Package ‘topdownr’

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Title Investigation of Fragmentation Conditions in Top-Down Proteomics

Version 1.26.0

Description The topdownr package allows automatic and systemic investigation of fragment conditions. It creates Thermo Orbitrap Fusion Lumos method files to test hundreds of fragmentation conditions. Additionally it provides functions to analyse and process the generated MS data and determine the best conditions to maximise overall fragment coverage.

Depends R (>= 3.5), methods, BiocGenerics (>= 0.20.0), ProtGenerics (>= 1.10.0), Biostrings (>= 2.42.1), S4Vectors (>= 0.12.2)

Imports grDevices, stats, tools, utils, Biobase, Matrix (>= 1.4-2), MSnbase (>= 2.3.10), PSMatch (>= 1.6.0), ggplot2 (>= 2.2.1), mzR (>= 2.27.5)

Suggests topdownrdata (>= 0.2), knitr, rmarkdown, ranger, testthat, BiocStyle, xml2

License GPL (>= 3)

URL https://github.com/sgibb/topdownr/

BugReports https://github.com/sgibb/topdownr/issues/

LazyData true

VignetteBuilder knitr

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Investigation of Fragmentation Conditions in Top-Down Proteomics

Description

The topdownr package allows automatic and systemic investigation of fragment conditions. It creates Thermo Orbitrap Fusion Lumos method files to test hundreds of fragmentation conditions. Additionally, it provides functions to analyse and process the generated MS data and determine the best conditions to maximise overall fragment coverage.

Details

The usage of the topdownr package is demonstrated in the following vignettes:

- Generate .meth files prior data acquisition for the Thermo Orbitrap Fusion Lumos MS device: vignette("data-generation", package="topdownr")
- How to analyse top-down fragmentation data: vignette("analysis", package="topdownr")

Author(s)

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The `AbstractTopDownSet` class

**Description**

*Abstract/VIRTUAL* parent class for `TopDownSet` and `NCBSet` to provide common interface.

**Usage**

```r
## S4 method for signature 'AbstractTopDownSet,ANY,ANY,ANY'
x[i, j, ..., drop = FALSE]
## S4 method for signature 'AbstractTopDownSet,ANY,missing'
x[[i, j, ...]]
## S4 replacement method for signature 'AbstractTopDownSet,ANY,missing'
x[[i, j, ...]] <- value
## S4 method for signature 'AbstractTopDownSet'
x$name
## S4 replacement method for signature 'AbstractTopDownSet'
x$name <- value
## S4 method for signature 'AbstractTopDownSet'
assayData(object)
## S4 method for signature 'AbstractTopDownSet'
colData(object)
## S4 replacement method for signature 'AbstractTopDownSet'
colData(object, ...) <- value
## S4 method for signature 'AbstractTopDownSet,AbstractTopDownSet'
combine(x, y)
```
AbstractTopDownSet-class

## S4 method for signature 'AbstractTopDownSet'
conditionData(object, ...)

## S4 replacement method for signature 'AbstractTopDownSet'
conditionData(object, ...) <- value

## S4 method for signature 'AbstractTopDownSet'
conditionNames(object)

## S4 method for signature 'AbstractTopDownSet'
dim(x)

## S4 method for signature 'AbstractTopDownSet'
dimnames(x)

## S4 method for signature 'AbstractTopDownSet'
removeEmptyConditions(object)

## S4 method for signature 'AbstractTopDownSet'
rowViews(object, ...)

## S4 method for signature 'AbstractTopDownSet'
show(object)

## S4 method for signature 'AbstractTopDownSet'
summary(object, what = c("rows", "columns"), ...)

## S4 method for signature 'AbstractTopDownSet'
updateConditionNames(
  object,
  sampleColumns = c("Mz", "AgcTarget", "EtdReagentTarget", "EtdActivation",
                    "CidActivation", "HcdActivation", "UvpdActivation"),
  verbose = interactive()
)

## S4 method for signature 'AbstractTopDownSet'
updateMedianInjectionTime(
  object,
  by = list(Mz = object$Mz, AgcTarget = object$AgcTarget)
)

**Arguments**

- **i,j**: numeric, logical or character, indices specifying elements to extract or replace.
- **drop**: logical, currently ignored.
- **value**: replacement value.
- **name**: character name of an (non)existing column in colData.
AbstractTopDownSet-class

object, x  AbstractTopDownSet
y          AbstractTopDownSet
what       character, specifies whether "rows" or "columns" should be summarized.
sampleColumns character, column names of the colData() used to define a sample (technical replicate). This is used to add the Sample column (used for easier aggregation, etc.).
verbose    logical, verbose output?
by         list, grouping information.
...        arguments passed to internal/other methods.

Details

This class just provides a common interface. It is not intended for direct use by the user. Please see TopDownSet for an example usage of its child class.

Value

This is an Abstract/VIRTUAL class to provide a common interface for TopDownSet and NCBSet. It is not possible to create an AbstractTopDownSet object.

