

Package ‘flowType’

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Type Package

Title Phenotyping Flow Cytometry Assays

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Description Phenotyping Flow Cytometry Assays using multidimensional expansion of single dimensional partitions.

Imports Biobase, graphics, grDevices, methods, flowCore, flowMeans, sfsmisc, rrcov, flowClust, flowMerge, stats

Depends R (>= 2.10), Rcpp (>= 0.10.4), BH (>= 1.51.0-3)

LinkingTo Rcpp, BH

Suggests xtable

biocViews FlowCytometry

License Artistic-2.0

LazyLoad yes

NeedsCompilation yes

R topics documented:

flowType-package	2
calcMemUse	3
calcNumPops	4
decodePhenotype	5
DLBCLExample	6
encodePhenotype	6
flowType	7
getLabels	9
HIVData	10
HIVMetaData	10
Phenotypes-class	11
plot	12
summary-methods	12

Index	14
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flowType-package *flowType: Phenotyping Flow Cytometry Assays*

Description

flowType uses a simple threshold, Kmeans, flowMeans, or flowClust to partition every channel to a positive and a negative cell population. These partitions are then combined to generate a set of multi-dimensional phenotypes.

Details

```

Package:    flowType
Type:      Package
Version:   0.0.1
Date:     2011-04-27
License:   Artistic-2.0
LazyLoad: yes
Depends:   methods

```

For a given FCS file, the flowType function extracts a the phenotypes and reports their cell frequencies (number of cells) and mean fluorescence intensity (MFI)s.

Author(s)

Nima Aghaeepour, Kieran O'Neill, Adrin Jalali

References

Please cite the following for the current version of flowType:

O'Neill K, Jalali A, Aghaeepour N, Hoos H, Brinkman RR. Enhanced flowType/RchyOptimyx: a BioConductor pipeline for discovery in high-dimensional cytometry data. *Bioinformatics*. 2014 May 1;30(9):1329-30. doi: 10.1093/bioinformatics/btt770

The original paper and description can be found at:

Nima Aghaeepour, Pratip K. Chattopadhyay, Anuradha Ganesan, Kieran O'Neill, Habil Zare, Adrin Jalali, Holger H. Hoos, Mario Roederer, and Ryan R. Brinkman. Early Immunologic Correlates of HIV Protection can be Identified from Computational Analysis of Complex Multivariate T-cell Flow Cytometry Assays. *Bioinformatics*, 2011.

Examples

```

#Load the library
library(flowType)
data(DLBCLExample)
MarkerNames <- c('Time', 'FSC-A', 'FSC-H', 'SSC-A', 'IgG', 'CD38', 'CD19', 'CD3', 'CD27', 'CD20', 'NA', 'NA')

#These markers will be analyzed
PropMarkers <- 3:5
MFIMarkers <- PropMarkers
MarkerNames <- c('FS', 'SS', 'CD3', 'CD5', 'CD19')

```

```

#Run flowType
Res <- flowType(DLBCLExample, PropMarkers, MFIMarkers, 'kmeans', MarkerNames);

MFIs=Res@MFIs;
Proportions=Res@CellFreqs;
Proportions <- Proportions / max(Proportions)
names(Proportions) <- unlist(lapply(Res@PhenoCodes,
                                function(x){return(decodePhenotype(
x,Res@MarkerNames[PropMarkers],
Res@PartitionsPerMarker))}))

#Select the 30 largest phenotypes
index=order(Proportions,decreasing=TRUE)[1:30]
bp=barplot(Proportions[index], axes=FALSE, names.arg=FALSE)
text(bp+0.2, par("usr")[3]+0.02, srt = 90, adj = 0, labels = names(Proportions[index]), xpd = TRUE, cex=0.8)
axis(2);
axis(1, at=bp, labels=FALSE);
title(xlab='Phenotype Names', ylab='Cell Proportion')

#These phenotype can be analyzed using a predictive model (e.g., classification or regression)

```

calcMemUse

*Function calcMemUse in Package **flowType***

Description

Estimates the memory usage in bytes for running flowType with a given set of parameters.

