

Package: lemur (via r-universe)

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Type Package

Title Latent Embedding Multivariate Regression

Version 1.10.0

Description Fit a latent embedding multivariate regression (LEMUR) model to multi-condition single-cell data. The model provides a parametric description of single-cell data measured with treatment vs. control or more complex experimental designs. The parametric model is used to (1) align conditions, (2) predict log fold changes between conditions for all cells, and (3) identify cell neighborhoods with consistent log fold changes. For those neighborhoods, a pseudobulked differential expression test is conducted to assess which genes are significantly changed.

URL <https://github.com/const-ae/lemur>

BugReports <https://github.com/const-ae/lemur/issues>

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.DollarNames.lemur_fit
Access values from a lemur_fit

Description

Access values from a lemur_fit

Usage

```
## S3 method for class 'lemur_fit'
.DollarNames(x, pattern = "")

## S4 method for signature 'lemur_fit'
x$name

## S4 replacement method for signature 'lemur_fit'
x$name <- value
```

Arguments

x	the lemur_fit
pattern	the pattern from looking up potential values interactively
name	the name of the value behind the dollar
value	the replacement value. This only works for colData and rowData.

Value

The respective value stored in the lemur_fit object.

See Also

[lemur_fit](#) for more documentation on the accessor functions.

align_harmony	<i>Enforce additional alignment of cell clusters beyond the direct differential embedding</i>
---------------	---

Description

Enforce additional alignment of cell clusters beyond the direct differential embedding

Usage

```
align_harmony(
  fit,
  design = fit$alignment_design,
  ridge_penalty = 0.01,
  max_iter = 10,
  ...,
  verbose = TRUE
)

align_by_grouping(
  fit,
  grouping,
  design = fit$alignment_design,
  ridge_penalty = 0.01,
  preserve_position_of_NAs = FALSE,
  verbose = TRUE
)
```

Arguments

fit	a lemur_fit object
design	a specification of the design (matrix or formula) that is used for the transformation. Default: fit\$design_matrix
ridge_penalty	specification how much the flexibility of the transformation should be regularized. Default: 0.01
max_iter	argument specific for align_harmony. The number of iterations. Default: 10
...	additional parameters that are passed on to relevant functions
verbose	Should the method print information during the fitting. Default: TRUE.
grouping	argument specific for align_by_grouping. Either a vector which assigns each cell to one group or a matrix with ncol(fit) columns where the rows are a soft-assignment to a cluster (i.e., columns sum to 1). NA's are allowed.
preserve_position_of_NAs	argument specific for align_by_grouping. Boolean flag to decide if NAs in the grouping mean that these cells should stay where they are (if possible) or if they are free to move around. Default: FALSE

Value

The fit object with the updated fit\$embedding and fit\$alignment_coefficients.

Examples

```
data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)
# Creating some grouping for illustration
cell_types <- sample(c("tumor cell", "neuron", "leukocyte"), size = ncol(fit), replace = TRUE)
fit_al1 <- align_by_grouping(fit, grouping = cell_types)

# Alternatively, use harmony to automatically group cells
fit_al2 <- align_harmony(fit)
fit_al2

# The alignment coefficients are a 3D array
fit_al2$alignment_coefficients
```

find_de_neighborhoods *Find differential expression neighborhoods*

Description

Find differential expression neighborhoods

Usage

```

find_de_neighborhoods(
  fit,
  group_by,
  contrast = fit$contrast,
  selection_procedure = c("zscore", "contrast"),
  directions = c("random", "contrast", "axis_parallel"),
  min_neighborhood_size = 50,
  de_mat = SummarizedExperiment::assays(fit)[["DE"]],
  test_data = fit$test_data,
  test_data_col_data = NULL,
  test_method = c("glmGamPoi", "edgeR", "limma", "none"),
  continuous_assay_name = fit$use_assay,
  count_assay_name = "counts",
  size_factor_method = NULL,
  design = fit$design,
  alignment_design = fit$alignment_design,
  add_diff_in_diff = TRUE,
  make_neighborhoods_consistent = FALSE,
  skip_confounded_neighborhoods = FALSE,
  control_parameters = NULL,
  verbose = TRUE
)

