

Package ‘LowMACA’

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Type Package

Title LowMACA - Low frequency Mutation Analysis via Consensus Alignment

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Description The LowMACA package is a simple suite of tools to investigate and analyze the mutation profile of several proteins or pfam domains via consensus alignment. You can conduct an hypothesis driven exploratory analysis using our package simply providing a set of genes or pfam domains of your interest.

License GPL-3

Depends R (>= 2.10), Biostrings, motifStack

Imports cgdsr, parallel, stringr, reshape2, data.table, RColorBrewer, methods, LowMACAAnnotation

Suggests knitr, rmarkdown

VignetteBuilder knitr

biocViews SomaticMutation, SequenceMatching, WholeGenome, Sequencing, Alignment, DataImport, MultipleSequenceAlignment

SystemRequirements clustalo, gs, perl

NeedsCompilation no

R topics documented:

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LowMACA-package	<i>LowMACA : Low frequency Mutations Analysis via Consensus Alignment</i>
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Description

The LowMACA package is a simple suite of tools to investigate and analyze the mutation profile of several proteins or pfam domains via consensus alignment. You can conduct an hypothesis driven exploratory analysis using our package simply providing a set of genes or pfam domains of your interest.

Details

Package:	LowMACA
Type:	Package
Version:	0.99.3
Date:	2015-02-20
License:	GPL-3
Depends:	methods

LowMACA allows to collect, align and visualize mutations from different proteins or pfam domains.

1. *newLowMACA*: construct a LowMACA object with your proteins or pfam
2. *setup*: align sequences, get mutations and map mutations on the consensus sequence
3. *entropy*: calculate entropy score and pvalues for every position
4. *lmPlot*: visualize mutations on the consensus sequence, conservation and significant clusters

Author(s)

Stefano de Pretis , Giorgio Melloni

Maintainer: <ste.depo@gmail.com> <melloni.giorgio@gmail.com>

References

doi:10.1186/gm563 923 Melloni et al.: DOTS-Finder: a comprehensive tool for assessing driver genes in cancer genomes. Genome Medicine 2014 6:44

See Also

[LowMACA website](#)

Examples

```
#Create an object of class LowMACA for RAS domain family
lm <- newLowMACA(pfam="PF00071" , genes=c("KRAS" , "NRAS" , "HRAS"))
#Select melanoma, breast cancer and colorectal cancer
lmParams(lm)$tumor_type <- c("skcm" , "brca" , "coadread")
#Align sequences, get mutation data and map them on consensus
lm <- setup(lm)
#Calculate statistics
lm <- entropy(lm)
#Retrieve original mutations
lfm(lm)
#Plot
bpAll(lm)
lmPlot(lm)
protter(lm)
```

alignSequences

Align sequences via clustalo

Description

Align sequences for an object of class LowMACA

Usage

```
alignSequences(object, clustalo_filename=NULL , mail=NULL , perlCommand="perl")
```

Arguments

object an object of class LowMACA containing at least 2 sequences.
clustalo_filename a character string that contains the file name where clustal omega alignment file will be stored. In case it's NULL no file will be written. Default=NULL

mail	a character string indicating the email address where error report should be sent in web mode
perlCommand	a character string containing the path to Perl executable. if missing, "perl" will be used as default

Details

This method launches a system call to clustalo aligner and optionally creates a fasta file in clustal format. A warning is returned if at least one sequence has a pairwise similarity below 20% to any other sequence. If only one sequence is passed to alignSequences, the alignment will be skipped, but no warning will be raised. If mail is not NULL, a local installation of clustal omega is no longer required and the alignment is performed using clustal omega EBI web service. A limit of 2000 sequences is set in this case and perl must be installed in the system

Value

The method returns an object of class LowMACA updating the slot alignment. See [lmAlignment](#)

Warning

When a sequence has a similarity below 20%, a warning is raised. In order to produce strong results in terms of conservation of multiple mutations, consider to remove that sequence from the analysis. The alignment will obviously change.

