# Package 'FRASER'

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**Type** Package

**Title** Find RAre Splicing Events in RNA-Seq Data

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**Description** Detection of rare aberrant splicing events in transcriptome profiles. Read count ratio expectations are modeled by an autoencoder to control for confounding factors in the data. Given these expectations, the ratios are assumed to follow a beta-binomial distribution with a junction specific dispersion. Outlier events are then identified as read-count ratios that deviate significantly from this distribution. FRASER is able to detect alternative splicing, but also intron retention. The package aims to support diagnostics in the field of rare diseases where RNA-seq is performed to identify aberrant splicing defects.

**biocViews** RNASeq, AlternativeSplicing, Sequencing, Software, Genetics, Coverage

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**BugReports** https://github.com/gagneurlab/FRASER/issues

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**LinkingTo** RcppArmadillo, Rcpp

**Collate** variables.R getNSetterFuns.R FRASER-package.R FraserDataSet-class.R AllGenerics-definitions.R AllGenerics.R Fraser-pipeline.R annotationOfRanges.R beta-binomial-testing.R calculatePSIValue.R countRNAseqData.R example_functions.R filterExpression.R find_encoding_dimensions.R getURLs.R helper-functions.R mergeExternalData.R saveHDF5Objects.R RcppExports.R autoencoder.R updateD.R updateE.R updateRho.R pvalsNzscore.R makeSimulatedDataset.R fitCorrectionMethods.R plotMethods.R resultAnnotations.R zzz.R

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---

`annotateRanges` *Annotates the given FraserDataSet with the HGNC symbol with biomaRt*

---

## Description

Annotates the given FraserDataSet with the HGNC symbol with biomaRt

## Usage

```
annotateRanges(
  fds,
  feature = "hgnc_symbol",
  featureName = feature,
  biotype = list("protein_coding"),
  ensembl = NULL,
  GRCh = 37
)

annotateRangesWithTxDb(
  fds,
  feature = "SYMBOL",
  featureName = "hgnc_symbol",
  keytype = "ENTREZID",
  txdb = NULL,
  orgDb = NULL,
  filter = list()
)
```

## Arguments

| | |
|---|---|
| fds | FraserDataSet |
| feature | Defines which feature (default is HGNC symbol) should be annotated. Has to be the biomaRt feature name or a column name in orgDb. |
| featureName | The column name of the feature in the FraserDataSet mcols. |
| biotype | The biotype for biomaRt. |
| ensembl | The ensembl that should be used. If NULL, the default one is used (hsapiens_gene_ensembl, GRCh37). |
| GRCh | GRCh version to connect to. If this is NULL, then the current GRCh38 is used. Otherwise, this can only be 37 (default) at the moment (see useEnsembl). |
| keytype | The keytype or column name of gene IDs in the TxDb object (see keytypes for a list of available ID types). |
| txdb | A TxDb object. If this is NULL, then the default one is used, currently this is TxDb.Hsapiens.UCSC.hg19.knownGene. |
| orgDb | An orgDb object or a data table to map the feature names. If this is NULL, then org.Hs.eg.db is used as the default. |
| filter | A named list specifying the filters which should be applied to subset to e.g. only protein-coding genes for annotation. names(filter) needs to be column names in the given orgDb object (default: no filtering). |

## Value

FraserDataSet

## Examples

```
fds <- createTestFraserDataSet()

### Two ways to annotage ranges with gene names:
# either using biomart with GRCh38
try({
  fds <- annotateRanges(fds, GRCh=38)
  rowRanges(fds, type="j")[,c("hgnc_symbol")]
})

# either using biomart with GRCh37
try({
  fds <- annotateRanges(fds, featureName="hgnc_symbol_37", GRCh=37)
  rowRanges(fds, type="j")[,c("hgnc_symbol_37")]
})

# or with a provided TxDb object
require(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
require(org.Hs.eg.db)
orgDb <- org.Hs.eg.db
fds <- annotateRangesWithTxDb(fds, txdb=txdb, orgDb=orgDb)
```

```
rowRanges(fds, type="j")[,"hgnc_symbol"]
```

---

## assayNames,FraserDataSet-method

*Returns the assayNames of FRASER*

---

### Description

Returns the assayNames of FRASER

### Usage

```
## S4 method for signature 'FraserDataSet'
assayNames(x)
```

### Arguments

| | |
|---|---|
| x | FraserDataSet |

### Value

Character vector

---

## assays,FraserDataSet-method

*Returns the assay for the given name/index of the FraserDataSet*

---

### Description

Returns the assay for the given name/index of the FraserDataSet

### Usage

```
## S4 method for signature 'FraserDataSet'
assays(x, withDimnames = TRUE, ...)

## S4 replacement method for signature 'FraserDataSet,SimpleList'
assays(x, withDimnames = TRUE, HDF5 = TRUE, type = NULL, ...) <- value

## S4 replacement method for signature 'FraserDataSet,list'
assays(x, withDimnames = TRUE, HDF5 = TRUE, type = NULL, ...) <- value

## S4 replacement method for signature 'FraserDataSet,DelayedMatrix'
assays(x, withDimnames = TRUE, HDF5 = TRUE, type = NULL, ...) <- value
```

### Arguments

| | |
|---|---|
| x | FraserDataSet |
| withDimnames | Passed on to SummarizedExperiment::assays() |
| ... | Parameters passed on to SummarizedExperiment::assays() |
| HDF5 | Logical value indicating whether the assay should be stored as a HDF5 file. |
| type | The psi type. |
| value | The new value to which the assay should be set. |

### Value

(Delayed) matrix.

---

## calculatePSIValues *PSI value calculation*

---

### Description

This function calculates the PSI values for each junction and splice site based on the FraserDataSet object

### Usage

```
calculatePSIValues(
  fds,
  types = psiTypes,
  overwriteCts = FALSE,
  BPPARAM = bpparam()
)
```

### Arguments

| | |
|---|---|
| fds | A FraserDataSet object |
| types | A vector with the psi types which should be calculated. Default is all of jaccard, psi5, psi3 and theta. |
| overwriteCts | FALSE or TRUE (the default) the total counts (aka N) will be recalculated based on the existing junction counts (aka K) |
| BPPARAM | the BiocParallel parameters for the parallelization |

### Value

FraserDataSet

## Examples

```
fds <- createTestFraserDataSet()
fds <- calculatePSIValues(fds, types="jaccard")

### usually one would run this function for all psi types by using:
# fds <- calculatePSIValues(fds)
```

---

countRNA *Count RNA-seq data*

---

## Description

The FRASER package provides multiple functions to extract and count both split and non-spliced reads from bam files. See Detail and Functions for more information.

## Usage

```
countRNAData(
  fds,
  NcpuPerSample = 1,
  minAnchor = 5,
  recount = FALSE,
  BPPARAM = bpparam(),
  genome = NULL,
  junctionMap = NULL,
  filter = TRUE,
  minExpressionInOneSample = 20,
  keepNonStandardChromosomes = TRUE,
  countDir = file.path(workingDir(fds), "savedObjects", nameNoSpace(name(fds))),
  ...
)

getSplitReadCountsForAllSamples(
  fds,
  NcpuPerSample = 1,
  junctionMap = NULL,
  recount = FALSE,
  BPPARAM = bpparam(),
  genome = NULL,
  countFiles = NULL,
  keepNonStandardChromosomes = TRUE,
  outDir = file.path(workingDir(fds), "savedObjects", nameNoSpace(name(fds)),
    "splitCounts")
)

getNonSplitReadCountsForAllSamples(
  fds,
```

```
  splitCountRanges,
  NcpuPerSample = 1,
  minAnchor = 5,
  recount = FALSE,
  BPPARAM = bpparam(),
  longRead = FALSE,
  outDir = file.path(workingDir(fds), "savedObjects", nameNoSpace(name(fds)),
    "nonSplitCounts")
)

addCountsToFraserDataSet(fds, splitCounts, nonSplitCounts)

countSplitReads(
  sampleID,
  fds,
  NcpuPerSample = 1,
  genome = NULL,
  recount = FALSE,
  keepNonStandardChromosomes = TRUE,
  bamfile = bamFile(fds[, sampleID]),
  pairedend = pairedEnd(fds[, sampleID]),
  strandmode = strandSpecific(fds[, sampleID]),
  cacheFile = getSplitCountCacheFile(sampleID, fds),
  scanbamparam = scanBamParam(fds),
  coldata = colData(fds)
)

mergeCounts(
  countList,
  fds,
  junctionMap = NULL,
  assumeEqual = FALSE,
  spliceSiteCoords = NULL,
  BPPARAM = SerialParam()
)

countNonSplicedReads(
  sampleID,
  splitCountRanges,
  fds,
  NcpuPerSample = 1,
  minAnchor = 5,
  recount = FALSE,
  spliceSiteCoords = NULL,
  longRead = FALSE
)
```