```

Arguments

<code>fit</code>	the <code>lemur_fit</code> generated by <code>lemur()</code>
<code>group_by</code>	Defines how the pseudobulks are formed. This is typically the variable in the column data that represents the independent unit of replication of the experiment (e.g., the mouse or patient ID). The argument has to be wrapped in <code>vars(...)</code> . The function automatically includes all variables that are referenced in the original design formula, so they don't need to be explicitly mentioned.
<code>contrast</code>	a specification which contrast to fit. This defaults to the contrast argument that was used for <code>test_de</code> and is stored in <code>fit\$contrast</code> .
<code>selection_procedure</code>	specify the algorithm that is used to select the neighborhoods for each gene. Broadly, <code>selection_procedure = "zscore"</code> is faster but less precise than <code>selection_procedure = "contrast"</code> .
<code>directions</code>	a string to define the algorithm to select the direction onto which the cells are projected before searching for the neighborhood. <code>directions = "random"</code> produces denser neighborhoods, whereas <code>directions = "contrast"</code> has usually more power. Alternatively, this can also be a matrix with one direction for each gene (i.e., a matrix of size <code>nrow(fit) * fit\$n_embedding</code>).
<code>min_neighborhood_size</code>	the minimum number of cells per neighborhood. Default: 50.

de_mat	the matrix with the differential expression values and is only relevant if selection_procedure = "zscore" or directions = "random". Defaults to an assay called "DE" that is produced by <code>lemur::test_de()</code> .
test_data	a SummarizedExperiment object or a named list of matrices. The data is used to test if the neighborhood inferred on the training data contain a reliable significant change. If test_method is "glmGamPoi" or "edgeR" a test using raw counts is conducted and two matching assays are needed: (1) the continuous assay (with continuous_assay_name) is projected onto the LEMUR fit to find the latent position of each cell and (2) the count assay (count_assay_name) is used for forming the pseudobulk. If test_method == "limma", only the continuous assay is needed. The arguments defaults to the test data split of when calling <code>lemur()</code> .
test_data_col_data	additional column data for the test_data argument.
test_method	choice of test for the pseudobulked differential expression. <code>glmGamPoi</code> and <code>edgeR</code> work on an count assay. <code>limma</code> works on the continuous assay.
continuous_assay_name, count_assay_name	the names of the assays that are used for the statistical test. By default, continuous_assay_name is the name that was specified in the original <code>lemur()</code> call and count_assay_name, which is used if test_method is "glmGamPoi" or "edgeR", is "counts".
size_factor_method	Set the procedure to calculate the size factor after pseudobulking. This argument is only relevant if test_method is "glmGamPoi" or "edgeR". If fit is subsetted, using a vector with the sequencing depth per cell ensures reasonable results. Default: NULL which means that <code>colSums(assay(fit\$test_data, count_assay_name))</code> is used.
design, alignment_design	the design to use for the fit. Default: <code>fit\$design</code>
add_diff_in_diff	a boolean to specify if the log-fold change (plus significance) of the DE in the neighborhood against the DE in the complement of the neighborhood is calculated. If TRUE, the result includes three additional columns starting with "did_" short for difference-in-difference. Default: TRUE.
make_neighborhoods_consistent	Include cells from outside the neighborhood if they are at least 10 times in the k-nearest neighbors of the cells inside the neighborhood. Secondly, remove cells from the neighborhood which are less than 10 times in the k-nearest neighbors of the other cells in the neighborhood. Default FALSE
skip_confounded_neighborhoods	Sometimes the inferred neighborhoods are not limited to a single cell state; this becomes problematic if the cells of the conditions compared in the contrast are unequally distributed between the cell states. Default: FALSE
control_parameters	named list with additional parameters passed to underlying functions.
verbose	Should the method print information during the fitting. Default: TRUE.

Value

a data frame with one entry per gene

name The gene name.

neighborhood A list column where each element is a vector with the cell names included in that neighborhood.

n_cells the number of cells in the neighborhood (lengths(neighborhood)).

sel_statistic The statistic that is maximized by the selection_procedure.

pval, adj_pval, t_statistic, lfc The p-value, Benjamini-Hochberg adjusted p-value (FDR), the t-statistic, and the log2 fold change of the differential expression test defined by contrast for the cells inside the neighborhood (calculated using test_method). Only present if test_data is not NULL.

did_pval, did_adj_pval, did_lfc The measurement if the differential expression of the cells inside the neighborhood is significantly different from the differential expression of the cells outside the neighborhood. Only present if add_diff_in_diff = TRUE.

