

# Package ‘NanoStringNCTools’

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**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.

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**Imports** BiocGenerics, Biostrings, ggbeeswarm, ggiraph, ggthemes, grDevices, IRanges, methods, pheatmap, RColorBrewer, stats, utils

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NanoStringRccSet-validity.R NanoStringRccSet-accessors.R  
NanoStringRccSet-signatures.R NanoStringRccSet-subset.R  
NanoStringRccSet-utils.R NanoStringRccSet-summary.R  
NanoStringRccSet-qc.R NanoStringRccSet-normalize.R  
NanoStringRccSet-munge.R NanoStringRccSet-ggplot.R  
NanoStringRccSet-autoplot.R readRccFile.R readRlfFile.R  
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Contents

|                                     |           |
|-------------------------------------|-----------|
| geom_beeswarm_interactive . . . . . | 2         |
| log2t . . . . .                     | 3         |
| NanoStringRccSet-autoplot . . . . . | 4         |
| NanoStringRccSet-class . . . . .    | 7         |
| normalize . . . . .                 | 11        |
| readNanoStringRccSet . . . . .      | 13        |
| readRccFile . . . . .               | 14        |
| readRlffFile . . . . .              | 15        |
| setQCFlags . . . . .                | 16        |
| SignatureSet-class . . . . .        | 18        |
| sThresh . . . . .                   | 19        |
| writeNanoStringRccSet . . . . .     | 21        |
| <b>Index</b>                        | <b>23</b> |

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|                                                  |
|--------------------------------------------------|
| geom_beeswarm_interactive                        |
| <i>Geometry for Interactive Bee Swarm Points</i> |

---

Description

The interactive version of [geom\\_beeswarm](#) from **ggbeeswarm**.

Usage

```
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 <a href="#">geom_beeswarm</a> .                  |
| data     | The data to be displayed at this layer. See <a href="#">geom_beeswarm</a> . |
| priority | Method used to perform point layout. See <a href="#">geom_beeswarm</a> .    |
| cex      | Scaling for adjusting point spacing. See <a href="#">geom_beeswarm</a> .    |

|             |                                                                                                       |
|-------------|-------------------------------------------------------------------------------------------------------|
| groupOnX    | Indicator for jittering on x-axis. See <a href="#">geom_beeswarm</a> .                                |
| dodge.width | Dodge amount for points from different aesthetic groups. See <a href="#">geom_beeswarm</a> .          |
| stat        | The statistical transformation to use on the data for this layer. See <a href="#">geom_beeswarm</a> . |
| na.rm       | Indicator for removing missing values with a warning. See <a href="#">geom_beeswarm</a> .             |
| show.legend | Indicator for including this layer in the legend. See <a href="#">geom_beeswarm</a> .                 |
| inherit.aes | Indicator for inheriting the aesthetics. See <a href="#">geom_beeswarm</a> .                          |
| ...         | Additional arguments. See <a href="#">geom_beeswarm</a> .                                             |

**Value**

The interactive geometry based on [geom\\_beeswarm](#).

**Author(s)**

Patrick Aboyoun

**See Also**

[geom\\_beeswarm](#)

**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)

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**

```
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

*Plot NanoStringRccSet Data*

---

**Description**

Generate common plots to visualize and QC NanoStringRccSet data.

**Usage**

```
## S3 method for class 'NanoStringRccSet'
autoplot(object,
  type = c("boxplot-feature",
    "boxplot-signature",
    "bindingDensity-mean",
    "bindingDensity-sd",
    "ercc-linearity",
    "ercc-lod",
    "heatmap-genes",
```

```

        "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 <a href="#">AnnotatedDataFrame</a> containing the phenotypic data.                                                                                                                                                                                                                                                    |
| featureData    | An <a href="#">AnnotatedDataFrame</a> containing columns "CodeClass", "GeneName", "Accession", "IsControl", and "ControlConc".                                                                                                                                                                                           |
| experimentData | An optional <a href="#">MIAME</a> instance with meta-data about the experiment.                                                                                                                                                                                                                                          |
| annotation     | A character string for the "GeneRLF".                                                                                                                                                                                                                                                                                    |
| protocolData   | An <a href="#">AnnotatedDataFrame</a> containing columns "FileVersion", "SoftwareVersion", "SystemType", "SampleID", "SampleOwner", "SampleComments", "SampleDate", "SystemAPF", "AssayType", "LaneID", "FovCount", "FovCounted", "ScannerID", "StagePosition", "BindingDensity", "CartridgeID", and "CartridgeBarcode". |
| 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 <a href="#">SignatureSet</a> object containing signature definitions.                                                                                                                                                                                                                                        |
| design         | An optional one-sided formula representing the experimental design based on columns from <a href="#">phenoData</a>                                                                                                                                                                                                       |
| ...            | Additional arguments for <a href="#">ExpressionSet</a> .                                                                                                                                                                                                                                                                 |

**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, heatmap)**

**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
design(solidTumor)
munge(solidTumor)

# 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.

## 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  $2^{\log_2}$  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  $2^{\log_2}$ . 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**

NanoString nSolver User Manual [https://www.nanostring.com/download\\_file/view/1168](https://www.nanostring.com/download_file/view/1168)

**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")
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.       |
| "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

*Read RLF File*

---

**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 |

**Author(s)**

Patrick Aboyoun

**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, ERCLinearity, and ERCLoD

**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")
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)

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

#Update cutoffs
newQCCutoffs <- 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**

```
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.

