

# Package ‘NanoStringQCPro’

December 5, 2022

**Title** Quality metrics and data processing methods for NanoString mRNA gene expression data

**Version** 1.30.0

**Date** 2018-04-10

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**Description** NanoStringQCPro provides a set of quality metrics that can be used to assess the quality of NanoString mRNA gene expression data -- i.e. to identify outlier probes and outlier samples. It also provides different background subtraction and normalization approaches for this data. It outputs suggestions for flagging samples/probes and an easily sharable html quality control output.

**Depends** R (>= 3.2), methods

**Imports** AnnotationDbi (>= 1.26.0), org.Hs.eg.db (>= 2.14.0), Biobase (>= 2.24.0), knitr (>= 1.12), NMF (>= 0.20.5), RColorBrewer (>= 1.0-5), png (>= 0.1-7)

**Suggests** roxygen2 (>= 4.0.1), testthat, BiocStyle, knitr, rmarkdown

**License** Artistic-2.0

**biocViews** Microarray, mRNAMicroarray, Preprocessing, Normalization, QualityControl, ReportWriting

**LazyData** true

**RoxygenNote** 6.0.1

**VignetteBuilder** knitr

**git\_url** <https://git.bioconductor.org/packages/NanoStringQCPro>

**git\_branch** RELEASE\_3\_16

**git\_last\_commit** d6922c8

**git\_last\_commit\_date** 2022-11-01

**Date/Publication** 2022-12-05

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---

addCodesetAnnotation,RccSet-method

*Add NanoString codeset annotation to an RccSet*

---

## Description

Returns a copy of the input RccSet where the codeset annotation has been merged into its fData slot. The merge key for each is a string formed from the concatenation of their CodeClass, GeneName, and Accession columns ("`<CodeClass>_<GeneName>_<Accession>`"). For creating the codeset annotation object, see buildCodesetAnnotation().

## Usage

```
## S4 method for signature 'RccSet'
addCodesetAnnotation(rccSet, annot, reorder = TRUE,
  showWarnings = TRUE)
```

## Arguments

rccSet	An RccSet object.
annot	Data frame containing the codeset annotation.

reorder	Logical indicating whether the probes should be reordered according to their barcodes (this can help in identifying barcode-specific artifacts – i.e. background noise).
showWarnings	Logical indicating whether or not warnings should be shown, if any.

**Value**

A copy of the input RccSet where the codeset annotation has been merged into its fData slot.

**Author(s)**

Dorothee Nickles, Robert Ziman

**Examples**

```
rccDir <- system.file("extdata", "RCC", package="NanoStringQCPro")
rccSet <- newRccSet(rccFiles = dir(rccDir, full.names=TRUE))
rlf <- system.file("extdata", "RLF", "NQCP_example.rlf", package="NanoStringQCPro")
annot <- buildCodesetAnnotation(rlf)
rccSet.annotated <- addCodesetAnnotation(rccSet, annot)
```

---

addQCFlags,RccSet-method

*Add sample QC flags to an rccSet*

---

**Description**

Returns a copy of the input RccSet with columns added to pData from the provided sample QC flag annotation file. (That file is produced by makeQCReport(); see its help page for more details.)

**Usage**

```
## S4 method for signature 'RccSet'
addQCFlags(rccSet, flagFile)
```

**Arguments**

rccSet	An RccSet object
flagFile	Path to a sample QC flag file as generated by the NanoStringQCPro QC report (see makeQCReport())

**Value**

A copy of the input RccSet with columns added to pData from the QC flag file.

**Author(s)**

Dorothee Nickles

---

allSumPlot,RccSet-method  
*allSumPlot*

---

### Description

Plot the sum of all counts (endogenous and housekeeping genes only) for each sample in an RccSet object.

### Usage

```
## S4 method for signature 'RccSet'  
allSumPlot(rccSet, method = c("cutoffByMMAD",  
  "cutoffByVar"), stringency = 4)
```

### Arguments

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar).

### Details

The sum of counts for each sample in the RccSet are plotted and outliers (as determined the cutoff function specified by the method argument) are marked in red (thresholds for outlier definition are plotted as red dashed lines).

### Value

A plot

### Author(s)

Dorothee Nickles

---

assessHousekeeping,RccSet-method  
*assessHousekeeping*

---

## Description

Assess correlation and variance/variability of housekeeping genes

## Usage

```
## S4 method for signature 'RccSet'  
assessHousekeeping(rccSet, hk, covar, annotate = TRUE,  
  plot = TRUE, digits = 2)
```

## Arguments

rccSet	An RccSet object
hk	Either a boolean vector of length <code>nrow(exprs(rccSet))</code> or a numeric vector of indices which genes in <code>exprs(rccSet)</code> are housekeeping genes
covar	character; colname in <code>fData(rccSet)</code> that can be used to label genes by a category of interest
annotate	Scalar boolean; if TRUE (default), probes will be "annotated" using the "Gene-Name" column in the <code>fData(rccSet)</code> slot
plot	Scalar boolean, plot pairwise relationships ?
digits	Scalar integer, the number of decimal places

## Details

Pairwise correlations of all defined housekeeping genes will be assessed and pairwise scatterplots will be generated. This function does not only output pairwise correlation coefficients, but also - for each housekeeping gene - the variance, the interquartile range (IQR) and median expression level across all samples in the experiment.

## Value

A dataframe with one row per housekeeping genes and several columns with metrics suggested to assess performance of defined housekeeping genes.

## Author(s)

Dorothee Nickles

---

bdPlot,RccSet-method    *Binding density plot*

---

### Description

Plot the binding density of each sample in an RccSet object. Samples with a binding density < 0.05 or > 2.25 (thresholds defined by NanoString) are marked in red (dashed red line indicates threshold).

### Usage

```
## S4 method for signature 'RccSet'  
bdPlot(rccSet)
```

### Arguments

rccSet            An RccSet object

### Value

A plot

### Author(s)

Dorothee Nickles

---

buildCodesetAnnotation  
                          *Build NanoString codeset annotation*

---

### Description

This function returns a data frame whose content is the combination of the NanoString-provided codeset annotation (.RLF file and the "Design Data" tab of the CDR spreadsheet) with gene annotation in the org.Hs.eg.db package.

### Usage

```
buildCodesetAnnotation(rlf = NULL, cdrDesignData = NULL,  
                          removeRedundantCols = TRUE, addEgAnnotations = FALSE)
```

**Arguments**

<code>rlf</code>	Path to the RLF file
<code>cdrDesignData</code>	Path to a manually prepared .CSV export of the "Design Data" tab of the CDR file (optional; see 'details' section below for how the export should be prepared)
<code>removeRedundantCols</code>	Logical. If TRUE, cols in the CDR that are redundant with those in the RLF will be omitted from the output.
<code>addEgAnnotations</code>	Logical indicating whether or not to add EntrezGene IDs and HGNC symbols from the <code>org.Hs.eg.db</code> package.

**Details**

The original NanoString provided .RLF file is expected as input. This file is the master (i.e. only probes listed here will be annotated; any extra ones in the CDR export will be dropped). If the CDR "Design Data" .CSV is specified, the function expects this .CSV file to be generated from the "Design Data" tab of the original NanoString provided Excel CDR file. This tab needs to be trimmed by skipping the NanoString header and first column containing only integers; the resulting .CSV should contain the actual table (including its header – beginning with "Customer Identifier"). The function will match and join the .RLF and CDR .CSV using their "ProbeID" and "NSID" fields, and then it will add gene annotation (EntrezGene ID, HGNC symbol, and chromosomal position) by doing lookups in the `org.Hs.eg.db` package using the RefSeq accessions from the RLF.

**Value**

A data frame whose content is the combination of the NanoString-provided codeset annotation with gene annotation in the `org.Hs.eg.db` package.

**Author(s)**

Dorothee Nickles, Robert Ziman

**Examples**

```
rlf <- system.file("extdata", "RLF", "NQCP_example.rlf", package="NanoStringQCPro")
cdrDesignData <- system.file("extdata", "CDR", "CDR-DesignData.csv", package="NanoStringQCPro")
annot <- buildCodesetAnnotation(rlf, cdrDesignData)
```

---

checkRccSet,RccSet-method

*Check an RccSet*

---

**Description**

Provides additional checks and generates warnings for unexpected or unusual conditions which, though permitted by the `RccSet` class, may indicate data import errors.



