

# 10 things (maybe) you didn't know about GenomicRanges, Biostrings, and Rsamtools

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## 1. Inner vs outer metadata columns

```
> mcols(gr1)$id <- paste0("ID", seq_along(gr1))
```

```
> gr1
```

GRangesList object of length 3:

\$gr1

GRanges object with 1 range and 2 metadata columns:

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom2   | 3-6       | +      | 5         | 0.45      |

-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths

\$gr2

GRanges object with 2 ranges and 2 metadata columns:

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 7-9       | +      | 3         | 0.3       |
| [2] | Chrom1   | 13-15     | -      | 4         | 0.5       |

-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths

\$gr3

GRanges object with 2 ranges and 2 metadata columns:

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 1-3       | -      | 6         | 0.4       |
| [2] | Chrom2   | 4-9       | -      | 2         | 0.1       |

## 1. Inner vs outer metadata columns

```
> mcols(gr1) # outer mcols
DataFrame with 3 rows and 1 column
      id
  <character>
gr1      ID1
gr2      ID2
gr3      ID3

> mcols(unlist(gr1, use.names=FALSE)) # inner mcols
DataFrame with 5 rows and 2 columns
      score      GC
  <integer> <numeric>
1          5      0.45
2          3      0.30
3          4      0.50
4          6      0.40
5          2      0.10
```

## 2. invertStrand()

Works out-of-the-box on any object that has a strand() getter and setter ==> no need to implement specific methods.

```
> gr
```

```
GRanges object with 10 ranges and 2 metadata columns:
```

|   | seqnames | ranges    | strand | score     | GC        |
|---|----------|-----------|--------|-----------|-----------|
|   | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| a | chr2     | 1-10      | -      | 1         | 1.000000  |
| b | chr2     | 2-10      | +      | 2         | 0.888889  |
| c | chr2     | 3-10      | +      | 3         | 0.777778  |
| . | ...      | ...       | ...    | ...       | ...       |
| h | chr3     | 8-10      | +      | 8         | 0.222222  |
| i | chr3     | 9-10      | -      | 9         | 0.111111  |
| j | chr3     | 10        | -      | 10        | 0.000000  |

```
-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

## 2. invertStrand()

```
> invertStrand(gr)
```

```
GRanges object with 10 ranges and 2 metadata columns:
```

|   | seqnames | ranges    | strand | score     | GC        |
|---|----------|-----------|--------|-----------|-----------|
|   | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| a | chr2     | 1-10      | +      | 1         | 1.000000  |
| b | chr2     | 2-10      | -      | 2         | 0.888889  |
| c | chr2     | 3-10      | -      | 3         | 0.777778  |
| . | ...      | ...       | ...    | ...       | ...       |
| h | chr3     | 8-10      | -      | 8         | 0.222222  |
| i | chr3     | 9-10      | +      | 9         | 0.111111  |
| j | chr3     | 10        | +      | 10        | 0.000000  |

-----

```
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

## 2. invertStrand()

```
> gr1
```

```
GRangesList object of length 3:
```

```
$gr1
```

```
GRanges object with 1 range and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom2   | 3-6       | +      | 5         | 0.45      |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
$gr2
```

```
GRanges object with 2 ranges and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 7-9       | +      | 3         | 0.3       |
| [2] | Chrom1   | 13-15     | -      | 4         | 0.5       |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
$gr3
```

```
GRanges object with 2 ranges and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 1-3       | -      | 6         | 0.4       |
| [2] | Chrom2   | 4-9       | -      | 2         | 0.1       |

## 2. invertStrand()

```
> invertStrand(gr1)
```

```
GRangesList object of length 3:
```

```
$gr1
```

```
GRanges object with 1 range and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom2   | 3-6       | -      | 5         | 0.45      |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
$gr2
```

```
GRanges object with 2 ranges and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 7-9       | -      | 3         | 0.3       |
| [2] | Chrom1   | 13-15     | +      | 4         | 0.5       |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
$gr3
```

```
GRanges object with 2 ranges and 2 metadata columns:
```

|     | seqnames | ranges    | strand | score     | GC        |
|-----|----------|-----------|--------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| [1] | Chrom1   | 1-3       | +      | 6         | 0.4       |
| [2] | Chrom2   | 4-9       | +      | 2         | 0.1       |

### 3. extractList()

Extract groups of elements from a vector-like object and return them in a list-like object.

