

# Package ‘gCrisprTools’

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**Description** Set of tools for evaluating pooled high-throughput screening experiments, typically employing CRISPR/Cas9 or shRNA expression cassettes. Contains methods for interrogating library and cassette behavior within an experiment, identifying differentially abundant cassettes, aggregating signals to identify candidate targets for empirical validation, hypothesis testing, and comprehensive reporting.

**License** Artistic-2.0

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---

gCrisprTools-package    *gCrisprTools*

---

**Description**

Pipeline for using CRISPR data at Genentech

---

aln                            *Precalculated alignment statistics of a crispr screen*

---

**Description**

Example alignment matrix file for the provided example Crispr screen. All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Genentech, Inc.

**See Also**

Please see ‘vignettes/Crispr\_example\_workflow.R’ for details.

**Examples**

```
data("aln")
head(aln)
```

---

ann                            *Annotation file for a mouse Crispr library*

---

**Description**

Example annotation file for the screen data provided in es. All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Genentech, Inc.

**See Also**

Please see ‘vignettes/Crispr\_example\_workflow.R’ for details.

**Examples**

```
data("ann")
head(ann)
```

---

ct.alignmentChart      *View a Barchart Summarizing Alignment Statistics for a Crispr Screen*

---

### Description

This function displays the alignment statistics for a pooled Crispr screen, reported directly from an alignment statistic matrix.

### Usage

```
ct.alignmentChart(aln, sampleKey = NULL)
```

### Arguments

aln	A numeric matrix of alignment statistics for a Crispr experiment. Corresponds to a 4xN matrix of read counts, with columns indicating samples and rows indicating the number of "targets", "nomatch", "rejections", and "double_match" reads. Details about these classes may be found in the best practices vignette or as part of the report generated with <code>ct.makeReport()</code> .
sampleKey	An optional ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in aln.

### Value

A grouped barplot displaying the alignment statistics for each sample included in the alignment matrix, which usually corresponds to all of the samples in the experiment.

### Author(s)

Russell Bainer

### Examples

```
data('aln')
ct.alignmentChart(aln)
```

---

ct.applyAlpha      *Apply RRA 'alpha' cutoff to RRAalpha input*

---

### Description

The 'alpha' part of RRAalpha is used to consider only the top guide-level scores for gene-level statistics. Practically, all guides failing the cutoff get a pvalue of 1. There are three ways of determining which guides fail. See 'scoring' below.

### Usage

```
ct.applyAlpha(
  stats,
  RRAalphaCutoff = 0.1,
  scoring = c("combined", "pvalue", "fc")
)
```

**Arguments**

stats	three-column numeric matrix with pvalues for down and up one-sided test with guide-level fold changes (coefficients from the relevant contrast).
RRAalphaCutoff	A cutoff to use when defining gRNAs with significantly altered abundance during the RRAa aggregation step, which may be specified as a single numeric value on the unit interval or as a logical vector. When supplied as a logical vector (of length equal to <code>nrows(fit)</code> ), this parameter directly indicates the gRNAs to include during RRAa aggregation. Otherwise, if <code>scoring</code> is set to <code>pvalue</code> or <code>combined</code> , this parameter is interpreted as the maximum nominal p-value required to consider a gRNA's abundance meaningfully altered during the aggregation step. If <code>scoring</code> is <code>fc</code> , this parameter is interpreted as the proportion of the list to be considered meaningfully altered in the experiment (e.g., if <code>RRAalphaCutoff</code> is set to 0.05, only consider the rankings of the 5 (or down-regulated) gRNAs for the purposes of RRAa calculations).
scoring	The gRNA ranking method to use in RRAa aggregation. May take one of three values: <code>pvalue</code> , <code>fc</code> , or <code>'combined'</code> . <code>pvalue</code> indicates that the gRNA ranking statistic should be created from the (one-sided) p-values in the fit object. <code>fc</code> indicates that the ranks of the gRNA coefficients should be used instead, and <code>combined</code> indicates that the coefficients should be used as the ranking statistic but gRNAs are discarded in the aggregation step based on the corresponding nominal p-value in the fit object.