Usage

```
calcMemUse(NumPops, NumPropMarkers, NumMFIMarkers, NumCells, MaxMarkersPerPop, PartitionsPerChannel)
```

Arguments

NumPops	Number of cell types which will be returned. Can be computed using calcNumPops
NumPropMarkers	Numer of markers to use for combinatorial gating
NumMFIMarkers	Number of markers to determine the MFIs of for every cell type
NumCells	Number of cells in the flowFrame passed to flowType
MaxMarkersPerPop	Maximum number of markers to use at once in combinatorial gating (ie all cell types over 1:MaxMarkersPerPop will be counted)
PartitionsPerChannel	Number of partitions per channel.

Details

If you use different numbers of partitions for different channels, try providing the highest number as PartitionsPerChannel, and expect an over-estimate.

Value

Estimated memory use in bytes.

Author(s)

Kieran O'Neill

See Also

[calcNumPops](#), [flowType](#)

calcNumPops

*Function calcNumPops in Package **flowType***

Description

Compute the number of populations that will be produced by running `flowType` with a given set of parameters. This is especially useful for estimating memory but is also used internally to determine the size of return objects to pass down to C++. This may also be useful for determining the cutoff for number of markers to use to make phenotypes (in terms of statistical power for later testing).

Usage

```
calcNumPops(PartitionsPerMarker, MaxMarkersPerPop)
```

Arguments

PartitionsPerMarker

Integer vector specifying the number of partitions for each marker, in order.

MaxMarkersPerPop

Integer specifying the threshold chosen

Value

Integer specifying the number of populations the given parameters would produce.

Author(s)

Kieran O'Neill

See Also

[calcMemUse](#), [flowType](#)

Examples

```
calcNumPops(c(2,2,3,2,2,4), 5)
```

decodePhenotype	<i>Method decodePhenotype in Package flowType</i>
-----------------	---

Description

Method to decode phenotypes back to a human-readable string.

Details

FlowType's encoding is as follows:

0 – marker not considered in phenotype (don't care about its value) 1 – marker is negative (e.g. CD4-) 2 – marker is positive (e.g. CD4+) 3 – marker is positive, but brighter than 2 (CD4++) 4 – marker is even brighter (CD4+++)

Note that this encoding system does not allow for "dim" markers – dim positivity is denoted by the first level of positivity.

Also note that the encoding is performed from the dimmest to the brightest partition, but the location of thresholds will dictate the interpretation of the code. (e.g. if you only set one threshold, but you place it between the positive and the bright population, then both positive and negative events will be considered negative.)

Methods

`signature(pheno.code = "character", marker.names = "character", partitions.per.marker = "numeric")`

In **flowType**, phenotypes themselves are represented by codes (e.g. 012) rather than full strings (CD4+CD8-), in order to save memory when a very large number of phenotypes are considered. `decodePhenotype` serves to translate the codes back to a human-readable string.

Author(s)

Kieran O'Neill

See Also

[encodePhenotype](#), [flowType](#)

Examples

```
decodePhenotype('1034',c('CD34','CD3','CD45','CD19'), 4)
decodePhenotype('20013',c('CD34','CD3','CD45','CD19','CD20'), 4)
```

DLBCLExample

*DLBCLExample***Description**

A flow cytometry sample from a patient with DLBC lymphoma. The full dataset is available through the FlowCAP project (<http://flowcap.flowsite.org>).

Usage

```
data(DLBCLExample)
```

Format

A flowFrame describing expression values of 3 markers and 3796 cells. Each column represents a marker and each row represents a cell.

Examples

```
data(DLBCLExample)
```

encodePhenotype

*Function encodePhenotype in Package flowType***Description**

In **flowType**, phenotypes themselves are represented by codes (e.g. 012) rather than full strings (CD4+CD8-), in order to save memory when a very large number of phenotypes are considered. encodePhenotype serves to translate a human-readable string down to flowType's internal coded representation.

