF}) / (\text{reads outside ORF and within transcript})$$

A pseudo-count of one is added to both the ORF and outside sums.

**Usage**

```
insideOutsideORF(
  gr1,
  RFP,
  GtfOrTx,
```

```

ds = NULL,
RFP.sorted = FALSE,
weight = 1L,
overlapGr1 = NULL
)

```

### Arguments

gr1	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs.
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
GtfOrTx	If it is <a href="#">TxDb</a> object transcripts will be extracted using <code>exonsBy(Gtf, by = "tx", use.names = TRUE)</code> . Else it must be <a href="#">GRangesList</a>
ds	numeric vector (NULL), disengagement score. If you have already calculated <a href="#">disengagementScore</a> , input here to save time.
RFP.sorted	logical (FALSE), an optimizer, have you ran this line: <code>RFP &lt;- sort(RFP[countOverlaps(RFP, tx, type = "within") &gt; 0])</code> Normally not touched, for internal optimization purposes.
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <code>countOverlaps()</code> presumes, if single number ( $\neq 1$ ), it repeats for all ranges, if vector with length > 1, it must be equal size of the reads object.
overlapGr1	an integer, (default: NULL), if defined must be <code>countOverlaps(gr1, RFP)</code> , added for speed if you already have it

### Value

a named vector of numeric values of scores

### References

doi: 10.1242/dev.098345

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```

# Check inside outside score of a ORF within a transcript
ORF <- GRanges("1",
               ranges = IRanges(start = c(20, 30, 40),

```

```
                                end = c(25, 35, 45)),
                                strand = "+")

gr1 <- GRangesList(tx1_1 = ORF)

tx1 <- GRanges(seqnames = "1",
              ranges = IRanges(start = c(1, 10, 20, 30, 40, 50),
                              end = c(5, 15, 25, 35, 45, 200)),
              strand = "+")
tx <- GRangesList(tx1 = tx1)
RFP <- GRanges(seqnames = "1",
              ranges = IRanges(start = c(1, 4, 30, 60, 80, 90),
                              end = c(30, 33, 63, 90, 110, 120)),
              strand = "+")

insideOutsideORF(gr1, RFP, tx)
```

---

install.fastp

*Download and prepare fastp trimmer*

---

## Description

On Linux, will not run "make", only use precompiled fastp file.

On Mac OS it will use precompiled binaries.

For windows must be installed through WSL (Windows Subsystem Linux)

## Usage

```
install.fastp(folder = "~/bin")
```

## Arguments

folder	path to folder for download, file will be named "fastp", this should be most recent version. On mac it will search for a folder called fastp-master inside folder given. Since there is no precompiled version of fastp for Mac OS.
--------	---

## Value

path to runnable fastp

## References

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6129281/>

## See Also

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#)

## Examples

```
## With default folder:
#install.fastp()

## Or set manual folder:
folder <- "~/I/WANT/IT/HERE/"
#install.fastp(folder)
```

---

install.sratoolkit      *Download sra toolkit*

---

## Description

Currently supported for Linux (64 bit centos and ubuntu is tested to work) and Mac-OS(64 bit). If other linux distro, centos binaries will be used.

## Usage

```
install.sratoolkit(folder = "~/bin", version = "2.11.3")
```

## Arguments

folder	default folder, "~/bin"
version	a string, default "2.11.3"

## Value

path to fastq-dump in sratoolkit

## References

<https://ncbi.github.io/sra-tools/fastq-dump.html>

## See Also

Other sra: [browseSRA\(\)](#), [download.SRA\(\)](#), [download.SRA.metadata\(\)](#), [download.ebi\(\)](#), [get\\_bioproject\\_candidates](#), [rename.SRA.files\(\)](#)

## Examples

```
# install.sratoolkit()
## Custom folder and version (not advised)
folder <- "~/I/WANT/IT/HERE/"
# install.sratoolkit(folder, version = "2.10.9")
```

---

`isInFrame`*Find frame for each orf relative to cds*

---

**Description**

Input of this function, is the output of the function [`distToCds()`], or any other relative ORF frame.

**Usage**

```
isInFrame(dists)
```

**Arguments**

`dists` a vector of integer distances between ORF and cds. 0 distance means equal frame

**Details**

possible outputs: 0: orf is in frame with cds 1: 1 shifted from cds 2: 2 shifted from cds

**Value**

a logical vector

**References**

doi: 10.1074/jbc.R116.733899

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
# simple example
isInFrame(c(3,6,8,11,15))

# GRangesList example
gr1 <- GRangesList(tx1_1 = GRanges("1", IRanges(1,10), "+"))
fiveUTRs <- GRangesList(tx1 = GRanges("1", IRanges(1,20), "+"))
dist <- distToCds(gr1, fiveUTRs)
isInFrame <- isInFrame(dist)
```

---

isOverlapping                      *Find frame for each orf relative to cds*

---

### Description

Input of this function, is the output of the function [distToCds()]

### Usage

```
isOverlapping(dists)
```

### Arguments

dists                      a vector of distances between ORF and cds

### Value

a logical vector

### References

doi: 10.1074/jbc.R116.733899

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
# simple example
isOverlapping(c(-3,-6,8,11,15))

# GRangesList example
gr1 <- GRangesList(tx1_1 = GRanges("1", IRanges(1,10), "+"))
fiveUTRs <- GRangesList(tx1 = GRanges("1", IRanges(1,20), "+"))
dist <- distToCds(gr1, fiveUTRs)
isOverlapping <- isOverlapping(dist)
```

---

kozak_IR_ranking	<i>Rank kozak initiation sequences</i>
------------------	--

---

### Description

Defined as region (-4, -1) relative to TIS

### Usage

```
kozak_IR_ranking(cds_k, mrna, dt.ir, faFile, group.min = 10, species = "human")
```

### Arguments

cds_k	cds ranges (GRangesList)
mrna	mrna ranges (GRangesList)
dt.ir	data.table with a column called IR, initiation rate
faFile	<a href="#">FaFile</a> , BSgenome, fasta/index file path or an ORFik <a href="#">experiment</a> . This file is usually used to find the transcript sequences from some GRangesList.
group.min	numeric, default 10. Minimum transcripts per initiation group to be included
species	("human"), which species to use, currently supports human (Homo sapiens), zebrafish (Danio rerio) and mouse (Mus musculus). Both scientific or common name for these species will work. You can also specify a pfm for your own species. Syntax of pfm is an rectangular integer matrix, where all columns must sum to the same value, normally 100. See example for more information. Rows are in order: c("A", "C", "G", "T")

### Value

a ggplot grid object

---

kozakHeatmap	<i>Make sequence region heatmap relative to scoring</i>
--------------	---

---

### Description

Given sequences, DNA or RNA. And some score, ribo-seq fpkm, TE etc. Create a heatmap divided per letter in seqs, by how strong the score is.

**Usage**

```
kozakHeatmap(
  seqs,
  rate,
  start = 1,
  stop = max(nchar(seqs)),
  center = ceiling((stop - start + 1)/2),
  min.observations = ">q1",
  skip.startCodon = FALSE,
  xlab = "TIS",
  type = "ribo-seq"
)
```

**Arguments**

seqs	the sequences (character vector, DNASTringSet)
rate	a scoring vector (equal size to seqs)
start	position in seqs to start at (first is 1), default 1.
stop	position in seqs to stop at (first is 1), default max(nchar(seqs)), that is the longest sequence length
center	position in seqs to center at (first is 1), center will be +1 in heatmap
min.observations	How many observations per position per letter to accept? numeric or quantile, default (">q1", bigger than quartile 1 (25 percentile)). You can do (10), to get all with more than 10 observations.
skip.startCodon	startCodon is defined as after centering (position 1, 2 and 3). Should they be skipped? default (FALSE). Not relevant if you are not doing Translation initiation sites (TIS).
xlab	Region you are checking, default (TIS)
type	What type is the rate scoring? default (ribo-seq)

**Details**

It will create blocks around the highest rate per position

**Value**

a ggplot of the heatmap

**Examples**

```
## Not run:
if (requireNamespace("BSgenome.Hsapiens.UCSC.hg19")) {
  txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
    package = "GenomicFeatures")
  #Extract sequences of Coding sequences.
```

```

cds <- loadRegion(txdbFile, "cds")
tx <- loadRegion(txdbFile, "mrna")

# Get region to check
kozakRegions <- startRegionString(cds, tx, BSgenome.Hsapiens.UCSC.hg19::Hsapiens
                                , upstream = 4, 5)

# Some toy ribo-seq fpkm scores on cds
set.seed(3)
fpkm <- sample(1:115, length(cds), replace = TRUE)
kozakHeatmap(kozakRegions, fpkm, 1, 9, skip.startCodon = F)
}

## End(Not run)

```

---

kozakSequenceScore      *Make a score for each ORFs start region by proximity to Kozak*

---

### Description

The closer the sequence is to the Kozak sequence the higher the score, based on the experimental pwms from article referenced. Minimum score is 0 (worst correlation), max is 1 (the best base per column was chosen).

### Usage

```
kozakSequenceScore(grl, tx, faFile, species = "human", include.N = FALSE)
```

### Arguments

grl	a <a href="#">GRangesList</a> grouped by ORF
tx	a <a href="#">GRangesList</a> , the reference area for ORFs, each ORF must have a corresponding tx.
faFile	<a href="#">FaFile</a> , <a href="#">BSgenome</a> , fasta/index file path or an <a href="#">ORFik</a> <a href="#">experiment</a> . This file is usually used to find the transcript sequences from some <a href="#">GRangesList</a> .
species	("human"), which species to use, currently supports human ( <i>Homo sapiens</i> ), zebrafish ( <i>Danio rerio</i> ) and mouse ( <i>Mus musculus</i> ). Both scientific or common name for these species will work. You can also specify a pfm for your own species. Syntax of pfm is an rectangular integer matrix, where all columns must sum to the same value, normally 100. See example for more information. Rows are in order: c("A", "C", "G", "T")
include.N	logical (F), if TRUE, allow N bases to be counted as hits, score will be average of the other bases. If True, N bases will be added to pfm, automatically, so dont include them if you make your own pfm.

**Details**

Ranges that does not have minimum 15 length (the kozak requirement as a sliding window of size 15 around grl start), will be set to score 0. Since they should not have the possibility to make an efficient ribosome binding.

**Value**

a numeric vector with values between 0 and 1

an integer vector, one score per orf

**References**

doi: <https://doi.org/10.1371/journal.pone.0108475>

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
# Usually the ORFs are found in orfik, which makes names for you etc.
# Here we make an example from scratch
seqName <- "Chromosome"
ORF1 <- GRanges(seqnames = seqName,
                ranges = IRanges(c(1007, 1096), width = 60),
                strand = c("+", "+"))
ORF2 <- GRanges(seqnames = seqName,
                ranges = IRanges(c(400, 100), width = 30),
                strand = c("-", "-"))
ORFs <- GRangesList(tx1 = ORF1, tx2 = ORF2)
ORFs <- makeORFNames(ORFs) # need ORF names
tx <- extendLeaders(ORFs, 100)
# get faFile for sequences
faFile <- FaFile(system.file("extdata/references/danio_rerio", "genome_dummy.fasta",
                             package = "ORFik"))
kozakSequenceScore(ORFs, tx, faFile)
# For more details see vignettes.
```

---

lastExonEndPerGroup    *Get last end per granges group*

---

**Description**

Get last end per granges group

**Usage**

```
lastExonEndPerGroup(gr1, keep.names = TRUE)
```

**Arguments**

```
gr1          a GRangesList
keep.names   a boolean, keep names or not, default: (TRUE)
```

**Value**

a Rle(keep.names = T), or integer vector(F)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                  ranges = IRanges(c(4, 1), c(9, 3)),
                  strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
lastExonEndPerGroup(gr1)
```

---

lastExonPerGroup	<i>Get last exon per GRangesList group</i>
------------------	--

---

**Description**

gr1 must be sorted, call ORFik:::sortPerGroup if needed

**Usage**

```
lastExonPerGroup(gr1)
```

**Arguments**

```
gr1          a GRangesList
```

**Value**

a GRangesList of the last exon per group

**Examples**

```

gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
lastExonPerGroup(grl)

```

---

lastExonStartPerGroup *Get last start per granges group*

---

**Description**

Get last start per granges group

**Usage**

```
lastExonStartPerGroup(grl, keep.names = TRUE)
```

**Arguments**

grl                    a [GRangesList](#)

keep.names            a boolean, keep names or not, default: (TRUE)

**Value**

a Rle(keep.names = T), or integer vector(F)

**Examples**

```

gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
lastExonStartPerGroup(grl)

```

---

*length,covRle-method*    *length covRle*

---

**Description**

Number of chromosomes

**Usage**

```
## S4 method for signature 'covRle'  
length(x)
```

**Arguments**

x                    a covRle object

**Value**

an integer, number of chromosomes in covRle object

---

*length,covRleList-method*  
*length covRleList*

---

**Description**

Number of covRle objects

**Usage**

```
## S4 method for signature 'covRleList'  
length(x)
```

**Arguments**

x                    a covRleList object

**Value**

an integer, number of covRle objects

length,GRangesList-method

*Get length of GRangesList*

---

**Description**

Faster version than S4Vector generic caller

**Usage**

```
## S4 method for signature 'GRangesList'  
length(x)
```

**Arguments**

x                    a GRangesList

**Value**

an integer (length 1)

---

lengths,covRle-method *lengths covRle*

---

**Description**

Lengths of each chromosome

**Usage**

```
## S4 method for signature 'covRle'  
lengths(x)
```

**Arguments**

x                    a covRle object

**Value**

a named integer vector of chromosome lengths

---

```
lengths,covRleList-method
      lengths covRleList
```

---

**Description**

Lengths of each chromosome

**Usage**

```
## S4 method for signature 'covRleList'
lengths(x)
```

**Arguments**

x                    a covRle object

**Value**

a named integer vector of chromosome lengths

---

```
libFolder                    Get path to ORFik experiment library folder
```

---

**Description**

Get path to ORFik experiment library folder

**Usage**

```
libFolder(x, mode = "first", unique_mappers = uniqueMappers(x))
```

**Arguments**

x                    an ORFik [experiment](#)

mode                character, default "first". Alternatives: "unique", "all". Unique means the unique directories, not to be confused with unique\_mappers argument below.

unique\_mappers    logical, default uniqueMappers(x) If true appends unique\_mappers to path

**Value**

a character path

---

```
libFolder, experiment-method
```

*Get path to ORFik experiment library folder*

---

**Description**

Get path to ORFik experiment library folder

**Usage**

```
## S4 method for signature 'experiment'
libFolder(x, mode = "first", unique_mappers = uniqueMappers(x))
```

**Arguments**

`x` an ORFik [experiment](#)

`mode` character, default "first". Alternatives: "unique", "all". Unique means the unique directories, not to be confused with `unique_mappers` argument below.

`unique_mappers` logical, default `uniqueMappers(x)` If true appends `unique_mappers` to path

**Value**

a character path

---

```
libraryTypes
```

*Which type of library type in [experiment](#)?*

---

**Description**

Which type of library type in [experiment](#)?

**Usage**

```
libraryTypes(df, uniqueTypes = TRUE)
```

**Arguments**

`df` an ORFik [experiment](#)

`uniqueTypes` logical, default TRUE. Only return unique lib types.

**Value**

library types (character vector)

**See Also**

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
libraryTypes(df)
libraryTypes(df, uniqueTypes = FALSE)
```

---

list.experiments	<i>List current experiment available</i>
------------------	--

---

**Description**

Will only search .csv extension, also exclude any experiment with the word template.

**Usage**

```
list.experiments(
  dir = ORFik::config()["exp"],
  pattern = "*",
  libtypeExclusive = NULL,
  validate = TRUE,
  BPPARAM = if (!validate) {
    BiocParallel::SerialParam()
  } else
    BiocParallel::bpparam()
)
```

**Arguments**

dir	directory for ORFik experiments: default: ORFik::config()["exp"], which by default is: "~/Bio_data/ORFik_experiments/"
pattern	allowed patterns in experiment file name: default ("*", all experiments)
libtypeExclusive	search for experiments with exclusively this libtype, default (NULL, all)
validate	logical, default TRUE. Abort if any library files does not exist. Do not set this to FALSE, unless you know what you are doing!
BPPARAM	how many cores/threads to use? Default single thread if validate is FALSE, else use bpparam. default: if(!validate){ BiocParallel::SerialParam()} else BiocParallel::bpparam()

**Value**

a data.table, 1 row per experiment with columns:

- experiment (name),
- organism
- author
- libtypes
- number of samples

**Examples**

```
## Make your experiments
df <- ORFik.template.experiment(TRUE)
df2 <- df[1:6,] # Only first 2 libs
## Save them
# save.experiment(df, "~/Bio_data/ORFik_experiments/exp1.csv")
# save.experiment(df2, "~/Bio_data/ORFik_experiments/exp1_subset.csv")
## List all experiment you have:
## Path above is default path, so no dir argument needed
#list.experiments()
#list.experiments(pattern = "subset")
## For non default directory experiments
#list.experiments(dir = "MY/CUSTOM/PATH")
```

---

list.genomes

*List genomes created with ORFik*

---

**Description**

Given the reference.folder, list all valid references. An ORFik genome is defined as a folder with a file called output.rds that is a named R vector with names gtf and genome, where the values are character paths to those files inside that folder. This makes sure that this reference was made by ORFik and not some other program.

**Usage**

```
list.genomes(reference.folder = ORFik::config()["ref"])
```

**Arguments**

```
reference.folder
      character path, default: ORFik::config()["ref"].
```

**Value**

a data.table with 5 columns:

- character (name of folder)
- logical (does it have a gtf)
- logical (does it have a fasta genome)

- logical (does it have a STAR index)
- logical (only displayed if some are TRUE, does it have protein structure predictions of ORFs from alphafold etc, in folder called 'protein\_structure\_predictions')
- logical (only displayed if some are TRUE, does it have gene symbol fst file from bioMart etc, in file called 'gene\_symbol\_tx\_table.fst')

### Examples

```
## Run with default config path
#list.genomes()
## Run with custom config path
list.genomes(tempdir())
## Get the path to fasta genome of first organism in list
#readRDS(file.path(config()["ref"], list.genomes()$name, "outputs.rds")[1])["genome"]
```

---

loadRegion	<i>Load transcript region</i>
------------	-------------------------------

---

### Description

Usefull to simplify loading of standard regions, like cds' and leaders. Adds another safety in that seqlevels will be set

### Usage

```
loadRegion(
  txdb,
  part = "tx",
  names.keep = NULL,
  by = "tx",
  skip.optimized = FALSE
)
```

### Arguments

- |            |  |
|------------|--|
| txdb       | a TxDb object, ORFik experiment object or a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite), Only in the loadRegion function: if it is a GRangesList, it will return it self.   |
| part       | a character, one of: tx, ncRNA, mrna, leader, cds, trailer, intron, NOTE: difference between tx and mrna is that tx are all transcripts, while mrna are all transcripts with a cds, respectively ncRNA are all tx without a cds.   |
| names.keep | a character vector of subset of names to keep. Example: loadRegions(txdb, names = "ENST1000005"), will return only that transcript. Remember if you set by to "gene", then this list must be with gene names.  |
| by         | a character, default "tx" Either "tx" or "gene". What names to output region by, the transcript name "tx" or gene names "gene". NOTE: this is not the same as cdsBy(txdb, by = "gene"), cdsBy would then only give 1 cds per Gene, loadRegion gives all isoforms, but with gene names. |

`skip.optimized` logical, default FALSE. If TRUE, will not search for optimized rds files to load created from `ORFik::makeTxdbFromGenome(..., optimize = TRUE)`. The optimized files are ~ 100x faster to load for human genome.

### Details

Load as `GRangesList` if input is not already `GRangesList`.

### Value

a `GRangesList` of region

### Examples

```
# GTF file is slow, but possible to use
gtf <- system.file("extdata", "hg19_knownGene_sample.sqlite",
  package = "GenomicFeatures")

txdb <- loadTxdb(gtf)
loadRegion(txdb, "cds")
loadRegion(txdb, "intron")
# Use txdb from experiment
df <- ORFik.template.experiment()
txdb <- loadTxdb(df)
loadRegion(txdb, "leaders")
# Use ORFik experiment directly
loadRegion(df, "mrna")
```

---

loadRegions

*Get all regions of transcripts specified to environment*

---

### Description

By default loads all parts to `.GlobalEnv` (global environment) Useful to not spend time on finding the functions to load regions.

### Usage

```
loadRegions(
  txdb,
  parts = c("mrna", "leaders", "cds", "trailers"),
  extension = "",
  names.keep = NULL,
  by = "tx",
  skip.optimized = FALSE,
  envir = .GlobalEnv
)
```

**Arguments**

txdb	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .db or .sqlite) or an ORFik experiment
parts	the transcript parts you want, default: c("mrna", "leaders", "cds", "trailers"). See ?loadRegion for more info on this argument.
extension	What to add on the name after leader, like: B -> leadersB
names.keep	a character vector of subset of names to keep. Example: loadRegions(txdb, names = "ENST1000005"), will return only that transcript. Remember if you set by to "gene", then this list must be with gene names.
by	a character, default "tx" Either "tx" or "gene". What names to output region by, the transcript name "tx" or gene names "gene". NOTE: this is not the same as cdsBy(txdb, by = "gene"), cdsBy would then only give 1 cds per Gene, loadRegion gives all isoforms, but with gene names.
skip.optimized	logical, default FALSE. If TRUE, will not search for optimized rds files to load created from ORFik::makeTxdbFromGenome(..., optimize = TRUE). The optimized files are ~ 100x faster to load for human genome.
envir	Which environment to save to, default: .GlobalEnv

**Value**

invisible(NULL) (regions saved in envir)

**Examples**

```
# Load all mrna regions to Global environment
gtf <- system.file("extdata", "hg19_knownGene_sample.sqlite",
  package = "GenomicFeatures")
loadRegions(gtf, parts = c("mrna", "leaders", "cds", "trailers"))
```

---

loadTranscriptType      *Load transcripts of given biotype*

---

**Description**

Like rRNA, snoRNA etc. NOTE: Only works on gtf/gff, not .db object for now. Also note that these annotations are not perfect, some rRNA annotations only contain 5S rRNA etc. If your gtf does not contain everything you need, use a resource like repeatmasker and download a gtf: <https://genome.ucsc.edu/cgi-bin/hgTables>

**Usage**

```
loadTranscriptType(object, part = "rRNA", tx = NULL)
```

**Arguments**

object	a TxDb, ORFik experiment or path to gtf/gff,
part	a character, default rRNA. Can also be: snoRNA, tRNA etc. As long as that biotype is defined in the gtf.
tx	a GRangesList of transcripts (Optional, default NULL, all transcript of that type), else it must be names a list to subset on.

**Value**

a GRangesList of transcript of that type

**References**

doi: 10.1002/0471250953.bi0410s25

**Examples**

```
gtf <- "path/to.gtf"
#loadTranscriptType(gtf, part = "rRNA")
#loadTranscriptType(gtf, part = "miRNA")
```

---

loadTxdb

*General loader for txdb*

---

**Description**

Useful to allow fast TxDb loader like .db

**Usage**

```
loadTxdb(txdb, chrStyle = NULL, organism = NA, chrominfo = NULL)
```

**Arguments**

txdb	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite) or an ORFik experiment
chrStyle	a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -> pick "NCBI"
organism	character, default NA. Scientific name of organism. Only used if input is path to gff.
chrominfo	Seqinfo object, default NULL. Only used if input is path to gff.

**Value**

a TxDb object

**Examples**

```
library(GenomicFeatures)
# Get the gtf txdb file
txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
                        package = "GenomicFeatures")
txdb <- loadTxdb(txdbFile)
```

---

longestORFs

*Get longest ORF per stop site*


---

**Description**

Rule: if seqname, strand and stop site is equal, take longest one. Else keep. If IRangesList or IRanges, seqnames are groups, if GRanges or GRangesList seqnames are the seqlevels (e.g. chromosomes/transcripts)

**Usage**

```
longestORFs(gr1)
```

**Arguments**

gr1                    a [GRangesList](#)/[IRangesList](#), [GRanges](#)/[IRanges](#) of ORFs

**Value**

a [GRangesList](#)/[IRangesList](#), [GRanges](#)/[IRanges](#) (same as input)

**See Also**

Other ORFHelpers: [defineTrailer\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

**Examples**

```
ORF1 = GRanges("1", IRanges(10,21), "+")
ORF2 = GRanges("1", IRanges(1,21), "+") # <- longest
gr1 <- GRangesList(ORF1 = ORF1, ORF2 = ORF2)
longestORFs(gr1) # get only longest
```

---

`makeGRangesFromDataFrameFast`*Faster version (also less safe) of makeGRangesFromDataFrame*

---

**Description**

Faster version (also less safe) of `makeGRangesFromDataFrame`

**Usage**

```
makeGRangesFromDataFrameFast(df, keep.extra.columns = TRUE, seqinfo = NULL)
```

**Arguments**

`df` a data.frame/data.table with columns minimum 4 columns: seqnames, start, strand  
Additional specific columns are:  
- width (if not set, width is set to 1 for all reads)  
Additional columns will be assigned as meta columns

`keep.extra.columns` logical, default TRUE, keep meta cols.

`seqinfo` Seqinfo object, default NULL (created from ranges). Add to avoid warnings later on differences in seqinfo.

**Value**

GRanges object

**Examples**

```
df <- data.frame(start = rep(1L, 1e5), end = 10L, strand = "+", seqnames = "1")

system.time(res <- makeGRangesFromDataFrame(df))
system.time(res_fast <- makeGRangesFromDataFrameFast(df))
identical(res, res_fast)

# Use width instead of end, does not work in original
df2 <- data.frame(start = rep(1L, 1e5), width = 10L, strand = "+", seqnames = "1")
system.time(makeGRangesFromDataFrameFast(df2))
df_small <- data.frame(start = 1L, end = 10L, strand = "+", seqnames = "1")
system.time(res <- makeGRangesFromDataFrame(df_small))
system.time(res_fast <- makeGRangesFromDataFrameFast(df_small))
identical(res, res_fast)
```

---

makeGRangesListFromCharacter  
*Convert a character vector to GRangesList*

---

**Description**

Convert a character vector to GRangesList

**Usage**

```
makeGRangesListFromCharacter(x)
```

**Arguments**

x                    a character vector

**Value**

a GRangesList

**Examples**

```
vec <- c("1:14598834-14598914:+", "1:15210514-15210562:+;1:15214895-15215025:+")
makeGRangesListFromCharacter(vec)
```

---

makeORFNames                    *Make ORF names per orf*

---

**Description**

grl must be grouped by transcript If a list of orfs are grouped by transcripts, but does not have ORF names, then create them and return the new GRangesList

**Usage**

```
makeORFNames(grl, groupByTx = TRUE)
```

**Arguments**

grl                    a [GRangesList](#)  
groupByTx            logical (T), should output GRangesList be grouped by transcripts (T) or by ORFs (F)?

**Value**

(GRangesList) with ORF names, grouped by transcripts, sorted.

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(7, 14), width = 3),
  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
  ranges = IRanges(c(4, 1), c(9, 3)),
  strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
makeORFNames(grl)
```

---

```
makeSummarizedExperimentFromBam
```

*Make a count matrix from a library or experiment*

---

**Description**

Make a summarizedExperiment / matrix object from bam files or other library formats specified by lib.type argument. Works like HTSeq, to give you count tables per library.

**Usage**

```
makeSummarizedExperimentFromBam(
  df,
  saveName = NULL,
  longestPerGene = FALSE,
  geneOrTxNames = "tx",
  region = "mrna",
  type = "count",
  lib.type = "ofst",
  weight = "score",
  forceRemake = FALSE,
  force = TRUE,
  library.names = bamVarName(df),
  libraries = outputLibs(df, chrStyle = seqinfo(df), paths = filepath(df, lib.type), type
    = lib.type, force = force, library.names = library.names, BPPARAM = BPPARAM),
  format = "qs",
  BPPARAM = BiocParallel::SerialParam()
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
saveName	a character (default NULL), if set save experiment to path given. Always saved as .rds., it is optional to add .rds, it will be added for you if not present. Also used to load existing file with that name.

longestPerGene	a logical (default FALSE), if FALSE all transcript isoforms per gene. Ignored if "region" is not a character of either: "mRNA", "tx", "cds", "leaders" or "trailers".
geneOrTxNames	a character vector (default "tx"), should row names keep transcript names ("tx") or change to gene names ("gene")
region	a character vector (default: "mrna"), make raw count matrices of whole mrnas or one of (leaders, cds, trailers). Can also be a <a href="#">GRangesList</a> , then it uses this region directly. Can then be uORFs or a subset of CDS etc.
type	default: "count" (raw counts matrix), alternative is "fpkm", "log2fpkm" or "log10fpkm"
lib.type	a character(default: "default"), load files in experiment or some precomputed variant, either "ofst", "pshifted" or "cov" These are made with <code>ORFik::convertLibs()</code> or <code>shiftFootprintsByExperiment()</code> . Can also be custom user made folders inside the experiments bam folder. Format "cov" (i.e. covRle format) is by far the fastest to use if existing.
weight	numeric or character, a column to score overlaps by. Default "score", will check for a metacolumn called "score" in libraries. If not found, will not use weights.
forceRemake	logical, default FALSE. If TRUE, will not look for existing file count table files.
force	logical, default TRUE If TRUE, reload library files even if matching named variables are found in environment used by experiment (see <a href="#">envExp</a> ) A simple way to make sure correct libraries are always loaded. FALSE is faster if data is loaded correctly already.
library.names	character, default: <code>bamVarName(df)</code> . Names to load libraries as to environment and names to display in plots.
libraries	The call to output libraries, the input is not used! Default: <code>outputLibs(df, chrStyle = seqinfo(df), paths = filepath(df, lib.type), type = lib.type, force = force, library.names = library.names, BPPARAM = BPPARAM)</code>
format	character, default "qs", alternative: "rds". Which format to save summarizedExperiment.
BPPARAM	how many cores/threads to use? default: <code>BiocParallel::SerialParam()</code>

## Details

If txdb or gtf path is added, it is a `rangedSummarizedExperiment` NOTE: If the file called `saveName` exists, it will then load file, not remake it!