**Value**

data.frame with guide-level pvals, fold change, and `scores.deplete` and `scores.enrich` which are the input the `RRAalpha`

**Author(s)**

Russell Bainer

---

 ct.buildSE

---

*Package Screen Data into a 'SummarizedExperiment' Object*


---

**Description**

Convenience function to package major components of a screen into a 'SummarizedExperiment' container for downstream visualization and analysis. All arguments are optional except for 'es'.

**Usage**

```
ct.buildSE(
  es,
  sampleKey = NULL,
  ann = NULL,
  vm = NULL,
  fit = NULL,
  summaryList = NULL
)
```

**Arguments**

es	An 'ExpressionSet' of screen data. Required.
sampleKey	a gCrisprTools 'sampleKey' object, to be added to the 'colData'.
ann	Annotation object to be packaged into the 'rowData'
vm	A 'voom'-derived normalized object
fit	a 'MAarrayLM' object containing the contrast information and model results
summaryList	A named list of data.frames, returned by ct.generateResults. if you need to generate one of these by hand for some reason, see the example resultsDF object loaded in the example below.

**Value**

A 'SummarizedExperiment' object.

**Author(s)**

Russell Bainer

**Examples**

```
data('ann', 'es', 'fit', 'resultsDF')
ct.buildSE(es, ann = ann, fit = 'fit', summaryList = list('resA' = resultsDF, 'resB' = resultsDF))
```

---

ct.CAT

---

*Compare Two CRISPR Screens via a CAT plot*


---

**Description**

This is a function for comparing the results of two screening experiments. Given two summaryDF, the function places them in register with one another, generates a Concordance At The Top (CAT) plot, and returns an invisible dataframe containing the relevant gene-level signals.

This function is conceptually similar to the 'ct.ROC' and 'ct.PRC()' functions, but is appropriate when considering consistency of ranked values rather than an interchangeable set; the most common use case is for comparing primary and replication screens, where the underlying technology and selection criteria are expected to be highly similar. CAT plots are fundamentally about comparing rankings, and so only targets in common between the two provided screens are considered. If the totality of list overlap is important, consider using 'ct.PRC()' or 'ct.ROC()'.

Note that ranking statistics in CRISPR screens are (usually) permutation-based, and so some granularity in the rankings is expected. This function does a little extra work to limit the influence of this granularity and to ensure that hits are counted as soon as the requisite value of the ranking statistic is reached regardless of where the target is located within the block of equally-significant hits. Functionally, this means that the drawn curve is somewhat anticonservative in cases where the target ranks are not well differentiated.

## Usage

```
ct.CAT(  
  df1,  
  df2,  
  targets = c("geneSymbol", "geneID"),  
  enrich,  
  plot.it = TRUE,  
  plot.rho = TRUE  
)
```

## Arguments

df1	A dataframe summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
df2	A dataframe summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
targets	Column of the provided summaryDF to consider. Must be geneID or geneSymbol.
enrich	Logical indicating whether to test for enrichment or depletion.
plot.it	Logical indicating whether to compose the plot on the default device.
plot.rho	Logical indicating whether to plot the Rho values in addition to the P-values, which sometimes have better ranking properties.

## Value

Invisibly, a data.frame containing the relevant summary stats for each target in both screens.

## Author(s)

Russell Bainer

## Examples

```
data('resultsDF')  
cat <- ct.CAT(resultsDF, resultsDF[1:2000,], enrich = TRUE)  
head(cat)
```

---

ct.DirectionalityTests     *Compute Directional P-values from eBayes Output*

---

## Description

This function produces two sets of one-sided P-values derived from the moderated t-statistics produced by eBayes.

## Usage

```
ct.DirectionalityTests(fit, contrast.term = NULL)
```

**Arguments**

<code>fit</code>	An object of class <code>MArrayLM</code> containing, at minimum, a <code>df.residual</code> slot containing the appropriate degrees of freedom for each test, and a <code>t</code> slot containing <code>t</code> statistics.
<code>contrast.term</code>	If a <code>fit</code> object with multiple coefficients is passed in, a string indicating the coefficient of interest.