Author(s)

Stefano de Pretis, Giorgio Melloni

References

[Trident Score Clustal Omega Clustal Omega Web Service](#)

See Also

[getMutations](#), [mapMutations](#), [setup](#)

Examples

```
#Create an object of class LowMACA for RAS domain family
lm <- newLowMACA(pfam="PF00071" , genes=c("KRAS" , "NRAS" , "HRAS"))
#Align sequences using local installation of clustalo
lm <- alignSequences(lm)
#Web service clustalomega aligner
lm <- alignSequences(lm , mail="lowmaca@gmail.com")
```

bpAll	<i>Draw a mutation barplot</i>
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Description

bpAll draws a stacked barplot of the mutations mapped on the consensus sequence

Usage

```
bpAll(object)
```

Arguments

object an object of class LowMACA

Details

Returns a barplot in which mutations are stacked per position on the consensus sequence. Every color represent mutations taht map on the same input sequence (either a protein or a pfam) The Low-MACA object must pass through the methods `alignSequences` , `getMutations` , `mapMutations`

Value

NULL

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[lmPlot](#)

Examples

```
#Load homeobox example and draw plot
data(lmObj)
lmObj <- entropy(lmObj)
bpAll(lmObj)
```

 entropy

Calculate LowMACA statistics

Description

entropy is a method for objects of class LowMACA. It calculates global entropy score of the mutation profile of the alignment and a test for every position in the consensus comparing the number of observed mutations against a weighed random uniform distribution.

Usage

```
entropy(object, bw = NULL)
```

Arguments

object	an object of class LowMACA
bw	a character string or a numeric positive value representing the desired bandwidth to launch the function density for the uniform distribution. 0 will not launch density (every position is not aggregated to the surrounded ones) , 'auto' will let the simulation decide according to the Silverman's rule of thumb and every other number is a user defined bandwidth passed to the function density.

Details

The parameter bw overwrites the bandwidth set with lmParams. Therefore, if bw is set to NULL, the method entropy uses the predefined bandwidth of the LowMACA object.

Value

entropy returns an object of class LowMACA updating the slot entropy and the slot alignment in the df data.frame by adding 6 new columns

alignment slot update:

- mean a numeric vector representing the mean value of the empirical uniform function at every position in the consensus
- lTsh a numeric vector representing the limit inferior of the 95% confidence interval of the empirical uniform function at every position in the consensus
- uTsh a numeric vector representing the limit superior of the 95% confidence interval of the empirical uniform function at every position in the consensus
- profile a numeric vector representing the density of mutations at every position in the sample normalized by the number of position. In case of bandwidth 0, this vector is equal to the number of mutations divided by the total number of mutations
- pvalue a numeric vector representing the pvalue of the number of mutations found at every position against the weighed random uniform distribution of mutations
- qvalue a numeric vector representing the corrected pvalues using FDR method. Only positions with a conservation score $\geq 10\%$ are considered

Author(s)

Stefano de Pretis , Giorgio Melloni

References

doi:10.1186/gm563 923 Melloni et al.: *DOTS-Finder: a comprehensive tool for assessing driver genes in cancer genomes*. *Genome Medicine* 2014 6:44

Silverman, B. W. (1986) *Density Estimation*. London: Chapman and Hall.

See Also

[alignSequences](#) [lmParams](#) [lmEntropy](#)

Examples

```
#Load homeobox example and run entropy
data(lmObj)
lmObj <- entropy(lmObj)
lmEntropy(lmObj)
```

getMutations

Retrieve mutation data for a LowMACA object

Description

Exploiting the capabilities of the `cgdsr` package, this method downloads and parse the mutation data of the specified genes in the selected tumor types. It also aggregates and show the frequencies of mutations of every gene in the different tumor types.

