Package ‘NestLink’

May 2, 2024

Type Package

Title NestLink an R data package to guide through Engineered Peptide Barcodes for In-Depth Analyzes of Binding Protein Ensembles

Version 1.20.0

Depends R (>= 3.6), AnnotationHub (>= 2.15), ExperimentHub (>= 1.0), Biostrings (>= 2.51), gplots (>= 3.0), protViz (>= 0.4), ShortRead (>= 1.41)

Imports grDevices, graphics, stats, utils

Description Provides next-generation sequencing (NGS) and mass spectrometry (MS) sample data, code snippets and replication material used for developing NestLink. The NestLink approach is a protein binder selection and identification technology able to biophysically characterize thousands of library members at once without handling individual clones at any stage of the process. Data were acquired on NGS and MS platforms at the Functional Genomics Center Zurich.

License GPL

VignetteBuilder knitr

Suggests BiocStyle (>= 2.2), DT, ggplot2, knitr, rmarkdown, testthat, specL, lattice, scales

NeedsCompilation no

biocViews ExperimentHub, ExperimentData, SequencingData, MassSpectrometryData, ReproducibleResearch

RoxygenNote 6.1.1

git_url https://git.bioconductor.org/packages/NestLink

git_branch RELEASE_3_19

git_last_commit 2f8800a

git_last_commit_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-02
.ssrc.mascot computes the correlation of predicted and measured retention time

Description

this helper function computes a linear model between predicted and measured retention time of the input set given identified peptides.

TODO(cp): consider moving this method to the protViz package.

Usage

```r
.ssrc.mascot(x, scores = c(10, 20, 40, 50), ...)
```
compose_GPGx8cTerm

**Arguments**

- `x` as.data.frame.mascot generated data.frame object.
- `scores` default is `c(10, 20, 40, 50)`.
- `...` passed to the plot function.

**Value**

a plot and summary

**Author(s)**

Christian Panse <cp@fgcz.ethz.ch>, 2017,2019

**Examples**

```r
library(ExperimentHub)
eh <- ExperimentHub()
load(query(eh, c("NestLink", "F255744.RData"))[[1]])
:ssrc.mascot(F255744, scores = 15)
```

---

**compose_GPGx8cTerm**  
Compose a peptide with a defined AA sequence frequency

**Description**

composes, out of an as input given amino acid distribution, a randomly sampled amino acid sequence. `compose_GPGx8cTerm`, `compose_GSx7cTerm`, and `compose_GPx10R` belong to three groups composing different flycode (peptide) construction. The construction is given in the function name. For example, GPGx8cTerm, composes a flycode having as prefix GPG followed by eight (x8) amino acids followed by a cTerm sequence. The different construction will have different detectability properties as mass range and hydrophobicity values.

**Usage**

```r
```

**Arguments**

- `pool` AA distributen.
- `cTerm` c-Terms

**Value**

a AA sequence
Author(s)
Christian Panse <cp@fgcz.ethz.ch> 2015

Examples
```r
set.seed(1)
compose_GPGx8cTerm()
(FlyCodes <- replicate(10, compose_GPGx8cTerm()))
plot(parentIonMass(FlyCodes) ~ ssr(FlyCodes))
```

compose_GPx10R  Compose a peptide with a defined AA sequence

Description
composes, out of an as input given amino acid distribution, a randomly sampled amino acid sequence. `compose_GPGx8cTerm`, `compose_GSx7cTerm`, and `compose_GPx10R` belong to three groups composing different flycode (peptide) construction. The construction is given in the function name. For example, GPGx8cTerm, composes a flycode having as prefix GPG followed by eight (x8) amino acids followed by a cTerm sequence. The different construction will have different detectability properties as mass range and hydrophobicity values.

Usage
```r
compose_GPx10R(aa_pool1, aa_pool2)
```

Arguments
- `aa_pool1` AA distributen.
- `aa_pool2` AA distributen.