**Value**

A matrix object with two numeric columns, indicating the p-values quantifying the evidence for enrichment and depletion of each feature in the relevant model contrast.

**Author(s)**

Russell Bainer

**Examples**

```
data('fit')
ct.DirectionalityTests(fit)
```

---

`ct.filterReads`

*Remove low-abundance elements from an ExpressionSet object*

---

**Description**

This function removes gRNAs only present in very low abundance across all samples of a pooled Crispr screening experiment. In most cases very low-abundance guides are the result of low-level contamination from other libraries, and often distort standard normalization approaches. This function trims gRNAs in a largely heuristic way, assuming that the majority of 'real' gRNAs within the library are comparably abundant in at least some of the samples (such as unexpanded controls), and that contaminants are present at negligible levels. Specifically, the function trims the `trim` most abundant guides from the upper tail of each log-transformed sample distribution, and then omits gRNAs whose abundances are always less than  $1/(2^{\log_2 \text{ratio}})$  of this value.

**Usage**

```
ct.filterReads(
  eset,
  trim = 1000,
  log2.ratio = 4,
  sampleKey = NULL,
  plot.it = TRUE,
  read.floor = NULL
)
```



**Arguments**

eset	An unnormalized ExpressionSet object containing, at minimum, a matrix of gRNA counts accessible with <code>exprs()</code> .
trim	The number of gRNAs to be trimmed from the top of the distribution before estimating the abundance range. Empirically, this usually should be equal to about 2 to 5 percent of the guides in the library.
log2.ratio	Maximum abundance of contaminant gRNAs, expressed on the log2 scale from the top of the trimmed range of each sample. That is, <code>log2.ratio = 4</code> means to discard all gRNAs whose abundance is $(1/2)^4$ of the trimmed maximum.
sampleKey	An (optional) sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
plot.it	Logical value indicating whether to plot the adjusted gRNA densities on the default device.
read.floor	Optionally, the minimum number of reads required for each gRNA.

**Value**

An ExpressionSet object, with trace-abundance gRNAs omitted.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')
ct.filterReads(es)
```

---

ct.GCbias

*Visualization of gRNA GC Content Trends*

---

**Description**

This function visualizes relationships between gRNA GC content and their measured abundance or various differential expression model estimates.

**Usage**

```
ct.GCbias(data.obj, ann, sampleKey = NULL, lib.size = NULL)
```

**Arguments**

data.obj	An ExpressionSet or fit (MArrayLM) object to be analyzed for the presence of GC content bias.
ann	An annotation data.frame, used to estimate GC content for each guide. Guides are annotated by row, and the object must minimally contain a target column containing a character vector that indicates the corresponding nucleotide sequences.

sampleKey	An optional sample key, supplied as a factor linking the samples to experimental variables. The names attribute should exactly match those present in eset, and the control set is assumed to be the first level. Ignored in the analysis of model fit objects.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <code>calcNormFactors</code> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the <code>exprs</code> slot of the <code>eset</code> .

**Value**

An image relating GC content to experimental observations on the default device. If the provided `data.obj` is an `ExpressionSet`, this takes the form of a scatter plot where the GC with a smoothed trendline within each sample. If `data.obj` is a fit object describing a linear model contrast, then four panels are returned describing the GC content distribution and its relationship to guide-level fold change, standard deviation, and P-value estimates.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')
data('ann')
data('fit')

ct.GCbias(es, ann)
ct.GCbias(fit, ann)
```

---

`ct.generateResults`     *Calculate results of a crispr screen from a contrast*

---

**Description**

This is a wrapper function that enables direct generation of target-level p-values from a crispr screen.