## Arguments

| | |
|---|---|
| fds | A FraserDataSet object |
| NcpuPerSample | A BiocParallel param object or a positive integer to configure the parallel backend of the internal loop per sample |
| minAnchor | Minimum overlap around the Donor/Acceptor for non spliced reads. Default to 5 |
| recount | if TRUE the cache is ignored and the bam file is recounted. |
| BPPARAM | the BiocParallel parameters for the parallelization |
| genome | NULL (default) or a character vector specifying the names of the reference genomes that were used to align the reads for each sample. The names have to be in a way accepted by the getBSgenome function. Available genomes can be listed using the available.genomes function from the BSgenome package. If genome is of length 1, the same reference genome will be used for all samples. If genome is supplied and strandSpecific(fds) == 0L (unstranded), then the strand information will be estimated by checking the dinucleotides found at the intron boundaries (see summarizeJunctions in GenomicAlignments package for details). This can e.g. help to avoid ambiguities when adding gene names from a gene annotation to the introns in a later step. |
| junctionMap | A object or file containing a map of all junctions of interest across all samples |
| filter | If TRUE, splice sites of introns with low read support in all samples are not considered when calculating the non-split reads. This helps to speed up the subsequent steps. |
| minExpressionInOneSample | The minimal split read count in at least one sample that is required for an intron to pass the filter. |
| keepNonStandardChromosomes | Logical value indicating if non standard chromosomes should also be counted. Defaults to TRUE. |
| countDir | The directory in which the tsv containing the position and counts of the junctions should be placed. |
| ... | Further parameters passed on to Rsubread::featureCounts. |
| countFiles | If specified, the split read counts for all samples are read from the specified files. Should be a vector of paths to files containing the split read counts for the individual samples. Reading from files is only supported for tsv(.gz) or RDS files containing GRranges objects. The order of the individual sample files should correspond to the order of the samples in the fds. |
| outDir | The full path to the output folder containing the merged counts. If the given folder already exists and stores a SummarizedExperiment object, the counts from this folder will be read in and used in the following (i.e. the reads are not recounted), unless the option recount=TRUE is used. If this folder doesn't exist or if recount=TRUE, then it will be created after counting has finished. |
| splitCountRanges | The merged GRanges object containing the positions of all the introns in the dataset over all samples. |

| | |
|---|---|
| longRead | If TRUE, then the isLongRead option of Rsubread::featureCounts is used when counting the non spliced reads overlapping splice sites. |
| splitCounts | The SummarizedExperiment object containing the position and counts of all the introns in the dataset for all samples. |
| nonSplitCounts | The SummarizedExperiment object containing the position and non split read counts of all splice sites present in the dataset for all samples. |
| sampleID | The ID of the sample to be counted. |
| bamfile | The BAM file to be used to extract the counts. Defaults to the BAM file defined in the FraserDataSet object. |
| pairedend | TRUE or FALSE if the BAM file is paired end. Defaults to the value specified in the FraserDataSet object. |
| strandmode | 0 (no, default), 1 (stranded), or 2 (revers) to specify the used protocol for the RNA-seq experiment. |
| cacheFile | File path to the cache, where counts are stored. |
| scanbamparam | The ScanBamParam object which is used for loading the reads from the BAM file before counting. Defaults to the params stored in the FraserDataSet object. |
| coldata | The colData as given by the FraserDataSet object. |
| countList | A list of GRanges objects containing the counts that should be merged into one object. |
| assumeEqual | Logical indicating whether all objects in countList can be assumed to contain counts for the same ranges. If FALSE, merging of the ranges is performed. |
| spliceSiteCoords | A GRanges object containing the positions of the splice sites. If it is NULL, then splice sites coordinates are calculated first based on the positions of the junctions defined from the split reads. |

## Details

The functions described in this file extract and count both the split and the non-spliced reads from bam files.

countRNAData is the main function that takes care of all counting steps and returns a FraserDataSet containing the counts for all samples in the fds.

getSplitReadCountsForAllSamples counts split reads for all samples and getNonSplitReadCountsForAllSamples counts non split reads overlapping splice sites for all samples. addCountsToFraserDataSet adds these counts to an existing fds.

countSplitReads calculates the split read counts for a single sample. countNonSplicedReads counts the non split reads overlapping with splice sites for a single sample.

mergeCounts merges the counts from different samples into a single count object, where the counts for junctions that are not present in a sample are set to zero.

## Value

countRNAData returns a FraserDataSet.

getSplitReadCountsForAllSamples returns a GRanges object.

getNonSplitReadCountsForAllSamples returns a GRanges object.

addCountsToFraserDataSet returns a FraserDataSet.

countSplitReads returns a GRanges object.

mergeCounts returns a SummarizedExperiment object.

countNonSplicedReads returns a GRanges object.

## Functions

- countRNAData(): This method extracts and counts the split reads and non spliced reads from RNA bam files.
- getSplitReadCountsForAllSamples(): This method creates a GRanges object containing the split read counts from all specified samples.
- getNonSplitReadCountsForAllSamples(): This method creates a GRanges object containing the non split read counts at the exon-intron boundaries inferred from the GRanges object containing the positions of all the introns in this dataset.
- addCountsToFraserDataSet(): This method adds the split read and non split read counts to a existing FraserDataSet containing the settings.
- countSplitReads(): This method counts all split reads in a bam file for a single sample.
- mergeCounts(): This method merges counts for multiple samples into one SummarizedExperiment object.
- countNonSplicedReads(): This method counts non spliced reads based on the given target (acceptor/donor) regions for a single sample.

## Examples

```
# On Windows SNOW is the default for the parallele backend, which can be
# very slow for many but small tasks. Therefore, we will use
# for the example the SerialParam() backend.
if(.Platform$OS.type != "unix") {
    register(SerialParam())
}

fds <- countRNAData(createTestFraserSettings())
```

---

`createTestFraserSettings` *Create a test dataset*

---

## Description

Create a test case dataset based on the test sample annotation to be used in the vignette and to explore the functionality of the FRASER package. Dependent on the request only the sample annotation or a full fitted model is returned.

## Usage

```
createTestFraserSettings(workingDir = "FRASER_output")

createTestFraserDataSet(
  workingDir = "FRASER_output",
  rerun = FALSE,
  metrics = "jaccard"
)
```

## Arguments

| | |
|---|---|
| workingDir | Directory where to store HDF5 and RDS files. Defaults to FRASER_output in the current working directory. |
| rerun | Defaults to FALSE. If set to TRUE it reruns the full fit of the model. |
| metrics | The splice metrics that should be included in the test fds. One or several of 'jaccard', 'psi5', 'psi3' or 'theta'. |

## Value

A FraserDataSet object that contains a test case

## Examples

```
fds <- createTestFraserSettings()
fds

fds <- createTestFraserDataSet()
fds
```

---

# estimateBestQ *Find optimal encoding dimension*

---

## Description

Finds the optimal encoding dimension by either Optimal Hard Thresholding or injecting artificial splicing outlier ratios while maximizing the precision-recall curve.

## Usage

```
estimateBestQ(
  fds,
  type = "jaccard",
  useOHT = TRUE,
  implementation = "PCA",
  q_param = getEncDimRange(fds),
  noise_param = 0,
```

```
  minDeltaPsi = 0.1,
  iterations = 5,
  setSubset = 50000,
  injectFreq = 0.01,
  BPPARAM = bpparam(),
  internalThreads = 1,
  plot = TRUE,
  delayed = ifelse(ncol(fds) <= 300, FALSE, TRUE),
  ...
)
```

## Arguments

| | |
|---|---|
| fds | A FraserDataSet object |
| type | The type of PSI (jaccard, psi5, psi3 or theta for theta/splicing efficiency) |
| useOHT | If TRUE (default), Optimal Hard Thresholding (OHT) is used to estimate the optimal encoding dimension. OHT is only supported for the Intron Jaccard Index. |
| implementation | The method that should be used to correct for confounders. |
| q_param | Vector specifying which values of q should be tested |
| noise_param | Vector specifying which noise levels should be tested. |
| minDeltaPsi | Minimal delta psi of an intron to be be considered a variable intron. |
| iterations | The maximal number of iterations. When the autoencoder has not yet converged after these number of iterations, the fit stops anyway. |
| setSubset | The size of the subset of the most variable introns that should be used for the hyperparameter optimization. |
| injectFreq | The frequency with which outliers are injected into the data. |
| BPPARAM | the BiocParallel parameters for the parallelization |
| internalThreads | The number of threads used internally. |
| plot | If TRUE, a plot of the singular values (OHT) or the area under the curve and the model loss for each evaluated parameter combination (grid-search) will be displayed after the estimation procedure. |
| delayed | If FALSE, count matrices will be loaded into memory (faster calculations), otherwise the function works on the delayedMatrix representations (more memory efficient). The default value depends on the number of samples in the fds-object. |
| ... | Additional parameters passed to injectOutliers. |

## Value

FraserDataSet

## See Also

FRASER

## Examples

```
# generate data
fds <- makeSimulatedFraserDataSet(m=15, j=20)
fds <- calculatePSIValues(fds)

# run OHT
fds <- estimateBestQ(fds, type="jaccard", useOHT=TRUE)

# run hyperparameter optimization
fds <- estimateBestQ(fds, type="jaccard", useOHT=FALSE, q_param=c(2, 5))

# get estimated optimal dimension of the latent space
bestQ(fds, type="jaccard")
hyperParams(fds, type="jaccard")
```

---

`filterVariability` *Filtering FraserDataSets*

---

## Description

This method can be used to filter out introns that are not reliably detected and to remove introns with no variablity between samples.