Methods (by generic)

- `x[i]`: Subset operator.
  For i numeric, logical or character vectors or empty (missing) or NULL are supported. Subsetting is done on the fragment/bond (row) level. character indices could be names (e.g. c("a1", "b1", "c1", "c2", "c3")) or types (e.g. c("c", "x")) of the fragments for TopDownSet objects, or names of the bonds (e.g. c("bond001")) for NCBSet objects.
  j could be a numeric or logical vector and subsetting is done on the condition/run (column) level.
- `x[[i]`: Subset operator.
  i could be a numeric or logical vector and subsetting is done on the condition/run (column) level.
- `'[i` (x = AbstractTopDownSet, i = ANY, j = missing) <- value: Setter for a column in the colData slot.
  The `[<- operator is used to add/replace a single column of the colData DataFrame.
- `$`: Accessor for columns in the colData slot.
  The $ simplifies the accession of a single column of the colData. It is identical to the [[ operator.
- `'$` (AbstractTopDownSet) <- value: Setter for a column in the colData slot.
  The `$<- operator is used to add/replace a single column of the colData DataFrame. It is identical to the [[<- operator.
- assayData(AbstractTopDownSet): Accessor for the assay slot.
  Returns a Matrix::dgCMatrix that stores the intensity/coverage information of AbstractTopDownSet object.
AbstractTopDownSet-class

- **colData(AbstractTopDownSet):** Accessor for the colData slot.  
  Returns a S4Vectors::DataFrame that stores metadata for the conditions/runs (columns) of the AbstractTopDownSet object.

- **colData(AbstractTopDownSet) <- value:** Setter for the colData slot.  
  Replaces metadata for the conditions/runs (columns) of the AbstractTopDownSet object.

- **combine(x = AbstractTopDownSet, y = AbstractTopDownSet):** Combine AbstractTopDownSet objects.  
  combine allows to combine two or more AbstractTopDownSet objects. Please note that it uses the default sampleColumns to define technical replicates (see readTopDownFiles()). and the default by argument to group ion injection times for the calculation of the median time (see updateMedianInjectionTime()). Both could be modified after combine by calling updateConditionNames() (with modified sampleColumns argument) and updateMedianInjectionTime() (with modified by argument).

- **conditionData(AbstractTopDownSet):** Accessor for the colData slot.  
  An alias for colData.

- **conditionData(AbstractTopDownSet) <- value:** Setter for the colData slot.  
  An alias for colData<-.  

- **conditionNames(AbstractTopDownSet):** Accessor for condition names.  
  Returns a character with names for the conditions/runs (columns).

- **dim(AbstractTopDownSet):** Accessor for dimensions.  
  Returns a numeric with number of fragments/bonds (rows) and conditions/runs (columns).

- **dimnames(AbstractTopDownSet):** Accessor for dimension names.  
  Returns a list with names for the fragments/bonds (rows) and for the conditions/runs (columns).

- **removeEmptyConditions(AbstractTopDownSet):** Remove empty conditions/runs.  
  Removes conditions/runs (columns) without any intensity/coverage information from the AbstractTopDownSet object. It returns a modified AbstractTopDownSet object.

- **rowViews(AbstractTopDownSet):** Accessor for the rowViews slot.  
  Depending on the implementation it returns an FragmentViews object for TopDownSet objects or an Biostrings::XStringViews object for NCBSSet objects.

- **summary(AbstractTopDownSet):** Summary statistics.  
  Returns a matrix with some statistics: number of fragments, total/min/first quartile/median/mean/third quartile/maximum of intensity values.

- **updateConditionNames(AbstractTopDownSet):** Update condition names.  
  Updates condition names based on sampleColumns from conditionData/colData. Columns with just identical entries are ignored. This method will create/update the colData(object)$Sample column that identifies technical replicates and could be used in other methods.

- **updateMedianInjectionTime(AbstractTopDownSet):** Update median ion injection times.  
  Recalculates median ion injection times by a user given grouping variable (default: Mz, AgcTarget). This is useful if you acquire new data and the ion injection time differs across the runs. Use the by argument to provide a list/data.frame of grouping variables, e.g. by=colData(object)[, c("Mz", "AgcTarget", "File")].
## Slots

rowViews Biostrings::XStringViews, information about fragments/bonds (name, type, sequence, mass, charge), see Biostrings::XStringViews and FragmentViews for details.

colData S4Vectors::DataFrame, information about the MS2 experiments and the fragmentation conditions.

assay Matrix::dgCMatrix, intensity/coverage values of the fragments/bonds. The rows correspond to the fragments/bonds and the columns to the condition/run. It just stores values that are different from zero.

files character, files that were imported.

processing character, log messages.

## Author(s)

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## See Also

- TopDownSet and NCBSSet which both implement/use this interface. These manual pages also provide some example code.
- FragmentViews (and Biostrings::XStringViews) for the row view interface.
- Matrix::dgCMatrix for technical details about the intensity/coverage storage.

## Examples

```r
## Because AbstractTopDownSet is a VIRTUAL class we could not create any object of it. Here we demonstrate the usage with an TopDownSet that implements the AbstractTopDownSet interface. See `?"TopDownSet-class"` for more details an further examples.

## Example data
data(tds, package="topdownr")

tds

head(summary(tds))

# Accessing slots
rowViews(tds)
colData(tds)
head(assayData(tds))

# Accessing colData
tds$Mz
tds$FilterString

# Subsetting

# First 100 fragments
tds[1:100]
```
createExperimentsFragmentOptimisation

Create fragment optimisation experiment

Description

This function is used to create a tree-like list of all combinations of a user-given set of MS1 and TMS2 settings for a fragment optimisation experiment. The list could be written to an Orbitrap Fusion Lumos method xml file using writeMethodXmIs().