**Usage**

```
ct.generateResults(
  fit,
  annotation,
  RRAalphaCutoff = 0.1,
  permutations = 1000,
  contrast.term = NULL,
  scoring = c("combined", "pvalue", "fc"),
  permutation.seed = NULL
)
```

**Arguments**

<code>fit</code>	An object of class <code>MArrayLM</code> containing, at minimum, a <code>t</code> slot with t-statistics from the comparison, a <code>df.residual</code> slot with the corresponding residuals for the model fits, and an <code>Amean</code> slot with the respective mean abundances.
<code>annotation</code>	An annotation file for the experiment. gRNAs are annotated by row, and must minimally contain columns <code>geneSymbol</code> and <code>geneID</code> .
<code>RRAalphaCutoff</code>	A cutoff to use when defining gRNAs with significantly altered abundance during the RRAa aggregation step, which may be specified as a single numeric value on the unit interval or as a logical vector. When supplied as a logical vector (of length equal to <code>nrows(fit)</code> ), this parameter directly indicates the gRNAs to include during RRAa aggregation. Otherwise, if <code>scoring</code> is set to <code>pvalue</code> or <code>combined</code> , this parameter is interpreted as the maximum nominal p-value required to consider a gRNA's abundance meaningfully altered during the aggregation step. If <code>scoring</code> is <code>fc</code> , this parameter is interpreted as the proportion of the list to be considered meaningfully altered in the experiment (e.g., if <code>RRAalphaCutoff</code> is set to 0.05, only consider the rankings of the 5 (or down-regulated) gRNAs for the purposes of RRAa calculations).  Note that this function uses directional tests to identify enriched or depleted targets, and when <code>RRAalphaCutoff</code> is provided as a logical vector, only one of these hypotheses is implicitly specified; this means that enrichment and depletion cannot be .
<code>permutations</code>	The number of permutations to use during the RRAa aggregation step.
<code>contrast.term</code>	If a fit object with multiple coefficients is passed in, a string indicating the coefficient of interest.
<code>scoring</code>	The gRNA ranking method to use in RRAa aggregation. May take one of three values: <code>pvalue</code> , <code>fc</code> , or <code>'combined'</code> . <code>pvalue</code> indicates that the gRNA ranking statistic should be created from the (one-sided) p-values in the fit object. <code>fc</code> indicates that the ranks of the gRNA coefficients should be used instead, and <code>combined</code> indicates that the coefficients should be used as the ranking statistic but gRNAs are discarded in the aggregation step based on the corresponding nominal p-value in the fit object.
<code>permutation.seed</code>	numeric seed for permutation reproducibility. Default: <code>NULL</code> means to not set any seed. This argument is passed through to <code>ct.RRAaPvals</code> .

**Value**

A dataframe containing gRNA-level and target-level statistics. In addition to the information present in the supplied annotation object, the returned object indicates P-values and Q-values for the depletion and enrichment of each gRNA and associated target, the median log2 fold change estimate among all gRNAs associated with the target, and Rho statistics that are calculated internally by the RRAa algorithm that may be useful in ranking targets that are considered significant at a given alpha or false discovery threshold.

A 'resultsDF' formatted dataframe containing gene-level statistics.

**Author(s)**

Russell Bainer

**Examples**

```

data('fit')
data('ann')
output <- ct.generateResults(fit, ann, permutations = 10)
head(output)
  p = seq(0, 1, length.out=20)
  fc = seq(-3, 3, length.out=20)
  fc[2] = NA
  fc[3] = -20
  stats = data.frame(
    Depletion.P=p,
    Enrichment.P=rev(p),
    fc=fc
  )
ct.applyAlpha(stats,scoring="combined")

```

---

ct.GREATdb

*Update a gene-centric msdb object for GREAT-style enrichment analysis using a specified CRISPR annotation.*

---

**Description**

Update a gene-centric msdb object for GREAT-style enrichment analysis using a specified CRISPR annotation.

**Usage**

```

ct.GREATdb(
  annotation,
  gsdb = getMSigGeneSetDb(c("h", "c2"), "human"),
  minsize = 10
)

```

**Arguments**

annotation	an annotation object returned by ct.prepareAnnotation().
gsdb	A gene-centric GeneSetDb object to conform to the relevant peakwise dataset.
minsize	Minimum number of targets required to consider a geneset valid for analysis.

