Package ‘monocle’

March 28, 2017

Type Package

Title Clustering, differential expression, and trajectory analysis for single-cell RNA-Seq

Version 2.2.0

Date 2016-06-06

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Description Monocle performs differential expression and time-series analysis for single-cell expression experiments. It orders individual cells according to progress through a biological process, without knowing ahead of time which genes define progress through that process. Monocle also performs differential expression analysis, clustering, visualization, and other useful tasks on single cell expression data. It is designed to work with RNA-Seq and qPCR data, but could be used with other types as well.

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Depends R (>= 2.10.0), methods, Matrix (>= 1.2-6), Biobase, ggplot2 (>= 1.0.0), VGAM (>= 1.0-1), DDRTree (>= 0.1.4)

Imports parallel, igraph (>= 1.0.1), BiocGenerics, HSMM, gisSingleCell (>= 0.10.1.5), plyr, cluster, combinat, fastICA, grid, irIba (>= 2.0.0), matrixStats, MASS, reshape2, limma, dplyr, qlcMatrix, pheatmap, stringr, proxy, slam, stats

VignetteBuilder knitr

Suggests knitr, Hmisc, testthat

Roxygen list(wrap = FALSE)

LazyData true

biocViews Sequencing, RNASeq, GeneExpression, DifferentialExpression, Infrastructure, DataImport, DataRepresentation, Visualization, Clustering, MultipleComparison, QualityControl

RoxygenNote 5.0.1

NeedsCompilation no

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BEAM

Identification of genes with branch-dependent expression

Usage

```r
BEAM(cds, fullModelFormulaStr = "~sm.ns(Pseudotime, df = 3)*Branch",
    reducedModelFormulaStr = "~sm.ns(Pseudotime, df = 3)",
    branch_states = NULL, branch_point = 1, relative_expr = TRUE,
    branch_labels = NULL, verbose = FALSE, cores = 1, ...)
```

Arguments

- **cds**: a CellDataSet object upon which to perform this operation
- **fullModelFormulaStr**: a formula string specifying the full model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
- **reducedModelFormulaStr**: a formula string specifying the reduced model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
- **branch_states**: ids for the immediate branch branch which obtained from branch construction based on MST
- **branch_point**: The ID of the branch point to analyze. Can only be used when reduceDimension is called with method = "DDRTree".
- **relative_expr**: a logic flag to determine whether or not the relative gene expression should be used
- **branch_labels**: the name for each branch, for example, "AT1" or "AT2"
branchTest

| verbose | Whether to generate verbose output |
| cores   | the number of cores to be used while testing each gene for differential expression |
| ...     | additional arguments to be passed to differentialGeneTest |

Details

Branches in single-cell trajectories are generated by cell fate decisions in development and also arise when analyzing genetic, chemical, or environmental perturbations. Branch expression analysis modeling is a statistical approach for finding genes that are regulated in a manner that depends on the branch. Consider a progenitor cell that generates two distinct cell types. A single-cell trajectory that includes progenitor cells and both differentiated cell types will capture the “decision” as a branch point, with progenitors upstream of the branch and the differentiated cells positioned along distinct branches. These branches will be characterized by distinct gene expression programs. BEAM aims to find all genes that differ between the branches. Such “branch-dependent” genes can help identify the mechanism by which the fate decision is made.

BEAM() Takes a CellDataSet and either a specified branch point, or a pair of trajectory outcomes (as States). If a branch point is provided, the function returns a dataframe of test results for dependence on that branch. If a pair of outcomes is provided, it returns test results for the branch that unifies those outcomes into a common path to the trajectory’s root state.

BEAM() compares two models with a likelihood ratio test for branch-dependent expression. The full model is the product of smooth Pseudotime and the Branch a cell is assigned to. The reduced model just includes Pseudotime. You can modify these to include arbitrary additional effects in the full or both models.

Value

a data frame containing the p values and q-values from the BEAM test, with one row per gene.

branchTest Test for branch-dependent expression

Description

Testing for branch-dependent expression with BEAM() first involves constructing a CellDataSet that assigns each cell to a branch, and then performing a likelihood ratio test to see if the branch assignments significantly improves the fit over a null model that does not split the cells. branchTest() implements these two steps.

Usage

branchTest(cds, fullModelFormulaStr = "~sm.ns(Pseudotime, df = 3)*Branch", reducedModelFormulaStr = "~sm.ns(Pseudotime, df = 3)", branch_states = NULL, branch_point = 1, relative_expr = TRUE, cores = 1, branch_labels = NULL, verbose = FALSE, ...)
**Arguments**

- **cds**: a `CellDataSet` object upon which to perform this operation
- **fullModelFormulaStr**: a formula string specifying the full model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
- **reducedModelFormulaStr**: a formula string specifying the reduced model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
- **branch_states**: states corresponding to two branches
- **branch_point**: The ID of the branch point to analyze. Can only be used when reduceDimension is called with method = "DDRTree".
- **relative_expr**: a logic flag to determine whether or not the relative gene expression should be used
- **cores**: the number of cores to be used while testing each gene for differential expression
- **branch_labels**: the name for each branch, for example, AT1 or AT2
- **verbose**: Whether to show VGAM errors and warnings. Only valid for cores = 1.
- **...**: Additional arguments passed to differentialGeneTest

**Value**

a data frame containing the p values and q-values from the likelihood ratio tests on the parallel arrays of models.

---

### Description

Analyzing branches with `BEAM()` requires fitting two models to the expression data for each gene. The full model assigns each cell to one of the two outcomes of the branch, and the reduced model excludes this assignment. `buildBranchCellDataSet()` takes a `CellDataSet` object and returns a version where the cells are assigned to one of two branches. The branch for each cell is encoded in a new column, "Branch", in the pData table in the returned `CellDataSet`.

### Usage

```r
buildBranchCellDataSet(cds, progenitor_method = c("sequential_split", "duplicate"), branch_states = NULL, branch_point = 1, branch_labels = NULL, stretch = TRUE)
```

### Arguments

- **cds**: `CellDataSet` for the experiment
- **progenitor_method**: The method to use for dealing with the cells prior to the branch
- **branch_states**: The states for two branching branches
calABCs

branch_point The ID of the branch point to analyze. Can only be used when `reduceDimension()` is called with `reduction_method = "DRRTree"`.

branch_labels The names for each branching branch

stretch A logical flag to determine whether or not the pseudotime trajectory for each branch should be stretched to the same range or not

Value

a CellDataSet with the duplicated cells and stretched branches

---

calABCs | Compute the area between curves (ABC) for branch-dependent genes

**Description**

This function is used to calculate the ABC score based on the the nature spline curves fitted for each branch. ABC score is used to quantify the total magnitude of divergence between two branches. By default, the ABC score is the area between two fitted spline curves. The ABC score can be used to rank gene divergence. When coupled with p-val calculated from the branchTest, it can be used to identify potential major regulators for branch bifurcation.

**Usage**

```r
calABCs(cds, trend_formula = "~sm.ns(Pseudotime, df = 3)*Branch", trajectory_states = c(2, 3), relative_expr = TRUE, stretch = TRUE, cores = 1, verbose = F, min_expr = 0.5, integer_expression = FALSE, num = 5000, branch_labels = NULL, ...)
```

**Arguments**

cds a CellDataSet object upon which to perform this operation
trend_formula a formula string specifying the full model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.

trajectory_states States corresponding to two branches

relative_expr a logic flag to determine whether or not the relative gene expression should be used

stretch a logic flag to determine whether or not each branch should be stretched

cores the number of cores to be used while testing each gene for differential expression

verbose a logic flag to determine whether or not we should output detailed running information

min_expr the lower limit for the expressed gene

integer_expression the logic flag to determine whether or not the integer numbers are used for calculating the ABCs. Default is False.

num number of points on the fitted branch trajectories used for calculating the ABCs. Default is 5000.

branch_labels the name for each branch, for example, AT1 or AT2

... Additional arguments passed to buildBranchCellDataSet
calibrate_per_cell_total_proposal

Description
Calibrate_per_cell_total_proposal

Usage

```r
calibrate_per_cell_total_proposal(relative_exprs_matrix, t_estimate, expected_capture_rate)
```

Arguments

- `relative_exprs_matrix`: The matrix of relative TPM expression values
- `t_estimate`: the TPM value that corresponds to 1 cDNA copy per cell
- `expected_capture_rate`: The fraction of mRNAs captured as cDNAs

callILRs

Description

Calculate the Instantaneous Log Ratio between two branches

Usage

```r
callILRs(cds, trend_formula = "~sm.ns(Pseudotime, df = 3)*Branch", trajectory_states = c(2, 3), relative_expr = TRUE, stretch = TRUE, cores = 1, ILRs_limit = 3, label_by_short_name = TRUE, useVST = FALSE, round_exprs = FALSE, output_type = "all", branch_labels = NULL, file = NULL, return_all = F, verbose = FALSE, ...)
```
Arguments

cds CellDataSet for the experiment
trend_formula a formula string specifying the full model in differential expres-
sion tests (i.e. likelihood ratio tests) for each gene/feature.
trajectory_states states corresponding to two branches
relative_expr A logic flag to determine whether or not the relative expressed should be used
when we fitting the spline curves
stretch a logic flag to determine whether or not each branch should be stretched
cores Number of cores when fitting the spline curves
ILRs_limit the minimum Instant Log Ratio used to make the heatmap plot
label_by_short_name label the rows of the returned matrix by gene_short_name (TRUE) or feature id
(useVST) (FALSE)
round_exprs A logic flag to determine whether or not the expression value should be rounded
into integer
output_type A character either of "all" or "after_bifurcation". If "after_bifurcation" is used, only the
time points after the bifurcation point will be selected
branch_labels the name for each branch, for example, AT1 or AT2
file the name for storing the data. Since the calculation of the Instant Log Ratio is
very time consuming, so by default the result will be stored
return_all A logic flag to determine whether or not all the results from the analysis should
be returned, this includes a dataframe for the log fold change, normalized log
t fold change, raw divergence, normalized divergence, fitting curves for each
branch
verbose Whether or not detailed running information should be returned
... Additional arguments passed to buildBranchCellDataSet

Value

ea ggplot2 plot object

CellDataSet The CellDataSet class

Description

The main class used by Monocle to hold single cell expression data. CellDataSet extends the basic
Bioconductor ExpressionSet class.