**Usage**

```
## S4 method for signature 'RccSet'
checkRccSet(rccSet, reportWarnings = TRUE,
            showMessages = FALSE)
```

**Arguments**

rccSet            An RccSet to be checked.

reportWarnings   Logical. If TRUE, warnings are reported.

showMessages    Logical. If TRUE, notes are shown indicating any optional missing columns and the like.

**Value**

Returns TRUE if no warnings were generated and FALSE otherwise.

**Author(s)**

Robert Ziman

**Examples**

```
data(example_rccSet)
checkRccSet(example_rccSet)
```

---

colByCovar

*colByCovar*

---

**Description**

Define colors based on a covariate of an RccSet object

**Usage**

```
colByCovar(pdata, covar)
```

**Arguments**

pdata            pData() of an RccSet object

covar            character, colname in the pdata used to stratify (color) data

**Value**

A list of length 2, with `[["color"]]` being a character vector of colors (one color for each level of covar) of length=number of observations and `[["legend"]]` providing the levels of covar to map colors to covar

**Author(s)**

Dorothee Nickles

---

colByFun	<i>colByFun</i>
----------	-----------------

---

**Description**

Color x based on upper and lower thresholds

**Usage**

colByFun(x, thresholds)

**Arguments**

x	Numeric vector
thresholds	List of length 2, with a scalar numeric in each slot, one giving the lower the upper threshold (for outlier definition)

**Value**

A vector of colors, with "red" for all values of x exceeding thresholds and "black" for all other values

**Author(s)**

Dorothee Nickles

---

contentNorm,RccSet-method	<i>Content normalization</i>
---------------------------	------------------------------

---

**Description**

Performs content normalization on the given RccSet.

**Usage**

```
## S4 method for signature 'RccSet'
contentNorm(rccSet, method = c("global", "housekeeping"),
  summaryFunction = "median", hk = NULL, inputMatrix = c("bgCorrData",
  "posCtrlData", "exprs"), quietly = FALSE)
```

**Arguments**

rccSet	An RccSet.
method	Specifies the features to be used for normalization. "global" indicates that all features should be used and "housekeeping" indicates that only housekeeping features should be used. If "housekeeping" is specified and the 'hk' argument (below) is also specified, then the features indicated by 'hk' will be used. If "housekeeping" is specified and 'hk' is left NULL, then the default housekeeping features (i.e. those with CodeClass == "Housekeeping") will be used.
summaryFunction	Character specifying the summary function to apply to the selected features (e.g. "mean" or "median"). User-defined functions similar to these can be specified here as well.
hk	Logical vector defining, for each feature, whether or not it shall be used for housekeeping normalization if housekeeping is specified as the normalization method.
inputMatrix	Name of the matrix in the RccSet's assayData to use as input for performing content normalization (one of "exprs", "posCtrlData", or "bgCorrData"). If posCtrlData or bgCorrData are specified but not found in the assayData, an error will be generated.
quietly	Boolean specifying whether or not messages and warnings should be omitted.

**Value**

A copy of the input is returned with a new matrix named 'normData' added to the assayData that contains the content-normalized counts. (**NOTE:** normData contains values on a log2 scale while all other matrices in assayData are on a linear scale.) If housekeeping is specified as the normalization method, then the housekeeping features used will be recorded in the returned RccSet in a new featureData column named 'Housekeeping'. Parameters specified in the function call are also recorded in the output's experimentData@preprocessing list.

**Author(s)**

Dorothee Nickles

**Examples**

```
data(example_rccSet)

pcnorm_example_rccSet <- posCtrlNorm(example_rccSet)
bg <- getBackground(pcnorm_example_rccSet)
bgcorr_example_rccSet <- subtractBackground(pcnorm_example_rccSet, bg)

gmnorm_example_rccSet <- contentNorm(bgcorr_example_rccSet, method="global",
  inputMatrix="exprs")
hknorm_example_rccSet <- contentNorm(bgcorr_example_rccSet, method="housekeeping",
  summaryFunction="mean")
```

copyRccSet,RccSet-method

*Deep-copy a NanoString RccSet*

---

### Description

Returns a copy of the input RccSet where the copy's assayData has been produced via copyEnv() rather than a simple assignment – hence deep-copying the environment pointed to by assayData rather than just copying the pointer. This guarantees that if the copy's assayData is affected later in the code, assayData for the original won't be affected.

### Usage

```
## S4 method for signature 'RccSet'  
copyRccSet(rccSet)
```

### Arguments

rccSet            A NanoString RccSet to be copied.

### Value

A new RccSet that is a deep copy of the original.

### Author(s)

Robert Ziman

### Examples

```
data(example_rccSet)  
example_rccSet_2 <- copyRccSet(example_rccSet)  
assayData(example_rccSet)  
assayData(example_rccSet_2) # Should be different
```

---

countsInBlankSamples\_verticalPlot

*Plot counts in blank samples (vertical orientation)*

---

### Description

Plot counts in blank samples (vertical orientation)

### Usage

```
countsInBlankSamples_verticalPlot(rccSet, outputFile)
```

**Arguments**

<code>rccSet</code>	An <code>RccSet</code>
<code>outputFile</code>	Output PNG filename

**Value**

A PNG file containing a boxplot of the gene-wise counts for the blank samples in the input. The PNG is set to a fixed resolution of 300 pixels per inch and a fixed width of 2250 pixels (i.e. 7.5" at 300ppi), but the height varies with the size of the input. The font size is also fixed so that the labels will be legible even for large datasets.

---

`ctrlsOverviewPlot,RccSet-method`  
*ctrlsOverviewPlot*

---

**Description**

Plot individual negative and positive controls across all samples in an `RccSet` object.

**Usage**

```
## S4 method for signature 'RccSet'  
ctrlsOverviewPlot(rccSet)
```

**Arguments**

<code>rccSet</code>	An <code>RccSet</code> object
---------------------	-------------------------------

**Value**

A plot with two panels, one for the negative controls, one for the positive controls.

**Author(s)**

Dorothee Nickles

---

ctrlsZprimePlot, RccSet-method  
*ctrlsZprimePlot*

---

### Description

Plot distribution of counts and the Z' Factors comparing the negative controls and the three highest input positive controls of an RccSet object.

### Usage

```
## S4 method for signature 'RccSet'
ctrlsZprimePlot(rccSet)
```

### Arguments

rccSet            An RccSet object

### Value

A plot

### Author(s)

Dorothee Nickles

---

cutoffByMMAD            *cutoffByMMAD*

---

### Description

Determine cutoffs of x (for outlier detection) based on median

### Usage

```
cutoffByMMAD(x, d, ...)
```

### Arguments

x                    numeric vector  
d                    scalar numeric, factor by which to multiply MAD of x  
...                   additional parameters passed on to median()

**Value**

A list of length 2, with a scalar numeric in each slot, one giving the lower threshold ( $\text{median}(x) - d * \text{mad}(x)$ ), the other giving the upper threshold ( $\text{median}(x) + d * \text{mad}(x)$ ) for outlier definition.

**Author(s)**

Dorothee Nickles

---

 cutoffByVar

*cutoffByVar*


---

**Description**

Determine cutoffs of  $x$  (for outlier detection) based on a certain percent CV

**Usage**

`cutoffByVar(x, d, ...)`

**Arguments**

$x$	numeric vector
$d$	scalar numeric, percent CV; passed on to <code>dCoVar</code>
$\dots$	additional parameters passed on to <code>mean()</code>

**Value**

A list of length 2, with a scalar numeric in each slot, one giving the lower threshold ( $\text{mean}(x) - \text{CV}$ ) the other giving the upper threshold ( $\text{mean}(x) + \text{percent CV based cutoff}$ ) for outlier definition.

