Package 'spade'

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Index 19

SPADE.addClusterToFCS Annotate observations in FCS file with cluster assignment

Description

Annotate observations in a FCS file with cluster assignment

Usage

SPADE.addClusterToFCS(infilename, outfilename, clusterfilename, cols = NULL, arcsinh_cofactor=NULL,

Arguments

infilename Name of input FCS file outfilename Name of output FCS file

clusterfilename

Name of FCS file with subset of cells used in clustering

cols Usually a vector of strings specifying the columns to be used in the density

calculation, e.g., c("(Cd110)D","(Cs111)D"). Strings will be matched against the parameter names extracted from the FCS file. The default=NULL will use

all parameters.

arcsinh_cofactor

DEPRECATED. Cofactor used in arcsinh transform asinh(data/arcsinh_cofactor)

of data.

transforms Transform object to apply to data. A single transform object will be applied

to all channels. To apply different transforms to specific channels use a named

vector of transform objects (where names are parameter names).

comp Apply compensation matrix if present in SPILL or SPILLOVER keywords

Value

The name of the written file is returned.

Note

Underlying implementations have been parallelized with OpenMP. Set OMP_NUM_THREADS in environment to control the number of threads used.

Author(s)

Michael Linderman

See Also

SPADE.FCSToTree

Examples

```
# Not run
## Load two-parameters sample data included in package
data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.F#
#output_dir <- tempdir()</pre>
## Compute and annotate FCS file with density
#density_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.addDensityToFCS(data_file_path, density_file_path, cols=c("marker1","marker2"))
## Downsample FCS file based on density
#downsample_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.downsampleFCS(density_file_path, downsample_file_path)
## Create tree from downsampled FCS file
#cells_file_path <- paste(output_dir,"clusters.fcs",sep="")</pre>
#clust_file_path <- paste(output_dir,"clusters.table",sep="")</pre>
#graph_file_path <- paste(output_dir,"mst.gml",sep="")</pre>
#SPADE.FCSToTree(downsample_file_path, cells_file_path, graph_file_path, clust_file_path, cols=c("marker1","mar
## Add cluster to FCS files (known as "upsampling")
#upsample_file_path <- paste(density_file_path, "cluster.fcs", sep=".")</pre>
#SPADE.addClusterToFCS(density_file_path, upsample_file_path, cells_file_path, cols = c("marker1","marker2"))
```

SPADE.addDensityToFCS Annotate FCS file with local density of each observation

Description

Compute the local density of observation and incorporate the result as a new parameter to the FCS file. The local density is modeled as an integer count of the number of other observations within a specified distance of the observation.

Usage

SPADE.addDensityToFCS(infilename, outfilename, cols = NULL, arcsinh_cofactor=NULL, transforms=flowCo

Arguments

infilename Name of the input FCS file outfilename Name of the output FCS file

cols Usually a vector of strings specifying the columns to be used in the density

calculation, e.g., c("(Cd110)D","(Cs111)D"). Strings will be matched against the parameter names extracted from the FCS file. The default=NULL will use

all parameters.

arcsinh_cofact	or
	DEPRECATED. Cofactor used in arcsinh transform asinh(data/arcsinh_cofactor) of data.
transforms	Transform object to apply to data. A single transform object will be applied to all channels. To apply different transforms to specific channels use a named vector of transform objects (where names are parameter names).
kernel_mult	Multiplier of the minimum median distance within which other observations are counted towards the density
apprx_mult	Multiplier of the minimum median distance within which observations are approximated to have the same density
med_samples	Number of observations used to estimate the minimum median distance
comp	Apply compensation matrix if present in SPILL or SPILLOVER keywords

Value

The name of the written file is returned

Author(s)

Michael Linderman

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.f
# #output_dir <- tempdir()
# ## Compute and annotate FCS file with density
#density_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.addDensityToFCS(data_file_path, density_file_path, cols=c("marker1","marker2"))</pre>
```

SPADE.annotateGraph Add attributes to graph

Description

Add specific and arbitrary attributes to a graph

Usage