Usage

createExperimentsFragmentOptimisation(
  ms1,
  ...,  
  groupBy = c("AgcTarget", "replication"),
  nMs2perMs1 = 10,
  scanDuration = 0,
  replications = 2,
  randomise = TRUE
)

Arguments

ms1  data.frame, MS1 settings.

...  further named arguments with data.frames containing the TMS2 settings.

groupBy  character, group experiments by columns in the TMS2 data.frames. The columns have to be present in all data.frames. Each group will be written to its own XML file.

nMs2perMs1  integer, how many TMS2 scans should be run after a MS1 scan?

scanDuration  double, if greater than zero (e.g. scanDuration=0.5) the Start/EndTimeMin are overwritten with a duration of scanDuration. If scanDuration is zero (default) Start/EndTimeMin are not overwritten.

replications  integer, number of replications.

randomise  logical, should the TMS2 scan settings randomised?
createExperimentsFragmentOptimisation

Value

list, able to be written via `xml2::as_xml_document`

See Also

`writeMethodXmIs()`, `expandMs1Conditions()`, `expandTms2Conditions()`

Examples

```r
## build experiments within R
ms1 <- expandMs1Conditions(
  FirstMass=400,
  LastMass=1200,
  Microscans=as.integer(10)
)

targetMz <- cbind(mz=c(560.6, 700.5, 933.7), z=rep(1, 3))
common <- list(
  OrbitrapResolution="R120K",
  IsolationWindow=1,
  MaxITT imeInMS=200,
  Microscans=as.integer(40),
  AgcTarget=c(1e5, 5e5, 1e6)
)

cid <- expandTms2Conditions( 
  MassList=targetMz,
  common,
  ActivationType="CID",
  CIDCollisionEnergy=seq(7, 35, 7)
)
hcd <- expandTms2Conditions( 
  MassList=targetMz,
  common,
  ActivationType="HCD",
  HCDCollisionEnergy=seq(7, 35, 7)
)
etd <- expandTms2Conditions( 
  MassList=targetMz,
  common,
  ActivationType="ETD",
  ETDReactionTime=as.double(1:2)
)
etcid <- expandTms2Conditions( 
  MassList=targetMz,
  common,
  ActivationType="ETD",
  ETDReactionTime=as.double(1:2),
  ETDSupplementalActivation="ETciD",
  ETDSupplementalActivationEnergy=as.double(1:2)
)

uvpd <- expandTms2Conditions(
```

```
createExperimentsFragmentOptimisation

```r
MassList=targetMz,
common,
ActivationType="UVPD"
)

exps <- createExperimentsFragmentOptimisation(
  ms1=ms1, cid, hcd, etd, etcid, uvpd,
groupBy=c("AgcTarget", "replication"), nMs2perMs1=10, scanDuration=0.5,
replications=2, randomise=TRUE
)

## use different settings for CID

cid560 <- expandTms2Conditions(
  MassList=cbind(560.6, 1),
  common,
  ActivationType="CID",
  CIDCollisionEnergy=seq(7, 21, 7)
)
cid700 <- expandTms2Conditions(
  MassList=cbind(700.5, 1),
  common,
  ActivationType="CID",
  CIDCollisionEnergy=seq(21, 35, 7)
)

exps <- createExperimentsFragmentOptimisation(
  ms1=ms1, cid560, cid700,
  groupBy=c("AgcTarget", "replication"), nMs2perMs1=10, scanDuration=0.5,
  replications=2, randomise=TRUE
)

## use a CSV (or excel) file as input
myCsvContent <- "
ActivationType, ETDReactionTime, UVPDActivationTime
UVPD,,1000
ETD,1000,"

myCsvSettings <- read.csv(text=myCsvContent, stringsAsFactors=FALSE)

myCsvSettings
# ActivationType ETDReactionTime UVPDActivationTime
# 1 UVPD NA 1000
# 2 ETD 1000 NA

exps <- createExperimentsFragmentOptimisation(
  ms1 = data.frame(FirstMass=500, LastMass=1000),
  TMS2
  myCsvSettings,
  other arguments
groupBy="ActivationType"
)
```
createTngFusionMethFiles

Windows specific functions.

**Description**

The functions `runXmlMethodChanger` and `runScanHeadsman` call `XmlMethodChanger.exe` and `ScanHeadsman.exe` with the corresponding arguments. The only work on Windows (maybe on Linux + wine as well but that was never tested).

**Usage**