Usage

```
getMutations(object, repos = NULL)
```

Arguments

object	a LowMACA class object
repos	a data.frame containing mutations for the specified genes in the LowMACA object in case of custom mutation data. Default NULL

Details

With `repos=NULL`, the method is a wrapper around `getMutationData` method from package `cgdsr-package`. The output of the method is moduled by the parameters in `lmParams("LowMACA_object")`. See [lmParams](#) for further information.

Value

An object of class LowMACA is returned with an update in the slot mutations. See [lmMutations](#) method.

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[lmParams](#) [getMutationData](#) [lmMutations](#)

Examples

```
#Create an object of class LowMACA
lm <- newLowMACA(pfam="PF12906")
#Change some paramters
#By default, LowMACA retrieve only missense mutations.
#We want all mutations
lmParams(lm)[['mutation_type']] <- 'all'
#By default, LowMACA takes mutations from all the kinds of tumor
#We want just prostate cancer samples
lmParams(lm)[['tumor_type']] <- 'prad'
lm <- getMutations(lm)
```

lfm

Show significant clusters of mutations

Description

The method lfm (low frequency mutations) retrieve the original mutations that created the significant clusters calculated with entropy on the consensus

Usage

```
lfm(object , metric='qvalue', threshold=.05, conservation=0.1)
```

Arguments

object	a LowMACA class object
metric	a character that defines whether to use 'pvalue' or 'qvalue' to select significant positions. Default: 'qvalue'
threshold	a numeric defining the threshold of significance for the defined metric. Default: 0.05
conservation	a numeric value in the range of 0-1 that defines the threshold of trident conservation score to include the specified position. Default: 0.1

Details

After the alignment, we lose every information about the original sequences used as input. The consensus sequence is in fact an alignment that could not represent the reality of human proteins. lfm allows to go back on the original dataset and retrieve the proteins and the real positions of the mutations that we consider 'conserved'.

Value

A data.frame with 11 columns and n rows corresponding to the mutations retrieved:

1. Gene_Symbol gene symbols of the mutations
2. Amino_Acid_Position amino acidic positions relative to original protein
3. Amino_Acid_Change amino acid changes in hgvs format
4. Sample Sample barcode where the mutation was found
5. Tumor_Type Tumor type of the Sample
6. Envelope_Start start of the pfam domain in the protein
7. Envelope_End end of the pfam domain in the protein
8. Multiple_Aln_pos positions in the consensus
9. Entrez entrez ids of the mutations
10. Entry Uniprot entry of the protein
11. UNIPROT other protein names for Uniprot
12. Chromosome cytobands of the genes
13. Protein.name extended protein names

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[entropy](#)

Examples

```
#Load homeobox example and draw the plot
data(lmObj)
significant_muts <- lfm(lmObj)
#Display original mutations that formed significant clusters (column Multiple_Aln_pos)
head(significant_muts)
#Position 4 has a qvalue<0.05
#What are the genes mutated in position 4 in the consensus?
cluster_4_genes <- significant_muts[ significant_muts[['Multiple_Aln_pos']]==4 , 'Gene_Symbol']
#Display the genes and their number of mutation in consensus position 4
sort(table(cluster_4_genes))
```

`lmAlignment`*Show Alignment Results from a LowMACA object*

Description

Method for objects of class LowMACA. It can show the results of the alignment procedure that has been performed on the LowMACA object

Usage

```
lmAlignment(object)
```

Arguments

`object` object of class LowMaca

Value

A list containing the following elements:

- ALIGNMENT an object of class data.frame containing the mapping of the position of the original amino acids to the consensus sequence
- SCORE a list of two objects
 - DIST_MAT a matrix of the pairwise similarities between sequences as resulted after the multiple alignment (from 0% to 100%)
 - SUMMARY_SCORE a data.frame containing summary descriptives of the distance matrix
 - CLUSTAL an object of class "AAMultipleAlignment" as provided by Biostrings R package
 - df a dataframe containing the predicted consensus sequence and the trident conservation score at every position

Author(s)

Stefano de Pretis, Giorgio Melloni

See Also

[alignSequences](#)