**Author(s)**

Dorothee Nickles

---

dCoVar	<i>dCoVar</i>
--------	---------------

---

**Description**

Determine standard deviation at a certain percent CV

**Usage**

```
dCoVar(x, d, ...)
```

**Arguments**

x	numeric vector
d	scalar numeric, percent CV
...	additional parameters passed on to mean()

**Value**

standard deviation of x at d percent of CV

**Author(s)**

Dorothee Nickles

---

densityPlot	<i>densityPlot</i>
-------------	--------------------

---

**Description**

Plot the density of counts for all endogenous and housekeeping genes for each sample in an RccSet object

**Usage**

```
densityPlot(M, log.transform = FALSE, pdata, covar, ...)
```

**Arguments**

M	One of the matrices from the assayData() of an RccSet object (make sure to set the log.transform parameter accordingly)
log.transform	Scalar boolean
pdata	pData() of the RccSet object
covar	character; colname in pData() that can be used to label genes by a category of interest (passed on to colByCovar)
...	additional plotting parameters



**Value**

A density plot

**Author(s)**

Dorothee Nickles

---

example_rccSet	<i>NanoStringQCPro example dataset</i>
----------------	--

---

**Description**

Example data for the NanoStringQCPro package

**Format**

An RccSet object

**Author(s)**

Dorothee Nickles

**Source**

This is an artificial dataset designed to resemble real data.

---

flagSamplesCount, RccSet-method
<i>flagSamplesCount</i>

---

**Description**

Flag samples based on overall counts

**Usage**

```
## S4 method for signature 'RccSet'  
flagSamplesCount(rccSet, method = c("cutoffByMMAD",  
  "cutoffByVar"), stringency = 4, maxMiss = 0.2)
```

**Arguments**

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar).
maxMiss	Numeric specifying the allowable fraction of genes below the lower limit of detection in a sample.

**Details**

The method and stringency arguments determine a cutoff value used to flag samples as outliers: samples will be flagged if the sum of counts of their endogenous genes exceeds the cutoff or if the ratio of the sums of their positive controls to the sums of their endogenous genes exceeds three times the cutoff. Samples will also be flagged if the fraction of genes below the lower limit of detection exceeds the maxMiss value.

**Value**

A numeric vector giving the indices of samples with outlier values according to the criteria described above.

**Author(s)**

Dorothee Nickles

---

flagSamplesCtrl,RccSet-method  
*flagSamplesCtrl*

---

**Description**

Flag samples based on the performance of their controls.

**Usage**

```
## S4 method for signature 'RccSet'
flagSamplesCtrl(rccSet, method = c("cutoffByMMAD",
  "cutoffByVar"), stringency = 4)
```

**Arguments**

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar).

**Details**

The method and stringency arguments determine a cutoff value used to flag outlier samples based on the interquartile range of negative and positive controls and in the slope of the linear fit of count versus input of positive controls. Outliers will also be flagged if their positive control scaling factor (see `posCtrlNorm`) is outside the NanoString recommended range (i.e. below 0.3 or greater than 3).

**Value**

A numeric vector giving the indices of samples with outlier values as described above.

**Author(s)**

Dorothee Nickles

---

`flagSamplesTech,RccSet-method`  
*flagSamplesTech*

---

**Description**

Flag samples based on their technical performance, i.e. field of vision (FOV) counted and binding density

**Usage**

```
## S4 method for signature 'RccSet'  
flagSamplesTech(rccSet)
```

**Arguments**

`rccSet`            An `RccSet` object

**Details**

Samples with a FOV counted/FOV count of less than 80

**Value**

A numeric vector giving the indices of samples with outlier values in FOV counted and binding density  $< 0.05$  or  $> 2.25$  (thresholds defined by NanoString) will be flagged.

**Author(s)**

Dorothee Nickles

---

fovPlot,RccSet-method *Fields of view (FOV) plot*

---

### Description

Plot the fraction of successfully imaged fields of view (FOV) in the given RccSet. The RccSet's phenoData should have 'FovCount' and 'FovCounted' columns populated with the total and successfully imaged FOV counts, respectively. Samples with a FOV counted/FOV count of less than 80

### Usage

```
## S4 method for signature 'RccSet'
fovPlot(rccSet)
```

### Arguments

rccSet            An RccSet object

### Value

A plot

### Author(s)

Dorothee Nickles

---

geneClustering            *Gene clustering heatmap*

---

### Description

Gene clustering heatmap

### Usage

```
geneClustering(rccSet, outputFile, main = "Gene clustering", covar = NULL)
```

### Arguments

rccSet            An RccSet  
outputFile        Output PNG filename  
main              Plot title  
covar             Colname in the rccSet's fData that can be used to label genes by a category of interest

**Value**

A PNG file showing clustering of genes by correlation across an experiment Positive and negative control probes and any zero-variance genes (typically housekeeping genes) are omitted from the heatmap. The width and height of the PNG file are set to vary with the size of the input.

**Author(s)**

Dorothee Nickles, Robert Ziman

---

getBackground,RccSet-method

*Get background estimates for a NanoString RccSet*

---

**Description**

Returns background estimates for a NanoString RccSet object. The function depends upon correct annotation in the RccSet: if the bgReference argument is set to "blanks", it expects blank measurements (i.e., water runs) to have their phenoData SampleType set to the value indicating blanks or an error will be thrown. (See getBlankLabel(); normally this value would have been set using an argument to newRccSet()). If bgReference is set to "negatives", then it expects to find the negative control probes via CodeClass == "Negative". If set to "both", it expects both of the above and will calculate initial background estimates using an algorithm that mimics the implementation in NanoString's nSolver Analysis Software (see the nSolverBackground() man page for details on the algorithm).

**Usage**

```
## S4 method for signature 'RccSet'
getBackground(rccSet, bgReference = c("both", "blanks",
  "negatives"), summaryFunction = "median", stringency = 0,
  nSolverBackground.shrink = TRUE, nSolverBackground.w1 = 2.18,
  inputMatrix = c("posCtrlData", "exprs"))
```

**Arguments**

rccSet	NanoString RccSet object.
bgReference	Measurements to use for background estimates: one of "blanks" (for blank samples), "negatives" (for negative control probes), or "both". Blanks are assumed to be indicated as in the description above.
summaryFunction	Summary function for background measurements (e.g. "mean" or "median"). User-defined functions similar to these can be specified here as well.
stringency	Factor by which deviation (SD or MAD) of the summarization output will be multiplied to obtain final background estimates.
nSolverBackground.shrink	Value to use for the 'shrink' argument to nSolverBackground().

`nSolverBackground.w1` Value to use for the 'w1' argument to `nSolverBackground()`.

`inputMatrix` Name of the matrix in the `RccSet`'s `assayData` to use as input for calculating background estimates (one of "exprs" or "posCtrlData"). If `posCtrlData` is specified but not present in the `assayData`, an error will be generated.

**Value**

A matrix containing background estimates for a NanoString `RccSet` object.

**Author(s)**

Dorothee Nickles

**See Also**

[subtractBackground](#)

**Examples**

```
data(example_rccSet)

## Calculate probe-specific background based on negative control probes
bg <- getBackground(example_rccSet, bgReference="negatives", summaryFunction="mean",
  inputMatrix="exprs")

## Calculate sample-specific background based on blanks
bg <- getBackground(example_rccSet, bgReference="blanks", inputMatrix="exprs")

## Calculate background that is both sample- and probe-specific
bg <- getBackground(example_rccSet, bgReference="both", stringency=1,
  inputMatrix="exprs")
```

---

`getBlankLabel,RccSet-method`

*Get the SampleType value that indicates blank samples*

---

**Description**

Returns the `phenoData` `SampleType` value that indicates blank samples (i.e. water runs). This value is parsed from the single-quoted string enclosed by "blankLabel='...'" in the `varMetadata` for `SampleType`.

**Usage**

```
## S4 method for signature 'RccSet'
getBlankLabel(rccSet, showWarnings = TRUE)
```

**Arguments**

rccSet            An RccSet  
showWarnings    Logical. If FALSE, no warnings will be generated (if any).

**Value**

NULL if the SampleType column is missing altogether, NA if the varMetadata doesn't have blankLabel recorded, or the blankLabel value otherwise.