## Usage

```
filterVariability(object, ...)

filterExpressionAndVariability(
  object,
  minExpressionInOneSample = 20,
  quantile = 0.75,
  quantileMinExpression = 10,
  minDeltaPsi = 0,
  filter = TRUE,
  delayed = ifelse(ncol(object) <= 300, FALSE, TRUE),
  filterOnJaccard = TRUE,
  BPPARAM = bpparam()
)

## S4 method for signature 'FraserDataSet'
filterExpression(
  object,
  minExpressionInOneSample = 20,
  quantile = 0.75,
  quantileMinExpression = 10,
  filter = TRUE,
  delayed = ifelse(ncol(object) <= 300, FALSE, TRUE),
```

```
  filterOnJaccard = TRUE,
  BPPARAM = bpparam()
)

## S4 method for signature 'FraserDataSet'
filterVariability(
  object,
  minDeltaPsi = 0,
  filter = TRUE,
  delayed = ifelse(ncol(object) <= 300, FALSE, TRUE),
  filterOnJaccard = TRUE,
  BPPARAM = bpparam()
)
```

## Arguments

| | |
|---|---|
| object | A FraserDataSet object |
| ... | Further parameters passed on to Rsubread::featureCounts. |
| minExpressionInOneSample | The minimal read count in at least one sample that is required for an intron to pass the filter. |
| quantile | Defines which quantile should be considered for the filter. |
| quantileMinExpression | The minimum read count an intron needs to have at the specified quantile to pass the filter. |
| minDeltaPsi | Only introns for which the maximal difference in the psi value of a sample to the mean psi of the intron is larger than this value pass the filter. |
| filter | If TRUE, a subsetted fds containing only the introns that passed all filters is returned. If FALSE, no subsetting is done and the information of whether an intron passed the filters is only stored in the mcols. |
| delayed | If FALSE, count matrices will be loaded into memory, otherwise the function works on the delayedMatrix representations. The default value depends on the number of samples in the fds-object. |
| filterOnJaccard | If TRUE, the Intron Jaccard Metric is used to define express introns during filtering. Otherwise, the psi5, psi3 and theta metrics are used (default: TRUE). |
| BPPARAM | the BiocParallel parameters for the parallelization |

## Value

A FraserDataSet with information about which junctions passed the filters. If `filter=TRUE`, the filtered FraserDataSet is returned.

## Functions

- `filterExpressionAndVariability()`: This functions filters out both introns with low read support and introns that are not variable across samples.

- `filterExpression(FraserDataSet)`: This function filters out introns and corresponding splice sites that have low read support in all samples.
- `filterVariability(FraserDataSet)`: This function filters out introns and corresponding splice sites that have low read support in all samples.

## Examples

```
fds <- createTestFraserDataSet()
fds <- filterExpressionAndVariability(fds, minDeltaPsi=0.1, filter=FALSE)
mcols(fds, type="jaccard")[, c(
        "maxCount", "passedExpression", "maxDJaccard", "passedVariability")]

plotFilterExpression(fds)
plotFilterVariability(fds)
```

---

`fit` *Fitting the denoising autoencoder*

---

## Description

This method corrects for confounders in the data and fits a beta-binomial distribution to the introns/splice sites.

For more details please see FRASER.

## Usage

```
## S3 method for class 'FraserDataSet'
fit(
  object,
  implementation = c("PCA", "PCA-BB-Decoder", "AE", "AE-weighted", "PCA-BB-full",
   "fullAE", "PCA-regression", "PCA-reg-full", "PCA-BB-Decoder-no-weights", "BB"),
  q,
  type = psiTypes,
  rhoRange = c(-30, 30),
  weighted = FALSE,
  noiseAlpha = 1,
  convergence = 1e-05,
  iterations = 15,
  initialize = TRUE,
  control = list(),
  BPPARAM = bpparam(),
  nSubset = 15000,
  minDeltaPsi = 0.1,
  ...
)
```

### Arguments

| | |
|---|---|
| object | A FraserDataSet object |
| implementation | The method that should be used to correct for confounders. |
| q | The encoding dimensions to be used during the fitting proceadure. Should be fitted using estimateBestQ if unknown. If a named vector is provided it is used for the different splicing types. |
| type | The type of PSI (jaccard, psi5, psi3 or theta for theta/splicing efficiency) |
| rhoRange | Defines the range of values that rho parameter from the beta-binomial distribution is allowed to take. For very small values of rho, the loss can be instable, so it is not recommended to allow rho < 1e-8. |
| weighted | If TRUE, the weighted implementation of the autoencoder is used |
| noiseAlpha | Controls the amount of noise that is added for the denoising autoencoder. |
| convergence | The fit is considered to have converged if the difference between the previous and the current loss is smaller than this threshold. |
| iterations | The maximal number of iterations. When the autoencoder has not yet converged after these number of iterations, the fit stops anyway. |
| initialize | If FALSE and a fit has been previoulsy run, the values from the previous fit will be used as initial values. If TRUE, (re-)initialization will be done. |
| control | List of control parameters passed on to optim(). |
| BPPARAM | the BiocParallel parameters for the parallelization |
| nSubset | The size of the subset to be used in fitting if subsetting is used. |
| minDeltaPsi | Minimal delta psi of an intron to be be considered a variable intron. |
| ... | Currently not used |

### Value

FraserDataSet

### See Also

FRASER

---

FRASER *FRASER: Find RAre Splicing Events in RNA-seq data*

---

### Description

This help page describes the FRASER function which can be used run the default FRASER pipeline. This pipeline combines the beta-binomial fit, the computation of Z scores and p values as well as the computation of delta-PSI values.

## Usage

```
FRASER(
  fds,
  q,
  type = fitMetrics(fds),
  implementation = c("PCA", "PCA-BB-Decoder", "AE-weighted", "AE", "BB"),
  iterations = 15,
  BPPARAM = bpparam(),
  correction,
  subsets = NULL,
  ...
)

calculateZscore(fds, type = currentType(fds), logit = TRUE)

calculatePvalues(
  fds,
  type = currentType(fds),
  implementation = "PCA",
  BPPARAM = bpparam(),
  distributions = c("betabinomial"),
  capN = 5 * 1e+05
)

calculatePadjValues(
  fds,
  type = currentType(fds),
  method = "BY",
  rhoCutoff = NA,
  geneLevel = TRUE,
  geneColumn = "hgnc_symbol",
  subsets = NULL,
  BPPARAM = bpparam()
)

calculatePadjValuesOnSubset(
  fds,
  genesToTest,
  subsetName,
  type = currentType(fds),
  method = "BY",
  geneColumn = "hgnc_symbol",
  BPPARAM = bpparam()
)
```

## Arguments

| | |
|---|---|
| fds | A FraserDataSet object |

| | |
|---|---|
| q | The encoding dimensions to be used during the fitting proceadure. Should be fitted using estimateBestQ if unknown. If a named vector is provided it is used for the different splicing types. |
| type | The type of PSI (jaccard, psi5, psi3 or theta for theta/splicing efficiency) |
| implementation | The method that should be used to correct for confounders. |
| iterations | The maximal number of iterations. When the autoencoder has not yet converged after these number of iterations, the fit stops anyway. |
| BPPARAM | A BiocParallel object to run the computation in parallel |
| correction | Deprecated. The name changed to implementation. |
| subsets | A named list of named lists specifying any number of gene subsets (can differ per sample). For each subset, FDR correction will be limited to genes in the subset, and the FDR corrected pvalues stored as an assay in the fds object in addition to the transcriptome-wide FDR corrected pvalues. See the examples for how to use this argument. |
| ... | Additional parameters passed on to the internal fit function |
| logit | Indicates if z scores are computed on the logit scale (default) or in the natural (psi) scale. |
| distributions | The distribution based on which the p-values are calculated. Possible are beta-binomial, binomial and normal. |
| capN | Counts are capped at this value to speed up the p-value calculation |
| method | The p.adjust method that should be used for genome-wide multiple testing correction. |
| rhoCutoff | The cutoff value on the fitted rho value (overdispersion parameter of the betabinomial) above which junctions are masked with NA during p value adjustment (default: NA, no masking). |
| geneLevel | Logical value indiciating whether gene-level p values should be calculated. Defaults to TRUE. |
| geneColumn | The column name of the column that has the gene annotation that will be used for gene-level pvalue computation. |
| genesToTest | A named list with the subset of genes to test per sample. The names must correspond to the sampleIDs in the given fds object. |
| subsetName | The name under which the resulting FDR corrected pvalues will be stored in metadata(fds). |

## Details

All computed values are returned as an FraserDataSet object. To have more control over each analysis step, one can call each function separately.

- fit to control for confounding effects and fit the beta binomial model parameters
- calculatePvalues to calculate the nominal p values
- calculatePadjValues to calculate adjusted p values (per sample)
- calculateZscore to calculate the Z scores

Available methods to correct for the confounders are currently: a denoising autoencoder with a BB loss ("AE" and "AE-weighted"), PCA ("PCA"), a hybrid approach where PCA is used to fit the latent space and then the decoder of the autoencoder is fit using the BB loss ("PCA-BB-Decoder"). Although not recommended, it is also possible to directly fit the BB distrbution to the raw counts ("BB").

## Value

FraserDataSet

## Functions

- `FRASER()`: This function runs the default FRASER pipeline combining the beta-binomial fit, the computation of Z scores and p values as well as the computation of delta-PSI values.
- `calculateZscore()`: This function calculates z-scores based on the observed and expected logit psi.
- `calculatePvalues()`: This function calculates two-sided p-values based on the beta-binomial distribution (or binomial or normal if desired). The returned p values are not yet adjusted with Holm's method per donor or acceptor site, respectively.
- `calculatePadjValues()`: This function adjusts the previously calculated p-values per sample for multiple testing. First, the previoulsy calculated junction-level p values are adjusted with Holm's method per donor or acceptor site, respectively. Then, if gene symbols have been annotated to junctions (and not otherwise requested), gene-level p values are computed.
- `calculatePadjValuesOnSubset()`: This function does FDR correction only for all junctions in a certain subset of genes which can differ per sample. Requires gene symbols to have been annotated to junctions. As with the full FDR correction across all junctions, first the previously calculated junction-level p values are adjusted with Holm's method per donor or acceptor site, respectively. Then, gene-level p values are computed.