There are different ways of counting hits on transcripts, ORFik does it as pure coverage (if a single read aligns to a region with 2 genes, both gets a count of 1 from that read). This is the safest way to avoid false negatives (genes with no assigned hits that actually have true hits).

## Value

a [SummarizedExperiment](#) object or `data.table` if "type" is not "count", with rownames as transcript / gene names.

## Examples

```
##Make experiment
df <- ORFik.template.experiment()
```

```

# makeSummarizedExperimentFromBam(df)
## Only cds (coding sequences):
# makeSummarizedExperimentFromBam(df, region = "cds")
## FPKM instead of raw counts on whole mrna regions
# makeSummarizedExperimentFromBam(df, type = "fpkm")
## Make count tables of pshifted libraries over uORFs
uorfs <- GRangesList(uorf1 = GRanges("chr23", 17599129:17599156, "-"))
#saveName <- file.path(dirname(df$filepath[1]), "uORFs", "countTable_uORFs")
#makeSummarizedExperimentFromBam(df, saveName, region = uorfs)
## To load the uORFs later
# countTable(df, region = "uORFs", count.folder = "uORFs")

```

---

makeSymbols

*Make Gene symbols from txdb*


---

## Description

Make Gene symbols from txdb

## Usage

```

makeSymbols(
  txdb,
  symbols_file_out_path = file.path(dirname(getGtfPathFromTxdb(txdb, stop.error = TRUE)),
    "gene_symbol_tx_table.fst"),
  uniprot_id = FALSE
)

```

## Arguments

txdb	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite) or an ORFik experiment
symbols_file_out_path	path to save, default file.path(dirname(getGtfPathFromTxdb(txdb, stop.error = TRUE)), "gene_symbol_tx_table.fst")
uniprot_id	logical default FALSE. If TRUE, will download and store all uniprot id for all transcripts (coding and noncoding)- In a file called: "gene_symbol_tx_table.fst" in same folder as txdb.

## Value

the data.table of tx\_ids, gene\_ids and gene symbols

---

makeTxdbFromGenome      *Make txdb from genome*

---

## Description

Make a Txdb with defined seqlevels and seqlevelsstyle from the fasta genome. This makes it more fail safe than standard Txdb creation. Example is that you can not create a coverage window outside the chromosome boundary, this is only possible if you have set the seqlengths.

## Usage

```
makeTxdbFromGenome(
  gtf,
  genome = NULL,
  organism,
  optimize = FALSE,
  gene_symbols = FALSE,
  uniprot_id = FALSE,
  pseudo_5UTRS_if_needed = NULL,
  minimum_5UTR_percentage = 30,
  return = is.null(txdb_file_out_path),
  txdb_file_out_path = paste0(gtf, ".db"),
  symbols_file_out_path = file.path(dirname(gtf), "gene_symbol_tx_table.fst")
)
```

## Arguments

gtf	path to gtf file
genome	character, default NULL. Path to fasta genome corresponding to the gtf. If NULL, can not set seqlevels. If value is NULL or FALSE, it will be ignored.
organism	Scientific name of organism, first letter must be capital! Example: Homo sapiens. Will force first letter to capital and convert any "_" (underscore) to " " (space)
optimize	logical, default FALSE. Create a folder within the output folder (defined by txdb_file_out_path), that includes optimized objects to speed up loading of annotation regions from up to 15 seconds on human genome down to 0.1 second. ORFik will then load these optimized objects instead. Currently optimizes filterTranscript() function and loadRegion() function for 5' UTRs, 3' UTRs, CDS, mRNA (all transcript with CDS) and tx (all transcripts).
gene_symbols	logical default FALSE. If TRUE, will download and store all gene symbols for all transcripts (coding and noncoding)- In a file called: "gene_symbol_tx_table.fst" in same folder as txdb. hgc for human, mouse symbols for mouse and rat, more to be added.
uniprot_id	logical default FALSE. If TRUE, will download and store all uniprot id for all transcripts (coding and noncoding)- In a file called: "gene_symbol_tx_table.fst" in same folder as txdb.

**pseudo\_5UTRS\_if\_needed**  
 integer, default NULL. If defined > 0, will add pseudo 5' UTRs of maximum this length if 'minimum\_5UTR\_percentage' (default 30 mRNAs (coding transcripts) do not have a leader. (NULL and 0 are both the ignore command)

**minimum\_5UTR\_percentage**  
 numeric, default 30. What minimum percentage of mRNAs most have a 5' UTRs (leaders), to not do the pseudo\_UTR addition. If percentage is higher, addition is ignored, set to 101 to always do it.

**return**  
 logical, default FALSE. If TRUE, return TXDB object, else invisible(NULL).

**txdb\_file\_out\_path**  
 character path, default paste0(gtf, ".db"). Set to NULL to not write file to disc.

**symbols\_file\_out\_path**  
 character path, default file.path(dirname(gtf), "gene\_symbol\_tx\_table.fst"). Must be defined as character if "gene\_symbols" is TRUE. Ignored if "gene\_symbols" is FALSE.

### Value

logical, default is.null(txdb\_file\_out\_path), Txdb saved to disc named default paste0(gtf, ".db"). Set 'return' argument to TRUE, to also get txdb back as an object.

### Examples

```

gtf <- "/path/to/local/annotation.gtf"
genome <- "/path/to/local/genome.fasta"
#makeTxdbFromGenome(gtf, genome, organism = "Saccharomyces cerevisiae")
# Runnable full example
df <- ORFik.template.experiment()
gtf <- sub("\\.db$", "", df@txdb)
genome <- df@fatile
txdb <- makeTxdbFromGenome(gtf, genome, organism = "Saccharomyces cerevisiae",
  txdb_file_out_path = NULL)
## Add pseudo UTRs if needed (< 30% of cds have a defined 5'UTR)

```

---

mergeFastq

*Merge groups of Fastq /Fasta files*

---

### Description

Will use multithreading to speed up process. Only works for Unix OS (Linux and Mac)

### Usage

```
mergeFastq(in_files_by_out_file_list, BPPARAM = bpparam())
```

**Arguments**

`in_files_by_out_file_list`  
list of character vectors, Per element of list are full path to the individual fastq.gz files to that output file.

`BPPARAM`  
how many cores/threads to use? default: `bpparam()`. To see number of threads used, do `bpparam()$workers`

**Value**

invisible(NULL).

**Examples**

```
# Make small example fastq files
fastq.folder <- tempdir() # <- Your fastq files
# Separate files into groups (here it is 4 output files from 12 input files)
in_files <- paste0(LETTERS[1:16], ".fastq.gz")
in_files <- file.path(fastq.folder, in_files)
samples_dna_letters <- vapply(seq_along(in_files), function(x)
  paste(sample(DNA_ALPHABET[1:4], 12, replace = TRUE), collapse = ""), character(1))
# Write example input files to temp
lapply(seq_along(in_files), function(i) {
  seq <- DNASTringSet(samples_dna_letters[i])
  names(seq) <- basename(in_files[i])
  writeXStringSet(seq, in_files[i])
})

out_files <- paste0(c("SSU_ribopool", "LSU_ribopool", "SSU_WT", "LSU_WT"), ".fastq.gz")
merged.fastq.folder <- file.path(fastq.folder, "merged/")
out_files <- file.path(merged.fastq.folder, out_files)

in_files_by_out_file_list <- split(in_files, rep(out_files, each = 4))
mergeFastq(in_files_by_out_file_list, BiocParallel::SerialParam())
lapply(out_files, readDNASTringSet)
```

---

mergeLibs

---

*Merge and save libraries of experiment*


---

**Description**

Aggregate count of reads (from the "score" column) by making a merged library. Only allowed for .ofst files!

**Usage**

```
mergeLibs(
  df,
  out_dir = file.path(libFolder(df), "ofst_merged"),
```

```

mode = "all",
type = "ofst",
keep_all_scores = TRUE,
paths = filepath(df, type),
lib_names_full = bamVarName(df, skip.libtype = FALSE),
max_splits = 20
)

```

## Arguments

<code>df</code>	an ORFik <a href="#">experiment</a>
<code>out_dir</code>	Output directory, default <code>file.path(dirname(df\$filepath[1]), "ofst_merged")</code> , saved as "all.ofst" in this folder if mode is "all". Use a folder called <code>pshifted_merged</code> , for default Ribo-seq ofst files.
<code>mode</code>	character, default "all". Merge all or "rep" for collapsing replicates only, or "lib" for collapsing all per library type.
<code>type</code>	<p>a character(default: "default"), load files in experiment or some precomputed variant, like "ofst" or "pshifted". These are made with <code>ORFik::convertLibs()</code>, <code>shiftFootprintsByExperiment()</code>, etc. Can also be custom user made folders inside the experiments bam folder. It acts in a recursive manner with priority: If you state "pshifted", but it does not exist, it checks "ofst". If no .ofst files, it uses "default", which always must exist.</p> <p>Presets are (folder is relative to default lib folder, some types fall back to other formats if folder does not exist):</p> <ul style="list-style-type: none"> <li>- "default": load the original files for experiment, usually bam.</li> <li>- "ofst": loads ofst files from the ofst folder, relative to lib folder (falls back to default)</li> <li>- "pshifted": loads ofst, wig or bigwig from pshifted folder (falls back to ofst, then default)</li> <li>- "cov": Load <code>covRle</code> objects from <code>cov_RLE</code> folder (fail if not found)</li> <li>- "covl": Load <code>covRleList</code> objects, from <code>cov_RLE_List</code> folder (fail if not found)</li> <li>- "bed": Load bed files, from bed folder (falls back to default)</li> <li>- Other formats must be loaded directly with <code>fimport</code></li> </ul>
<code>keep_all_scores</code>	logical, default TRUE, keep all library scores in the merged file. These score columns are named the libraries full name from <code>bamVarName(df)</code> .
<code>paths</code>	character vector, the filpaths to use, default <code>filepath(df, type, only_unique_mappers = only_unique_mappers)</code> . Change type argument if not what is wanted. If that is not enough, then you can also update this argument. But be careful about using this directly.
<code>lib_names_full</code>	character vector, default: <code>bamVarName(df, skip.libtype = FALSE)</code> . Name to assign to single libraries inside merged file, only kept if mode != "all"
<code>max_splits</code>	integer, default 20. If number of rows to merge > 2 <sup>31</sup> , how many times can you allow split merging to try to "rescue" the merging process?

**Value**

NULL, files saved to disc. A data.table with a score column that now contains the sum of scores per merge setting.

**Examples**

```
df2 <- ORFik.template.experiment()
df2 <- df2[df2$libtype == "RFP",]
# Merge all
mergeLibs(df2, tempdir(), mode = "all", type = "default")
# Read as GRanges with mcols
fimport(file.path(tempdir(), "all.ofst"))
# Only keep total score, Read as direct fst data.table
mergeLibs(df2, tempdir(), mode = "all", type = "default", keep_all_scores = FALSE)
fst::read_fst(file.path(tempdir(), "all.ofst"))
# Collapse replicates
mergeLibs(df2, tempdir(), mode = "rep", type = "default")
paths <- file.path(tempdir(), paste0("RFP_", c("Mutant", "WT"), ".ofst"))
lapply(paths, fimport)
# Collapse by lib types (same as "all" in this case)
#mergeLibs(df2, tempdir(), mode = "lib", type = "default")
```

---

metadata.autnaming      *Guess SRA metadata columns*

---

**Description**

Guess SRA metadata columns

**Usage**

```
metadata.autnaming(file)
```

**Arguments**

file                    a data.table of SRA metadata

**Value**

a data.table of SRA metadata with additional columns: LIBRARYTYPE, REPLICATE, STAGE, CONDITION, INHIBITOR

---

 metaWindow
 

---



---

*Calculate meta-coverage of reads around input GRanges/List object.*


---

### Description

Sums up coverage over set of GRanges objects as a meta representation.

### Usage

```
metaWindow(
  x,
  windows,
  scoring = "sum",
  withFrames = FALSE,
  zeroPosition = NULL,
  scaleTo = 100,
  fraction = NULL,
  feature = NULL,
  forceUniqueEven = !is.null(scoring),
  forceRescale = TRUE,
  weight = "score",
  drop.zero.dt = FALSE,
  append.zeroes = FALSE
)
```

### Arguments

x	GRanges/GAlignment object of your reads. Remember to resize them beforehand to width of 1 to focus on 5' ends of footprints etc, if that is wanted.
windows	GRangesList or GRanges of your ranges
scoring	a character, default: "sum", one of (zscore, transcriptNormalized, mean, median, sum, sumLength, NULL), see ?coverageScorings for info and more alternatives.
withFrames	a logical (TRUE), return positions with the 3 frames, relative to zeroPosition. zeroPosition is frame 0.
zeroPosition	an integer DEFAULT (NULL), the point if all windows are equal size, that should be set to position 0. Like leaders and cds combination, then 0 is the TIS and -1 is last base in leader. NOTE!: if not all windows have equal width, this will be ignored. If all have equal width and zeroPosition is NULL, it is set to as.integer(width / 2).
scaleTo	an integer (100), if windows have different size, a meta window can not directly be created, since a meta window must have equal size for all windows. Rescale (bin) all windows to scaleTo. i.e c(1,2,3) -> size 2 -> coverage of position c(1, mean(2,3)) etc.
fraction	a character/integer (NULL), the fraction i.e (27) for read length 27, or ("LSU") for large sub-unit TCP-seq.

feature	a character string, info on region. Usually either gene name, transcript part like cds, leader, or CpG motifs etc.
forceUniqueEven	a logical (TRUE), if TRUE; require that all windows are of same width and even. To avoid bugs. FALSE if score is NULL.
forceRescale	logical, default TRUE. If TRUE, if unique(widthPerGroup(windows)) has length > 1, it will force all windows to width of the scaleTo argument, making a binned meta coverage.
weight	(default: 'score'), if defined a character name of valid meta column in subject. GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGER CAGE files and many other package formats. You can also assign a score column manually.
drop.zero.dt	logical FALSE, if TRUE and as.data.table is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)
append zeroes	logical, default FALSE. If TRUE and drop.zero.dt is TRUE and all windows have equal length, it will add back 0 values after transformation. Sometimes needed for correct plots, if TRUE, will call abort if not all windows are equal length!

### Value

A data.table with scored counts (score) of reads mapped to positions (position) specified in windows along with frame (frame) per gene (genes) per library (fraction) per transcript region (feature). Column that does not apply is not given, but position and (score/count) is always returned.

### See Also

Other coverage: [coverageScorings\(\)](#), [regionPerReadLength\(\)](#), [scaledWindowPositions\(\)](#), [windowPerReadLength\(\)](#)

### Examples

```
library(GenomicRanges)
windows <- GRangesList(GRanges("chr1", IRanges(c(50, 100), c(80, 200)),
                              "-"))
x <- GenomicRanges::GRanges(
  seqnames = "chr1",
  ranges = IRanges::IRanges(c(100, 180), c(200, 300)),
  strand = "-")
metaWindow(x, windows, withFrames = FALSE)
```

`model.matrix,experiment-method`

*Get experiment design model matrix*

---

### Description

The function extends `stats::model.matrix`.

### Usage

```
## S4 method for signature 'experiment'  
model.matrix(object, design_formula = design(object, as.formula = TRUE))
```

### Arguments

`object` an ORFik [experiment](#)  
`design_formula` the experiment design, as formula, subset columns, to change the `model.matrix`,  
default: `design(object, as.formula = TRUE)`

### Value

a matrix with design and level attributes

### Examples

```
df <- ORFik.template.experiment()  
model.matrix(df) # Single factor, default  
model.matrix(df, design(df, as.formula = TRUE, multi.factor = TRUE))
```

---

`name`

*Get name of ORFik experiment*

---

### Description

Get name of ORFik experiment

### Usage

```
name(x)
```

### Arguments

`x` an ORFik [experiment](#)

### Value

character, name of experiment

---

name,experiment-method  
*Get name of ORFik experiment*

---

### Description

Get name of ORFik experiment

### Usage

```
## S4 method for signature 'experiment'  
name(x)
```

### Arguments

x                    an ORFik [experiment](#)

### Value

character, name of experiment

---

names,GRangesList-method  
*Get names of GRangesList*

---

### Description

Faster version than S4Vector generic caller

### Usage

```
## S4 method for signature 'GRangesList'  
names(x)
```

### Arguments

x                    a GRangesList

### Value

a character vector

---

names<- ,GRangesList-method  
*Get names of GRangesList*

---

**Description**

Faster version than S4Vector generic caller

**Usage**

```
## S4 replacement method for signature 'GRangesList'
names(x) <- value
```

**Arguments**

x	a GRangesList
value	character vector of names

**Value**

a GRangesList with updated names

---

nrow,experiment-method  
*Internal nrow function for ORFik experiment Number of runs in experiment*

---

**Description**

Internal nrow function for ORFik experiment Number of runs in experiment

**Usage**

```
## S4 method for signature 'experiment'
nrow(x)
```

**Arguments**

x	an ORFik <a href="#">experiment</a>
---	-------------------------------------

**Value**

number of rows in experiment (integer)

---

numExonsPerGroup	<i>Get list of the number of exons per group</i>
------------------	--

---

### Description

Can also be used generally to get number of GRanges object per GRangesList group

### Usage

```
numExonsPerGroup(gr1, keep.names = TRUE)
```

### Arguments

gr1            a [GRangesList](#)  
keep.names    a logical, keep names or not, default: (TRUE)

### Value

an integer vector of counts

### Examples

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),  
                  ranges = IRanges(c(7, 14), width = 3),  
                  strand = c("+", "+"))  
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),  
                    ranges = IRanges(c(4, 1), c(9, 3)),  
                    strand = c("-", "-"))  
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)  
numExonsPerGroup(gr1)
```

---

ofst_merge	<i>Merge multiple ofst file</i>
------------	---------------------------------

---

### Description

Collapses and sums the score column of each ofst file It is required that each file is of same ofst type. That is if one file has cigar information, all must have it.

**Usage**

```

ofst_merge(
  file_paths,
  lib_names = sub("\\.ofst$", "", basename(file_paths)),
  keep_all_scores = TRUE,
  keepCigar = TRUE,
  sort = TRUE,
  max_splits = 20L,
  dt_max_index_size = 2^31
)

```

**Arguments**

file_paths	Full path to .ofst files wanted to merge
lib_names	character, the name to give the resulting score columns. Default: sub(pattern = "\\.ofst\$", replacement = "", basename(file_paths))
keep_all_scores	logical, default TRUE, keep all library scores in the merged file. These score columns are named the libraries full name from bamVarName(df).
keepCigar	logical, default TRUE. If CIGAR is defined, keep column. Setting to FALSE compresses the file much more usually.
sort	logical, default TRUE. Sort the ranges. Will make the file smaller and faster to load, but some additional merging time is added.
max_splits	integer, default 20. If number of rows to merge > 2^31, how many times can you allow split merging to try to "rescue" the merging process?
dt_max_index_size	2^31, the number of rows data.table support, set lower to merge split on lower counts

**Value**

a data.table of merged result, it is merged on all columns except "score". The returned file will contain the scores of each file + the aggregate sum score.

---

optimizedTranscriptLengths

*Load length and names of all transcripts*

---

**Description**

A speedup wrapper around [transcriptLengths](#), default load time of lengths is ~ 15 seconds, if ORFik fst optimized lengths object has been made, load that file instead: load time reduced to ~ 0.1 second.

**Usage**

```

optimizedTranscriptLengths(
  txdb,
  with.utr5_len = TRUE,
  with.utr3_len = TRUE,
  create.fst.version = FALSE,
  optimized_path = optimized_txdb_path(txdb, stop.error = FALSE)
)

```

**Arguments**

txdb	a TxDb object, ORFik experiment object or a path to one of: (.gtf, .gff, .gff2, .gff2, .db or .sqlite), Only in the loadRegion function: if it is a GRangesList, it will return it self.
with.utr5_len	logical TRUE, include length of 5' UTRs, ignored if .fst exists
with.utr3_len	logical TRUE, include length of 3' UTRs, ignored if .fst exists
create.fst.version	logical, FALSE. If TRUE, creates a .fst version of the transcript length table (if it not already exists), reducing load time from ~ 15 seconds to ~ 0.01 second next time you run filterTranscripts with this txdb object. The file is stored in the same folder as the genome this txdb is created from, with the name: <code>paste0(ORFik::remove.file_ext(metadata(txdb)[3,2]), "_", gsub("\\(.*   : ", "", metadata(txdb)[metadata(txdb)[,1] == "Creation time", 2]), "_txLengths.fst")</code> Some error checks are done to see this is a valid location, if the txdb data source is a repository like UCSC and not a local folder, it will not be made.
optimized_path	character, path to optimized txdb objects, default: <code>optimized_txdb_path(txdb, stop.error = FALSE)</code> . If no existing file, will be slower and load lengths through <a href="#">transcriptLengths</a> .

**Value**

a data.table of loaded lengths 8 columns, 1 row per transcript isoform.

**Examples**

```

dt <- optimizedTranscriptLengths(ORFik.template.experiment())
dt
dt[cds_len > 0,] # All mRNA

```

---

optimizeTranscriptRegions

*Make optimized GRangesList objects saved to disc*

---

**Description**

Much faster to load

**Usage**

```
optimizeTranscriptRegions(
  txdb,
  base_path = optimized_txdb_path(txdb, create.dir = TRUE),
  regions = c("tx", "mrna", "leaders", "cds", "trailers", "ncRNA")
)
```

**Arguments**

txdb	a TxDb object, ORFik experiment object or a path to one of: (.gtf, .gff, .gff2, .db or .sqlite), Only in the loadRegion function: if it is a GRangesList, it will return it self.
base_path	Directory and file prefix for files, will append "_region.qs", where region is specific region.
regions	character, default: c("tx", "mrna", "leaders", "cds", "trailers", "ncRNA"). Valid options specified by loadRegion.

**Value**

invisible(NULL)

---

orfFrameDistributions *Find shifted Ribo-seq frame distributions*

---

**Description**

Per library: get coverage over CDS per frame per readlength Return as data.dataable with information and best frame found. Can be used to automatize re-shifting of read lengths (find read lengths where frame 0 is not the best frame over the entire cds)

**Usage**

```
orfFrameDistributions(
  df,
  type = "pshifted",
  weight = "score",
  orfs = loadRegion(df, part = "cds"),
  libraries = outputLibs(df, type = type, output.mode = "envirlist"),
  BPPARAM = BiocParallel::bpparam()
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
type	type of library loaded, default pshifted, warning if not pshifted might crash if too many read lengths!

weight	which column in reads describe duplicates, default "score".
orfs	GRangesList, default loadRegion(df, part = "cds")
libraries	a list of loaded libraries, default: outputLibs(df, type = type, output.mode = "envirlist")
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

**Value**

data.table with columns: fraction (library) frame (0, 1, 2) score (coverage) length (read length) percent (coverage percentage of library) percent\_length (coverage percentage of library and length) best\_frame (TRUE/FALSE, is this the best frame per length)

**Examples**

```
df <- ORFik.template.experiment()[9,]
dt <- orfFrameDistributions(df, BPPARAM = BiocParallel::SerialParam())
## Check that frame 0 is best frame for all
all(dt[frame == 0,]$best_frame)
```

---

ORFik.template.experiment

*An ORFik experiment to see how it looks*

---

**Description**

Toy-data created to resemble human genes:  
 Number of genes: 6  
 Genome size: 1161nt x 6 chromosomes = 6966 nt  
 Experimental design (2 replicates, Wild type vs Mutant)  
 CAGE: 4 libraries  
 PAS (poly-A): 4 libraries  
 Ribo-seq: 4 libraries  
 RNA-seq: 4 libraries

**Usage**

```
ORFik.template.experiment(as.temp = FALSE)
```

**Arguments**

as.temp logical, default FALSE, load as ORFik experiment. If TRUE, loads as data.frame template of the experiment.

**Value**

an ORFik [experiment](#)

**See Also**

Other ORFik\_experiment: [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

**Examples**

```
ORFik.template.experiment()
```

---

```
ORFik.template.experiment.zf
```

*An ORFik experiment to see how it looks*

---

**Description**

Toy-data created to resemble Zebrafish genes:

Number of genes: 150

Ribo-seq: 1 library

**Usage**

```
ORFik.template.experiment.zf(as.temp = FALSE)
```

**Arguments**

`as.temp` logical, default FALSE, load as ORFik experiment. If TRUE, loads as data.frame template of the experiment.

**Value**

an ORFik [experiment](#)

**See Also**

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

**Examples**

```
ORFik.template.experiment.zf()
```

**Description**

The ORFik QC uses the aligned files (usually bam files), fastp and STAR log files combined with annotation to create relevant statistics.

This report consists of several steps:

1. Convert bam file / Input files to ".ofst" format, if not already done. This format is around 400x faster to use in R than the bam format. Files are also outputted to R environment specified by `envExp(df)`
2. From this report you will get a summary csv table, with distribution of aligned reads and overlap counts over transcript regions like: leader, cds, trailer, lincRNAs, tRNAs, rRNAs, snoRNAs etc. It will be called `STATS.csv`. And can be imported with `QCstats` function.
3. It will also make correlation plots and meta coverage plots, so you get a good understanding of how good the quality of your NGS data production + aligner step were.
4. Count tables are produced, similar to HTseq count tables. Over mrna, leader, cds and trailer separately. This tables are stored as `SummarizedExperiment`, for easy loading into DEseq, conversion to normalized fpkm values, or collapsing replicates in an experiment. And can be imported with `countTable` function.

Everything will be outputted in the directory of your NGS data, inside the folder `./QC_STATS/`, relative to data location in 'df'. You can specify new out location with `out.dir` if you want.

To make a ORFik experiment, see `?ORFik::experiment`

To see some normal mrna coverage profiles of different RNA-seq protocols: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4310221/figure/F6/>

**Usage**

```
ORFikQC(
  df,
  out.dir = resFolder(df),
  plot.ext = ".pdf",
  create.ofst = TRUE,
  force.remake.count.tables = FALSE,
  complex.correlation.plots = TRUE,
  library.names = bamVarName(df),
  use_simplified_reads = TRUE,
  BPPARAM = bpparam()
)
```

**Arguments**

<code>df</code>	an ORFik <a href="#">experiment</a>
<code>out.dir</code>	character, output directory, default: <code>resFolder(df)</code> . Will make a folder within this called "QC_STATS" with all results in this directory. Warning: If you assign

	not default path, you will have a hazzle to load files later. Much easier to load count tables, statistics, ++ later with default. Update resFolder of df instead if needed.
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg". Note that in pdf format the complex correlation plots become very slow to load!
create.ofst	logical, default TRUE. Create ".ofst" files from the input libraries, ofst is much faster to load in R, for later use. Stored in ./ofst/ folder relative to experiment main folder.
force.remake.count.tables	logical, default FALSE. If TRUE and count tables already exists, delete and make new ones. Useful if you altered input libraries.
complex.correlation.plots	logical, default TRUE. Add in addition to simple correlation plot two computationally heavy dots + correlation plots. Useful for deeper analysis, but takes longer time to run, especially on low-quality gpu computers. Set to FALSE to skip these.
library.names	character, default: bamVarName(df). Names to load libraries as to environment and names to display in plots.
use_simplified_reads	logical, default TRUE. For count tables and coverage plots a speed up for GAlignments is to use 5' ends only. This will lose some detail for splice sites, but is usually irrelevant. Note: If reads are precollapsed GRanges, set to FALSE to avoid recollapsing.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

### Value

invisible(NULL) (objects are stored to disc)

### See Also

Other QC report: [QCplots\(\)](#), [QCstats\(\)](#)

### Examples

```
# Load an experiment
df <- ORFik.template.experiment()
# Run QC
#QCreport(df, tempdir())
# QC on subset
#QCreport(df[9,], tempdir())
```

---

orfScore                      *Get ORFscore for a GRangesList of ORFs*

---

### Description

ORFscore tries to check whether the first frame of the 3 possible frames in an ORF has more reads than second and third frame. IMPORTANT: Only use p-shifted libraries, see ([detectRibosomeShifts](#)). Else this score makes no sense.

### Usage

```
orfScore(
  grl,
  RFP,
  is.sorted = FALSE,
  weight = "score",
  overlapGr1 = NULL,
  coverage = NULL,
  stop3 = TRUE
)
```

### Arguments

grl	a <a href="#">GRangesList</a> of 5' utrs, CDS, transcripts, etc.
RFP	ribosomal footprints, given as <a href="#">Alignments</a> or <a href="#">GRanges</a> object, must be already shifted and resized to the p-site. Requires a \$size column with original read lengths.
is.sorted	logical (FALSE), is grl sorted. That is + strand groups in increasing ranges (1,2,3), and - strand groups in decreasing ranges (3,2,1)
weight	(default: 'score'), if defined a character name of valid meta column in subject. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGEr CAGE files and many other package formats. You can also assign a score column manually.
overlapGr1	an integer, (default: NULL), if defined must be <code>countOverlaps(grl, RFP)</code> , added for speed if you already have it.
coverage	a data.table from <code>coveragePerTiling</code> of length same as 'grl' argument. Save time if you have already computed it.
stop3	logical, default TRUE. Stop if any input is of width < 3.