**Author(s)**

Robert Ziman

**Examples**

```
data(example_rccSet)
blankLabel <- getBlankLabel(example_rccSet)
```

---

*getSpikeInInput*            *getSpikeInInput*

---

**Description**

Gets the RNA “spike-in” input levels for positive and negative control probes from the label in their GeneName. Note that this is a helper function for readRlf() and elsewhere and is not intended for external use.

**Usage**

```
getSpikeInInput(CodeClass, GeneName, nonCtrlProbeVal = NA)
```

**Arguments**

CodeClass            Character vector with code classes for each probe.  
GeneName            Character vector with gene names/symbols for each probe.  
nonCtrlProbeVal      Value to assign as the spike-in input for the non-control probes.

**Value**

A data frame with the input CodeClass and GeneName but where the latter has been split into two columns: one showing the GeneName for each probe with spike-in input labels removed – and another with the spike-in input levels.

**Author(s)**

Robert Ziman

---

*iqrPlot, RccSet-method* *iqrPlot*

---

### **Description**

Plot the interquartile range (IQR) for a certain code class of probes in an `RccSet` object.

### **Usage**

```
## S4 method for signature 'RccSet'  
iqrPlot(rccSet, codeClass = c("Negative", "Positive",  
  "Endogenous", "Housekeeping"), method = c("cutoffByMMAD", "cutoffByVar"),  
  stringency = 4)
```

### **Arguments**

<code>rccSet</code>	An <code>RccSet</code> object
<code>codeClass</code>	Character string specifying the code class (as annotated in the <code>fData(rccSet)\$CodeClass</code> column) for which the IQR shall be determined.
<code>method</code>	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
<code>stringency</code>	Numeric value passed to the cutoff function specified by the <code>method</code> argument (see the 'd' argument of <code>cutoffByMMAD</code> and <code>cutoffByVar</code> ).

### **Details**

IQR of the specified code class for each sample in the `RccSet` are plotted and outliers (as determined by the function specified in the `method` argument) are marked in red (thresholds for outlier definition are plotted as red dashed lines).

### **Value**

A plot

### **Author(s)**

Dorothee Nickles

### **See Also**

[cutoffByMMAD](#), [cutoffByVar](#)



---

lodAssess,RccSet-method  
*lodAssess*

---

**Description**

Assess how many genes in each sample in an RccSet object are below the limit of detection. (The current implementation does a straightforward column sum on the presence/absence matrix (paData) in assayData.)

**Usage**

```
## S4 method for signature 'RccSet'
lodAssess(rccSet)
```

**Arguments**

rccSet            An RccSet object

**Value**

A numeric vector giving the number of missing genes (endogenous and housekeeping genes) for each sample in an RccSet. If paData is not found in the input's assayData, NULL is returned.

**Author(s)**

Dorothee Nickles

---

lodPlot,RccSet-method *lodPlot*

---

**Description**

Function to plot the number of missing genes per sample in an RccSet object.

**Usage**

```
## S4 method for signature 'RccSet'
lodPlot(rccSet, maxMiss = 0.2)
```

**Arguments**

rccSet            An RccSet object  
maxMiss          Numeric specifying the allowable fraction of genes below the lower limit of detection in a sample.

**Details**

Samples with more than 50 measurements are present, they are represented as triangles.

**Value**

A plot

**Author(s)**

Dorothee Nickles

---

makeQCReport,RccSet-method

*Make NanoString QC report*

---

**Description**

Creates an html QC report for an RccSet object. Alongside the html file, a directory with matching filename is produced that contains additional files as well as high resolution versions of the various plots in the report. In addition to generating the QC report, the function returns a copy of the input RccSet with columns added to phenoData that show the QC flags for each sample.

The various plots in the report depend upon correct annotation and preprocessing in the input object. If the input is missing any elements required for a given plot, the plot will be replaced with a message indicating the missing elements. If the preprocOverride argument is set to TRUE, the input's preprocessing will be ignored and a default configuration will be used so that all applicable plots will be rendered.

**Usage**

```
## S4 method for signature 'RccSet'
makeQCReport(rccSet,
  outputBaseName = "NanoStringQCPro_QC_report", outputDir = getwd(),
  preprocOverride = FALSE,
  experimentTitle = expinfo(experimentData(rccSet))["title"],
  covar = "SampleType", method = c("cutoffByMMAD", "cutoffByVar"),
  stringency = 4, maxMiss = 0.2, sampleNameCol = "SampleID",
  heatmaps = FALSE, cleanMarkdown = TRUE, verbose = FALSE)
```

**Arguments**

rccSet	RccSet object for which to generate the QC report.
outputBaseName	Character string specifying the base filename (without extension) to use for the output file.
outputDir	Character string specifying the path to the output directory for the QC report and associated files.

preprocOverride	Logical. If TRUE, the input's preprocessing will be ignored, and a default preprocessing configuration (specifically, the defaults for <code>preprocRccSet()</code> ) will be applied so that all applicable plots can be rendered in the report.
experimentTitle	Character string specifying an easy to read identifier of the experiment.
covar	Character string specifying a covariate for stratifying samples (e.g. "Sample-Type").
method	Method to determine outlier samples: either "cutoffByVar" or "cutoffByMMAD".
stringency	Multiplier with which to adjust cutoff values for determining outlier samples.
maxMiss	Numeric specifying the allowable fraction of genes below the lower limit of detection in a sample.
sampleNameCol	Character string specifying the name of the phenoData column holding the sample names.
heatmaps	Logical: render and show heatmaps?
cleanMarkdown	Logical: upon completion, delete markdown files used to produce QC report?
verbose	Logical: print progress messages?

**Value**

An html report is written to disk and a copy of the input `RccSet` is invisibly returned with columns added to `phenoData` that show the QC flags for each sample.

**Author(s)**

Dorothee Nickles, Thomas Sandmann, Robert Ziman, Richard Bourgon

**Examples**

```
data(example_rccSet)
norm_example_rccSet <- preprocRccSet(example_rccSet)
qc_example_rccSet <- makeQCReport(norm_example_rccSet, "example_QC_report")
```

---

myCols

*myCols*


---

**Description**

Function that defines nice colors

**Usage**

```
myCols()
```

**Value**

A vector of colors

**Author(s)**

Dorothee Nickles

---

NanoStringQCPro	<i>NanoStringQCPro</i>
-----------------	------------------------

---

**Description**

NanoStringQCPro

---

<i>negCtrlsByLane,RccSet-method</i>	<i>negCtrlsByLane</i>
-------------------------------------	-----------------------

---

**Description**

Plot negative controls per lane in an RccSet object

**Usage**

```
## S4 method for signature 'RccSet'
negCtrlsByLane(rccSet)
```

**Arguments**

rccSet	An RccSet object
--------	------------------

**Details**

Boxplots are colored by lane (as specified in the pData slot). Bars on top of the panel indicate the stage position for each cartridge/sample (as specified in the pData slot).

**Value**

A plot with boxplots for the negative control counts for each individual sample (lane-specific background)

**Author(s)**

Dorothee Nickles

---

`negCtrlsByLane_verticalPlot`*Plot of negative controls by lane (vertical orientation)*

---

**Description**

Plot of negative controls by lane (vertical orientation)

**Usage**

```
negCtrlsByLane_verticalPlot(rccSet, outputFile)
```

**Arguments**

<code>rccSet</code>	An RccSet
<code>outputFile</code>	Output PNG filename

**Value**

A PNG file containing a boxplot of the counts for negative controls by lane in the input. The PNG is set to a fixed resolution of 300 pixels per inch and a fixed width of 2250 pixels (i.e. 7.5" at 300ppi), but the height varies with the size of the input. The font size is also fixed so that the labels will be legible even for large datasets.

---

`negCtrlsPairs, RccSet-method`*negCtrlsPairs*

---

**Description**

Pairs plot of negative controls across all samples in an RccSet object

**Usage**

```
## S4 method for signature 'RccSet'  
negCtrlsPairs(rccSet, log.transform = FALSE)
```

**Arguments**

<code>rccSet</code>	An RccSet object
<code>log.transform</code>	boolean, whether data needs to be log2 transformed

**Value**

Pairs plot of the negative controls with a scatter plot in the lower panel and correlation coefficients printed in the upper panel.