Details

This class is initialized from a matrix of expression values. Methods that operate on CellDataSet
objects constitute the basic Monocle workflow.
Slots

- `reducedDimS` Matrix of class numeric, containing the source values computed by Independent Components Analysis.
- `reducedDimW` Matrix of class numeric, containing the whitened expression values computed during Independent Components Analysis.
- `reducedDimA` Matrix of class numeric, containing the weight values computed by Independent Components Analysis.
- `reducedDimK` A Matrix of class numeric, containing the pre-whitening matrix computed by Independent Components Analysis.
- `minSpanningTree` An Object of class igraph, containing the minimum spanning tree used by Monocle to order cells according to progress through a biological process.
- `cellPairwiseDistances` A Matrix of class numeric, containing the pairwise distances between cells in the reduced dimension space.
- `expressionFamily` An Object of class vglmff, specifying the VGAM family function used for expression responses.
- `lowerDetectionLimit` A numeric value specifying the minimum expression level considered to be true expression.
- `dispFitInfo` An environment containing lists, one for each set of estimated dispersion values. See `estimateDispersions`.
- `dim_reduce_type` A string encoding how this CellDataSet has been reduced in dimensionality.
- `auxOrderingData` An environment of auxiliary data structures used by various steps in Monocle. Not to be accessed by users directly.

**Description**

Methods for the CellDataSet class

**Usage**

```r
## S4 method for signature 'CellDataSet'
sizeFactors(object)

## S4 replacement method for signature 'CellDataSet,numeric'
sizeFactors(object) <- value

## S4 method for signature 'CellDataSet'
estimateSizeFactors(object, locfunc = median, ...)

## S4 method for signature 'CellDataSet'
estimateDispersions(object, modelFormulaStr = "~ 1",
                      relative_expr = TRUE, min_cells_detected = 1, remove_outliers = TRUE,
                      cores = 1, ...)
```

---

---
cellPairwiseDistances

Arguments

object  The CellDataSet object
value  A vector of size factors, with length equal to the cells in object
locfunc  A function applied to the geometric-mean-scaled expression values to derive the size factor.
...  Additional arguments to be passed to estimateSizeFactorsForMatrix
modelFormulaStr  A model formula, passed as a string, specifying how to group the cells prior to estimated dispersion. The default groups all cells together.
relative_expr  Whether to transform expression into relative values
min_cells_detected  Only include genes detected above lowerDetectionLimit in at least this many cells in the dispersion calculation
remove_outliers  Whether to remove outliers (using Cook’s distance) when estimating dispersions
cores  The number of cores to use for computing dispersions

cellPairwiseDistances  Get the matrix of pairwise distances between cells

description

Retrieves a matrix capturing distances between each cell used during cell ordering.

Usage

        cellPairwiseDistances(cds)

Arguments

        cds  expression data matrix for an experiment

Value

        A square, symmetric matrix containing the distances between each cell in the reduced-dimensionality space.

Examples

        ## Not run:
        D <- cellPairwiseDistances(HSMM)

        ## End(Not run)
cellPairwiseDistances <-

Sets the matrix containing distances between each pair of cells used by Monocle during cell ordering. Not intended to be called directly.

**Description**

Sets the matrix containing distances between each pair of cells used by Monocle during cell ordering. Not intended to be called directly.

**Usage**

cellPairwiseDistances(cds) <- value

**Arguments**

cds A CellDataSet object.
value a square, symmetric matrix containing pairwise distances between cells.

**Value**

An updated CellDataSet object

**Examples**

```r
## Not run:
cds <- cellPairwiseDistances(D)
## End(Not run)
```

---

**CellType**

*The CellType class*

**Description**

Classifies cells using a criterion function.

**Details**

Classifies cells via a user-defined gating function. The gating function accepts as input the entire matrix of expression data from a CellDataSet, and return TRUE or FALSE for each cell in it, depending on whether each meets the criteria in the gating function.

**Slots**

classify_func: A function that accepts a matrix of expression values as input, and returns a logical vector (of length equal to the number of columns in the matrix) as output.
### The CellTypeHierarchy class

**Description**

Classifies cells according to a hierarchy of types.

**Details**

Classifies cells according to a hierarchy of types via user-defined gating functions.

**Slots**

- `classificationTree`: Object of class "igraph"

### clusterCells

**Cluster cells into a specified number of groups.**

**Description**

Unsupervised clustering of cells is a common step in many single-cell expression workflows. In an experiment containing a mixture of cell types, each cluster might correspond to a different cell type. This method takes a CellDataSet as input along with a requested number of clusters, clusters them with an unsupervised algorithm, and then returns the CellDataSet with the cluster assignments stored in the pData table.

**Usage**

```r
classicCells(cds, num_clusters, cell_type_hierarchy = NULL,
             frequency_thresh = 0.1, clustering_genes = NULL, max_components = 10,
             residualModelFormulaStr = NULL, param.gamma = 100, verbose = F, ...)
```

**Arguments**

- `cds` the CellDataSet upon which to perform this operation
- `num_clusters` number of desired cell clusters
- `cell_type_hierarchy` the CellTypeHierarchy that divides the cells from cds into different types of cells
- `frequency_thresh` When a CellTypeHierarchy is provided, cluster cells will impute cell types in clusters that are composed of at least this much of exactly one cell type.
- `clustering_genes` a vector of genes used to differentiate between the cell types in the CellType-Hierarchy
- `max_components` number of dimensions to project the data into via `reduceDimension()`
- `residualModelFormulaStr` A model formula specifying the effects to subtract from the data before clustering.
- `param.gamma` gamma parameter for DDRTree
- `verbose` Verbose parameter for DDRTree
- `...` Additional arguments passed to `reduceDimension()`
clusterGenes

Clusters genes by pseudotime trend.

Description
This function takes a matrix of expression values and performs k-means clustering on the genes.

Usage
clusterGenes(expr_matrix, k, method = function(x) { as.dist((1 - cor(Matrix::t(x)))/2} }, ...)

Arguments
- `expr_matrix`: A matrix of expression values to cluster together. Rows are genes, columns are cells.
- `k`: How many clusters to create
- `method`: The distance function to use during clustering
- `...`: Extra parameters to pass to pam() during clustering

Value
a pam cluster object

Examples
```
## Not run:
full_model_fits <- fitModel(HSMM[[sample(nrow(fData(HSMM_filtered)), 100),]],
                           modelFormulaStr="sm.ns(Pseudotime)"
expression_curve_matrix <- responseMatrix(full_model_fits)
clusters <- clusterGenes(expression_curve_matrix, k=4)
plot_clusters(HSMM_filtered[ordering_genes,], clusters)
## End(Not run)
```

compareModels

Compare model fits

Description
Performs likelihood ratio tests on nested vector generalized additive models

Usage
compareModels(full_models, reduced_models)
detectBifurcationPoint

Arguments

full_models a list of models, e.g. as returned by fitModels(), forming the numerators of the L.R.Ts.
reduced_models a list of models, e.g. as returned by fitModels(), forming the denominators of the L.R.Ts.

Value

a data frame containing the p values and q-values from the likelihood ratio tests on the parallel arrays of models.

detectBifurcationPoint

Calculate divergence times for branch-dependent genes

Description

Branch-dependent genes may diverge at different points in pseudotime. detectBifurcationPoint() calculates these times. Although the branch times will be shaped by and distributed around the branch point in the trajectory, upstream regulators tend to branch earlier in pseudotime than their targets.

Usage

detectBifurcationPoint(str_log_df = NULL, ILRs_threshold = 0.1, detect_all = T, cds = cds, Branch = "Branch", branch_point = NULL, branch_states = c(2, 3), stretch = T, cores = 1, trend_formula = ~sm.ns(Pseudotime, df = 3), ILRs_limit = 3, relative_expr = TRUE, label_by_short_name = TRUE, useVST = FALSE, round_exprs = FALSE, output_type = "all", return_cross_point = T, file = "bifurcation_heatmap", verbose = FALSE, ...)