### Details

Pseudocode: assume rff - is reads fraction in specific frame

$$\text{ORFscore} = \log(\text{rff1} + \text{rff2} + \text{rff3})$$

If rff2 or rff3 is bigger than rff1, negate the resulting value.

```
ORFScore[rff1Smaller] <- ORFScore[rff1Smaller] * -1
```

As result there is one value per ORF: - Positive values say that the first frame have the most reads, - zero values means it is uniform: (ORFScore between -2.5 and 2.5 can be considered close to uniform), - negative values say that the first frame does not have the most reads. NOTE non-shifted reads: If reads are not of width 1, then a read from 1-4 on range of 1-4, will get scores frame1 = 2, frame2 = 1, frame3 = 1. What could be logical is that only the 5' end is important, so that only frame1 = 1, to get this, you first resize reads to 5'end only.

General NOTES: 1. p shifting is not exact, so some functional ORFs will get a bad ORF score. 2. If a score column is defined, it will use it as weights, set to weight = 1L if you don't have weight, and score column is something else. 3. If needed a test for significance and critical values, use chi-squared. There are 3 degrees of freedom (3 frames), so critical 0.05 (3-1 degrees of freedom = 2), value is:  $\log_2(6) = 2.58$  see [getWeights](#)

## Value

a data.table with 4 columns, the orfscore (ORFScores) and score of each of the 3 tiles (frame\_zero\_RP, frame\_one\_RP, frame\_two\_RP)

## References

doi: 10.1002/emj.201488411

## See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

## Examples

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
names(ORF) <- c("tx1", "tx1", "tx1")
gr1 <- GRangesList(tx1_1 = ORF)
RFP <- GRanges("1", IRanges(25, 25), "+") # 1 width position based
score(RFP) <- 28 # original width
orfScore(gr1, RFP) # negative because more hits on frames 1,2 than 0.

# example with positive result, more hits on frame 0 (in frame of ORF)
RFP <- GRanges("1", IRanges(c(1, 1, 1, 25), width = 1), "+")
score(RFP) <- c(28, 29, 31, 28) # original width
orfScore(gr1, RFP)
```

---

organism,experiment-method

*Get ORFik experiment organism*

---

## Description

If not defined directly, checks the txdb / gtf organism information, if existing.

## Usage

```
## S4 method for signature 'experiment'  
organism(object)
```

## Arguments

object            an ORFik [experiment](#)

## Value

character, name of organism

## See Also

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

## Examples

```
# if you have set organism in txdb of ORFik experiment:  
df <- ORFik.template.experiment()  
organism(df)  
  
#' If you have not set the organism you can do:  
#gtf <- "pat/to/gff_or_gff"  
#txdb_path <- paste0(gtf, ".db") # This file is created in next step  
#txdb <- makeTxdbFromGenome(gtf, genome, organism = "Homo sapiens",  
# optimize = TRUE, return = TRUE)  
# then use this txdb in you ORFik experiment and load:  
# create.experiment(exper = "new_experiment",  
# txdb = txdb_path) ...  
# organism(read.experiment("new-experiment"))
```

---

 outputLibs
 

---



---

*Output NGS libraries to R as variables*


---

### Description

By default loads the original files of the experiment into the global environment, named by the rows of the experiment required to make all libraries have unique names.

Uses multiple cores to load, defined by multicoreParam

### Usage

```
outputLibs(
  df,
  type = "default",
  paths = filepath(df, type, only_unique_mappers = only_unique_mappers),
  param = NULL,
  strandMode = 0,
  naming = "minimum",
  library.names = name_decider(df, naming),
  output.mode = "envir",
  chrStyle = NULL,
  envir = envExp(df),
  verbose = TRUE,
  force = TRUE,
  validate_libs = TRUE,
  only_unique_mappers = uniqueMappers(df),
  BPPARAM = bpparam()
)
```

### Arguments

df	an ORFik <a href="#">experiment</a>
type	<p>a character(default: "default"), load files in experiment or some precomputed variant, like "ofst" or "pshifted". These are made with ORFik:::convertLibs(), shiftFootprintsByExperiment(), etc. Can also be custom user made folders inside the experiments bam folder. It acts in a recursive manner with priority: If you state "pshifted", but it does not exist, it checks "ofst". If no .ofst files, it uses "default", which always must exist.</p> <p>Presets are (folder is relative to default lib folder, some types fall back to other formats if folder does not exist):</p> <ul style="list-style-type: none"> <li>- "default": load the original files for experiment, usually bam.</li> <li>- "ofst": loads ofst files from the ofst folder, relative to lib folder (falls back to default)</li> <li>- "pshifted": loads ofst, wig or bigwig from pshifted folder (falls back to ofst, then default)</li> <li>- "cov": Load covRle objects from cov_RLE folder (fail if not found)</li> <li>- "covl": Load covRleList objects, from cov_RLE_List folder (fail if not found)</li> </ul>

	<ul style="list-style-type: none"> <li>- "bed": Load bed files, from bed folder (falls back to default)</li> <li>- Other formats must be loaded directly with fimport</li> </ul>
paths	character vector, the filpaths to use, default filepath(df, type, only_unique_mappers = only_unique_mappers). Change type argument if not what is wanted. If that is not enough, then you can also update this argument. But be careful about using this directly.
param	<p>NULL or a <a href="#">ScanBamParam</a> object. Like for <a href="#">scanBam</a>, this influences what fields and which records are imported. However, note that the fields specified thru this <a href="#">ScanBamParam</a> object will be loaded <i>in addition</i> to any field required for generating the returned object (<a href="#">GAlignments</a>, <a href="#">GAlignmentPairs</a>, or <a href="#">GappedReads</a> object), but only the fields requested by the user will actually be kept as meta-data columns of the object.</p> <p>By default (i.e. param=NULL or param=ScanBamParam()), no additional field is loaded. The flag used is scanBamFlag(isUnmappedQuery=FALSE) for readGAlignments, readGAlignmentsList, and readGappedReads. (i.e. only records corresponding to mapped reads are loaded), and scanBamFlag(isUnmappedQuery=FALSE, isPaired=TRUE, hasUnmappedMate=FALSE) for readGAlignmentPairs (i.e. only records corresponding to paired-end reads with both ends mapped are loaded).</p>
strandMode	numeric, default 0. Only used for paired end bam files. One of (0: strand = *, 1: first read of pair is +, 2: first read of pair is -). See ?strandMode. Note: Sets default to 0 instead of 1, as readGAlignmentPairs uses 1. This is to guarantee hits, but will also make mismatches of overlapping transcripts in opposite directions.
naming	a character (default: "minimum"). Name files as minimum information needed to make all files unique. Set to "full" to get full names. Set to "fullexp", to get full name with experiment name as prefix, the last one guarantees uniqueness.
library.names	character vector, names of libraries, default: name_decider(df, naming)
output.mode	character, default "envir". Output libraries to environment. Alternative: "list", return as list. "envirlist", output to envir and return as list. If output is list format, the list elements are named from: bamVarName(df.rfp) (Full or minimum naming based on 'naming' argument)
chrStyle	a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -> pick "NCBI"
envir	environment to save to, default envExp(df), which defaults to .GlobalEnv, but can be set with envExp(df) <- new.env() etc.
verbose	logical, default TRUE, message about library output status.
force	logical, default TRUE If TRUE, reload library files even if matching named variables are found in environment used by experiment (see <a href="#">envExp</a> ) A simple way to make sure correct libraries are always loaded. FALSE is faster if data is loaded correctly already.
validate_libs	logical, default TRUE. If FALSE, don't check that default files exists (i.e. bam files), useful if you are using pshifted ofst etc and don't have the bams anymore.

only_unique_mappers	logical, default uniqueMappers(df). Load file of only unique format type, located in './unique_mappers' relative to bam files / default files. See ?uniqueMappers for more information.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

### Details

The functions checks if the total set of libraries have already been loaded: i.e. Check if all names from 'library.names' exists as S4 objects in environment of experiment.

### Value

NULL (libraries set by envir assignment), unless output.mode is "list" or "envirlist": Then you get a list of the libraries. The library objects will have 3 additional attributes, "exp", "filepath" and "short\_name".

### See Also

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [read.experiment\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

### Examples

```
## Load a template ORFik experiment
df <- ORFik.template.experiment()
## Default library type load, usually bam files
outputLibs(df, type = "default")
RFP_WT_r1
attr(RFP_WT_r1, "filepath")
attr(RFP_WT_r1, "exp")
## .ofst file load, if ofst files does not exists
## it will load default
# outputLibs(df, type = "ofst")
## .wig file load, if wiggle files does not exists
## it will load default
# outputLibs(df, type = "wig")
## Load as list
outputLibs(df, output.mode = "list")
## Load libs to new environment (called ORFik in Global)
# outputLibs(df, envir = assign(name(df), new.env(parent = .GlobalEnv)))
## Load to hidden environment given by experiment
# envExp(df) <- new.env()
# outputLibs(df)
```

pcaExperiment

*Simple PCA analysis from ORFik experiment***Description**

Detect outlier libraries with PCA analysis. Will output PCA plot of PCA component 1 (x-axis) vs PCA component 2 (y-axis) for each library (colored by library), shape by replicate. Will be extended to allow batch correction in the future.

**Usage**

```
pcaExperiment(
  df,
  output.dir = NULL,
  table = countTable(df, "cds", type = "fpkm"),
  title = "PCA analysis by CDS fpkm",
  subtitle = paste("Numer of genes/regions:", nrow(table)),
  plot.ext = ".pdf",
  return.data = FALSE,
  color.by.group = TRUE,
  PCA_X = "PC1",
  PCA_Y = "PC2"
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
output.dir	default NULL, else character path to directory. File saved as "PCAplot_(experiment name)(plot.ext)"
table	data.table, e.g. countTable(df, "cds", type = "fpkm"), a data.table of counts per column (default normalized fpkm values).
title	character, default "CDS fpkm".
subtitle	character, default: paste("Numer of genes:", nrow(table))
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg".
return.data	logical, default FALSE. Return data instead of plot
color.by.group	logical, default TRUE. Colors in PCA plot represent unique library groups, if FALSE. Color each sample in seperate color (harder to distinguish for > 10 samples)
PCA_X	name of principle component to use for x axis: valid options: PC1-PC6
PCA_Y	name of principle component to use for y axis: valid options: PC1-PC6

**Value**

ggplot if return.data is false, data.table of PCAs if return.data is TRUE, if data has < 3 samples, returns (invisible(NULL))

**Examples**

```
df <- ORFik.template.experiment()
# Select only Ribo-seq and RNA-seq
pcaExperiment(df[df$libtype %in% c("RNA", "RFP"),])
```

pcaPlot

*Simple PCA analysis from table***Description**

Detect outlier libraries with PCA analysis. Will output PCA plot of PCA component 1 (x-axis) vs PCA component 2 (y-axis) for each library (colored by library), shape by replicate.

**Usage**

```
pcaPlot(
  table,
  path = NULL,
  group = sub("_r[0-9]+$", "", colnames(table)),
  replicate = sub(".*_r([0-9]+)$", "\\1", colnames(table)),
  PCA_X = "PC1",
  PCA_Y = "PC2",
  title = "PCA analysis by CDS fpkm",
  subtitle = paste("Numer of genes/regions:", nrow(table)),
  plot.ext = ".pdf",
  return.data = FALSE
)
```

**Arguments**

table	data.table, e.g. countTable(df, "cds", type = "fpkm"), a data.table of counts per column (default normalized fpkm values).
path	default NULL, else character path to file to save. File saved as "PCAplot_(experiment name)(plot.ext)"
group	character vector of equal size to nrow of dt, default group = sub("_r[0-9]+\$", "", colnames(table))
replicate	haracter vector of equal size to nrow of dt, sub(".*_r([0-9]+)\$", "\\1", colnames(table))
PCA_X	name of principle component to use for x axis: valid options: PC1-PC6
PCA_Y	name of principle component to use for y axis: valid options: PC1-PC6
title	character, default "CDS fpkm".
subtitle	character, default: paste("Numer of genes:", nrow(table))
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg".
return.data	logical, default FALSE. Return data instead of plot

**Value**

ggplot or invisible(NULL) if output.dir is defined or < 3 samples. Returns data.table with PCA analysis if return.data is TRUE.

**Examples**

```
df <- ORFik.template.experiment()
# Select only Ribo-seq and RNA-seq
df <- df[df$libtype %in% c("RNA", "RFP"),]
table <- countTable(df, "cds", type = "fpkm")
pcaPlot(table)
```

---

pmapFromTranscriptF *Faster pmapFromTranscript*

---

**Description**

Map range coordinates between features in the transcriptome and genome (reference) space. The length of x must be the same as length of transcripts. Only exception is if x have integer names like (1, 3, 3, 5), so that x[1] maps to 1, x[2] maps to transcript 3 etc.

**Usage**

```
pmapFromTranscriptF(x, transcripts, removeEmpty = FALSE)
```

**Arguments**

x	IRangesList/IRanges/GRanges to map to genomic coordinates
transcripts	a GRangesList to map against (the genomic coordinates)
removeEmpty	a logical, remove non hit exons, else they are set to 0. That is all exons in the reference that the transcript coordinates do not span.

**Details**

This version tries to fix the shortcomings of GenomicFeature's version. Much faster and uses less memory. Implemented as dynamic program optimized c++ code.

**Value**

a GRangesList of mapped reads, names from ranges are kept.

**Examples**

```

ranges <- IRanges(start = c( 5, 6), end = c(10, 10))
seqnames = rep("chr1", 2)
strands = rep("-", 2)
gr1 <- split(GRanges(seqnames, IRanges(c(85, 70), c(89, 82))), strands),
            c(1, 1))
ranges <- split(ranges, c(1,1)) # both should be mapped to transcript 1
pmapFromTranscriptF(ranges, gr1, TRUE)

```

---

pmapToTranscriptF      *Faster pmapToTranscript*

---

**Description**

Map range coordinates between features in the transcriptome and genome (reference) space. The length of x must be the same as length of transcripts. Only exception is if x have integer names like (1, 3, 3, 5), so that x[1] maps to 1, x[2] maps to transcript 3 etc.

**Usage**

```

pmapToTranscriptF(
  x,
  transcripts,
  ignore.strand = FALSE,
  x.is.sorted = TRUE,
  tx.is.sorted = TRUE
)

```

**Arguments**

x	GRangesList/GRanges/IRangesList/IRanges to map to transcriptomic coordinates
transcripts	a GRangesList/GRanges/IRangesList/IRanges to map against (the genomic coordinates). Must be of lower abstraction level than x. So if x is GRanges, transcripts can not be IRanges etc.
ignore.strand	When ignore.strand is TRUE, strand is ignored in overlaps operations (i.e., all strands are considered "+") and the strand in the output is '*'. When ignore.strand is FALSE (default) strand in the output is taken from the transcripts argument. When transcripts is a GRangesList, all inner list elements of a common list element must have the same strand or an error is thrown. Mapped position is computed by counting from the transcription start site (TSS) and is not affected by the value of ignore.strand.
x.is.sorted	if x is a GRangesList object, are "-" strand groups pre-sorted in decreasing order within group, default: TRUE
tx.is.sorted	if transcripts is a GRangesList object, are "-" strand groups pre-sorted in decreasing order within group, default: TRUE

**Details**

This version tries to fix the shortcomings of GenomicFeature's version. Much faster and uses less memory. Implemented as dynamic program optimized c++ code.

**Value**

object of same class as input x, names from ranges are kept.

**Examples**

```
library(GenomicFeatures)
# Need 2 ranges object, the target region and whole transcript
# x is target region
x <- GRanges("chr1", IRanges(start = c(26, 29), end = c(27, 29)), "+")
names(x) <- rep("tx1_ORF1", length(x))
x <- groupGRangesBy(x)
# tx is the whole region
tx_gr <- GRanges("chr1", IRanges(c(5, 29), c(27, 30)), "+")
names(tx_gr) <- rep("tx1", length(tx_gr))
tx <- groupGRangesBy(tx_gr)
pmapToTranscriptF(x, tx)
pmapToTranscripts(x, tx)

# Reuse names for matching
x <- GRanges("chr1", IRanges(start = c(26, 29, 5), end = c(27, 29, 18)), "+")
names(x) <- c(rep("tx1_1", 2), "tx1_2")
x <- groupGRangesBy(x)
tx1_2 <- GRanges("chr1", IRanges(c(4, 28), c(26, 31)), "+")
names(tx1_2) <- rep("tx1", 2)
tx <- c(tx, groupGRangesBy(tx1_2))

a <- pmapToTranscriptF(x, tx[txNames(x)])
b <- pmapToTranscripts(x, tx[txNames(x)])
identical(a, b)
seqinfo(a)
# A note here, a & b only have 1 seqlength, even though the 2 "tx1"
# are different in size. This is an artifact of using duplicated names.

## Also look at the asTx for a similar useful function.
```

---

pseudoIntronsPerGroup *Get pseudo introns per Group*

---

**Description**

If an intron is of length < 'width' \* 2, it will not be split into pseudo.

**Usage**

```
pseudoIntronsPerGroup(gr1, width = 100)
```

**Arguments**

gr1                    a GRangesList of length 1  
width                  numeric, default 100. The size of pseudo flanks.

**Value**

a GRangesList

**Examples**

```
tx <- GRangesList(GRanges("1", IRanges(c(1, 150, 1e5, 1e6)), "+"))
pseudoIntronsPerGroup(tx) # See intron 1 is not split
tx_2 <- rep(GRangesList(GRanges("1", IRanges(c(1, 150, 1e5, 1e6)), "+")), 2)
pseudoIntronsPerGroup(tx_2)
pseudoIntronsPerGroup(tx_2, 1e6)
```

---

pSitePlot

*Plot area around TIS as histogram*


---

**Description**

Usefull to validate p-shifting is correct Can be used for any coverage of region around a point, like TIS, TSS, stop site etc.

**Usage**

```
pSitePlot(
  hitMap,
  length = unique(hitMap$fraction),
  region = "start",
  output = NULL,
  type = "canonical CDS",
  scoring = "Averaged counts",
  forHeatmap = FALSE,
  title = "auto",
  facet = FALSE,
  frameSum = FALSE
)
```

**Arguments**

hitMap                a data.frame/data.table, given from metaWindow (must have columns: position, (score or count) and frame)  
length                an integer (29), which read length is this for?  
region                a character (start), either "start or "stop"

output	character (NULL), if set, saves the plot as pdf or png to path given. If no format is given, is save as pdf.
type	character (canonical CDS), type for plot
scoring	character, default: (Averaged counts), which scoring did you use ? see ?coverageScorings for info and more alternatives.
forHeatmap	a logical (FALSE), should the plot be part of a heatmap? It will scale it differently. Removing title, x and y labels, and truncate spaces between bars.
title	character, title of plot. Default "auto", will make it: paste("Length", length, "over", region, "of", type). Else set your own (set to NULL to remove all together).
facet	logical, default FALSE. If you input multiple read lengths, specified by fraction column of hitMap, it will split the plots for each read length, putting them under each other. Ignored if forHeatmap is TRUE.
frameSum	logical default FALSE. If TRUE, add an addition plot to the right, sum per frame over all positions per length.

## Details

The region is represented as a histogram with different colors for the 3 frames. To make it easy to see patterns in the reads. Remember if you want to change anything like colors, just return the ggplot object, and reassign like: obj + scale\_color\_brewer() etc.

## Value

a ggplot object of the coverage plot, NULL if output is set, then the plot will only be saved to location.

## See Also

Other coveragePlot: [coverageHeatMap\(\)](#), [savePlot\(\)](#), [windowCoveragePlot\(\)](#)

## Examples

```
# An ORF
gr1 <- GRangesList(tx1 = GRanges("1", IRanges(1, 6), "+"))
# Ribo-seq reads
range <- IRanges(c(rep(1, 3), 2, 3, rep(4, 2), 5, 6), width = 1 )
reads <- GRanges("1", range, "+")
coverage <- coveragePerTiling(gr1, reads, TRUE, as.data.table = TRUE,
                             withFrames = TRUE)

pSitePlot(coverage)
pSitePlot(coverage, frameSum = TRUE)
# See vignette for more examples
```

---

QCfolder

*Get path to ORFik experiment QC folder*

---

**Description**

Get path to ORFik experiment QC folder

**Usage**

QCfolder(x)

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

---

QCfolder,experiment-method

*Get path to ORFik experiment QC folder*

---

**Description**

Get path to ORFik experiment QC folder

**Usage**

```
## S4 method for signature 'experiment'  
QCfolder(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

## Description

The ORFik QC uses the aligned files (usually bam files), fastp and STAR log files combined with annotation to create relevant statistics.

This report consists of several steps:

1. Convert bam file / Input files to ".ofst" format, if not already done. This format is around 400x faster to use in R than the bam format. Files are also outputted to R environment specified by `envExp(df)`
2. From this report you will get a summary csv table, with distribution of aligned reads and overlap counts over transcript regions like: leader, cds, trailer, lincRNAs, tRNAs, rRNAs, snoRNAs etc. It will be called `STATS.csv`. And can be imported with `QCstats` function.
3. It will also make correlation plots and meta coverage plots, so you get a good understanding of how good the quality of your NGS data production + aligner step were.
4. Count tables are produced, similar to HTseq count tables. Over mrna, leader, cds and trailer separately. This tables are stored as `SummarizedExperiment`, for easy loading into DEseq, conversion to normalized fpkm values, or collapsing replicates in an experiment. And can be imported with `countTable` function.

Everything will be outputted in the directory of your NGS data, inside the folder `./QC_STATS/`, relative to data location in 'df'. You can specify new out location with `out.dir` if you want.

To make a ORFik experiment, see `?ORFik::experiment`

To see some normal mrna coverage profiles of different RNA-seq protocols: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4310221/figure/F6/>

## Usage

```
QCreport(  
  df,  
  out.dir = resFolder(df),  
  plot.ext = ".pdf",  
  create.ofst = TRUE,  
  force.remake.count.tables = FALSE,  
  complex.correlation.plots = TRUE,  
  library.names = bamVarName(df),  
  use_simplified_reads = TRUE,  
  BPPARAM = bpparam()  
)
```

## Arguments

<code>df</code>	an ORFik <a href="#">experiment</a>
<code>out.dir</code>	character, output directory, default: <code>resFolder(df)</code> . Will make a folder within this called "QC_STATS" with all results in this directory. Warning: If you assign

	not default path, you will have a hazzle to load files later. Much easier to load count tables, statistics, ++ later with default. Update resFolder of df instead if needed.
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg". Note that in pdf format the complex correlation plots become very slow to load!
create.ofst	logical, default TRUE. Create ".ofst" files from the input libraries, ofst is much faster to load in R, for later use. Stored in ./ofst/ folder relative to experiment main folder.
force.remake.count.tables	logical, default FALSE. If TRUE and count tables already exists, delete and make new ones. Useful if you altered input libraries.
complex.correlation.plots	logical, default TRUE. Add in addition to simple correlation plot two computationally heavy dots + correlation plots. Useful for deeper analysis, but takes longer time to run, especially on low-quality gpu computers. Set to FALSE to skip these.
library.names	character, default: bamVarName(df). Names to load libraries as to environment and names to display in plots.
use_simplified_reads	logical, default TRUE. For count tables and coverage plots a speed up for GAlignments is to use 5' ends only. This will lose some detail for splice sites, but is usually irrelevant. Note: If reads are precollapsed GRanges, set to FALSE to avoid recollapsing.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

### Value

invisible(NULL) (objects are stored to disc)

### See Also

Other QC report: [QCplots\(\)](#), [QCstats\(\)](#)

### Examples

```
# Load an experiment
df <- ORFik.template.experiment()
# Run QC
#QCreport(df, tempdir())
# QC on subset
#QCreport(df[9,], tempdir())
```

---

QCstats	<i>Load ORFik QC Statistics report</i>
---------	--

---

**Description**

Loads the pre / post alignment statistics made in ORFik.

**Usage**

```
QCstats(df, path = file.path(QCfolder(df), "STATS.csv"))
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
path	path to QC statistics report, default: <code>file.path(dirname(df\$filepath[1]), "/QC_STATS/STATS.csv")</code>

**Details**

The ORFik QC uses the aligned files (usually bam files), fastp and STAR log files combined with annotation to create relevant statistics.

**Value**

data.table of QC report or NULL if not exists

**See Also**

Other QC report: [QCplots\(\)](#), [QCreport\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
## First make QC report
# QCreport(df)
# stats <- QCstats(df)
```

---

QCstats.plot	<i>Make plot of ORFik QCreport</i>
--------------	------------------------------------

---

**Description**

From post-alignment QC relative to annotation, make a plot for all samples. Will contain among others read lengths, reads overlapping leaders, cds, trailers, mRNA / rRNA etc.

**Usage**

```
QCstats.plot(stats, output.dir = NULL, plot.ext = ".pdf", as_gg_list = FALSE)
```

**Arguments**

<code>stats</code>	the experiment object or path to custom ORFik QC folder where a file called "STATS.csv" is located.
<code>output.dir</code>	NULL or character path, default: NULL, plot not saved to disc. If defined saves plot to that directory with the name "/STATS_plot.pdf".
<code>plot.ext</code>	character, default: ".pdf". Alternatives: ".png" or ".jpg".
<code>as_gg_list</code>	logical, default FALSE. Return as a list of ggplot objects instead of as a grob. Gives you the ability to modify plots more directly.

**Value**

the plot object, a grob of ggplot objects of the the statistics data

**Examples**

```
df <- ORFik.template.experiment()[3,]
## First make QC report
# QCreport(df)
## Now you can get plot
# QCstats.plot(df)
```

---

r

*strandMode covRle*


---

**Description**

`strandMode covRle`

**Usage**

`r(x)`

**Arguments**

`x` a `covRle` object

**Value**

the forward `RleList`

---

r,covRle-method	<i>strandMode covRle</i>
-----------------	--------------------------

---

**Description**

strandMode covRle

**Usage**

```
## S4 method for signature 'covRle'  
r(x)
```

**Arguments**

x                    a covRle object

**Value**

the forward RleList

---

rankOrder	<i>ORF rank in transcripts</i>
-----------	--------------------------------

---

**Description**

Creates an ordering of ORFs per transcript, so that ORF with the most upstream start codon is 1, second most upstream start codon is 2, etc. Must input a grl made from ORFik, txNames\_2 -> 2.

**Usage**

```
rankOrder(grl)
```

**Arguments**

grl                    a [GRangesList](#) object with ORFs

**Value**

a numeric vector of integers

**References**

doi: 10.1074/jbc.R116.733899

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
grl <- ORFik:::makeORFNames(grl)
rankOrder(grl)
```

---

read.experiment

*Read ORFik experiment*


---

**Description**

Read in runs / samples from an experiment as a single R object. To read an ORFik experiment, you must of course make one first. See [create.experiment](#) The file must be csv and be a valid ORFik experiment

**Usage**

```
read.experiment(
  file,
  in.dir = ORFik:::config()["exp"],
  validate = TRUE,
  output.env = .GlobalEnv
)
```

**Arguments**

file	relative path to a ORFik experiment. That is a .csv file following ORFik experiment style ("," as separator). , or a template data.frame from <a href="#">create.experiment</a> . Can also be full path to file, then in.dir argument is ignored.
in.dir	Directory to load experiment csv file from, default: <code>ORFik:::config()["exp"]</code> , which has default <code>"~/Bio_data/ORFik_experiments/"</code> Set to NULL if you don't want to save it to disc. Does not apply if file argument is not a path (can also be a data.frame). Also does not apply if file argument was given as full path.

validate	logical, default TRUE. Abort if any library files does not exist. Do not set this to FALSE, unless you know what you are doing!
output.env	an environment, default .GlobalEnv. Which environment should ORFik output libraries to (if this is done), can be updated later with <code>envExp(df) &lt;- new.env()</code> .

**Value**

an ORFik [experiment](#)

**See Also**

Other ORFik\_experiment: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-method](#), [outputLibs\(\)](#), [save.experiment\(\)](#), [validateExperiments\(\)](#)

**Examples**

```
# From file
## Not run:
# Read from file
df <- read.experiment(filepath) # <- valid ORFik .csv file

## End(Not run)
## Read from (create.experiment() template)
df <- ORFik.template.experiment()

## To save it, do:
# save.experiment(df, file = "path/to/save/experiment")
## You can then do:
# read.experiment("path/to/save/experiment")
# or (identical):
# read.experiment("experiment", in.dir = "path/to/save/")
```

---

read\_RDSQS

*Read RDS or QS format file*


---

**Description**

Read RDS or QS format file

**Usage**

```
read_RDSQS(file, nthread = 5)
```

**Arguments**

file	path to file with "rds" or "qs" file extension
nthread	numeric, number of threads for qs2::qs_read

**Value**

R object loaded from file

**Examples**

```
df <- ORFik::ORFik.template.experiment()
path <- ORFik::countTablePath(df)
read_RDSQS(path)
```

---

readBam

*Custom bam reader*

---

**Description**

Read in Bam file from either single end or paired end. Safer combined version of [readGAlignments](#) and [readGAlignmentPairs](#) that takes care of some common errors.