Examples

```
data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)
# Optional alignment
# fit <- align_harmony(fit)
fit <- test_de(fit, contrast = cond(condition = "panobinostat") - cond(condition = "ctrl"))
nei <- find_de_neighborhoods(fit, group_by = vars(patient_id))
head(nei)
```

glioblastoma_example_data

The glioblastoma_example_data dataset

Description

The dataset is a [SingleCellExperiment](#) object subset to 5,000 cells and 300 genes. The colData contain an entry for each cell from which patient it came and to which treatment condition it belonged ("ctrl" or "panobinostat").

Details

The original data was collected by Zhao et al. (2021).

Value

A [SingleCellExperiment](#) object.

References

- Zhao, Wenting, Athanassios Dovas, Eleonora Francesca Spinazzi, Hanna Mendes Levitin, Matei Alexandru Banu, Pavan Upadhyayula, Tejaswi Sudhakar, et al. “Deconvolution of Cell Type-Specific Drug Responses in Human Tumor Tissue with Single-Cell RNA-Seq.” *Genome Medicine* 13, no. 1 (December 2021): 82. <https://doi.org/10.1186/s13073-021-00894-y>.

lemur	<i>Main function to fit the latent embedding multivariate regression (LEMUR) model</i>
-------	--

Description

The main function of this package, to fit the latent embedding multivariate regression (LEMUR) model.

Usage

```
lemur(
  data,
  design = ~1,
  col_data = NULL,
  n_embedding = 15,
  linear_coefficient_estimator = c("linear", "mean", "cluster_median", "zero"),
  use_assay = "logcounts",
  test_fraction = 0.2,
  ...,
  verbose = TRUE
)
```

Arguments

data	a matrix with observations in the columns and features in the rows, or a <code>SummarizedExperiment</code> / <code>SingleCellExperiment</code> object
design	a formula referring to global objects, <code>colData</code> (column annotations) of data if it is a <code>SummarizedExperiment</code> / <code>SingleCellExperiment</code> object, or column names of the <code>col_data</code> argument
col_data	an optional data frame with <code>ncol(data)</code> rows, with annotations ("metadata") on the observations, i.e, on the columns of data.
n_embedding	the dimension of the linear subspace (latent space).
linear_coefficient_estimator	specify which estimator is used to center the conditions. "linear" runs simple regression. It works well in many circumstances, but can produce unsatisfactory results if the composition of the cell types changes drastically between conditions (e.g., one cell type disappears). "mean", "cluster_median" and "zero" are alternative estimators, which are each supposed to be more robust against

	compositional changes, but cannot account for genes that change for all cells between conditions. "linear" is the default as it works best with subsequent alignment steps.
use_assay	if data is a SummarizedExperiment / SingleCellExperiment object: which of its assay slots should be used?
test_fraction	the fraction of cells (observations) that are set aside before the model fit, to keep an independent set of test observations. Alternatively, a logical vector of length ncol(data).
...	additional parameters that are passed on to the internal function lemur_impl.
verbose	Should the method print information during the fitting.

Value

An object of class `lemur_fit`, which extends `SingleCellExperiment`. Accordingly, all functions that work for that class also work for `lemur_fit` objects. In addition, slots of these objects can be accessed using the dollar notation, e.g., `fit$embedding`. For details see the `lemur_fit` help page.

References

- Ahlmann-Eltze, C. & Huber, W. (2023). Analysis of multi-condition single-cell data with latent embedding multivariate regression. bioRxiv <https://doi.org/10.1101/2023.03.06.531268>

See Also

[align_by_grouping](#), [align_harmony](#), [test_de](#), [find_de_neighborhoods](#)

Examples

```
data("glioblastoma_example_data")
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition)
fit
```

lemur_fit-class	<i>The lemur_fit class</i>
-----------------	----------------------------

Description

The `lemur_fit` class extends `SingleCellExperiment` and provides additional accessors to get the values of the values produced by `lemur`.

Usage

```
## S4 method for signature 'lemur_fit,ANY,ANY,ANY'
x[i, j, ..., drop = TRUE]

## S4 method for signature 'lemur_fit'
design(object)
```

Arguments

`x, i, j, ...`, `drop` the `lemur_fit` object and indices for the `[` subsetting operator
`object` the `lemur_fit` object for the `BiocGenerics::design` generic

Details

To access the values produced by `lemur`, use the dollar notation (`$`):

`fit$n_embedding` the number of embedding dimensions.

`fit$design` the specification of the design in `lemur`. Usually this is a `stats::formula`.