Arguments

str_log_df the ILRs dataframe calculated from calILRs function. If this data.frame is provided, all the following parameters are ignored. Note that we need to only use the ILRs after the bifurcation point if we duplicated the progenitor cell state.
ILRs_threshold the ILR value used to determine the earliest divergence time point
detect_all a logic flag to determine whether or not genes without ILRs pass the threshold will still report a bifurcation point
cds CellDataSet for the experiment
Branch The column in pData used for calculating the ILRs (If not equal to "Branch", a warning will report)
branch_point The ID of the branch point to analyze. Can only be used when reduceDimension is called with method = "DDRTree".
branch_states The states for two branching branches
stretch a logic flag to determine whether or not each branch should be stretched
cores Number of cores when fitting the spline curves
detectGenes

Sets the global expression detection threshold to be used with this CellDataSet. Counts how many cells each feature in a CellDataSet object that are detectably expressed above a minimum threshold. Also counts the number of genes above this threshold are detectable in each cell.

**Description**

Sets the global expression detection threshold to be used with this CellDataSet. Counts how many cells each feature in a CellDataSet object that are detectably expressed above a minimum threshold. Also counts the number of genes above this threshold are detectable in each cell.

**Usage**

detectGenes(cds, min_expr = NULL)

**Arguments**

cds the CellDataSet upon which to perform this operation

min_expr the expression threshold
differentialGeneTest

Value

an updated CellDataSet object

Examples

## Not run:
HSMM <- detectGenes(HSMM, min_expr=0.1)
## End(Not run)

differentialGeneTest  Test genes for differential expression

Description

Tests each gene for differential expression as a function of pseudotime or according to other covariates as specified. differentialGeneTest is Monocle’s main differential analysis routine. It accepts a CellDataSet and two model formulae as input, which specify generalized lineage models as implemented by the VGAM package.

Usage

differentialGeneTest(cds, fullModelFormulaStr = "~sm.ns(Pseudotime, df=3)", reducedModelFormulaStr = "~1", relative_expr = TRUE, cores = 1, verbose = FALSE)

Arguments

cds a CellDataSet object upon which to perform this operation
fullModelFormulaStr a formula string specifying the full model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
reducedModelFormulaStr a formula string specifying the reduced model in differential expression tests (i.e. likelihood ratio tests) for each gene/feature.
relative_expr Whether to transform expression into relative values.
cores the number of cores to be used while testing each gene for differential expression.
verbose Whether to show VGAM errors and warnings. Only valid for cores = 1.

Value

a data frame containing the p values and q-values from the likelihood ratio tests on the parallel arrays of models.

See Also

vglm
diff_test_helper  

Helper function for parallel differential expression testing

Description

test

Usage

diff_test_helper(x, fullModelFormulaStr, reducedModelFormulaStr,  
expressionFamily, relative_expr, weights, disp_func = NULL,  
verbose = FALSE)

Arguments

  x  
test
fullModelFormulaStr  
a formula string specifying the full model in differential expression tests (i.e.  
likelihood ratio tests) for each gene/feature.
reducedModelFormulaStr  
a formula string specifying the reduced model in differential expression tests  
(i.e. likelihood ratio tests) for each gene/feature.
expressionFamily  
specifies the VGAM family function used for expression responses
relative_expr  
Whether to transform expression into relative values
weights  
test
disp_func  
test
verbose  
Whether to show VGAM errors and warnings. Only valid for cores = 1.

dispersionTable  

Retrieve a table of values specifying the mean-variance relationship

Description

Calling estimateDispersions computes a smooth function describing how variance in each gene’s  
expression across cells varies according to the mean. This function only works for CellDataSet  
objects containing count-based expression data, either transcripts or reads.

Usage

dispersionTable(cds)

Arguments

  cds  
The CellDataSet from which to extract a dispersion table.

Value

A data frame containing the empirical mean expression, empirical dispersion, and the value esti-
mated by the dispersion model.
estimateDispersionsForCellDataSet

*Helper function to estimate dispersions*

**Description**

Helper function to estimate dispersions

**Usage**

```r
estimateDispersionsForCellDataSet(cds, modelFormulaStr, relative_expr, min_cells_detected, removeOutliers, cores)
```

**Arguments**

- **cds**: a `CellDataSet` that contains all cells user wants evaluated
- **modelFormulaStr**: a formula string specifying the model to fit for the genes.
- **relative_expr**: Whether to transform expression into relative values
- **min_cells_detected**: Only include genes detected above lowerDetectionLimit in at least this many cells in the dispersion calculation
- **removeOutliers**: a boolean it determines whether or not outliers from the data should be removed
- **cores**: the number of cores to be used while testing each gene for differential expression.

estimateSizeFactorsForMatrix

*Function to calculate the size factor for the single-cell RNA-seq data*

@importFrom stats median

**Description**

Function to calculate the size factor for the single-cell RNA-seq data

@importFrom stats median

**Usage**

```r
estimateSizeFactorsForMatrix(counts, locfunc = median, round_exprs = TRUE, method = "mean-geometric-mean-total")
```

**Arguments**

- **counts**: The matrix for the gene expression data, either read counts or FPKM values or transcript counts
- **locfunc**: The location function used to find the representative value
- **round_exprs**: A logic flag to determine whether or not the expression value should be rounded
- **method**: A character to specify the size factor calculation approaches. It can be either "mean-geometric-mean-total" (default), "weighted-median", "median-geometric-mean", "median", "mode", "geometric-mean-total".

**estimate_t**

*Find the most commonly occurring relative expression value in each cell*

**Description**

Converting relative expression values to mRNA copies per cell requires knowing the most commonly occurring relative expression value in each cell. This value typically corresponds to an RPC value of 1. This function finds the most commonly occurring (log-transformed) relative expression value for each column in the provided expression matrix.

**Usage**

```r
estimate_t(relative_expr_matrix, relative_expr_thresh = 0.1)
```

**Arguments**

- `relative_expr_matrix`
  - a matrix of relative expression values for values with each row and column representing genes/isoforms and cells, respectively. Row and column names should be included. Expression values should not be log-transformed.

- `relative_expr_thresh`
  - Relative expression values below this threshold are considered zero.

**Details**

This function estimates the most abundant relative expression value \( t^* \) using a gaussian kernel density function. It can also optionally output the \( t^* \) based on a two gaussian mixture model based on the `smsn.mixture` from `mixsmsn` package.

**Value**

- a vector of most abundant relative_expr value corresponding to the RPC 1.

**Examples**

```r
## Not run:
HSMM_fpkm_matrix <- exprs(HSMM)
t_estimate = estimate_t(HSMM_fpkm_matrix)
## End(Not run)
```
extract_good_branched_ordering

*Extract a linear ordering of cells from a PQ tree*

**Description**

Extract a linear ordering of cells from a PQ tree

**Usage**

```r
extract_good_branched_ordering(orig_pq_tree, curr_node, dist_matrix, 
    num_branches, reverse_main_path = FALSE)
```

**Arguments**

- `orig_pq_tree`: The PQ object to use for ordering
- `curr_node`: The node in the PQ tree to use as the start of ordering
- `dist_matrix`: A symmetric matrix containing pairwise distances between cells
- `num_branches`: The number of outcomes allowed in the trajectory.
- `reverse_main_path`: Whether to reverse the direction of the trajectory

---

**fitModel**

*Fits a model for each gene in a CellDataSet object.*

**Description**

Fits a model for each gene in a CellDataSet object.

**Usage**

```r
fitModel(cds, modelFormulaStr = "~sm.ns(Pseudotime, df=3)", 
    relative_expr = TRUE, cores = 1)
```

**Arguments**

- `cds`: the CellDataSet upon which to perform this operation
- `modelFormulaStr`: a formula string specifying the model to fit for the genes.
- `relative_expr`: Whether to fit a model to relative or absolute expression. Only meaningful for count-based expression data. If TRUE, counts are normalized by Size_Factor prior to fitting.
- `cores`: the number of processor cores to be used during fitting.
Details

This function fits a vector generalized additive model (VGAM) from the VGAM package for each gene in a CellDataSet. By default, expression levels are modeled as smooth functions of the Pseudo-time value of each cell. That is, expression is a function of progress through the biological process. More complicated formulae can be provided to account for additional covariates (e.g. day collected, genotype of cells, media conditions, etc).

Value

a list of VGAM model objects

Description

Helper function for parallel VGAM fitting

Usage

fit_model_helper(x, modelFormulaStr, expressionFamily, relative_expr, disp_func = NULL, verbose = FALSE, ...)

Arguments

- x: test
- modelFormulaStr: a formula string specifying the model to fit for the genes.
- expressionFamily: specifies the VGAM family function used for expression responses
- relative_expr: Whether to transform expression into relative values
- disp_func: test
- verbose: Whether to show VGAM errors and warnings. Only valid for cores = 1.
- ...: test

Description

Fit smooth spline curves and return the residuals matrix

Usage

genSmoothCurveResiduals(cds, trend_formula = "~sm.ns(Pseudotime, df = 3)", relative_expr = T, residual_type = "response", cores = 1)
genSmoothCurves

Arguments

cds a CellDataSet object upon which to perform this operation
trend_formula a formula string specifying the model formula used in fitting the spline curve for each gene/feature.
relative_expr a logic flag to determine whether or not the relative gene expression should be used
residual_type the response desired, as accepted by VGAM’s predict function
cores the number of cores to be used while testing each gene for differential expression

Value

a data frame containing the data for the fitted spline curves.