```r
createTngFusionMethFiles(
  template,
  xml = list.files(pattern = ".*\.xml$"),
  executable = "XmlMethodChanger.exe",
  verbose = interactive()
)
unXmlMethodChanger(
  template,
  xml = list.files(pattern = ".*\.xml$"),
  executable = "XmlMethodChanger.exe",
  verbose = interactive()
)

runScanHeadsman(path = ".", executable = "ScanHeadsman.exe")
```

**Arguments**

- `template` character, path to template .meth file.
- `xml` character, vector of path to .xml files.
- `executable` character, path to the `XmlMethodChanger.exe` or `ScanHeadsman.exe` executable.
- `verbose` logical, if TRUE a progress bar is shown.
- `path` character, path to the directory containing the .raw files.

**Details**

`runXmlMethodChanger` applies `XmlMethodChanger.exe` on all given XML files generated with `writeMethodXmls()` to create .meth files from a template.

`runScanHeadsman` calls `ScanHeadsman.exe` on a given directory containing .raw files. `ScanHeadsman.exe` extracts the method and scan header data into .experiments.csv and .txt files, respectively.

**Value**

Nothing. Used for its side effects.
References
XmlMethodChanger source code: https://github.com/thermofisherisms/meth-modifications/
ScanHeadsman source code: https://bitbucket.org/caetera/scanheadsman

See Also
writeMethodXmls()

Examples
## Not run:
runXmlMethodChanger(templateMeth="TMS2IndependentTemplate240Extended.meth",
                     modificationXml=list.files(pattern="^method.*\.xml$"),
                     executable="..\XmlMethodChanger.exe")
## End(Not run)

## Not run:
runScanHeadsman("raw", executable="..\ScanHeadsman.exe")
## End(Not run)

expandMs1Conditions  Expand MS Conditions

Description
Create a data.frame of all possible combinations of the given arguments. It ensures that just
arguments are applied that yield a valid MethodModification.xml file.

Usage
expandMs1Conditions(..., family = "Calcium", version = "3.2")
expandTms2Conditions(
  ActivationType = c("CID", "HCD", "ETD", "UVPD"),
  ...
)

Arguments
... further named arguments, used to create the combination of conditions.
family character, currently just Calcium is supported
version character, currently 3.1, 3.2 (default), 3.3 are supported
ActivationType character, ActivationType for TMS2, either CID, HCD, ETD, or UVPD.
FragmentViews-class

The FragmentViews class

Description

The FragmentViews class is a basic container for storing a set of views (start/end locations) on the same peptides/protein sequence. Additionally it keeps information about mass, type and charge of the fragments.

Usage

FragmentViews(
    sequence,
    mass,
    type,
    z = 1L,
    start = NULL,
    end = NULL,
    width = NULL,
    names = NULL,
    metadata = list()
)
## S4 method for signature 'FragmentViews,FragmentViews'
combine(x, y)

## S4 method for signature 'FragmentViews'
mz(object, ...)

## S4 method for signature 'FragmentViews'
show(object)

### Arguments

- **sequence** character/Biostrings::AAString, complete protein/peptide sequence.
- **mass** double, mass of the fragments, same length as start/end/width.
- **type** character, type of the fragments, same length as start/end/width.
- **z** integer, charge of the fragments, length one or same length as start/end/width.
- **start** integer, start positions of the fragments. At least two of start/end/width has to be given.
- **end** integer, end positions of the fragments. At least two of start/end/width has to be given.
- **width** integer, width positions of the fragments. At least two of start/end/width has to be given.
- **names** character, names of the fragments, same length as start/end/width.
- **metadata** list, metadata like modifications.
- **object, x, y** FragmentViews
- **...** arguments passed to internal/other methods.

### Details

FragmentViews extends Biostrings::XStringViews. In short it combines an IRanges::IRanges object to store start/end location on a sequence, an Biostrings::AAString object.

### Value

An FragmentViews object.

### Functions

- **FragmentViews()**: Constructor
  
  In general it is not necessary to call the constructor manually. See readTopDownFiles() instead.

### Coercion

- as(object, "data.frame"): Coerce an FragmentViews object into a data.frame.
NCBSet-class

Description

The NCBSet class is a container for a top-down proteomics experiment similar to the TopDownSet but instead of intensity values it just stores the information if a bond is covered by a N-terminal (encoded as 1), a C-terminal (encoded as 2) and/or bidirectional fragments (encoded as 3).

Usage

## S4 method for signature 'NCBSet'
bestConditions(
  object,
  n = ncol(object),
  minN = 0L,
  maximise = c("fragments", "bonds"),
  ...
)

## S4 method for signature 'NCBSet'
fragmentationMap(
  object,
  nCombinations = 10,
  cumCoverage = TRUE,
  maximise = c("fragments", "bonds"),
  labels = colnames(object),
  alphaIntensity = TRUE,
  ...
)
## S4 method for signature 'NCBSet'

show(object)

## S4 method for signature 'NCBSet'

summary(object, what = c("conditions", "bonds"), ...)

### Arguments

- **object**: NCBSet
- **n**: integer, max number of combinations/iterations.
- **minN**: integer, stop if there are less than minN additional fragments
- **maximise**: character, optimisation targeting for the highest number of "fragments" (default) or "bonds".
- **nCombinations**: integer, number of combinations to show (0 to avoid plotting them at all).
- **cumCoverage**: logical, if TRUE (default) cumulative coverage of combinations is shown.
- **labels**: character, overwrite x-axis labels.
- **alphaIntensity**: logical, if TRUE (default) the alpha level of the color is used to show the colData(object)$AssignedIntensity. If FALSE the alpha is set to 1.
- **what**: character, specifies whether "conditions" (columns; default) or "bonds" (rows) should be summarized.
- **...**: arguments passed to internal/other methods. added.

### Value

An NCBSet object.

### Methods (by generic)

- **bestConditions(NCBSet)**: Best combination of conditions.
  Finds the best combination of conditions for highest coverage of fragments or bonds. If there are two (or more conditions) that would add the same number of fragments/bonds the one with the highest assigned intensity is used. Use n to limit the number of iterations and combinations that should be returned. If minN is set at least minN fragments have to be added to the combinations. The function returns a 7-column matrix. The first column contains the index (Index) of the condition (column number). The next columns contain the newly added number of fragments or bonds (FragmentsAddedToCombination, BondsAddedToCombination), the fragments or bonds in the condition (FragmentsInCondition, BondsInCondition), and the cumulative coverage fragments or bonds (FragmentCoverage, BondCoverage).

- **fragmentationMap(NCBSet)**: Plot fragmentation map.
  Plots a fragmentation map of the Protein. Use nCombinations to add another plot with nCombinations combined conditions. If cumCoverage is TRUE (default) these combinations increase the coverage cumulatively.

- **summary(NCBSet)**: Summary statistics.
  Returns a matrix with some statistics: number of fragments, total/min/first quartile/median/mean/third quartile/maximum of intensity values.
Slots

rowViews Biostrings::XStringViews, information about bonds (name, start, end, width, sequence), see Biostrings::XStringViews for details.

colData S4Vectors::DataFrame, information about the MS2 experiments and the fragmentation conditions.

assay Matrix::dgCMatrix, coverage values of the bonds. The rows correspond to the bonds and the columns to the condition/run. It just stores values that are different from zero. If a bond is covered by an N-terminal fragment its encoded with 1, by an C-terminal fragment with 2 and by both fragment types/bidirectional by 3 respectively.

files character, files that were imported.

processing character, log messages.

Author(s)

Sebastian Gibb <mail@sebastiangibb.de>

See Also

• An NCBSet is generated from an TopDownSet object.
• Biostrings::XStringViews for the row view interface.
• Matrix::dgCMatrix for technical details about the coverage storage.

Examples

## Example data
data(tds, package="topdown")

## Aggregate technical replicates
tds <- aggregate(tds)

## Coercion into an NCBSet object
ncb <- as(tds, "NCBSet")

ncb

head(summary(ncb))

# Accessing slots
rowViews(ncb)
colData(ncb)
head(assayData(ncb))

# Accessing colData
ncb$Mz

# Subsetting
# First 100 bonds
ncb[1:100]
# Just bond 152
ncb["bond152"]

# Condition 1 to 10
ncb[, 1:10]

# Plot fragmentation map
fragmentationMap(ncb)

## readTopDownFiles

**Read top-down files.**

**Description**

It creates a TopDownSet object and is its only constructor.

**Usage**