If QNAMES of the aligned reads are from collapsed fasta files (if the names are formatted from collapsing in either (ORFik, ribotoolkit or fastx)), the bam file will contain a meta column called "score" with the counts of duplicates per read. Only works for single end reads, as perfect duplication events for paired end is more rare and therefor not supported!.

**Usage**

```
readBam(
  path,
  chrStyle = NULL,
  param = NULL,
  strandMode = 0,
  only_unique_mappers = FALSE
)
```

**Arguments**

path	<p>a character / data.table with path to .bam file. There are 3 input file possibilities.</p> <ul style="list-style-type: none"> <li>• single end : a character path (length 1)</li> <li>• paired end (1 file) : Either a character path (length of 2), where path[2] is "paired-end", or a data.table with 2 columns, forward = path &amp; reverse = "paired-end"</li> <li>• paired end (2 files) : Either a character path (length of 2), where path[2] is path to R2, or a data.table with 2 columns, forward = path to R1 &amp; reverse = path to R2. (This one is not used often)</li> </ul>
chrStyle	<p>a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a>. (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -&gt; pick "NCBI"</p>

param	<p>NULL or a <a href="#">ScanBamParam</a> object. Like for <a href="#">scanBam</a>, this influences what fields and which records are imported. However, note that the fields specified thru this <a href="#">ScanBamParam</a> object will be loaded <i>in addition</i> to any field required for generating the returned object (<a href="#">GAlignments</a>, <a href="#">GAlignmentPairs</a>, or <a href="#">GappedReads</a> object), but only the fields requested by the user will actually be kept as meta-data columns of the object.</p> <p>By default (i.e. param=NULL or param=ScanBamParam()), no additional field is loaded. The flag used is scanBamFlag(isUnmappedQuery=FALSE) for readGAlignments, readGAlignmentsList, and readGappedReads. (i.e. only records corresponding to mapped reads are loaded), and scanBamFlag(isUnmappedQuery=FALSE, isPaired=TRUE, hasUnmappedMate=FALSE) for readGAlignmentPairs (i.e. only records corresponding to paired-end reads with both ends mapped are loaded).</p>
strandMode	<p>numeric, default 0. Only used for paired end bam files. One of (0: strand = *, 1: first read of pair is +, 2: first read of pair is -). See ?strandMode. Note: Sets default to 0 instead of 1, as readGAlignmentPairs uses 1. This is to guarantee hits, but will also make mismatches of overlapping transcripts in opposite directions.</p>
only_unique_mappers	<p>logical, default FALSE. Only load unique mappers. For bam files it extracts NH flag, for other formats, it presumes the presence of a directory './unique_mappers' relative to bam file directory.</p>

### Details

In the future will use a faster .bam loader for big .bam files in R.

### Value

a [GAlignments](#) or [GAlignmentPairs](#) object of bam file

### See Also

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBigWig\(\)](#), [readWig\(\)](#)

### Examples

```
bam_file <- system.file("extdata/Danio_rerio_sample", "ribo-seq.bam", package = "ORFik")
readBam(bam_file, "UCSC")
```

---

readBamIsUniqueMapper *Read unique mapper status from bam*

---

### Description

The 'seq' flag of the bam, with a specified number of rows

**Usage**

```
readBamIsUniqueMapper(bam_paths, yieldSize = NA_integer_)
```

**Arguments**

`bam_paths` paths to bam files

`yieldSize` integer, default `NA_integer_`, number of reads to read in, set to `NA_integer_` to get full file.

**Value**

a list of logical elements, 1 for each bam file, TRUE is unique mapper.

**Examples**

```
df <- ORFik.template.experiment.zf()
bam_file_path <- filepath(df, "default")
readBamIsUniqueMapper(bam_file_path, 1e2)
```

---

readBamSeqs	<i>Read sequences from bam</i>
-------------	--------------------------------

---

**Description**

The 'seq' flag of the bam, with a specified number of rows

**Usage**

```
readBamSeqs(path, yieldSize = NA_integer_)
```

**Arguments**

`path` path to bam file

`yieldSize` integer, default `NA_integer_`, number of reads to read in, set to `NA_integer_` to get full file.

**Value**

a DNASTringSet of length `yieldSize` (all in file if NA was specified)

**Examples**

```
df <- ORFik.template.experiment.zf()
bam_file_path <- filepath(df, "default")
readBamSeqs(bam_file_path, 1e2)
```

---

readBigWig	<i>Custom bigWig reader</i>
------------	-----------------------------

---

### Description

Given 2 bigWig files (.bw, .bigWig), first is forward second is reverse. Merge them and return as GRanges object. If they contain name reverse and forward, first and second order does not matter, it will search for forward and reverse.

### Usage

```
readBigWig(path, chrStyle = NULL, as = "GRanges")
```

### Arguments

path	a character path to two .bigWig files, or a data.table with 2 columns, (forward, filepath) and reverse, only 1 row.
chrStyle	a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -> pick "NCBI"
as	Specifies the class of the return object. Default is GRanges, which has one range per range in the file, and a score column holding the value for each range. For NumericList, one numeric vector is returned for each range in the selection argument. For RleList, there is one Rle per sequence, and that Rle spans the entire sequence.

### Value

a [GRanges](#) object of the file/s

### See Also

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readWig\(\)](#)

---

readWidths	<i>Get read widths</i>
------------	------------------------

---

### Description

Input any reads, e.g. ribo-seq object and get width of reads, this is to avoid confusion between width, qwidth and meta column containing original read width.

### Usage

```
readWidths(reads, after.softclips = TRUE, along.reference = FALSE)
```

### Arguments

`reads` a GRanges, GAlignment, GAlignmentPairs or covRleList object.

`after.softclips` logical (TRUE), include softclips in width. Does not apply if `along.reference` is TRUE.

`along.reference` logical (FALSE), example: The cigar "26MI2" is by default width 28, but if `along.reference` is TRUE, it will be 26. The length of the read along the reference. Also "1D20M" will be 21 if by `along.reference` is TRUE. Intronic regions (cigar: N) will be removed. So: "1M200N19M" is 20, not 220.

### Details

If input is p-shifted and GRanges, the "\$size" or "\$score" column must exist, and the column must contain the original read widths. In ORFik "\$size" have higher priority than "\$score" for defining length. ORFik P-shifting creates a \$size column, other softwares like shoelaces creates a score column.

Remember to think about how you define length. Like the question: is a Illumina error mismatch sufficient to reduce size of read and how do you know what is biological variance and what are Illumina errors?

### Value

an integer vector of widths

### Examples

```
gr <- GRanges("chr1", 1)
readWidths(gr)

# GAlignment with hit (1M) and soft clipped base (1S)
ga <- GAlignments(seqnames = "1", pos = as.integer(1), cigar = "1M1S",
  strand = factor("+", levels = c("+", "-", "*")))
readWidths(ga) # Without soft-clip bases
```

```
readWidths(ga, after.softclips = FALSE) # With soft-clip bases
```

---

readWig	<i>Custom wig reader</i>
---------	--------------------------

---

### Description

Given 2 wig files, first is forward second is reverse. Merge them and return as GRanges object. If they contain name reverse and forward, first and second order does not matter, it will search for forward and reverse.

### Usage

```
readWig(path, chrStyle = NULL)
```

### Arguments

path	a character path to two .wig files, or a data.table with 2 columns, (forward, filepath) and reverse, only 1 row.
chrStyle	a GRanges object, TxDb, FaFile, , a <a href="#">seqlevelsStyle</a> or <a href="#">Seqinfo</a> . (Default: NULL) to get seqlevelsStyle from. In addition if it is a Seqinfo object, seqinfo will be updated. Example of seqlevelsStyle update: Is chromosome 1 called chr1 or 1, is mitochondrial chromosome called MT or chrM etc. Will use 1st seqlevel-style if more are present. Like: c("NCBI", "UCSC") -> pick "NCBI"

### Value

a [GRanges](#) object of the file/s

### See Also

Other utils: [bedToGR\(\)](#), [convertToOneBasedRanges\(\)](#), [export.bed12\(\)](#), [export.bigWig\(\)](#), [export.fstwig\(\)](#), [export.wiggle\(\)](#), [fimport\(\)](#), [findFa\(\)](#), [fread.bed\(\)](#), [optimizeReads\(\)](#), [readBam\(\)](#), [readBigWig\(\)](#)

---

reassignTSSbyCage	<i>Reassign all Transcript Start Sites (TSS)</i>
-------------------	--

---

### Description

Given a GRangesList of 5' UTRs or transcripts, reassign the start sites using max peaks from CageSeq data. A max peak is defined as new TSS if it is within boundary of 5' leader range, specified by 'extension' in bp. A max peak must also be higher than minimum CageSeq peak cutoff specified in 'filterValue'. The new TSS will then be the positioned where the cage read (with highest read count in the interval). If removeUnused is TRUE, leaders without cage hits, will be removed, if FALSE the original TSS will be used.

**Usage**

```
reassignTSSbyCage(
  fiveUTRs,
  cage,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
  removeUnused = FALSE,
  preCleanup = TRUE,
  cageMcol = FALSE
)
```

**Arguments**

fiveUTRs	(GRangesList) The 5' leaders or full transcript sequences
cage	Either a filePath for the CageSeq file as .bed .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: <code>convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE)</code> The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
extension	The maximum number of bases upstream of the TSS to search for CageSeq peak.
filterValue	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.
restrictUpstreamToTx	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
removeUnused	logical (FALSE), if False: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.
preCleanup	logical (TRUE), if TRUE, remove all reads in region (-5:-1, 1:5) of all original tss in leaders. This is to keep original TSS if it is only +/- 5 bases from the original.
cageMcol	a logical (FALSE), if TRUE, add a meta column to the returned object with the raw CAGE counts in support for new TSS.

**Details**

Note: If you used CAGER, you will get reads of a probability region, with always score of 1. Remember then to set filterValue to 0. And you should use the 5' end of the read as input, use: `ORFik:::convertToOneBasedRanges(cage)` NOTE on filtervalue: To get high quality TSS, set filtervalue to median count of reads overlapping per leader. This will make you discard a lot of new TSS positions though. I usually use 10 as a good standard.

TIP: do `summary(countOverlaps(fiveUTRs, cage))` so you can find a good cutoff value for noise.

**Value**

a GRangesList of newly assigned TSS for fiveUTRs, using CageSeq data.

**See Also**

Other CAGE: [assignTSSByCage\(\)](#), [reassignTxDbByCage\(\)](#)

**Examples**

```
# example 5' leader, notice exon_rank column
fiveUTRs <- GenomicRanges::GRangesList(
  GenomicRanges::GRanges(seqnames = "chr1",
    ranges = IRanges::IRanges(1000, 2000),
    strand = "+",
    exon_rank = 1))
names(fiveUTRs) <- "tx1"

# make fake CAGE data from promoter of 5' leaders, notice score column
cage <- GenomicRanges::GRanges(
  seqnames = "1",
  ranges = IRanges::IRanges(500, width = 1),
  strand = "+",
  score = 10) # <- Number of tags (reads) per position
# notice also that seqnames use different naming, this is fixed by ORFik
# finally reassign TSS for fiveUTRs
reassignTSSbyCage(fiveUTRs, cage)
# See vignette for example using gtf file and real CAGE data.
```

---

reassignTxDbByCage      *Input a txdb and reassign the TSS for each transcript by CAGE*

---

**Description**

Given a TxDb object, reassign the start site per transcript using max peaks from CageSeq data. A max peak is defined as new TSS if it is within boundary of 5' leader range, specified by 'extension' in bp. A max peak must also be higher than minimum CageSeq peak cutoff specified in 'filterValue'. The new TSS will then be the positioned where the cage read (with highest read count in the interval).

**Usage**

```
reassignTxDbByCage(
  txdb,
  cage,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
```

```

    removeUnused = FALSE,
    preCleanup = TRUE
  )

```

### Arguments

<code>txdb</code>	a TxDb file, a path to one of: (.gtf, .gff, .gff2, .db or .sqlite) or an ORFik experiment
<code>cage</code>	Either a filePath for the CageSeq file as .bed, .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: <code>convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE)</code> The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
<code>extension</code>	The maximum number of bases upstream of the TSS to search for CageSeq peak.
<code>filterValue</code>	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.
<code>restrictUpstreamToTx</code>	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
<code>removeUnused</code>	logical (FALSE), if FALSE: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.
<code>preCleanup</code>	logical (TRUE), if TRUE, remove all reads in region (-5:-1, 1:5) of all original tss in leaders. This is to keep original TSS if it is only +/- 5 bases from the original.

### Details

Note: If you used CAGER, you will get reads of a probability region, with always score of 1. Remember then to set `filterValue` to 0. And you should use the 5' end of the read as input, use: `ORFik::convertToOneBasedRanges(cage)`

### Value

a TxDb object of reassigned transcripts

### See Also

Other CAGE: [assignTSSByCage\(\)](#), [reassignTSSbyCage\(\)](#)

### Examples

```

## Not run:
library(GenomicFeatures)
# Get the gtf txdb file
txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",

```

```

package = "GenomicFeatures")
cagePath <- system.file("extdata", "cage-seq-heart.bed.bgz",
package = "ORFik")
reassignTxDbByCage(txdbFile, cagePath)

## End(Not run)

```

---

reduceKeepAttr	<i>Reduce GRanges / GRangesList</i>
----------------	-------------------------------------

---

## Description

Reduce away all GRanges elements with 0-width.

## Usage

```

reduceKeepAttr(
  grl,
  keep.names = FALSE,
  drop.empty.ranges = FALSE,
  min.gapwidth = 1L,
  with.revmap = FALSE,
  with.inframe.attrib = FALSE,
  ignore.strand = FALSE,
  min.strand.decreasing = TRUE
)

```

## Arguments

grl	a <a href="#">GRangesList</a> or GRanges object
keep.names	(FALSE) keep the names and meta columns of the GRangesList
drop.empty.ranges	(FALSE) if a group is empty (width 0), delete it.
min.gapwidth	(1L) how long gap can it be between two ranges, to merge them.
with.revmap	(FALSE) return info on which mapped to which
with.inframe.attrib	(FALSE) For internal use.
ignore.strand	(FALSE), can different strands be reduced together.
min.strand.decreasing	(TRUE), if GRangesList, return minus strand group ranges in decreasing order (1-5, 30-50) -> (30-50, 1-5)

## Details

Extends function [reduce](#) by trying to keep names and meta columns, if it is a GRangesList. It also does not lose sorting for GRangesList, since original reduce sorts all by ascending position. If keep.names == FALSE, it's just the normal GenomicRanges::reduce with sorting negative strands descending for GRangesList.

**Value**

A reduced GRangesList

**See Also**

Other ExtendGenomicRanges: [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

**Examples**

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 2, 3), end = c(1, 2, 3)),
               strand = "+")
# For GRanges
reduceKeepAttr(ORF, keep.names = TRUE)
# For GRangesList
grl <- GRangesList(tx1_1 = ORF)
reduceKeepAttr(grl, keep.names = TRUE)
```

---

refFolder

*Get path to ORFik experiment genome reference folder*

---

**Description**

Get path to ORFik experiment genome reference folder

**Usage**

```
refFolder(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

---

 refFolder,experiment-method

*Get path to ORFik experiment genome reference folder*


---

**Description**

Get path to ORFik experiment genome reference folder

**Usage**

```
## S4 method for signature 'experiment'
refFolder(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

---

 regionPerReadLength    *Find proportion of reads per position per read length in region*


---

**Description**

This is defined as: Given some transcript region (like CDS), get coverage per position. By default only returns positions that have hits, set drop.zero.dt to FALSE to get all 0 positions.

**Usage**

```
regionPerReadLength(
  grl,
  reads,
  acceptedLengths = NULL,
  withFrames = TRUE,
  scoring = "transcriptNormalized",
  weight = "score",
  exclude.zero.cov.grl = TRUE,
  drop.zero.dt = TRUE,
  BPPARAM = bpparam()
)
```

**Arguments**

grl	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs
reads	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> , or precomputed coverage as <a href="#">covRleList</a> (where names of covRle objects are readlengths) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better.
acceptedLengths	an integer vector (NULL), the read lengths accepted. Default NULL, means all lengths accepted.
withFrames	logical TRUE, add ORF frame (frame 0, 1, 2), starting on first position of every grl.
scoring	a character (transcriptNormalized), which meta coverage scoring ? one of (zscore, transcriptNormalized, mean, median, sum, sumLength, fracPos), see ?coverageScorings for more info. Use to decide a scoring of hits per position for metacoverage etc. Set to NULL if you do not want meta coverage, but instead want per gene per position raw counts.
weight	(default: 'score'), if defined a character name of valid meta column in subject. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGEr CAGE files and many other package formats. You can also assign a score column manually.
exclude.zero.cov.grl	logical, default TRUE. Do not include ranges that does not have any coverage (0 reads on them), this makes it faster to run.
drop.zero.dt	logical, default TRUE. If TRUE and as.data.table is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 count positions are used in some sense.
BPPARAM	how many cores/threads to use? default: <code>bpparam()</code>

**Value**

a data.table with lengths by coverage.

**See Also**

Other coverage: [coverageScorings\(\)](#), [metaWindow\(\)](#), [scaledWindowPositions\(\)](#), [windowPerReadLength\(\)](#)

**Examples**

```
# Raw counts per gene per position
cds <- GRangesList(tx1 = GRanges("1", 100:129, "+"))
reads <- GRanges("1", seq(79,129, 3), "+")
reads$size <- 28 # <- Set read length of reads
regionPerReadLength(cds, reads, scoring = NULL)
```

```
## Sum up reads in each frame per read length per gene
regionPerReadLength(cds, reads, scoring = "frameSumPerLG")
```

---

remove.experiments      *Remove ORFik experiment libraries load in R*

---

### Description

Variable names defined by df, in envir defined

### Usage

```
remove.experiments(df, envir = envExp(df))
```

### Arguments

df	an ORFik <a href="#">experiment</a>
envir	environment to save to, default envExp(df), which defaults to .GlobalEnv, but can be set with envExp(df) <- new.env() etc.

### Value

NULL (objects removed from envir specified)

### Examples

```
df <- ORFik.template.experiment()
# Output to .GlobalEnv with:
# outputLibs(df)
# Then remove them with:
# remove.experiments(df)
```

---

resFolder      *Get ORFik experiment main output directory*

---

### Description

Get ORFik experiment main output directory

### Usage

```
resFolder(x)
```

### Arguments

x	an ORFik <a href="#">experiment</a>
---	-------------------------------------

**Value**

a character path

---

resFolder, experiment-method

*Get ORFik experiment main output directory*

---

**Description**

Get ORFik experiment main output directory

**Usage**

```
## S4 method for signature 'experiment'
resFolder(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

---

ribo\_fft

*Get periodogram data per read length*

---

**Description**

A data.table of periods and amplitudes, great to detect ribosomal read lengths. Uses 5' end of reads to detect periodicity. Works both before and after p-shifting. Plot results with ribo\_fft\_plot.

**Usage**

```
ribo_fft(footprints, cds, read_lengths = 26:34, firstN = 150)
```

**Arguments**

footprints	Ribosome footprints in either <a href="#">GAlignments</a> or <a href="#">GRanges</a>
cds	a <a href="#">GRangesList</a> of coding sequences. Length must match length of argument mrna, and all must have length > argument firstN.
read_lengths	integer vector, default: 26:34, which read length to check for. Will exclude all read_lengths that does not exist for footprints.
firstN	(integer) Represents how many bases of the transcripts downstream of start codons to use for initial estimation of the periodicity.

**Value**

a data.table with read\_length, amplitude and periods

**Examples**

```
## Note, this sample data is not intended to be strongly periodic.
## Real data should have a cleaner peak for x = 3 (periodicity)
# Load sample data
df <- ORFik.template.experiment()
# Load annotation
loadRegions(df, "cds", names.keep = filterTranscripts(df))
# Select a riboseq library
df <- df[df$libtype == "RFP", ]
footprints <- fimport(filepath(df[1,], "default"))
fft_dt <- ribo_fft(footprints, cds)
ribo_fft_plot(fft_dt)
```

---

ribo\_fft\_plot

*Get periodogram plot per read length*


---

**Description**

Get periodogram plot per read length

**Usage**

```
ribo_fft_plot(fft_dt, period_window = c(0, 6))
```

**Arguments**

```
fft_dt          a data.table with read_length, amplitude and periods
period_window  x axis limits, default c(0,6)
```

**Value**

a ggplot, geom\_line plot facet by read length.

**Examples**

```
## Note, this sample data is not intended to be strongly periodic.
## Real data should have a cleaner peak for x = 3 (periodicity)
# Load sample data
df <- ORFik.template.experiment()
# Load annotation
cds <- loadRegion(df, "cds", names.keep = filterTranscripts(df))
# Select a riboseq library
df <- df[df$libtype == "RFP", ]
footprints <- fimport(filepath(df[1,], "default"))
fft_dt <- ribo_fft(footprints, cds)
ribo_fft_plot(fft_dt)
```

---

riboORFs	<i>Load Predicted translons</i>
----------	---------------------------------

---

**Description**

Load Predicted translons

**Usage**

```
riboORFs(df, type = "table", folder = riboORFsFolder(df))
```

**Arguments**

df	ORFik experiment
type	default "table", alternatives: c("table", "ranges_candidates", "ranges_predictions", "predictions")
folder	base folder to check for computed results, default: riboORFsFolder(df)

**Value**

a data.table, GRangesList or list of logical vector depending on input

**Examples**

```
df <- ORFik.template.experiment()
df <- df[df$libtype == "RFP",][c(1,2),]
# riboORFs(df) # Works when you have run prediction
```

---

riboORFsFolder	<i>Define folder for prediction output</i>
----------------	--

---

**Description**

Define folder for prediction output

**Usage**

```
riboORFsFolder(df, parent_dir = resFolder(df))
```

**Arguments**

df	ORFik experiment
parent_dir	Parent directory of computed study results, default: resFolder(df)

**Value**

a file path (full path)

**Examples**

```
df <- ORFik.template.experiment()
df <- df[df$libtype == "RFP",][c(1,2),]
riboORFsFolder(df)
riboORFsFolder(df, tempdir())
```

---

RiboQC.plot

*Quality control for pshifted Ribo-seq data*


---

**Description**

Combines several statistics from the pshifted reads into a plot:

- 1 Coding frame distribution per read length
- 2 Alignment statistics
- 3 Biotype of non-exonic pshifted reads
- 4 mRNA localization of pshifted reads

**Usage**

```
RiboQC.plot(
  df,
  output.dir = QCfolder(df),
  width = 6.6,
  height = 4.5,
  plot.ext = ".pdf",
  type = "pshifted",
  weight = "score",
  bar.position = "dodge",
  as_gg_list = FALSE,
  BPPARAM = BiocParallel::SerialParam(progressbar = TRUE)
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
output.dir	NULL or character path, default: NULL, plot not saved to disc. If defined saves plot to that directory with the name "/STATS_plot.pdf".
width	width of plot, default 6.6 (in inches)
height	height of plot, default 4.5 (in inches)
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg".

type	type of library loaded, default pshifted, warning if not pshifted might crash if too many read lengths!
weight	which column in reads describe duplicates, default "score".
bar.position	character, default "dodge". Should Ribo-seq frames per read length be positioned as "dodge" or "stack" (on top of each other).
as_gg_list	logical, default FALSE. Return as a list of ggplot objects instead of as a grob. Gives you the ability to modify plots more directly.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

### Value

the plot object, a grob of ggplot objects of the the data

### Examples

```
df <- ORFik.template.experiment()
df <- df[9,] #lets only p-shift RFP sample at index 9
#shiftFootprintsByExperiment(df)
#RiboQC.plot(df, tempdir())
```

---

ribosomeReleaseScore *Ribosome Release Score (RRS)*

---

### Description

Ribosome Release Score is defined as

$$\frac{\text{(RPFs over ORF)}}{\text{(RPFs over 3' utrs)}}$$

and additionally normalized by lengths. If RNA is added as argument, it will normalize by RNA counts to justify location of 3' utrs. It can be understood as a ribosome stalling feature. A pseudo-count of one was added to both the ORF and downstream sums.

### Usage

```
ribosomeReleaseScore(
  grl,
  RFP,
  GtfOrThreeUtrs,
  RNA = NULL,
  weight.RFP = 1L,
  weight.RNA = 1L,
  overlapGr1 = NULL
)
```

**Arguments**

<code>gr1</code>	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs.
<code>RFP</code>	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
<code>GtfOrThreeUtrs</code>	if <code>Gtf</code> : a <a href="#">TxDb</a> object of a gtf file transcripts is called from: <code>'threeUTRsByTranscript(Gtf, use.names = TRUE)'</code> , if object is <a href="#">GRangesList</a> , it is presumed to be the 3' utrs
<code>RNA</code>	RnaSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
<code>weight.RFP</code>	a vector (default: 1L). Can also be character name of column in RFP. As in <code>translationalEff(weight = "score")</code> for: <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean score column tells that this alignment region was found 5 times.
<code>weight.RNA</code>	Same as <code>weightRFP</code> but for RNA weights. (default: 1L)
<code>overlapGr1</code>	an integer, (default: NULL), if defined must be <code>countOverlaps(gr1, RFP)</code> , added for speed if you already have it

**Value**

a named vector of numeric values of scores, NA means that no 3' utr was found for that transcript.

**References**

doi: 10.1016/j.cell.2013.06.009

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
gr1 <- GRangesList(tx1_1 = ORF)
threeUTRs <- GRangesList(tx1 = GRanges("1", IRanges(40, 50), "+"))
RFP <- GRanges("1", IRanges(25, 25), "+")
RNA <- GRanges("1", IRanges(1, 50), "+")
ribosomeReleaseScore(gr1, RFP, threeUTRs, RNA)
```

---

ribosomeStallingScore *Ribosome Stalling Score (RSS)*

---

### Description

Is defined as

$$\frac{\text{(RPFs over ORF stop sites)}}{\text{(RPFs over ORFs)}}$$

and normalized by lengths A pseudo-count of one was added to both the ORF and downstream sums.

### Usage

```
ribosomeStallingScore(gr1, RFP, weight = 1L, overlapGr1 = NULL)
```

### Arguments

gr1	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs.
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <a href="#">GRanges("chr1", 1, "+", score = 5)</a> , would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <a href="#">countOverlaps()</a> presumes, if single number ( $\neq 1$ ), it repeats for all ranges, if vector with length $> 1$ , it must be equal size of the reads object.
overlapGr1	an integer, (default: NULL), if defined must be <a href="#">countOverlaps(gr1, RFP)</a> , added for speed if you already have it

### Value

a named vector of numeric values of RSS scores

### References

doi: 10.1016/j.cels.2017.08.004

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
gr1 <- GRangesList(tx1_1 = ORF)
RFP <- GRanges("1", IRanges(25, 25), "+")
ribosomeStallingScore(gr1, RFP)
```

---

<code>rnaNormalize</code>	<i>Normalize a data.table of coverage by RNA seq per position</i>
---------------------------	---

---

**Description**

Normalizes per position per gene by this function:  $(\text{reads at position} / \min(\text{librarysize}, 1)) * \text{number of genes} / \text{fpkm of that gene's RNA-seq}$

**Usage**

```
rnaNormalize(coverage, df, dfr = NULL, tx, normalizeMode = "position")
```

**Arguments**

<code>coverage</code>	a data.table containing at least columns (count/score, position), it is possible to have additional: (genes, fraction, feature)
<code>df</code>	an ORFik <a href="#">experiment</a>
<code>dfr</code>	an ORFik <a href="#">experiment</a> of RNA-seq to normalize against. Will add RNA normalized to plot name if this is done.
<code>tx</code>	a <a href="#">GRangesList</a> of mrna transcripts
<code>normalizeMode</code>	a character (default: "position"), how to normalize library against rna library. Either on "position", normalize by number of genes, sum of reads and RNA seq, on tx "region" or "feature": same as position but RNA is split into the feature groups to normalize. Useful if you have a list of targets and background genes.

**Details**

Good way to compare libraries

**Value**

a data.table of normalized transcripts by RNA.

---

runIDs	<i>Get SRR/DRR/ERR run ids from ORFik experiment</i>
--------	--

---

**Description**

Get SRR/DRR/ERR run ids from ORFik experiment

**Usage**

```
runIDs(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character vector of runIDs, "" if not existing.