## Author(s)

Christian Mertes <mertes@in.tum.de>

Ines Scheller <scheller@in.tum.de>

## See Also

fit

## Examples

```
# set default parallel backend
register(SerialParam())

# preprocessing
fds <- createTestFraserDataSet()

# filtering not expressed introns
fds <- calculatePSIValues(fds)
fds <- filterExpressionAndVariability(fds)
```

```
# Run the full analysis pipeline: fits distribution and computes p values
fds <- FRASER(fds, q=2, implementation="PCA")

# afterwards, the fitted fds-object can be saved and results can
# be extracted and visualized, see ?saveFraserDataSet, ?results and
# ?plotVolcano

# The functions run inside the FRASER function can also be directly
# run themselves.
# To directly run the fit function:
fds <- fit(fds, implementation="PCA", q=2, type="jaccard")

# To directly run the nomial and adjusted p value and z score
# calculation, the following functions can be used:
fds <- calculatePvalues(fds, type="jaccard")
head(pVals(fds, type="jaccard"))
fds <- calculatePadjValues(fds, type="jaccard", method="BY")
head(padjVals(fds, type="jaccard"))
fds <- calculateZscore(fds, type="jaccard")
head(zScores(fds, type="jaccard"))

# example of restricting FDR correction to subsets of genes of interest
genesOfInterest <- list("sample1"=c("TIMMDC1"), "sample2"=c("MCOLN1"))
fds <- calculatePadjValues(fds, type="jaccard",
                subsets=list("exampleSubset"=genesOfInterest))
padjVals(fds, type="jaccard", subsetName="exampleSubset")
padjVals(fds, type="jaccard", level="gene", subsetName="exampleSubset")
fds <- calculatePadjValues(fds, type="jaccard",
                subsets=list("anotherExampleSubset"=c("TIMMDC1")))
padjVals(fds, type="jaccard", subsetName="anotherExampleSubset")

# only adding FDR corrected pvalues on a subset without calculating
# transcriptome-wide FDR again:
fds <- calculatePadjValuesOnSubset(fds, genesToTest=genesOfInterest,
         subsetName="setOfInterest", type="jaccard")
padjVals(fds, type="jaccard", subsetName="setOfInterest")
```

---

`FraserDataSet` *The FRASER dataset object*

---

## Description

Constructs an FRASER object based on the given input. It can take only the annotation (colData) or count tables (junctions/spliceSites).

## Usage

```
FraserDataSet(colData = NULL, junctions = NULL, spliceSites = NULL, ...)
```

### Arguments

| | |
|---|---|
| colData | A DataFrame containing the annotation of the samples |
| junctions, spliceSites | A data.frame like object containing the raw counts for each junction or splice site. It requires the columns startID and endID for the junctions and spliceSiteID and type for the splice sites. Those columns identifies the corresponding splice site for the given junction and map to the splice site. For each sample the counts are saved in a corresponding column with the same name. It can also be a GRange object. |
| ... | Any parameters corresponding to the slots and their possible values. See FraserDataSet |

### Value

A FraserDataSet object.

### Author(s)

Christian Mertes <mertes@in.tum.de>

### Examples

```
fraser <- FraserDataSet()

# example sample annoation
sampleTable <- data.table::fread(system.file("extdata",
        "sampleTable_countTable.tsv", package="FRASER", mustWork=TRUE))

# get raw counts
junctionCts   <- data.table::fread(system.file("extdata",
        "raw_junction_counts.tsv.gz", package="FRASER", mustWork=TRUE))
spliceSiteCts <- data.table::fread(system.file("extdata",
        "raw_site_counts.tsv.gz", package="FRASER", mustWork=TRUE))

# create FRASER object
fds <- FraserDataSet(colData=sampleTable, junctions=junctionCts,
        spliceSites=spliceSiteCts, name="Example Dataset")
```

---

## FraserDataSet-class *FraserDataSet*

---

### Description

This class is designed to store the whole FRASER data set needed for an analysis of a disease cohort

### Author(s)

Christian Mertes <mertes@in.tum.de>

getter_setter_functions *Getter/Setter functions*

## Description

This is a collection of small accessor/setter functions for easy access to the values within the FRASER model.

## Usage

```
featureExclusionMask(fds, type = currentType(fds))

featureExclusionMask(fds, type = currentType(fds)) <- value

rho(fds, type = currentType(fds))

zScores(fds, type = currentType(fds), byGroup = FALSE, ...)

pVals(
  fds,
  type = currentType(fds),
  level = "site",
  filters = list(),
  dist = "BetaBinomial",
  ...
)

padjVals(
  fds,
  type = currentType(fds),
  dist = c("BetaBinomial"),
  level = "site",
  subsetName = NULL,
  filters = list(),
  ...
)

availableFDRsubsets(fds)

predictedMeans(fds, type = currentType(fds))

deltaPsiValue(fds, type = currentType(fds))

currentType(fds)

currentType(fds) <- value
```

```
fitMetrics(fds)

fitMetrics(fds) <- value

pseudocount(value = NULL)

hyperParams(fds, type = currentType(fds), all = FALSE)

bestQ(fds, type = currentType(fds))

dontWriteHDF5(fds)

dontWriteHDF5(fds) <- value

verbose(fds)

verbose(fds) <- value
```

## Arguments

| | |
|---|---|
| fds | An FraserDataSet object. |
| type | The type of psi (psi5, psi3 or theta) |
| value | The new value to be assigned. |
| byGroup | If TRUE, aggregation by donor/acceptor site will be done. |
| ... | Internally used parameters. |
| level | Indicates if the retrieved p values should be adjusted on the donor/acceptor site-level (default) or if unadjusted junction-level p values should be returned. |
| filters | A named list giving the filters that were applied for masking during p value correction. Used for storing and retrieving the correct set of requested p values. |
| dist | Distribution for which the p-values should be extracted. |
| subsetName | The name of a subset of genes of interest for which FDR corrected pvalues were previously computed. Default is NULL (using transcriptome-wide FDR corrected pvalues). |
| all | Logical value indicating whether `hyperParams(fds)` should return the results of all evaluated parameter combinations or only for the optimal parameter combination. |

## Value

A (delayed) matrix or vector dependent on the type of data retrieved.

## Functions

- `featureExclusionMask()`: Retrieves a logical vector indicating for each junction whether it is included or excluded during the fitting procedure.

- `featureExclusionMask(fds, type = currentType(fds)) <- value`: To remove certain junctions from being used in the train step of the encoding dimension we can set the `featureExclusion` vector to `FALSE`. This can be helpfull if we have local linkage between features which we do not want to model by the autoencoder.
- `rho()`: Returns the fitted rho values for the beta-binomial distribution
- `zScores()`: This returns the calculated z-scores.
- `pVals()`: This returns the calculated p-values.
- `padjVals()`: This returns the adjusted p-values.
- `availableFDRsubsets()`: This returns the names of FDR subsets for which adjusted p values have been calculated.
- `predictedMeans()`: This returns the fitted mu (i.e. psi) values.
- `deltaPsiValue()`: Returns the difference between the observed and the fitted psi values.
- `currentType()`: Returns the psi type that is used within several methods in the FRASER package (defaults to jaccard).
- `currentType(fds) <- value`: Sets the psi type that is to be used within several methods in the FRASER package.
- `fitMetrics()`: Returns the splice metrics that will be fitted (defaults to jaccard, used within several methods in the FRASER package).
- `fitMetrics(fds) <- value`: Sets the splice metrics that will be fitted (used within several methods in the FRASER package).
- `pseudocount()`: Sets and returns the pseudo count used within the FRASER fitting procedure.
- `hyperParams()`: This returns the results of OHT or the hyperparameter optimization. It returns NULL if `estimateBestQ` was not run yet.
- `bestQ()`: This returns the optimal latent space dimension estimated by OHT or a hyperparameter optimization. If `estimateBestQ` has not been run before, a simple estimate of about a tenth of the number of samples is returned.
- `dontWriteHDF5()`: Gets the current value of whether the assays should be stored as hdf5 files.
- `dontWriteHDF5(fds) <- value`: Sets whether the assays should be stored as hdf5 files.
- `verbose()`: Dependent on the level of verbosity the algorithm reports more or less to the user. 0 means being quiet and 10 means everything.
- `verbose(fds) <- value`: Sets the verbosity level to a value between 0 and 10. 0 means being quiet and 10 means reporting everything.

## Examples

```
fds <- createTestFraserDataSet()

# should assays be saved as hdf5?
dontWriteHDF5(fds)
dontWriteHDF5 <- TRUE

# get/set the splice metric for which results should be retrieved
currentType(fds) <- "jaccard"
```

```
currentType(fds)

# get fitted parameters
bestQ(fds)
predictedMeans(fds)
rho(fds)

# get statistics
pVals(fds)
padjVals(fds)

# zscore not calculated by default
fds <- calculateZscore(fds, type="jaccard")
zScores(fds)

# set and get pseudocount
pseudocount(4L)
pseudocount()

# retrieve or set a mask to exclude certain junctions in the fitting step
featureExclusionMask(fds, type="jaccard") <- sample(
        c(FALSE, TRUE), nrow(mcols(fds, type="jaccard")), replace=TRUE)
featureExclusionMask(fds, type="jaccard")

# controlling the verbosity level of the output of some algorithms
verbose(fds) <- 2
verbose(fds)
```

---

`injectOutliers` *Inject artificial outliers in an existing fds*

---

## Description

Inject artificial outliers in an existing fds

## Usage

```
injectOutliers(
  fds,
  type = psiTypes,
  freq = 0.001,
  minDpsi = 0.2,
  minCoverage = 2,
  deltaDistr = "uniformDistr",
  verbose = FALSE,
  method = c("samplePSI", "meanPSI", "simulatedPSI"),
  BPPARAM = bpparam()
)
```

### Arguments

| | |
|---|---|
| fds | FraserDataSet |
| type | The psi type |
| freq | The injection frequency. |
| minDpsi | The minimal delta psi with which outliers will be injected. |
| minCoverage | The minimal total coverage (i.e. N) required for a junction to be considered for injection of an outlier. |
| deltaDistr | The distribution from which the delta psi value of the injections is drawn (default: uniform distribution). |
| verbose | Should additional information be printed during computation? |
| method | Defines by which method the new psi of injections is computed, i.e. to which value the delta psi of the injection is added: "meanPSI" for adding to the mean psi of the junction over all samples or "samplePSI" to add to the psi value of the junction in the specific sample. "simulatedPSI" is only possible if a simulated dataset is used. |
| BPPARAM | A BiocParallel object to run the computation in parallel |