```r
readTopDownFiles(
  path,
  pattern = ".*",
  type = c("a", "b", "c", "x", "y", "z"),
  modifications = c("Carbamidomethyl", "Acetyl", "Met-loss"),
  customModifications = data.frame(),
  adducts = data.frame(),
  neutralLoss = PSMatch::defaultNeutralLoss(),
  sequenceOrder = c("original", "random", "inverse"),
  tolerance = 5e-06,
  redundantIonMatch = c("remove", "closest"),
  redundantFragmentMatch = c("remove", "closest"),
  dropNonInformativeColumns = TRUE,
  sampleColumns = c("Mz", "AgcTarget", "EtdReagentTarget", "EtdActivation",
                    "CidActivation", "HcdActivation", "UvpdActivation"),
  conditions = "ScanDescription",
  verbose = interactive()
)
```

**Arguments**

- **path** character, path to directory that contains the top-down files.
- **pattern** character, a filename pattern, the default .* means all files.
- **type** character, type of fragments, currently a-c and x-z are supported, see `PSMatch::calculateFragments()` for details.
- **modifications** character, unimod names of modifications that should be applied. Currently just **Acetyl** (Unimod:1 but just protein N-term), **Carbamidomethyl** (Unimod:4) and **Met-loss** (Unimod:765) are supported. **Met-loss** removes M (if followed by

customModifications
data.frame, with 4 columns, namely: mass, name, location, variable, see details section.

adducts
data.frame, with 3 columns, namely: mass, name, to, see details section.

neutralLoss
list, neutral loss that should be applied, see PSMatch::calculateFragments() and PSMatch::defaultNeutralLoss() for details.

sequenceOrder
character, order of the sequence before fragment calculation and matching is done. "original" doesn't change anything. "inverse" reverse the sequence and "random" arranges the amino acid sequence at random.

tolerance
double, tolerance in ppm that is used to match the theoretical fragments with the observed ones.

redundantIonMatch
character, a mz could be matched to one, two or more fragments. If it is matched against more than one fragment the match could be "remove"d or the match to the "closest" fragment could be chosen.

redundantFragmentMatch
character, one or more mz could be matched to the same fragment, these matches could be "remove"d or the match to the "closest" mz is chosen.

dropNonInformativeColumns
logical, should columns with just one identical value across all runs be removed?

sampleColumns
character, column names of the colData() used to define a sample (technical replicate). This is used to add the Sample column (used for easier aggregation, etc.).

conditions
character/numeric, one of:

- "ScanDescription" (default): create condition IDs based on the given "Scan Description" parameter (set automatically by createExperimentsFragmentOptimisation()).
- "FilterString": create condition IDs based on mass labels in the FilterString column (included for backward-compatibility, used in writeMethodXmIls() prior version 1.5.2 in Dec 2018).
- A single numeric value giving the number of conditions.

verbose
logical, verbose output?

Details

readTopDownFiles reads and processes all top-down files, namely:

- .fasta (protein sequence)
- .mzML (spectra)
- .experiments.csv (method/fragmentation conditions)
- .txt (scan header information)
customModifications: additional to the provided unimod modifications available through the modifications argument customModifications allow to apply user-defined modifications to the output of `PSMatch::calculateFragments()`. The customModifications argument takes a data.frame with the mass to add, the name of the modification, the location (could be the position of the amino acid or "N-term"/"C-term"), whether the modification is always seen \(\text{(variable=FALSE)}\) or both, the modified and unmodified amino acid are present \(\text{(variable=TRUE)}\), e.g. for Activation \(\text{(which is available via modification="Acetyl"\)}}\) data.frame(mass=42.010565, name="Acetyl", location="N-term", variable=FALSE) or variable one (that could be present or not): data.frame(mass=365.132, name="Custom", location=10, variable=TRUE)

If the customModifications data.frame contains multiple columns the modifications are applied from row one to the last row one each time.

adducts: *Thermo's Xtract* allows some mistakes in deisotoping, mostly it allows +/- C13-C12 and +/- H+. The adducts argument takes a data.frame with the mass to add, the name that should assign to these new fragments and an information to whom the modification should be applied, e.g. for H+ on z. data.frame(mass=1.008, name="zpH", to="z").

*Please note:* The adducts are added to the output of `PSMatch::calculateFragments()`. That has some limitations, e.g. neutral loss calculation could not be done in topdownr-package. If neutral loss should be applied on adducts you have to create additional rows, e.g.: data.frame(mass=c(1.008, 1.008), name=c("cpH", "cpH_"), to=c("c", "c_")).

Value

A TopDownSet object.

See Also

`PSMatch::calculateFragments()`, `PSMatch::defaultNeutralLoss()`

Examples

