# Package 'lemur'

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Type Package

Title Latent Embedding Multivariate Regression

Version 1.7.0

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.

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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

2

#### align\_harmony

#### 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

the lemur_fit
the pattern from looking up potential values interactively
the name of the value behind the dollar
the replacement value. This only works for $\verb"colData"$ and <code>rowData</code> .

# Value

The respective value stored in the lemur\_fit object.

# See Also

lemur\_fit for more documentation on the accessor functions.

align_harmony	Enforce additional alignment of cell clusters beyond the direct differ-
	ential embedding

## 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 transforma- tion. Default: fit\$design_matrix	
ridge_penalty	specification how much the flexibility of the transformation should be regular- ized. 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

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

align\_impl

Align the points according to some grouping

# Description

Align the points according to some grouping

#### find\_de\_neighborhoods

#### Usage

```
align_impl(
   embedding,
   grouping,
   design_matrix,
   ridge_penalty = 0.01,
   preserve_position_of_NAs = FALSE,
   calculate_new_embedding = TRUE
)
```

#### Value

A list with the new embedding and the 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

fit

the lemur\_fit generated by lemur()

group_by	If the independent_matrix is provided, group_by defines how the pseudob- ulks 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 vars().
contrast	a specification which contrast to fit. This defaults to the contrast argument that was used for test_de and is stored in fit\$contrast.
selection_proce	
	<pre>specify the algorithm that is used to select the neighborhoods for each gene. Broadly, selection_procedure = "zscore" is faster but less precise than selection_procedure = "contrast".</pre>
directions	a string to define the algorithm to select the direction onto which the cells are projected before searching for the neighborhood. directions = "random" pro- duces denser neighborhoods, whereas directions = "contrast" has usually more power. Alternatively, this can also be a matrix with one direction for each gene (i.e., a matrix of size nrow(fit) * fit\$n_embedding).
min_neighborhoo	od_size
	the minimum number of cells per neighborhood. Default: 50.
de_mat	<pre>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 lemur::test_de().</pre>
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 lemur().
test_data_col_c	lata
	additional column data for the test_data argument.
test_method	choice of test for the pseudobulked differential expression. glmGamPoi and edgeR work on an count assay. limma works on the continuous assay.
continuous_assa	ay_name, count_assay_name
	the assay or list names of independent_data.
<pre>size_factor_met</pre>	chod
	Set the procedure to calculate the size factor after pseudobulking. This argu- ment 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 colSums(assay(fit\$test_data, count_assay_name)) is used.
design, alignmer	nt_design
	the design to use for the fit. Default: fit\$design
add_diff_in_dif	
	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_neighborh	pods_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_paramet	ters	
	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

fold\_left

Fold left over a sequence

#### Description

Fold left over a sequence

Fold right over a sequence

#### Usage

fold\_left(init)

fold\_right(init)

## Arguments

init	initial value. If not specified NULL
х	the sequence to iterate over
FUN	a function with first argument named elem and second argument named accum

### Value

The final value of accum.

## Examples

```
## Not run:
    # This produces ...
    fold_left(0)(1:10, \(elem, accum) accum + elem)
    # ... the same as
    sum(1:10)
```

```
## End(Not run)
```

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.

```
grassmann_geodesic_regression
```

Solve  $d(P, exp_p(V * x))^2$  for V

## Description

Solve  $d(P, exp_p(V * x))^2$  for V

## Usage

```
grassmann_geodesic_regression(
   coordsystems,
   design,
   base_point,
   weights = 1,
   tangent_regression = FALSE
)
```

# Value

A three-dimensional array with the coefficients V.

grassmann_lm Solve $  Y - exp_p(V * x) Y  ^2_2$ for V	$p(V * x) Y \parallel^{2} 2 \text{ for } V$
---	---

## Description

```
Solve ||Y - exp_p(V * x) Y ||^2_2 for V
```

#### Usage

```
grassmann_lm(data, design, base_point, tangent_regression = FALSE)
```

## Value

A three-dimensional array with the coefficients V.

harmony\_new\_object Create an arbitrary Harmony object so that I can modify it later

## Description

Create an arbitrary Harmony object so that I can modify it later

# Usage

```
harmony_new_object()
```

## Value

The full harmony object (R6 reference class type).

lemur

Main function to fit the latent embedding multivariate regression (LEMUR) model

## Description

Main function 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 SummarizedExperiment / SingleCellExperiment object
design	a formula referring to global objects or column in the colData of data and col_data argument
col_data	an optional data frame with ncol(data) rows.
n_embedding	the dimension of the \$k\$-plane that is rotated through space.
linear_coeffici	ient_estimator
	specify which estimator is used to center the conditions. "linear" runs simple
	regression it works well in many circumstances but can produce poor results if
	the composition of the cell types changes 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 assay should be used.
test_fraction	the fraction of cells that are split of before the model fit to keep an independent
	set of test observations. Alternatively, a logical vector of length ncol(data).
	Default: 20% (0.2).
	additional parameters that are passed on to the internal function lemur_impl.
verbose	Should the method print information during the fitting. Default: TRUE.

# Value

An object of class lemur\_fit which extends SingleCellExperiment. Accordingly, all functions that work for sce's also work for lemur\_fit's. In addition, we give easy access to the fitted values using the dollar notation (e.g., fit\$embedding). For details see the lemur\_fit help page.

#### lemur\_fit-class

#### 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, n_emb = 5)
fit</pre>
```

lemur\_fit-class The lemur\_fit class

#### 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\$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\_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

mply\_dbl

Iterating function that returns a matrix

### Description

The length of x determines the number of rows. The length of FUN(x[i]) determines the number of columns. Must match ncol.

#### Usage

mply\_dbl(x, FUN, ncol = 1, ...)
stack\_rows(x)
stack\_cols(x)

# Arguments

х	the sequence that is mapped to a matrix
FUN	the function that returns a vector of length ncol
ncol	the length of the output vector
	additional arguments that are passed to FUN

# Value

A matrix with length(x) / nrow(x) rows and ncol columns. For msply\_dbl the number of columns depends on the output of FUN.

#### Functions

- stack\_rows(): Each list element becomes a row in a matrix
- stack\_cols(): Each list element becomes a row in a matrix

one\_hot\_encoding Take a vector and convert it to a one-hot encoded matrix

#### Description

Take a vector and convert it to a one-hot encoded matrix

#### Usage

one\_hot\_encoding(groups)

## Value

A matrix with length(unique(groups)) rows and length(groups) columns.

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,
   newdesign = NULL,
   newcondition = NULL,
   embedding = object$embedding,
   with_linear_model = TRUE,
   with_embedding = TRUE,
   ...
)
```

## Arguments

object	an lemur_fit object
newdata	a data.frame which passed to ${\tt model.matrix}$ with design to make the newdesign matrix
newdesign	a matrix with the covariates for which the output is predicted. If NULL, the object\$design_matrix is used. If it is a vector it is repeated ncol(embedding) times to create a design matrix with the same entry for each cell.
newcondition	an unquoted expression with a call to cond() specifying the covariates of the prediction. See the contrast argument in test_de for more details. Note that combinations of multiple calls to cond() are not allowed (e.g., cond( $a = 1$ ) - cond( $a = 2$ )). If specified, newdata and newdesign are ignored.
embedding	the low-dimensional cell position for which the output is predicted.
with_linear_mod	del
	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 predict_impl.

## Value

A matrix with the same dimension nrow(object) \* nrow(newdesign).

## See Also

residuals

## Examples

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

#### project\_on\_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(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.		the design formulas or design matrices that are used to project the data on the
	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

pseudoinverse

# Description

In the simplest case, the pseudoinverse is

$$X^+ = (X^T X)^{-1} X^T.$$

# Usage

pseudoinverse(X)

# Arguments X

a matrix X

# Details

To handle the more general case, the pseudoinverse can expressed using a SVD  $X = UDV^T$ :

$$X^+ = V D^{-1} U^T$$

## Value

The matrix  $X^+$ .

recursive\_least\_squares

Iteratively calculate the least squares solution

## Description

Both functions are for testing purposes. There is a faster implementation called cum\_brls\_which\_abs\_max.

#### Usage

```
recursive_least_squares(y, X)
bulked_recursive_least_squares_contrast(
    y,
    X,
    group,
```

contrast, ridge\_penalty = 1e-06

)

## Arguments

У	a vector with observations
Х	a design matrix

#### reexports

#### Value

a matrix where column i is the solution to  $y[1:i] \sim X[1:i,]$ .

```
reexports
```

Objects exported from other packages

## Description

These objects are imported from other packages. Follow the links below to see their documentation.

glmGamPoi vars

# Value

see glmGamPoi::vars.

## Examples

```
# `vars` quotes expressions (just like in dplyr)
vars(condition, sample)
```

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

ridge\_regression Ridge regression

# Description

The function does not treat the intercept special.

# Usage

```
ridge_regression(Y, X, ridge_penalty = 0, weights = rep(1, nrow(X)))
```

# Arguments

Υ	the observations matrix (features x samples)
Х	the design matrix (samples x covariates)
ridge_penalty	a numeric vector or matrix of size (covariates or covariates x covariates respectively)
weights	a vector of observation weights

#### Value

The matrix of coefficients.

<pre>stack_slice</pre>	Make a cube from a list of matrices	
------------------------	-------------------------------------	--

## Description

The length of the list will become the third dimension of the cube.

# Usage

```
stack_slice(x)
```

destack\_slice(x)

## Arguments

```
х
```

a list of vectors/matrices that are stacked

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#### test\_de

# Value

A three-dimensional array.

# Functions

• destack\_slice(): Make a list of matrices from a cube

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

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

<code>new\_assay\_name </code> the name of the assay added to the fit 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

```
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 lemur()
contrast	Specification of the contrast: a call to cond() specifying a full observation (e.g. cond(treatment = "A", sex = "male") - cond(treatment = "C", sex = "male") 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 consider = "linear". 'resampling' is the most flexible (to adapt the number of resampling iterations, set n_resampling_iter. Default: 100)

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## %zero\_dom\_mat\_mult%

verbose should the method print information during the fitting. Default: TRUE. ... additional arguments.

#### Value

a data.frame

%zero\_dom\_mat\_mult% Helper function that makes sure that NA \* 0 = 0 in matrix multiply

# Description

Helper function that makes sure that NA \* 0 = 0 in matrix multiply

# Usage

X %zero\_dom\_mat\_mult% Y

# Arguments

Х	a matrix of size n*m
Υ	a matrix of size m*p

#### Value

a matrix of size n\*p

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