```
SPADE.annotateGraph(graph, layout = NULL, anno)
```

SPADE.annotateGraph 5

Arguments

graph The graph object to work on. Note that the original graph is never modified, a

new graph object is returned instead; if you don't assign it to a variable your

modifications will be lost!

layout Optional numeric matrix with vertex x,y positions with the same number of rows

as vertices and at least two columns, the x and y positions.

anno List of annotations to add to the graph. Each entry in list must have a name and

must be a matrix. All matrices must have the same number of rows as vertices. List entry name plus column names are used as attribute names (unless they

match, then just the column name is used).

Details

Add specific arbitrary attributes to a graph.

Value

A new graph object with the attributes added.

Author(s)

Michael Linderman

See Also

```
set.graph.attribute, set.vertex.attribute, set.edge.attribute
```

Examples

```
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
## Run basic SPADE analyses, clustering on two parameters. Annotated graphs will be
## in output_dir. See SPADE.plot.trees to generate PDFs of annotated graphs.
#output_dir <- tempdir()
#SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))
## Add additional parameters to output graphs using SPADE.annotateGraph
#old_graph <- igraph:::read.graph(paste(output_dir, "SimulatedRawData.fcs.density.fcs.cluster.fcs.medians.gml",s
#new_graph <- SPADE.annotateGraph(old_graph, layout=igraph:::layout.kamada.kawai(old_graph), anno=list(demo=mate)</pre>
```

SPADE.downsampleFCS	Downsample observcations in a FCS file according to density param-
	eter

Description

Downsample the observations in a FCS file according to a previously computed density parameter. The goal is to produce a smaller set of observations with similar density. Downsampling is independent of how the density is modeled.

Usage

```
SPADE.downsampleFCS(infilename, outfilename,
    exclude_pctile = 0.01, target_pctile = 0.05,
    desired_samples = NULL)
```

Arguments

infilename Name of the input FCS file. Must have a parameter named "density".

outfilename Name of the output FCS file

exclude_pctile Numeric value in [0,1]. Densities below this percentile will be excluded.

target_pctile Numeric value in [0,1]. Densities below this percentile, but above 'exclude_pctile' will be retained. Only meaningful if 'desired_samples' is NULL.

desired_samples

Desired number of samples. If set to integer value, the target percentile will be set internally to downsample to approximately the desired number of samples.

Value

The name of the written file is returned

Note

Underlying implementations have been parallelized with OpenMP. Set OMP_NUM_THREADS in environment to control the number of threads used.

Author(s)

Michael Linderman

See Also

```
SPADE.addDensityToFCS
```

SPADE.driver 7

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
#output_dir <- tempdir()
#
## Compute and annotate FCS file with density
#density_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.addDensityToFCS(data_file_path, density_file_path, cols=c("marker1","marker2"))
## Downsample FCS file based on density
#downsample_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.downsampleFCS(density_file_path, downsample_file_path)</pre>
```

SPADE.driver

SPADE workflow driver

Description

A function to drive the SPADE workflow. Produces graphs annoated with parameter medians and fold change.

Usage

```
SPADE.driver(files, file_pattern="*.fcs", out_dir=".", cluster_cols=NULL, panels=NULL, comp=TRUE, are
```

Arguments

Either a vector of FCS files, or a directory. If a directory, all of the *.fcs files in files the directory are processed. file_pattern Wildcard pattern to match file if files is a director out_dir Directory where output files are written. Will be created if it does not exist. cluster_cols Usually a vector of strings specifying the columns to be used in the clustering, e.g., c("(Cd110)D","(Cs111)D"). Strings will be matched against the parameter names extracted from the FCS file. The default=NULL will use all parameters. panels List of panels for median and fold change calculations. See details for specific structure. If NULL, medians are computed for all markers in all files. Apply compensation matrix if present in SPILL or SPILLOVER keywords comp arcsinh_cofactor DEPRECATED. Cofactor used in arcsinh transform asinh(data/arcsinh_cofactor) of data. transforms Transform object to apply to data. A single transform object will be applied to all channels. To apply different transforms to specific channels use a named vector of transform objects (where names are parameter names).