**Author(s)**

Dorothee Nickles

---

negCtrlsPlot,RccSet-method  
*negCtrlsPlot*

---

**Description**

Plot negative controls across all samples in an RccSet object

**Usage**

```
## S4 method for signature 'RccSet'  
negCtrlsPlot(rccSet)
```

**Arguments**

rccSet            An RccSet object

**Details**

In the second panel, boxplots are colored by lane (as specified in the pData slot). Bars on top of the panel indicate the stage position for each cartridge/sample (as specified in the pData slot).

**Value**

A plot with two panels: one showing boxplots for the individual negative controls across all samples, and one showing boxplots for the negative control counts for each individual sample (lane-specific background).

**Author(s)**

Dorothee Nickles

newRccSet

*Create a new RccSet object***Description**

This is the main wrapper function for generating an RccSet from NanoString data. The function takes as input a vector of NanoString .RCC files with the raw data or a .CSV file generated via the RCC Collector Tool Export feature of NanoString's nSolver Analysis Software, an optional path to the .RLF file describing the codeset used, optional paths to additional annotation about the features and samples, and details about the experiment. It returns an RccSet object.

**Usage**

```
newRccSet(rccFiles, rccCollectorToolExport = NULL, rlf = NULL,
  cdrDesignData = NULL, extraPdata = NULL, blankLabel = "blank",
  addEgAnnotations = FALSE, dropPdataCols = c("FileVersion",
  "SoftwareVersion", "Owner", "SystemAPF", "ScannerID", "CartridgeBarcode"),
  dropFdataCols = c("CodeClass_codesetAnnot", "Accession_codesetAnnot",
  "GeneName_codesetAnnot", "Accession_CDR"), experimentData.name = "",
  experimentData.lab = "", experimentData.contact = "",
  experimentData.title = "", experimentData.abstract = "",
  experimentData.url = "", experimentData.other = list())
```

**Arguments**

rccFiles	Vector of paths to .RCC files with the raw count data.
rccCollectorToolExport	Path to a .CSV file generated via the RCC Collector Tool Export feature of NanoString's nSolver Analysis Software. (Note that this is an alternative to rccFiles, and if both arguments are specified at the same time, the function will throw an error.)
rlf	Path to the NanoString .RLF file describing the codeset used in generating the .RCCs.
cdrDesignData	Path to a .CSV extract of the "Design Data" tab of a CDR spreadsheet corresponding to the rest of the input files. See 'Details' section of the buildCodesetAnnotation() help page for more info on how this extract should be prepared.
extraPdata	Vector of paths to files containing additional annotation about the samples which will be added to the phenoData of the output RccSet. All files should be tab-separated and should contain a column labelled "FileName" whose values correspond exactly to the basenames (including .RCC extension) of the files specified in rccFiles or listed in the RCC Collector Tool Export. More than one such file may be used. A SampleType column should be present in at most one file.
blankLabel	Value for the output's phenoData SampleType column that will indicate blank samples. This will be recorded in the varMetadata for SampleType. Blank samples, if available, play an important role in preprocessing.

<code>addEgAnnotations</code>	Logical indicating whether or not to add EntrezGene annotations from the <code>org.Hs.eg.db</code> package.
<code>dropPdataCols</code>	Character vector specifying <code>phenoData</code> columns to be dropped from the output object (if empty or <code>NULL</code> , no columns will be dropped).
<code>dropFdataCols</code>	Character vector specifying <code>featureData</code> columns to be dropped from the output object (if empty or <code>NULL</code> , no columns will be dropped).
<code>experimentData.name</code>	String passed to the 'name' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.lab</code>	String passed to the 'lab' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.contact</code>	String passed to the 'contact' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.title</code>	String passed to the 'title' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.abstract</code>	String passed to the 'abstract' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.url</code>	String passed to the 'url' slot of the output <code>RccSet</code> 's <code>experimentData</code> .
<code>experimentData.other</code>	List passed to the 'other' slot of the output <code>RccSet</code> 's <code>experimentData</code> .

## Details

In the `.RLF` (and sometimes in the `.RCC` files), the `GeneName` field for positive and negative control probes contains a parenthesized label indicating the RNA “spike-in” levels for each probe. These labels are removed from the control probe `GeneNames` in the output and recorded instead in `SpikeInInput` in the output's `featureData`.

A pseudocount of 1 is added to all measurements to enable subsequent log transformation of the data.

If the `phenoData SampleType` column is not specified via an annotation file passed in through `extraPdata`, it will be created and assigned `NA` for all samples.

## Value

An `RccSet` containing the raw `NanoString` data and annotations.

## Author(s)

Robert Ziman

## Examples

```
rccDir <- system.file("extdata", "RCC", package="NanoStringQCPro")
rccSet <- newRccSet(
  rccFiles = dir(rccDir, full.names=TRUE),
  rlf = system.file("extdata", "RLF", "NQCP_example.rlf", package="NanoStringQCPro"),
```



```

extraPdata = system.file("extdata", "extraPdata", "SampleType.txt", package="NanoStringQCPro"),
blankLabel = "blank",
experimentData.name = "Robert Ziman",
experimentData.lab = "Richard Bourgon",
experimentData.contact = "ziman.robert@gene.com",
experimentData.title = "NanoStringQCPro example dataset",
experimentData.abstract = "Example data for the NanoStringQCPro package"
)

```

---

nSolverBackground,RccSet-method

*nSolver Analysis Software background estimation*


---

### Description

Calculates initial probe- and lane-specific background estimates using an algorithm that mimics the implementation in NanoString's nSolver Analysis Software (see details below for the exact algorithm).

### Usage

```

## S4 method for signature 'RccSet'
nSolverBackground(rccSet, stringency = 1, shrink = TRUE,
  w1 = 2.18, inputMatrix = c("posCtrlData", "exprs"))

```

### Arguments

rccSet	NanoString RccSet object
stringency	Multiplier with which to adjust final values.
shrink	Boolean specifying if probe-specific estimates should be shrunken towards their global mean.
w1	Shrink weight "w1".
inputMatrix	Name of the matrix in the RccSet's assayData to use as input for calculating background estimates (one of "exprs" or "posCtrlData"). If posCtrlData is specified but not present in the assayData, an error will be generated.

### Details

The mean values for each blank lane (not including positive control probes) are computed from the original data, and a vector of probe-specific background is established by taking the rowMeans of the blank measurements for each probe after subtracting out these values. If shrink=TRUE, the vector is adjusted via the following formula (where 'probe.bg' represents the vector):

```

w2 <- 1/length(blanks)
probe.bg <- (w1*probe.bg + w2*mean(probe.bg)) / (w1 + w2)

```

This probe-specific background is further adjusted by subtracting the mean of its values for the negative control probes. A lane-specific "affinity" is calculated for all lanes in the original data by taking the colMeans of the negative control probe values in the original data, and background estimates for each probe and lane in the original data are computed by summing the corresponding probe-specific background and lane-specific affinity. Any resulting values less than zero are set to zero, and the last step before returning these values is to multiply them by the given stringency.

**Value**

A matrix containing lane- and probe-specific background estimates.

**Author(s)**

Dorothee Nickles, Thomas Sandmann

**See Also**

[getBackground](#), [subtractBackground](#)

---

`nSolverCsv.to.pdata_fdata_adata`

*nSolverCsv.to.pdata\_fdata\_adata*

---

**Description**

First stage of `readRccCollectorToolExport()`: produces a list containing matrices (for `pdata` and `adata`) and a data frame (for `fdata`) that `pdata_fdata_adata.to.rccSet` then transforms into a full `RccSet` (after some further checks and adjustments). Not intended for external use; see also `rccFiles.to.pdata_fdata_adata()`.

**Usage**

```
nSolverCsv.to.pdata_fdata_adata(rccCollectorToolExport)
```

**Arguments**

```
rccCollectorToolExport
```

Path to the nSolver RCC Collector Tool .CSV export.

**Value**

A list containing matrices (for `pdata` and `adata`) and a data frame (for `fdata`) that `pdata_fdata_adata.to.rccSet()` then transforms into a full `ExpressionSet`.

