Package ‘NanoStringNCTools’

February 23, 2024

Title  NanoString nCounter Tools
Description  Tools for NanoString Technologies nCounter Technology. Provides support for reading RCC files into an ExpressionSet derived object. Also includes methods for QC and normalization of NanoString data.
Version  1.10.0
Encoding  UTF-8
Depends  R (>= 3.6), Biobase, S4Vectors, ggplot2
Imports  BiocGenerics, Biostrings, ggbeeswarm, ggiraph, ggthemes, grDevices, IRanges, methods, pheatmap, RColorBrewer, stats, utils
Suggests  biovizBase, ggbio, RUnit, rmarkdown, knitr, qpdf
License  MIT
biocViews  GeneExpression, Transcription, CellBasedAssays, DataImport, Transcriptomics, Proteomics, mRNAMicroarray, ProprietaryPlatforms, RNASeq
VignetteEngine  knitr
VignetteBuilder  knitr
git_url  https://git.bioconductor.org/packages/NanoStringNCTools
git_branch  RELEASE_3_18
git_last_commit  f70ebcf
git_last_commit_date  2023-10-24
Repository  Bioconductor 3.18
Date/Publication  2024-02-22
**Description**

The interactive version of `geom_beeswarm` from `ggbeeswarm`.

**Usage**

```r
geom_beeswarm_interactive(mapping = NULL, data = NULL,
                           priority = c("ascending", "descending", "density",
                           "random", "none"),
                           cex = 1, groupOnX = NULL, dodge.width = 0,
                           stat = "identity", na.rm = FALSE, show.legend = NA,
                           inherit.aes = TRUE, ...)
```

**Arguments**

- `mapping` The aesthetic mapping. See `geom_beeswarm`.
- `data` The data to be displayed at this layer. See `geom_beeswarm`.
- `priority` Method used to perform point layout. See `geom_beeswarm`.
- `cex` Scaling for adjusting point spacing. See `geom_beeswarm`.
- `groupOnX` Indicator for jittering on x-axis. See `geom_beeswarm`. 
dodge.width  Dodge amount for points from different aesthetic groups. See `geom_beeswarm`.
stat          The statistical transformation to use on the data for this layer. See `geom_beeswarm`.
na.rm         Indicator for removing missing values with a warning. See `geom_beeswarm`.
show.legend   Indicator for including this layer in the legend. See `geom_beeswarm`.
inherit.aes   Indicator for inheriting the aesthetics. See `geom_beeswarm`.
...           Additional arguments. See `geom_beeswarm`.

Value

The interactive geometry based on `geom_beeswarm`.

Author(s)

Patrick Aboyoun

See Also

`geom_beeswarm`

Examples

```r
# Create NanoStringRccSet from data files
datadir <- system.file("extdata", "3D_Bio_Example_Data", package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC\", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_PhenoData.csv")
solidTumor <-
  readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno)

eg_data <- as.data.frame(assayDataElement(solidTumor, "exprs")[1:5, 1])
eg_data["tooltip"] <- names(eg_data)
geom_beeswarm_interactive(aes_string(tooltip = "tooltip"), data=eg_data)
```

---

### log2t

*Logarithm With Thresholding*

**Description**

Safe log and log2 calculations where values within [0, thresh) are thresholded to thresh prior to the transformation.

**Usage**

```r
logt(x, thresh = 0.5)
log2t(x, thresh = 0.5)
```
Arguments

x  a numeric or complex vector.
thresh  a positive number specifying the threshold.

Details

For non-negative elements in x, calculates \( \log(\text{pmax}(x, \text{thresh})) \) or \( \log_2(\text{pmax}(x, \text{thresh})) \).

Value

A vector of the same length as x containing the transformed values.