---

runIDs,experiment-method	<i>Get SRR/DRR/ERR run ids from ORFik experiment</i>
--------------------------	--

---

**Description**

Get SRR/DRR/ERR run ids from ORFik experiment

**Usage**

```
## S4 method for signature 'experiment'  
runIDs(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character vector of runIDs, "" if not existing.

---

save.experiment	Save <a href="#">experiment</a> to disc
-----------------	---

---

**Description**

Save [experiment](#) to disc

**Usage**

```
save.experiment(df, file)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
file	name of file to save df as

**Value**

NULL (experiment save only)

**See Also**

Other ORFik\_experimnet: [ORFik.template.experiment\(\)](#), [ORFik.template.experiment.zf\(\)](#), [bamVarName\(\)](#), [create.experiment\(\)](#), [experiment-class](#), [filepath\(\)](#), [libraryTypes\(\)](#), [organism](#), [experiment-meth](#), [outputLibs\(\)](#), [read.experiment\(\)](#), [validateExperiments\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
## Save with:
#save.experiment(df, file = "path/to/save/experiment.csv")
## Identical (.csv not needed, can be added):
#save.experiment(df, file = "path/to/save/experiment")
```

---

save_RDSQS	Read RDS or QS format file
------------	----------------------------

---

**Description**

Read RDS or QS format file

**Usage**

```
save_RDSQS(object, file, nthread = 5)
```

**Arguments**

object            the object to save  
 file              path to file with "rds" or "qs" file extension  
 nthread          numeric, number of threads for qs2::qs\_save

**Value**

R object loaded from file

**Examples**

```
path <- tempfile(fileext = ".qs")
# Simple numeric save
x <- 1
save_RDSQS(x, path)
read_RDSQS(path)
# Save a list
x <- list(a = 1, b = c(1,2,3))
save_RDSQS(x, path)
read_RDSQS(path)
```

---

scaledWindowPositions *Scale (bin) windows to a meta window of given size*

---

**Description**

For example scale a coverage table of a all human CDS to width 100

**Usage**

```
scaledWindowPositions(
  grl,
  reads,
  scaleTo = 100,
  scoring = "meanPos",
  weight = "score",
  is.sorted = FALSE,
  drop.zero.dt = FALSE
)
```

**Arguments**

grl                a [GRangesList](#) of 5' utrs, CDS, transcripts, etc.  
 reads             a [GAlignments](#), [GRanges](#), or precomputed coverage as [covRle](#) (one for each strand) of RiboSeq, RnaSeq etc.  
 Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a

	few genes, then loading the entire files is usually better. File streaming is still in beta, so use with care!
scaleTo	an integer (100), if windows have different size, a meta window can not directly be created, since a meta window must have equal size for all windows. Rescale all windows to scaleTo. i.e c(1,2,3) -> size 2 -> c(1, mean(2,3)) etc. Can also be a vector, 1 number per grl group.
scoring	a character, one of (zscore, transcriptNormalized, mean, median, sum, log2sum, log10sum, sumLength, meanPos and frameSum, periodic, NULL). More info in details
weight	(default: 'score'), if defined a character name of valid meta column in subject. GRanges("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGER CAGE files and many other package formats. You can also assign a score column manually.
is.sorted	logical (FALSE), is grl sorted. That is + strand groups in increasing ranges (1,2,3), and - strand groups in decreasing ranges (3,2,1)
drop.zero.dt	logical FALSE, if TRUE and as.data.table is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)

### Details

Nice for making metaplots, the score will be mean of merged positions.

### Value

A data.table with scored counts (counts) of reads mapped to positions (position) specified in windows along with frame (frame).

### See Also

Other coverage: [coverageScorings\(\)](#), [metaWindow\(\)](#), [regionPerReadLength\(\)](#), [windowPerReadLength\(\)](#)

### Examples

```
library(GenomicRanges)
windows <- GRangesList(GRanges("chr1", IRanges(1, 200), "-"))
x <- GenomicRanges::GRanges(
  seqnames = "chr1",
  ranges = IRanges::IRanges(c(1, 100, 199), c(2, 101, 200)),
  strand = "-")
scaledWindowPositions(windows, x, scaleTo = 100)
```

---

scoreSummarizedExperiment

*Helper function for makeSummarizedExperimentFromBam*

---

### Description

If txdb or gtf path is added, it is a rangedSummerizedExperiment For FPKM values, DESeq2::fpkm(robust = FALSE) is used

### Usage

```
scoreSummarizedExperiment(
  final,
  score = "transcriptNormalized",
  collapse = FALSE
)
```

### Arguments

final	ranged summarized experiment object
score	default: "transcriptNormalized" (row normalized raw counts matrix), alternative is "fpkm", "log2fpkm" or "log10fpkm"
collapse	a logical/character (default FALSE), if TRUE all samples within the group SAMPLE will be collapsed to one. If "all", all groups will be merged into 1 column called merged_all. Collapse is defined as rowSum(elements_per_group) / ncol(elements_per_group)

### Value

a DEseq summerizedExperiment object (transcriptNormalized) or matrix (if fpkm input)

---

seqinfo,covRle-method *Seqinfo covRle Extracted from forward RleList*

---

### Description

Seqinfo covRle Extracted from forward RleList

### Usage

```
## S4 method for signature 'covRle'
seqinfo(x)
```

### Arguments

x	a covRle object
---	-----------------

**Value**

integer vector with names

---

seqinfo,covRleList-method

*Seqinfo covRle Extracted from forward RleList*

---

**Description**

Seqinfo covRle Extracted from forward RleList

**Usage**

```
## S4 method for signature 'covRleList'  
seqinfo(x)
```

**Arguments**

x                    a covRle object

**Value**

integer vector with names

---

seqinfo,experiment-method

*Seqinfo ORFik experiment Extracted from fasta genome index*

---

**Description**

Seqinfo ORFik experiment Extracted from fasta genome index

**Usage**

```
## S4 method for signature 'experiment'  
seqinfo(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

integer vector with names

---

seqlevels,covRle-method

*Seqlevels covRle Extracted from forward RleList*

---

**Description**

Seqlevels covRle Extracted from forward RleList

**Usage**

```
## S4 method for signature 'covRle'  
seqlevels(x)
```

**Arguments**

x                    a covRle object

**Value**

integer vector with names

---

seqlevels,covRleList-method

*Seqlevels covRleList Extracted from forward RleList*

---

**Description**

Seqlevels covRleList Extracted from forward RleList

**Usage**

```
## S4 method for signature 'covRleList'  
seqlevels(x)
```

**Arguments**

x                    a covRle object

**Value**

integer vector with names

---

seqlevels,experiment-method

*Seqlevels ORFik experiment Extracted from fasta genome index*

---

**Description**

Seqlevels ORFik experiment Extracted from fasta genome index

**Usage**

```
## S4 method for signature 'experiment'  
seqlevels(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

integer vector with names

---

seqnames,experiment-method

*Seqnames ORFik experiment Extracted from fasta genome index*

---

**Description**

Seqnames ORFik experiment Extracted from fasta genome index

**Usage**

```
## S4 method for signature 'experiment'  
seqnames(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

integer vector with names

---

seqnamesPerGroup	<i>Get first seqname per GRangesList group</i>
------------------	--

---

**Description**

Get first seqname per GRangesList group

**Usage**

```
seqnamesPerGroup(gr1, keep.names = TRUE)
```

**Arguments**

gr1	a <a href="#">GRangesList</a>
keep.names	a boolean, keep names or not, default: (TRUE)

**Value**

a character vector or Rle of seqnames(if seqnames == T)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(7, 14), width = 3),
  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
  ranges = IRanges(c(4, 1), c(9, 3)),
  strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
seqnamesPerGroup(gr1)
```

---

shiftFootprints	<i>Shift footprints by selected offsets</i>
-----------------	---

---

**Description**

Function shifts footprints (GRanges) using specified offsets for every of the specified lengths. Reads that do not conform to the specified lengths are filtered out and rejected. Reads are resized to single base in 5' end fashion, treated as p site. This function takes account for junctions and soft clips in cigars of the reads. Length of the footprint is saved in size' parameter of GRanges output. Footprints are also sorted according to their genomic position, ready to be saved as a ofst, covRle, bed or wig file.

**Usage**

```
shiftFootprints(footprints, shifts, sort = TRUE)
```

**Arguments**

footprints	<a href="#">GAlignments</a> object of RiboSeq reads - footprints, can also be path to the .bam / .ofst file. If GAlignment object has a meta column called "score", this will be used as replicate numbering for that read. So be careful if you have custom files with score columns, with another meaning.
shifts	a data.frame / data.table with minimum 2 columns, fraction (selected read lengths) and offsets_start (relative position in nt). Output from <a href="#">detectRibosomeShifts</a> . Run <code>ORFik::shifts_load(df)[[1]]</code> for an example of input.
sort	logical, default TRUE. If False will keep original order of reads, and not sort output reads in increasing genomic location per chromosome and strand.

**Details**

The two columns in the shift data.frame/data.table argument are:

- fraction Numeric vector of lengths of footprints you select for shifting.
- offsets\_start Numeric vector of shifts for corresponding selected\_lengths. eg. `c(-10, -10)` with selected\_lengths of `c(31, 32)` means length of 31 will be shifted left by 10. Footprints of length 32 will be shifted right by 10.

NOTE: It will remove softclips from valid width, the CIGAR 3S30M is qwidth 33, but will remove 3S so final read width is 30 in ORFik.

**Value**

A [GRanges](#) object of shifted footprints, sorted and resized to 1bp of p-site, with metacolumn "size" indicating footprint size before shifting and resizing, sorted in increasing order.

**References**

<https://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-018-4912-6>

**See Also**

Other pshifting: [changePointAnalysis\(\)](#), [detectRibosomeShifts\(\)](#), [shiftFootprintsByExperiment\(\)](#), [shiftPlots\(\)](#), [shifts\\_load\(\)](#), [shifts\\_save\(\)](#)

**Examples**

```
## Basic run
# Transcriptome annotation ->
gtf_file <- system.file("extdata/references/danio_rerio", "annotations.gtf", package = "ORFik")
# Ribo seq data ->
riboSeq_file <- system.file("extdata/Danio_rerio_sample", "ribo-seq.bam", package = "ORFik")
## Not run:
footprints <- readBam(riboSeq_file)

# detect the shifts automagically
shifts <- detectRibosomeShifts(footprints, gtf_file)
# shift the RiboSeq footprints
shiftedReads <- shiftFootprints(footprints, shifts)
```

```
## End(Not run)
```

---

```
shiftFootprintsByExperiment
  Shift footprints of each file in experiment
```

---

### Description

A function that combines the steps of periodic read length detection, p-site shift detection and p-shifting into 1 function. For more details, see: [detectRibosomeShifts](#)

Saves files to a specified location as .ofst and .wig, The .ofst file will include a 'score' column with read count at that position per read width (read width column is called 'size')

The .wig files, will be saved in pairs of +/- strand, and score column will be replicates of reads starting at that position, score = 5 means 5 reads.

Remember that different species might have different default Ribosome read lengths, for human, mouse etc, normally around 27:30.

### Usage

```
shiftFootprintsByExperiment(
  df,
  out.dir = pasteDir(libFolder(df), "/pshifted/"),
  start = TRUE,
  stop = FALSE,
  top_tx = 10L,
  minFiveUTR = 30L,
  minCDS = 150L,
  minThreeUTR = if (stop) {
    30
  } else NULL,
  firstN = 150L,
  min_reads = 1000,
  min_reads_TIS = 50,
  accepted.lengths = 26:34,
  output_format = c("ofst", "wig"),
  BPPARAM = bpparam(),
  tx = NULL,
  shift.list = NULL,
  log = TRUE,
  heatmap = FALSE,
  must.be.periodic = TRUE,
  strict.fft = TRUE,
  verbose = FALSE
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
out.dir	output directory for files, default: pasteDir(libFolder(df), "/pshifted/"), making a /pshifted folder inside default bam file location
start	(logical) Whether to include predictions based on the start codons. Default TRUE.
stop	(logical) Whether to include predictions based on the stop codons. Default FALSE. Only use if there exists 3' UTRs for the annotation. If periodicity around stop codon is stronger than at the start codon, use stop instead of start region for p-shifting.
top_tx	(integer), default 10. Specify which % of the top TIS coverage transcripts to use for estimation of the shifts. By default we take top 10 top covered transcripts as they represent less noisy data-set. This is only applicable when there are more than 1000 transcripts.
minFiveUTR	(integer) minimum bp for 5' UTR during filtering for the transcripts. Set to NULL if no 5' UTRs exists for annotation.
minCDS	(integer) minimum bp for CDS during filtering for the transcripts
minThreeUTR	(integer) minimum bp for 3' UTR during filtering for the transcripts. Set to NULL if no 3' UTRs exists for annotation.
firstN	(integer) Represents how many bases of the transcripts downstream of start codons to use for initial estimation of the periodicity.
min_reads	default (1000), how many reads must a read-length have in total to be considered for periodicity.
min_reads_TIS	default (50), how many reads must a read-length have in the TIS region to be considered for periodicity.
accepted.lengths	accepted read lengths, default 26:34, usually ribo-seq is strongest between 27:32.
output_format	default c("ofst", "wig"), use export.ofst or wiggle format (wig) using <a href="#">export.wiggle</a> ? Default is both. Options are: c("ofst", "bigWig", "wig", "bed", "bedo") For future coverage per nucleotide, we advice to do here ofst and bigWig for other genome browsers, then call <a href="#">convert_to_covRleList</a> to get much faster R objects. The wig format version can be used in IGV, the score column is counts of that read with that read length, the cigar reference width is lost, ofst is much faster to save and load in R, and retain cigar reference width, but can not be used in IGV. Also for larger tracks, you can use "bigWig".
BPPARAM	how many cores/threads to use? default: bpparam()
tx	a GRangesList, if you do not have 5' UTRs in annotation, send your own version. Example: extendLeaders(tx, 30) Where 30 bases will be new "leaders". Since each original transcript was either only CDS or non-coding (filtered out).
shift.list	default NULL, or a list containing named data.frames / data.tables with minimum 2 columns, fraction (selected read lengths) and offsets_start (relative position in nt). 1 named data.frame / data.table per library. Output from <a href="#">detectRibosomeShifts</a> .

Run `ORFik::shifts_load(df)` for an example of input. The names of the list must be the file.paths of the Ribo-seq libraries. Use this to edit the shifts, if you suspect some of them are wrong in an experiment.  
Can be a subset of libraries, i.e. all other libraries will use auto-detect.

log	logical, default (TRUE), output a log file with parameters used and a .rds file with all shifts per library (can be loaded with <code>shifts_load</code> )
heatmap	a logical or character string, default FALSE. If TRUE, will plot heatmap of raw reads before p-shifting to console, to see if shifts given make sense. You can also set a filepath to save the file there.
must.be.periodic	logical TRUE, if FALSE will not filter on periodic read lengths. (The Fourier transform filter will be skipped). This is useful if you are not going to do periodicity analysis, that is: for you more coverage depth (more read lengths) is more important than only keeping the high quality periodic read lengths.
strict.fft	logical, TRUE. Use a FFT without noise filter. This means keep only reads lengths that are "periodic for the human eye". If you want more coverage, set to FALSE, to also get read lengths that are "messy", but the noise filter detects the periodicity of 3. This should only be done when you do not need high quality periodic reads! Example would be differential translation analysis by counts over each ORF.
verbose	logical, default FALSE. Report details of analysis/periodogram. Good if you are not sure if the analysis was correct.

### Value

NULL (Objects are saved to `out.dir/pshited/"name_pshifted.ofst"`, `wig`, `bedo` or `.bedo`)

### References

<https://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-018-4912-6>

### See Also

Other pshifting: `changePointAnalysis()`, `detectRibosomeShifts()`, `shiftFootprints()`, `shiftPlots()`, `shifts_load()`, `shifts_save()`

### Examples

```
df <- ORFik.template.experiment.zf()
df <- df[1,] #lets only p-shift first RFP sample
## Output files as both .ofst and .wig(can be viewed in IGV/UCSC)
shiftFootprintsByExperiment(df)
# If you only need in R, do: (then you get no .wig files)
#shiftFootprintsByExperiment(df, output_format = "ofst")
## With debug info:
#shiftFootprintsByExperiment(df, verbose = TRUE)
## Re-shift, if you think some are wrong
## Here as an example we update library 1, third read length to shift 12
shift.list <- shifts_load(df)
```

```

shift.list[[1]]$offsets_start[3] <- -12
#shiftFootprintsByExperiment(df, shift.list = shift.list)
## For additional speedup in R for nucleotide coverage (coveragePerTiling etc)

```

---

shiftPlots

*Plot shifted heatmaps per library*


---

## Description

Around CDS TISs, plot coverage. A good validation for you p-shifting, to see shifts are corresponding and close to the CDS TIS.

## Usage

```

shiftPlots(
  df,
  output = NULL,
  title = "Ribo-seq",
  scoring = "transcriptNormalized",
  pShifted = TRUE,
  upstream = if (pShifted) 5 else 20,
  downstream = if (pShifted) 20 else 5,
  type = "bar",
  addFracPlot = TRUE,
  plot.ext = ".pdf",
  dpi = ifelse(nrow(df) < 22, 300, 200),
  height_scaler = ifelse(type == "heatmap", 85, 95),
  BPPARAM = bpparam()
)

```

## Arguments

df	an ORFik <a href="#">experiment</a>
output	name to save file, full path. (Default NULL) No saving. Sett to "auto" to save to QC_STATS folder of experiment named: "pshifts_barplots.png" or "pshifts_heatmaps.png" depending on type argument. Folder must exist!
title	Title for top of plot, default "Ribo-seq". A more informative name could be "Ribo-seq zebrafish Chew et al. 2013"
scoring	which scoring scheme to use for heatmap, default "transcriptNormalized". Some alternatives: "sum", "zscore".
pShifted	a logical (TRUE), are Ribo-seq reads p-shifted to size 1 width reads? If upstream and downstream is set, this argument is irrelevant. So set to FALSE if this is not p-shifted Ribo-seq.
upstream	an integer (5), relative region to get upstream from. Default: <code>ifelse(!is.null(tx), ifelse(pShifted, 5, 20), min(ifelse(pShifted, 5, 20), 0))</code>

downstream	an integer (20), relative region to get downstream from. Default: <code>ifelse(pShifted, 20, 5)</code>
type	character, default "bar". Plot as faceted bars, gives more detailed information of read lengths, but harder to see patterns over multiple read lengths. Alternative: "heatmap", better overview of patterns over multiple read lengths.
addFracPlot	logical, default TRUE, add positional sum plot on top per heatmap.
plot.ext	default ".pdf". Alternative ".png". Only added if output is "auto".
dpi	numeric, default: <code>ifelse(nrow(df) &lt; 22, 300, 200)</code>
height_scaler	numeric default: <code>ifelse(type == "heatmap", 85, 95)</code> . The total height of plot in unit "mm" is <code>(length(res) - 1) * height_scaler</code> . Increase if many readlengths are used.
BPPARAM	how many cores/threads to use? default: <code>bpparam()</code>

**Value**

a ggplot2 grob object

**See Also**

Other pshifting: [changePointAnalysis\(\)](#), [detectRibosomeShifts\(\)](#), [shiftFootprints\(\)](#), [shiftFootprintsByExperiment\(\)](#), [shifts\\_load\(\)](#), [shifts\\_save\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
df <- df[df$libtype == "RFP",][1,] #lets only p-shift first RFP sample
#shiftFootprintsByExperiment(df, output_format = "ofst")
#grob <- shiftPlots(df, title = "Ribo-seq Human ORFik et al. 2020")
#plot(grob) #Only plot in RStudio for small amount of files!
```

---

shifts\_load

*Load the shifts from experiment*

---

**Description**

When you p-shift using the function `shiftFootprintsByExperiment`, you will get a list of shifts per library. To automatically load them, you can use this function. Defaults to loading pshifts, if you made a-sites or e-sites, change the path argument to `ashifted/eshifted` folder instead.

**Usage**

```
shifts_load(
  df,
  path = file.path(libFolder(df), "pshifted", "shifting_table.rds")
)
```

**Arguments**

`df` an ORFik [experiment](#)

`path` path, default `file.path(libFolder(df), "pshifted", "shifting_table.rds")`. Path to .rds file containing the shifts as a list, one list element per shifted bam file.

**Value**

a list of the shifts, one list element per shifted bam file.

**See Also**

Other pshifting: [changePointAnalysis\(\)](#), [detectRibosomeShifts\(\)](#), [shiftFootprints\(\)](#), [shiftFootprintsByExperiment\(\)](#), [shiftPlots\(\)](#), [shifts\\_save\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
# subset on Ribo-seq
df <- df[df$libtype == "RFP",][[1,]
#shiftFootprintsByExperiment(df)
#shifts_load(df)
```

---

<code>shifts_save</code>	<i>Save shifts for Ribo-seq</i>
--------------------------	---------------------------------

---

**Description**

Should be stored in pshifted folder relative to default files

**Usage**

```
shifts_save(shifts, folder)
```

**Arguments**

`shifts` a list of data.table/data.frame objects. Must be named with the full path to ofst/bam files that defines the shifts.

`folder` directory to save file, Usually: `file.path(libFolder(df), "pshifted")`, where `df` is the ORFik experiment / or your path of default file types. It will be named `file.path(folder, "shifting_table.rds")`. For ORFik to work optimally, the folder should be the `/pshifted/` folder relative to default files.

**Value**

invisible(NULL), file saved to disc as "shifting\_table.rds".

**See Also**

Other pshifting: [changePointAnalysis\(\)](#), [detectRibosomeShifts\(\)](#), [shiftFootprints\(\)](#), [shiftFootprintsByExperiment\(\)](#), [shiftPlots\(\)](#), [shifts\\_load\(\)](#)

**Examples**

```
df <- ORFik.template.experiment.zf()
shifts <- shifts_load(df)
original_shifts <- file.path(libFolder(df), "pshifted", "shifting_table.rds")
# Move to temp
new_shifts_path <- file.path(tempdir(), "shifting_table.rds")
new_shifts <- c(shifts, shifts)
names(new_shifts)[2] <- file.path(tempdir(), "RiboSeqTemp.ofst")
saveRDS(new_shifts, new_shifts_path)
new_shifts[[1]][1,2] <- -10
# Now update the new shifts, here we input only first
shifts_save(new_shifts[1], tempdir())
readRDS(new_shifts_path) # You still get 2 outputs
```

---

show,covRle-method      *covRle show definition*

---

**Description**

Show a simplified version of the covRle

**Usage**

```
## S4 method for signature 'covRle'
show(object)
```

**Arguments**

object            [acovRle](#)

**Value**

print state of covRle

---

show,covRleList-method

*covRleList show definition*

---

### Description

Show a simplified version of the covRleList.

### Usage

```
## S4 method for signature 'covRleList'  
show(object)
```

### Arguments

object            [acovRleList](#)

### Value

print state of covRleList

---

show,experiment-method

*experiment show definition*

---

### Description

Show a simplified version of the experiment. The show function simplifies the view so that any column of data (like replicate or stage) is not shown, if all values are identical in that column. Filepaths are also never shown.

### Usage

```
## S4 method for signature 'experiment'  
show(object)
```

### Arguments

object            an ORFik [experiment](#)

### Value

print state of experiment

simpleLibs

*Converted format of NGS libraries***Description**

Export as either .ofst, .wig, .bigWig, .bedo (legacy format) or .bedoc (legacy format) files:

Export files as .ofst for fastest load speed into R.

Export files as .wig / bigWig for use in IGV or other genome browsers.

The input files are checked if they exist from: envExp(df).

**Usage**

```
simpleLibs(
  df,
  out.dir = libFolder(df),
  addScoreColumn = TRUE,
  addSizeColumn = TRUE,
  must.overlap = NULL,
  method = "None",
  type = "ofst",
  input.type = "ofst",
  reassign.when.saving = FALSE,
  envir = envExp(df),
  force = TRUE,
  library.names = bamVarName(df),
  libs = outputLibs(df, type = input.type, chrStyle = must.overlap, library.names =
    library.names, output.mode = "list", force = force, BPPARAM = BPPARAM),
  BPPARAM = bpparam()
)
```

**Arguments**

df	an ORFik <a href="#">experiment</a>
out.dir	optional output directory, default: libFolder(df), if it is NULL, it will just reassign R objects to simplified libraries. Will then create a final folder specified as: paste0(out.dir, "/", type, "/"). Here the files will be saved in format given by the type argument.
addScoreColumn	logical, default TRUE, if FALSE will not add replicate numbers as score column, see ORFik::convertToOneBasedRanges.
addSizeColumn	logical, default TRUE, if FALSE will not add size (width) as size column, see ORFik::convertToOneBasedRanges. Does not apply for (GAlignment version of.ofst) or .bedoc. Since they contain the original cigar.
must.overlap	default (NULL), else a GRanges / GRangesList object, so only reads that overlap (must.overlap) are kept. This is useful when you only need the reads over transcript annotation or subset etc.

method	character, default "None", the method to reduce ranges, for more info see <a href="#">convertToOneBasedRanges</a>
type	character, output format, default "ofst". Alternatives: "ofst", "bigWig", "wig", "bedo" or "bedoc". Which format you want. Will make a folder within out.dir with this name containing the files.
input.type	character, input type "ofst". Remember this function uses the loaded libraries if existing, so this argument is usually ignored. Only used if files do not already exist.
reassign.when.saving	logical, default FALSE. If TRUE, will reassign library to converted form after saving. Ignored when out.dir = NULL.
envir	environment to save to, default envExp(df), which defaults to .GlobalEnv, but can be set with envExp(df) <- new.env() etc.
force	logical, default TRUE. If TRUE, reload library files even if matching named variables are found in environment used by experiment (see <a href="#">envExp</a> ) A simple way to make sure correct libraries are always loaded. FALSE is faster if data is loaded correctly already.
library.names	character vector, names of libraries, default: name_decider(df, naming)
libs	list, output of outputLibs as list of GRanges/GAlignments/GAlignmentPairs objects. Set input.type and force arguments to define parameters.
BPPARAM	how many cores/threads to use? default: bpparam(). To see number of threads used, do bpparam()\$workers. You can also add a time remaining bar, for a more detailed pipeline.

## Details

We advice you to not use this directly, as other function are more safe for library type conversions. See family description below. This is mostly used internally in ORFik. It is only advised to use if large bam files are already loaded in R and conversions are wanted from those.

See [export.ofst](#), [export.wiggle](#), [export.bedo](#) and [export.bedoc](#) for information on file formats.

If libraries of the experiment are already loaded into environment (default: .globalEnv) is will export using those files as templates. If they are not in environment the .ofst files from the bam files are loaded (unless you are converting to .ofst then the .bam files are loaded).

## Value

invisible NULL (saves files to disc or R .GlobalEnv)

## See Also

Other lib\_converters: [convert\\_bam\\_to\\_ofst\(\)](#), [convert\\_to\\_bigWig\(\)](#), [convert\\_to\\_covRle\(\)](#), [convert\\_to\\_covRleList\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
#convertLibs(df, out.dir = NULL)
# Keep only 5' ends of reads
#convertLibs(df, out.dir = NULL, method = "5prime")
```

---

sortPerGroup	<i>Sort a GRangesList</i>
--------------	---------------------------

---

**Description**

A faster, more versatile reimplementaion of `sort.GenomicRanges` for `GRangesList`, needed since the original works poorly for more than 10k groups. This function sorts each group, where "+" strands are increasing by starts and "-" strands are decreasing by ends.

**Usage**

```
sortPerGroup(grl, ignore.strand = FALSE, quick.rev = FALSE)
```

**Arguments**

<code>grl</code>	a <a href="#">GRangesList</a>
<code>ignore.strand</code>	a boolean, (default FALSE): should minus strands be sorted from highest to lowest ends. If TRUE: from lowest to highest ends.
<code>quick.rev</code>	default: FALSE, if TRUE, given that you know all ranges are sorted from min to max for both strands, it will only reverse coordinates for minus strand groups, and only if they are in increasing order. Much quicker

**Details**

Note: will not work if groups have equal names.

**Value**

an equally named `GRangesList`, where each group is sorted within group.

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(14, 7), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(1, 4), c(3, 9)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
sortPerGroup(grl)
```

---

STAR.align.folder	<i>Align all libraries in folder with STAR</i>
-------------------	--

---

### Description

Does either all files as paired end or single end, so if you have mix, split them in two different folders.

If STAR halts at .... loading genome, it means the STAR index was aborted early, then you need to run: STAR.remove.crashed.genome(), with the genome that crashed, and rerun.