```r
if (require("topdownrdata")) {
  # add H+ to z and no neutral loss of water
  tds <- readTopDownFiles(
    topdownrdata::topDownDataPath("myoglobin"),
    # Use an artificial pattern to load just the fasta
    # file and files from m/z == 1211, ETD reagent
    # target 1e6 and first replicate to keep runtime
    # of the example short
    pattern=".*fasta.gz$|1211_.*1e6_1",
    adducts=data.frame(mass=1.008, name="zpH", to="z"),
    neutralLoss=PSMatch::defaultNeutralLoss(
      disableWaterLoss=c("Cterm", "D", "E", "S", "T")),
    tolerance=25e-6
  )
}
```
tds

TopDownSet Example Data

Description

An example data set for topdownr. It is just a subset of the myoglobin dataset available in topdownr::topdownrdata-package.

Usage

tds

Format

A TopDownSet with 14901 fragments (1949 rows, 351 columns).

Details

It was created as follows:

```r
tds <- readTopDownFiles(
  topdownrdata::topDownDataPath("myoglobin"),
  ## Use an artificial pattern to load just the fasta
  ## file and files from m/z == 1211, ETD reagent
  ## target 1e6 and first replicate to keep runtime
  ## of the example short
  pattern=".*fasta.gz$|1211_.*1e6_1",
  adducts=data.frame(mass=1.008, name="zpH", to="z"),
  neutralLoss=PSMatch::defaultNeutralLoss(
    disableWaterLoss=c("Cterm", "D", "E", "S", "T")),
  tolerance=25e-6)
```

Source

Subset taken from the topdownr::topdownrdata-package package.

topdownr-deprecated  Deprecated functions in topdownr

Description

These functions are provided for compatibility with older versions of ‘MyPkg’ only, and will be defunct at the next release.
Details

The following functions are deprecated and will be made defunct; use the replacement indicated below:

- defaultMs1Settings: expandMs1Conditions() in combination with createExperimentsFragmentOptimisation()
- defaultMs2Settings: expandTms2Conditions() in combination with createExperimentsFragmentOptimisation()

TopDownSet-class

The TopDownSet class

Description

The TopDownSet class is a container for a whole top-down proteomics experiment.

Usage

```r
## S4 method for signature 'TopDownSet'
aggregate(x, by = x$Sample, ...)