Author(s)

Patrick Aboyoun

See Also

log, log2

Examples

logt(0:8)
identical(logt(0:8), log(c(0.5, 1:8)))

log2t(0:8)
identical(log2t(0:8), log2(c(0.5, 1:8)))
NanoStringRccSet-autoplot

"heatmap-signatures",
"housekeep-geom",
"lane-bindingDensity",
"lane-fov",
"mean-sd-features",
"mean-sd-samples"),
log2scale = TRUE,
elt = "exprs",
index = 1L,
geomParams = list(),
tooltipDigits = 4L,
heatmapGroup = NULL,
blacklist = NULL,
tooltipID = NULL,
qcCutoffs = list(
  Housekeeper = c("failingCutoff" = 32,"passingCutoff" = 100),
  Imaging = c("fovCutoff" = 0.75),
  BindingDensity = c("minimumBD" = 0.1, "maximumBD" = 2.25,
                    "maximumBDSprint" = 1.8),
  ERCCLinearity = c("correlationValue" = 0.95),
  ERCCLoD = c("standardDeviations" = 2)),
scalingFactor=1L,
show_rownames_gene_limit=60L,
show_colnames_gene_limit=36L,
show_rownames_sig_limit=60L,
show_colnames_sig_limit=36L,
subSet = NULL,
...)

Arguments

object A NanoStringRccSet object
type Character string referencing the type of plot to generate
log2scale An optional boolean indicating expression data is on log2 scale
elt An optional character string of the expression matrix name
index An optional integer giving the feature of interest row location
geomParams An option list of parameters for geometry
tooltipDigits An optional integer for number of tooltip decimal places to display
heatmapGroup An optional character string referencing pData column to color samples by in heatmap
blacklist An optional character vector of features not to plot
tooltipID An optional character string referencing pData column to use for sample ID in the tooltip
qcCutoffs An optional list of QC cutoffs
scalingFactor An optional numeric value indicating a scaling factor to apply to plot drawing
show_rownames_gene_limit
An optional integer limit on number of features to display row-wise
show_colnames_gene_limit
An optional integer limit on number of features to display column-wise
show_rownames_sig_limit
An optional integer limit on number of signatures to display row-wise
show_colnames_sig_limit
An optional integer limit on number of signatures to display column-wise
subSet
An optional subset to plot on
... Additional arguments to pass on to autoplot function

Details
"boxplot-feature" Generate feature boxplots
"boxplot-signature" Generate signature boxplots
"bindingDensity-mean" Plot binding density displayed as average expression
"bindingDensity-sd" Plot binding density displayed as standard deviation of expression
"ercc-linearity" Assess linearity of ERCCs
"ercc-lod" Assess limit of detection based on ERCC expression
"heatmap-genes" Generate a heatmap from feature expression
"heatmap-signatures" Generate a heatmap from signature expression
"housekeep-geom" Plot geometric mean of housekeeper genes
"lane-bindingDensity“ View binding density by lane
"lane-fov“ Assess image quality by lane
"mean-sd-features” Plot mean versus standard deviation feature-wise
"mean-sd-samples” Plot mean versus standard deviation sample-wise

Value
A ggplot or pheatmap plot depending on the type of plot generated

Examples
# Create NanoStringRccSet from data files
datadir <- system.file("extdata", "3D_Bio_Example_Data",   
  package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC$", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_PhenoData.csv")
solidTumor <-
  readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno)

# Assess experiment linearity
#autoplot(solidTumor, "ercc-linearity")

# Plot a feature's expression across all samples
#autoplot(solidTumor, "boxplot-feature", index=2)
NanoStringRccSet-class

Class to Contain NanoString Expression Level Assays

Description

The NanoStringRccSet class extends the ExpressionSet class for NanoString Reporter Code Count (RCC) data.