### Usage

```
STAR.align.folder(  
  input.dir,  
  output.dir,  
  index.dir,  
  star.path = STAR.install(),  
  fastp = install.fastp(),  
  paired.end = FALSE,  
  steps = "tr-ge",  
  adapter.sequence = "auto",  
  quality.filtering = FALSE,  
  min.length = 20,  
  mismatches = 3,  
  trim.front = 0,  
  trim.tail = 0,  
  max.multimap = 10,  
  alignment.type = "Local",  
  allow.introns = TRUE,  
  max.cpus = min(90, BiocParallel::bpparam()$workers),  
  include.subfolders = "n",  
  resume = NULL,  
  multiQC = TRUE,  
  keep.contaminants = FALSE,  
  keep.contaminants.type = c("bam", "fastq")[1],  
  keep.unaligned.genome = FALSE,  
  keep.index.in.memory = FALSE,  
  script.folder = system.file("STAR_Aligner", "RNA_Align_pipeline_folder.sh", package =  
    "ORFik"),  
  script.single = system.file("STAR_Aligner", "RNA_Align_pipeline.sh", package = "ORFik")  
)
```

### Arguments

input.dir	path to fast files to align, the valid input files will be search for from formats: (".fasta", ".fastq", ".fq", or ".fa") with or without compression of .gz. Also either paired end or single end reads. Pairs will automatically be detected from
-----------	---

similarity of naming, separated by something as .1 and .2 in the end. If files are renamed, where pairs are not similarly named, this process will fail to find correct pairs!

output.dir	directory to save indices, default: <code>paste0(dirname(arguments[1]), "/STAR_index/")</code> , where arguments is the arguments input for this function.
index.dir	path to STAR index folder. Path returned from ORFik function <code>STAR.index</code> , when you created the index folders.
star.path	path to STAR, default: <code>STAR.install()</code> , if you don't have STAR installed at default location, it will install it there, set path to a runnable star if you already have it.
fastp	path to fastp trimmer, default: <code>install.fastp()</code> , if you have it somewhere else already installed, give the path. Only works for unix (linux or Mac OS), if not on unix, use your favorite trimmer and give the output files from that trimmer as <code>input.dir</code> here.
paired.end	a logical: default FALSE, alternative TRUE. If TRUE, will auto detect pairs by names. Can not be a combination of both TRUE and FALSE! If running in folder mode: The folder must then contain an even number of files and they must be named with the same prefix and suffix of either <code>_1</code> and <code>_2</code> , <code>1</code> and <code>2</code> , etc. If SRR numbers are used, it will start on lowest and match with second lowest etc.
steps	a character, default: "tr-ge", trimming then genome alignment steps of depletion and alignment wanted: The possible candidates you can use are: <ul style="list-style-type: none"> <li>• tr : trim reads</li> <li>• co : contamination merged depletion</li> <li>• ph : phix depletion</li> <li>• rR : rRNA depletion</li> <li>• nc : ncRNA depletion</li> <li>• tR : tRNA depletion (Mature tRNA, so no intron checks done)</li> <li>• ge : genome alignment</li> <li>• all: run steps: "tr-co-ge" or "tr-ph-rR-nc-tR-ge", depending on if you have merged contaminants or not</li> </ul> <p>If not "all", a subset of these ("tr-co-ph-rR-nc-tR-ge") If co (merged contaminants) is used, non of the specific contaminants can be specified, since they should be a subset of co. The step where you align to the genome is usually always included, unless you are doing pure contaminant analysis or only trimming. For Ribo-seq and TCP(RCP-seq) you should do rR (ribosomal RNA depletion), so when you made the STAR index you need the rRNA step, either use rRNA from <code>.gtf</code> or manual download. (usually just download a Silva rRNA database for SSU&amp;LSU at: <a href="https://www.arb-silva.de/">https://www.arb-silva.de/</a>) for your species.</p>
adapter.sequence	character, default: "auto". Auto detect adapter using fastp adapter auto detection, checking first 1.5M reads. (Auto detection of adapter will not work 100%

of the time (if the library is of low quality), then you must rerun this function with specified adapter from fastp adapter analysis. , using FASTQC or other adapter detection tools, else alignment will most likely fail!). If already trimmed or trimming not wanted: adapter.sequence = "disable" .You can manually assign adapter like: "ATCTCGTATGCCGTCTTCTGCTTG" or "AAAAAAAAAAAAA". You can also specify one of the three presets:

- illumina (TrueSeq ~75/100 bp sequencing) : AGATCGGAAGAGC
- small\_RNA (standard for ~50 bp sequencing): TGGAAATTCTCGG
- nextera: CTGTCTCTTATA

Paired end auto detection uses overlap sequence of pairs, to use the slower more secure paired end adapter detection, specify as: "autoPE".

quality.filtering	logical, default FALSE. Not needed for modern library prep of RNA-seq, Ribo-seq etc (usually < ~ 0.5 If you are aligning bad quality data, set this to TRUE. These filters will then be applied (default of fastp), filter if: <ul style="list-style-type: none"> <li>• Number of N bases in read : &gt; 5</li> <li>• Read quality : &gt; 40% of bases in the read are &lt;Q15</li> </ul>
min.length	20, minimum length of aligned read without mismatches to pass filter. Anything under 20 is dangerous, as chance of random hits will become high!
mismatches	3, max non matched bases. Excludes soft-clipping, this only filters reads that have defined mismatches in STAR. Only applies for genome alignment step.
trim.front	0, default trim 0 bases on 5' ends. Ignored if tr (trim) is not one of the arguments in "steps". For Ribo-seq use default 0, unless you have 5' end custom barcodes to remove. Alignment to STAR might fail if you have large barcodes, which are not removed!
trim.tail	0, default trim 0 bases on 3' ends. Ignored if tr (trim) is not one of the arguments in "steps". For Ribo-seq use default 0, unless you have 3' end custom barcodes to remove. Alignment to STAR might fail if you have large barcodes, which are not removed!
max.multimap	numeric, default 10. If a read maps to more locations than specified, will skip the read. Set to 1 to only get unique mapping reads. Only applies for genome alignment step. The depletions are allowing for multimapping.
alignment.type	default: "Local": standard local alignment with soft-clipping allowed, "End-ToEnd" (global): force end-to-end read alignment, does not soft-clip.
allow.introns	logical, default TRUE. Allow large gaps of N in reads during genome alignment, if FALSE: sets -alignIntronMax to 1 (no introns). NOTE: You will still get some spliced reads if you assigned a gtf at the index step.
max.cpus	integer, default: min(90, BiocParallel:::bpparam()\$workers), number of threads to use. Default is minimum of 90 and maximum cores - 2. So if you have 8 cores it will use 6. Note: FASTP will use maximum 16 threads as from testing I see performance actually degrades using anything higher. From testing I also see STAR gets no performance gain after ~50 threads. I do suspect this will change when hard drives gets better in the future.

include.subfolders	"n" (no), do recursive search downwards for fast files if "y".
resume	default: NULL, continue from step, lets say steps are "tr-ph-ge": (trim, phix depletion, genome alignment) and resume is "ge", you will then use the assumed already trimmed and phix depleted data and start at genome alignment, useful if something crashed. Like if you specified wrong STAR version, but the trimming step was completed. Resume mode can only run 1 step at the time.
multiQC	logical, default TRUE. Do mutliQC comparison of STAR alignment between all the samples. Outputted in aligned/LOGS folder. See ?STAR.multiQC
keep.contaminants	logical, default FALSE. Create and keep contaminant aligning bam files, default is to only keep unaliged fastq reads, which will be further processed in "ge" genome alignment step. Useful if you want to do further processing on contaminants, like specific coverage of specific tRNAs etc.
keep.contaminants.type	logical, default "bam". If aligned files of contaminants are kept, which format to output as, only supports "bam" for now. Fasta / Fastq will be implemented later.
keep.unaligned.genome	logical, default FALSE. Create and keep reads that did not align at the genome alignment step, default is to only keep the aliged bam file. Useful if you want to do further processing on plasmids/custom sequences.
keep.index.in.memory	logical or character, default FALSE (i.e. LoadAndRemove). For STAR.align.single: If TRUE, will keep index in memory, useful if you need to loop over single calls, instead of using STAR.align.folder (remember last run should use FALSE, to remove index). For STAR.align.folder: Only applies to last library, will always keep for all libraries before last. Alternative useful for MAC machines especially is "noShared", for machines that do not support shared memory index, usually gives error: "abort trap 6".
script.folder	location of STAR index script, default internal ORFik file. You can change it and give your own if you need special alignments.
script.single	location of STAR single file alignment script, default internal ORFik file. You can change it and give your own if you need special alignments.

## Details

Can only run on unix systems (Linux, Mac and WSL (Windows Subsystem Linux)), and requires a minimum of 30GB memory on genomes like human, rat, zebrafish etc.

If for some reason the internal STAR alignment bash script will not work for you, like if you want more customization of the STAR/fastp arguments. You can copy the internal alignment script, edit it and give that as the script used for this function.

The trimmer used is fastp (the fastest I could find), also works on (Linux, Mac and WSL (Windows Subsystem Linux)). If you want to use your own trimmer set file1/file2 to the location of the trimmed files from your program.

A note on trimming from creator of STAR about trimming: "adapter trimming it definitely needed for short RNA sequencing. For long RNA-seq, I would agree with Devon that in most cases adapter trimming is not advantageous, since, by default, STAR performs local (not end-to-end) alignment, i.e. it auto-trims." So trimming can be skipped for longer reads.

**Value**

output.dir, can be used as as input in ORFik::create.experiment

**See Also**

Other STAR: [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
# First specify directories wanted (temp directory here)
config_file <- tempfile()
#config.save(config_file, base.dir = tempdir())
#config <- ORFik::config(config_file)

## Yeast RNA-seq samples (small genome)
#project <- ORFik::config.exper("chalmers_2012", "Saccharomyces_cerevisiae", "RNA-seq", config)
#annotation.dir <- project["ref"]
#fastq.input.dir <- project["fastq RNA-seq"]
#bam.output.dir <- project["bam RNA-seq"]

## Download some SRA data and metadata (subset to 50k reads)
# info <- download.SRA.metadata("SRP012047", outdir = conf["fastq RNA-seq"])
# info <- info[1:2,] # Subset to 2 first libraries
# download.SRA(info, fastq.input.dir, rename = FALSE, subset = 50000)

## No contaminant depletion:
# annotation <- getGenomeAndAnnotation("Saccharomyces cerevisiae", annotation.dir)
# index <- STAR.index(annotation)
# STAR.align.folder(fastq.input.dir, bam.output.dir,
#                  index, paired.end = FALSE) # Trim, then align to genome

## Human Ribo-seq sample (NB! very large genome and libraries!)
## Requires >= 32 GB memory
#project <- ORFik::config.exper("subtelny_2014", "Homo_sapiens", "Ribo-seq", config)
#annotation.dir <- project["ref"]
#fastq.input.dir <- project["fastq Ribo-seq"]
#bam.output.dir <- project["bam Ribo-seq"]

## Download some SRA data and metadata (full libraries)
# info <- download.SRA.metadata("DRR041459", fastq.input.dir)
# download.SRA(info, fastq.input.dir, rename = FALSE)
## Now align 2 different ways, without and with contaminant depletion

## No contaminant depletion:
# annotation <- getGenomeAndAnnotation("Homo sapiens", annotation.dir)
# index <- STAR.index(annotation)
# STAR.align.folder(fastq.input.dir, bam.output.dir,
#                  index, paired.end = FALSE)

## All contaminants merged:
# annotation <- getGenomeAndAnnotation(
```

```

# organism = "Homo_sapiens",
# phix = TRUE, ncRNA = TRUE, tRNA = TRUE, rRNA = TRUE,
# output.dir = annotation.dir
# )
# index <- STAR.index(annotation)
# STAR.align.folder(fastq.input.dir, bam.output.dir,
#                   index, paired.end = FALSE,
#                   steps = "tr-ge")

```

---

STAR.align.single      *Align single or paired end pair with STAR*

---

### Description

Given a single NGS fastq/fasta library, or a paired setup of 2 mated libraries. Run either combination of fastq trimming, contamination removal and genome alignment. Works for (Linux, Mac and WSL (Windows Subsystem Linux))

### Usage

```

STAR.align.single(
  file1,
  file2 = NULL,
  output.dir,
  index.dir,
  star.path = STAR.install(),
  fastp = install.fastp(),
  steps = "tr-ge",
  adapter.sequence = "auto",
  quality.filtering = FALSE,
  min.length = 20,
  mismatches = 3,
  trim.front = 0,
  trim.tail = 0,
  max.multimap = 10,
  alignment.type = "Local",
  allow.introns = TRUE,
  max.cpus = min(90, BiocParallel::bpparam()$workers),
  resume = NULL,
  multiQC = FALSE,
  keep.contaminants = FALSE,
  keep.unaligned.genome = FALSE,
  keep.index.in.memory = FALSE,
  script.single = system.file("STAR_Aligner", "RNA_Align_pipeline.sh", package = "ORFik")
)

```

**Arguments**

file1	library file, if paired must be R1 file. Allowed formats are: (.fasta, .fastq, .fq, or.fa) with or without compression of .gz. This filename usually contains a suffix of .1
file2	default NULL, set if paired end to R2 file. Allowed formats are: (.fasta, .fastq, .fq, or.fa) with or without compression of .gz. This filename usually contains a suffix of .2
output.dir	directory to save indices, default: paste0(dirname(arguments[1]), "/STAR_index/"), where arguments is the arguments input for this function.
index.dir	path to STAR index folder. Path returned from ORFik function STAR.index, when you created the index folders.
star.path	path to STAR, default: STAR.install(), if you don't have STAR installed at default location, it will install it there, set path to a runnable star if you already have it.
fastp	path to fastp trimmer, default: install.fastp(), if you have it somewhere else already installed, give the path. Only works for unix (linux or Mac OS), if not on unix, use your favorite trimmer and give the output files from that trimmer as input.dir here.
steps	<p>a character, default: "tr-ge", trimming then genome alignment  steps of depletion and alignment wanted: The possible candidates you can use are:</p> <ul style="list-style-type: none"> <li>• tr : trim reads</li> <li>• co : contamination merged depletion</li> <li>• ph : phix depletion</li> <li>• rR : rRNA depletion</li> <li>• nc : ncRNA depletion</li> <li>• tR : tRNA depletion (Mature tRNA, so no intron checks done)</li> <li>• ge : genome alignment</li> <li>• all: run steps: "tr-co-ge" or "tr-ph-rR-nc-tR-ge", depending on if you have merged contaminants or not</li> </ul> <p>If not "all", a subset of these ("tr-co-ph-rR-nc-tR-ge")  If co (merged contaminants) is used, non of the specific contaminants can be specified, since they should be a subset of co.  The step where you align to the genome is usually always included, unless you are doing pure contaminant analysis or only trimming. For Ribo-seq and TCP(RCP-seq) you should do rR (ribosomal RNA depletion), so when you made the STAR index you need the rRNA step, either use rRNA from .gtf or manual download. (usually just download a Silva rRNA database for SSU&amp;LSU at: <a href="https://www.arb-silva.de/">https://www.arb-silva.de/</a>) for your species.</p>
adapter.sequence	character, default: "auto". Auto detect adapter using fastp adapter auto detection, checking first 1.5M reads. (Auto detection of adapter will not work 100% of the time (if the library is of low quality), then you must rerun this function

with specified adapter from fastp adapter analysis. , using FASTQC or other adapter detection tools, else alignment will most likely fail!). If already trimmed or trimming not wanted: adapter.sequence = "disable" .You can manually assign adapter like: "ATCTCGTATGCCGTCTTCTGCTTG" or "AAAAAAAAAAAAA". You can also specify one of the three presets:

- illumina (TrueSeq ~75/100 bp sequencing) : AGATCGGAAGAGC
- small\_RNA (standard for ~50 bp sequencing): TGGAATTCTCGG
- nextera: CTGTCTCTTATA

Paired end auto detection uses overlap sequence of pairs, to use the slower more secure paired end adapter detection, specify as: "autoPE".

quality.filtering	logical, default FALSE. Not needed for modern library prep of RNA-seq, Ribo-seq etc (usually < ~ 0.5 If you are aligning bad quality data, set this to TRUE. These filters will then be applied (default of fastp), filter if: <ul style="list-style-type: none"> <li>• Number of N bases in read : &gt; 5</li> <li>• Read quality : &gt; 40% of bases in the read are &lt;Q15</li> </ul>
min.length	20, minimum length of aligned read without mismatches to pass filter. Anything under 20 is dangerous, as chance of random hits will become high!
mismatches	3, max non matched bases. Excludes soft-clipping, this only filters reads that have defined mismatches in STAR. Only applies for genome alignment step.
trim.front	0, default trim 0 bases on 5' ends. Ignored if tr (trim) is not one of the arguments in "steps". For Ribo-seq use default 0, unless you have 5' end custom barcodes to remove. Alignment to STAR might fail if you have large barcodes, which are not removed!
trim.tail	0, default trim 0 bases on 3' ends. Ignored if tr (trim) is not one of the arguments in "steps". For Ribo-seq use default 0, unless you have 3' end custom barcodes to remove. Alignment to STAR might fail if you have large barcodes, which are not removed!
max.multimap	numeric, default 10. If a read maps to more locations than specified, will skip the read. Set to 1 to only get unique mapping reads. Only applies for genome alignment step. The depletions are allowing for multimapping.
alignment.type	default: "Local": standard local alignment with soft-clipping allowed, "End-ToEnd" (global): force end-to-end read alignment, does not soft-clip.
allow.introns	logical, default TRUE. Allow large gaps of N in reads during genome alignment, if FALSE: sets -alignIntronMax to 1 (no introns). NOTE: You will still get some spliced reads if you assigned a gtf at the index step.
max.cpus	integer, default: min(90, BiocParallel::bpparam()\$workers), number of threads to use. Default is minimum of 90 and maximum cores - 2. So if you have 8 cores it will use 6. Note: FASTP will use maximum 16 threads as from testing I see performance actually degrades using anything higher. From testing I also see STAR gets no performance gain after ~50 threads. I do suspect this will change when hard drives gets better in the future.

resume	default: NULL, continue from step, lets say steps are "tr-ph-ge": (trim, phix depletion, genome alignment) and resume is "ge", you will then use the assumed already trimmed and phix depleted data and start at genome alignment, useful if something crashed. Like if you specified wrong STAR version, but the trimming step was completed. Resume mode can only run 1 step at the time.
multiQC	logical, default TRUE. Do mutliQC comparison of STAR alignment between all the samples. Outpouted in aligned/LOGS folder. See ?STAR.multiQC
keep.contaminants	logical, default FALSE. Create and keep contaminant aligning bam files, default is to only keep unaligned fastq reads, which will be further processed in "ge" genome alignment step. Useful if you want to do further processing on contaminants, like specific coverage of specific tRNAs etc.
keep.unaligned.genome	logical, default FALSE. Create and keep reads that did not align at the genome alignment step, default is to only keep the aliged bam file. Useful if you want to do further processing on plasmids/custom sequences.
keep.index.in.memory	logical or character, default FALSE (i.e. LoadAndRemove). For STAR.align.single: If TRUE, will keep index in memory, useful if you need to loop over single calls, instead of using STAR.align.folder (remember last run should use FALSE, to remove index). For STAR.align.folder: Only applies to last library, will always keep for all libraries before last. Alternative useful for MAC machines especially is "noShared", for machines that do not support shared memory index, usually gives error: "abort trap 6".
script.single	location of STAR single file alignment script, default internal ORFik file. You can change it and give your own if you need special alignments.

## Details

Can only run on unix systems (Linux, Mac and WSL (Windows Subsystem Linux)), and requires a minimum of 30GB memory on genomes like human, rat, zebrafish etc.

If for some reason the internal STAR alignment bash script will not work for you, like if you want more customization of the STAR/fastp arguments. You can copy the internal alignment script, edit it and give that as the script used for this function.

The trimmer used is fastp (the fastest I could find), also works on (Linux, Mac and WSL (Windows Subsystem Linux)). If you want to use your own trimmer set file1/file2 to the location of the trimmed files from your program.

A note on trimming from creator of STAR about trimming: "adapter trimming it definitely needed for short RNA sequencing. For long RNA-seq, I would agree with Devon that in most cases adapter trimming is not advantageous, since, by default, STAR performs local (not end-to-end) alignment, i.e. it auto-trims." So trimming can be skipped for longer reads.

## Value

output.dir, can be used as as input in ORFik::create.experiment

**See Also**

Other STAR: [STAR.align.folder\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
## Specify output libraries (using temp config)
config_file <- tempfile()
#config.save(config_file, base.dir = tempdir())
#config <- ORFik::config(config_file)
#project <- ORFik::config.exper("yeast_1", "Saccharomyces_cerevisiae", "RNA-seq", config)
# Get genome of yeast (quite small)
# arguments <- getGenomeAndAnnotation("Saccharomyces cerevisiae", project["ref"])
# index <- STAR.index(arguments)

## Make fake reads
#genome <- readDNASTringSet(arguments["genome"])
#which_chromosomes <- sample(seq_along(genome), 1000, TRUE, prob = width(genome))
#nt50_windows <- lapply(which_chromosomes, function(x)
# {window <- sample(width(genome[x]) - 51, 1); genome[[x]][seq(window, window+49)]})
#nt50_windows <- DNASTringSet(nt50_windows)
#names(nt50_windows) <- paste0("read_", seq_along(nt50_windows))
#dir.create(project["fastq RNA-seq"], recursive = TRUE)
#fake_fasta <- file.path(project["fastq RNA-seq"], "fake-RNA-seq.fasta")
#writeXStringSet(nt50_windows, fake_fasta, format = "fasta")
## Align the fake reads and import bam
# STAR.align.single(fake_fasta, NULL, project["bam RNA-seq"], index, steps = "ge")
#bam_file <- list.files(file.path(project["bam RNA-seq"], "aligned"),
# pattern = "\.bam$", full.names = TRUE)
#fimport(bam_file)
```

---

STAR.allsteps.multiQC *Create STAR multiQC plot and table*

---

**Description**

Takes a folder with multiple Log.final.out files from STAR, and create a multiQC report. This is automatically run with STAR.align.folder function.

**Usage**

```
STAR.allsteps.multiQC(
  folder,
  steps = "auto",
  plot.ext = ".pdf",
  output.file = file.path(folder, "full_process.csv")
)
```

**Arguments**

folder	path to main output folder of STAR run. The folder that contains /aligned/, /trim/, "contaminants_depletion" etc. To find the LOGS folders in, to use for summarized statistics.
steps	a character, default "auto". Find which steps you did. If manual, a combination of "tr-co-ge". See STAR alignment functions for description.
plot.ext	character, default ".pdf". Which format to save QC plot. Alternative: ".png".
output.file	character, file path, default: file.path(folder, "full_process.csv")

**Value**

data.table of main statistics, plots and data saved to disc. Named: "/00\_STAR\_LOG\_plot.pdf" and "/00\_STAR\_LOG\_table.csv"

**See Also**

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
process_dir <- system.file("extdata/test_processing/", package = "ORFik")
STAR.allsteps.multiQC(process_dir)
STAR.allsteps.multiQC(process_dir, steps = "tr-ge")
```

---

STAR.index	<i>Create STAR genome index</i>
------------	---------------------------------

---

**Description**

Used as reference when aligning data  
Get genome and gtf by running `getGenomeAndFasta()`

**Usage**

```
STAR.index(
  arguments,
  output.dir = paste0(dirname(arguments[1]), "/STAR_index/"),
  star.path = STAR.install(),
  max.cpus = min(90, BiocParallel::bpparam()$workers),
  max.ram = 30,
  SAsparse = 1,
  tmpDirStar = "-",
  remake = FALSE,
  script = system.file("STAR_Aligner", "STAR_MAKE_INDEX.sh", package = "ORFik"),
  notify_load_existing = TRUE
)
```

**Arguments**

arguments	a named character vector containing paths wanted to use for index creation. They must be named correctly: names must be a subset of: c("gtf", "genome", "contaminants", "phix", "rRNA", "tRNA", "ncRNA")
output.dir	directory to save indices, default: paste0(dirname(arguments[1]), "/STAR_index/"), where arguments is the arguments input for this function.
star.path	path to STAR, default: STAR.install(), if you don't have STAR installed at default location, it will install it there, set path to a runnable star if you already have it.
max.cpus	integer, default: min(90, BiocParallel::bpparam()\$workers), number of threads to use. Default is minimum of 90 and maximum cores - 2. So if you have 8 cores it will use 6. Note: FASTP will use maximum 16 threads as from testing I see performance actually degrades using anything higher. From testing I also see STAR gets no performance gain after ~50 threads. I do suspect this will change when hard drives gets better in the future.
max.ram	integer, default 30, in Giga Bytes (GB). Maximum amount of RAM allowed for STAR limitGenomeGenerateRAM argument. RULE: ideally 10x genome size, but do not set too close to machine limit. Default fits well for human genome size (3 GB * 10 = 30 GB)
SAsparse	int > 0, default 1. If you do not have at least 64GB RAM, you might need to set this to 2. suffix array sparsity, i.e. distance between indices: use bigger numbers to decrease needed RAM at the cost of mapping speed reduction. Only applies to genome, not conaminants.
tmpDirStar	character, default "-". STAR automatic temp folder creation, deleted when done. The directory can not exists, as a safety STAR must make it!. If you are on a NFS file share drive, and you have a non NFS tmp dir, set this to tempfile() or the manually specified folder to get a considerable speedup!
remake	logical, default: FALSE, if TRUE remake everything specified
script	location of STAR index script, default internal ORFik file. You can change it and give your own if you need special alignments.
notify_load_existing	logical, default TRUE. If annotation exists (defined as: locally (a file called outputs.rds) exists in outputdir), print a small message notifying the user it is not redownloading. Set to FALSE, if this is not wanted

**Details**

Can only run on unix systems (Linux and Mac), and requires minimum 30GB memory on genomes like human, rat, zebrafish etc.

If for some reason the internal STAR index bash script will not work for you, like if you have a very small genome. You can copy the internal index script, edit it and give that as the Index script used for this function. It is recommended to run through the RStudio local job tab, to give full info about the run. The system console will not stall, as can happen in happen in normal RStudio console.

**Value**

output.dir, can be used as as input for STAR.align..

## See Also

Other STAR: `STAR.align.folder()`, `STAR.align.single()`, `STAR.allsteps.multiQC()`, `STAR.install()`, `STAR.multiQC()`, `STAR.remove.crashed.genome()`, `getGenomeAndAnnotation()`, `install.fastp()`

## Examples

```
## Manual way, specify all paths yourself.
#arguments <- c(path.GTF, path.genome, path.phix, path.rrna, path.trna, path.ncrna)
#names(arguments) <- c("gtf", "genome", "phix", "rRNA", "tRNA", "ncRNA")
#STAR.index(arguments, "output.dir")

## Or use ORFik way:
output.dir <- "/Bio_data/references/Human"
# arguments <- getGenomeAndAnnotation("Homo sapiens", output.dir)
# STAR.index(arguments, output.dir)
```

---

STAR.install

*Download and prepare STAR*

---

## Description

Will not run "make", only use precompiled STAR file.

Can only run on unix systems (Linux and Mac), and requires minimum 30GB memory on genomes like human, rat, zebrafish etc.

## Usage

```
STAR.install(folder = "~/bin", version = "2.7.4a")
```

## Arguments

folder	path to folder for download, file will be named "STAR-version", where version is version wanted.
version	default "2.7.4a"

## Details

ORFik for now only uses precompiled STAR binaries, so if you already have a STAR version it is advised to redownload the same version, since STAR genome indices usually does not work between STAR versions.

## Value

path to runnable STAR

## References

<https://www.ncbi.nlm.nih.gov/pubmed/23104886>

**See Also**

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.multiQC\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
## Default folder install:
#STAR.install()
## Manual set folder:
folder <- "/I/WANT/IT/HERE"
#STAR.install(folder, version = "2.7.4a")
```

---

STAR.multiQC

*Create STAR multiQC plot and table*


---

**Description**

Takes a folder with multiple Log.final.out files from STAR, and create a multiQC report

**Usage**

```
STAR.multiQC(
  folder,
  type = "aligned",
  plot.ext = ".pdf",
  log_files = dir(folder, "Log.final.out", full.names = TRUE),
  simplified_table = TRUE
)
```

**Arguments**

folder	path to LOGS folder of ORFik STAR runs. Can also be the path to the aligned/ (parent directory of LOGS), then it will move into LOG from there. Only if no files with pattern Log.final.out are found in parent directory. If no LOGS folder is found it can check for a folder /aligned/LOGS/ so to go 2 folders down.
type	a character path, default "aligned". Which subfolder to check for. If you want log files for contamination do type = "contaminants_depletion"
plot.ext	character, default ".pdf". Which format to save QC plot. Alternative: ".png".
log_files	character, path to "Log.final.out" STAR files, default: dir(folder, "Log.final.out", full.names = TRUE)
simplified_table	logical, default TRUE. Subset columns, to the most important ones.