Value
a AA sequence

Author(s)
Christian Panse <cp@fgcz.ethz.ch> 2015

Examples
```r
set.seed(1)
```

```r
```
compose_GSx7cTerm

Compose a FlyCode GSx7cTerm Amino Acid Sequence

Description

composes, out of an as input given amino acid distribution, a randomly sampled amino acid sequence. compose_GPGx8cTerm, compose_GSx7cTerm, and compose_GPx10R belong to three groups composing different flycode (peptide) construction. The construction is given in the function name. For example, GPGx8cTerm, composes a flycode having as prefix GPG followed by eight (x8) amino acids followed by a cTerm sequence. The different construction will have different detectability properties as mass range and hydrophobicity values.

Usage


Arguments

pool a vector of amino acids.
cTerm a vector of a sequence suffix.

Value

a amino acid sequence, e.g., GSAPTTVFGWLTVR.

Author(s)

Christian Panse <cp@fgcz.ethz.ch> 2015

Examples

```r
sample.size <- 100
# # Compose a GSXXXXXXXX(WR|WLTVR|WQGGER|WQSR|WLR) peptide
set.seed(2)
FC.GSx7cTerm <- replicate(sample.size, compose_GSx7cTerm())
# # Some Sanity Checks
table(FC.GSx7cTerm)
stopifnot(length(FC.GSx7cTerm) == 100)
FC.PATTERN <- "^GS[ASTNQDEFVLYWGP]{7}(WR|WLTVR|WQEGGR|WQSR|WLR)$"
```
getExperimentHubFilename

```r
stopifnot(
  length(FC.GSx7cTerm[grepl(FC.PATTERN, FC.GSx7cTerm)])
  == sample.size)
```

---

F255744  

**F255744 Mascot Search results**

**Description**

F255744 Mascot Search results

**Author(s)**

Pascal Egloff <p.egloff@imm.uzh.ch>

**See Also**

F255744

**Examples**

```r
library(ExperimentHub)
eh <- ExperimentHub(); load(query(eh, c("NestLink", "F255744.RData"))[[1]])
class(F255744)
hist(F255744$RTINSECONDS)
hist(F255744$RTINSECONDS[F255744$pep_score > 20])
```

---

getExperimentHubFilename

```r
getExperimentHubFilename
```

**Description**

getExperimentHubFilename

**Usage**

getExperimentHubFilename(filename)

**Arguments**

filename of the aws s3 blob.

**Value**

the file name of the local ExperimentHub.
getFC

Examples

```r
fl <- system.file("extdata", "metadata.csv", package="NestLink")
metadata <- read.csv(fl, stringsAsFactors=FALSE)
metadata>Title
lapply(metadata$RDataPath, getExperimentHubFilename)
```

getFC  Read FlyCodes (FCs)

Description

A wrapper function for reading the flycodes using ExperimentHub. The files are used for demonstrating the detectability of the AA sequences. The wrapper functions are extended by columns `ssrc prediction` and the `parentIonMass`. The column ESP_Prediction was generated by using the service from [https://genepattern.broadinstitute.org](https://genepattern.broadinstitute.org).

Usage

```r
getFC(pattern = "^GS\[ASTNQDEFVLYWGP\]{7}(WR|WLTVR|WQEGGR|WLR|WQSR)$", 
      filename = NULL)
```

Arguments

- **pattern**: a regular expression FlyCode pattern
- **filename**: a two column tab separated file containing a peptide sequence and an ESP value. default is NULL which reads the data provided by the package through ExperimentHub.

Value

a `data.frame` object of Flycodes

Author(s)

Christian Panse <cp@fgcz.ethz.ch> 2015, 2018

Source

- [https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-4_S4.extendedFrags_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-4_S4.extendedFrags_uniqNB2FC.txt)
- [https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-5_S5.extendedFrags_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-5_S5.extendedFrags_uniqNB2FC.txt)

Examples

```r
FC <- getFC()
dim(FC)
```
**Description**

A wrapper function for reading the flycodes using ExperimentHub. The files are used for demonstrating the detectability of the AA sequences. The wrapper functions are extended by columns `ssrc` prediction and the `parentIonMass`. The column ESP_Prediction was generated by using the service from https://genepattern.broadinstitute.org.

