

# Package ‘NestLink’

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**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

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.ssrc.mascot	<i>computes the correlation of predicted and measured retention time</i>
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### 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

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

**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**

```
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**

```
compose_GPGx8cTerm(pool = c(rep("A", 12), rep("S", 0), rep("T", 12),
  rep("N", 12), rep("Q", 12), rep("D", 8), rep("E", 0), rep("V", 12),
  rep("L", 0), rep("F", 0), rep("Y", 8), rep("W", 0), rep("G", 12),
  rep("P", 12)), cTerm = c("VFR", "VSR", "VFGIR", "VSGER"))
```

**Arguments**

pool AA distributen.  
 cTerm c-Terms

**Value**

a AA sequence

**Author(s)**

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

**Examples**

```
set.seed(1)
compose_GPGx8cTerm()
(FlyCodes <- replicate(10, compose_GPGx8cTerm()))
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_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**

```
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**

```
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),
```

```
rep('Y', 6), rep('W', 4), rep('G', 12), rep('P', 28))

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(parentIonMass(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\\_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

```
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),
  rep("L", 2), rep("F", 1), rep("Y", 4), rep("W", 1), rep("G", 8), rep("P",
  12)), cTerm = c("WR", "WLTVR", "WQEGGR", "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 GSXXXXXX(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|WLR|WQSR)$"
```

```
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

```
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

*getExperimentHubFilename*


---

### Description

getExperimentHubFilename

### Usage

```
getExperimentHubFilename(filename)
```

### Arguments

filename            of the aws s3 blob.

### Value

the file name of the local ExperimentHub.

## Examples

```
f1 <- system.file("extdata", "metadata.csv", package="NestLink")
metadata <- read.csv(f1, stringsAsFactors=FALSE)
  metadata$Title

lapply(metadata$RDataPath, getExperimentHubFilename)
```

---

getFC	<i>Read FlyCodes (FCs)</i>
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---

## 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

```
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.extendedFragments\\_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-4_S4.extendedFragments_uniqNB2FC.txt)
- [https://fgcz-gstore.uzh.ch/projects/p1644/analysis\\_20170609\\_o3040/p1644o3482-5\\_S5.extendedFragments\\_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-5_S5.extendedFragments_uniqNB2FC.txt)

## Examples

```
FC <- getFC()
dim(FC)
```

---

`getNB`*Read NanoBodies (NBs)*

---

## 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

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

## Arguments

<code>filename</code>	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.extendedFragments\\_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-4_S4.extendedFragments_uniqNB2FC.txt)
- [https://fgcz-gstore.uzh.ch/projects/p1644/analysis\\_20170609\\_o3040/p1644o3482-5\\_S5.extendedFragments\\_uniqNB2FC.txt](https://fgcz-gstore.uzh.ch/projects/p1644/analysis_20170609_o3040/p1644o3482-5_S5.extendedFragments_uniqNB2FC.txt)

## Examples

```
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 <a href="#">runNGSAnalysis</a> .
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(nanobodyFlycodeLinkage.sample)
nanobodyFlycodeLinking.as.fasta(nanobodyFlycodeLinkage.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 `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	<i>Determine unambiguous NBs</i>
----------------	----------------------------------

---

**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	<i>make NB table unique</i>
-----------	-----------------------------

---

**Description**

make NB table unique

**Usage**

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

**Arguments**

x                    a data.frame

**Value**

a data.frame

**Examples**

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

---

PGexport	<i>PGexport results</i>
----------	-------------------------

---

**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\_nl5idx1-3\_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$Modifications == '', ]
P <- P[,c('Accession', 'Sequence',
"X20170919_05_62465_n15idx1.3_6titratecoli",
"X20170919_16_62465_n15idx1.3_6titratecoli",
"X20170919_09_62466_n15idx1.3_7titratesmeg",
"X20170919_14_62466_n15idx1.3_7titratesmeg")]
names(P)<-c('Accession', 'Sequence', 'coli1', 'coli2', 'smeg1', 'smeg2')
P<- P[grep("^P[0-9][A-Z][0-9]", P$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)
axis(3, 1:6, b$n)
outliers.idx <- sapply(1:length(b$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**

<code>peptides</code>	a vector of peptides.
<code>...</code>	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']] <- "GGCCggcggGGCC"
param[['NB_Linker2']] <- "GCAGGAGGA"
param[['ProteaseSite']] <- "TTAGTCCAAGA"
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**

```
reads <- DNASTringSet(c('ACTGGGTTT','ACCCTGGGTTT'))
leftPattern <- 'CT'
rightPattern <- 'TTT'
maxMismatch <- 0
twoPatternReadFilter(reads, leftPattern, rightPattern, maxMismatch)
```

---

WU160118

*WU160118 Mascot Search results*

---

**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')
```



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