**Value**

a data.table with all information from STAR runs, plot and data saved to disc. Named: "/00\_STAR\_LOG\_plot.pdf" and "/00\_STAR\_LOG\_table.csv"

**See Also**

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.remove.crashed.genome\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
#' @examples
process_dir <- system.file("extdata/test_processing/", package = "ORFik")
STAR.multiQC(process_dir)
```

---

```
STAR.remove.crashed.genome
```

*Remove crashed STAR genome*

---

**Description**

This happens if you abort STAR run early, and it halts at: ..... loading genome

**Usage**

```
STAR.remove.crashed.genome(index.path, star.path = STAR.install())
```

**Arguments**

<code>index.path</code>	path to index folder of genome
<code>star.path</code>	path to STAR, default: <code>STAR.install()</code> , if you don't have STAR installed at default location, it will install it there, set path to a runnable star if you already have it.

**Value**

return value from system call, 0 if all good.

**See Also**

Other STAR: [STAR.align.folder\(\)](#), [STAR.align.single\(\)](#), [STAR.allsteps.multiQC\(\)](#), [STAR.index\(\)](#), [STAR.install\(\)](#), [STAR.multiQC\(\)](#), [getGenomeAndAnnotation\(\)](#), [install.fastp\(\)](#)

**Examples**

```
index.path = "/home/data/human_GRCh38/STAR_INDEX/genomeDir/"
# STAR.remove.crashed.genome(index.path = index.path)
## If you have the index argument from STAR.index function:
# index.path <- STAR.index()
# STAR.remove.crashed.genome(file.path(index.path, "genomeDir"))
# STAR.remove.crashed.genome(file.path(index.path, "contaminants_genomeDir"))
```

---

start, GRanges-method    *Get starts of GRanges*

---

### Description

Faster version than S4Vector generic caller

### Usage

```
## S4 method for signature 'GRanges'
start(x)
```

### Arguments

x                    a GRanges

### Value

an integer (length equal to x)

---

startCodons            *Get the Start codons(3 bases) from a GRangesList of orfs grouped by orfs*

---

### Description

In ATGTTTTGA, get the positions ATG. It takes care of exons boundaries, with exons < 3 length.

### Usage

```
startCodons(grl, is.sorted = FALSE)
```

### Arguments

grl                    a [GRangesList](#) object  
 is.sorted            a boolean, a speedup if you know the ranges are sorted

### Value

a GRangesList of start codons, since they might be split on exons

### See Also

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

## Examples

```
gr_plus <- GRanges(seqnames = "chr1",
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = "+")
gr_minus <- GRanges(seqnames = "chr2",
                  ranges = IRanges(c(4, 1), c(9, 3)),
                  strand = "-")
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
startCodons(grl, is.sorted = FALSE)
```

---

startDefinition	Returns start codon definitions
-----------------	---------------------------------

---

## Description

According to: <<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1>> ncbi genetic code number for translation. This version is a cleaned up version, unknown indices removed.

## Usage

```
startDefinition(transl_table)
```

## Arguments

transl\_table    numeric. NCBI genetic code number for translation.

## Value

A string of START sites separated with "|".

## See Also

Other findORFs: [findMapORFs\(\)](#), [findORFs\(\)](#), [findORFsFasta\(\)](#), [findUORFs\(\)](#), [stopDefinition\(\)](#)

## Examples

```
startDefinition
startDefinition(1)
```

---

startRegion	<i>Start region as GRangesList</i>
-------------	------------------------------------

---

### Description

Get the start region of each ORF. If you want the start codon only, set `upstream = 0` or just use [startCodons](#). Standard is 2 upstream and 2 downstream, a width 5 window centered at start site. since p-shifting is not 100 usually the reads from the start site.

### Usage

```
startRegion(grl, tx = NULL, is.sorted = TRUE, upstream = 2L, downstream = 2L)
```

### Arguments

<code>grl</code>	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs
<code>tx</code>	default NULL, a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all grl names. The names of grl can also be the ORFik orf names. that is "txName_id"
<code>is.sorted</code>	logical (TRUE), is grl sorted.
<code>upstream</code>	an integer (2), relative region to get upstream from.
<code>downstream</code>	an integer (2), relative region to get downstream from

### Details

If tx is null, then upstream will be forced to 0 and downstream to a maximum of grl width (3' UTR end for mRNAs). Since there is no reference for splicing.

### Value

a [GRanges](#), or [GRangesList](#) object if any group had > 1 exon.

### See Also

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

### Examples

```
## ORF start region
orf <- GRangesList(tx1 = GRanges("1", 200:300, "+"))
tx <- GRangesList(tx1 = GRanges("1",
                               IRanges(c(100, 200), c(195, 400)), "+"))
startRegion(orf, tx, upstream = 6, downstream = 6)
```

```
## 2nd codon of ORF
startRegion(orf, tx, upstream = -3, downstream = 6)
```

---

```
startRegionCoverage  Start region coverage
```

---

## Description

Get the number of reads in the start region of each ORF. If you want the start codon coverage only, set upstream = 0. Standard is 2 upstream and 2 downstream, a width 5 window centered at start site. since p-shifting is not 100 start site.

## Usage

```
startRegionCoverage(
  grl,
  RFP,
  tx = NULL,
  is.sorted = TRUE,
  upstream = 2L,
  downstream = 2L,
  weight = 1L
)
```

## Arguments

grl	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs
RFP	ribo seq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
tx	default NULL, a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all grl names. The names of grl can also be the ORFik orf names. that is "txName_id"
is.sorted	logical (TRUE), is grl sorted.
upstream	an integer (2), relative region to get upstream from.
downstream	an integer (2), relative region to get downstream from
weight	a numeric/integer vector or metacolumn name. (default: 1L, no differential weighting). If weight is name of defined meta column in reads object, it gives the number of times a read was found at that position. <a href="#">GRanges</a> ("chr1", 1, "+", score = 5), would mean "score" column tells that this alignment region was found 5 times. if 1L it means each read is weighted equal as 1, this is what among others <a href="#">countOverlaps()</a> presumes, if single number (!= 1), it repeats for all ranges, if vector with length > 1, it must be equal size of the reads object.

## Details

If tx is null, then upstream will be force to 0 and downstream to a maximum of grl width. Since there is no reference for splicing.

**Value**

a numeric vector of counts

**See Also**

Other features: `computeFeatures()`, `computeFeaturesCage()`, `countOverlapsW()`, `disengagementScore()`, `distToCds()`, `distToTSS()`, `entropy()`, `floss()`, `fpkm()`, `fpkm_calc()`, `fractionLength()`, `initiationScore()`, `insideOutsideORF()`, `isInFrame()`, `isOverlapping()`, `kozakSequenceScore()`, `orfScore()`, `rankOrder()`, `ribosomeReleaseScore()`, `ribosomeStallingScore()`, `startRegion()`, `stopRegion()`, `subsetCoverage()`, `translationalEff()`

**Examples**

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(21, 40),
               strand = "+")
names(ORF) <- c("tx1")
gr1 <- GRangesList(tx1 = ORF)
tx <- extendLeaders(gr1, 20)
# 1 width p-shifted reads
reads <- GRanges("1", IRanges(c(21, 23, 50, 50, 50, 53, 53, 56, 59),
                              width = 1), "+")
score(reads) <- 28 # original width
startRegionCoverage(gr1, reads, tx)
```

---

startRegionString      *Get start region as DNA-strings per GRanges group*

---

**Description**

One window per start site, if upstream and downstream are both 0, then only the startsite is returned.

**Usage**

```
startRegionString(gr1, tx, faFile, upstream = 20, downstream = 20)
```

**Arguments**

gr1	a <a href="#">GRangesList</a> of ranges to find regions in.
tx	a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all gr names. The names of gr can also be the ORFik orf names. that is "tx-Name_id".
faFile	<a href="#">FaFile</a> , BSgenome, fasta/index file path or an ORFik <a href="#">experiment</a> . This file is usually used to find the transcript sequences from some <a href="#">GRangesList</a> .
upstream	an integer, default (0), relative region to get upstream end from. (0 means start site, +1 is one upstream, -1 is one downstream)
downstream	an integer, default (0), relative region to get downstream end from (0 means start site, +1 is one downstream, -1 is one upstream)

**Value**

a character vector of start regions

---

startSites	<i>Get the start sites from a GRangesList of orfs grouped by orfs</i>
------------	---

---

**Description**

In ATGTTTTGG, get the position of the A.

**Usage**

```
startSites(grl, asGR = FALSE, keep.names = FALSE, is.sorted = FALSE)
```

**Arguments**

grl	a <a href="#">GRangesList</a> object
asGR	a boolean, return as GRanges object
keep.names	a logical (FALSE), keep names of input.
is.sorted	a speedup, if you know the ranges are sorted

**Value**

if asGR is False, a vector, if True a GRanges object

**See Also**

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
grl <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
startSites(grl, is.sorted = FALSE)
```

---

stopCodons	<i>Get the Stop codons (3 bases) from a GRangesList of orfs grouped by orfs</i>
------------	---

---

### Description

In ATGTTTGA, get the positions TGA. It takes care of exons boundaries, with exons < 3 length.

### Usage

```
stopCodons(gr1, is.sorted = FALSE)
```

### Arguments

gr1	a <a href="#">GRangesList</a> object
is.sorted	a boolean, a speedup if you know the ranges are sorted

### Value

a [GRangesList](#) of stop codons, since they might be split on exons

### See Also

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

### Examples

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
stopCodons(gr1, is.sorted = FALSE)
```

---

stopDefinition	Returns stop codon definitions
----------------	--------------------------------

---

**Description**

According to: <<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1>> ncbi genetic code number for translation. This version is a cleaned up version, unknown indices removed.

**Usage**

```
stopDefinition(transl_table)
```

**Arguments**

transl\_table    numeric. NCBI genetic code number for translation.

**Value**

A string of STOP sites separated with "|".

**See Also**

Other findORFs: [findMapORFs\(\)](#), [findORFs\(\)](#), [findORFsFasta\(\)](#), [findUORFs\(\)](#), [startDefinition\(\)](#)

**Examples**

```
stopDefinition
stopDefinition(1)
```

---

stopRegion	Stop region as GRangesList
------------	----------------------------

---

**Description**

Get the stop region of each ORF / region. If you want the stop codon only, set downstream = 0 or just use [stopCodons](#). Standard is 2 upstream and 2 downstream, a width 5 window centered at stop site.

**Usage**

```
stopRegion(gr1, tx = NULL, is.sorted = TRUE, upstream = 2L, downstream = 2L)
```

**Arguments**

grl	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs
tx	default NULL, a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all grl names. The names of grl can also be the ORFik orf names. that is "txName_id"
is.sorted	logical (TRUE), is grl sorted.
upstream	an integer (2), relative region to get upstream from.
downstream	an integer (2), relative region to get downstream from

**Details**

If tx is null, then downstream will be forced to 0 and upstream to a minimum of -grl width (to the TSS). . Since there is no reference for splicing.

**Value**

a [GRanges](#), or [GRangesList](#) object if any group had > 1 exon.

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [subsetCoverage\(\)](#), [translationalEff\(\)](#)

**Examples**

```
## ORF stop region
orf <- GRangesList(tx1 = GRanges("1", 200:300, "+"))
tx <- GRangesList(tx1 = GRanges("1",
                               IRanges(c(100, 305), c(300, 400)), "+"))
stopRegion(orf, tx, upstream = 6, downstream = 6)
## 2nd last codon of ORF
stopRegion(orf, tx, upstream = 6, downstream = -3)
```

---

stopSites

*Get the stop sites from a GRangesList of orfs grouped by orfs*


---

**Description**

In ATGTTTTGC, get the position of the C.

**Usage**

```
stopSites(grl, asGR = FALSE, keep.names = FALSE, is.sorted = FALSE)
```

**Arguments**

gr1            a [GRangesList](#) object  
 asGR            a boolean, return as GRanges object  
 keep.names    a logical (FALSE), keep names of input.  
 is.sorted      a speedup, if you know the ranges are sorted

**Value**

if asGR is False, a vector, if True a GRanges object

**See Also**

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [txNames\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(7, 14), width = 3),
  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
  ranges = IRanges(c(4, 1), c(9, 3)),
  strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
stopSites(gr1, is.sorted = FALSE)
```

---

strandBool

*Get logical list of strands*


---

**Description**

Helper function to get a logical list of True/False, if GRangesList group have + strand = T, if - strand = F Also checks for \* strands, so a good check for bugs

**Usage**

```
strandBool(gr1)
```

**Arguments**

gr1            a [GRangesList](#) or GRanges object

**Value**

a logical vector

**Examples**

```
gr <- GRanges(Rle(c("chr2", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)),
              IRanges(1:10, width = 10:1),
              Rle(strand(c("-", "+", "*", "+", "-")), c(1, 2, 2, 3, 2)))
strandBool(gr)
```

---

strandMode,covRle-method

*strandMode covRle*


---

**Description**

strandMode covRle

**Usage**

```
## S4 method for signature 'covRle'
strandMode(x)
```

**Arguments**

x                    a covRle object

**Value**

integer vector with names

---

strandMode,covRleList-method

*strandMode covRle*


---

**Description**

strandMode covRle

**Usage**

```
## S4 method for signature 'covRleList'
strandMode(x)
```

**Arguments**

x                    a covRle object

**Value**

integer vector with names

---

strandPerGroup	<i>Get first strand per GRangesList group</i>
----------------	---

---

**Description**

Get first strand per GRangesList group

**Usage**

```
strandPerGroup(gr1, keep.names = TRUE)
```

**Arguments**

gr1	a <a href="#">GRangesList</a>
keep.names	a boolean, keep names or not, default: (TRUE)

**Value**

a vector named/unnamed of characters

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(7, 14), width = 3),
  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
  ranges = IRanges(c(4, 1), c(9, 3)),
  strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
strandPerGroup(gr1)
```

---

subsetToFrame	<i>Subset GRanges to get desired frame.</i>
---------------	---

---

**Description**

Usually used for ORFs to get specific frame (0-2): frame 0, frame 1, frame 2

**Usage**

```
subsetToFrame(x, frame)
```

**Arguments**

x	A tiled to size of 1 GRanges object
frame	A numeric indicating which frame to extract

**Details**

GRanges object should be beforehand tiled to size of 1. This subsetting takes account for strand.

**Value**

GRanges object reduced to only first frame

**Examples**

```
subsetToFrame(GRanges("1", IRanges(1:10, width = 1), "+"), 2)
```

---

sum, covRle-method	<i>sum covRle</i>
--------------------	-------------------

---

**Description**

Sum coverage per chromosome

**Usage**

```
## S4 method for signature 'covRle'
sum(x)
```

**Arguments**

x                    a covRle object

**Value**

an integer, sum of coverage per chromosomes in covRle object

---

symbols	<i>Get ORFik experiment gene symbols</i>
---------	--

---

**Description**

Loads premade fst table at path: file.path(refFolder(x), "gene\_symbol\_tx\_table.fst")

**Usage**

```
symbols(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a data.table with gene id, gene symbols and tx ids (3 columns)

**Examples**

```
df <- ORFik.template.experiment()
symbols(df)
```

---

symbols,experiment-method

*Get path to ORFik experiment QC folder*

---

**Description**

Get path to ORFik experiment QC folder

**Usage**

```
## S4 method for signature 'experiment'
symbols(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a character path

---

te.plot

*Translational efficiency plots*

---

**Description**

Create 2 TE plots of:

- Within sample (TE log2 vs mRNA fpkm) ("default")
- Between all combinations of samples (x-axis: rna1fpkm - rna2fpkm, y-axis rfp1fpkm - rfp2fpkm)

**Usage**

```
te.plot(
  df.rfp,
  df.rna,
  output.dir = QCfolder(df.rfp),
  type = c("default", "between"),
  filter.rfp = 1,
  filter.rna = 1,
  collapse = FALSE,
  plot.title = "",
  plot.ext = ".pdf",
  width = 6,
  height = "auto"
)
```

**Arguments**

df.rfp	a <a href="#">experiment</a> of Ribo-seq or 80S from TCP-seq.
df.rna	a <a href="#">experiment</a> of RNA-seq
output.dir	directory to save plots, plots will be named "TE_between.pdf" and "TE_within.pdf"
type	which plots to make, default: c("default", "between"). Both plots.
filter.rfp	numeric, default 1. minimum fpkm value to be included in plots
filter.rna	numeric, default 1. minimum fpkm value to be included in plots
collapse	a logical/character (default FALSE), if TRUE all samples within the group SAMPLE will be collapsed to one. If "all", all groups will be merged into 1 column called merged_all. Collapse is defined as <code>rowSum(elements_per_group) / ncol(elements_per_group)</code>
plot.title	title for plots, usually name of experiment etc
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg".
width	numeric, default 6 (in inches)
height	numeric or character, default "auto", which is: $3 + (\text{ncol}(\text{RFP\_CDS\_FPKM}) - 2)$ . Else a numeric value of height (in inches)

**Details**

Ribo-seq and RNA-seq must have equal nrows, with matching samples. Only exception is if RNA-seq is 1 single sample. Then it will use that for each of the Ribo-seq samples. Same stages, conditions etc, with a unique pairing 1 to 1. If not you can run `collapse = "all"`. It will then merge all and do combined of all RNA-seq vs all Ribo-seq

**Value**

a data.table with TE values, fpkm and log fpkm values, library samples melted into rows with split variable called "variable".

**Examples**

```
##
# df.rfp <- read.experiment("zf_baz14_RFP")
# df.rna <- read.experiment("zf_baz14_RNA")
# te.plot(df.rfp, df.rna)
## Collapse replicates:
# te.plot(df.rfp, df.rna, collapse = TRUE)
```

te.table

*Create a TE table***Description**

Creates a data.table with 6 columns, column names are:  
variable, rfp\_log2, rna\_log2, rna\_log10, TE\_log2, id

**Usage**

```
te.table(df.rfp, df.rna, filter.rfp = 1, filter.rna = 1, collapse = FALSE)
```

**Arguments**

df.rfp	a <a href="#">experiment</a> of usually Ribo-seq or 80S from TCP-seq. (the numerator of the experiment, usually having a primary role)
df.rna	a <a href="#">experiment</a> of usually RNA-seq. (the denominator of the experiment, usually having a normalizing function)
filter.rfp	numeric, default 1. What is the minimum fpkm value?
filter.rna	numeric, default 1. What is the minimum fpkm value?
collapse	a logical/character (default FALSE), if TRUE all samples within the group SAMPLE will be collapsed to one. If "all", all groups will be merged into 1 column called merged_all. Collapse is defined as rowSum(elements_per_group) / ncol(elements_per_group)

**Value**

a data.table with 6 columns

**See Also**

Other DifferentialExpression: [DEG.plot.static\(\)](#), [DEG\\_model\(\)](#), [DTEG.analysis\(\)](#), [DTEG.plot\(\)](#), [te\\_rna.plot\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()
df.rfp <- df[df$libtype == "RFP",]
df.rna <- df[df$libtype == "RNA",]
#te.table(df.rfp, df.rna)
```

te\_rna.plot

*Translational efficiency plots***Description**

Create TE plot of:  
 - Within sample (TE log2 vs mRNA fpkm)

**Usage**

```
te_rna.plot(
  dt,
  output.dir = NULL,
  filter.rfp = 1,
  filter.rna = 1,
  plot.title = "",
  plot.ext = ".pdf",
  width = 6,
  height = "auto",
  dpi = 300,
  dot.size = 0.4,
  xlim = c(filter.rna, filter.rna + 2.5)
)
```

**Arguments**

dt	a data.table with the results from <a href="#">te.table</a>
output.dir	a character path, default NULL(no save), or a directory to save to a file will be called "TE_within.pdf"
filter.rfp	numeric, default 1. What is the minimum fpkm value?
filter.rna	numeric, default 1. What is the minimum fpkm value?
plot.title	title for plots, usually name of experiment etc
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg". Multiple values allowed, if so will save file in each format specified.
width	numeric, default 6 (in inches)
height	a numeric, width of plot in inches. Default "auto".
dpi	numeric, default 300.
dot.size	numeric, default 0.4, size of point dots in plot.
xlim	numeric vector of length 2. X-axis limits. Default: c(filter.rna, filter.rna + 2.5)

**Value**

a ggplot object

## See Also

Other DifferentialExpression: [DEG.plot.static\(\)](#), [DEG\\_model\(\)](#), [DTEG.analysis\(\)](#), [DTEG.plot\(\)](#), [te.table\(\)](#)

## Examples

```
df <- ORFik.template.experiment()
df.rfp <- df[df$libtype == "RFP",]
df.rna <- df[df$libtype == "RNA",]
#dt <- te.table(df.rfp, df.rna)
#te_rna.plot(dt, filter.rfp = 0, filter.rna = 5, dot.size = 1)
```

---

template\_shift\_table *Make template for shift table of experiment*

---

## Description

Must have ofst files already created!

## Usage

```
template_shift_table(df, accepted.lengths = 26:34)
```

## Arguments

df                    an ORFik [experiment](#)  
accepted.lengths     accepted read lengths, default 26:34, usually ribo-seq is strongest between 27:32.

## Value

a list with names of elements as file path to the ofst, the data.table elements 2 columns fraction: read length and offset\_start, the shifts in negative coordinates.

## Examples

```
df <- ORFik::ORFik.template.experiment()
template_shift_table(df)
```

---

tile1	<i>Tile each GRangesList group to 1-base resolution.</i>
-------	--

---

### Description

Will tile a GRangesList into single bp resolution, each group of the list will be splited by positions of 1. Returned values are sorted as the same groups as the original GRangesList, except they are in bp resolutions. This is not supported originally by GenomicRanges for GRangesList.

### Usage

```
tile1(
  grl,
  sort.on.return = TRUE,
  matchNaming = TRUE,
  is.sorted = TRUE,
  mergeEqualNamed = TRUE
)
```

### Arguments

grl	a <a href="#">GRangesList</a> object with names.
sort.on.return	logical (TRUE), should the groups be sorted before return (Negative ranges should be in decreasing order). Makes it a bit slower, but much safer for downstream analysis.
matchNaming	logical (TRUE), should groups keep unlisted names and meta data.(This make the list very big, for > 100K groups)
is.sorted	logical (TRUE), grl is presorted (negative coordinates are decreasing). Set to FALSE if they are not, else output will most likely be wrong!
mergeEqualNamed	logical, default TRUE. Merge groups with equal name

### Value

a GRangesList grouped by original group, tiled to 1. Groups with identical names will be merged unless mergeEqualNamed is FALSE!

### See Also

Other ExtendGenomicRanges: [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [txSeqsFromFa\(\)](#), [windowPerGroup\(\)](#)

**Examples**

```

gr1 <- GRanges("1", ranges = IRanges(start = c(1, 10, 20),
                                     end = c(5, 15, 25)),
               strand = "+")
gr2 <- GRanges("1", ranges = IRanges(start = c(20, 30, 40),
                                     end = c(25, 35, 45)),
               strand = "+")
names(gr1) = rep("tx1_1", 3)
names(gr2) = rep("tx1_2", 3)
gr1 <- GRangesList(tx1_1 = gr1, tx1_2 = gr2)
tile1(gr1)

```

TOP.Motif.ecdf

*TOP Motif ecdf plot***Description**

Given sequences, DNA or RNA. And some score, scanning efficiency (SE), ribo-seq fpkm, TE etc.

**Usage**

```

TOP.Motif.ecdf(
  seqs,
  rate,
  start = 1,
  stop = max(nchar(seqs)),
  xlim = c("q10", "q99"),
  type = "Scanning efficiency",
  legend.position.1st = c(0.75, 0.28),
  legend.position.motif = c(0.75, 0.28)
)

```

**Arguments**

seqs	the sequences (character vector, DNASTringSet), of 5' UTRs (leaders). See example below for input.
rate	a scoring vector (equal size to seqs)
start	position in seqs to start at (first is 1), default 1.
stop	position in seqs to stop at (first is 1), default max(nchar(seqs)), that is the longest sequence length
xlim	What interval of rate values you want to show type: numeric or quantile of length 2, 1. default c("q10","q99"). bigger than 10 percentile and less than 99 percentile. 2. Set to numeric values, like c(5, 1000), 3. Set to NULL if you want all values. Backend uses coord_cartesian.
type	What type is the rate scoring ? default ("Scanning efficiency")

`legend.position.1st`  
adjust left plot label position, default `c(0.75, 0.28)`, ("none", "left", "right", "bottom", "top", or two-element numeric vector)

`legend.position.motif`  
adjust right plot label position, default `c(0.75, 0.28)`, ("none", "left", "right", "bottom", "top", or two-element numeric vector)

### Details

Top motif defined as a TSS of C and 4 T's or C's (pyrimidins) downstream of TSS C.

The right plot groups: C nucleotide, TOP motif (C, then 4 pyrimidines) and OTHER (all other TSS variants).

### Value

a ggplot gtable of the TOP motifs in 2 plots

### Examples

```
## Not run:
if (requireNamespace("BSgenome.Hsapiens.UCSC.hg19")) {
  txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
                        package = "GenomicFeatures")
  #Extract sequences of Coding sequences.
  leaders <- loadRegion(txdbFile, "leaders")

  # Should update by CAGE if not already done
  cageData <- system.file("extdata", "cage-seq-heart.bed.bgz",
                        package = "ORFik")
  leadersCage <- reassignTSSbyCage(leaders, cageData)
  # Get region to check
  seqs <- startRegionString(leadersCage, NULL,
                          BSgenome.Hsapiens.UCSC.hg19::Hsapiens, 0, 4)
  # Some toy ribo-seq fpkm scores on cds
  set.seed(3)
  fpkm <- sample(1:115, length(leadersCage), replace = TRUE)
  # Standard arguments
  TOP.Motif.ecdf(seqs, fpkm, type = "ribo-seq FPKM",
                legend.position.1st = "bottom",
                legend.position.motif = "bottom")
  # with no zoom on x-axis:
  TOP.Motif.ecdf(seqs, fpkm, xlim = NULL,
                legend.position.1st = "bottom",
                legend.position.motif = "bottom")
}

## End(Not run)
```

---

topMotif	<i>TOP Motif detection</i>
----------	----------------------------

---

### Description

Per leader, detect if the leader has a TOP motif at TSS (5' end of leader) TOP motif defined as: (C, then 4 pyrimidines)

### Usage

```
topMotif(seqs, start = 1, stop = max(nchar(seqs)), return.sequence = TRUE)
```

### Arguments

seqs	the sequences (character vector, DNASTringSet), of 5' UTRs (leaders) start region. seqs must be of minimum widths start - stop + 1 to be included. See example below for input.
start	position in seqs to start at (first is 1), default 1.
stop	position in seqs to stop at (first is 1), default max(nchar(seqs)), that is the longest sequence length
return.sequence	logical, default TRUE, return as data.table with sequence as columns in addition to TOP class. If FALSE, return character vector.

### Value

default: return.sequence == FALSE, a character vector of either TOP, C or OTHER. C means leaders started on C, Other means not TOP and did not start on C. If return.sequence == TRUE, a data.table is returned with the base per position in the motif is included as additional columns (per position called seq1, seq2 etc) and a id column called X.gene\_id (with names of seqs).

### Examples

```
## Not run:
if (requireNamespace("BSgenome.Hsapiens.UCSC.hg19")) {
  txdbFile <- system.file("extdata", "hg19_knownGene_sample.sqlite",
    package = "GenomicFeatures")
  #Extract sequences of Coding sequences.
  leaders <- loadRegion(txdbFile, "leaders")

  # Should update by CAGE if not already done
  cageData <- system.file("extdata", "cage-seq-heart.bed.bgz",
    package = "ORFik")
  leadersCage <- reassignTSSbyCage(leaders, cageData)
  # Get region to check
  seqs <- startRegionString(leadersCage, NULL,
    BSgenome.Hsapiens.UCSC.hg19::Hsapiens, 0, 4)
  topMotif(seqs)
```

```

    }
    ## End(Not run)

```

---

transcriptWindow      *Make 100 bases size meta window for all libraries in experiment*

---

## Description

Gives you binned meta coverage plots, either saved separately or all in one.

## Usage

```

transcriptWindow(
  leaders,
  cds,
  trailers,
  df,
  outdir = NULL,
  scores = c("sum", "transcriptNormalized"),
  allTogether = TRUE,
  colors = experiment.colors(df),
  title = "Coverage metaplot",
  windowSize = min(min(c(wanted_window_size, widthPerGroup(leaders, FALSE))),
    min(c(wanted_window_size, widthPerGroup(cds, FALSE))), min(c(wanted_window_size,
      widthPerGroup(trailers, FALSE))))),
  wanted_window_size = 100,
  returnPlot = is.null(outdir),
  dfr = NULL,
  idName = "",
  plot.ext = ".pdf",
  type = "ofst",
  is.sorted = FALSE,
  drop.zero.dt = TRUE,
  verbose = TRUE,
  force = TRUE,
  library.names = bamVarName(df),
  BPPARAM = bpparam()
)

```

## Arguments

leaders	a <a href="#">GRangesList</a> of leaders (5' UTRs)
cds	a <a href="#">GRangesList</a> of coding sequences
trailers	a <a href="#">GRangesList</a> of trailers (3' UTRs)
df	an ORFik <a href="#">experiment</a>

outdir	directory to save to (default: NULL, no saving)
scores	scoring function (default: c("sum", "transcriptNormalized")), see ?coverageScorings for possible scores.
allTogether	plot all coverage plots in 1 output? (default: TRUE)
colors	Which colors to use, default auto color from function <code>experiment.colors</code> , new color per library type. Else assign colors yourself.
title	title of ggplot
windowSize	size of binned windows, minimum of 'wanted_window_size' and minimum of ranges given. Will inform you if windowSize is < wanted_window_size.
wanted_window_size	numeric, default 100. The wanted window size to bin on.
returnPlot	return plot from function, default is.null(outdir), so TRUE if outdir is not defined.
dfr	an ORFik <code>experiment</code> of RNA-seq to normalize against. Will add RNA normalized to plot name if this is done.
idName	A character ID to add to saved name of plot, if you make several plots in the same folder, and same experiment, like splitting transcripts in two groups like targets / nontargets etc. (default: "")
plot.ext	character, default: ".pdf". Alternatives: ".png" or ".jpg".
type	a character(default: "default"), load files in experiment or some precomputed variant, like "ofst" or "pshifted". These are made with <code>ORFik::convertLibs()</code> , <code>shiftFootprintsByExperiment()</code> , etc. Can also be custom user made folders inside the experiments bam folder. It acts in a recursive manner with priority: If you state "pshifted", but it does not exist, it checks "ofst". If no .ofst files, it uses "default", which always must exist. Presets are (folder is relative to default lib folder, some types fall back to other formats if folder does not exist): - "default": load the original files for experiment, usually bam. - "ofst": loads ofst files from the ofst folder, relative to lib folder (falls back to default) - "pshifted": loads ofst, wig or bigwig from pshifted folder (falls back to ofst, then default) - "cov": Load covRle objects from cov_RLE folder (fail if not found) - "covl": Load covRleList objects, from cov_RLE_List folder (fail if not found) - "bed": Load bed files, from bed folder (falls back to default) - Other formats must be loaded directly with <code>fimport</code>
is.sorted	logical (FALSE), is grl sorted. That is + strand groups in increasing ranges (1,2,3), and - strand groups in decreasing ranges (3,2,1)
drop.zero.dt	logical FALSE, if TRUE and <code>as.data.table</code> is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)
verbose	logical, default TRUE, message about library output status.

force logical, default TRUE If TRUE, reload library files even if matching named variables are found in environment used by experiment (see [envExp](#)) A simple way to make sure correct libraries are always loaded. FALSE is faster if data is loaded correctly already.

library.names character vector, names of libraries, default: name\_decider(df, naming)

BPPARAM how many cores/threads to use? default: bpparam()

**Value**

NULL, or ggplot object if returnPlot is TRUE

**See Also**

Other experiment plots: [transcriptWindow1\(\)](#), [transcriptWindowPer\(\)](#)

**Examples**

```
df <- ORFik.template.experiment()[3,] # Only third library
loadRegions(df) # Load leader, cds and trailers as GRangesList
#transcriptWindow(leaders, cds, trailers, df, outdir = "directory/to/save")
```

---

translationalEff	<i>Translational efficiency</i>
------------------	---------------------------------

---

**Description**

Uses RnaSeq and RiboSeq to get translational efficiency of every element in 'grl'. Translational efficiency is defined as:

$$(\text{density of RPF within ORF}) / (\text{RNA expression of ORFs transcript})$$
**Usage**