```
> cvg <- Rle(c(OL, 2L, 5L, 1L, 0L), c(10, 6, 3, 4, 15))
> cvg
integer-Rle of length 38 with 5 runs
  Lengths: 10  6  3  4 15
  Values  :  0  2  5  1  0
> i <- IRanges(c(16, 19, 9), width=5, names=letters[1:3])
> i
IRanges object with 3 ranges and 0 metadata columns:
      start      end      width
  <integer> <integer> <integer>
a         16         20         5
b         19         23         5
c          9         13         5
```

### 3. `extractList()`

```
> extractList(cvg, i)
RleList of length 3
$a
integer-Rle of length 5 with 3 runs
  Lengths: 1 3 1
  Values  : 2 5 1

$b
integer-Rle of length 5 with 2 runs
  Lengths: 1 4
  Values  : 5 1

$c
integer-Rle of length 5 with 2 runs
  Lengths: 2 3
  Values  : 0 2
```

### 3. extractList()

i can be an IntegerList object:

```
> i <- IntegerList(c(25:20), NULL, seq(from=2, to=length(cvg), by=2))  
> i
```

```
IntegerList of length 3
```

```
[[1]] 25 24 23 22 21 20
```

```
[[2]] integer(0)
```

```
[[3]] 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
```

```
> extractList(cvg, i)
```

```
RleList of length 3
```

```
[[1]]
```

```
integer-Rle of length 6 with 2 runs
```

```
  Lengths: 2 4
```

```
  Values : 0 1
```

```
[[2]]
```

```
integer-Rle of length 0 with 0 runs
```

```
  Lengths:
```

```
  Values :
```

```
[[3]]
```

```
integer-Rle of length 19 with 5 runs
```

```
  Lengths: 5 3 1 2 8
```

```
  Values : 0 2 5 1 0
```

#### 4. 'with.revmap' arg for reduce() and (now) disjoin()

```
> ir
```

IRanges object with 6 ranges and 2 metadata columns:

|     | start     | end       | width     | id          | score     |
|-----|-----------|-----------|-----------|-------------|-----------|
|     | <integer> | <integer> | <integer> | <character> | <integer> |
| [1] | 11        | 13        | 3         | a           | 3         |
| [2] | 12        | 14        | 3         | b           | 2         |
| [3] | 13        | 15        | 3         | c           | 1         |
| [4] | 2         | 4         | 3         | d           | 0         |
| [5] | 7         | 9         | 3         | e           | -1        |
| [6] | 6         | 8         | 3         | f           | -2        |

```
> ir2 <- reduce(ir, with.revmap=TRUE)
```

```
> ir2
```

IRanges object with 3 ranges and 1 metadata column:

|     | start     | end       | width     | revmap        |
|-----|-----------|-----------|-----------|---------------|
|     | <integer> | <integer> | <integer> | <IntegerList> |
| [1] | 2         | 4         | 3         | 4             |
| [2] | 6         | 9         | 4         | 6,5           |
| [3] | 11        | 15        | 5         | 1,2,3         |

#### 4. 'with.revmap' arg for reduce() and disjoin()

```
> revmap <- mcols(ir2)$revmap
> extractList(mcols(ir)$id, revmap)
CharacterList of length 3
[[1]] d
[[2]] f e
[[3]] a b c
> extractList(mcols(ir)$score, revmap)
IntegerList of length 3
[[1]] 0
[[2]] -2 -1
[[3]] 3 2 1
> mcols(ir2) <- DataFrame(id=extractList(mcols(ir)$id, revmap),
+                           score=extractList(mcols(ir)$score, revmap))
> ir2
```

IRanges object with 3 ranges and 2 metadata columns:

|     | start     | end       | width     | id              | score         |
|-----|-----------|-----------|-----------|-----------------|---------------|
|     | <integer> | <integer> | <integer> | <CharacterList> | <IntegerList> |
| [1] | 2         | 4         | 3         | d               | 0             |
| [2] | 6         | 9         | 4         | f,e             | -2,-1         |
| [3] | 11        | 15        | 5         | a,b,c           | 3,2,1         |

## 5. Zero-width ranges

`findOverlaps/countOverlaps` support zero-width ranges.