Description

This function will fit smooth spline curves for the gene expression dynamics along pseudotime in a gene-wise manner and return the corresponding response matrix. This function is build on other functions (fit_models and responseMatrix) and used in calILRs and calABCs functions.

Usage

genSmoothCurves(cds, new_data, trend_formula = "~sm.ns(Pseudotime, df = 3)", relative_expr = T, response_type = "response", cores = 1)

Arguments

cds a CellDataSet object upon which to perform this operation
new_data a data.frame object including columns (for example, Pseudotime) with names specified in the model formula. The values in the data.frame should be consist with the corresponding values from cds object.
trend_formula a formula string specifying the model formula used in fitting the spline curve for each gene/feature.
relative_expr a logic flag to determine whether or not the relative gene expression should be used
response_type the response desired, as accepted by VGAM’s predict function
cores the number of cores to be used while testing each gene for differential expression

Value

a data frame containing the data for the fitted spline curves.
get\_classic\_muscle\_markers

_description_
Return the names of classic muscle genes

_usage_
get\_classic\_muscle\_markers()

load\_HSMM

_build a CellDataSet from the HSMM\_SingleCell package_

_description_
Build a CellDataSet from the HSMM\_SingleCell package

_usage_
load\_HSMM()

load\_HSMM\_markers

_return a CellDataSet of classic muscle genes_

_description_
Return a CellDataSet of classic muscle genes

_usage_
load\_HSMM\_markers()

_value_
A CellDataSet object

load\_lung

_build a CellDataSet from the data stored in inst/\_extdata directory_

_description_
Build a CellDataSet from the data stored in inst/\_extdata directory

_usage_
load\_lung()
markerDiffTable  Test genes for cell type-dependent expression

Description
Test genes for cell type-dependent expression

Usage
markerDiffTable(cds, cth, residualModelFormulaStr = "~1", balanced = FALSE, reclassify_cells = TRUE, remove_ambig = TRUE, remove_unknown = TRUE, verbose = FALSE, cores = 1)

Arguments
cds A CellDataSet object containing cells to classify
cth The CellTypeHierarchy object to use for classification
residualModelFormulaStr A model formula string specify effects you want to exclude when testing for cell type dependent expression
balanced Whether to downsample the cells so that there's an equal number of each type prior to performing the test
reclassify_cells a boolean that indicates whether or not the cds and cth should be run through classifyCells again
remove_ambig a boolean that indicates whether or not ambiguous cells should be removed the cds
remove_unknown a boolean that indicates whether or not unknown cells should be removed from the cds
verbose Whether to emit verbose output during the the search for cell-type dependent genes
cores The number of cores to use when testing

Value
A table of differential expression test results

mcesApply  Multicore apply-like function for CellDataSet

Description
mcesApply computes the row-wise or column-wise results of FUN, just like esApply. Variables in pData from X are available in FUN.
**minSpanningTree**

**Usage**

```
mcesApply(X, MARGIN, FUN, required_packages, cores = 1,
          convert_to_dense = TRUE, ...)```

**Arguments**

- **X**: a CellDataSet object
- **MARGIN**: The margin to apply to, either 1 for rows (samples) or 2 for columns (features)
- **FUN**: Any function
- **required_packages**: A list of packages FUN will need. Failing to provide packages needed by FUN will generate errors in worker threads.
- **cores**: The number of cores to use for evaluation
- **convert_to_dense**: Whether to force conversion a sparse matrix to a dense one before calling FUN
- **...**: Additional parameters for FUN

**Value**

The result of `with(pData(X), apply(exprs(X), MARGIN, FUN, ...))`

---

**Description**

Retrieves the minimum spanning tree (MST) that Monocle constructs during orderCells(). This MST is mostly used in `plot_spanning_tree` to help assess the accuracy of Monocle’s ordering.

**Usage**

```
minSpanningTree(cds)
```

**Arguments**

- **cds**: expression data matrix for an experiment

**Value**

An igraph object representing the CellDataSet’s minimum spanning tree.

**Examples**

```r
## Not run:
T <- minSpanningTree(HSMM)
## End(Not run)
```
minSpanningTree <- Set the minimum spanning tree generated by Monocle during cell ordering.

Description
Sets the minimum spanning tree used by Monocle during cell ordering. Not intended to be called directly.

Usage
minSpanningTree(cds) <- value

Arguments
cds A CellDataSet object.
value an igraph object describing the minimum spanning tree.

Value
An updated CellDataSet object

Examples
### Not run:
cds <- minSpanningTree(T)
### End(Not run)

newCellDataSet Creates a new CellDataSet object.

Description
Creates a new CellDataSet object.

Usage
newCellDataSet(cellData, phenoData = NULL, featureData = NULL,
lowerDetectionLimit = 0.1, expressionFamily = VGAM::tobit(Lower =
log10(lowerDetectionLimit), lmu = "identitylink"))

Arguments
cellData expression data matrix for an experiment
phenoData data frame containing attributes of individual cells
featureData data frame containing attributes of features (e.g. genes)
lowerDetectionLimit the minimum expression level that constitutes true expression
expressionFamily the VGAM family function to be used for expression response variables
newCellTypeHierarchy

Classify cells according to a set of markers

Value

a new CellDataSet object

Examples

```r
# Not run:
sample_sheet_small <- read.delim("../data/sample_sheet_small.txt", row.names=1)
sample_sheet_small$Time <- as.factor(sample_sheet_small$Time)
gene_annotations_small <- read.delim("../data/gene_annotations_small.txt", row.names=1)
fpkm_matrix_small <- read.delim("../data/fpkm_matrix_small.txt")
pd <- new("AnnotatedDataFrame", data = sample_sheet_small)
f <- new("AnnotatedDataFrame", data = gene_annotations_small)
HSMM <- new("CellDataSet", exprs = as.matrix(fpkm_matrix_small), phenoData = pd, featureData = f)
```

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

Description

CellTypeHierarchy objects are Monocle’s mechanism for classifying cells into types based on known markers. To classify the cells in a CellDataSet object according to known markers, first construct a CellTypeHierarchy with `newCellTypeHierarchy()` and `addCellType()` and then provide both the CellDataSet and the CellTypeHierarchy to `classifyCells()`. Each call to `addCellType()` registers a classification function that accepts the expression data from a CellDataSet object as input, and returns a boolean vector indicating whether each cell is of the given type. When you call `classifyCells()`, each cell will be checked against the classification functions in the CellTypeHierarchy. If you wish to make a cell type a subtype of another that’s already been registered with a CellType-Hierarchy object, make that one the “parent” type with the `cell_type_name` argument. If you want two types to be mutually exclusive, make them “siblings” by giving them the same parent.

Usage

```r
newCellTypeHierarchy()
addCellType(cth, cell_type_name, classify_func,
    parent_cell_type_name = "root")
classifyCells(cds, cth, frequency_thresh = NULL, ...)
calculateMarkerSpecificity(cds, cth, remove_ambig = TRUE,
    remove_unknown = TRUE)
```

Arguments

- `cth` : The CellTypeHierarchy object
- `cell_type_name` : The name of the new cell type. Can’t already exist in `cth`
- `classify_func` : A function that returns true when a cell is of the new type
- `parent_cell_type_name` : If this cell type is a subtype of another, provide its name here
newCellTypeHierarchy

cds The CellDataSet you want to classify
frequency_thresh If at least this fraction of group of cells meet a cell types marker criteria, impute them all to be of that type.
... character strings that you wish to pass to dplyr's group_by_ routine
remove_ambig a boolean that determines if ambiguous cells should be removed
remove_unknown a boolean that determines whether unknown cells should be removed

Details

The classification functions in a CellTypeHierarchy must take a single argument, a matrix of expression values, as input. Note that this matrix could either be a sparseMatrix or a dense matrix. Explicitly casting the input to a dense matrix inside a classification function is likely to drastically slow down classifyCells and other routines that use CellTypeHierarchy objects.

Successive calls to addCellType build up a tree of classification functions inside a CellTypeHierarchy. When two functions are siblings in the tree, classifyCells expects that a cell will meet the classification criteria for at most one of them. For example, you might place classification functions for T cells and B cells as siblings, because a cell cannot be both of these at the same time. When a cell meets the criteria for more than one function, it will be tagged as "Ambiguous". If classifyCells reports a large number of ambiguous cells, consider adjusting your classification functions. For example, some cells are defined by very high expression of a key gene that is expressed at lower levels in other cell types. Raising the threshold for this gene in a classification could resolve the ambiguities.

A classification function can also have child functions. You can use this to specify subtypes of cells. For example, T cells express the gene CD3, and there are many subtypes. You can encode each subset by first adding a general T cell classification function that recognizes CD3, and then adding an additional function that recognizes CD4 (for CD4+ helper T cells), one for CD8 (to identify CD8+ cytotoxic T cells), and so on. classifyCells will aim to assign each cell to its most specific subtype in the "CellType" column.

By default, classifyCells applies the classification functions to individual cells, but you can also apply it to cells in a "grouped" mode to impute the type of cells that are missing expression of your known markers. You can specify additional (quoted) grouping variables to classifyCells. The function will group the cells according to these factors, and then classify the cells. It will compute the frequency of each cell type in each group, and if a cell type is present at the frequency specified in frequency_thresh, all the cells in the group are classified as that type. If group contains more one cell type at this frequency, all the cells are marked "Ambiguous". This allows you to impute cell type based on unsupervised clustering results (e.g. with clusterCells()) or some other grouping criteria.

Value

newCellTypeHierarchy and addCellType both return an updated CellTypeHierarchy object. classifyCells returns an updated CellDataSet with a new column, "CellType", in the pData table.

For a CellDataset with N genes, and a CellTypeHierarchy with k types, returns a dataframe with N x k rows. Each row contains a gene and a specificity score for one of the types.

Functions

- addCellType: Add a cell type to a CellTypeHierarchy
- classifyCells: Add a cell type to a CellTypeHierarchy
orderCells

- `calculateMarkerSpecificity`: Calculate each gene's specificity for each cell type. Computes the Jensen-Shannon distance between the distribution of a gene's expression across cells and a hypothetical gene that is perfectly restricted to each cell type. The Jensen-Shannon distance is an information theoretic metric between two probability distributions. It is a widely accepted measure of cell-type specificity. For a complete description see Cabili et al., Genes & Development (2011).

Examples