## S4 method for signature 'TopDownSet,TopDownSet'
combine(x, y)

## S4 method for signature 'TopDownSet'
filterCv(object, threshold, by = object$Sample, ...)

## S4 method for signature 'TopDownSet'
filterInjectionTime(
  object,
  maxDeviation = log2(3),
  keepTopN = 2,
  by = object$Sample,
...
)

## S4 method for signature 'TopDownSet'
filterIntensity(object, threshold, relative = TRUE, ...)

## S4 method for signature 'TopDownSet'
filterNonReplicatedFragments(object, minN = 2, by = object$Sample, ...)

## S4 method for signature 'TopDownSet'
normalize(object, method = "TIC", ...)

## S4 method for signature 'TopDownSet,missing'
plot(x, y, ..., verbose = interactive())
```
## S4 method for signature 'TopDownSet'
show(object)

## S4 method for signature 'TopDownSet'
summary(object, what = c("conditions", "fragments"), ...)

**Arguments**

- **x, object**: TopDownSet
- **by**: list, grouping variable, in general it refers to technical
- **y**: missing, not used.
- **threshold**: double, threshold variable.
- **maxDeviation**: double, maximal allowed deviation in the \( \log_2 \) injection time in ms in comparison to the median ion injection time.
- **keepTopN**: integer, how many technical replicates should be kept?
- **relative**: logical, if relative is TRUE all fragments with intensity below \( \text{threshold} \times \max(\text{intensity}) \) per fragment are removed, otherwise all fragments below \( \text{threshold} \) are removed.
- **minN**: numeric, if less than \( \minN \) of a fragment are found across technical replicates it is removed.
- **method**: character, normalisation method, currently just "TIC" for Total Ion Current normalisation of the scans/conditions (column-wise normalisation) is supported.
- **verbose**: logical, verbose output?
- **what**: character, specifies whether "conditions" (columns; default) or "fragments" (rows) should be summarized.
- **...**: arguments passed to internal/other methods. replicates (that’s why the default is the "Sample" column in colData).

**Details**

See vignette("analysis", package="topdownr") for a detailed example how to work with TopDownSet objects.

**Value**

An TopDownSet object.

**Methods (by generic)**

- **aggregate(TopDownSet)**: Aggregate conditions/runs.
  
  Aggregates conditions/runs (columns) in an TopDownSet object by a user-given value (default is the "Sample" column of colData which has the same value for technical replicates). It combines intensity values and numeric metadata of the grouped conditions/runs (columns) by mean and returns a reduced TopDownSet object.
combine(x = TopDownSet, y = TopDownSet): Combine TopDownSet objects.

combine allows to combine two or more TopDownSet objects. Please note that it uses the
default sampleColumns to define technical replicates (see readTopDownFiles()) and the
default by argument to group ion injection times for the calculation of the median time (see
updateMedianInjectionTime()). Both could be modified after combine by calling updateConditionNames()
(with modified sampleColumns argument) and updateMedianInjectionTime() (with mod-
ified by argument).

filterCv(TopDownSet): Filter by CV.

Filtering is done by coefficient of variation across technical replicates (defined by the by argu-
ment). All fragments below a given threshold are removed. The threshold is the maximal
allowed CV in percent (sd/mean * 100 < threshold).

filterInjectionTime(TopDownSet): Filter by ion injection time.

Filtering is done by maximal allowed deviation and just the technical keepTopN replicates
with the lowest deviation from the median ion injection time are kept.

filterIntensity(TopDownSet): Filter by intensity.

Filtering is done by removing all fragments that are below a given (absolute/relative) intensity
threshold.

filterNonReplicatedFragments(TopDownSet): Filter by non-replicated fragments.

Filtering is done by removing all fragments that don’t replicate across technical replicates.

normalize(TopDownSet): Normalise.

Applies Total Ion Current normalisation to a TopDownSet object. The normalisation ist done
per scans/conditions (column-wise normalisation).

plot(x = TopDownSet, y = missing): Plotting.

Plots an TopDownSet object. The function returns a list of ggplot objects (one item per
condition). Use pdf or another non-interactive device to plot the list of ggplot objects (see
example section).

summary(TopDownSet): Summary statistics.

Returns a matrix with some statistics: number of fragments, total/min/first quartile/median/mean/third
quartile/maximum of intensity values.

Slots

rowViews FragmentViews, information about fragments (name, type, sequence, mass, charge), see
FragmentViews for details.

colData S4Vectors::DataFrame, information about the MS2 experiments and the fragmentation
conditions.

assay Matrix::dgCMatrix, intensity values of the fragments. The rows correspond to the fragments
and the columns to the condition/run. It just stores values that are different from zero.

files character, files that were imported.

tolerance double, tolerance in ppm that were used for matching the experimental mz values to
the theoretical fragments.

redundantMatching character, matching strategies for redundant ion/fragment matches. See
redundantIonMatch and redundantFragmentMatch in readTopDownFiles() for details.

processing character, log messages.
Coercion

`as(object, "MSnSet")`: Coerce an `TopDownSet` object into an `MSnbase::MSnSet` object.

`as(object, "NCBSet")`: Coerce an `TopDownSet` object into an `NCBSet` object.

Author(s)

Sebastian Gibb <mail@sebastiangibb.de>

See Also

- `FragmentViews` for the row view interface.
- `Matrix::dgCMatrix` for technical details about the intensity storage.
- `?vignette("analysis", package="topdownr")` for a full documented example of an analysis using `topdownr`.

Examples

```r
## Example data
data(tds, package="topdownr")

tds

head(summary(tds))