**Author(s)**

Dorothee Nickles, Thomas Sandmann, Robert Ziman

---

panelCor	<i>panelCor</i>
----------	-----------------

---

**Description**

Helper function for printing correlation coefficients inside a pairs plots

**Usage**

```
panelCor(x, y, digits = 2, cex.cor = 0.75, doTest = FALSE)
```

**Arguments**

x	integer
y	integer, same length as x
digits	scalar integer, indicating the number of decimal positions for displaying the correlation coefficient
cex.cor	scalar numeric to specify relative font size for printing the correlation coefficient
doTest	boolean, whether a results of cor.test should be displayed as well

**Value**

Prints correlation coefficients (and p-values if doTest = TRUE) within a pairs plot.

---

pcaPlot	<i>pcaPlot</i>
---------	----------------

---

**Description**

Wrapper function to perform a PCA analysis on the exprs slot of an RccSet object and plot some results

**Usage**

```
pcaPlot(exx, ...)
```

**Arguments**

exx	exprs() of an RccSet object
...	additional parameters passed on to the plotting functions

**Value**

PCA screeplot and a plot with two panels, one plotting PC1 versus PC2, the other plotting PC1 versus PC3.

**Author(s)**

Dorothee Nickles

---

`pdata_fdata_adata.to.rccSet`  
*pdata\_fdata\_adata.to.rccSet*

---

**Description**

Second stage of `readRccBatch()/readRccCollectorToolExport()` – not intended for external use.

**Usage**

```
pdata_fdata_adata.to.rccSet(pdata_fdata_adata)
```

**Arguments**

`pdata_fdata_adata`  
List containing the `pdata`, `fdata`, and `adata` returned by `rccFiles.to.pdata_fdata_adata()` or `nSolverCsv.to.pdata_fdata_adata()`.

**Details**

Note that a pseudo-count of 1 is always added to all measurements, to enable subsequent log transformation of the data in cases where zero-counts are present.

N.B. The function currently expects certain columns to be present in `pdata_fdata_adata$pdata.m`, and it converts these to numerics. These expectations should be incorporated into the class definition, and conversion should only take place with a warning. Future updates will address this.

**Value**

An [RccSet](#) whose contents reflect the input data.

**Author(s)**

Robert Ziman

---

posCtrlNorm,RccSet-method

*Positive control normalization*

---

## Description

Applies positive control normalization to the data in an RccSet object.

## Usage

```
## S4 method for signature 'RccSet'  
posCtrlNorm(rccSet, summaryFunction = "sum",  
            quietly = FALSE)
```

## Arguments

rccSet	An RccSet object.
summaryFunction	Function to be used for the normalization (e.g. "mean", "median", or "sum"). User-defined functions similar to these can be specified here as well.
quietly	Logical. If TRUE, messages and warnings will not be shown.

## Value

A copy of the input RccSet that has count data adjusted by positive control counts. The positive control scaling factor is recorded in PosFactor in the output's phenoData (if this column already exists in the input, it will be overwritten in the output copy).

## Author(s)

Dorothee Nickles

## Examples

```
data(example_rccSet)  
pcnorm_example_rccSet <- posCtrlNorm(example_rccSet)
```

---

*posNormFactPlot,RccSet-method*  
*posNormFactPlot*

---

**Description**

Plot positive control scaling factor for each sample in an RccSet object

**Usage**

```
## S4 method for signature 'RccSet'  
posNormFactPlot(rccSet)
```

**Arguments**

rccSet            An RccSet object

**Value**

A plot of the positive control scaling factor for each sample in an RccSet object. Samples with a positive control scaling factor  $< 0.3$  or  $> 3$  (thresholds defined by NanoString) are marked in red (dashed red line indicates threshold).

**Author(s)**

Dorothee Nickles

---

*posR2Plot,RccSet-method*  
*posR2Plot*

---

**Description**

Plot the R squared of linear fit of counts versus input for positive controls in an RccSet object.

**Usage**

```
## S4 method for signature 'RccSet'  
posR2Plot(rccSet)
```

**Arguments**

rccSet            RccSet object

**Details**

R squared for each sample in the RccSet are plotted and samples with R squared < 0.95 are marked in red (threshold indicated by dashed red line).

**Value**

A plot

**Author(s)**

Dorothee Nickles

---

posRatioPlot,RccSet-method  
*posRatioPlot*

---

**Description**

Plot the ratio of the mean of positive control counts for each sample and the overall mean of positive control counts in an RccSet object.

**Usage**

```
## S4 method for signature 'RccSet'
posRatioPlot(rccSet, method = c("cutoffByMMAD",
  "cutoffByVar"), stringency = 4)
```

**Arguments**

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar).

**Details**

The ratio for each sample in the RccSet is plotted and outliers (as determined the cutoff function specified by the method argument) are marked in red (thresholds for outlier definition are plotted as red dashed lines).

**Value**

A plot

**Author(s)**

Dorothee Nickles

---

posSlopePlot,RccSet-method  
*posSlopePlot*

---

**Description**

Plot the slope of linear fit of counts versus input for positive controls in an RccSet object.

**Usage**

```
## S4 method for signature 'RccSet'
posSlopePlot(rccSet, method = c("cutoffByMMAD",
  "cutoffByVar"), stringency = 4)
```

**Arguments**

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar).

**Details**

The slope for each sample in the RccSet are plotted and outliers (as determined by the function specified by the method argument) are marked in red (thresholds for outlier definition are plotted as red dashed lines).

**Value**

A plot

**Author(s)**

Dorothee Nickles

---

posSumVsAllSumPlot,RccSet-method  
*posSumVsAllSumPlot*

---

**Description**

Plot the ratio of sums of positive control counts to all counts for all samples in an RccSet object.



**Usage**

```
## S4 method for signature 'RccSet'  
posSumVsAllSumPlot(rccSet, method = c("cutoffByMMAD",  
  "cutoffByVar"), stringency = 4)
```

**Arguments**

rccSet	An RccSet object
method	Character string specifying the method for outlier detection: either "cutoffByMMAD" or "cutoffByVar".
stringency	Numeric value passed to the cutoff function specified by the method argument (see the 'd' argument of cutoffByMMAD and cutoffByVar). (If the median ratio is less than 1, three times this value will be used.)

**Details**

The ratio for each sample in the RccSet is plotted and outliers (as determined by the cutoff function specified by the method argument) are marked in red (thresholds for outlier definition are plotted as red dashed lines).

**Value**

A plot

**Author(s)**

Dorothee Nickles

---

preprocRccSet,RccSet-method

*Preprocess an RccSet*

---

**Description**

This function is a wrapper to perform any combination of positive control normalization, background correction, and content normalization on the input RccSet. For each completed preprocessing step, a matrix is added to the assayData of the resulting RccSet object:

- posCtrlData: expression data after positive control normalization
- bgEstimates: background estimates
- bgCorrData: expression data after positive control normalization and background correction
- normData: expression data after positive control normalization, background correction, and content normalization

(NOTE: normData is on a log2 scale while all the other matrices are on a linear scale.)

If any step is omitted, the corresponding matrix will not be present in the output's assayData. The parameters for all steps are recorded in the output's experimentData@preprocessing list (accessible through preproc(rccSet) where rccSet is an RccSet output by this function). In addition:

- If blanks are not present in the data, use bgReference="negatives" to prevent the function from throwing an error.
- If positive control normalization is performed, a column named 'PosCtrl' is added to the output's phenoData to record the positive control scaling factors.
- If the presence/absence call is performed, a matrix named 'paData' is added to the output's assayData to indicate the presence/absence of each feature in each sample. See the 'pa' argument for details.
- If housekeeping normalization is performed, a column labeled 'Housekeeping' is added to the featureData to indicate which features were used for it.