```r
## Not run:
# Initialize a new CellTypeHierarchy

# Register a set of classification functions. There are multiple types of T cells
# A cell cannot be both a B cell and a T cell, a T cell and a Monocyte, or
# a B cell and a Monocyte.
cth <- newCellTypeHierarchy()

cth <- addCellType(cth, "T cell",
                classify_func=function(x) {x["CD3D",] > 0})

cth <- addCellType(cth, "CD4+ T cell",
                classify_func=function(x) {x["CD4",] > 0},
                parent_cell_type_name = "T cell")

cth <- addCellType(cth, "CD8+ T cell",
                classify_func=function(x) {
                    x["CD8A",] > 0 | x["CD8B",] > 0
                },
                parent_cell_type_name = "T cell")

cth <- addCellType(cth, "B cell",
                classify_func=function(x) {x["MS4A1",] > 0})

cth <- addCellType(cth, "Monocyte",
                classify_func=function(x) {x["CD14",] > 0})

# Classify each cell in the CellDataSet "mix" according to these types
mix <- classifyCells(mix, cth)

# Group the cells by the pData table column "Cluster". Apply the classification
# functions to the cells groupwise. If a group is at least 5% of a type, make
# them all that type. If the group is 5% one type, and 5% a different, mutually
# exclusive type, mark the whole cluster "Ambiguous"
mix <- classifyCells(mix, Cluster, 0.05)

## End(Not run)
```

`orderCells`  
*Orders cells according to pseudotime.*
Description

Learns a "trajectory" describing the biological process the cells are going through, and calculates where each cell falls within that trajectory. Monocle learns trajectories in two steps. The first step is reducing the dimensionality of the data with `reduceDimension()`. The second is this function, `orderCells()`. This function takes as input a `CellDataSet` and returns it with two new columns: `Pseudotime` and `State`, which together encode where each cell maps to the trajectory. `orderCells()` optionally takes a “root” state, which you can use to specify the start of the trajectory. If you don’t provide a root state, one is selected arbitrarily.

Usage

```
orderCells(cds, root_state = NULL, num_paths = NULL, reverse = NULL)
```

Arguments

- `cds`: the `CellDataSet` upon which to perform this operation
- `root_state`: The state to use as the root of the trajectory. You must already have called `orderCells()` once to use this argument.
- `num_paths`: the number of end-point cell states to allow in the biological process.
- `reverse`: whether to reverse the beginning and end points of the learned biological process.

Details

The `reduction_method` argument to `reduceDimension()` determines which algorithm is used by `orderCells()` to learn the trajectory. If `reduction_method == "ICA"`, this function uses `polygonal reconstruction` to learn the underlying trajectory. If `reduction_method == "DDRTree"`, the trajectory is specified by the principal graph learned by the `DDRTree()` function.

Whichever algorithm you use, the trajectory will be composed of segments. The cells from a segment will share the same value of `State`. One of these segments will be selected as the root of the trajectory arbitrarily. The most distal cell on that segment will be chosen as the “first” cell in the trajectory, and will have a `Pseudotime` value of zero. `orderCells()` will then "walk" along the trajectory, and as it encounters additional cells, it will assign them increasingly large values of `Pseudotime`.

Value

an updated `CellDataSet` object, in which `phenoData` contains values for `State` and `Pseudotime` for each cell

---

### order_p_node

Return an ordering for a P node in the PQ tree

Description

Return an ordering for a P node in the PQ tree

Usage

```
order_p_node(q_level_list, dist_matrix)
```
**plot_cell_trajectory**

**Arguments**

- `q_level_list` A list of Q nodes in the PQ tree
- `dist_matrix` A symmetric matrix of pairwise distances between cells

**Description**

Plots the minimum spanning tree on cells.

**Usage**

```r
plot_cell_trajectory(cds, x = 1, y = 2, color_by = "State", 
                      show_tree = TRUE, show_backbone = TRUE, backbone_color = "black", 
                      markers = NULL, show_cell_names = FALSE, cell_size = 1.5, 
                      cell_link_size = 0.75, cell_name_size = 2, show_branch_points = TRUE)
```

**Arguments**

- `cds` CellDataSet for the experiment
- `x` the column of `reducedDimS(cds)` to plot on the horizontal axis
- `y` the column of `reducedDimS(cds)` to plot on the vertical axis
- `color_by` the cell attribute (e.g. the column of `pData(cds)`) to map to each cell’s color
- `show_tree` whether to show the links between cells connected in the minimum spanning tree
- `show_backbone` whether to show the diameter path of the MST used to order the cells
- `backbone_color` the color used to render the backbone.
- `markers` a gene name or gene id to use for setting the size of each cell in the plot
- `show_cell_names` draw the name of each cell in the plot
- `cell_size` The size of the point for each cell
- `cell_link_size` The size of the line segments connecting cells (when used with ICA) or the principal graph (when used with DDRTree)
- `cell_name_size` the size of cell name labels
- `show_branch_points` Whether to show icons for each branch point (only available when `reduceDimension` was called with DDRTree)

**Value**

A `ggplot2` plot object
plot_clusters

Plots kinetic clusters of genes.

Description

Plots kinetic clusters of genes.

Usage

plot_clusters(cds, clustering, drawSummary = TRUE, sumFun = mean_cl_boot,
ncol = NULL, nrow = NULL, row_samples = NULL, callout_ids = NULL)

Arguments

cds CellDataSet for the experiment
clustering a clustering object produced by clusterCells
drawSummary whether to draw the summary line for each cluster
sumFun whether the function used to generate the summary for each cluster
ncol number of columns used to layout the faceted cluster panels
nrow number of columns used to layout the faceted cluster panels
row_samples how many genes to randomly select from the data
callout_ids a vector of gene names or gene ids to manually render as part of the plot

Value

a ggplot2 plot object

Examples

## Not run:
full_model_fits <- fitModel(HSMM_filtered[sample(nrow(fData(HSMM_filtered)), 100),],
   modelFormulaStr="VGAM::bs(Pseudotime)"
expression_curve_matrix <- responseMatrix(full_model_fits)
clusters <- clusterGenes(expression_curve_matrix, k=4)
plot_clusters(HSMM_filtered[ordering_genes,], clusters)
## End(Not run)
plot_coexpression_matrix

Not sure we’re ready to release this one quite yet: Plot the branch genes in pseudotime with separate branch curves

Description

Not sure we’re ready to release this one quite yet: Plot the branch genes in pseudotime with separate branch curves

Usage

plot_coexpression_matrix(cds, rowgenes, colgenes, relative_expr = TRUE,
min_expr = NULL, cell_size = 0.85, label_by_short_name = TRUE,
show_density = TRUE, round_expr = FALSE)

Arguments

cds
CellDataSet for the experiment

rowgenes
Gene ids or short names to be arrayed on the vertical axis.

colgenes
Gene ids or short names to be arrayed on the horizontal axis

relative_expr
Whether to transform expression into relative values

min_expr
The minimum level of expression to show in the plot

cell_size
A number how large the cells should be in the plot

label_by_short_name
a boolean that indicates whether cells should be labeled by their short name

show_density
a boolean that indicates whether a 2D density estimation should be shown in the plot

round_expr
a boolean that indicates whether cds_expr values should be rounded or not

Value

a ggplot2 plot object

plot_genes_branched_heatmap

Create a heatmap to demonstrate the bifurcation of gene expression along two branches

Description

Create a heatmap to demonstrate the bifurcation of gene expression along two branches
Usage

plot_genes_branched_heatmap(cds_subset, branch_point = 1,
branch_states = NULL, branch_labels = c("Cell fate 1", "Cell fate 2"),
cluster_rows = TRUE, hclust_method = "ward.D2", num_clusters = 6,
hmcols = NULL, branch_colors = c("#979797", "#F05662", "#7990C8"),
add_annotation_row = NULL, add_annotation_col = NULL,
show_rownames = FALSE, use_gene_short_name = TRUE, scale_max = 3,
scale_min = -3, norm_method = c("vstExprs", "log"),
trend_formula = "~sm.ns(Pseudotime, df=3) * Branch",
return_heatmap = FALSE, cores = 1, ...)

Arguments

cds_subset | CellDataSet for the experiment (normally only the branching genes detected
            | with branchTest)
branch_point | The ID of the branch point to visualize. Can only be used when reduceDimension
             | is called with method = "DDRTree".
branch_states | The two states to compare in the heatmap. Mutually exclusive with branch_point.
branch_labels | The labels for the branches.
cluster_rows | Whether to cluster the rows of the heatmap.
hclust_method | The method used by pheatmap to perform hierarchical clustering of the rows.
num_clusters | Number of clusters for the heatmap of branch genes
hmcols | The color scheme for drawing the heatmap.
branch_colors | The colors used in the annotation strip indicating the pre- and post-branch cells.
add_annotation_row | Additional annotations to show for each row in the heatmap. Must be a dataframe
                  | with one row for each row in the fData table of cds_subset, with matching IDs.
add_annotation_col | Additional annotations to show for each column in the heatmap. Must be a
dataframe with one row for each cell in the pData table of cds_subset, with
                  | matching IDs.
show_rownames | Whether to show the names for each row in the table.
use_gene_short_name | Whether to use the short names for each row. If FALSE, uses row IDs from the
                   | fData table.
scale_max | The maximum value (in standard deviations) to show in the heatmap. Values
           | larger than this are set to the max.
scale_min | The minimum value (in standard deviations) to show in the heatmap. Values
           | smaller than this are set to the min.
norm_method | Determines how to transform expression values prior to rendering
trend_formula | A formula string specifying the model used in fitting the spline curve for each
genue/feature.
return_heatmap | Whether to return the heatmap object to the user.
cores | Number of cores to use when smoothing the expression curves shown in the
       | heatmap.
... | Additional arguments passed to buildBranchCellDataSet
**Value**

A list of `heatmap_matrix` (expression matrix for the branch commitment), `ph` (pheatmap heatmap object), `annotation_row` (annotation data.frame for the row), `annotation_col` (annotation data.frame for the column).

---

**plot_genes_branched_pseudotime**

*Plot the branch genes in pseudotime with separate branch curves.*

**Description**

This plotting function is used to make the branching plots for a branch dependent gene goes through the progenitor state and bifurcating into two distinct branches (Similar to the pitch-fork bifurcation in dynamic systems). In order to make the bifurcation plot, we first duplicated the progenitor states and by default stretch each branch into maturation level 0-100. Then we fit two nature spline curves for each branches using VGAM package.

**Usage**