Examples

```
data('lmObj')
str(lmAlignment(lmObj))
```

`lmEntropy`*Show Entropy Information Contained in a LowMACA object*

Description

Method for objects of class LowMACA. It can show the results of entropy analysis performed on the LowMACA object by the function [entropy](#)

Usage

```
lmEntropy(object)
```

Arguments

`object` object of class LowMaca

Value

A list containing the following elements:

- `bw` a numeric value that represents the bandwidth used to calculate the Shannon entropy score
- `uniform` an object of class function that was used to calculate the score
- `absval` a numeric value representing the Shannon entropy of the sample data
- `log10pval` a numeric value representing the pvalue of the Shannon entropy score against a gamma distribution with same mean and variance as the empirical uniform distribution in $-\log_{10}$ scale
- `pvalue` a numeric value representing the pvalue of the Shannon entropy score against a gamma distribution with same mean and variance as the empirical uniform distribution

Author(s)

Stefano de Pretis, Giorgio Melloni

See Also

[entropy](#)

Examples

```
data('lmObj')
lmObj <- entropy(lmObj)
lmEntropy(lmObj)
```

`lmMutations`*Show Mutation Data Contained in a LowMACA object*

Description

Method for objects of class LowMACA. It can show the mutation data contained within the LowMACA object that has been retrieved from [getMutations](#) method.

Usage

```
lmMutations(object)
```

Arguments

`object` object of class LowMaca

Value

A list containing the following elements:

- `data` a data.frame describing the mutations on every genes and their effect the amino acids they belong to
- `freq` a data.frame containing the absolute number of mutated patients by gene and selected tumor types (this is useful to explore the mutational landscape of your genes in the different tumor types)
- `aligned` a matrix where rows represent proteins/pfam, and columns report the number of mutations on every position of the consensus

Author(s)

Stefano de Pretis, Giorgio Melloni

See Also

[getMutations](#)

Examples

```
data('lmObj')  
str(lmMutations(lmObj))
```

lmObj

Example of a LowMACA object

Description

An object of class LowMACA of the alignment and mapping of the homeobox domain. It is the example used in the vignette.

Usage

```
data("lmObj")
```

Format

An object of class LowMACA

Source

Created by LowMACA package

Examples

```
#Load lmObj and show its structure  
data(lmObj)  
str(lmObj)
```

lmParams

Show and set parameters

Description

Method for objects of class LowMACA. It can show the most important user-definable parameters for a LowMACA analysis and allows to change them.

Usage

```
lmParams(object)  
lmParams(object) <- value
```

Arguments

object	an object of class LowMaca
value	a named list containing: <ol style="list-style-type: none"> 1. <code>mutation_type</code> a character string among: 'missense', 'truncating', 'silent', 'all'. Default 'missense' 2. <code>tumor_type</code> a character vector or string containing the tumor type barcode of the data in cBioPortal. Default 'all'. 3. <code>min_mutation_number</code> an integer value describing the minimum number of mutations accepted for a sequence. If a sequence does not harbor a sufficient number of mutations is discarded from the analysis. Default is 1 4. <code>density_bw</code> either a numeric value or 'auto'. A numeric value is passed directly to the function <code>density</code> while putting 0 will not launch density at all (every position is not aggregated to the surrounded ones). 'auto' will let the simulation decide according to the Silverman's rule of thumb the correct bandwidth. Default is 0. 5. <code>clustal_cmd</code> path to clustalo executable

Details

LowMACA is a suite of tool that analyze conserved mutations, so it looks for clusters of gain of function alterations. With 'missense' `mutation_type` we intend all those mutations that change the original DNA but do not create stop codon nor alter the reading frame (these mutations are collectively defined as 'truncating' mutations). In addition we let the possibility to also choose 'silent' mutations even though they are currently not supported by the cBioPortal. To see all the available tumor types to run a LowMACA analysis, simply run `showTumorType`. The parameter `density_bw` has a strong effect on the statistical analysis of LowMACA. With the default bandwidth (0), the Shannon entropy calculation becomes discrete, while the continuous version is used in all the other cases.