### Usage

```
## S4 method for signature 'RccSet'
preprocRccSet(rccSet, doPosCtrlNorm = TRUE,
  doBackground = TRUE, doPresAbs = TRUE, doContentNorm = TRUE,
  pcnSummaryFunction = "sum", bgReference = c("both", "blanks",
  "negatives"), bgSummaryFunction = "median", bgStringency = 1,
  nSolverBackground.w1 = 2.18, nSolverBackground.shrink = TRUE,
  paStringency = 2, normMethod = c("global", "housekeeping"),
  normSummaryFunction = "median", hkgenes = NULL, hkfeatures = NULL,
  quietly = FALSE)
```

### Arguments

rccSet	An RccSet.
doPosCtrlNorm	Boolean specifying whether or not to perform positive control normalization. ('pcd' is short for 'posCtrlData', the matrix which gets added to assayData when this step is performed.)
doBackground	Boolean specifying whether or not to perform background correction.
doPresAbs	Boolean specifying whether or not the presence/absence call should be performed. For details, see presAbsCall().
doContentNorm	Boolean specifying whether or not content normalization should be performed.
pcnSummaryFunction	Function to be used for the positive control normalization (e.g. "mean", "median", or "sum"). User-defined functions similar to these can be specified here as well.
bgReference	Measurements to use for background estimates: either "blank" (for blank samples), "negatives" (for negative control probes), or "both". For details on exactly how the background estimates are computed in each case, see getBackground().
bgSummaryFunction	Summary function for background measurements (e.g. "mean" or "median"). User-defined functions similar to these can be specified here as well.

bgStringency	Factor by which deviation (SD or MAD) of the summarization output will be multiplied to obtain final background estimates.
nSolverBackground.w1	Value to use for the 'w1' argument to nSolverBackground(). (Only takes effect if bgReference == "both"; see getBackground().)
nSolverBackground.shrink	Value to use for the 'shrink' argument to nSolverBackground(). (Only takes effect if bgReference == "both"; see getBackground().)
paStringency	Multiplier to use in establishing the presence/absence call. For details, see presAbsCall().
normMethod	Specifies the features to be used for content normalization. "global" indicates that all features should be used and "housekeeping" indicates that only housekeeping features should be used. If "housekeeping" is specified and the 'hk' argument (below) is also specified, then the features indicated by 'hk' will be used. If "housekeeping" is specified and 'hk' is left NULL, then the default housekeeping features (i.e. those with CodeClass == "Housekeeping") will be used.
normSummaryFunction	Character specifying the summary function to apply to the selected features (e.g. "mean" or "median") during the content normalization step. User-defined functions similar to these can be specified here as well.
hkgenes	Character vector with gene symbols to be used for content normalization if housekeeping is specified as the normalization method. If specified, all features that match any of the specified symbols will be used. (To specify specific features, use the 'hkfeatures' argument instead; see below.)
hkfeatures	Character vector with full feature names (" <code>&lt;CodeClass&gt;_&lt;GeneName&gt;_&lt;Accession&gt;</code> ", e.g. "Endogenous_ACTG1_NM_001614.1") to be used for content normalization if housekeeping is specified as the normalization method. (Note: if this argument is specified at the same time as 'hkgenes', an error will be thrown.)
quietly	Boolean specifying whether or not messages and warnings should be omitted.

## Details

For more information on the rationale behind the recommended preprocessing and normalization steps, please see the vignette.

## Value

A copy of the input RccSet with additional matrices in the assayData for each successive preprocessing step along with parameters for each step recorded in the experimentData@preprocessing list.

## Author(s)

Dorothee Nickles, Robert Ziman

## References

NanoString nCounter(R) Expression Data Analysis Guide (2012)

## Examples

```
data(example_rccSet)
hknorm_example_rccSet <- preprocRccSet(example_rccSet)
```

---

presAbsCall,RccSet-method

*Presence/absence call*

---

## Description

Adds a matrix to assayData ('paData') which indicates the presence/absence call for each gene in each sample using the background estimates and a stringency value. A gene is considered present in a sample if its count in that sample exceeds the corresponding background estimate times the stringency. The count values can be taken from either the positive control normalized data or the raw data (see the inputMatrix argument). If the input doesn't contain background-corrected data, an error will be generated.

## Usage

```
## S4 method for signature 'RccSet'
presAbsCall(rccSet, stringency = 2,
  inputMatrix = c("posCtrlData", "exprs"), quietly = FALSE)
```

## Arguments

rccSet	An RccSet with background-corrected data.
stringency	Multiplier to use in establishing the presence/absence call as mentioned in the description.
inputMatrix	Name of the matrix in the RccSet's assayData on which to apply the presence/absence call (either "posCtrlData" or "exprs").
quietly	Logical. If TRUE, messages and warnings will not be shown.

## Value

A copy of the input is returned with a new matrix named 'paData' added to the assayData that contains the presence/absence calls.

**Examples**

```

data(example_rccSet)
pcnorm_rccSet <- posCtrlNorm(example_rccSet)
bgEst <- getBackground(pcnorm_rccSet)
bgcorr_rccSet <- subtractBackground(pcnorm_rccSet, bgEst)
pa_rccset <- presAbsCall(bgcorr_rccSet)

```

---

```

previewPNG          Create a preview of a PNG

```

---

**Description**

Generates a resized, vertically-cropped preview version of the input PNG.

**Usage**

```
previewPNG(inputFile, outputFile, width, cropHeight, res = 72)
```

**Arguments**

inputFile	Input PNG filename
outputFile	Output PNG filename
width	Width (in pixels) for the preview image
cropHeight	Height (in pixels) for the preview image (if the rescaled input is larger than this, it will be cropped)
res	Output PNG resolution (passed to the 'res' argument of png())

**Value**

A resized, vertically-cropped preview version of the input PNG.

---

```

rccFiles.to.pdata_fdata_adata
          rccFiles.to.pdata_fdata_adata

```

---

**Description**

First stage of readRccBatch(): produces a list containing matrices (for pdata and adata) and a data frame (for fdata) that pdata\_fdata\_adata.to.rccSet() then transforms into a full RccSet (after some further checks and adjustments). See also nSolverCsv.to.pdata\_fdata\_adata().

**Usage**

```
rccFiles.to.pdata_fdata_adata(rccFiles)
```

**Arguments**

rccFiles            Vector of .RCC paths

**Value**

A list containing matrices (for pdata and adata) and a data frame (for fdata) that `pdata_fdata_adata.to.rccSet()` then transforms into a full `ExpressionSet`.

**Author(s)**

Robert Ziman

---

RccSet	<i>RccSet constructor methods</i>
--------	-----------------------------------

---

**Description**

Constructor methods for making new `RccSet` objects.

**Usage**

```
RccSet(obj, ...)

## S4 method for signature 'ExpressionSet'
RccSet(obj, ...)

## S4 method for signature 'environment'
RccSet(obj, ...)

## S4 method for signature 'matrix'
RccSet(obj, ...)

## S4 method for signature 'missing'
RccSet(obj, ...)
```

**Arguments**

obj            An object of appropriate class  
 ...           Passed to methods.

**Details**

Arguments accepted by constructors are identical to those for the `ExpressionSet` constructors.

See `RccSet` class documentation for examples of constructor use.

Constructor calls for which mandatory `phenoData` or `featureData` columns are missing will successfully create objects that include mandatory columns, but with NA values. See `RccSet` documentation for a list of mandatory columns.

**Value**

A new [RccSet](#) object.

---

RccSet-class

*RccSet class, derived from ExpressionSet*

---

**Description**

The RccSet class is a trivial extension of [ExpressionSet](#), but with additional validation criteria. RccSet is a class generator function.

**Details**

A valid RccSet object must have the following columns in featureData: "CodeClass", "GeneName", and "Accession". It must also have the following phenoData columns: "FileName", "SampleID", "LaneID", "FovCount", "FovCounted", "StagePosition", "BindingDensity", "CartridgeID", and "SampleType". A final requirement is that the "FovCount" column of phenoData have at most one distinct value.

**See Also**

See [checkRccSet](#), which provides additional checks and generates warnings for unexpected or unusual conditions which, though permitted by the class, may indicate data import errors.