```
> sliding_query <- IRanges(1:6, width=0)
> sliding_query
IRanges object with 6 ranges and 0 metadata columns:
      start      end      width
  <integer> <integer> <integer>
[1]      1      0      0
[2]      2      1      0
[3]      3      2      0
[4]      4      3      0
[5]      5      4      0
[6]      6      5      0
> countOverlaps(sliding_query, IRanges(3, 4))
[1] 0 0 0 1 0 0
```

But you have to specify `minoverlap=0` for this to work (default is 1).

```
> countOverlaps(sliding_query, IRanges(3, 4), minoverlap=0)
[1] 0 0 0 1 0 0
```

## 6. Biostrings::replaceAt()

Perform multiple substitutions at arbitrary positions in a set of sequences.

```
> library(Biostrings)
> library(hgu95av2probe)
> probes <- DNASTringSet(hgu95av2probe)
> probes
```

DNASTringSet object of length 201800:

|          | width | seq                        |
|----------|-------|----------------------------|
| [1]      | 25    | TGGCTCCTGCTGAGGTCCCCTTTCC  |
| [2]      | 25    | GGCTGTGAATTCCTGTACATATTC   |
| [3]      | 25    | GCTTCAATTCCATTATGTTTTAATG  |
| ...      | ...   | ...                        |
| [201798] | 25    | TTCTGTCAAAGCATCATCTCAACAA  |
| [201799] | 25    | CAAAGCATCATCTCAACAAGCCCTC  |
| [201800] | 25    | GTGCTCCTTGTC AACAGCGCACCCA |

## 6. Biostrings::replaceAt()

Replace 3rd and 4th nucleotides by pattern -++-.

```
> replaceAt(probes, at=IRanges(3, 4), value="-++-")
```

DNASTringSet object of length 201800:

```
      width seq
[1]      27 TG-++-TCCTGCTGAGGTCCCCTTTCC
[2]      27 GG-++-GTGAATTCCTGTACATATTTTC
[3]      27 GC-++-CAATTCATTATGTTTTAATG
...      ...
[201798] 27 TT-++-GTCAAAGCATCATCTCAACAA
[201799] 27 CA-++-GCATCATCTCAACAAGCCCTC
[201800] 27 GT-++-TCCTTGTC AACAGCGCACCCA
```

## 6. Biostrings::replaceAt()

If supplied pattern is empty, then performs deletions.

```
> replaceAt(probes, at=IRanges(3, 4), value="")
```

DNASTringSet object of length 201800:

|          | width | seq                     |
|----------|-------|-------------------------|
| [1]      | 23    | TGTCCTGCTGAGGTCCCCTTTCC |
| [2]      | 23    | GGGTGAATTCCTGTACATATTTC |
| [3]      | 23    | GCCAATTCCATTATGTTTAAATG |
| ...      | ...   | ...                     |
| [201798] | 23    | TTGTCAAAGCATCATCTCAACAA |
| [201799] | 23    | CAGCATCATCTCAACAAGCCCTC |
| [201800] | 23    | GTTCTTGTCAACAGCGCACCCA  |

## 6. Biostrings::replaceAt()

If `at` is a zero-width range, then performs insertions.

```
> replaceAt(probes, at=IRanges(4, 3), value="--+-")
```

DNASTringSet object of length 201800:

```
      width seq
[1]      29 TGG--+-CTCCTGCTGAGGTCCCCTTTCC
[2]      29 GGC--+-TGTGAATTCCTGTACATATTC
[3]      29 GCT--+-TCAATTCATTATGTTTTAATG
...      ...
[201798] 29 TTC--+-TGTCAAAGCATCATCTCAACAA
[201799] 29 CAA--+-AGCATCATCTCAACAAGCCCTC
[201800] 29 GTG--+-CTCCTTGTC AACAGCGCACCCA
```

## 6. Biostrings::replaceAt()

Use it in combination with `vmatchPattern` to replace all the occurrences of a given pattern with another pattern:

```
> midx <- vmatchPattern("VCGTT", probes, fixed=FALSE)
> replaceAt(probes, at=midx, value="-++-")
```

DNASTringSet object of length 201800:

```
      width seq
[1]      25 TGGCTCCTGCTGAGGTCCCCTTTCC
[2]      25 GGCTGTGAATTCCTGTACATATTTTC
[3]      25 GCTTCAATTCATTATGTTTTAATG
...      ...
[201798] 25 TTCTGTCAAAGCATCATCTCAACAA
[201799] 25 CAAAGCATCATCTCAACAAGCCCTC
[201800] 25 GTGCTCCTTGTC AACAGCGCACCCA
```

## 7. GRanges as a subscript