`fit$base_point` a matrix (`nrow(fit) * fit$n_embedding`) with the base point for the Grassmann exponential map.

`fit$coefficients` a three-dimensional tensor (`nrow(fit) * fit$n_embedding * ncol(fit$design_matrix)`) with the coefficients for the exponential map.

`fit$embedding` a matrix (`fit$n_embedding * ncol(fit)`) with the low dimensional position for each cell.

`fit$design_matrix` a matrix with covariates for each cell (`ncol(fit) * ncol(fit$design_matrix)`).

`fit$linear_coefficients` a matrix (`nrow(fit) * ncol(fit$design_matrix)`) with the coefficients for the linear regression.

`fit$alignment_coefficients` a 3D tensor with the coefficients for the alignment (`fit$n_embedding * fit$n_embedding * ncol(fit$design_matrix)`)

`fit$alignment_design` an alternative design specification for the alignment. This is typically a `stats::formula`.

`fit$alignment_design_matrix` an alternative design matrix specification for the alignment.

`fit$contrast` a parsed version of the contrast specification from the `test_de` function or `NULL`.

`fit$colData` the column annotation `DataFrame`.

`fit$rowData` the row annotation `DataFrame`.

Value

An object of class `lemur_fit`.

See Also

[lemur](#), [predict](#), [residuals](#)

Examples

```
# The easiest way to make a lemur_fit object, is to call `lemur`
data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)
```

```
fit$n_embedding
fit$embedding[,1:10]
fit$n_embedding
```

```
fit$embedding[,1:10]
fit$design_matrix[1:10,]
fit$coefficients[1:3,,]
```

predict.lemur_fit *Predict values from lemur_fit object*

Description

Predict values from lemur_fit object

Usage

```
## S3 method for class 'lemur_fit'
predict(
  object,
  newdata = NULL,
  newdesign = NULL,
  newcondition = NULL,
  embedding = object$embedding,
  with_linear_model = TRUE,
  with_embedding = TRUE,
  with_alignment = TRUE,
  ...
)
```

Arguments

object	an lemur_fit object
newdata	a data.frame which passed to <code>model.matrix</code> with design to make the newdesign matrix
newdesign	a matrix with the covariates for which the output is predicted. If NULL, the <code>object\$design_matrix</code> is used. If it is a vector it is repeated <code>ncol(embedding)</code> times to create a design matrix with the same entry for each cell.
newcondition	an unquoted expression with a call to <code>cond()</code> specifying the covariates of the prediction. See the contrast argument in <code>test_de</code> for more details. Note that combinations of multiple calls to <code>cond()</code> are not allowed (e.g., <code>cond(a = 1) - cond(a = 2)</code>). If specified, <code>newdata</code> and <code>newdesign</code> are ignored.
embedding	the low-dimensional cell position for which the output is predicted.
with_linear_model	a boolean to indicate if the linear regression offset is included in the prediction.
with_embedding	a boolean to indicate if the embedding contributes to the output.
with_alignment	a boolean to indicate if the alignment effect is removed from the output.
...	additional parameters passed to <code>predict_impl</code> .

Value

A matrix with the same dimension $nrow(\text{object}) * nrow(\text{newdesign})$.

See Also

[residuals](#)

Examples

```
data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)

pred <- predict(fit)

pred_ctrl <- predict(fit, newdesign = c(1, 0, 0, 0, 0, 0))
pred_trt <- predict(fit, newdesign = c(1, 0, 0, 0, 0, 1))
# This is the same as the test_de result
fit <- test_de(fit, cond(condition = "panobinostat") - cond(condition = "ctrl"))
all.equal(SummarizedExperiment::assay(fit, "DE"), pred_trt - pred_ctrl,
          check.attributes = FALSE)
```

project_on_lemur_fit *Project new data onto the latent spaces of an existing lemur fit*

Description

Project new data onto the latent spaces of an existing lemur fit

Usage

```
project_on_lemur_fit(
  fit,
  data,
  col_data = NULL,
  use_assay = "logcounts",
  design = fit$design,
  alignment_design = fit$alignment_design,
  return = c("matrix", "lemur_fit")
)
```

Arguments

fit	an lemur_fit object
data	a matrix with observations in the columns and features in the rows. Or a SummarizedExperiment / SingleCellExperiment object. The features must match the features in fit.
col_data	col_data an optional data frame with $ncol(\text{data})$ rows.

use_assay if data is a SummarizedExperiment / SingleCellExperiment object, which assay should be used.
design, alignment_design the design formulas or design matrices that are used to project the data on the correct latent subspace. Both default to the designs from the fit object.
return which data structure is returned.