```
translationalEff(
  grl,
  RNA,
  RFP,
  tx,
  with.fpkm = FALSE,
  pseudoCount = 0,
  librarySize = "full",
  weight.RFP = 1L,
  weight.RNA = 1L
)
```

**Arguments**

grl	a <a href="#">GRangesList</a> object can be either transcripts, 5' utrs, cds', 3' utrs or ORFs as a special case (uORFs, potential new cds' etc). If regions are not spliced you can send a <a href="#">GRanges</a> object.
RNA	RnaSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
RFP	RiboSeq reads as <a href="#">GAlignments</a> , <a href="#">GRanges</a> or <a href="#">GRangesList</a> object
tx	a <a href="#">GRangesList</a> of the transcripts. If you used cage data, then the tss for the the leaders have changed, therefor the tx lengths have changed. To account for that call: ' translationalEff(grl, RNA, RFP, tx = extendLeaders(tx, cageFiveUTRs)) ' where cageFiveUTRs are the reannotated by CageSeq data leaders.
with.fpkm	logical, default: FALSE, if true return the fpkm values together with translational efficiency as a data.table
pseudoCount	a numeric, default 0, set it to 1 if you want to avoid NA and inf values.
librarySize	either numeric value or character vector. Default ("full"), number of alignments in library (reads). If you just have a subset, you can give the value by librarySize = length(wholeLib) or sum(wholeLib\$score), if you want lib size to be only number of reads overlapping grl, do: librarySize = "overlapping" sum(countOverlaps(reads, grl) > 0), if reads[1] has 3 hits in grl, and reads[2] has 2 hits, librarySize will be 2, not 5. You can also get the inverse overlap, if you want lib size to be total number of overlaps, do: librarySize = "DESeq" This is standard fpkm way of DESeq2::fpkm(robust = FALSE) sum(countOverlaps(grl, reads)) if grl[1] has 3 reads and grl[2] has 2 reads, librarySize is 5, not 2.
weight.RFP	a vector (default: 1L). Can also be character name of column in RFP. As in translationalEff(weight = "score") for: <a href="#">GRanges</a> ("chr1", 1, "+", score = 5), would mean score column tells that this alignment region was found 5 times.
weight.RNA	Same as weightRFP but for RNA weights. (default: 1L)

**Value**

a numeric vector of fpkm ratios, if with.fpkm is TRUE, return a data.table with te and fpkm values (total 3 columns then)

**References**

doi: 10.1126/science.1168978

**See Also**

Other features: [computeFeatures\(\)](#), [computeFeaturesCage\(\)](#), [countOverlapsW\(\)](#), [disengagementScore\(\)](#), [distToCds\(\)](#), [distToTSS\(\)](#), [entropy\(\)](#), [floss\(\)](#), [fpkm\(\)](#), [fpkm\\_calc\(\)](#), [fractionLength\(\)](#), [initiationScore\(\)](#), [insideOutsideORF\(\)](#), [isInFrame\(\)](#), [isOverlapping\(\)](#), [kozakSequenceScore\(\)](#), [orfScore\(\)](#), [rankOrder\(\)](#), [ribosomeReleaseScore\(\)](#), [ribosomeStallingScore\(\)](#), [startRegion\(\)](#), [startRegionCoverage\(\)](#), [stopRegion\(\)](#), [subsetCoverage\(\)](#)

**Examples**

```

ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20), end = c(5, 15, 25)),
               strand = "+")
grl <- GRangesList(tx1_1 = ORF)
RFP <- GRanges("1", IRanges(25, 25), "+")
RNA <- GRanges("1", IRanges(1, 50), "+")
tx <- GRangesList(tx1 = GRanges("1", IRanges(1, 50), "+"))
# grl must have same names as cds + _1 etc, so that they can be matched.
te <- translationalEff(grl, RNA, RFP, tx, with.fpkm = TRUE, pseudoCount = 1)
te$fpkmRFP
te$te

```

---

trimming.table	<i>Create trimming table</i>
----------------	------------------------------

---

**Description**

From fastp runs in ORFik alignment process

**Usage**

```

trimming.table(
  trim_folder,
  raw_libraries = dir(trim_folder, "\\*.json$", full.names = TRUE),
  include_adapter = FALSE
)

```

**Arguments**

trim_folder	folder of trimmed files, only reads fastp .json files. Can be NULL if raw_libraries is defined
raw_libraries	character, default: dir(trim_folder, "\.json", full.names = TRUE), file paths of all json file paths.
include_adapter	logical, default FALSE. If TRUE, will add an extra column: adapter, with adapter found. If not found, it will specify: "passed".

**Value**

a data.table with 6 columns, raw\_library (names of library), raw\_reads (numeric, number of raw reads), trim\_reads (numeric, number of trimmed reads), raw\_mean\_length (numeric, raw mean read length), trim\_mean\_length (numeric, trim mean read length). Optional columns: adapter (character, adapter, if not found "passed")

**Examples**

```
# Location of fastp trimmed .json files
trimmed_file <- system.file("extdata/test_processing/trim",
  "output_template.json", package = "ORFik")
trimmed_folder <- dirname(trimmed_file)
trimming.table(trimmed_folder)
trimming.table(NULL, trimmed_file)
trimming.table(NULL, trimmed_file, include_adapter = TRUE)
```

txNames

*Get transcript names from orf names***Description**

Using the ORFik definition of orf name, which is: example ENSEMBL:  
tx name: ENST0909090909090  
orf id: \_1 (the first of on that tx)  
orf\_name: ENST0909090909090\_1  
So therefor txNames("ENST0909090909090\_1") = ENST0909090909090

**Usage**

```
txNames(grl, ref = NULL, unique = FALSE)
```

**Arguments**

grl            a [GRangesList](#) grouped by ORF , GRanges object or IRanges object.  
ref            a reference [GRangesList](#). The object you want grl to subset by names. Add to make sure naming is valid.  
unique         a boolean, if true unique the names, used if several orfs map to same transcript and you only want the unique groups

**Details**

The names must be extracted from a column called names, or the names of the grl object. If it is already tx names, it returns the input

NOTE! Do not use \_123 etc in end of transcript names if it is not ORFs. Else you will get errors. Just \_ will work, but if transcripts are called ENST\_123124124000 etc, it will crash, so substitute "\_ " with "." gsub("\_ ", ".", names)

**Value**

a character vector of transcript names, without \*\_ naming

**See Also**

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [uniqueGroups\(\)](#), [uniqueOrder\(\)](#)

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
gr1 <- GRangesList(tx1_1 = gr_plus, tx2_1 = gr_minus)
# there are 2 orfs, both the first on each transcript
txNames(gr1)
```

---

txNamesToGeneNames	<i>Convert transcript names to gene names</i>
--------------------	---

---

**Description**

Works for ensembl, UCSC and other standard annotations.

**Usage**

```
txNamesToGeneNames(txNames, txdb)
```

**Arguments**

txNames	character vector, the transcript names to convert. Can also be a named object with tx names (like a GRangesList), will then extract names.
txdb	the transcript database to use or gtf/gff path to it.

**Value**

character vector of gene names

**Examples**

```
df <- ORFik.template.experiment()
txdb <- loadTxdb(df)
loadRegions(txdb, "cds") # using tx names
txNamesToGeneNames(cds, txdb)
# Identical to:
loadRegions(txdb, "cds", by = "gene")
```

---

txSeqsFromFa	<i>Get transcript sequence from a GRangesList and a faFile or BSgenome</i>
--------------	--

---

## Description

For each GRanges object, find the sequence of it from faFile or BSgenome.

## Usage

```
txSeqsFromFa(grl, faFile, is.sorted = FALSE, keep.names = TRUE)
```

## Arguments

grl	a <a href="#">GRangesList</a> object
faFile	<a href="#">FaFile</a> , <a href="#">BSgenome</a> , fasta/index file path or an <a href="#">ORFik experiment</a> . This file is usually used to find the transcript sequences from some <a href="#">GRangesList</a> .
is.sorted	a speedup, if you know the grl ranges are sorted
keep.names	a logical, default (TRUE), if FALSE: return as character vector without names.

## Details

A wrapper around [extractTranscriptSeqs](#) that works for [DNAStringSet](#) and [ORFik experiment](#) input. For debug of errors do: `which(!(unique(seqnamesPerGroup(grl, FALSE)))` This happens usually when the grl contains chromosomes that the fasta file does not have. A normal error is that mitochondrial chromosome is called MT vs chrM even though they have same seqlevelsStyle. The above line will give you which chromosome it is missing.

## Value

a [DNAStringSet](#) of the transcript sequences

## See Also

Other [ExtendGenomicRanges](#): [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [windowPerGroup\(\)](#)

---

uniqueGroups	<i>Get the unique set of groups in a GRangesList</i>
--------------	--

---

### Description

Sometimes [GRangesList](#) groups might be identical, for example ORFs from different isoforms can have identical ranges. Use this function to reduce these groups to unique elements in [GRangesList](#) `gr1`, without names and metacolumns.

### Usage

```
uniqueGroups(gr1)
```

### Arguments

`gr1` a [GRangesList](#)

### Value

a [GRangesList](#) of unique orfs

### See Also

Other ORFHelpers: [defineTrailer\(\)](#), [longestORFs\(\)](#), [mapToGRanges\(\)](#), [orfID\(\)](#), [startCodons\(\)](#), [startSites\(\)](#), [stopCodons\(\)](#), [stopSites\(\)](#), [txNames\(\)](#), [uniqueOrder\(\)](#)

### Examples

```
gr1 <- GRanges("1", IRanges(1,10), "+")
gr2 <- GRanges("1", IRanges(20, 30), "+")
# make a gr1 with duplicated ORFs (gr1 twice)
gr1 <- GRangesList(tx1_1 = gr1, tx2_1 = gr2, tx3_1 = gr1)
uniqueGroups(gr1)
```

---

uniqueMappers	<i>Get ORFik uniqueMappers status</i>
---------------	---------------------------------------

---

### Description

Do you want to load/save libraries with unique mappers only, for bam it subsets from file, for other formats it presumes a directory `./unique_mappers` relative to bam directory.

### Usage

```
uniqueMappers(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a logical (length 1)

---

uniqueMappers,experiment-method  
*Get ORFik uniqueMappers status*

---

**Description**

Do you want to load/save libraries with unique mappers only, for bam it subsets from file, for other formats it presumes a directory `./unique_mappers` relative to bam directory.

**Usage**

```
## S4 method for signature 'experiment'
uniqueMappers(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a logical (length 1)

---

uniqueMappers,NULL-method  
*Get ORFik uniqueMappers status*

---

**Description**

Do you want to load/save libraries with unique mappers only, for bam it subsets from file, for other formats it presumes a directory `./unique_mappers` relative to bam directory.

**Usage**

```
## S4 method for signature 'NULL'
uniqueMappers(x)
```

**Arguments**

x                    an ORFik [experiment](#)

**Value**

a logical (length 1)

---

uniqueMappers<-            *Set ORFik uniqueMappers status*

---

**Description**

Do you want to load/save libraries with unique mappers only, for bam it subsets from file, for other formats it presumes a directory './unique\_mappers' relative to bam directory.

**Usage**

```
uniqueMappers(x) <- value
```

**Arguments**

x                    an ORFik [experiment](#)  
value                a logical (length 1) (NA values not allowed)

**Value**

an ORFik [experiment](#) with updated uniqueMappers

---

uniqueMappers<-,experiment-method  
                                 *Set ORFik uniqueMappers status*

---

**Description**

Do you want to load/save libraries with unique mappers only, for bam it subsets from file, for other formats it presumes a directory './unique\_mappers' relative to bam directory.

**Usage**

```
## S4 replacement method for signature 'experiment'  

uniqueMappers(x) <- value
```

**Arguments**

x                    an ORFik [experiment](#)  
value                a logical (length 1) (NA values not allowed)

**Value**

an ORFik [experiment](#) with updated uniqueMappers

---

`uniqueOrder`*Get unique ordering for GRangesList groups*

---

### Description

This function can be used to calculate unique numerical identifiers for each of the `GRangesList` elements. Elements of `GRangesList` are unique when the `GRanges` inside are not duplicated, so ranges differences matter as well as sorting of the ranges.

### Usage

```
uniqueOrder(gr1)
```

### Arguments

`gr1`            a `GRangesList`

### Value

an integer vector of indices of unique groups

### See Also

`uniqueGroups`

Other ORFHelpers: `defineTrailer()`, `longestORFs()`, `mapToGRanges()`, `orfID()`, `startCodons()`, `startSites()`, `stopCodons()`, `stopSites()`, `txNames()`, `uniqueGroups()`

### Examples

```
gr1 <- GRanges("1", IRanges(1,10), "+")
gr2 <- GRanges("1", IRanges(20, 30), "+")
# make a gr1 with duplicated ORFs (gr1 twice)
gr1 <- GRangesList(tx1_1 = gr1, tx2_1 = gr2, tx3_1 = gr1)
uniqueOrder(gr1) # remember ordering

# example on unique ORFs
uniqueORFs <- uniqueGroups(gr1)
# now the orfs are unique, let's map back to original set:
reMappedGr1 <- uniqueORFs[uniqueOrder(gr1)]
```

---

unlistGrl	<i>Safe unlist</i>
-----------	--------------------

---

### Description

Same as [AnnotationDbi::unlist2()], keeps names correctly. Two differences is that if grl have no names, it will not make integer names, but keep them as null. Also if the GRangesList has names , and also the GRanges groups, then the GRanges group names will be kept.

### Usage

```
unlistGrl(grl)
```

### Arguments

grl                    a GRangesList

### Value

a GRanges object

### Examples

```
ORF <- GRanges(seqnames = "1",
               ranges = IRanges(start = c(1, 10, 20),
                               end = c(5, 15, 25)),
               strand = "+")
# GRL named, GR not named
grl <- GRangesList(tx1_1 = ORF)
res <- unlistGrl(grl)
res_original <- unlist(grl)
identical(res, res_original) # TRUE

# GRL not named, GR not named
grl_no_names <- grl
names(grl_no_names) <- NULL
unlistGrl(grl_no_names)
res <- unlistGrl(grl_no_names)
res_original <- unlist(grl_no_names)
identical(res, res_original) # TRUE

# GRL named, GR named
grl_names_gr_names <- unlistGrl(grl)
grl_names_gr_names <-
  split(grl_names_gr_names, names(grl_names_gr_names))
res <- unlistGrl(grl_names_gr_names)
res_original <- unlist(grl_names_gr_names)
identical(res, res_original) # FALSE

# GRL not named, GR named
```

```
grl_not_names_gr_names <- grl_names_gr_names
names(grl_not_names_gr_names) <- NULL
res <- unlistGrl(grl_not_names_gr_names)
res_original <- unlist(grl_not_names_gr_names)
identical(res, res_original) # TRUE
```

---

unlistToExtremities     *Get flanks as GRanges*

---

### Description

For a GRangesList, get start and end site, return back as GRanges.

### Usage

```
unlistToExtremities(grl)
```

### Arguments

grl                    a [GRangesList](#)

### Value

a GRang object with meta column "group", which gives

---

uORFSearchSpace     *Create search space to look for uORFs*

---

### Description

Given a GRangesList of 5' UTRs or transcripts, reassign the start sites using max peaks from CageSeq data (if CAGE is given). A max peak is defined as new TSS if it is within boundary of 5' leader range, specified by 'extension' in bp. A max peak must also be higher than minimum CageSeq peak cutoff specified in 'filterValue'. The new TSS will then be the positioned where the cage read (with highest read count in the interval). If you want to include uORFs going into the CDS, add this argument too.

**Usage**

```

uORFSearchSpace(
  fiveUTRs,
  cage = NULL,
  extension = 1000,
  filterValue = 1,
  restrictUpstreamToTx = FALSE,
  removeUnused = FALSE,
  cds = NULL
)

```

**Arguments**

fiveUTRs	(GRangesList) The 5' leaders or full transcript sequences
cage	Either a filePath for the CageSeq file as .bed .bam or .wig, with possible compressions (".gzip", ".gz", ".bgz"), or already loaded CageSeq peak data as GRanges or GAlignment. NOTE: If it is a .bam file, it will add a score column by running: <code>convertToOneBasedRanges(cage, method = "5prime", addScoreColumn = TRUE)</code> The score column is then number of replicates of read, if score column is something else, like read length, set the score column to NULL first.
extension	The maximum number of bases upstream of the TSS to search for CageSeq peak.
filterValue	The minimum number of reads on cage position, for it to be counted as possible new tss. (represented in score column in CageSeq data) If you already filtered, set it to 0.
restrictUpstreamToTx	a logical (FALSE). If TRUE: restrict leaders to not extend closer than 5 bases from closest upstream leader, set this to TRUE.
removeUnused	logical (FALSE), if False: (standard is to set them to original annotation), If TRUE: remove leaders that did not have any cage support.
cds	(GRangesList) CDS of relative fiveUTRs, applicable only if you want to extend 5' leaders downstream of CDS's, to allow upstream ORFs that can overlap into CDS's.

**Value**

a GRangesList of newly assigned TSS for fiveUTRs, using CageSeq data.

**See Also**

Other uorfs: [addCdsOnLeaderEnds\(\)](#), [filterUORFs\(\)](#), [removeORFsWithSameStartAsCDS\(\)](#), [removeORFsWithSameStopAsCDS\(\)](#), [removeORFsWithStartInsideCDS\(\)](#), [removeORFsWithinCDS\(\)](#)

**Examples**

```

# example 5' leader, notice exon_rank column
fiveUTRs <- GenomicRanges::GRangesList(

```

```

GenomicRanges::GRanges(seqnames = "chr1",
                        ranges = IRanges::IRanges(1000, 2000),
                        strand = "+",
                        exon_rank = 1))
names(fiveUTRs) <- "tx1"

# make fake CageSeq data from promoter of 5' leaders, notice score column
cage <- GenomicRanges::GRanges(
  seqnames = "chr1",
  ranges = IRanges::IRanges(500, 510),
  strand = "+",
  score = 10)

# finally reassign TSS for fiveUTRs
uORFSearchSpace(fiveUTRs, cage)

```

---

width,GRanges-method *Get widths of GRanges*

---

### Description

Faster version than S4Vector generic caller

### Usage

```
## S4 method for signature 'GRanges'
width(x)
```

### Arguments

x                    a GRanges

### Value

an integer (length equal to x)

---

widthPerGroup            *Get total widths per GRangesList group*

---

### Description

Get total widths per GRangesList group

### Usage

```
widthPerGroup(grl, keep.names = TRUE)
```

**Arguments**

gr1                    a [GRangesList](#)  
 keep.names           a boolean, keep names or not, default: (TRUE)

**Value**

an integer vector (named/unnamed) of widths

**Examples**

```
gr_plus <- GRanges(seqnames = c("chr1", "chr1"),
                  ranges = IRanges(c(7, 14), width = 3),
                  strand = c("+", "+"))
gr_minus <- GRanges(seqnames = c("chr2", "chr2"),
                   ranges = IRanges(c(4, 1), c(9, 3)),
                   strand = c("-", "-"))
gr1 <- GRangesList(tx1 = gr_plus, tx2 = gr_minus)
widthPerGroup(gr1)
```

---

windowCoveragePlot      *Get meta coverage plot of reads*

---

**Description**

Spanning a region like a transcripts, plot how the reads distribute.

**Usage**

```
windowCoveragePlot(
  coverage,
  output = NULL,
  scoring = "zscore",
  colors = c("skyblue4", "orange"),
  title = "Coverage metaplot",
  type = "transcripts",
  scaleEqual = FALSE,
  setMinToZero = FALSE
)
```

**Arguments**

coverage              a data.table, e.g. output of scaledWindowCoverage  
 output                character string (NULL), if set, saves the plot as pdf or png to path given. If no format is given, is save as pdf.  
 scoring                character vector, default "zscore", either of zscore, transcriptNormalized, sum, mean, median, .. or NULL. Set NULL if already scored. see ?coverageScorings for info and more alternatives.

colors	character vector colors to use in plot, will fix automatically, using binary splits with colors <code>c('skyblue4', 'orange')</code> .
title	a character (metaplot) (what is the title of plot?)
type	a character (transcripts), what should legends say is the whole region? Transcripts, genes, non coding rnas etc.
scaleEqual	a logical (FALSE), should all fractions (rows), have same max value, for easy comparison of max values if needed.
setMinToZero	a logical (FALSE), should minimum y-value be 0 (TRUE). With FALSE minimum value is minimum score at any position. This parameter overrides scaleEqual.

### Details

If coverage has a column called feature, this can be used to subdivide the meta coverage into parts as (5' UTRs, cds, 3' UTRs) These are the columns in the plot. The fraction column divide sequence libraries. Like ribo-seq and rna-seq. These are the rows of the plot. If you return this function without assigning it and output is NULL, it will automatically plot the figure in your session. If output is assigned, no plot will be shown in session. NULL is returned and object is saved to output.

Colors: Remember if you want to change anything like colors, just return the ggplot object, and reassign like: `obj + scale_color_brewer()` etc.

### Value

a ggplot object of the coverage plot, NULL if output is set, then the plot will only be saved to location.

### See Also

Other coveragePlot: [coverageHeatMap\(\)](#), [pSitePlot\(\)](#), [savePlot\(\)](#)

### Examples

```
library(data.table)
coverage <- data.table(position = seq(20),
                      score = sample(seq(20), 20, replace = TRUE))
windowCoveragePlot(coverage)

#Multiple plots in one frame:
coverage2 <- copy(coverage)
coverage$fraction <- "Ribo-seq"
coverage2$fraction <- "RNA-seq"
dt <- rbindlist(list(coverage, coverage2))
windowCoveragePlot(dt, scoring = "log10sum")

# See vignette for a more practical example
```

---

windowPerGroup      *Get window region of GRanges object*

---

### Description

Per GRanges input (*gr*) of single position inputs (center point), create a GRangesList window output of specified upstream, downstream region relative to some transcript "tx".

If downstream is 20, it means the window will start 20 downstream of *gr* start site (-20 in relative transcript coordinates.) If upstream is 20, it means the window will start 20 upstream of *gr* start site (+20 in relative transcript coordinates.) It will keep exon structure of tx, so if -20 is on next exon, it jumps to next exon.

### Usage

```
windowPerGroup(gr, tx, upstream = 0L, downstream = 0L)
```

### Arguments

<i>gr</i>	a GRanges/IRanges object (startSites or others, must be single point per in genomic coordinates)
<i>tx</i>	a GRangesList of transcripts or (container region), names of tx must contain all <i>gr</i> names. The names of <i>gr</i> can also be the ORFik orf names. that is "tx-Name_id".
<i>upstream</i>	an integer, default (0), relative region to get upstream end from. (0 means start site, +1 is one upstream, -1 is one downstream)
<i>downstream</i>	an integer, default (0), relative region to get downstream end from (0 means start site, +1 is one downstream, -1 is one upstream)

### Details

If a region has a part that goes out of bounds, E.g if you try to get window around the CDS start site, goes longer than the 5' leader start site, it will set start to the edge boundary (the TSS of the transcript in this case). If region has no hit in bound, a width 0 GRanges object is returned. This is useful for things like countOverlaps, since 0 hits will then always be returned for the correct object index. If you don't want the 0 width windows, use reduce() to remove 0-width windows.

### Value

a GRanges, or GRangesList object if any group had > 1 exon.

### See Also

Other ExtendGenomicRanges: [asTX\(\)](#), [coveragePerTiling\(\)](#), [extendLeaders\(\)](#), [extendTrailers\(\)](#), [reduceKeepAttr\(\)](#), [tile1\(\)](#), [txSeqsFromFa\(\)](#)

**Examples**

```

# find 2nd codon of an ORF on a spliced transcript
ORF <- GRanges("1", c(3), "+") # start site
names(ORF) <- "tx1_1" # ORF 1 on tx1
tx <- GRangesList(tx1 = GRanges("1", c(1,3,5,7,9,11,13), "+"))
windowPerGroup(ORF, tx, upstream = 0, downstream = 0) # <- TIS
windowPerGroup(ORF, tx, upstream = 0, downstream = 1) # <- first and second base
windowPerGroup(ORF, tx, upstream = -1, downstream = 1) # <- second base
# find 2nd codon of an ORF on a spliced transcript
windowPerGroup(ORF, tx, upstream = -3, downstream = 5) # <- 2nd codon

# With multiple extensions downstream
ORF <- rep(ORF, 2)
names(ORF)[2] <- "tx1_2"
windowPerGroup(ORF, tx, upstream = 0, downstream = c(2, 5))
# The last one gives 2nd for first ORF and (1st and 2nd) codon for
# second ORF, returned as two groups of class GRanges/GRangList

```

---

windowPerReadLength     *Find proportion of reads per position per read length in window*

---

**Description**

This is defined as: Fraction of reads per read length, per position in whole window (defined by upstream and downstream) If tx is not NULL, it gives a metaWindow, centered around startSite of grl from upstream and downstream. If tx is NULL, it will use only downstream, since it has no reference on how to find upstream region. The exception is when upstream is negative, that is, going into downstream region of the object.

**Usage**

```

windowPerReadLength(
  grl,
  tx = NULL,
  reads,
  pShifted = TRUE,
  upstream = ifelse(!is.null(tx), ifelse(pShifted, 5, 20), min(ifelse(pShifted, 5, 20),
    0)),
  downstream = ifelse(pShifted, 20, 5),
  acceptedLengths = NULL,
  zeroPosition = upstream,
  scoring = "transcriptNormalized",
  weight = "score",
  drop.zero.dt = FALSE,
  append.zeroes = FALSE,
  windows = startRegion(grl, tx, TRUE, upstream, downstream)
)

```

**Arguments**

grl	a <a href="#">GRangesList</a> object with usually either leaders, cds', 3' utrs or ORFs
tx	default NULL, a <a href="#">GRangesList</a> of transcripts or (container region), names of tx must contain all grl names. The names of grl can also be the ORFik orf names. that is "txName_id"
reads	a <a href="#">GAlignments</a> , <a href="#">GRanges</a> , or precomputed coverage as <a href="#">covRlelist</a> (where names of covRle objects are readlengths) of RiboSeq, RnaSeq etc. Weights for scoring is default the 'score' column in 'reads'. Can also be random access paths to bigWig or fstwig file. Do not use random access for more than a few genes, then loading the entire files is usually better.
pShifted	a logical (TRUE), are Ribo-seq reads p-shifted to size 1 width reads? If upstream and downstream is set, this argument is irrelevant. So set to FALSE if this is not p-shifted Ribo-seq.
upstream	an integer (5), relative region to get upstream from. Default: <code>ifelse(!is.null(tx), ifelse(pShifted, 5, 20), min(ifelse(pShifted, 5, 20), 0))</code>
downstream	an integer (20), relative region to get downstream from. Default: <code>ifelse(pShifted, 20, 5)</code>
acceptedLengths	an integer vector (NULL), the read lengths accepted. Default NULL, means all lengths accepted.
zeroPosition	an integer DEFAULT (upstream), what is the center point? Like leaders and cds combination, then 0 is the TIS and -1 is last base in leader. NOTE!: if windows have different widths, this will be ignored.
scoring	a character (transcriptNormalized), which meta coverage scoring ? one of (zscore, transcriptNormalized, mean, median, sum, sumLength, fracPos), see ?coverageScorings for more info. Use to decide a scoring of hits per position for metacoverage etc. Set to NULL if you do not want meta coverage, but instead want per gene per position raw counts.
weight	(default: 'score'), if defined a character name of valid meta column in subject. <code>GRanges("chr1", 1, "+", score = 5)</code> , would mean score column tells that this alignment region was found 5 times. Formats which loads a score column like this: Bigwig, wig, ORFik ofst, collapsed bam, bedoc and .bedo. As do CAGEr CAGE files and many other package formats. You can also assign a score column manually.
drop.zero.dt	logical FALSE, if TRUE and <code>as.data.table</code> is TRUE, remove all 0 count positions. This greatly speeds up and most importantly, greatly reduces memory usage. Will not change any plots, unless 0 positions are used in some sense. (mean, median, zscore coverage will only scale differently)
append.zeroes	logical, default FALSE. If TRUE and <code>drop.zero.dt</code> is TRUE and all windows have equal length, it will add back 0 values after transformation. Sometimes needed for correct plots, if TRUE, will call abort if not all windows are equal length!
windows	the <a href="#">GRangesList</a> windows to actually check, default: <code>startRegion(grl, tx, TRUE, upstream, downstream)</code> .

**Details**

Careful when you create windows where not all transcripts are long enough, this function usually is used first with `filterTranscripts` to make sure they are of all of valid length!

**Value**

a `data.table` with 4 columns: position (in window), score, fraction (read length). If score is `NULL`, will also return genes (index of `grl`). A note is that if no coverage is found, it returns an empty `data.table`.

**See Also**

Other coverage: [coverageScorings\(\)](#), [metaWindow\(\)](#), [regionPerReadLength\(\)](#), [scaledWindowPositions\(\)](#)

**Examples**

```
cds <- GRangesList(tx1 = GRanges("1", 100:129, "+"))
tx <- GRangesList(tx1 = GRanges("1", 80:129, "+"))
reads <- GRanges("1", seq(79,129, 3), "+")
windowPerReadLength(cds, tx, reads, scoring = "sum")
windowPerReadLength(cds, tx, reads, scoring = "transcriptNormalized")
```

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