### Value

FraserDataSet

### Examples

```
# A generic dataset
fds <- makeSimulatedFraserDataSet()
fds <- calculatePSIValues(fds)
fds <- injectOutliers(fds, minDpsi=0.2, freq=1E-3)
```

---

## K *Getter/setter for count data*

---

### Description

Getter/setter for count data

setter for count data

### Usage

```
K(fds, type = currentType(fds))

N(fds, type = currentType(fds))

## S4 method for signature 'FraserDataSet'
counts(object, type = currentType(object), side = c("ofInterest", "otherSide"))
```

```
## S4 replacement method for signature 'FraserDataSet,ANY'
counts(
  object,
  type = currentType(object),
  side = c("ofInterest", "otherSide"),
  ...
) <- value
```

## Arguments

| | |
|---|---|
| fds, object | FraserDataSet |
| type | The psi type. |
| side | "ofInterest" for junction counts, "other" for sum of counts of all other junctions at the same donor site (psi5) or acceptor site (psi3), respectively. |
| ... | Further parameters that are passed to assays(object,...) |
| value | An integer matrix containing the counts. |

## Value

FraserDataSet

## Examples

```
fds <- createTestFraserDataSet()

counts(fds, side="ofInterest")
counts(fds, type="jaccard", side="other")
head(K(fds))
head(K(fds, type="psi5"))
head(K(fds, type="psi3"))
head(N(fds, type="theta"))
```

---

# length,FraserDataSet-method

*retrieve the length of the object (aka number of junctions)*

---

## Description

retrieve the length of the object (aka number of junctions)

## Usage

```
## S4 method for signature 'FraserDataSet'
length(x)
```

### Arguments

| | |
|---|---|
| x | FraserDataSet |

### Value

Length of the object.

---

`loadFraserDataSet` *Loading/Saving FraserDataSets*

---

### Description

This is a convenient function to load and save a FraserDataSet object. It looks and saves the FraserDataSet objects and HDF5 files on disk under the given working dir. Internally it uses HDF5 files for all assays.

### Usage

```
loadFraserDataSet(dir, name = NULL, file = NULL, upgrade = FALSE)

saveFraserDataSet(fds, dir = NULL, name = NULL, rewrite = FALSE)
```

### Arguments

| | |
|---|---|
| dir | A path where to save the objects (replaces the working directory) |
| name | The analysis name of the project (saved within the 'dir') |
| file | The file path to the fds-object.RDS file that should be loaded. |
| upgrade | Should the version of the loaded object be updated? |
| fds | A FraserDataSet object ot be saved |
| rewrite | logical if the object should be rewritten. This makes sense if you have filtered or subsetted the object and want to save only the subsetted version |

### Value

FraserDataSet

### Examples

```
fds <- createTestFraserSettings()
name(fds) <- "saveing_test"

# make sure the object is saved to disc
dontWriteHDF5(fds) <- FALSE
fdsSaved <- saveFraserDataSet(fds)
fdsSaved
```

```
# load object from disc
fdsLoaded <- loadFraserDataSet(dir=workingDir(fds), name=name(fds))
fdsLoaded

all.equal(fdsSaved, fdsLoaded)
```

---

`makeSimulatedFraserDataSet` *Create an simulated example data set for FRASER*

---

## Description

Simulates a data set based on random counts following a beta binomial (or Dirichlet-Multinomial) distribution.

## Usage

```
makeSimulatedFraserDataSet(
  m = 100,
  j = 500,
  q = 10,
  distribution = c("BB", "DM"),
  ...
)
```

## Arguments

| | |
|---|---|
| `m` | Number of simulated samples |
| `j` | Number of simulated junctions |
| `q` | number of simulated latent variables. |
| `distribution` | Either "BB" for a beta-binomial simulation or "DM" for a dirichlet-multinomial simulation. |
| `...` | Further arguments used to construct the FraserDataSet. |

## Value

An FraserDataSet containing an example dataset based on simulated data

## Examples

```
# A generic dataset
fds1 <- makeSimulatedFraserDataSet()
fds1

# A generic dataset with specificed sample size and injection method
fds2 <- makeSimulatedFraserDataSet(m=10, j=100, q=3)
fds2
```

`mergeExternalData` *Merge external data*

## Description

To boost its own sequencing data, one can download existing and precounted data. This function merges the existing `FraserDataSet` with external count data.

## Usage

```
mergeExternalData(fds, countFiles, sampleIDs, annotation = NULL)
```

## Arguments

| | |
|---|---|
| `fds` | A `FraserDataSet` |
| `countFiles` | A character vector of file names pointing to the external count data. The vector has to be names or the files have to start with `k_j`, `k_theta`, `n_psi3`, `n_psi5`, `n_theta`. |
| `sampleIDs` | The samples to be merged from the external data. |
| `annotation` | A sample annotation of the external data (optional). |

## Details

For more details on existing datasets have a look at: <https://github.com/gagneurlab/drop#datasets>

Since FRASER can not hand NA values, the merge will return only the intersecting regions and will drop any non overlapping features. This has to be kept in mind when analysing rare disease samples.

## Value

Merged `FraserDataSet` object.

## Examples

```
anno <- data.table::fread(system.file("extdata", "externalCounts",
        "annotation.tsv.gz", package="FRASER"))
ctsFiles <- list.files(full.names = TRUE, pattern="counts",
        system.file("extdata", "externalCounts", package="FRASER"))

fds <- createTestFraserDataSet()
fds_merged <- mergeExternalData(fds, ctsFiles, anno[,sampleID], anno)

K(fds, "psi5")
K(fds_merged, "psi5")
```

plotManhattan *Visualization functions for FRASER*

## Description

The FRASER package provides mutliple functions to visualize the data and the results of a full data set analysis.

Plots the p values over the delta psi values, known as volcano plot. Visualizes per sample the outliers. By type and aggregate by gene if requested.

Plot the number of aberrant events per samples

Plots the observed split reads of the junction of interest over all reads coming from the given donor/acceptor.

Plots the observed values of the splice metric across samples for a junction of interest.

Plots the expected psi value over the observed psi value of the given junction.

Plots the quantile-quantile plot

Histogram of the geometric mean per junction based on the filter status

Histogram of minimal delta psi per junction

Count correlation heatmap function

## Usage

```
plotManhattan(object, ...)

## S4 method for signature 'FraserDataSet'
plotVolcano(
  object,
  sampleID,
  type = fitMetrics(object),
  basePlot = TRUE,
  aggregate = FALSE,
  main = NULL,
  label = NULL,
  deltaPsiCutoff = 0.1,
  padjCutoff = 0.1,
  subsetName = NULL,
  ...
)

## S4 method for signature 'FraserDataSet'
plotAberrantPerSample(
  object,
  main,
  type = fitMetrics(object),
  padjCutoff = 0.1,
```

```
  deltaPsiCutoff = 0.1,
  aggregate = TRUE,
  subsetName = NULL,
  BPPARAM = bpparam(),
  ...
)

plotExpression(
  fds,
  type = fitMetrics(fds),
  idx = NULL,
  result = NULL,
  colGroup = NULL,
  basePlot = TRUE,
  main = NULL,
  label = "aberrant",
  subsetName = NULL,
  ...
)

plotSpliceMetricRank(
  fds,
  type = fitMetrics(fds),
  idx = NULL,
  result = NULL,
  colGroup = NULL,
  basePlot = TRUE,
  main = NULL,
  label = "aberrant",
  subsetName = NULL,
  ...
)

plotExpectedVsObservedPsi(
  fds,
  type = fitMetrics(fds),
  idx = NULL,
  result = NULL,
  colGroup = NULL,
  main = NULL,
  basePlot = TRUE,
  label = "aberrant",
  subsetName = NULL,
  ...
)

## S4 method for signature 'FraserDataSet'
plotQQ(
```

```
  object,
  type = NULL,
  idx = NULL,
  result = NULL,
  aggregate = FALSE,
  global = FALSE,
  main = NULL,
  conf.alpha = 0.05,
  samplingPrecision = 3,
  basePlot = TRUE,
  label = "aberrant",
  Ncpus = min(3, getDTthreads()),
  subsetName = NULL,
  ...
)

## S4 method for signature 'FraserDataSet'
plotEncDimSearch(object, type = psiTypes, plotType = c("sv", "auc", "loss"))

plotFilterExpression(
  fds,
  bins = 200,
  legend.position = c(0.8, 0.8),
  onlyVariableIntrons = FALSE
)

plotFilterVariability(
  fds,
  bins = 200,
  legend.position = c(0.8, 0.8),
  onlyExpressedIntrons = FALSE
)

## S4 method for signature 'FraserDataSet'
plotCountCorHeatmap(
  object,
  type = psiTypes,
  logit = FALSE,
  topN = 50000,
  topJ = 5000,
  minMedian = 1,
  minCount = 10,
  main = NULL,
  normalized = FALSE,
  show_rownames = FALSE,
  show_colnames = FALSE,
  minDeltaPsi = 0.1,
  annotation_col = NA,
```

```
  annotation_row = NA,
  border_color = NA,
  nClust = 5,
  plotType = c("sampleCorrelation", "junctionSample"),
  sampleClustering = NULL,
  plotMeanPsi = TRUE,
  plotCov = TRUE,
  ...
)

plotBamCoverage(
  fds,
  gr,
  sampleID,
 control_samples = sample(samples(fds[, which(samples(fds) != sampleID)]), min(3,
    ncol(fds) - length(sampleID))),
  txdb = NULL,
  min_junction_count = 20,
  highlight_range = NULL,
  highlight_range_color = "firebrick",
  color_annotated = "gray",
  color_novel = "goldenrod3",
  color_sample_interest = "firebrick",
  color_control_samples = "dodgerblue4",
  toscale = c("exon", "gene", "none"),
  mar = c(2, 10, 0.1, 5),
  curvature_splicegraph = 1,
  curvature_coverage = 1,
  cex = 1,
  splicegraph_labels = c("genomic_range", "id", "name", "none"),
  splicegraph_position = c("top", "bottom"),
  ...
)

plotBamCoverageFromResultTable(
  fds,
  result,
  show_full_gene = FALSE,
  txdb = NULL,
  orgDb = NULL,
  res_gene_col = "hgncSymbol",
  res_geneid_type = "SYMBOL",
  txdb_geneid_type = "ENTREZID",
  left_extension = 1000,
  right_extension = 1000,
  ...
)
```

```
## S4 method for signature 'FraserDataSet'
plotManhattan(
  object,
  sampleID,
  value = "pvalue",
  type = fitMetrics(object),
  chr = NULL,
  main = paste0("sample: ", sampleID),
  chrColor = c("black", "darkgrey"),
  subsetName = NULL,
  ...
)
```