**Usage**

```r
getNB(filename = NULL)
```

**Arguments**

- `filename` a two column tab separated file containing a peptide sequence and an ESP value. default is NULL which reads the data provided by the package through ExperimentHub.

**Value**

a `data.frame` object of NBs

**Author(s)**

Christian Panse <cp@fgcz.ethz.ch> 2015, 2018, 2019

**Source**

- https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-4_S4.extendedFrags_uniqNB2FC.txt
- https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-5_S5.extendedFrags_uniqNB2FC.txt

**Examples**

```r
NB <- getNB()
dim(NB)
```
nanobodyFlycodeLinking.as.fasta

```
Write FASTA
```

**Description**

Write FASTA

**Usage**

```r
nanobodyFlycodeLinking.as.fasta(x, file = NULL, ...)
```

**Arguments**

- `x`: a nanobodyFlycodeLinking S3 object computed by `runNGSAnalysis`
- `file`: a filename
- `...`: just passed

**Value**

`sprintf` stream

**Author(s)**

Lennart Opitz, Christian Panse 2018

**Examples**

```r
library(ExperimentHub)
eh <- ExperimentHub()
f <- query(eh, c("NestLink", "nanobodyFlycodeLinkage.RData"))[1]
load(f)
summary(nanobodyFlycodeLinkage.sample)
nanobodyFlycodeLinking.as.fasta(nanobodyFlycodeLinkage.sample)
```

```
nanobodyFlycodeLinking.summary

Object Summaries of S3 class nanobodyFlycodeLinking
```

**Description**

Object Summaries of S3 class nanobodyFlycodeLinking

**Usage**

```r
nanobodyFlycodeLinking.summary(object)
```
Arguments

object  a nanobodyFlycodeLinking class computed by runNGSAnalysis.

Value

a data.frame object

Examples

library(ExperimentHub)
eh <- ExperimentHub()
f <- query(eh, c("NestLink", "nanobodyFlycodeLinkage.RData"))[1]
load(f)
summary(nanobodyFlycodeLinkage.sample)

---

NB.unambiguous  Determine unambiguous NBs

Description

Determine unambiguous NBs

Usage

NB.unambiguous(x = getNB())

Arguments

x  a data.frame containing a column peptide

Value

a data.frame a data.frame of unambiguously assignable peptides (those, which occur only on one nanobody)

