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)
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R topics documented:

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flowType-package

**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**

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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:


The original paper and description can be found at:


**Examples**

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

#These markers will be analyzed
PropMarkers <- 3:5
MFIMarkers <- PropMarkers
MarkerNames <- c("FS", "SS","CD3","CDS","CD19")
```
calcMemUse

#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** Number 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.
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

\[ \text{calcNumPops(PartitionsPerMarker, MaxMarkersPerPop)} \]

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

\[ \text{calcNumPops(c(2,2,3,2,2,4), 5)} \]
**decodePhenotype**

*Method decodePhenotype in Package flowType*

**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+++ etc

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)
**DLBCEExample**

**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(DLBCEExample)

**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(DLBCEExample)

---

**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.
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, Thresholds=NULL, MemLimit=4,verbose=FALSE)

#If upgrading from flowType 1.x to 2.x, please check documentation as some arguments have changed slightly.

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.

Examples

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

See Also

decodePhenotype, flowType

Author(s)

Kieran O’Neill
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.

MFIs: 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 vigenette for more details and examples.

Author(s)
Nima Aghaeepour, Kieran O’Neill

References
Please cite the following for the current version of flowType:

The original paper and description can be found at:
getLabels

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

Examples
#See the vignette

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)

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 \texttt{flowType}, containing counts

Objects from the Class

Objects can be created by calls of the form \texttt{new("Phenotypes", ...)}.

Slots

\texttt{CellFreqs}: Numeric vector containing counts of the number of cells belonging to each cell type.
\texttt{MFIs}: Matrix of MFIs, with rows for cell types and columns for markers.
\texttt{PhenoCodes}: Vector of character strings representing the codes of each cell type (phenotype).
\texttt{PropMarkers}: Numeric vector specifying which markers were used for combinatorial gating.
\texttt{MFIMarkers}: Numeric vector specifying for which markers MFIs were computed for each cell type.
\texttt{MarkerNames}: A character vector of the names of all markers in the flowFrame given
\texttt{Partitions}: The first level partitions that each cell in the flowFrame belong to in each channel.
\texttt{MaxPopSize}: \texttt{MaxMarkersPerPop}
\texttt{PartitionsPerMarker}: Vector of number of partitions used for each marker
\texttt{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

\texttt{flowType}

Examples

showClass("Phenotypes")
Methods for Function plot

## 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 vignette

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Methods for Function summary

### Methods

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

### See Also

flowType
Examples

#See the vignette
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