```r
plot_genes_branched_pseudotime(cds, branch_states = NULL, branch_point = 1,
                                branch_labels = NULL, method = "fitting", min_expr = NULL,
                                cell_size = 0.75, nrow = NULL, ncol = 1, panel_order = NULL,
                                color_by = "State", expression_curve_linetype_by = "Branch",
                                trend_formula = ~ sm.ns(Pseudotime, df=3) * Branch,
                                reducedModelFormulaStr = NULL, label_by_short_name = TRUE,
                                relative_expr = TRUE, ...)```

**Arguments**

- `cds`: CellDataSet for the experiment
- `branch_states`: The states for two branching branches
- `branch_point`: The ID of the branch point to analyze. Can only be used when `reduceDimension` is called with method = "DDRTree".
- `branch_labels`: The names for each branching branch
- `method`: The method to draw the curve for the gene expression branching pattern, either loess (’loess’) or VGLM fitting (’fitting’)
- `min_expr`: The minimum (untransformed) expression level to use in plotted the genes.
- `cell_size`: The size (in points) of each cell used in the plot
- `nrow`: Number of columns used to layout the faceted cluster panels
- `ncol`: Number of columns used to layout the faceted cluster panels
- `panel_order`: The a character vector of gene short names (or IDs, if that’s what you’re using), specifying order in which genes should be layed out (left-to-right, top-to-bottom)
- `color_by`: The cell attribute (e.g. the column of pData(cds)) to be used to color each cell
- `expression_curve_linetype_by`: The cell attribute (e.g. the column of pData(cds)) to be used for the linetype of each branch curve
plot_genes_in_pseudotime

Plots expression for one or more genes as a function of pseudotime

Description

Plots expression for one or more genes as a function of pseudotime

Usage

plot_genes_in_pseudotime(cds_subset, min_expr = NULL, cell_size = 0.75, nrow = NULL, ncol = 1, panel_order = NULL, color_by = "State", trend_formula = "~ sm.ns(Pseudotime, df=3)", label_by_short_name = TRUE, relative_expr = TRUE, vertical_jitter = NULL, horizontal_jitter = NULL)

Arguments

cds_subset CellDataSet for the experiment
min_expr the minimum (untransformed) expression level to use in plotted the genes.
cell_size the size (in points) of each cell used in the plot
nrow the number of rows used when laying out the panels for each gene’s expression
ncol the number of columns used when laying out the panels for each gene’s expression
panel_order the order in which genes should be layed out (left-to-right, top-to-bottom)
color_by the cell attribute (e.g. the column of pData(cds)) to be used to color each cell
trend_formula the model formula to be used for fitting the expression trend over pseudotime
label_by_short_name label figure panels by gene_short_name (TRUE) or feature id (FALSE)
relative_expr Whether to transform expression into relative values
vertical_jitter A value passed to ggplot to jitter the points in the vertical dimension. Prevents overplotting, and is particularly helpful for rounded transcript count data.
horizontal_jitter A value passed to ggplot to jitter the points in the horizontal dimension. Prevents overplotting, and is particularly helpful for rounded transcript count data.

Value

a ggplot2 plot object
plot_genes_jitter

Value

a ggplot2 plot object

Examples

```r
## Not run:
data(HSMM)
my_genes <- row.names(subset(fData(HSMM), gene_short_name %in% c("CDK1", "MEF2C", "MYH3")))
cds_subset <- HSMM[my_genes,]
plot_genes_in_pseudotime(cds_subset, color_by="Time")
## End(Not run)
```

plot_genes_jitter

Plots expression for one or more genes as a jittered, grouped points

Description

Plots expression for one or more genes as a jittered, grouped points

Usage

```r
plot_genes_jitter(cds_subset, grouping = "State", min_expr = NULL, cell_size = 0.75, nrow = NULL, ncol = 1, panel_order = NULL, color_by = NULL, plot_trend = FALSE, label_by_short_name = TRUE, relative_expr = TRUE)
```

Arguments

- `cds_subset`: CellDataSet for the experiment
- `grouping`: the cell attribute (e.g. the column of pData(cds)) to group cells by on the horizontal axis
- `min_expr`: the minimum (untransformed) expression level to use in plotted the genes.
- `cell_size`: the size (in points) of each cell used in the plot
- `nrow`: the number of rows used when laying out the panels for each gene’s expression
- `ncol`: the number of columns used when laying out the panels for each gene’s expression
- `panel_order`: the order in which genes should be layed out (left-to-right, top-to-bottom)
- `color_by`: the cell attribute (e.g. the column of pData(cds)) to be used to color each cell
- `plot_trend`: whether to plot a trendline tracking the average expression across the horizontal axis.
- `label_by_short_name`: label figure panels by gene_short_name (TRUE) or feature id (FALSE)
- `relative_expr`: Whether to transform expression into relative values

Value

a ggplot2 plot object
plot_genes_positive_cells

Plots the number of cells expressing one or more genes as a barplot

Description

Plots the number of cells expressing one or more genes as a barplot

Usage

plot_genes_positive_cells(cds_subset, grouping = "State", min_expr = 0.1, nrow = NULL, ncol = 1, panel_order = NULL, plot_as_fraction = TRUE, label_by_short_name = TRUE, relative_expr = TRUE, plot_limits = c(0, 100))

Arguments

cds_subset CellDataSet for the experiment
grouping the cell attribute (e.g. the column of pData(cds)) to group cells by on the horizontal axis
min_expr the minimum (untransformed) expression level to use in plotted the genes.
nrow the number of rows used when laying out the panels for each gene’s expression
ncol the number of columns used when laying out the panels for each gene’s expression
panel_order the order in which genes should be layed out (left-to-right, top-to-bottom)
plot_as_fraction whether to show the percent instead of the number of cells expressing each gene
label_by_short_name label figure panels by gene_short_name (TRUE) or feature id (FALSE)
relative_expr Whether to transform expression into relative values
plot_limits A pair of number specifying the limits of the y axis. If NULL, scale to the range of the data.

Value

a ggplot2 plot object
Examples

```r
## Not run:
data(HSMM)
MYOG_ID1 <- HSMM[row.names(subset(fData(HSMM), gene_short_name %in% c("MYOG", "ID1"))),]
plot_genes_positive_cells(MYOG_ID1, grouping="Media", ncol=2)
## End(Not run)
```

Description

Each gray point in the plot is a gene. The black dots are those that were included in the last call to 
setOrderingFilter. The red curve shows the mean-variance model learning by estimateDispersions().