## Arguments

| | |
|---|---|
| object, fds | An FraserDataSet object. |
| ... | Additional parameters passed to plot() or plot_ly() if not stated otherwise in the details for each plot function |
| sampleID | A sample ID which should be plotted. Can also be a vector. Integers are treated as indices. |
| type | The psi type: either psi5, psi3 or theta (for SE). |
| basePlot | if TRUE (default), use the R base plot version, else use the plotly framework. |
| aggregate | If TRUE, the pvalues are aggregated by gene (default), otherwise junction level pvalues are used (default for Q-Q plot). |
| main | Title for the plot, if missing a default title will be used. |
| label | Indicates the genes or samples that will be labelled in the plot (only for basePlot=TRUE). Setting label="aberrant" will label all aberrant genes or samples. Labelling can be turned off by setting label=NULL. The user can also provide a custom list of gene symbols or sampleIDs. |
| padjCutoff, deltaPsiCutoff | Significance or delta psi cutoff to mark outliers |
| subsetName | The name of a subset of genes of interest for which FDR corrected pvalues were previously computed. Those FDR values on the subset will then be used to determine aberrant status. Default is NULL (using transcriptome-wide FDR corrected pvalues). |
| BPPARAM | BiocParallel parameter to use. |
| idx | A junction site ID or gene ID or one of both, which should be plotted. Can also be a vector. Integers are treated as indices. |
| result | The result table to be used by the method. |
| colGroup | Group of samples that should be colored. |
| global | Flag to plot a global Q-Q plot, default FALSE |
| conf.alpha | If set, a confidence interval is plotted, defaults to 0.05 |
| samplingPrecision | Plot only non overlapping points in Q-Q plot to reduce number of points to plot. Defines the digits to round to. |

`Ncpus` Number of cores to use.

`plotType` The type of plot that should be shown as character string. For plotEncDimSearch, it has to be either `"sv"` for a plot of the singular values against their rank, `"auc"` for a plot of the area under the curve (AUC) or `"loss"` for the model loss. For the correlation heatmap, it can be either `"sampleCorrelation"` for a sample-sample correlation heatmap or `"junctionSample"` for a junction-sample correlation heatmap.

`bins` Set the number of bins to be used in the histogram.

`legend.position`
Set legend position (x and y coordinate), defaults to the top right corner.

`onlyVariableIntrons`
Logical value indicating whether to show only introns that also pass the variability filter. Defaults to FALSE.

`onlyExpressedIntrons`
Logical value indicating whether to show only introns that also pass the expression filter. Defaults to FALSE.

`logit` If TRUE, the default, psi values are plotted in logit space.

`topN` Top x most variable junctions that should be used for the calculation of sample x sample correlations.

`topJ` Top x most variable junctions that should be displayed in the junction-sample correlation heatmap. Only applies if plotType is "junctionSample".

`minMedian`, `minCount`, `minDeltaPsi`
Minimal median ($m \geq 1$), delta psi ($|\Delta\psi| > 0.1$), read count ($n \geq 10$) value of a junction to be considered for the correlation heatmap.

`normalized` If TRUE, the normalized psi values are used, the default, otherwise the raw psi values

`show_rownames`, `show_colnames`
Logical value indicating whether to show row or column names on the heatmap axes.

`annotation_col`, `annotation_row`
Row or column annotations that should be plotted on the heatmap.

`border_color` Sets the border color of the heatmap

`nClust` Number of clusters to show in the row and column dendrograms.

`sampleClustering`
A clustering of the samples that should be used as an annotation of the heatmap.

`plotMeanPsi`, `plotCov`
If `TRUE`, then the heatmap is annotated with the mean psi values or the junction coverage.

`gr` A GRanges object indicating the genomic range that should be shown in `plotBamCoverage`.

`control_samples`
The sampleIDs of the samples used as control in `plotBamCoverage`.

`txdb` A TxDb object giving the gene/transcript annotation to use.

`min_junction_count`
The minimal junction count across samples required for a junction to appear in the splicegraph and coverage tracks of `plotBamCoverage`.

highlight_range
: A GenomicRanges or GenomicRangesList object of ranges to be highlighted in the splicegraph of plotBamCoverage.

highlight_range_color
: The color of highlighted ranges in the splicegraph of plotBamCoverage.

color_annotated
: The color for exons and junctions present in the given annotation (in the splicegraph of plotBamCoverage).

color_novel
: The color for novel exons and junctions not present in the given annotation (in the splicegraph of plotBamCoverage).

color_sample_interest
: The color in plotBamCoverage for the sample of interest.

color_control_samples
: The color in plotBamCoverage for the samples used as controls.

toscale
: In plotBamCoverage, indicates which part of the plotted region should be drawn to scale. Possible values are 'exon' (exonic regions are drawn to scale), 'gene' (both exonic and intronic regions are drawn to scale) or 'none' (exonic and intronic regions have constant length) (see SGSeq package).

mar
: The margin of the plot area for plotBamCoverage (b,l,t,r).

curvature_splicegraph
: The curvature of the junction arcs in the splicegraph in plotBamCoverage. Decrease this value for flatter arcs and increase it for steeper arcs.

curvature_coverage
: The curvature of the junction arcs in the coverage tracks of plotBamCoverage. Decrease this value for flatter arcs and increase it for steeper arcs.

cex
: For controlling the size of text and numbers in plotBamCoverage.

splicegraph_labels
: Indicated the format of exon/splice junction labels in the splicegraph of plotBamCoverage. Possible values are 'genomic_range' (gives the start position of the first exon and the end position of the last exon that are shown), 'id' (format E1,... J1,...), 'name' (format type:chromosome:start-end:strand for each feature), 'none' for no labels (see SGSeq package).

splicegraph_position
: The position of the splicegraph relative to the coverage tracks in plotBamCoverage. Possible values are 'top' (default) and 'bottom'.

show_full_gene
: Should the full genomic range of the gene be shown in plotBamCoverageFromResultTable (default: FALSE)? If FALSE, only a certain region (see parameters left_extension and right_extension) around the outlier junction is shown.

orgDb
: A OrgDb object giving the mapping of gene ids and symbols.

res_gene_col
: The column name in the given results table that contains the gene annotation.

res_geneid_type
: The type of gene annotation in the results table in res_gene_col (e.g. SYMBOL or ENTREZID etc.). This information is needed for mapping between the results table and the provided annotation in the txdb object.

`txdb_geneid_type`
: The type of gene_id present in `genes(txdb)` (e.g. ENTREZID). This information is needed for mapping between the results table and the provided annotation in the txdb object.

`left_extension`
: Indicating how far the plotted range around the outlier junction should be extended to the left in `plotBamCoverageFromResultTable`.

`right_extension`
: Indicating how far the plotted range around the outlier junction should be extended to the right in `plotBamCoverageFromResultTable`.

`value`
: Indicates which assay is shown in the manhattan plot. Defaults to 'pvalue'. Other options are 'deltaPsi' and 'zScore'.

`chr`
: Vector of chromosome names to show in `plotManhattan`. The default is to show all chromosomes.

`chrColor`
: Interchanging colors by chromosome for `plotManhattan`.

## Details

This is the list of all plotting function provided by FRASER:

- plotAberrantPerSample()
- plotVolcano()
- plotExpression()
- plotQQ()
- plotExpectedVsObservedPsi()
- plotCountCorHeatmap()
- plotFilterExpression()
- plotFilterVariability()
- plotEncDimSearch()
- plotBamCoverage()
- plotBamCoverageFromResultTable()
- plotManhattan()
- plotSpliceMetricRank()

For a detailed description of each plot function please see the details. Most of the functions share the same parameters.

`plotAberrantPerSample`: The number of aberrant events per sample are plotted sorted by rank. The ... parameters are passed on to the `aberrant` function.

`plotVolcano`: the volcano plot is sample-centric. It plots for a given sample and psi type the negative log10 nominal P-values against the delta psi values for all splice sites or aggregates by gene if requested.

`plotExpression`: This function plots for a given site the read count at this site (i.e. K) against the total coverage (i.e. N) for the given psi type ($\psi_5, \psi_3, or \theta$ (SE)) for all samples.