8 SPADE.driver

downsampling_samples

Desired number of samples remaining after downsampling files

downsampling_exclude_pctile

Numeric value in [0,1]. Densities below this percentile will be excluded during downsampling.

downsampling_target_pctile

Numeric value in [0,1]. Densities below this percentile, but above 'exclude_pctile' will be retained during downsampling. Only meaningful if 'downsampling_samples' in 'NULL'.

is 'NULL'.

k Desirec number of clusters. Algorithm might create between [k/2,3k/2] clusters.

clustering_samples

Desired number of samples to be used in clustering.

layout Layout function

pctile_color A two element vector specifying lower and upper percentiles that should be used

to set the color scale. Values below and above these percentiles will be forced to the 'smallest' and 'largest' color respectively. Not in effect if 'scale' is specified. Relevant for downstream tools that used global value ranges produced by driver.

Details

The panels argument must be null or a list of panel descriptors, which are themselves lists containing at minimum a vector of panel files and median cols. An example minimum panels argument would be list(list(panel_files="basal.fcs", median_cols=NULL)) . panel_files is a single file name or vector of file names in the experiment. median_cols is similar to the cluster_cols argument. Each panel descriptor can optionally specify reference_files and columns for fold change analysis. An example full panel descriptor would be list(list(panel_files=c("basal.fcs", "stim.f fold_cols is similar to the cluster_cols argument. reference_files is a single file name or vector of file names in the experiment and in the panel_files for this experiment. median_cols and fold_cols are only interpreted in the context on their panel files, and so partially overlapping panels are possible. However, all the files specified within a panel must have the cluster, median and fold change parameters specified.

Value

NULL

Author(s)

Michael Linderman

Examples

```
# Load two-parameters sample data included in package
data_file_path = system.file(file.path("extdata","SimulatedRawData.fcs"), package = "spade")
# Run basic SPADE analyses, clustering on two parameters. Annotated graphs will be
# in output_dir. See SPADE.plot.trees to generate PDFs of annotated graphs.
output_dir <- tempdir()
SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))</pre>
```

SPADE.FCSToTree 9

SPADE.FCSToTree	Cluster and build minimum spanning tree from data in FCS files

Description

Hierarchically cluster observations in a set of FCS files and build a minimum spanning tree connecting those clusters.

Usage

```
SPADE.FCSToTree(infilenames, outfilename, graphfilename, clusterfilename,
  cols = NULL, k = 200, arcsinh_cofactor=NULL, transforms=flowCore::arcsinhTransform(a=0, b=0.2),
  desired_samples = 50000, comp=TRUE)
```

Arguments

infilenames Vector of FCS file names that should be used as input

outfilename Name of FCS file to write subset of cells used for clustering along with their

cluster assignment

graphfilename Name of file to write gml graph description

clusterfilename

Name of file to write table of cluster centers

cols Usually a vector of strings specifying the columns to be used in the density

calculation, e.g., c("(Cd110)D","(Cs111)D"). Strings will be matched against the parameter names extracted from the FCS file. The default=NULL will use

all parameters.

k Desired number of clusters. Algorithm might create between [k/2,3k/2] clusters.

arcsinh_cofactor

DEPRECATED. Cofactor used in arcsinh transform asinh(data/arcsinh_cofactor)

of data.

transforms Transform object to apply to data. A single transform object will be applied

to all channels. To apply different transforms to specific channels use a named

vector of transform objects (where names are parameter names).

desired_samples

Desired number of samples to be used in clustering. Usually leave at default.

comp Apply compensation matrix if present in SPILL or SPILLOVER keywords

Value

None.

Note

Underlying implementations have been parallelized with OpenMP. Set OMP_NUM_THREADS in environment to control the number of threads used. Implementation can be very memory intensive.