Usage

NanoStringRccSet(assayData,
    phenoData = annotatedDataFrameFrom(assayData, byrow = FALSE),
    featureData = annotatedDataFrameFrom(assayData, byrow = TRUE),
    experimentData = MIAME(),
    annotation = character(),
    protocolData = annotatedDataFrameFrom(assayData, byrow = FALSE),
    dimLabels = c("GeneName", "SampleID"),
    signatures = SignatureSet(),
    design = NULL,
    ...
)

Arguments

assayData  A matrix or environment containing the RCCs.
phenoData  An AnnotatedDataFrame containing the phenotypic data.
featureData  An AnnotatedDataFrame containing columns "CodeClass", "GeneName", "Accession", "IsControl", and "ControlConc".
experimentData  An optional MIAME instance with meta-data about the experiment.
annotation  A character string for the "GeneRLF".
dimLabels  A character vector of length 2 that provides the column names to use as labels for the features and samples respectively in the autoplot method.
signatures  An optional SignatureSet object containing signature definitions.
design  An optional one-sided formula representing the experimental design based on columns from phenoData
...

Value

An S4 class containing NanoString Expression Level Assays
**Accessing**

In addition to the standard ExpressionSet accessor methods, NanoStringRccSet objects have the following:

- `sData(object)`: extracts the data.frame containing the sample data, `cbind(pData(object), pData(protocolData(object)))`.
- `svarLabels(object)`: extracts the sample data column names, `c(varLabels(object), varLabels(protocolData(object)))`.
- `dimLabels(object)`: extracts the column names to use as labels for the features and samples in the autoplot method.
- `dimLabels(object) <- value`: replaces the `dimLabels` of the object.
- `signatures(object)`: extracts the SignatureSet of the object.
- `signatures(object) <- value`: replaces the SignatureSet of the object.
- `signatureScores(object, elt = "exprs")`: extracts the matrix of computed signature scores.
- `design(object)`: extracts the one-sided formula representing the experimental design based on columns from `phenoData`.
- `design(object) <- value`: replaces the one-sided formula representing the experimental design based on columns from `phenoData`.
- `setSignatureFuncs(object)`: returns the signature functions.
- `setSignatureFuncs(object) <- value`: replaces the signature functions.
- `setSignatureGroups(object) <- value`: returns the signature groups.
- `setSignatureGroups(object) <- value`: replaces the signature groups.

**Summarizing**

- `summary(object, MARGIN = 2L, GROUP = NULL, log2scale = TRUE, elt = "exprs", signatureScores = FALSE)`: When `signatureScores = FALSE`, the marginal summaries of the elt assayData matrix along either the feature (MARGIN = 1) or sample (MARGIN = 2) dimension. When `signatureScores = TRUE`, the marginal summaries of the elt signatureScores matrix along either the signature (MARGIN = 1) or sample (MARGIN = 2) dimension. When `log2scale = FALSE`, the summary statistics are Mean, Standard Deviation, Skewness, Excess Kurtosis, Minimum, First Quartile, Median, Third Quartile, and Maximum. When `log2scale = TRUE`, the summary statistics are Geometric Mean with thresholding at 0.5, Size Factor ($2^{(\text{MeanLog2} - \text{mean}(\text{MeanLog2}))}$), Mean of Log2 with thresholding at 0.5, Standard Deviation of Log2 with thresholding at 0.5, Minimum, First Quartile, Median, Third Quartile, and Maximum.

**Subsetting**

In addition to the standard ExpressionSet subsetting methods, NanoStringRccSet objects have the following:

- `subset(x, subset, select, ...)`: Subset the feature and sample dimensions using the subset and select arguments respectively. The subset argument will be evaluated with respect to the `featureData`, while the select argument will be evaluated with respect to the `phenoData` and `protocolData`.

endogenousSubset(x, subset, select): Extracts the endogenous barcode class feature subset of x with optional additional subsetting using subset and select.

housekeepingSubset(x, subset, select): Extracts the housekeeping barcode class feature subset of x with optional additional subsetting using subset and select.

negativeControlSubset(x, subset, select): Extracts the negative control barcode class feature subset of x with optional additional subsetting using subset and select.

positiveControlSubset(x, subset, select): Extracts the positive control barcode class feature subset of x with optional additional subsetting using subset and select.

controlSubset(x, subset, select): Extracts the feature subset representing the controls of x with optional additional subsetting using subset and select.

nonControlSubset(x, subset, select): Extracts the feature subset representing the non-controls of x with optional additional subsetting using subset and select.

signatureSubset(x, subset, select): Extracts the feature subset representing the genes in the signatures of x with optional additional subsetting using subset and select.