`plotQQ`: the quantile-quantile plot for a given gene or if `global` is set to `TRUE` over the full data set. Here the observed P-values are plotted against the expected ones in the negative log10 space.

plotExpectedVsObservedPsi: A scatter plot of the observed psi against the predicted psi for a given site.

plotSpliceMetricRank: This function plots for a given intron the observed values of the selected splice metrix against the sample rank.

plotCountCorHeatmap: The correlation heatmap of the count data either of the full data set (i.e. sample-sample correlations) or of the top x most variable junctions (i.e. junction-sample correlations). By default the values are log transformed and row centered. The ... arguments are passed to the pheatmap function.

plotFilterExpression: The distribution of FPKM values. If the FraserDataSet object contains the passedFilter column, it will plot both FPKM distributions for the expressed introns and for the filtered introns.

plotFilterVariability: The distribution of maximal delta Psi values. If the FraserDataSet object contains the passedFilter column, it will plot both maximal delta Psi distributions for the variable introns and for the filtered (i.e. non-variable) introns.

plotEncDimSearch: Visualization of the hyperparameter optimization. It plots the encoding dimension against the achieved loss (area under the precision-recall curve). From this plot the optimum should be choosen for the q in fitting process.

plotManhattan: A Manhattan plot showing the junction pvalues by genomic position. Useful to identify if outliers cluster by genomic position.

plotBamCoverage: A sashimi plot showing the read coverage from the underlying bam files for a given genomic range and sampleIDs.

plotBamCoverageFromResultTable: A sashimi plot showing the read coverage from the underlying bam files for a row in the results table. Can either show the full range of the gene with the outlier junction or only a certain region around the outlier.

## Value

If base R graphics are used nothing is returned else the plotly or the gplot object is returned.

## Examples

```
# create full FRASER object
fds <- makeSimulatedFraserDataSet(m=40, j=200)
fds <- calculatePSIValues(fds)
fds <- filterExpressionAndVariability(fds, filter=FALSE)
# this step should be done for more dimensions in practice
fds <- estimateBestQ(fds, type="jaccard", useOHT=FALSE, q_param=c(2,5,10,25))

# assign gene names to show functionality on test dataset
# use fds <- annotateRanges(fds) on real data
mcols(fds, type="j")$hgnc_symbol <-
    paste0("gene", sample(1:25, nrow(fds), replace=TRUE))

# fit and calculate pvalues
genesOfInterest <- rep(list(paste0("gene", sample(1:25, 10))), 4)
names(genesOfInterest) <- c("sample1", "sample6", "sample15", "sample23")
fds <- FRASER(fds, subsets=list("testSet"=genesOfInterest))
```

```
# QC plotting
plotFilterExpression(fds)
plotFilterVariability(fds)
plotCountCorHeatmap(fds, "jaccard")
plotCountCorHeatmap(fds, "jaccard", normalized=TRUE)
plotEncDimSearch(fds, type="jaccard")

# extract results
plotAberrantPerSample(fds, aggregate=FALSE)
plotAberrantPerSample(fds, aggregate=TRUE, subsetName="testSet")
plotVolcano(fds, "sample2", "jaccard", label="aberrant")
plotVolcano(fds, "sample1", "jaccard", aggregate=TRUE, subsetName="testSet")

# dive into gene/sample level results
res <- data.table::as.data.table(results(fds))
res
plotExpression(fds, result=res[1])
plotQQ(fds, result=res[1])
plotExpectedVsObservedPsi(fds, res=res[1])
plotSpliceMetricRank(fds, res=res[1])

# other ways to call these plotting functions
plotExpression(fds, idx=10, sampleID="sample1", type="jaccard")
plotExpression(fds, result=res[1], subsetName="testSet")
plotQQ(fds, idx=10, sampleID="sample1", type="jaccard")
plotQQ(fds, result=res[1], subsetName="testSet")
plotExpectedVsObservedPsi(fds, idx=10, sampleID="sample1", type="jaccard")
plotExpectedVsObservedPsi(fds, result=res[1], subsetName="testSet")
plotSpliceMetricRank(fds, idx=10, sampleID="sample1", type="jaccard")
plotSpliceMetricRank(fds, result=res[1], subsetName="testSet")

# create manhattan plot of pvalues by genomic position
if(require(ggbio)){
    plotManhattan(fds, type="jaccard", sampleID="sample10")
}

# plot splice graph and coverage from bam files in a given region
if(require(SGSeq)){
    fds <- createTestFraserSettings()
    gr <- GRanges(seqnames="chr19",
        IRanges(start=7587496, end=7598895),
        strand="+")
    plotBamCoverage(fds, gr=gr, sampleID="sample3",
        control_samples="sample2", min_junction_count=5,
        curvature_splicegraph=1, curvature_coverage=1,
        mar=c(1, 7, 0.1, 3))

    # plot coverage from bam file for a row in the result table
    fds <- createTestFraserDataSet()
    require(TxDb.Hsapiens.UCSC.hg19.knownGene)
    txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
    require(org.Hs.eg.db)
    orgDb <- org.Hs.eg.db
```

```
    res <- results(fds, padjCutoff=NA, deltaPsiCutoff=NA)
    res_dt <- data.table::as.data.table(res)
    res_dt <- res_dt[sampleID == "sample2",]

    # plot full range of gene containing outlier junction
    plotBamCoverageFromResultTable(fds, result=res_dt[1,], show_full_gene=TRUE,
        txdb=txdb, orgDb=orgDb, control_samples="sample3")

    # plot only certain range around outlier junction
    plotBamCoverageFromResultTable(fds, result=res_dt[1,], show_full_gene=FALSE,
        control_samples="sample3", curvature_splicegraph=0.5, txdb=txdb,
        curvature_coverage=0.5, right_extension=5000, left_extension=5000,
        splicegraph_labels="id")
}
```

---

`potentialImpactAnnotations` *Additional result annotations*

---

## Description

These functions work on the result table and add additional annotations to the reported introns: the type of potential impact on splicing (e.g. exon skipping, exon truncation, ...), potential occurence of frameshift, overlap with UTR regions as well as a flag for introns that are located in blacklist regions of the genome.

`annotateIntronReferenceOverlap` adds basic annotations to the fds for each intron based on the overlap of the intron's location with the reference annotation. Has to be run before the result table is created so that the new column can be included in it (see examples).

`annotatePotentialImpact` annotates each intron in the results table with the type of potential impact on splicing and potential occurence of frameshift (likely, unlikely, inconclusive). Can also calculate overlap with annotated UTR regions. Potential impact can be: annotatedIntron_increasedUsage, annotatedIntron_reducedUsage, exonTruncation, exonElongation, exonTruncation&Elongation, exonSkipping, splicingBeyondGene, multigenicSplicing, downstreamOfNearestGene, upstreamOfNearestGene, complex (everything else). Splice sites (theta metric) annotations indicate how the splice site is located with respect to the reference annotation. The annotated types are: annotatedSpliceSite, exonicRegion, intronicRegion.

`flagBlacklistRegions` flags introns in the results table on whether or not they are located in a blacklist region of the genome. By default, the blacklist regions as reported in *Amemiya, Kundaje & Boyle (2019)* and downloaded from here are used.

## Usage

```
annotateIntronReferenceOverlap(fds, txdb, BPPARAM = bpparam())

annotatePotentialImpact(
```

```
  result,
  txdb,
  fds,
  addPotentialImpact = TRUE,
  addUTRoverlap = TRUE,
  minoverlap = 5,
  BPPARAM = bpparam()
)

flagBlacklistRegions(
  result,
  blacklist_regions = NULL,
  assemblyVersion = c("hg19", "hg38"),
  minoverlap = 5
)
```

## Arguments

| | |
|---|---|
| fds | A FraserDataSet |
| txdb | A txdb object providing the reference annotation. |
| BPPARAM | For controlling parallelization behavior. Defaults to bpparam(). |
| result | A result table as generated by FRASER, including the column annotatedJunction as generated by the function annotateIntronReferenceOverlap. |
| addPotentialImpact | Logical, indicating if the type of the potential impact should be added to the results table. Defaults to TRUE. |
| addUTRoverlap | Logical, indicating if the overlap with UTR regions should checked and added to the results table. Defaults to TRUE. |
| minoverlap | Integer value defining the number of base pairs around the splice site that need to overlap with UTR or blacklist region, respectivly, to be considered matching. Defaults to 5 bp. |
| blacklist_regions | A BED file that contains the blacklist regions. If NULL (default), the BED files that are packaged with FRASER are used (see Details for more information). |
| assemblyVersion | Indicates the genome assembly version of the intron coordinates. Only used if blacklist_regions is NULL. For other versions, please provide the BED file containing the blacklist regions directly. |

## Value

An annotated FraserDataSet or results table, respectively

## Functions

- annotateIntronReferenceOverlap(): This method calculates basic annotations based on overlap with the reference annotation (start, end, none, both) for the full fds. The overlap type is added as a new column annotatedJunction in mcols(fds).

- annotatePotentialImpact(): This method annotates the splice event type to junctions in the given results table.
- flagBlacklistRegions(): This method flags all introns and splice sites in the given results table for which at least one splice site (donor or acceptor) is located in a blacklist region. Blacklist regions of the genome are determined from the provided BED file.

## Examples

```
# get data, fit and compute p-values and z-scores
fds <- createTestFraserDataSet()

# load reference annotation
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene

# add basic annotations for overlap with the reference annotation
# run this function before creating the results table
fds <- annotateIntronReferenceOverlap(fds, txdb)

# extract results: for this small example dataset, no cutoffs used
# to get some results
res <- results(fds, padjCutoff=NA, deltaPsiCutoff=NA)

# annotate the type of potential impact on splicing and UTR overlap
res <- annotatePotentialImpact(result=res, txdb=txdb, fds=fds)

# annotate overlap with blacklist regions
res <- flagBlacklistRegions(result=res, assemblyVersion="hg19")

# show results table containing additional annotations
res
```

---

# psiTypes *Available splice metrics*

---

## Description

Available splice metrics

## Usage

```
psiTypes
```

## Format

An object of class character of length 4.