Usage

```
encodePhenotype(pheno.string, marker.names)
```

Arguments

pheno.string character vector containing the phenotype string to be encoded
marker.names vector of character vectors each specifying the name of a channel, in order

Details

FlowType's encoding is as follows:

0 – marker not considered in phenotype (don't care about its value) 1 – marker is negative (e.g. CD4-) 2 – marker is positive (e.g. CD4+) 3 – marker is positive, but brighter than 2 (CD4++) 4 – marker is even brighter (CD4+++)

Note that this encoding system does not allow for "dim" markers – dim positivity is denoted by the first level of positivity.

Also note that the encoding is performed from the dimmest to the brightest partition, but the location of thresholds will dictate the interpretation of the code. (e.g. if you only set one threshold, but you place it between the positive and the bright population, then both positive and negative events will be considered negative.)

Value

Character vector containing the encoded phenotype, with one character per channel.

Author(s)

Kieran O'Neill

See Also

[decodePhenotype](#), [flowType](#)

Examples

```
encodePhenotype('CD34++CD3-CD45+++',c('CD34','CD3','CD45','CD19'))
```

flowType

flowType: Phenotyping Flow Cytometry Assays

Description

flowType uses a simple threshold, Kmeans, flowMeans or flowClust to partition every channel to a positive and a negative cell population. These partitions are then combined to generate a set of multi-dimensional phenotypes.

Usage

```
flowType(Frame, PropMarkers=NULL, MFIMarkers=NULL, Methods='kmeans', MarkerNames=NULL, MaxMarkersPerPop=NULL)
#If upgrading from flowType 1.x to 2.x, please check documentation as some arguments have changed
```

Arguments

Frame	A flowFrame (after transformation) that is going to be phenotyped.
PropMarkers	A vector of the indexes or names of the markers to partition to specify phenotypes. If NULL, all markers in the frame will be used.
MFIMarkers	A vector of the indexes or names of the markers for which MFIs must be measured. If NULL, no markers will be used.
Methods	A single string specifying the method to use to determine thresholds for partitioning of markers. Values can be "kmeans", "flowMeans", "flowClust", or "Thresholds". If "Thresholds" is specified, user-specified thresholds must be provided via the Thresholds parameter.
MarkerNames	A vector of names for the channels. If NULL, the names in Frame will be used.
MaxMarkersPerPop	An integer specifying the maximum number of markers to use to define populations (how "deep" to phenotype). This should be less than or equal to PropMarkers. If NULL, will default to the length of PropMarkers.

PartitionsPerMarker	An integer or vector of integers specifying the number of partitions per marker. If a single integer, this number will be used for all markers. If a vector, the numbers will be matched with PropMarkers in order.
Thresholds	A list of vectors specifying per-channel thresholds. Each list item corresponds to one marker, and contains the threshold(s) for that marker. If only one vector is provided in the list, then those thresholds will be used for all markers. Otherwise, the list must be of the same length as PropMarkers. Note: if Methods == 'thresholds', then Thresholds must be specified. If not, it is ignored.
MemLimit	Memory limit in GB. flowType will do a sanity check before executing, and if the total size of counts plus MFI values for all populations would exceed MemLimit, will not run.
verbose	Boolean variable. If TRUE, information about different processing tasks will be printed into the standard output.

Value

CellFreqs:	Object of class "numeric" containing the cell frequencies measured for each phenotype. Phenotype names are assigned as labels.
MFI:	Object of class "matrix" containing the measured MFIs for each phenotype. Phenotype names are assigned as column labels and marker names as row labels.
PropMarkers	A vector of the indexes or names of the markers for which cell proportions must be measured.
MFIMarkers	A vector of the indexes or names of the markers for which MFIs must be measured.
MarkerNames	A vector of names for the channels. If NULL, the names provided in Frame will be used.
Partitions	A matrix where each column shows the partitioning of the respective channel. 1 and 2 correspond to negative and positive, respectively.
PhenoCodes	A vector of strings of length N (the number of markers) for each phenotype measured. For every phenotype, the character corresponding to a given marker can be 0, 1, 2, etc for neutral, negative, positive, bright, etc. See the provided vignette for more details and examples.

