Package ‘NestLink’

June 27, 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), Bioststrings (>= 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 2024-06-27
.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 as input set given identified peptides.

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

Usage

.ssrmascot(x, scores = c(10, 20, 40, 50), ...)

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## 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 distribution.
- `cTerm`: c-Terms

### Value

A AA sequence
compose\_GPx10R

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

Examples

```r
set.seed(1)
compose\_GPx8cTerm()
(FlyCodes <- replicate(10, compose\_GPx8cTerm()))
plot(parentIonMass(FlyCodes) ~ ssrc(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\_GPx8cTerm, 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

```r
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)
aa\_pool\_1\_2\_9\_10 <- c(rep('A', 8), rep('S', 7), rep('T', 7), rep('N', 6),
    rep('Q', 6), rep('D', 8), rep('E', 8), rep('V', 9), rep('L', 6), rep('F', 5),
    rep('Y', 9), rep('W', 6), rep('G', 15), rep('P', 0))
aa\_pool\_3\_8 <- c(rep('A', 5), rep('S', 4), rep('T', 5), rep('N', 2),
    rep('Q', 2), rep('D', 8), rep('E', 8), rep('V', 7), rep('L', 5), rep('F', 4),
```
compose\_GSx7cTerm 5


compose\_GPx10R(aa\_pool\_1\_2\_9\_10, aa\_pool\_3\_8)
(FlyCodes <- replicate(10, compose\_GPx10R(aa\_pool\_1\_2\_9\_10, aa\_pool\_3\_8)))
plot(parent\_Ion\_Mass(FlyCodes) ~ ssrc(FlyCodes))

---

**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\_GPx8cTerm, compose\_GSx7cTerm, and compose\_GPx10R belong to three groups composing different flycode (peptide) construction. The construction is given in the function name. For example, GPx8cTerm, composes a flycode having as prefix GP 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**
compose\_GSx7cTerm(pool = c(rep("A", 18), rep("S", 6), rep("T", 12),
rep("N", 1), rep("Q", 1), rep("D", 11), rep("E", 11), rep("V", 12),
12)), cTerm = c("WR", "WLTVR", "WQGGER", "WQSR", "WLR"))

**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**
sample.size <- 100
# ## Compose a GSXXXXXXX(WR|WLTVR|WQGER|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\{ASTNQDEFWL\}7\{WR|WLTVR|WQGGER|WQSR\}$"
getExperimentHubFilename

**Description**

getExperimentHubFilename

**Usage**

getExperimentHubFilename(filename)

**Arguments**

filename of the aws s3 blob.

**Value**

the file name of the local ExperimentHub.

---

describe(

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**

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

---
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

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

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

nanobodyFlycodeLinking.summary

Object Summaries of S3 class nanobodyFlycodeLinking

Description

Object Summaries of S3 class nanobodyFlycodeLinking

Usage

nanobodyFlycodeLinking.summary(object)
Arguments

object a nanobodyFlycodeLinking class computed by runNGSA

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

**Description**

PGexport results

**Author(s)**

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

**Source**

[https://fgcz-bfabric.uzh.ch](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

# 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$Modifications == '', ]
P <- P[,c('Accession', 'Sequence',
    "X20170919_05_62465_nl5idx1.3_6titratecoli",
    "X20170919_16_62465_nl5idx1.3_6titratecoli",
    "X20170919_09_62466_nl5idx1.3_7titratesmeg",
    "X20170919_14_62466_nl5idx1.3_7titratesmeg")]
names(P)<-c('Accession', 'Sequence',
    'coli1', 'coli2', 'smeg1', 'smeg2')
P<- P[grepl("P[0-9][A-Z][0-9]", P$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$smeg2),
  ylab='normalized log2ratios', ylim = c(-1,1), axes=FALSE,
  main=paste("ConcGr = all"))
axis(1, 1:6, c('coli12', 'coli1-smeg1', 'coli1-smeg2', 'coli2-smeg1',
  'coli2-smeg2', 'smeg12'))
abline(h=0, col='red')
box()
axis(2)
outliers.idx <- sapply(1:length(b$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_Linker1 nucleotide sequence of the linker left to the nanobody.
- NB_Linker2 nucleotide sequence of the linker right to the nanobody.
- ProteaseSite nucleotide sequence left to the flycode.
- FC_Linker 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 nanobody 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

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']] <- "GGCCgcggGGCC"
param[['NB_Linker2']] <- "GCAGGAGGGA"
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)

twoPatternReadFilter  Filter input sequences for two patterns

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

```r
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

```r
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')
```
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