# Accessing slots
rowViews(tds)
colData(tds)
head(assayData(tds))

# Accessing colData
tds$Mz
tds$FilterString

# Subsetting

# First 100 fragments
tds[1:100]

# All c fragments
tds["c"]

# Just c 152
tds["c152"]

# Condition 1 to 10
tds[, 1:10]

# Filtering
# Filter all intensities that don't have at least 10 % of the highest
# intensity per fragment.
```
validMs1Settings

List valid MS settings.

Description
These functions list settings for MS1 or TMS2 that are supported by Thermo’s XmlMethodChanger.

Usage
validMs1Settings(family = "Calcium", version = "3.2")
validTms2Settings
writeMethodXmls

```r
type = c("All", "TMS2", "ETD", "CID", "HCD", "UVPD"),
family = "Calcium",
version = "3.2"
)
```

**Arguments**

- `family` character, currently just Calcium is supported
- `version` character, currently 3.1, 3.2 (default), 3.3 are supported
- `type` character, type of activation.

**Value**

matrix with three columns:

- name: element name
- class: expected R class of the value
- type: MS/ActivationType, e.g. MS1/TMS2/ETD/...

**Examples**

```r
validMs1Settings()
validTms2Settings()
validTms2Settings("TMS2")
validTms2Settings("ETD")
validTms2Settings(c("TMS2", "ETD"))
```

---

**writeMethodXmls**

*Create Orbitrap Fusion Lumos method.xml files.*

**Description**

This function is used to create Orbitrap Fusion Lumos method files from a tree-like list experiment generated by e.g. `createExperimentsFragmentOptimisation()`.

**Usage**

```r
writeMethodXmls(exps, pattern = "method-%s.xml", verbose = interactive())
```

**Arguments**

- `exps` list, generated by e.g. `createExperimentsFragmentOptimisation()`
- `pattern` character, file name pattern for the method.xml files.
- `verbose` logical, verbose output?
Details

- **exps**: a named tree-like list object generated by e.g. `createExperimentsFragmentOptimisation()`. Its names are used as filename.
- **pattern**: The file name pattern used to name different method files. It must contain a "%s" that is replaced by the conditions defined in `groupBy`.

**DEFUNCT options:**

- **ms1Settings**: A list of MS1 settings. This has to be a named list. Valid MS1 settings are:
  
  ```
  c("FirstMass", "LastMass", "Microscans", "MaxITTimeInMS", "AgcTarget")
  ```

- **ms2Settings**: A list of MS2 settings. This has to be a named list. Valid MS2 settings are:
  
  ```
  c("ActivationType", "IsolationWindow", "EnableMultiplexIons", 
  "EnableMSXIds", "MaxNoOfMultiplexIons", "OrbitrapResolution", 
  "AgcTarget", "MinAgcTarget", "MaxITTimeInMS", 
  "Microscans", "ETDReactionTime", "ETDReagentTarget", 
  "MaximumETDReagentInjectionTime", "UseInternalCalibratedETD", 
  "ETDSupplementalActivationEnergy", "ETDSupplementalActivation")
  ```

- **groupBy**: The `groupBy` parameter is used to split methods into different files. Valid entries are all settings that could be used in `ms2Settings` and "replication".

- **massLabeling**: The Orbitrap Fusion devices seems not to respect the start and end times of the runs given in the method.xml files. That's why it is nearly impossible to identify the run with its conditions based on the timings. If `massLabeling` is `TRUE` (default) the mass values given in `mz` are rounded to the first decimal place and the second to fourth decimal place is used as numeric identifier.

**Author(s)**

Sebastian Gibb <mail@sebastiangibb.de>, Pavel V. Shliaha <pavels@bmb.sdu.dk>

**See Also**

`createExperimentsFragmentOptimisation()`

**Examples**

```r
ms1 <- expandMs1Conditions(FirstMass=400, LastMass=1200, Microscans=as.integer(10))

targetMz <- cbind(mz=c(560.6, 700.5, 933.7), z=rep(1, 3))
common <- list(
  OrbitrapResolution="R120K", 
  IsolationWindow=1, 
  MaxITTimeInMS=200, 
  Microscans=as.integer(40), 
  AgcTarget=c(1e5, 5e5, 1e6)
)

cid <- expandTms2Conditions(
  MassList=targetMz, 
  common, 
  ActivationType="CID", 
  CIDCollisionEnergy=seq(7, 35, 7)
)

hcd <- expandTms2Conditions(
```

```r
writeMethodXmls

MassList=targetMz,
common,
ActivationType="HCD",
HCDCollisionEnergy=seq(7, 35, 7)
)

etd <- expandTms2Conditions(
    MassList=targetMz,
    common,
    ActivationType="ETD",
    ETDReagentTarget=c(1e6, 5e6, 1e7),
    ETDReactionTime=c(2.5, 5, 10, 15, 30, 50),
    ETDSupplementalActivation=c("None", "ETciD", "EThcD"),
    ETDSupplementalActivationEnergy=seq(7, 35, 7)
)

exps <- createExperimentsFragmentOptimisation(ms1=ms1, cid, hcd, etd,
    groupBy=c("AgcTarget", "replication"), nMs2perMs1=10, scanDuration=0.5,
    replications=2, randomise=TRUE
)

writeMethodXmls(exps=exps)
```
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