Author(s)

Michael Linderman

See Also

```
SPADE.downsampleFCS
```

Examples

```
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
#output_dir <- tempdir()
#
## Compute and annotate FCS file with density
#density_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.addDensityToFCS(data_file_path, density_file_path, cols=c("marker1","marker2"))
## Downsample FCS file based on density
#downsample_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs",sep="")
#SPADE.downsampleFCS(density_file_path, downsample_file_path)
## Create tree from downsampled FCS file
#cells_file_path <- paste(output_dir,"clusters.fcs",sep="")
#clust_file_path <- paste(output_dir,"clusters.table",sep="")
#graph_file_path <- paste(output_dir,"clusters.table",sep="")
#graph_file_path <- paste(output_dir,"mst.gml",sep="")
#graph_file_path, clust_file_path, cols=c("marker1","marker1","marker1","marker2")</pre>
```

SPADE.flattenAnnotations

Flatten list of annotations to matrix

Description

Helper function for flattening list of annotations

Usage

```
SPADE.flattenAnnotations(annotations)
```

Arguments

annotations A list of annotation matrices. All matrices must have the same number of rows.

Value

Single matrix of annotations

SPADE.installPlugin 11

Author(s)

Michael Linderman

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
## Run basic SPADE analyses, clustering on two parameters.
#output_dir <- tempdir()
#SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))

## Compute medians, counts and other parameters from processed files
#upsampled_file_path <- paste(output_dir,.Platform$file.sep,basename(data_file_path),".density.fcs.cluster.fcs'
#mst_graph <- igraph:::read.graph(paste(output_dir,"mst.gml",sep=.Platform$file.sep),format="gml")
#anno <- SPADE.markerMedians(upsampled_file_path, igraph:::vcount(mst_graph), cols = c("marker1","marker2"))

## Flatten annotations so they can easily be saved in table
#flat_anno <- SPADE.flattenAnnotations(anno)</pre>
```

Description

Install, CytoSPADE, the Cytoscape plugin for working with SPADE that is distributed with the SPADE R package. CytSPADE provides a GUI for setting-up SPADE analyses and interactively visualizing the results.

Usage

```
SPADE.installPlugin(cytoscape_path)
```

Arguments

cytoscape_path Path to your Cytoscape install, e.g., on OSX it is typically something like '/Applications/Cytoscape_v2.8.1'

Details

Copies the Cytoscape plugin file distributed with the SPADE R package to the Cytoscape plugin directory.

Value

Logical indicating success of the copy operation.

12 SPADE.layout.arch

Author(s)

Michael Linderman

Examples

```
# On OSX:
# SPADE.installPlugin("/Applications/Cytoscape_v2.8.1/")
```

SPADE.layout.arch

Generate coordinates for plotting graphs

Description

Performing "arch" layouts of graph vertices

Usage

```
SPADE.layout.arch(mst_graph)
```

Arguments

mst_graph

The graph to layout. Must be acyclic and undirected.

Details

These functions calculate the coordinates of the vertices for a graph.

layout.arch lays out the longest chain of the graph, the "backbone", on an arch, and the "side chains" as trees normal to that backbone.

Value

All these functions return a numeric matrix with at least two columns, x and y positions, and the same number of lines as the number of vertices.

Author(s)

Michael Linderman

See Also

SPADE.annotateGraph

SPADE.markerMedians 13

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.f
## Run basic SPADE analyses, clustering on two parameters.
#output_dir <- tempdir()
#SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))
## Generate PDFs of annotated graphs (into output_dir/pdf) using arch layout
#mst_graph <- igraph:::read.graph(paste(output_dir,"mst.gml",sep=.Platform$file.sep),format="gml")
#SPADE.plot.trees(mst_graph, output_dir, out_dir=paste(output_dir,"pdf",sep=.Platform$file.sep), layout=SPADE.]</pre>
```

SPADE.markerMedians Comp

Compute marker medians, coefficient of variations and counts for clusters

Description

Compute the marker medians, coefficients of variation and observations counts for cluster annoated FCS files.