```
> cvg <- RleList(chr1=101:120, chr2=2:-8, chr3=31:40)
> gr
```

GRanges object with 10 ranges and 2 metadata columns:

|   | seqnames | ranges    | strand | score     | GC        |
|---|----------|-----------|--------|-----------|-----------|
|   | <Rle>    | <IRanges> | <Rle>  | <integer> | <numeric> |
| a | chr2     | 1-10      | -      | 1         | 1.000000  |
| b | chr2     | 2-10      | +      | 2         | 0.888889  |
| c | chr2     | 3-10      | +      | 3         | 0.777778  |
| . | ...      | ...       | ...    | ...       | ...       |
| h | chr3     | 8-10      | +      | 8         | 0.222222  |
| i | chr3     | 9-10      | -      | 9         | 0.111111  |
| j | chr3     | 10        | -      | 10        | 0.000000  |

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

## 7. GRanges as a subscript

```
> cvg[gr]

RleList of length 10
$chr2
integer-Rle of length 10 with 10 runs
  Lengths:  1  1  1  1  1  1  1  1  1  1
  Values  :  2  1  0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 9 with 9 runs
  Lengths:  1  1  1  1  1  1  1  1  1
  Values  :  1  0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 8 with 8 runs
  Lengths:  1  1  1  1  1  1  1  1
  Values  :  0 -1 -2 -3 -4 -5 -6 -7

$chr2
integer-Rle of length 7 with 7 runs
  Lengths:  1  1  1  1  1  1  1
  Values  : -1 -2 -3 -4 -5 -6 -7

$chr1
integer-Rle of length 6 with 6 runs
  Lengths:  1  1  1  1  1  1
  Values  : 105 106 107 108 109 110

...
<5 more elements>
```

## 8. BSgenomeViews objects

```
> library(BSgenome.Mmusculus.UCSC.mm10)
> genome <- BSgenome.Mmusculus.UCSC.mm10
> library(TxDb.Mmusculus.UCSC.mm10.knownGene)
> txdb <- TxDb.Mmusculus.UCSC.mm10.knownGene
> ex <- exons(txdb, columns=c("exon_id", "tx_name", "gene_id"))
> v <- Views(genome, ex)
```

## 8. BSgenomeViews objects

```
> v
```

```
BSgenomeViews object with 447558 views and 3 metadata columns:
```

|          | seqnames       | ranges               | strand          | dna                       |
|----------|----------------|----------------------|-----------------|---------------------------|
|          | <Rle>          | <IRanges>            | <Rle>           | <DNAStrngSet>             |
| [1]      | chr1           | 3073253-3074322      | +               | [AAGGAAAGAG...TAGAGAAATG] |
| [2]      | chr1           | 3102016-3102125      | +               | [GTGCTTGCTT...ACAAAAATAT] |
| [3]      | chr1           | 3252757-3253236      | +               | [TTCTTCTGTG...TACCTTCAAT] |
| ...      | ...            | ...                  | ...             | ...                       |
| [447556] | chrUn_JH584304 | 58564-58835          | -               | [CTGTGGTCCT...CAGAGAAATG] |
| [447557] | chrUn_JH584304 | 58564-59690          | -               | [CTCTCTGCTG...CAGAGAAATG] |
| [447558] | chrUn_JH584304 | 59592-59667          | -               | [AGCTGTCCCG...GCCTTCTCAG] |
|          | exon_id        | tx_name              | gene_id         |                           |
|          | <integer>      | <CharacterList>      | <CharacterList> |                           |
| [1]      | 1              | ENSMUST00000193812.1 |                 |                           |
| [2]      | 2              | ENSMUST00000082908.1 |                 |                           |
| [3]      | 3              | ENSMUST00000192857.1 |                 |                           |
| ...      | ...            | ...                  | ...             | ...                       |
| [447556] | 447556         | ENSMUST00000179505.7 | 66776           |                           |
| [447557] | 447557         | ENSMUST00000178343.1 | 66776           |                           |
| [447558] | 447558         | ENSMUST00000179505.7 | 66776           |                           |

```
-----  
seqinfo: 239 sequences (1 circular) from mm10 genome
```

## 8. BSgenomeViews objects