Usage

```
plot_ordering_genes(cds)
```

Arguments

cds
The CellDataSet to be used for the plot.

Description

Plots a pseudotime-ordered, row-centered heatmap

Usage

```
plot_pseudotime_heatmap(cds_subset, cluster_rows = TRUE, 
hclust_method = "ward.D2", num_clusters = 6, hmcols = NULL, 
add_annotation_row = NULL, add_annotation_col = NULL, 
show_rownames = FALSE, use_gene_short_name = TRUE, 
norm_method = c("vstExprs", "log"), scale_max = 3, scale_min = -3, 
trend_formula = "~sm.ns(Pseudotime, df=3)", return_heatmap = FALSE, 
cores = 1)
```
Arguments

cds_subset  
CellDataSet for the experiment (normally only the branching genes detected with branchTest)

cluster_rows  
Whether to cluster the rows of the heatmap.

hclust_method  
The method used by pheatmap to perform hierarchical clustering of the rows.

num_clusters  
Number of clusters for the heatmap of branch genes

hmcols  
The color scheme for drawing the heatmap.

add_annotation_row  
Additional annotations to show for each row in the heatmap. Must be a dataframe with one row for each row in the fData table of cds_subset, with matching IDs.

add_annotation_col  
Additional annotations to show for each column in the heatmap. Must be a dataframe with one row for each cell in the pData table of cds_subset, with matching IDs.

show_rownames  
Whether to show the names for each row in the table.

use_gene_short_name  
Whether to use the short names for each row. If FALSE, uses row IDs from the fData table.

norm_method  
Determines how to transform expression values prior to rendering

scale_max  
The maximum value (in standard deviations) to show in the heatmap. Values larger than this are set to the max.

scale_min  
The minimum value (in standard deviations) to show in the heatmap. Values smaller than this are set to the min.

trend_formula  
A formula string specifying the model used in fitting the spline curve for each gene/feature.

return_heatmap  
Whether to return the heatmap object to the user.

cores  
Number of cores to use when smoothing the expression curves shown in the heatmap.

Value

A list of heatmap_matrix (expression matrix for the branch commitment), ph (pheatmap heatmap object), annotation_row (annotation data.frame for the row), annotation_col (annotation data.frame for the column).

plot_spanning_tree  
Plots the minimum spanning tree on cells. This function is deprecated.

Description

This function arranges all of the cells in the cds in a tree and predicts their location based on their pseudotime value.

Usage

plot_spanning_tree(cds, x = 1, y = 2, color_by = "State", show_tree = TRUE, show_backbone = TRUE, backbone_color = "black", markers = NULL, show_cell_names = FALSE, cell_size = 1.5, cell_link_size = 0.75, cell_name_size = 2, show_branch_points = TRUE)
pq_helper

Recursively builds and returns a PQ tree for the MST

Description
Recursively builds and returns a PQ tree for the MST

Usage
pq_helper(mst, use_weights = TRUE, root_node = NULL)
Arguments

mst The minimum spanning tree, as an igraph object.
use_weights Whether to use edge weights when finding the diameter path of the tree.
root_node The name of the root node to use for starting the path finding.

Description

Retrieves the weights that transform the cells’ coordinates in the reduced dimension space back to the full (whitened) space.

Usage

reducedDimA(cds)

Arguments

cds A CellDataSet object.

Value

A matrix that when multiplied by a reduced-dimension set of coordinates for the CellDataSet, recovers a matrix in the full (whitened) space

Examples

## Not run:
A <- reducedDimA(HSMM)
## End(Not run)

Description

Sets the weights transform the cells’ coordinates in the reduced dimension space back to the full (whitened) space.

Usage

reducedDimA(cds) <- value
reducedDimK

Arguments

  cds        A CellDataSet object.
  value      A whitened expression data matrix

Value

  An updated CellDataSet object

Examples

  ## Not run:
  cds <- reducedDimA(A)
  ## End(Not run)

reducedDimK

  Retrieves the whitening matrix during independent component analysis.

Description

  Retrieves the whitening matrix during independent component analysis.

Usage

  reducedDimK(cds)

Arguments

  cds        A CellDataSet object.

Value

  A matrix, where each row is a set of whitened expression values for a feature and columns are cells.

Examples

  ## Not run:
  K <- reducedDimW(HSMM)
  ## End(Not run)
reducedDimK <- Sets the whitening matrix during independent component analysis.

Description
Sets the whitening matrix during independent component analysis.

Usage
reducedDimK(cds) <- value

Arguments
- cds: A CellDataSet object.
- value: a numeric matrix

Value
A matrix, where each row is a set of whitened expression values for a feature and columns are cells.

Examples
## Not run:
cds <- reducedDimK(K)
## End(Not run)

reducedDimS
Retrieves the coordinates of each cell in the reduced-dimensionality space generated by calls to reduceDimension.

Description
Reducing the dimensionality of the expression data is a core step in the Monocle workflow. After you call reduceDimension(), this function will return the new coordinates of your cells in the reduced space.

Usage
reducedDimS(cds)

Arguments
- cds: A CellDataSet object.

Value
A matrix, where rows are cell coordinates and columns correspond to dimensions of the reduced space.
Examples

```r
## Not run:
S <- reducedDimS(HSMM)
## End(Not run)
```

```
reducedDimS<-  Set embedding coordinates of each cell in a CellDataSet.
```

Description

This function sets the coordinates of each cell in a new (reduced-dimensionality) space. Not intended to be called directly.

Usage

```r
cds <- reducedDimS(cds) <- value
```

Arguments

- `cds` A CellDataSet object.
- `value` A matrix of coordinates specifying each cell’s position in the reduced-dimensionality space.

Value

An update CellDataSet object

Examples

```r
## Not run:
cds <- reducedDimS(S)
## End(Not run)
```

```
reducedDimW Get the whitened expression values for a CellDataSet.
```

Description

Retrieves the expression values for each cell (as a matrix) after whitening during dimensionality reduction.

Usage

```r
reducedDimW(cds)
```

Arguments

- `cds` A CellDataSet object.
**reducedDimW**

A matrix, where each row is a set of whitened expression values for a feature and columns are cells.

**Examples**

```r
## Not run:
W <- reducedDimW(HSMM)
## End(Not run)
```

**Description**

Sets the whitened expression values for each cell prior to independent component analysis. Not intended to be called directly.

**Usage**

```r
reducedDimW(cds) <- value
```

**Arguments**

- **cds**: A CellDataSet object.
- **value**: A whitened expression data matrix

**Value**

An updated CellDataSet object

**Examples**

```r
## Not run:
#' cds <- reducedDimA(A)
## End(Not run)
```
reduceDimension

Compute a projection of a CellDataSet object into a lower dimensional space

Description
Monocle aims to learn how cells transition through a biological program of gene expression changes in an experiment. Each cell can be viewed as a point in a high-dimensional space, where each dimension describes the expression of a different gene in the genome. Identifying the program of gene expression changes is equivalent to learning a trajectory that the cells follow through this space. However, the more dimensions there are in the analysis, the harder the trajectory is to learn. Fortunately, many genes typically co-vary with one another, and so the dimensionality of the data can be reduced with a wide variety of different algorithms. Monocle provides two different algorithms for dimensionality reduction via reduceDimension. Both take a CellDataSet object and a number of dimensions allowed for the reduced space. You can also provide a model formula indicating some variables (e.g. batch ID or other technical factors) to "subtract" from the data so it doesn’t contribute to the trajectory.

Usage
reduceDimension(cds, max_components = 2, reduction_method = c("DDRTree", "ICA"), norm_method = c("vstExprs", "log", "none"), residualModelFormulaStr = NULL, pseudo_expr = NULL, verbose = FALSE, ...)

Arguments
cds            the CellDataSet upon which to perform this operation
max_components the dimensionality of the reduced space
reduction_method A character string specifying the algorithm to use for dimensionality reduction.
norm_method Determines how to transform expression values prior to reducing dimensionality
residualModelFormulaStr A model formula specifying the effects to subtract from the data before clustering.
pseudo_expr amount to increase expression values before dimensionality reduction
verbose        Whether to emit verbose output during dimensionality reduction
...            additional arguments to pass to the dimensionality reduction function

Details
You can choose two different reduction algorithms: Independent Component Analysis (ICA) and Discriminative Dimensionality Reduction with Trees (DDRTree). The choice impacts numerous downstream analysis steps, including orderCells. Choosing ICA will execute the ordering procedure described in Trapnell and Cacchiarelli et al., which was implemented in Monocle version 1. DDRTree is a more recent manifold learning algorithm developed by Qi Mao and colleagues. It is substantially more powerful, accurate, and robust for single-cell trajectory analysis than ICA, and is now the default method.
Often, experiments include cells from different batches or treatments. You can reduce the effects of these treatments by transforming the data with a linear model prior to dimensionality reduction. To do so, provide a model formula through `residualModelFormulaStr`.