Usage

SPADE.markerMedians(files, num.clusters, cols = NULL, arcsinh_cofactor=NULL, transforms=flowCore::arcsinh_cofactor = 5)

Arguments

files Name of input FCS file or vector of input FCS file names. FCS files must have

"cluster" column.

num. clusters Number of clusters. Note not all clusters need to be present in all files.

Cols Usually a vector of strings specifying the columns to be used in the density

calculation, e.g., c("(Cd110)D","(Cs111)D"). Strings will be matched against the parameter names extracted from the FCS file. The default=NULL will use

all parameters.

arcsinh_cofactor

DEPRECATED. Cofactor used in arcsinh transform asinh(data/arcsinh_cofactor)

of data.

transforms Transform object to apply to data. A single transform object will be applied

to all channels. To apply different transforms to specific channels use a named

vector of transform objects (where names are parameter names).

cluster_cols A vector of strings specifying columns that should be marked as having been

used in clustering

comp Apply compensation matrix if present in SPILL or SPILLOVER keywords

SPADE.plot.trees

Details

SPADE.annotateMarkers is deprecated.

Value

List with:

count Matrix of observation count for clusters

percenttotal Matrix of percent of total number of cells [0-100] in each cluster

medians Matrix of medians for specified columns

cvs Matrix of coefficient of variation (CV), 100*sd(data)/abs(mean(data)), for spec-

ified columns

Author(s)

Michael Linderman

See Also

```
SPADE.addClusterToFCS, SPADE.annotateGraph
```

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
## Run basic SPADE analyses, clustering on two parameters.
#output_dir <- tempdir()
#SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))
## Compute medians, counts and other parameters from processed files
#upsampled_file_path <- paste(output_dir, .Platform$file.sep,basename(data_file_path),".density.fcs.cluster.fcs'
#mst_graph <- igraph:::read.graph(paste(output_dir, "mst.gml", sep=.Platform$file.sep),format="gml")
#anno <- SPADE.markerMedians(upsampled_file_path, igraph:::vcount(mst_graph), cols = c("marker1","marker2"))</pre>
```

SPADE.plot.trees

Plot trees with annotated vertices

Description

Plot trees for each vertex annotation setting vertex size and color based on the particular annotation.

Usage

```
SPADE.plot.trees(graph, files, file_pattern = "*anno.Rsave", out_dir = ".", layout = SPADE.layout.arc
```

SPADE.plot.trees 15

Arguments

graph iGraph graph object

files Either a vector of save annotation files or a directory. If a directory, all of the

files matching the pattern wildcard pattern are processesd.

file_pattern Wildcard pattern to match files if files is a directory.

out_dir Directory where output files are written. Will be created if it does not exist.

layout Either a function or a numeric matrix specifying how vertices are placed on plot.

If it is a matrix, the matrix must have two columns, x and y position, and as many rows as vertices. If layout is a function, it will be called with an igraph graph

as the single parameter.

attr_pattern A regular expression that matches the attributes that should be plotted for each

graph. Parameter names matching regex "medianlfractionlcvs" will be plotted with a scale range set to [min, max] for that attribute, while all other parameters will be plotted on a centered scale with the range [abs(min(parameter values),max(parameter values)), abs(min(parameter values),max(parameter values)).

ues))]

scale A two element vector, e.g. c(-1,1), specifying low and upper bound for color

scale. Values below and above these bounds will be forced to the 'smallest' and

'largest' color respectively. If specified, overrides 'pctile_color'.

pctile_color A two element vector specifying lower and upper percentiles that should be used

to set the color scale. Values below and above these percentiles will be forced to the 'smallest' and 'largest' color respectively. Not in effect if 'scale' is specified.

normalize A string (either "global" or "local"), specifying color scale normalization. Set-

ting to "global" will set the scale range to the global min/max of all GML files in the folder, while "local" will set the scale range to the min/max of the particular

GML file being plotted.

size_scale_factor

A scale factor for node size in drawing. Current function for node size: percenttotal[i]/(max(percent

edge.color Set the edge color. See igraph.plotting for more details.

bare Boolean specifying whether to omit titles and gradient legend.

palette A string (either "jet" or "bluered"), specifying color palette for nodes. "bluered"

tends to show up better on LCD projectors.

Author(s)

Michael Linderman

See Also

SPADE.driver

16 SPADE.read.FCS

Examples