Value

If `ImParams` is used as a `show` method it returns a named list of 5 elements: `mutation_type='missense'`, `tumor_type='all'`, `min_mutation_number=1`, `density_bw=0`, `clustal_cmd='clustalo'`

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[showTumorType](#) [getMutations](#) [entropy](#) [density](#)

Examples

```
#Construct a LowMACA object
lm <- newLowMACA(pfam="PF12906")
#Show default parameters
lmParams(lm)
```

```
#Change all parameters
lmParams(lm) <- list(mutation_type='all'
                    , tumor_type=c('skcm','brca')
                    , min_mutation_number=0
                    , density_bw=0
                    , clustal_cmd='clustalo')
#Change just one parameter
lmParams(lm)[['tumor_type']] <- 'prad'
```

lmPlot

Draw a comprehensive LowMACA plot

Description

LowMACA comprehensive plot is a four layers plot the summarize the entire LowMACA output

Usage

```
lmPlot(object)
```

Arguments

object a LowMACA class object

Details

The method returns a plot, which is divided into four layers. The LowMACA object must have been passed through the methods `alignSequences`, `getMutations`, `mapMutations` and `entropy`. The four layers of the plot are:

1. The bar plot visualized by `bpAll`
2. The distribution of mutations against the 95% confidence interval superior limit of the null hypothesis (dotted line) with orange bars representing a position with a pvalue <0.05 and a red star for qvalue<0.05
3. The Trident score distribution
4. The logo plot representing the consensus sequence

If this plot is used on a LowMACA object with a single protein, the result is formed by three layers only:

1. The bar plot visualized by `bpAll`
2. The Pfam domains structure inside the protein
3. The distribution of mutations against the 95% confidence interval superior limit of the null hypothesis (dotted line) with orange bars representing a position with a pvalue <0.05 and a red star for qvalue<0.05

Value

NULL

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also[alignSequences](#) [getMutations](#) [mapMutations](#) [entropy](#) [bpAll](#)**Examples**

```
#Load homeobox example and draw the plot
data(lmObj)
#Calculate statistics for nullProfile
lmObj <- entropy(lmObj)
lmPlot(lmObj)
```

LowMACA-class

Class "LowMACA"

Description

LowMACA class object describing the properties of mutations mapped on pfam domains or proteins

Objects from the Class

Objects can be created by calls of the form `newLowMACA(genes, pfam)`.

Constructor

```
newLowMACA(genes=character_vector , pfam=character_vector)
```

Slots

arguments Object of class "list" with 6 elements:

- `genes` : vector of selected genes for the analysis in Hugo names format. NULL if `mode="pfam"`.
- `pfam` : vector of selected domains for the analysis in pfam ids format. NULL if `mode="genes"`.
- `input` : data.frame describing the input data as gene symbols, pfam ids, entrez ids, envelope start and end of the domain relative to the protein, name of the canonical protein in uniprot format, amino acidic sequence.
- `mode` : character. automatically set by the constructor as either "pfam" or "genes". If `pfam=NULL` then `mode="genes"`, "pfam" otherwise.
- `params` : named list of starting parameters for the LowMaca analysis. Call `lmParams(object)` to show default. See [lmParams](#) for further details.

- `parallelize` : named list of logicals. `getMutations=FALSE` is the default for the `getMutations` method and `makeAlignment=TRUE` is the default for the `alignSequences` method. See `parallelize` for further details.

alignment Object of class "list" with 4 elements:

- `ALIGNMENT` : data.frame of the result of the alignment. Every row represents a position of a sequence and the relative mapping to the consensus sequence.
- `SCORE` : list of two elements. `DIST_MAT` is a matrix of pairwise similarities between sequences as described by clustalo. `SUMMARY_SCORE` is a dataframe of summary descriptive statistics of the `DIST_MAT` matrix
- `CLUSTAL` : an object of class `AAMultipleAlignment` from package Biostrings
- `df` : a data.frame describing the consensus sequence, its per-position degree of conservation and its mutations null profile density. See `entropy` and `lmPlot` for further details

mutations Object of class "list" with 3 elements:

- `data` : data.frame derived from the query to the cBioPortal query, `getMutationData`. Every row represents a mutation stratified by position, gene and tumor type.
- `freq` : data.frame of absolute frequency of mutation stratified by gene and tumor type.
- `aligned` : matrix representing the number of mutations at every position in the consensus sequence (columns) and in each original sequence (rows)

entropy Object of class "list" with 5 elements:

- `bw` : numeric value. user defined bandwidth for the function `entropy`
- `uniform` : function that generate the uniform null profile
- `absval` : numeric value. Shannon entropy of the mutation data profile according to the defined bandwidth
- `log10pval` : numeric value. pvalue of the entropy test in -log10 scale
- `pvalue` : numeric value. pvalue of the entropy test

Methods

alignSequences `alignSequences(object = "LowMACA"): ...`

bpAll `bpAll(object = "LowMACA"): ...`

entropy `entropy(object = "LowMACA"): ...`

getMutations `getMutations(object = "LowMACA"): ...`

lfm `lfm(object = "LowMACA"): ...`

lmPlot `lmPlot(object = "LowMACA"): ...`

mapMutations `mapMutations(object = "LowMACA"): ...`

nullProfile `signature(object = "LowMACA"): ...`

parallelize `parallelize(object = "LowMACA"): ...`

parallelize<- `signature(object = "LowMACA"): ...`

lmParams `params(x = "LowMACA"): ...`

lmParams<- `signature(object = "LowMACA"): ...`

protter `protter(object = "LowMACA"): ...`

setup `setup(object = "LowMACA"): ...`

show `show(object = "LowMACA"): ...`

Author(s)

Stefano de Pretis, Giorgio Melloni

References

[LowMACA website](#)

See Also

[newLowMACA](#)

Examples

```
#ANALYSIS OF SOME OF THE PROTEINS THAT SHARE THE HOMEBOX DOMAIN
#Genes to analyze
Genes <- c("ADNP", "ALX1", "ALX4", "ARGFX", "CDX4", "CRX"
           , "CUX1", "CUX2", "DBX2", "DLX5", "DMBX1", "DRGX"
           , "DUXA", "ESX1", "EVX2", "HDX", "HLX", "HNF1A"
           , "HOXA1", "HOXA2", "HOXA3", "HOXA5", "HOXB1", "HOXB3"
           , "HOXD3", "ISL1", "ISX", "LHX8")
#Pfam to analyze
Pfam <- "PF00046"
#Construct a new LowMACA object
lm <- newLowMACA(genes=Genes , pfam=Pfam)
#Change some parameters
lmParams(lm)[['tumor_type']] <- c("skcm" , "stad" , "ucec" , "luad" , "lusc" , "coadread" , "brca")
lmParams(lm)[['min_mutation_number']] <- 1
lmParams(lm)[['density_bw']] <- 0
#Run if you have clustalo installed
lm <- setup(lm)
#Calculate staistics
lm <- entropy(lm)
#Retrieve original mutations
lfm(lm)
#Plot
bpAll(lm)
lmPlot(lm)
protter(lm)
```

mapMutations

Map mutations on consensus sequence

Description

mapMutations is a method for the class LowMACA that re-maps the mutations on a sequence to the relative position in a consensus sequence.

Usage

```
mapMutations(object)
```

Arguments

object an object of class LowMACA

Details

Every position in the consensus alignment correspond to different positions in the single aligned sequences. The mutations are mapped according to this scheme that can be evinced from the slot alignment. mapMutations must be called after alignSequences and getMutations

Value

An object of class LowMACA with an update in the slot mutations. mapMutations add a object named aligned of class matrix in this slot that represents the absolute number of mutations in each sequence/position in the consensus as a matrix.