Examples

NB <- getNB()
dim(NB.unambiguous(NB))
**NB.unique**

**Description**

make NB table unique

**Usage**

```r
NB.unique(x = getNB())
```

**Arguments**

- `x` : a data.frame

**Value**

a data.frame

**Examples**

```r
NB <- getNB()
dim(NB.unique(NB))
```

---

**PGexport**

**PGexport results**

**Description**

PGexport results

**Author(s)**

Pascal Egloff <p.egloff@imm.uzh.ch>

**Source**

https://fgcz-bfabric.uzh.ch

- Workunit : 158716 - QEXACTIVEHF_1 20170919_16_62465_nl5idx1-3_6titratecoli.raw 20170919_05_62465_nl5idx3_6titratecoli.raw
- Workunit : 158717 - QEXACTIVEHF_1 20170919_14_62466_nl5idx1-3_7titratesmeg.raw 20170919_09_62466_nl5idx1-3_7titratesmeg.raw
Examples

```r
# filename <- system.file(
# "extdata/PGexport2_normalizedAgainstSBstandards_Peptides.csv",
# package = "NestLink")
library(ExperimentHub)
eh <- ExperimentHub()
filename <- query(eh,
c("NestLink", "PGexport2_normalizedAgainstSBstandards_Peptides.csv"))[1]
P <- read.csv(filename, header = TRUE, sep=';
P <- P[P$Modifications == '"', ]
P <- P[,c('Accession', 'Sequence',
"X20170919_05_62465_nl5idx1.3_6titratecoli",
"X20170919_16_62465_nl5idx1.3_6titratecoli",
"X20170919_09_62466_nl5idx1.3_7titratesmeg",
"X20170919_14_62466_nl5idx1.3_7titratesmeg")]
names(P)<-c('Accession', 'Sequence',
"coli1", "coli2", 'smeg1', 'smeg2')
P<- P[grep("^P[0-9][A-Z][0-9]", P$Accession), ]
P$FCset_ng <- NA
P$FCset_ng[P$Accession %in% c('P1A4', 'P1B4', 'P1C4',
'P1D4', 'P1E4', 'P1F4')] <- 92
P$FCset_ng[P$Accession %in% c('P1A5', 'P1B5', 'P1C5',
'P1D5', 'P1G4', 'P1H4')] <- 295
P$FCset_ng[P$Accession %in% c('P1A6', 'P1B6', 'P1E5',
'P1F5', 'P1G5', 'P1H5')] <- 943
P$FCset_ng[P$Accession %in% c('P1C6', 'P1D6', 'P1E6',
'P1F6', 'P1G6', 'P1H6')] <- 3017
P$coli1 <- (log(P$coli1,2) - mean(log(P$coli1,2))) / sd(log(P$coli1,2))
P$coli2 <- (log(P$coli2,2) - mean(log(P$coli2,2))) / sd(log(P$coli2,2))
P$smeg1 <- (log(P$smeg1,2) - mean(log(P$smeg1,2))) / sd(log(P$smeg1,2))
P$smeg2 <- (log(P$smeg2,2) - mean(log(P$smeg2,2))) / sd(log(P$smeg2,2))
O <- P
b <- boxplot(df<-cbind(P$coli1 - P$coli2, P$coli1 - P$smeg1,
P$coli1 - P$smeg2,P$coli2 - P$smeg1, P$coli2 - P$smeg2,
P$smeg1 - P$smeg2),
ylab='normalized log2ratios', ylim = c(-1,1), axes=FALSE,
main=paste("ConcGr = all"))
axis(1, 1:6, c('coli[12]', 'coli1-smeg1', 'coli1-smeg2', 'coli2-smeg1',
'coli2- smeg2','smeg[12]'))
abline(h=0, col='red')
box() 
axis(2)
outliers.idx <- sapply(1:length(b$group), function(i){
q <- df[, b$group[i]] == b$out[i];
text(b$group[i], b$out[i], P[q, 2], pos=4, cex=0.4);
text(b$group[i], b$out[i], P[q, 1], pos=2, cex=0.4);
which(q) })
```

plot_in_silico_LCMS_map

plot a LC-MS map of a given set of amino acid sequences

Description

plot a LC-MS map of a given set of amino acid sequences

Usage

plot_in_silico_LCMS_map(peptides, ...)

Arguments

peptides a vector of peptides.
...

pass through the plot method.

Details

TODO(cp): consider using hexbin using ggplot2 ggplot facet_wrap aes geom_point

Value

gplots::hist2d a gplot 2d histogram

Author(s)

Christian Panse

Examples

set.seed(1)
par(mfrow=c(2,1));
FlyCodes <- replicate(10000, compose_GPGx8cTerm())
rv <- plot_in_silico_LCMS_map(FlyCodes)
runNGSAnalysis

NGS linkage workflow

Description

performs the NGS filtering workflow to get high quality FlyCode and Nanobody sequences linkage.

Usage

runNGSAnalysis(file, param)

Arguments

file sequence file path
param list of input parameters, explained in details paragraph below.

Details

The elements of the parameter list object is described as follows:

- **NB_Link1** nucleotide sequence of the linker left to the nanobody.
- **NB_Link2** nucleotide sequence of the linker right to the nanobody.
- **ProteaseSite** nucleotide sequence left to the flycode.
- **FC_Link** nucleotide sequence right to the flycode.
- **knownNB** known nanobody sequences in the experiment.
- **nReads** number of Reads from the start of fastq file to process.
- **minRelBestHitFreq** minimal fraction of the dominant nanobody for a specific flycode.
- **minConsensusScore** minimal fraction per sequence position in nanabody consensus sequence calculation.
- **maxMismatch** number of accepted mismatches for all pattern search steps.
- **minNanobodyLength** minimal nanobody length in [nt].
- **minFlycodeLength** minimal flycode length in [nt].
- **FCminFreq** minimal number of subreads for a specific flycode to keep it in the analysis.

missing elements are replace by the example provided values.