Author(s)

Nima Aghaeepour, Kieran O'Neill

References

Please cite the following for the current version of flowType:

O'Neill K, Jalali A, Aghaeepour N, Hoos H, Brinkman RR. Enhanced flowType/RchyOptimyx: a BioConductor pipeline for discovery in high-dimensional cytometry data. *Bioinformatics*. 2014 May 1;30(9):1329-30. doi: 10.1093/bioinformatics/btt770

The original paper and description can be found at:

Nima Aghaeepour, Pratip K. Chattopadhyay, Anuradha Ganesan, Kieran O'Neill, Habil Zare, Adrin Jalali, Holger H. Hoos, Mario Roederer, and Ryan R. Brinkman. Early Immunologic Correlates of HIV Protection can be Identified from Computational Analysis of Complex Multivariate T-cell Flow Cytometry Assays. *Bioinformatics*, 2011.

Examples

```

#Load the library
library(flowType)
data(DLBCLExample)

#These markers will be analyzed
PropMarkers <- 3:5
MFIMarkers <- PropMarkers
MarkerNames <- c('FS', 'SS', 'CD3', 'CD5', 'CD19')

#Run flowType
Res <- flowType(DLBCLExample, PropMarkers, MFIMarkers, 'kmeans', MarkerNames);

MFIs=Res@MFIs;
Proportions=Res@CellFreqs;
Proportions <- Proportions / max(Proportions)
names(Proportions) <- unlist(lapply(Res@PhenoCodes,
                                function(x){return(decodePhenotype(
                                    x, Res@MarkerNames[PropMarkers],
                                    Res@PartitionsPerMarker))}))

#Select the 30 largest phenotypes
index=order(Proportions,decreasing=TRUE)[1:30]
bp=barplot(Proportions[index], axes=FALSE, names.arg=FALSE)
text(bp+0.2, par("usr")[3]+0.02, srt = 90, adj = 0, labels = names(Proportions[index]), xpd = TRUE, cex=0.8)
axis(2);
axis(1, at=bp, labels=FALSE);
title(xlab='Phenotype Names', ylab='Cell Proportion')

#These phenotype can be analyzed using a predictive model (e.g., classification or regression)

```

getLabels

getLabels: Returns the labels of the cells in a given phenotype.

Description

Returns the labels of the cells in a given phenotype in a Phenotypes object.

Usage

```
getLabels(Phenotypes, PhenotypeNumber)
```

Arguments

Phenotypes An object of class Phenotypes as produced by the flowType function.

PhenotypeNumber A numeric or character value representing the phenotypes number of name, respectively.

Value

Membership Labels:

A vector of length of the number of events. 1 and 2 represent the cells that are not-included and included in the phenotype respectively.

Author(s)

Nima Aghaeepour

References

Nima Aghaeepour, Pratip K. Chattopadhyay, Anuradha Ganesan, Kieran O'Neill, Habil Zare, Adrin Jalali, Holger H. Hoos, Mario Roederer, and Ryan R. Brinkman. Early Immunologic Correlates of HIV Protection can be Identified from Computational Analysis of Complex Multivariate T-cell Flow Cytometry Assays. submitted to Bioinformatics, 2011.

Examples

#See the vignette

HIVData

HIVData

Description

A flow cytometry dataset from a HIV+ patients PBMC by the Scott lab of the Simon Fraser University and the Spina lab of the University of California San Diego.

Usage

data(HIVData)

Format

A flowSet describing expression values of 11 markers and 500 cells (sampled uniformly) for 19 HIV+ and 12 normal subjects.

Examples

data(HIVData)

HIVMetaData

HIVMetaData

Description

The meta-data of a flow cytometry dataset from a HIV+ patients PBMC by the Scott lab of the Simon Fraser University and the Spina lab of the University of California San Diego.

Usage

data(HIVMetaData)

Format

A matrix describing the FCS filename, patient label (HIV+ or normal) and tube number of every assay.