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[getMutations](#) [alignSequences](#) [LowMACA-class](#)

Examples

```
#Create an object of class LowMACA
lm <- newLowMACA(pfam="PF12906")
#Align the sequences, requires clustalo
## Not run: lm <- alignSequences(lm)
#Get mutations from the corresponding genes
## Not run: lm <- getMutations(lm)
#Map mutations on the consensus sequence
## Not run: lm <- mapMutations(lm)
```

newLowMACA

Construct a LowMACA object

Description

Constructor for the class LowMACA. It initializes a LowMACA object with default parameters

Usage

```
newLowMACA(genes = NULL, pfam = NULL)
```

Arguments

genes	a character vector of gene symbols in HGNC format or a integer vector of Entrez IDs. If pfam is defined, it can be set to NULL
pfam	a character vector of pfam IDs. If genes is defined, it can be set to NULL

Details

When a LowMACA object is initialized, the arguments slot is filled with the input data and default parameters and path to clustalomega aligner. See `lmParams` and `parallelize` to change them.

Value

An object of class "LowMACA". See [LowMACA-class](#)

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[lmParams](#) [parallelize](#)

Examples

```
#Set Genes and pfam for the analysis
Genes <- c("ADNP", "ALX1", "ALX4", "ARGFX", "CDX4", "CRX"
          , "CUX1", "CUX2", "DBX2", "DLX5", "DMBX1", "DRGX"
          , "DUXA", "ESX1", "EVX2", "HDX", "HLX", "HNF1A"
          , "HOXA1", "HOXA2", "HOXA3", "HOXA5", "HOXB1", "HOXB3"
          , "HOXD3", "ISL1", "ISX", "LHX8")
Pfam <- "PF00046"
#LowMACA object of pfam PF00046 filtered by Genes
lm <- newLowMACA(genes=Genes , pfam=Pfam)
#LowMACA object of the entire pfam PF00046
lm <- newLowMACA(pfam=Pfam)
#LowMACA object of entire canonical proteins associated to Genes
lm <- newLowMACA(genes=Genes)
```

nullProfile

Draw a mutational profile plot

Description

nullProfile is a method for objects of class LowMACA that draw a barplot highlighting the significant clusters of mutations found by LowMACA

Usage

```
nullProfile(object)
```

Arguments

object an object of class LowMACA

Details

This method draw the second layer of the `lmPlot` of LowMACA. The blue dotted line is a curve that pass through all the points of the upper limit of the 95% confidence interval for the single position test performed by entropy (one point per position in the consensus). The black bars represents the density of mutations in our sample. If a bar passes the blue line, it will be depicted in orange (significant pvalue). After the correction for multiple testing, red stars appears at the top of the orange bars if a cluster is below 0.05 for the qvalue and has a conservation trident score of at least 0.1.

Value

NULL

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[lmPlot entropy](#)

Examples

```
#Load homeobox example
data(lmObj)
#Calculate statistics
lmObj <- entropy(lmObj)
nullProfile(lmObj)
```

parallelize

Show and set parallelization options

Description

Method for objects of class LowMACA. It can show parallelization parameters of an object of class LowMACA and switch off and on parallelization of [alignSequences](#) and [getMutations](#) method

Usage

```
parallelize(object)
parallelize(object) <- value
```

Arguments

object	object of class LowMaca
value	a named list containing logical values. Default list(getMutations=FALSE , makeAlignment=TRUE)

Details

With getMutations=TRUE, the `getMutations` method runs in parallel during the queries to the different tumor_types. This can result in an overload to the cBioPortal database and the function returns error. With makeAlignment=TRUE, clustalo should run in parallel. Nevertheless, clustalo can be parallelized only if the OpenMP C library is correctly functioning.