```
## Not run:
# Load two-parameters sample data included in package
data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.Pl
# Run basic SPADE analyses, clustering on two parameters.
output_dir <- tempdir()
SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))
# Generate PDFs of annotated graphs (into output_dir/pdf)
mst_graph <- igraph:::read.graph(paste(output_dir,"mst.gml",sep=.Platform$file.sep),format="gml")
SPADE.plot.trees(mst_graph, output_dir, out_dir=paste(output_dir,"pdf",sep=.Platform$file.sep), layout=igraph::
## End(Not run)</pre>
```

SPADE.read.FCS

Read an FCS File

Description

Read and FCS file, correcting problematic column names and applying compensation if requested. A wrapper around read.FCS.

Usage

```
SPADE.read.FCS(file, comp = TRUE, verbose = FALSE, ...)
```

Arguments

file FCS filename

comp boolean indicating whether to apply compensation

verbose boolean indicating whether to not suppress warnings from upstream FCS file

reader.

... additional arguments are passed through to read.FCS

Details

A wrapper around read.FCS that copies FCS parameter "short name" to description when none is supplied, and applies compensation matrix stored in the SPILL or SPILLOVER keyword.

Value

a flowFrame object.

Author(s)

Michael Linderman <michael.linderman@mssm.edu>

SPADE.write.graph 17

See Also

```
read.FCS
```

Examples

```
fcs <- SPADE.read.FCS(system.file(file.path("extdata", "SimulatedRawData.fcs"), package = "spade"))</pre>
```

SPADE.write.graph

Writing the graph to a file in some format

Description

General function for exporting graphs to foreign file formats, however at present only the GML format is implemented.

Usage

```
SPADE.write.graph(graph, file = "", format = c("gml"))
```

Arguments

graph The graph to export

file A connection or a string giving the file name to write the graph to.

format Character string giving the file format.

Details

GML is general textual format for graphs.

The vertex and edge attributes are written to the file if they are numeric or strings. Currently only the graphics struct is supported, and only for vertices; graphics.x indicates an x attribute in the graphics struct.

Value

A NULL, invisibly

Author(s)

Michael Linderman

See Also

```
write.graph
```

SPADE.write.graph

Examples

```
# Not run
## Load two-parameters sample data included in package
#data_file_path = paste(installed.packages()["spade","LibPath"],"spade","extdata","SimulatedRawData.fcs",sep=.ff
## Run basic SPADE analyses, clustering on two parameters.
#output_dir <- tempdir()
#SPADE.driver(data_file_path, out_dir=output_dir, cluster_cols=c("marker1","marker2"))
## Read and write minimum spanning tree graph
#mst_graph <- igraph:::read.graph(paste(output_dir,"mst.gml",sep=.Platform$file.sep),format="gml")
#SPADE.write.graph(mst_graph, file = paste(output_dir,"new_mst.gml",sep=.Platform$file.sep), format = c("gml"))</pre>
```

Index

```
flowFrame, 16
read. FCS, 16, 17
set.edge.attribute, 5
set.graph.attribute, 5
set.vertex.attribute, 5
SPADE.addClusterToFCS, 2, 14
SPADE.addDensityToFCS, 3, 6
SPADE.annotateGraph, 4, 12, 14
SPADE.annotateMarkers
        (SPADE.markerMedians), 13
SPADE.downsampleFCS, 6, 10
SPADE.driver, 7, 15
SPADE.FCSToTree, 2, 9
SPADE.flattenAnnotations, 10
SPADE.installPlugin, 11
SPADE.layout.arch, 12
{\tt SPADE.markerMedians}, 13
SPADE.plot.trees, 14
SPADE.read.FCS, 16
SPADE.write.graph, 17
write.graph, 17
```