**Examples**

```
data("example_rccSet")
e <- example_rccSet

# "ExpressionSet" constructor makes a new assayData environment
r1 <- RccSet(e)
validObject(r1)
assayData(e)
assayData(r1)
head(pData(r1))
head(fData(r1))

# For other constructors, if not explicitly supplied, blank phenoData and
# featureData objects are populated with mandatory columns (and NA values).
r2 <- RccSet(assayData(e))
validObject(r2)
head(pData(r2))
head(fData(r2))

r3 <- RccSet(assayData(e), phenoData(e), featureData(e))
identical(pData(r1), pData(r3))
identical(fData(r1), fData(r3))
identical(annotation(r1), annotation(r3)) # We forgot it!
annotation(e)
```

```
r3 <- RccSet(assayData(e), phenoData(e), featureData(e), annotation = annotation(e))
identical(annotation(r1), annotation(r3)) # Better
identical(r1, r3) # False, due to assayData environments
assayData(r1)
assayData(r3)

# Matrix constructor is similar
r4 <- RccSet(exprs(e), phenoData(e), featureData(e), annotation = annotation(e))
identical(exprs(r1), exprs(r4))

# Blank object constructor
r0 <- RccSet()
dim(r0)
pData(r0)
fData(r0)
```

---

readCdrDesignData      *Read .CSV containing CDR 'Design Data' extract*

---

## Description

Return a data frame containing the contents of the 'Design Data' tab extracted from a CDR spreadsheet. The extract, a .CSV file, must be manually prepared in advance (see 'details' section in the buildCodesetAnnotation() help page for more info).

## Usage

```
readCdrDesignData(cdrDesignData)
```

## Arguments

cdrDesignData      Path to the .CSV file containing the content extracted from the CDR's 'Design Data' tab

## Value

A data frame containing the contents of the CDR 'Design Data' tab.

## Author(s)

Robert Ziman

## Examples

```
path <- system.file("extdata", "CDR", "CDR-DesignData.csv", package="NanoStringQCPro")
cdr <- readCdrDesignData(path)
```



---

readRcc	<i>Read an .RCC file</i>
---------	--------------------------

---

**Description**

Parse an .RCC file into a list with each part of the file (Header, Sample\_Attributes, Lane\_Attributes, Code\_Summary, etc) stored as a vector or data frame.

**Usage**

```
readRcc(rcc, removeSpikeInLabels = TRUE)
```

**Arguments**

rcc	Path to the .RCC file.
removeSpikeInLabels	Logical. If TRUE (the default), RNA “spike-in” input labels (if any) in the GeneName for positive and negative control probes will be removed.

**Value**

A list where each element holds the contents of one part of the .RCC file (Header, Sample\_Attributes, Lane\_Attributes, Code\_Summary, etc) as a vector or data frame.

**Author(s)**

Robert Ziman

**Examples**

```
rcc <- system.file("extdata", "RCC", "20140604_C1-unstim_C1-unstim_01.RCC", package="NanoStringQCPro")  
rcc.ls <- readRcc(rcc)
```

---

readRccBatch	<i>Read RCC files</i>
--------------	-----------------------

---

**Description**

Reads the contents of all .RCC files from a given directory into a new RccSet object. Note: this function is not intended for external use. For that, see newRccSet().

**Usage**

```
readRccBatch(rccFiles)
```

**Arguments**

rccFiles            Vector of .RCC file paths

**Value**

An RccSet object that has raw counts in assayData, probe information in fData, and sample annotation in pData.

**Author(s)**

Robert Ziman

---

readRccCollectorToolExport

*Read RCC Collector Tool Export*

---

**Description**

Reads the contents of a .CSV file generated from the RCC Collector Tool Export feature of NanoString's nSolver Analysis software into a new RccSet object. (Note: this function is not intended for external use. For that, see newRccSet().)

**Usage**

```
readRccCollectorToolExport(file)
```

**Arguments**

file                Path to the NSolver .CSV file to be read.

**Details**

See 'details' in the readRccBatch() help page.

**Value**

An RccSet object that has count data in exprs, probe information in fData and sample annotation in pData.

**Author(s)**

Dorothee Nickles, Thomas Sandmann

---

readRlf	<i>Read RLF file</i>
---------	----------------------

---

**Description**

Reads the contents of an .RLF file into a data frame. RNA “spike-in” concentrations recorded in the GeneName for positive and negative control probes are stripped and stored in a separate column in the output. An error will be generated for any recognized deviations from the expected file format.

**Usage**

```
readRlf(rlf)
```

**Arguments**

rlf                    Path to the .RLF file

**Value**

A data frame containing the contents of the .RLF file.

**Author(s)**

Robert Ziman

**Examples**

```
rlf <- system.file("extdata", "RLF", "NQCP_example.rlf", package="NanoStringQCPro")
rlf.df <- readRlf(rlf)
```

---

sampleClustering,RccSet-method	<i>Clustering by sample correlation</i>
--------------------------------	---

---

**Description**

Clustering by sample correlation

**Usage**

```
## S4 method for signature 'RccSet'
sampleClustering(rccSet, outputFile,
  main = "Sample correlations in raw data", annCol = NULL,
  covar = "SampleType")
```

**Arguments**

rccSet	An RccSet
outputFile	Output PNG filename
main	Plot title
annCol	See <a href="#">aheatmap</a>
covar	Covariate (e.g. "SampleType")

**Value**

A PNG file showing clustering of samples by correlation. Any zero-variance samples are omitted from the heatmap. The width and height of the PNG file are set to vary with the size of the input.

**Author(s)**

Dorothee Nickles, Robert Ziman

---

scatterPair

*scatterPair*

---

**Description**

Helper function for a scatter plot inside a pairs plots

**Usage**

```
scatterPair(x, y)
```

**Arguments**

x	integer, x positions
y	integer, y positions

**Value**

A scatter plot x versus y.

---

 subtractBackground,RccSet-method

*Subtract background estimates for a NanoString RccSet*


---

### Description

Returns a NanoString [RccSet](#) with background-corrected count data. During subtraction, any counts below 1 will be truncated to 1 to enable subsequent log transformation of the data.

### Usage

```
## S4 method for signature 'RccSet'
subtractBackground(rccSet, bgEstimates,
  bgEstimatesParams = list(), inputMatrix = c("posCtrlData", "exprs"),
  quietly = FALSE)
```

### Arguments

rccSet	NanoString RccSet object
bgEstimates	Matrix containing the background estimates to subtract.
bgEstimatesParams	A list with the parameters that were used to generate the background estimates (see <code>getBackground()</code> ): <ul style="list-style-type: none"> <li>• bgReference</li> <li>• summaryFunction</li> <li>• stringency</li> <li>• nSolverBackground.w1</li> <li>• nSolverBackground.shrink</li> <li>• inputMatrix</li> </ul> The values of these list elements will be assigned to corresponding elements in the output's <code>experimentData@preprocessing</code> list. If any element is NULL, the corresponding element in the output's preprocessing list will be NA.
inputMatrix	Name of the matrix in the RccSet's <code>assayData</code> to use as input for subtracting background estimates (one of "exprs" or "posCtrlData"). If <code>posCtrlData</code> is specified but not found in the <code>assayData</code> , an error will be generated.
quietly	Boolean specifying whether or not messages and warnings should be omitted.

### Value

A NanoString `linkS4class{RccSet}` object with background estimates subtracted from the count data.

### Author(s)

Dorothee Nickles

**See Also**[getBackground](#)**Examples**

```
data(example_rccSet)

pcnorm_rccSet <- posCtrlNorm(example_rccSet)

bg1 <- getBackground(pcnorm_rccSet, bgReference="negatives", summaryFunction="mean")
bg2 <- getBackground(pcnorm_rccSet, bgReference="blanks")
bg3 <- getBackground(pcnorm_rccSet, bgReference="both", stringency=1)

bgCor1 <- subtractBackground(pcnorm_rccSet, bgEstimates=bg1)
bgCor2 <- subtractBackground(pcnorm_rccSet, bgEstimates=bg2)
bgCor3 <- subtractBackground(pcnorm_rccSet, bgEstimates=bg3)
```

---

zfacFun

*zfacFun*

---

**Description**

Calculate Z' Factor

**Usage**

```
zfacFun(p, n)
```

**Arguments**

p	numeric vector: measurements for the positive controls (or actual measurement)
n	numeric vector: measurements for the negative controls

**Value**

Scalar numeric: the Z' Factor

**Author(s)**

Dorothee Nickles

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