Value

If parallelize is used as a show method it returns a named list of two elements: getMutations and makeAlignment

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[getMutations](#)

Examples

```
#Construct a LowMACA object
lm <- newLowMACA(pfam="PF12906")
#Show parallelize default
parallelize(lm)
#Change all parameters
parallelize(lm) <- list(getMutations=TRUE , makeAlignment=FALSE)
#Change just one parameter
parallelize(lm)[['getMutations']] <- TRUE
```

protter

Draw a Protter plot

Description

This is a wrapper around Protter web service for LowMACA class objects that draw a protter style plot.

Usage

```
protter(object, filename = "protter.png", threshold = 0.05)
```

Arguments

object	an object of class LowMACA
filename	a character string that identifies the file name where protter plot will be stored. Default "protter.png"
threshold	a numeric value in the interval (0 , 1] that identifies the significant mutations. Default 0.05

Details

Using the information in the slot alignment, a request is send to Protter server. Protter will predict a possible sencondary structure for the consensus sequence (if possible) and highlights the significant clusters of mutations found by LowMACA (if any). A significant pvalue is colored in orange, a significant qvalue is colored in red.

Value

NULL

Author(s)

Stefano de Pretis , Giorgio Melloni

References

[Protter website](#)

See Also

[LowMACA-class entropy](#)

Examples

```
#Load homeobox example
data(lmObj)
#Calculate statistics
lmObj <- entropy(lmObj)
#Create protter.png
protter(lmObj)
```

setup

Setup of a LowMACA object

Description

A wrapper around `alignSequences`, `getMutations` and `mapMutations` in order to execute all these methods at once.

Usage

```
setup(object, repos = NULL, clustalo_filename=NULL  
, mail=NULL , perlCommand="perl")
```

Arguments

<code>object</code>	an object of class <code>LowMACA</code>
<code>repos</code>	a <code>data.frame</code> containing mutations for the specified genes in the <code>LowMACA</code> object in case of custom mutation data. Default <code>NULL</code>
<code>clustalo_filename</code>	a character string that contains the file name where clustal omega alignment file will be stored. In case it's <code>NULL</code> no file will be written. Default= <code>NULL</code>
<code>mail</code>	a character string indicating the email address where error report should be sent in web mode
<code>perlCommand</code>	a character string containing the path to Perl executable. if missing, "perl" will be used as default

Details

If `mail` is not `NULL`, a local installation of clustal omega is no longer required and the alignment is performed using clustal omega EBI web service. A limit of 2000 sequences is set in this case and perl is required with `XML::Simple` and `LWP` modules installed

Value

An object of class `LowMACA` with all the updates provided by `alignSequences`, `getMutations` and `mapMutations` methods.

Author(s)

Stefano de Pretis , Giorgio Melloni

References

[Trident Score Clustal Omega Clustal Omega Web Service](#)

See Also

[alignSequences](#) [getMutations](#) [mapMutations](#)

Examples

```
#Create an object of class LowMACA for RAS domain family
lm <- newLowMACA(pfam="PF00071" , genes=c("KRAS" , "NRAS" , "HRAS"))
#Select a few tumor types
lmParams(lm)$tumor_type <- c("skcm" , "brca" , "coadread")
#Align sequences, get mutation data and map them on consensus
lm <- setup(lm)
#Same as above, but using web service
lm <- setup(lm , mail="lowmaca@gmail.com")
```

showTumorType	<i>List of tumor types</i>
---------------	----------------------------

Description

Show all the possible tumor types accepted by LowMACA

Usage

```
showTumorType()
```

Details

This method is a wrapper around [getCancerStudies](#) and show all the barcodes for the tumor types as used by cBioPortal.

Value

A named vector of all the tumor types available in cgdsr package that can be passed to the method `lmParams`. Every element is the aggregation of all the available sequenced data from all the studies involved in a particular tumor type.

Author(s)

Stefano de Pretis , Giorgio Melloni

See Also

[lmParams](#) [getCancerStudies](#)

Examples

```
data('lmObj')
out <- showTumorType()
chosenTumors <- out[1:3]
lmParams(lmObj)$tumor_type <- chosenTumors
```

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