**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"))
```

sThresh

*Convenience Functions for Assay Data Element Sweep Operations***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
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.

### 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 *Write NanoString Reporter Code Count (RCC) files*

---

**Description**

Write NanoString Reporter Code Count (RCC) files from an instance of class [NanoStringRccSet](#).

**Usage**

```
writeNanoStringRccSet(x, dir = getwd())
```

**Arguments**

|     |                                                                                        |
|-----|----------------------------------------------------------------------------------------|
| x   | an instance of class <a href="#">NanoStringRccSet</a> .                                |
| 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])))))  
}
```

# Index

- \* **NanoStringRccSet**
  - readNanoStringRccSet, [13](#)
  - writeNanoStringRccSet, [21](#)
- \* **array**
  - sThresh, [19](#)
- \* **classes**
  - NanoStringRccSet-class, [7](#)
  - SignatureSet-class, [18](#)
- \* **datasets**
  - NanoStringRccSet-autoplot, [4](#)
  - setQCFlags, [16](#)
- \* **file**
  - readNanoStringRccSet, [13](#)
  - readRccFile, [14](#)
  - readRlffFile, [15](#)
  - writeNanoStringRccSet, [21](#)
- \* **graphics**
  - geom\_beeswarm\_interactive, [2](#)
- \* **iteration**
  - sThresh, [19](#)
- \* **manip**
  - readNanoStringRccSet, [13](#)
  - readRccFile, [14](#)
  - readRlffFile, [15](#)
  - writeNanoStringRccSet, [21](#)
- \* **math**
  - log2t, [3](#)
- \* **methods**
  - NanoStringRccSet-class, [7](#)
  - SignatureSet-class, [18](#)
- \* **normalize**
  - normalize, [11](#)
- [, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- AnnotatedDataFrame, [7](#)
- assayData, [8](#), [9](#)
- assayDataApply  
(NanoStringRccSet-class), [7](#)
- assayDataApply, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- autoplot (NanoStringRccSet-autoplot), [4](#)
- class: NanoStringRccSet  
(NanoStringRccSet-class), [7](#)
- class: SignatureSet  
(SignatureSet-class), [18](#)
- coerce, ExpressionSet, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- controlSubset (NanoStringRccSet-class),  
[7](#)
- controlSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- DataFrame, [15](#)
- design, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- design<-, NanoStringRccSet, ANY-method  
(NanoStringRccSet-class), [7](#)
- design<-, NanoStringRccSet, formula-method  
(NanoStringRccSet-class), [7](#)
- design<-, NanoStringRccSet, NULL-method  
(NanoStringRccSet-class), [7](#)
- dimLabels, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- dimLabels<-, NanoStringRccSet, character-method  
(NanoStringRccSet-class), [7](#)
- endogenousSubset  
(NanoStringRccSet-class), [7](#)
- endogenousSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- esBy (NanoStringRccSet-class), [7](#)
- esBy, NanoStringRccSet-method  
(NanoStringRccSet-class), [7](#)
- experimentData, [9](#)
- ExpressionSet, [7](#), [8](#), [10](#)
- fAbove (sThresh), [19](#)

- fAtLeast (sThresh), 19
- fAtMost (sThresh), 19
- fBelow (sThresh), 19
- fCenter (sThresh), 19
- featureData, 8, 9
- fIntCenter (sThresh), 19
- fIntScale (sThresh), 19
- fIntThresh (sThresh), 19
- fScale (sThresh), 19
- fThresh (sThresh), 19
  
- geom\_beeswarm, 2, 3
- geom\_beeswarm\_interactive, 2
- getSigFuncs (SignatureSet-class), 18
- getSigFuncs, SignatureSet-method  
(SignatureSet-class), 18
- ggplot.NanoStringRccSet  
(NanoStringRccSet-class), 7
- groups (SignatureSet-class), 18
- groups, SignatureSet-method  
(SignatureSet-class), 18
- groups<- (SignatureSet-class), 18
- groups<- , SignatureSet, ANY-method  
(SignatureSet-class), 18
- groups<- , SignatureSet, factor-method  
(SignatureSet-class), 18
- groups<- , SignatureSet, NULL-method  
(SignatureSet-class), 18
  
- housekeepingSubset  
(NanoStringRccSet-class), 7
- housekeepingSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
  