Value

Either a matrix with the low-dimensional embeddings of the data or an object of class `lemur_fit` wrapping that embedding.

Examples

```

data(glioblastoma_example_data)

subset1 <- glioblastoma_example_data[,1:2500]
subset2 <- glioblastoma_example_data[,2501:5000]

fit <- lemur(subset1, design = ~ condition, n_emb = 5,
             test_fraction = 0, verbose = FALSE)

# Returns a `lemur_fit` object with the projection of `subset2`
fit2 <- project_on_lemur_fit(fit, subset2, return = "lemur_fit")
fit2

```

residuals,lemur_fit-method

Predict values from lemur_fit object

Description

Predict values from `lemur_fit` object

Usage

```

## S4 method for signature 'lemur_fit'
residuals(object, with_linear_model = TRUE, with_embedding = TRUE, ...)

```

Arguments

object an `lemur_fit` object
with_linear_model a boolean to indicate if the linear regression offset is included in the prediction.
with_embedding a boolean to indicate if the embedding contributes to the output.
... ignored.

Value

A matrix with the same dimension `dim(object)`.

See Also

[predict.lemur_fit](#)

Examples

```
data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)

resid <- residuals(fit)
dim(resid)
```

test_de

Predict log fold changes between conditions for each cell

Description

Predict log fold changes between conditions for each cell

Usage

```
test_de(
  fit,
  contrast,
  embedding = NULL,
  consider = c("embedding+linear", "embedding", "linear"),
  new_assay_name = "DE"
)
```

Arguments

<code>fit</code>	the result of calling <code>lemur()</code>
<code>contrast</code>	Specification of the contrast: a call to <code>cond()</code> specifying a full observation (e.g. <code>cond(treatment = "A", sex = "male") - cond(treatment = "C", sex = "male")</code>) to compare treatment A vs C for male observations). Unspecified factors default to the reference level.
<code>embedding</code>	matrix of size <code>n_embedding × n</code> that specifies where in the latent space the differential expression is tested. It defaults to the position of all cells from the original fit.
<code>consider</code>	specify which part of the model are considered for the differential expression test.
<code>new_assay_name</code>	the name of the assay added to the <code>fit</code> object. Default: "DE".

Value

If `is.null(embedding)` the fit object with a new assay called "DE". Otherwise return a matrix with the differential expression values.

See Also

[find_de_neighborhoods](#)

Examples

```
library(SummarizedExperiment)
library(SingleCellExperiment)

data(glioblastoma_example_data)
fit <- lemur(glioblastoma_example_data, design = ~ patient_id + condition,
            n_emb = 5, verbose = FALSE)
# Optional alignment
# fit <- align_harmony(fit)
fit <- test_de(fit, contrast = cond(condition = "panobinostat") - cond(condition = "ctrl"))

# The fit object contains a new assay called "DE"
assayNames(fit)

# The DE assay captures differences between conditions
is_ctrl_cond <- fit$colData$condition == "ctrl"
mean(logcounts(fit)[1,!is_ctrl_cond]) - mean(logcounts(fit)[1,is_ctrl_cond])
mean(assay(fit, "DE")[1,])
```

test_global

Differential embedding for each condition

Description

Differential embedding for each condition

Usage

```
test_global(
  fit,
  contrast,
  reduced_design = NULL,
  consider = c("embedding+linear", "embedding", "linear"),
  variance_est = c("analytical", "resampling", "none"),
  verbose = TRUE,
  ...
)
```

Arguments

fit	the result of calling <code>lemur()</code>
contrast	Specification of the contrast: a call to <code>cond()</code> specifying a full observation (e.g. <code>cond(treatment = "A", sex = "male") - cond(treatment = "C", sex = "male")</code> to compare treatment A vs C for male observations). Unspecified factors default to the reference level.
reduced_design	an alternative specification of the null hypothesis.
consider	specify which part of the model are considered for the differential expression test.
variance_est	How or if the variance should be estimated. 'analytical' is only compatible with <code>consider = "linear"</code> . 'resampling' is the most flexible (to adapt the number of resampling iterations, set <code>n_resampling_iter</code> . Default: 100)
verbose	should the method print information during the fitting. Default: TRUE.
...	additional arguments.

Value

a `data.frame`

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