Looping

assayDataApply(X, MARGIN, FUN, ..., elt = "exprs"): Loop over the feature (MARGIN = 1) or sample (MARGIN = 2) dimension of assayDataElement(X, elt).

signatureScoresApply(X, MARGIN, FUN, ..., elt = "exprs"): Loop over the signature (MARGIN = 1) or sample (MARGIN = 2) dimension of signatureScores(X, elt).

esBy(X, GROUP, FUN, ..., simplify = TRUE): Split X by GROUP column within featureData, phenoData, or protocolData and apply FUN to each partition.

Transforming

munge(data, mapping = update(design(data), exprs ~ .), extradata = NULL, elt = "exprs", ...): munge argument data into a data.frame object for modeling and visualization using the mapping argument. Supplemental data can be specified using the extradata argument.

transform('_.data', ...): Similar to the transform generic in the base package, creates or modifies one or more assayData matrices based upon name = value pairs in .... The expressions in ... are appended to the preprocessing list in experimentData, which can be extracted using the preproc method.

Evaluating

with(data, expr, ...): Evaluate expression expr with respect to assayData, featureData, phenoData, and protocolData: c(as.list(assayData(data)), fData(data), sData(data)).

Normalizing

normalize(object, type, fromElt = "exprs", toElt = "exprs_norm", ...):
Plotting

ggplot(data, mapping = aes(), ..., extradata = NULL, tooltip_digits = 4L, environment = parent.frame()): the NanoStringRccSet method for ggplot.

autoplot(object, type, log2scale = TRUE, elt = "exprs", index = 1L, geomParams = list(), tooltipDigits = 4L, heatmapGroup = NULL, ...):

Author(s)

Patrick Aboyoun

See Also

readNanoStringRccSet, writeNanoStringRccSet, ExpressionSet

Examples

# Create NanoStringRccSet from data files
datadir <- system.file("extdata", "3D_Bio_Example_Data", package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC\$", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_Phenodata.csv")
solidTumor <-
    readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno)

# Create a deep copy of a NanoStringRccSet object
deepCopy <- NanoStringRccSet(solidTumor)
all.equal(solidTumor, deepCopy)
identical(solidTumor, deepCopy)

# Accessing sample data and column names
head(sData(solidTumor))
svarLabels(solidTumor)

# Set experimental design
design(solidTumor) <- ~ BRAFGenotype + Treatment

# Marginal summarizing of NanoStringRccSet assayData matrices
head(summary(solidTumor, 1)) # Marginal summaries along features
head(summary(solidTumor, 2)) # Marginal summaries along samples

# Subsetting NanoStringRccSet objects
# Extract the positive controls for wildtype BRAF
dim(solidTumor)
dim(subset(solidTumor, CodeClass == "Positive", BRAFGenotype == "wt/wt"))

# Extract by barcode class
with(solidTumor, table(CodeClass))
with(endogenousSubset(solidTumor), table(CodeClass))
with(housekeepingSubset(solidTumor), table(CodeClass))
with(negativeControlSubset(solidTumor), table(CodeClass))
with(positiveControlSubset(solidTumor), table(CodeClass))
with(controlSubset(solidTumor), table(CodeClass))
with(nonControlSubset(solidTumor), table(CodeClass))

# Looping over NanoStringRccSet assayData matrices
log1pCoefVar <- function(x){
  x <- log1p(x)
  sd(x) / mean(x)
}

# Log1p Coefficient of Variation along Features
head(assayDataApply(solidTumor, 1, log1pCoefVar))

# Log1p Coefficient of Variation along Samples
head(assayDataApply(solidTumor, 2, log1pCoefVar))

# Transforming NanoSetRccSet assayData matrices
# Subtract max count from each sample
# Create log1p transformation of adjusted counts
thresh <- assayDataApply(negativeControlSubset(solidTumor), 2, max)
solidTumor2 <-
  transform(solidTumor,
    negCtrlZeroed = sweep(exprs, 2, thresh),
    log1p_negCtrlZeroed = log1p(pmax(negCtrlZeroed, 0)))
assayDataElementNames(solidTumor2)

# Evaluating expression using NanoStringRccSet data
meanLog1pExprs <-
  with(solidTumor,
    {
      means <- split(apply(exprs, 1, function(x) mean(log1p(x))), CodeClass)
      means <- means[order(sapply(means, median))]  
      boxplot(means, horizontal = TRUE)
      means
    })

normalize

Normalize RCCSet

Description

This package performs normalization on NanoStringRccSet data using one of three methods.
normalize

Usage

normalize(object, ...)

Arguments

object object NanoStringRccSet object

... object additional arguments to pass on to normalize function

Details

Normalization is performed in one of three ways with data pulled from one slot of assayData and inserted into another. It is possible to overwrite the original slot of assayData if the fromElt and toElt are set to the same slot. nSolver normalization uses positive controls to scale and housekeepers to standardize the data and mimics the normalization performed by default in the nSolver software. The Housekeeping—Log2 normalization calculates the log2 sizeFactor of the housekeeping genes and then takes $2^\text{log}_2$ expression data centered by the log transformed sizeFactor. PositiveControl—Log2Log2 regresses the log2 positive control probes greater than 0.5 concentration on their geometric mean and then uses the intercept and slope to predict normalized values from the log2 transformed expression values. The predictions are then rescaled by $2^\text{log}_2$. Additional parameters with NanoStringRccSet method include:

- type normalization method to use. Options are nSolver, Housekeeping—Log2, and PositiveControl—Log2Log2
- fromElt assayData slot from which to pull raw data
- toElt assayData slot to which normalized data will be inserted

Value

The function returns a new NanoStringRccSet with either an additional assayData slot of normalized data, or overwrites the original assayData depending on whether fromElt and toElt are identical.

Author(s)

Patrick Aboyoun

References


Examples

datadir <- system.file("extdata", "3D_Bio_Example_Data", package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC\$", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_PhenoData.csv")
solidTumor <-
  readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno)
solidTumor <- normalize(solidTumor, "nSolver", fromElt = "exprs", toElt = "exprs_norm")
readNanoStringRccSet

head( assayDataElement( solidTumor, elt = "exprs_norm" ) )

readNanoStringRccSet  Read 'NanoStringRccSet'

Description

Create an instance of class NanoStringRccSet by reading data from NanoString Reporter Code Count (RCC) files.

Usage

readNanoStringRccSet(rccFiles, rlfFile = NULL, phenoDataFile = NULL, phenoDataRccColName = "^RCC", phenoDataColPrefix = "")

Arguments

rccFiles    A character vector containing the paths to the RCC files.
rlfFile     An optional character string representing the path to the corresponding RLF file.
phenoDataFile An optional character string representing the path to the corresponding phenotypic csv data file.
phenoDataRccColName The regular expression that specifies the RCC column in the phenoDataFile.
phenoDataColPrefix   An optional prefix to add to the phenoData column names to distinguish them from the names of assayData matrices, featureData columns, and protocolData columns.

Value

An instance of the NanoStringRccSet class.

Author(s)

Patrick Aboyoun

See Also

NanoStringRccSet, writeNanoStringRccSet
Examples

# Data file paths
datadir <- system.file("extdata", "3D_Bio_Example_Data", 
    package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC\$", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_PhenoData.csv")

# Just RCC data
solidTumorNoRlfPheno <- readNanoStringRccSet(rccs)
varLabels(solidTumorNoRlfPheno)
fvarLabels(solidTumorNoRlfPheno)

# RCC and RLF data
solidTumorNoPheno <- readNanoStringRccSet(rccs, rlfFile = rlf)
setdiff(fvarLabels(solidTumorNoPheno), fvarLabels(solidTumorNoRlfPheno))

# All data
solidTumor <- 
    readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno)
varLabels(solidTumor)
design(solidTumor) <- ~ BRAFGenotype + Treatment

# All data with phenoData prefix
solidTumorPhenoPrefix <- 
    readNanoStringRccSet(rccs, rlfFile = rlf, phenoDataFile = pheno, 
        phenoDataColPrefix = "PHENO_")
varLabels(solidTumorPhenoPrefix)
design(solidTumorPhenoPrefix) <- ~ PHENO_BRAFGenotype + PHENO_Treatment

---

readRccFile  Read RCC File

Description

Read a NanoString Reporter Code Count (RCC) file.

Usage

readRccFile(file)

Arguments

file A character string containing the path to the RCC file.

Value

An list object with five elements:

"Header" a data.frame object containing the header information.
readRlfFile

“Sample_Attributes”
   a data.frame object containing the attributes of the sample.

“Lane_Attributes”
   a data.frame object containing the attributes of the lane.

“Code_Summary”
   a data.frame object containing the reporter code counts.

“Messages”
   A character vector containing messages, if any.

Author(s)
   Patrick Aboyoun

See Also
   readNanoStringRccSet

Examples
   datadir <- system.file("extdata", "3D_Bio_Example_Data",
   package = "NanoStringNCTools")
   rccs <- dir(datadir, pattern = "SKMEL.*\.RCC$", full.names = TRUE)
   rccData <- lapply(rccs, readRccFile)

---

readRlfFile

Description
   Read a NanoString Reporter Library File (RLF) file.

Usage
   readRlfFile(file)

Arguments
   file
      A character string containing the path to the RLF file.

Value
   An instance of the DataFrame class containing columns:

   “CodeClass”    code class
   “GeneName”     gene name
   “Accession”    accession number
   ...           additional columns
setQCFlags

Author(s)
Patrick Aboyon

See Also
readNanoStringRccSet

Examples

datadir <- system.file("extdata", "3D_Bio_Example_Data",
package = "NanoStringNCTools")
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
rlfData <- readRlfFile(rlf)

setQCFlags

Set flags for QC of the assayData in a NanoStringRccSet.

Description
This function takes a list containing the quality control (QC) thresholds for data in a NanoStringRccSet and then returns a matrix of QC results by sample to protocolData.

Usage

setQCFlags(object, ...)

Arguments

object A valid NanoStringRccSet object with all housekeeping genes, positive control probes, and negative control probes present

... Additional arguments to pass

Details
This function checks that the housekeeping genes, positive control, and negative control probes or genes are within acceptable boundaries. Additional parameters with NanoStringRccSet method include:

qcCutoffs An optional list with members named Housekeeper, Imaging, BindingDensity, ERCCLinearity, and ERCCLoD

hkGenes An optional vector of housekeeping gene names if alternative genes to those defined in the panel are to be used

ReferenceSampleColumn An optional character string indicating the pData column containing reference sample information

Borderline thresholds and fail thresholds are defined and each sample receives a row in a matrix that contains flags indicating either borderline or failing performance.
Housekeeper is a vector with names members. failingCutoff sets the lower bound of housekeeper gene expression such that samples with a value below this threshold are labeled as failures. passingCutoff sets a lower bound of housekeeper gene expression such that samples with a value below this threshold are labeled as borderline. Values greater than or equal to either threshold are labeled as either borderline or passing. The default values are failingCutoff = 32 and passingCutoff = 100.

Imaging is a vector with a single named member fovCutoff. This threshold determines the minimum proportion of FOV to be counted. The default value is 0.75.

BindingDensity is a named vector with members minimumBD, maximumBD, and maximumBDSprint. minimumBD sets a minimum threshold for binding density across machine platforms. maximumBD sets a maximum binding density for non-Sprint machines while maximumBDSprint does the same for Sprint machines. The default values are minimumBD = 0.1, maximumBD = 2.25, and maximumBDSprint = 1.8.

ERCCLinearity is a named vector with a single member correlationValue. This member sets a minimum threshold for the correlation between the observed counts of positive controls and their theoretical concentration. The default value is 0.95.

ERCCLoD is a named vector with a single member standardDeviations. This sets a minimum threshold for the 0.5uMol concentration to be above the geoMean of the negative controls in units of standard deviation of the negative controls. The default value is 2.

Value

This function returns a new NanoStringRccSet with matrices of QC pass and QC borderline criteria added to the protocolData slots called QCFlags and QCBorderlineFlags, respectively.

Examples

# Create NanoStringRccSet from data files
datadir <- system.file("extdata", "3D_Bio_Example_Data", 
    package = "NanoStringNCTools")
rcs <- dir(datadir, pattern = "SKMEL.*\.RCC\$", full.names = TRUE)
rlf <- file.path(datadir, "3D_SolidTumor_Sig.rlf")
pheno <- file.path(datadir, "3D_SolidTumor_PhenoData.csv")
solidTumor <-
    readNanoStringRccSet(rcs, rlfFile = rlf, phenoDataFile = pheno)

#Set QC flags with default cutoffs
solidTumorDefaultQC <- setQCFlags(solidTumor)
head( protocolData( solidTumorDefaultQC )[["QCFlags"]])
head( protocolData( solidTumorDefaultQC )[["QCBorderlineFlags"]])

#Update cutoffs
newQCFlags <- list(
    Housekeeper = c("failingCutoff" = 32,"passingCutoff" = 100),
    Imaging = c("fovCutoff" = 0.75),
    BindingDensity = c("minimumBD" = 0.1, "maximumBD" = 2.25, "maximumBDSprint" = 1.8),
    ERCCLinearity = c("correlationValue" = 0.98),
    ERCCLoD = c("standardDeviations" = 2)
)
# Set QC flags with new cutoffs
solidTumorNewQC <- setQCFlags(solidTumor, qcCutoffs=newQCCutoffs)

# Compare QC results with default and new cutoffs
head( protocolData( solidTumorDefaultQC )[["QCFlags"]])
head( protocolData( solidTumorNewQC )[["QCFlags"]])

---

### SignatureSet-class

**Class to Contain Signature Definitions**

#### Description

The SignatureSet class defines gene-based signatures.

#### Usage

```r
SignatureSet(weights = NumericList(), groups = factor(), func = character(),
version = character(), ...)
```

#### Arguments

- `weights`: A named NumericList defining signatures based on linear combinations of genes.
- `groups`: A factor vector indicating groups in the SignatureSet
- `func`: Character indicating function to use
- `version`: Character indicating version to use
- `...`: Additional arguments for future use.

#### Value

A SignatureSet object

#### Utilities

- `length(x)`: returns the number of signatures in x.
- `lengths(x, use.names = TRUE)`: returns a named integer vector containing the number of genes in each of the signatures in x.
- `names(x)`: returns a character vector containing the signature names in x.
- `weights(object)`: returns a named NumericList that defines the linear combination based signatures.
- `weights(object) <- value`: replaces the NumericList that defines the linear combination based signatures.
- `getSigFuncs(object)`: returns the signature functions of an object.
- `groups(object)`: returns a factor vector representing the signature groups.
- `groups(object) <- value`: replaces the factor vector representing the signature groups.
- `version(object)`: returns the signature version.
- `version(object) <- value`: replaces the signature version.
sThresh

Author(s)
Patrick Aboyoun

See Also
NanoStringRccSet

Examples
SignatureSet(weights=list(x = c(a = 1),
y = c(b = 1/3, d = 2/3),
z = c(a = 2, c = 4)),
groups=factor("x", "y", "z"),
func = c(x="default", y="default", z="default"))

Description
Convenience functions for matrix thresholding, centering, and scaling based upon margin statistics.

Usage
# Loop over features
fThresh(x, STATS)
fCenter(x, STATS)
fScale(x, STATS)

## Round results to integers
fIntThresh(x, STATS)
fIntCenter(x, STATS)
fIntScale(x, STATS)

## Comparisons
fAbove(x, STATS)
fBelow(x, STATS)
fAtLeast(x, STATS)
fAtMost(x, STATS)

# Loop over samples
sThresh(x, STATS)
sCenter(x, STATS)
sScale(x, STATS)

# Round results to integers
sThresh(x, STATS)
sIntThresh(x, STATS)
sIntCenter(x, STATS)
sIntScale(x, STATS)

## Comparisons
sAbove(x, STATS)
sBelow(x, STATS)
sAtLeast(x, STATS)
sAtMost(x, STATS)

Arguments

- **x**: a numeric array.
- **STATS**: the summary statistic for thresholding, centering, or scaling.