```
> af <- alphabetFrequency(v, baseOnly=TRUE)
```

```
> head(af)
```

|      | A   | C   | G   | T   | other |
|------|-----|-----|-----|-----|-------|
| [1,] | 376 | 160 | 206 | 328 | 0     |
| [2,] | 45  | 20  | 20  | 25  | 0     |
| [3,] | 138 | 105 | 86  | 151 | 0     |
| [4,] | 28  | 14  | 30  | 29  | 0     |
| [5,] | 57  | 39  | 20  | 33  | 0     |
| [6,] | 208 | 258 | 204 | 256 | 0     |

## 9. Pile-up statistics on a BAM file with Rsamtools::pileup()

```
> library(Rsamtools)
> library(RNAseqData.HNRNPC.bam.chr14)
> fl <- RNAseqData.HNRNPC.bam.chr14_BAMFILES[1]
> sbp <- ScanBamParam(which=GRanges("chr14", IRanges(1, 53674770)))
> pp <- PileupParam(distinguish_nucleotides=FALSE,
+                   distinguish_strands=FALSE,
+                   min_mapq=13,
+                   min_base_quality=10,
+                   min_nucleotide_depth=4)
> res <- pileup(fl, scanBamParam=sbp, pileupParam=pp)
```

## 9. Pile-up statistics on a BAM file with Rsamtools::pileup()

```
> dim(res)
[1] 248409      4
> head(res)
```

|   | seqnames | pos      | count | which_label      |
|---|----------|----------|-------|------------------|
| 1 | chr14    | 19681651 | 4     | chr14:1-53674770 |
| 2 | chr14    | 19681655 | 4     | chr14:1-53674770 |
| 3 | chr14    | 19681657 | 4     | chr14:1-53674770 |
| 4 | chr14    | 19681658 | 4     | chr14:1-53674770 |
| 5 | chr14    | 19681661 | 4     | chr14:1-53674770 |
| 6 | chr14    | 19681662 | 4     | chr14:1-53674770 |

## 10. Merging 2 GRanges objects (added this week)

```
> x
```

```
GRanges object with 2 ranges and 3 metadata columns:
```

|     | seqnames | ranges    | strand | score     | a1        | a2        |
|-----|----------|-----------|--------|-----------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <numeric> | <integer> | <numeric> |
| [1] | chr1     | 1-1000    | *      | 0.45      | 5         | 6         |
| [2] | chr2     | 2000-3000 | *      | NA        | 7         | 8         |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

```
> y
```

```
GRanges object with 3 ranges and 3 metadata columns:
```

|     | seqnames | ranges    | strand | score     | b1        | b2        |
|-----|----------|-----------|--------|-----------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <numeric> | <integer> | <numeric> |
| [1] | chr2     | 150-151   | *      | 0.70      | 0         | 1         |
| [2] | chr1     | 1-10      | *      | 0.82      | 5         | -2        |
| [3] | chr2     | 2000-3000 | *      | 0.10      | 1         | 1         |

```
-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths
```

## 10. Merging 2 GRanges objects

```
> merge(x, y)
```

GRanges object with 1 range and 5 metadata columns:

|     | seqnames | ranges    | strand | score     | a1        | a2        | b1        |
|-----|----------|-----------|--------|-----------|-----------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <numeric> | <integer> | <numeric> | <integer> |
| [1] | chr2     | 2000-3000 | *      | 0.1       | 7         | 8         | 1         |
|     |          | b2        |        |           |           |           |           |
|     |          | <numeric> |        |           |           |           |           |
| [1] |          | 1         |        |           |           |           |           |

-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths

## 10. Merging 2 GRanges objects

```
> merge(x, y, all=TRUE)
```

GRanges object with 4 ranges and 5 metadata columns:

|     | seqnames | ranges    | strand | score     | a1        | a2        | b1        |
|-----|----------|-----------|--------|-----------|-----------|-----------|-----------|
|     | <Rle>    | <IRanges> | <Rle>  | <numeric> | <integer> | <numeric> | <integer> |
| [1] | chr1     | 1-10      | *      | 0.82      | <NA>      | NA        | 5         |
| [2] | chr1     | 1-1000    | *      | 0.45      | 5         | 6         | <NA>      |
| [3] | chr2     | 150-151   | *      | 0.70      | <NA>      | NA        | 0         |
| [4] | chr2     | 2000-3000 | *      | 0.10      | 7         | 8         | 1         |

b2

<numeric>

|     |    |
|-----|----|
| [1] | -2 |
| [2] | NA |
| [3] | 1  |
| [4] | 1  |

-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths