

# Package ‘slalom’

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**Type** Package

**Title** Factorial Latent Variable Modeling of Single-Cell RNA-Seq Data

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**Description** slalom is a scalable modelling framework for single-cell RNA-seq data that uses gene set annotations to dissect single-cell transcriptome heterogeneity, thereby allowing to identify biological drivers of cell-to-cell variability and model confounding factors. The method uses Bayesian factor analysis with a latent variable model to identify active pathways (selected by the user, e.g. KEGG pathways) that explain variation in a single-cell RNA-seq dataset. This an R/C++ implementation of the f-scLVM Python package. See the publication describing the method at <https://doi.org/10.1186/s13059-017-1334-8>.

**Depends** R (>= 4.0)

**Imports** Rcpp (>= 0.12.8), RcppArmadillo, BH, ggplot2, grid, GSEABase, methods, rsvd, SingleCellExperiment, SummarizedExperiment, stats

**Suggests** BiocStyle, knitr, rhdf5, rmarkdown, scater, testthat

**LinkingTo** Rcpp, RcppArmadillo, BH

**License** GPL-2

**VignetteBuilder** knitr

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**Encoding** UTF-8

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addResultsToSingleCellExperiment  
*Add results to SingleCellExperiment object*

---

## Description

Add results to SingleCellExperiment object

## Usage

```
addResultsToSingleCellExperiment(sce_object, slalom_object, n_active = 20,
  mad_filter = 0.4, annotated = TRUE, unannotated_dense = FALSE,
  unannotated_sparse = FALSE, add_loadings = TRUE, dimred = "slalom",
  check_convergence = TRUE)
```

**Arguments**

sce_object	an object of class <a href="#">SingleCellExperiment</a>
slalom_object	an object of class Rcpp_SlalomModel
n_active	number of terms (factors) to be added (default is 20)
mad_filter	numeric(1), filter factors by this mean absolute deviation to ensure variability in the factor states. For large datasets this can be set to 0
annotated	logical(1), should annotated factors be included? Default is TRUE
unannotated_dense	logical(1), should dense unannotated factors be included? Default is FALSE
unannotated_sparse	logical(1), should sparse unannotated factors be included? Default is FALSE
add_loadings	logical(1), should gene/feature loadings be added to the rowData of the object?
dimred	character(1), name of the reduced-dimension slot to save the factor states to. Default is "slalom"
check_convergence	logical(1), check that model has converged before adding slalom results. If TRUE and model has not converged it throws an error.

**Value**

a [SingleCellExperiment](#) object with factor states (X) in a reduced-dimension slot, and gene loadings for factors added to rowData.

**Examples**

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- trainSlalom(model, nIterations = 10)
mesc <- addResultsToSingleCellExperiment(mesc, model,
check_convergence = FALSE)
```

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initSlalom	<i>Initialize a SlalomModel object</i>
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**Description**

Initialize a SlalomModel with sensible starting values for parameters before training the model.

**Usage**

```
initSlalom(object, alpha_priors = NULL, epsilon_priors = NULL,
noise_model = "gauss", seed = NULL, pi_prior = NULL, n_hidden = NULL,
design = NULL, verbose = FALSE, save_init = FALSE)
```

## Arguments

object	a Rcpp_SlalomModel object
alpha_priors	numeric(2) giving alpha and beta hyperparameters for a gamma prior distribution for alpha parameters (precision of factor weights)
epsilon_priors	numeric(2) giving alpha and beta hyperparameters for a gamma prior distribution for noise precision parameters
noise_model	character(1) defining noise model, defaults to "gauss" for Gaussian noise model
seed	integer(1) value supplying a random seed to make results reproducible (default is NULL)
pi_prior	numeric matrix (genes x factors) giving prior probability of a gene being active for a factor
n_hidden	integer(1), number of hidden factors in model. Required if pi_prior is not NULL, ignored otherwise.
design	matrix of known factors (covariates) to fit in the model. Optional if pi_prior is not NULL, ignored otherwise.
verbose	logical(1), should messages be printed about what the function is doing? Default is TRUE.
save_init	logical(1), save the initial X values (factor states for each cell) in the object? Default is FALSE as this is potentially a large N (number of cell) by K (number of factors) matrix.

## Details

It is strongly recommended to use [newSlalomModel](#) to create the [SlalomModel](#) object prior to applying `initSlalom`.

## Value

an 'Rcpp\_SlalomModel' object

## Author(s)

Davis McCarthy

## Examples

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
```

---

mesc	<i>A single-cell expression dataset to demonstrate capabilities of slalom from mouse embryonic stem cells (mESCs)</i>
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### Description

This data set consists of an [SCESet](#) object with log<sub>2</sub>-counts-per-million expression values for 3635 genes for 182 cells. They are from a real experiment, studying cell cycle in mouse embryonic stem cells (mESCs). See Buettner et al (Nat. Biotech., 2015) for details. d.

### Usage

```
mesc
```

### Format

an SCESet instance, 1 row per gene.

### Value

NULL, but makes available an SCESet object containing expression data

### Author(s)

Davis McCarthy, Florian Buettner, 2016-12-02

### Source

EMBL-EBI, Hinxton, UK

### References

Buettner F, Natarajan KN, Paolo Casale F, Proserpio V, Scialdone A, Theis FJ, et al. Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. Nat Biotechnol. Nature Publishing Group; 2015;33: 155–160.

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newSlalomModel	<i>Create a new SlalomModel object.</i>
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### Description

Slalom fits relatively complicated hierarchical Bayesian factor analysis models with data and results stored in a "SlalomModel" object. This function builds a new "SlalomModel" object from minimal inputs.

**Usage**

```
newSlalomModel(object, genesets, n_hidden = 5, prune_genes = TRUE,
  min_genes = 15, design = NULL, anno_fpr = 0.01, anno_fnr = 0.001,
  assay_name = "logcounts", verbose = TRUE)
```

**Arguments**

object	"SingleCellExperiment" object N x G expression data matrix (cells x genes)
genesets	a "GeneSetCollection" object containing annotated gene sets
n_hidden	number of hidden factors to fit in the model (2-5 recommended)
prune_genes	logical, should genes that are not annotated to any gene sets be filtered out? If TRUE, then any genes with zero variance in expression are also filtered out.
min_genes	scalar, minimum number of genes required in order to retain a gene set for analysis
design	numeric design matrix providing values for covariates to fit in the model (rows represent cells)
anno_fpr	numeric(1), false positive rate (FPR) for assigning genes to factors (pathways); default is 0.01
anno_fnr	numeric(1), false negative rate (FNR) for assigning genes to factors (pathways); default is 0.001
assay_name	character(1), the name of the assay of the object to use as expression values. Default is logcounts, assumed to be normalised log2-counts-per-million values or equivalent.
verbose	logical(1), should information about what's going be printed to screen?

**Details**

This function builds and returns the object, checking for validity, which includes checking that the input data is of consistent dimensions.

**Value**

a new Rcpp\_SlalomModel object

**Examples**

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)

exprsfile <- system.file("extdata", "mesc.csv", package = "slalom")
mesc_mat <- as.matrix(read.csv(exprsfile))
sce <- SingleCellExperiment::SingleCellExperiment(assays = list(logcounts = mesc_mat))
# model2 <- newSlalomModel(mesc_mat, genesets, n_hidden = 5, min_genes = 10)
```

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plotLoadings	<i>Plot highest loadings of a factor</i>
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## Description

Plot highest loadings of a factor

## Usage

```
plotLoadings(object, term, n_genes = 10)
```

## Arguments

object	an object of class Rcpp_SlalomModel
term	integer(1) or character(1), providing either index for desired term (if an integer) or the term name (if character)
n_genes	integer(1), number of loadings (genes) to show

## Details

Show the factor loadings for a genes with the highest loadings for a given factor. Absolute weights are shown, with genes ordered by absolute weight. Indications are given on the plot as to whether the gene was originally in the factor geneset or added to it by the slalom model.

## Value

a ggplot plot object

## Examples

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- trainSlalom(model, nIterations = 10)
plotLoadings(model, term = 2)
```

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plotRelevance	<i>Plot results of a Slalom model</i>
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### Description

Plot results of a Slalom model

### Usage

```
plotRelevance(object, n_active = 20, mad_filter = 0.4, annotated = TRUE,  
              unannotated_dense = FALSE, unannotated_sparse = FALSE)
```

### Arguments

object	an object of class Rcpp_SlalomModel
n_active	number of terms (factors) to be plotted (default is 20)
mad_filter	numeric(1), filter factors by this mean absolute deviation to exclude outliers. For large datasets this can be set to 0
annotated	logical(1), should annotated factors be plotted? Default is TRUE
unannotated_dense	logical(1), should dense unannotated factors be plotted? Default is FALSE
unannotated_sparse	logical(1), should sparse unannotated factors be plotted? Default is FALSE

### Value

invisibly returns a list containing the two ggplot objects that make up the plot

### Examples

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")  
genesets <- GSEABase::getGmt(gmtfile)  
data("mesc")  
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)  
model <- initSlalom(model)  
model <- trainSlalom(model, nIterations = 10)  
plotRelevance(model)
```



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plotTerms	<i>Plot relevance for all terms</i>
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### Description

Plot relevance for all terms

### Usage

```
plotTerms(object, terms = NULL, order_terms = TRUE, mad_filter = 0.2,
          annotated = TRUE, unannotated_dense = TRUE, unannotated_sparse = FALSE)
```

### Arguments

object	an object of class Rcpp_SlalomModel
terms	integer or character vector, providing either indices for desired terms (if an integer) or the term names (if character); default is NULL, in which case all terms are plotted.
order_terms	logical(1), should factors be ordered by relevance ( TRUE, default), or in the order they come
mad_filter	numeric(1), filter factors by this mean absolute deviation to exclude outliers. For large datasets this can be set close to 0; default is 0.2.
annotated	logical(1), should annotated factors be plotted? Default is TRUE
unannotated_dense	logical(1), should dense unannotated factors be plotted? Default is TRUE
unannotated_sparse	logical(1), should sparse unannotated factors be plotted? Default is TRUE

### Value

a ggplot plot object

### Examples

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- trainSlalom(model, nIterations = 10)
plotTerms(model)
```

---

Rcpp\_SlalomModel      *The "Slalom Model" (Rcpp\_SlalomModel) class*

---

### Description

S4 class and the main class used by slalom to hold model data and results. SingleCellExperiment extends the Bioconductor SummarizedExperiment class.

### Details

This class is initialized from a matrix of expression values and a collection of genesets in a GeneSetCollection object from the GSEABase package.

Methods that operate on SingleCellExperiment objects constitute the basic scater workflow.

### Methods

train() void train() docstring : Train the SlalomModel  
 update() void update() docstring : Update the SlalomModel  
 updateAlpha(...) void updateAlpha(int) docstring : Update alpha  
 updateEpsilon() void updateEpsilon() docstring : Update Epsilon  
 updatePi(...) void updatePi(int) docstring : Update Pi  
 updateW(...) void updateW(int) docstring : Update W  
 updateX(...) void updateX(int) docstring : Update X

### Slots

.xData: Environment enabling access to the C++-level SlalomModel object.

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slalom      *Factorial single-cell latent variable models*

---

### Description

slalom

### Details

Factorial latent variable models for RNA-seq data.

### Author(s)

Davis McCarthy

---

SlalomModel	<i>SlalomModel C++ class</i>
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**Description**

A C++ class for SlalomModel models.

**Arguments**

Y_init	matrix of expression values
pi_init	G x K matrix with each entry being the prior probability for a gene g being active for factor k.
X_init	matrix of initial factor states (N x K)
W_init	G x K matrix of initial weights
prior_alpha	numeric vector of length two giving prior values for the gamma hyperparameters of the precisions
prior_epsilon	numeric vector of length two giving prior values for the gamma hyperparameters of the residual variances

**Value**

an object of the SlalomModel class

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topTerms	<i>Show results of a Slalom model</i>
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**Description**

Show results of a Slalom model

**Usage**

```
topTerms(object, n_active = 20, mad_filter = 0.4, annotated = TRUE,
unannotated_dense = FALSE, unannotated_sparse = FALSE)
```

**Arguments**

object	an object of class Rcpp_SlalomModel
n_active	number of terms (factors) to be plotted (default is 20)
mad_filter	numeric(1), filter factors by this mean absolute deviation to exclude outliers. For large datasets this can be set to 0
annotated	logical(1), should annotated factors be plotted? Default is TRUE
unannotated_dense	logical(1), should dense unannotated factors be plotted? Default is FALSE
unannotated_sparse	logical(1), should sparse unannotated factors be plotted? Default is FALSE

**Value**

data.frame with factors ordered by relevance, showing term (term names), relevance, type (factor type: known, annotated or unannotated), n\_prior (number of genes annotated to the gene set/factor), n\_gain (number of genes added/switched on for the factor), n\_loss (number of genes turned off for the factor).

**Examples**

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- trainSlalom(model, nIterations = 10)
topTerms(model)
```

---

trainSlalom	<i>Train a SlalomModel object</i>
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**Description**

Train a SlalomModel to infer model parameters.

**Usage**

```
trainSlalom(object, nIterations = 5000, minIterations = 700,
  tolerance = 1e-08, forceIterations = FALSE, shuffle = TRUE,
  pretrain = TRUE, verbose = TRUE, seed = NULL, drop_factors = TRUE)
```

**Arguments**

object	a Rcpp_SlalomModel object
nIterations	integer(1) maximum number of iterations to use in training the model (default: 5000)
minIterations	integer(1) minimum number of iterations to perform.
tolerance	numeric(1) tolerance to allow between iterations (default 1e-08)
forceIterations	logical(1) should the model be forced to update nIteration times?
shuffle	logical(1) should the order in which factors are updated be shuffled between iterations? Shuffling generally helps speed up convergence so is recommended and defaults is TRUE
pretrain	logical(1), should the model be "pre-trained" to achieve faster convergence and obtain an initial update order? Recommended; default is TRUE
verbose	logical(1), should messages be printed about what the function is doing? Default is TRUE.

seed	integer(1) value supplying a random seed to make results reproducible (default is NULL)
drop_factors	logical(1), should factors be dropped from the model if the model determines them not to be relevant? Default is TRUE.

**Details**

Train the model using variational Bayes methods to infer parameters.

**Value**

an 'Rcpp\_SlalomModel' object

**Author(s)**

Davis McCarthy

**Examples**

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- trainSlalom(model, nIterations = 10)
```

---

updateSlalom

*Update a SlalomModel object*

---

**Description**

Do one variational update of a SlalomModel to infer model parameters.

**Usage**

```
updateSlalom(object)
```

**Arguments**

object            a Rcpp\_SlalomModel object

**Details**

Update the model with one iteration using variational Bayes methods to infer parameters.

**Value**

an 'Rcpp\_SlalomModel' object

**Author(s)**

Davis McCarthy

**Examples**

```
gmtfile <- system.file("extdata", "reactome_subset.gmt", package = "slalom")
genesets <- GSEABase::getGmt(gmtfile)
data("mesc")
model <- newSlalomModel(mesc, genesets, n_hidden = 5, min_genes = 10)
model <- initSlalom(model)
model <- updateSlalom(model)
```

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