Details

These functions are convenience wrappers for the following code:

- **fThresh**: sweep(x, 1L, STATS, FUN = "pmax")
- **fCenter**: sweep(x, 1L, STATS, FUN = "-")
- **fScale**: sweep(x, 1L, STATS, FUN = "/")
- **fIntThresh**: round(sweep(x, 1L, STATS, FUN = "pmax"))
- **fIntCenter**: round(sweep(x, 1L, STATS, FUN = "+"))
- **fIntScale**: round(sweep(x, 1L, STATS, FUN = "/"))
- **fAbove**: sweep(x, 1L, STATS, FUN = ">")
- **fBelow**: sweep(x, 1L, STATS, FUN = "<")
- **fAtLeast**: sweep(x, 1L, STATS, FUN = ">=")
- **fAtMost**: sweep(x, 1L, STATS, FUN = "<=")
- **sThresh**: sweep(x, 2L, STATS, FUN = "pmax")
- **sCenter**: sweep(x, 2L, STATS, FUN = "-")
- **sScale**: sweep(x, 2L, STATS, FUN = "/")
- **sIntThresh**: round(sweep(x, 2L, STATS, FUN = "pmax"))
- **sIntCenter**: round(sweep(x, 2L, STATS, FUN = "+"))
- **sIntScale**: round(sweep(x, 2L, STATS, FUN = "/"))
- **sAbove**: sweep(x, 2L, STATS, FUN = ">")
- **sBelow**: sweep(x, 2L, STATS, FUN = "<")
- **sAtLeast**: sweep(x, 2L, STATS, FUN = ">=")
- **sAtMost**: sweep(x, 2L, STATS, FUN = "<=")

Value

An array with the same shape as x that has been modified by thresholding, centering, or scaling.
writeNanoStringRccSet

Author(s)
Patrick Aboyoun

See Also
sweep

Examples

# Find reasonable column minimums
thresh <- apply(stack.x, 2L, quantile, 0.05)

# Threshold column values
identical(sThresh(stack.x, thresh),
         sweep(stack.x, 2L, thresh, FUN = "pmax"))

# Subtract column values
identical(sCenter(stack.x, thresh),
         sweep(stack.x, 2L, thresh))

# Scale to common mean
identical(sScale(stack.x, colMeans(stack.x) / mean(colMeans(stack.x))),
         sweep(stack.x, 2L, colMeans(stack.x) / mean(colMeans(stack.x)),
               FUN = "/")

# Scale to common mean, rounded to the nearest integer
sIntScale(stack.x, colMeans(stack.x) / mean(colMeans(stack.x)))

writeNanoStringRccSet(x, dir = getwd())

Arguments

x      an instance of class NanoStringRccSet.
dir    An optional character string representing the path to the directory for the RCC files.

Details
Writes a set of NanoString Reporter Code Count (RCC) files based upon x in dir.
Value

A character vector containing the paths for all the newly created RCC files.

Author(s)

Patrick Aboyoun

See Also

`NanoStringRccSet`, `readNanoStringRccSet`

Examples

datadir <- system.file("extdata", "3D_Bio_Example_Data",
                        package = "NanoStringNCTools")
rccs <- dir(datadir, pattern = "SKMEL.*\.RCC$", full.names = TRUE)
solidTumorNoRlfPheno <- readNanoStringRccSet(rccs)
writeNanoStringRccSet(solidTumorNoRlfPheno, tempdir())
for (i in seq_along(rccs)) {
  stopifnot(identical(readLines(rccs[i]),
                        readLines(file.path(tempdir(), basename(rccs[i])))))
}
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