- length, SignatureSet-method  
(SignatureSet-class), 18
- lengths, SignatureSet-method  
(SignatureSet-class), 18
- log, 4
- log2, 4
- log2t, 3
- logt (log2t), 3
  
- MIAME, 7
- munge (NanoStringRccSet-class), 7
- munge, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
  
- names, SignatureSet-method  
(SignatureSet-class), 18
  
- NanoStringRccSet, 13, 17, 19, 21, 22
- NanoStringRccSet  
(NanoStringRccSet-class), 7
- NanoStringRccSet, environment-method  
(NanoStringRccSet-class), 7
- NanoStringRccSet, ExpressionSet-method  
(NanoStringRccSet-class), 7
- NanoStringRccSet, matrix-method  
(NanoStringRccSet-class), 7
- NanoStringRccSet, missing-method  
(NanoStringRccSet-class), 7
- NanoStringRccSet, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- NanoStringRccSet-autoplot, 4
- NanoStringRccSet-class, 7
- negativeControlSubset  
(NanoStringRccSet-class), 7
- negativeControlSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- nonControlSubset  
(NanoStringRccSet-class), 7
- nonControlSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- normalize, 11
- normalize, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- NumericList, 18
  
- phenoData, 7–9
- positiveControlSubset  
(NanoStringRccSet-class), 7
- positiveControlSubset, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- preproc, 9
- protocolData, 8, 9
  
- readNanoStringRccSet, 10, 13, 15, 22
- readRccFile, 14
- readRlffFile, 15
  
- sAbove (sThresh), 19
- sAtLeast (sThresh), 19
- sAtMost (sThresh), 19
- sBelow (sThresh), 19
- sCenter (sThresh), 19
- sData (NanoStringRccSet-class), 7
- sData, NanoStringRccSet-method  
(NanoStringRccSet-class), 7
- setQCFlags, 16

setQCFlags, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 setSignatureFuncs<-  
     (NanoStringRccSet-class), 7  
 setSignatureFuncs<-, NanoStringRccSet, character-method  
     (NanoStringRccSet-class), 7  
 setSignatureGroups<-  
     (NanoStringRccSet-class), 7  
 setSignatureGroups<-, NanoStringRccSet, character-method  
     (NanoStringRccSet-class), 7  
 setSignatureGroups<-, NanoStringRccSet, factor-method  
     (NanoStringRccSet-class), 7  
 show, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 show, SignatureSet-method  
     (SignatureSet-class), 18  
 signatureFuncs  
     (NanoStringRccSet-class), 7  
 signatureFuncs, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 signatureGroups  
     (NanoStringRccSet-class), 7  
 signatureGroups, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 signatures (NanoStringRccSet-class), 7  
 signatures, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 signatures<- (NanoStringRccSet-class), 7  
 signatures<-, NanoStringRccSet, SignatureSet-method  
     (NanoStringRccSet-class), 7  
 signatureScores  
     (NanoStringRccSet-class), 7  
 signatureScores, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 signatureScoresApply  
     (NanoStringRccSet-class), 7  
 signatureScoresApply, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 SignatureSet, 7, 8  
 SignatureSet (SignatureSet-class), 18  
 SignatureSet-class, 18  
 signatureSubset  
     (NanoStringRccSet-class), 7  
 signatureSubset, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 sIntCenter (sThresh), 19  
 sIntScale (sThresh), 19  
 sIntThresh (sThresh), 19  
 sScale (sThresh), 19  
 sThresh, 19  
 subset, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 summary, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 svarLabels (NanoStringRccSet-class), 7  
 svarLabels, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 sweep, 21  
 transform, 9  
 transform, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 update\_geom\_params  
     (NanoStringRccSet-class), 7  
 version (SignatureSet-class), 18  
 version, SignatureSet-method  
     (SignatureSet-class), 18  
 version<- (SignatureSet-class), 18  
 version<-, SignatureSet, ANY-method  
     (SignatureSet-class), 18  
 version<-, SignatureSet, character-method  
     (SignatureSet-class), 18  
 version<-, SignatureSet, NULL-method  
     (SignatureSet-class), 18  
 weights, SignatureSet-method  
     (SignatureSet-class), 18  
 weights<- (SignatureSet-class), 18  
 weights<-, SignatureSet, ANY-method  
     (SignatureSet-class), 18  
 weights<-, SignatureSet, CompressedNumericList-method  
     (SignatureSet-class), 18  
 weights<-, SignatureSet, list-method  
     (SignatureSet-class), 18  
 weights<-, SignatureSet, NULL-method  
     (SignatureSet-class), 18  
 weights<-, SignatureSet, NumericList-method  
     (SignatureSet-class), 18  
 with, NanoStringRccSet-method  
     (NanoStringRccSet-class), 7  
 writeNanoStringRccSet, 10, 13, 21