Examples

```
data(HIVMetaData)
```

Phenotypes-class	Class "Phenotypes"
------------------	--------------------

Description

The return data from running [flowType](#), containing counts

Objects from the Class

Objects can be created by calls of the form `new("Phenotypes", ...)`.

Slots

CellFreqs: Numeric vector containing counts of the number of cells belonging to each cell type.

MFIs: Matrix of MFIs, with rows for cell types and columns for markers.

PhenoCodes: Vector of character strings representing the codes of each cell type (phenotype).

PropMarkers: Numeric vector specifying which markers were used for combinatorial gating.

MFIMarkers: Numeric vector specifying for which markers MFIs were computed for each cell type.

MarkerNames: A character vector of the names of all markers in the flowFrame given

Partitions: The first level partitions that each cell in the flowFrame belong to in each channel.

MaxPopSize: MaxMarkersPerPop

PartitionsPerMarker: Vector of number of partitions used for each marker

Thresholds: A list of vectors with the calculated thresholds for each marker (if a clustering algorithm was used) or the thresholds provided by the user.

See Also

[flowType](#)

Examples

```
showClass("Phenotypes")
```

plot *Methods for Function plot*

Description

Methods for function plot

Usage

```
## S4 method for signature 'Phenotypes,flowFrame'
plot(x, y, ...)
## S4 method for signature 'Phenotypes,numeric'
plot(x, y, Frame,...)
## S4 method for signature 'Phenotypes,character'
plot(x, y, Frame,...)
```

Arguments

x	An object of class Phenotypes as generated by the flowType package.
y	A flowFrame or a numeric/character value representing the phenotype that needs to be plotted depending on the signature of the function
Frame	A flowFrame (might be optional depending on the signature of the function)
...	Extra parameters that will be passed to the generic plot function

Author(s)

Nima Aghaeepour <<naghaeep@gmail.com>>

See Also

[flowType](#)

Examples

```
#See the vigentte
```

summary-methods *~~ Methods for Function summary ~~*

Description

~~ Methods for function summary ~~

Methods

signature(object = "Phenotypes") Prints basic characteristics of a Phenotypes object.

See Also

[flowType](#)

Examples

#See the vigentte

Index

*Topic **FlowCytData**

flowType, 7
flowType-package, 2
getLabels, 9

*Topic **HIV**

flowType, 7
flowType-package, 2
getLabels, 9

*Topic **classes**

Phenotypes-class, 11

*Topic **classification**

flowType, 7
flowType-package, 2
getLabels, 9

*Topic **clustering**

flowType, 7
flowType-package, 2
getLabels, 9

*Topic **datasets**

DLBCLExample, 6
HIVData, 10
HIVMetaData, 10

*Topic **print**

plot, 12
summary-methods, 12

*Topic **utilities**

calcMemUse, 3
calcNumPops, 4
decodePhenotype, 5
encodePhenotype, 6

calcMemUse, 3, 4

calcNumPops, 3, 4, 4

decodePhenotype, 5, 7

decodePhenotype, character,
character, numeric-method
(decodePhenotype), 5

decodePhenotype, character, character, numeric-method
(decodePhenotype), 5

decodePhenotype-methods
(decodePhenotype), 5

DLBCLExample, 6

encodePhenotype, 5, 6

encodePhenotype, character, character-method
(encodePhenotype), 6

encodePhenotype-methods
(encodePhenotype), 6

flowType, 4, 5, 7, 7, 11, 12

flowType-package, 2

getLabels, 9

HIVData, 10

HIVMetaData, 10

Phenotypes (Phenotypes-class), 11

Phenotypes-class, 11

plot, 12

plot, Phenotypes, character (plot), 12

plot, Phenotypes, character-method
(plot), 12

plot, Phenotypes, flowFrame (plot), 12

plot, Phenotypes, flowFrame-method
(plot), 12

plot, Phenotypes, numeric (plot), 12

plot, Phenotypes, numeric-method (plot),
12

summary, Phenotypes-method
(summary-methods), 12

summary-methods, 12