Value

uniqNB2FC dataframe

Author(s)

Lennart Opitz <lopitz@fgcz.ethz.ch>, 2019
Examples

```r
library(ExperimentHub)
eh <- ExperimentHub()
expFile <- query(eh, c("NestLink", "NL42_100K.fastq.gz"))[1]
knownNB_File <- query(eh, c("NestLink", "knownNB.txt"))[1]
knownNB_data <- read.table(knownNB_File, sep='\t', header = TRUE,
  row.names = 1, stringsAsFactors = FALSE)
knownNB <- Biostrings::translate(DNAStringSet(knownNB_data$Sequence))
names(knownNB) <- rownames(knownNB_data)
knownNB <- sapply(knownNB, toString)
param <- list()
param[['NB_Linker1']] <- "GGCCggcggGGCC"
param[['NB_Linker2']] <- "GCAGGAGGA"
param[['ProteaseSite']] <- "TTAGTCCCAAGA"
param[['FC_Linker']] <- "GGCCaaggaggcCGG"
param[['knownNB']] <- knownNB
param[['nReads']] <- 10000
param[['minRelBestHitFreq']] <- 0.8
param[['minConsensusScore']] <- 0.9
param[['maxMismatch']] <- 1
param[['minNanobodyLength']] <- 348
param[['minFlycodeLength']] <- 33
param[['FCminFreq']] <- 1
runNGSAnalysis(file = expFile[1], param)
```

Description

Filter input sequences for two patterns

Usage

twoPatternReadFilter(reads, leftPattern, rightPattern, maxMismatch, prevPatternPos = NULL)

Arguments

- **reads**: input sequences
- **leftPattern**: left pattern motive.
- **rightPattern**: right pattern motive.
- **maxMismatch**: maximal number of miss matches.
- **prevPatternPos**: prev pattern position; default is set to NULL.

Value

list object
Examples

    reads <- DNAStringSet(c('ACTGGGTTT', 'ACCTGGGTTT'))
    leftPattern <- 'CT'
    rightPattern <- 'TTT'
    maxMismatch <- 0
    twoPatternReadFilter(reads, leftPattern, rightPattern, maxMismatch)

Description

WU160118 Mascot Search results

Author(s)

Christian Panse

References

https://fgcz-bfabric.uzh.ch/bfabric/userlab/show-workunit.html?id=160118

See Also

please read the vignette summaryFASTA.Rmd.

Examples

library(ExperimentHub)
eh <- ExperimentHub();
load(query(eh, c("NestLink", "WU160118.RData"))[[1]])
class(WU160118)
PATTERN <- "^GS[ASTNQDEFVLYWGP]{7}(WR|WLTVR|WQEGGR|WLR|WQSR)$"
idx <- grepl(PATTERN, WU160118$pep_seq)
WU <- WU160118[idx & WU160118$pep_score > 25,]

library(lattice)
histogram(~RTINSECONDS| datfilename, data = WU, type='count')
Index

* data
  F255744, 6
  PGexport, 11
  WU160118, 16
  .ssrc.mascot, 2

  compose_GPGx8cTerm, 3, 4, 5
  compose_GPx10R, 3, 4, 5
  compose_GSx7cTerm, 3–5, 5

  F255744, 6
  getExperimentHubFilename, 6
  getFC, 7
  getNB, 8

  mascot, 3

  nanobodyFlycodeLinking.as.fasta, 9
  nanobodyFlycodeLinking.summary, 9
  NB.unambiguous, 10
  NB.unique, 11

  parentIonMass, 7, 8
  PGexport, 11
  plot_in_silico_LCMS_map, 13

  runNGSAnalysis, 9, 10, 14

  ssrc, 7, 8

  twoPatternReadFilter, 15

  WU160118, 16