## Examples

```
# to show all available splice metrics:
psiTypes
```

---

`results,FraserDataSet-method`

*Extracting results and aberrant splicing events*

---

## Description

The result function extracts the results from the given analysis object based on the given options and cutoffs. The aberrant function extracts aberrant splicing events based on the given cutoffs.

## Usage

```
## S4 method for signature 'FraserDataSet'
results(
  object,
  sampleIDs = samples(object),
  padjCutoff = 0.1,
  deltaPsiCutoff = 0.1,
  rhoCutoff = NA,
  aggregate = FALSE,
  collapse = FALSE,
  minCount = 5,
  psiType = psiTypes,
  geneColumn = "hgnc_symbol",
  all = FALSE,
  returnTranscriptomewideResults = TRUE,
  additionalColumns = NULL,
  BPPARAM = bpparam()
)

## S4 method for signature 'FraserDataSet'
aberrant(
  object,
  type = fitMetrics(object),
  padjCutoff = 0.1,
  deltaPsiCutoff = 0.1,
  minCount = 5,
  rhoCutoff = NA,
  by = c("none", "sample", "feature"),
  aggregate = FALSE,
  geneColumn = "hgnc_symbol",
  subsetName = NULL,
  all = FALSE,
```

```
  ...
)
```

## Arguments

| | |
|---|---|
| object | A FraserDataSet object |
| sampleIDs | A vector of sample IDs for which results should be retrieved |
| padjCutoff | The FDR cutoff to be applied or NA if not requested. |
| deltaPsiCutoff | The cutoff on delta psi or NA if not requested. |
| rhoCutoff | The cutoff value on the fitted rho value (overdispersion parameter of the betabinomial) above which junctions are filtered |
| aggregate | If TRUE the returned object is aggregated to the feature level (i.e. gene level). |
| collapse | Only takes effect if aggregate=TRUE. If TRUE, collapses results across the different psi types to return only one row per feature (gene) and sample. |
| minCount | The minimum count value of the total coverage of an intron to be considered as significant. result |
| psiType | The psi types for which the results should be retrieved. |
| geneColumn | The column name of the column that has the gene annotation that will be used for gene-level pvalue computation. |
| all | By default FALSE, only significant introns (or genes) are listed in the results. If TRUE, results are assembled for all samples and introns/genes regardless of significance. |
| returnTranscriptomewideResults | If FDR corrected pvalues for subsets of genes of interest have been calculated, this parameter indicates whether additionally the transcriptome-wide results should be returned as well (default), or whether only results for those subsets should be retrieved. |
| additionalColumns | Character vector containing the names of additional columns from mcols(fds) that should appear in the result table (e.g. ensembl_gene_id). Default is NULL, so no additional columns are included. |
| BPPARAM | The BiocParallel parameter. |
| type | Splicing type (psi5, psi3 or theta) |
| by | By default none which means no grouping. But if sample or feature is specified the sum by sample or feature is returned |
| subsetName | The name of a subset of genes of interest for which FDR corrected pvalues were previously computed. Those FDR values on the subset will then be used to determine aberrant status. Default is NULL (using transcriptome-wide FDR corrected pvalues). |
| ... | Further arguments can be passed to the method. If "n", "padjVals", "dPsi" or "rhoVals" are given, the values of those arguments are used to define the aberrant events. |

## Value

For `results`: GRanges object containing significant results. For `aberrant`: Either a of logical values of size introns/genes x samples if "by" is NA or a vector with the number of aberrant events per sample or feature depending on the vaule of "by"

## Examples

```
# get data, fit and compute p-values and z-scores
fds <- createTestFraserDataSet()

# extract results: for this example dataset, no cutoffs are used to
# show the output of the results function
res <- results(fds, all=TRUE)
res

# aggregate the results by genes (gene symbols need to be annotated first
# using annotateRanges() function)
results(fds, padjCutoff=NA, deltaPsiCutoff=0.1, aggregate=TRUE)

# aggregate the results by genes and collapse over all psi types to obtain
# only one row per gene in the results table
results(fds, padjCutoff=NA, deltaPsiCutoff=0.1, aggregate=TRUE,
        collapse=TRUE)

# get aberrant events per sample: on the example data, nothing is aberrant
# based on the adjusted p-value
aberrant(fds, type="jaccard", by="sample")

# get aberrant events per gene (first annotate gene symbols)
fds <- annotateRangesWithTxDb(fds)
aberrant(fds, type="jaccard", by="feature", padjCutoff=NA, aggregate=TRUE)

# find aberrant junctions/splice sites
aberrant(fds, type="jaccard")

# retrieve results limiting FDR correction to only a subset of genes
# first, we need to create a list of genes per sample that will be tested
geneList <- list('sample1'=c("TIMMDC1"), 'sample2'=c("MCOLN1"))
fds <- calculatePadjValues(fds, type="jaccard",
                 subsets=list("exampleSubset"=geneList))
results(fds, all=TRUE, returnTranscriptomewideResults=FALSE)
```

---

`samples` *Getter/Setter methods for the FraserDataSet*

---

## Description

The following methods are getter and setter methods to extract or set certain values of a FraserDataSet object.

samples sets or gets the sample IDs; condition ; nonSplicedReads return a RangedSummarizedExperiment object containing the counts for the non spliced reads overlapping splice sites in the fds.

## Usage

```
samples(object)

samples(object) <- value

condition(object)

condition(object) <- value

bamFile(object)

bamFile(object) <- value

name(object)

name(object) <- value

strandSpecific(object)

strandSpecific(object) <- value

pairedEnd(object)

pairedEnd(object) <- value

workingDir(object)

workingDir(object) <- value

scanBamParam(object)

scanBamParam(object) <- value

nonSplicedReads(object)

nonSplicedReads(object) <- value

## S4 method for signature 'FraserDataSet'
samples(object)

## S4 replacement method for signature 'FraserDataSet'
samples(object) <- value

## S4 method for signature 'FraserDataSet'
```

```
condition(object)

## S4 replacement method for signature 'FraserDataSet'
condition(object) <- value

## S4 method for signature 'FraserDataSet'
bamFile(object)

## S4 replacement method for signature 'FraserDataSet'
bamFile(object) <- value

## S4 method for signature 'FraserDataSet'
name(object)

## S4 replacement method for signature 'FraserDataSet'
name(object) <- value

## S4 method for signature 'FraserDataSet'
workingDir(object)

## S4 replacement method for signature 'FraserDataSet'
workingDir(object) <- value

## S4 method for signature 'FraserDataSet'
strandSpecific(object)

## S4 replacement method for signature 'FraserDataSet'
strandSpecific(object) <- value

## S4 method for signature 'FraserDataSet'
pairedEnd(object)

## S4 replacement method for signature 'FraserDataSet'
pairedEnd(object) <- value

## S4 method for signature 'FraserDataSet'
scanBamParam(object)

## S4 replacement method for signature 'FraserDataSet'
scanBamParam(object) <- value

## S4 method for signature 'FraserDataSet'
nonSplicedReads(object)

## S4 replacement method for signature 'FraserDataSet'
nonSplicedReads(object) <- value

FRASER.mcols.get(x, type = NULL, ...)
```

```
FRASER.rowRanges.get(x, type = NULL, ...)

mapSeqlevels(fds, style = "UCSC", ...)
```

## Arguments

| | |
|---|---|
| object | A FraserDataSet object. |
| value | The new value that should replace the current one. |
| x | A FraserDataSet object. |
| type | The psi type (psi3, psi5 or theta) |
| ... | Further parameters. For mapSeqLevels: further parameters passed to GenomeInfoDb::mapSeqlevels(). |
| fds | FraserDataSet |
| style | The style of the chromosome names. |

## Value

Getter method return the respective current value.

## Author(s)

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## Examples

```
fds <- createTestFraserDataSet()
samples(fds)
samples(fds) <- 1:dim(fds)[2]
condition(fds)
condition(fds) <- 1:dim(fds)[2]
bamFile(fds) # file.paths or objects of class BamFile
bamFile(fds) <- file.path("bamfiles", samples(fds), "rna-seq.bam")
name(fds)
name(fds) <- "My Analysis"
workingDir(fds)
workingDir(fds) <- tempdir()
strandSpecific(fds)
strandSpecific(fds) <- TRUE
strandSpecific(fds) <- "reverse"
strandSpecific(fds)
scanBamParam(fds)
scanBamParam(fds) <- ScanBamParam(mapqFilter=30)
nonSplicedReads(fds)
rowRanges(fds)
rowRanges(fds, type="theta")
mcols(fds, type="psi5")
mcols(fds, type="theta")
```

```
seqlevels(fds)
seqlevels(mapSeqlevels(fds, style="UCSC"))
seqlevels(mapSeqlevels(fds, style="Ensembl"))
seqlevels(mapSeqlevels(fds, style="dbSNP"))
```

---

`subset.FRASER` *Subsetting by indices for junctions*

---

## Description

Providing subsetting by indices through the single-bracket operator

## Usage

```
## S3 method for class 'FRASER'
subset(x, i, j, by = c("j", "ss"), ..., drop = FALSE)

## S4 method for signature 'FraserDataSet,ANY,ANY,ANY'
x[i, j, by = c("j", "ss"), ..., drop = FALSE]
```

## Arguments

| | |
|---|---|
| `x` | A `FraserDataSet` object |
| `i` | A integer vector to subset the rows/ranges |
| `j` | A integer vector to subset the columns/samples |
| `by` | a character (j or ss) defining if we subset by junctions or splice sites |
| `...` | Parameters currently not used or passed on |
| `drop` | No dimension reduction is done. And the `drop` parameter is currently not used at all. |

## Value

A subsetted `FraserDataSet` object

## Examples

```
fds <- createTestFraserDataSet()
fds[1:10,2:3]
fds[,samples(fds) %in% c("sample1", "sample2")]
fds[1:10,by="ss"]
```

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