Prior to reducing the dimensionality of the data, it usually helps to normalize it so that highly expressed or highly variable genes don’t dominate the computation. `reduceDimension()` automatically transforms the data in one of several ways depending on the `expressionFamily` of the `CellDataSet` object. If the `expressionFamily` is `negbinomial` or `negbinomial.size`, the data are variance-stabilized. If the `expressionFamily` is `Tobit`, the data are adjusted by adding a pseudo-count (of 1 by default) and then log-transformed. If you don’t want any transformation at all, set `norm_method` to “none” and `pseudo_expr` to 0. This maybe useful for single-cell qPCR data, or data you’ve already transformed yourself in some way.

**Value**

an updated `CellDataSet` object

---

**relative2abs**

Transform relative expression values into absolute transcript counts.

**Description**

Transform a relative expression matrix to absolute transcript matrix based on the inferred linear regression parameters from most abundant isoform relative expression value. This function takes a relative expression matrix and a vector of estimated most abundant expression value from the isoform-level matrix and transform it into absolute transcript number. It is based on the observation that the recovery efficient of the single-cell RNA-seq is relative low and that most expressed isoforms of gene in a single cell therefore only sequenced one copy so that the most abundant isoform log10-FPKM (t^*) will corresponding to 1 copy transcript. It is also based on the fact that the spikein regression parameters k/b for each cell will fall on a line because of the intrinsic properties of spikein experiments. We also assume that if we perform the same spikein experiments as Treutlein et al. did, the regression parameters should also fall on a line in the same way. The function takes the the vector t^* and the detection limit as input, then it uses the t^* and the m/c value corresponding to the detection limit to calculate two parameters vectors k^* and b^* (corresponding to each cell) which correspond to the slope and intercept for the linear conversion function between log10 FPKM and log10 transcript counts. The function will then apply a linear transformation to convert the FPKM to estimated absolute transcript counts based on the the k^* and b^*. The default m/c values used in the algorithm are 3.652201, 2.263576, respectively.

**Usage**

```r
relative2abs(relative_cds, t_estimate = estimate_t(exprs(relative_cds)),
modelFormulaStr = '~1', ERCC_controls = NULL, ERCC_annotation = NULL,
volume = 10, dilution = 40000, mixture_type = 1,
detection_threshold = 800, expected_capture_rate = 0.25,
verbose = FALSE, return_all = FALSE, cores = 1)
```

**Arguments**

- `relative_cds`: the cds object of relative expression values for single cell RNA-seq with each row and column representing genes/isoforms and cells. Row and column names should be included.
Relative2Abs

**t_estimate**

An vector for the estimated most abundant FPKM value of isoform for a single cell. Estimators based on gene-level relative expression can also give good approximation but estimators based on isoform FPKM will give better results in general.

**modelFormulaStr**

Model formula used to grouping cells for transcript counts recovery. Default is "~ 1", which means to recover the transcript counts from all cells.

**ERCC_controls**

The FPKM matrix for each ERCC spike-in transcript in the cells if user wants to perform the transformation based on their spike-in data. Note that the row and column names should match up with the ERCC_annotation and relative Exprs_matrix respectively.

**ERCC_annotation**

The ERCC_annotation matrix from illumina USE GUIDE which will be used for calculating the ERCC transcript copy number for performing the transformation.

**volume**

The approximate volume of the lysis chamber (nanoliters). Default is 10

**dilution**

The dilution of the spike-in transcript in the lysis reaction mix. Default is 40,000. The number of spike-in transcripts per single-cell lysis reaction was calculated from

**mixture_type**

The type of spike-in transcripts from the spike-in mixture added in the experiments. By default, it is mixture 1. Note that m/c we inferred are also based on mixture 1.

**detection_threshold**

The lowest concentration of spike-in transcript considered for the regression. Default is 800 which will ensure (almost) all included spike-in transcripts expressed in all the cells. Also note that the value of c is based on this concentration.

**expected_capture_rate**

The expected fraction of RNA molecules in the lysate that will be captured as cDNAs during reverse transcription

**verbose**

A logical flag to determine whether or not we should print all the optimization details

**return_all**

Parameter for the intended return results. If setting TRUE, matrix of m, c, k^*, b^* as well as the transformed absolute cds will be returned in a list format

**cores**

Number of cores to perform the recovery. The recovery algorithm is very efficient so multiple cores only needed when we have very huge number of cells or genes.

**Value**

An matrix of absolute count for isoforms or genes after the transformation.

**Examples**

```r
## Not run:
HSMM_relative_expr_matrix <- exprs(HSMM)
HSMM_abs_matrix <- relative2abs(HSMM_relative_expr_matrix,
t_estimate = estimate_t(HSMM_relative_expr_matrix))
## End(Not run)
```
residualMatrix  

Description
Generates a matrix of response values for a set of fitted models

Usage
residualMatrix(models, residual_type = "response", cores = 1)

Arguments
- models: a list of models, e.g. as returned by fitModels()
- residual_type: the response desired, as accepted by VGAM’s predict function
- cores: number of cores used for calculation

Value
a matrix where each row is a vector of response values for a particular feature’s model, and columns are cells.

responseMatrix  

Description
Generates a matrix of response values for a set of fitted models

Usage
responseMatrix(models, newdata = NULL, response_type = "response", cores = 1)

Arguments
- models: a list of models, e.g. as returned by fitModels()
- newdata: a dataframe used to generate new data for interpolation of time points
- response_type: the response desired, as accepted by VGAM’s predict function
- cores: number of cores used for calculation

Value
a matrix where each row is a vector of response values for a particular feature’s model, and columns are cells.
scale_pseudotime

Scale pseudotime to be in the range from 0 to 100

Description
This function transforms the pseudotime scale so that it ranges from 0 to 100. If there are multiple branches, each leaf is set to be 100, with branches stretched accordingly.

Usage
scale_pseudotime(cds, verbose = F)

Arguments
- cds: the CellDataSet upon which to perform this operation
- verbose: Whether to emit verbose output

Value
an updated CellDataSet object which an

selectNegentropyGenes
Filter genes with extremely high or low negentropy

Description
Filter genes with extremely high or low negentropy

Usage
selectNegentropyGenes(cds, lower_negentropy_bound = "0%", upper_negentropy_bound = "99%", expression_lower_thresh = 0.1, expression_upper_thresh = Inf)

Arguments
- cds: a CellDataSet object upon which to perform this operation
- lower_negentropy_bound: the centile below which to exclude to genes
- upper_negentropy_bound: the centile above which to exclude to genes
- expression_lower_thresh: the expression level below which to exclude genes used to determine negentropy
- expression_upper_thresh: the expression level above which to exclude genes used to determine negentropy

Value
a vector of gene names
Examples

```r
## Not run:
reasonableNegentropy <- selectNegentropyGenes(HSMM, "07", "95", 1, 100)
## End(Not run)
```

selectTopMarkers  Select the most cell type specific markers

Description

This is a handy wrapper function around dplyr’s top_n function to extract the most specific genes for each cell type. Convenient, for example, for selecting a balanced set of genes to be used in semi-supervised clustering or ordering.

Usage

```r
selectTopMarkers(marker_specificities, num_markers = 10)
```

Arguments

- **marker_specificities**
  - The dataframe of specificity results produced by `calculateMarkerSpecificity()`
- **num_markers**
  - The number of markers that will be shown for each cell type

Value

A data frame of specificity results

setOrderingFilter  Sets the features (e.g. genes) to be used for ordering cells in pseudo-time.

Description

Sets the features (e.g. genes) to be used for ordering cells in pseudotime.

Usage

```r
setOrderingFilter(cds, ordering_genes)
```

Arguments

- **cds**
  - the CellDataSet upon which to perform this operation
- **ordering_genes**
  - a vector of feature ids (from the CellDataSet’s featureData) used for ordering cells

Value

an updated CellDataSet object
spike_df

Description
A dataset containing the information for the 92 ERCC spikein transcripts (This dataset is based on the data from the Nature paper from Stephen Quake group)

Usage
spike_df

Format
A data frame with 92 rows and 9 variables:

- **ERCC_ID**: ID for ERCC transcripts
- **subgroup**: Subgroup for ERCC transcript
- **conc_attomoles_ul_Mix1**: Concentration of Mix 1 (attomoles / ul)
- **conc_attomoles_ul_Mix2**: Concentration of Mix 2 (attomoles / ul)
- **exp_fch_ratio**: Expected fold change between mix 1 over mix 2
- **numMolecules**: Number of molecules calculated from concentration and volume
- **rounded_numMolecules**: Number in rounded digit of molecules calculated from concentration and volume

vstExprs

Description
This function was taken from the DESeq package (Anders and Huber) and modified to suit Monocle’s needs

Usage
vstExprs(cds, dispModelName = "blind", expr_matrix = NULL, round_vals = TRUE)

Arguments
- **cds**: A CellDataSet to use for variance stabilization.
- **dispModelName**: The name of the dispersion function to use for VST.
- **expr_matrix**: An matrix of values to transform. Must be normalized (e.g. by size factors) already. This function doesn’t do this for you.
- **round_vals**: Whether to round expression values to the nearest integer before applying the transformation.
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