**Value**

A new GeneSetDb object with the features annotated genewise to pathways.

---

`ct.gRNARankByReplicate`*Visualization of Ranked gRNA Abundances by Replicate*

---

### Description

This function median scales and log2 transforms the raw gRNA count data contained in an ExpressionSet, and then plots the ordered expression values within each replicate. The curve colors are assigned based on a user-specified column of the pData contained in the ExpressionSet. Optionally, this function can plot the location of Nontargeting control guides (or any guides, really) within the distribution.

### Usage

```
ct.gRNARankByReplicate(  
  eset,  
  sampleKey,  
  annotation = NULL,  
  geneSymb = NULL,  
  lib.size = NULL  
)
```

### Arguments

<code>eset</code>	An ExpressionSet object containing, at minimum, count data accessible by <code>exprs()</code> and some <code>phenoData</code> .
<code>sampleKey</code>	A sample key, supplied as a (possibly ordered) factor linking the samples to experimental variables. The names attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
<code>annotation</code>	An annotation dataframe indicating the nontargeting controls in the <code>geneID</code> column.
<code>geneSymb</code>	The <code>geneSymbol</code> identifier(s) in <code>annotation</code> that corresponds to gRNAs to be plotted on the curves. If the provided value is not present in the <code>geneSymbol</code> , nontargeting controls will be plotted instead.
<code>lib.size</code>	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <code>calcNormFactors</code> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the <code>exprs</code> slot of the <code>eset</code> .

### Value

A waterfall plot as specified, on the default device.

### Author(s)

Russell Bainer

**Examples**

```

data('es')
data('ann')

#Build the sample key
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))

ct.gRNARankByReplicate(es, sk, ann, 'Ripk3')

```

---

ct.guideCDF

*View CDFs of the ranked gRNAs or Targets present in a crispr screen*


---

**Description**

This function generates a plot relating the cumulative proportion of reads in each sample of a crispr screen to the abundance rank of the underlying guides (or Targets). The purpose of this algorithm is to detect potential distortions in the library composition that might not be properly controlled by sample normalization (see also: `ct.stackedGuides()`).

**Usage**

```
ct.guideCDF(eset, sampleKey = NULL, plotType = "gRNA", annotation = NULL)
```

**Arguments**

<code>eset</code>	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the <code>exprs()</code> function.
<code>sampleKey</code>	An optional sample key, supplied as an ordered factor linking the samples to experimental variables. The <code>names</code> attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
<code>plotType</code>	A string indicating whether the individual guides should be displayed ("gRNA"), or if they should be aggregated into target-level estimates ("Target") according to the <code>geneSymbol</code> column in the annotation object.
<code>annotation</code>	An optional data.frame containing an annotation object to be used to aggregate the guides into targets. gRNAs are annotated by row, and must minimally contain a column <code>geneSymbol</code> indicating the target elements.

**Value**

A CDF plot displaying the appropriate CDF curves on the default device.

**Author(s)**

Russell Bainer

**Examples**

```

data('es')
ct.guideCDF(es)

```

---

ct.inputCheck	<i>Check compatibility of a sample key with a supplied object</i>
---------------	---

---

### Description

For many gCrisprTools functions, a sample key must be provided that specifies sample mapping to experimental groups and specifies which of these contains control samples. This function checks whether the specified sample key is of the proper format and has properties consistent matching the specified object.

### Usage

```
ct.inputCheck(sampleKey, object)
```

### Arguments

sampleKey	A named factor, where the levels indicate the experimental replicate groups and the names match the colnames of the expression matrix contained in object. The first level should correspond to the control samples, but obviously there is no way to algorithmically control this.
object	An ExpressionSet, EList, or matrix.

### Value

A logical indicating whether the objects are compatible.

### Author(s)

Russell Bainer

### Examples

```
data('es')
library(limma)
library(Biobase)

#Build the sample key
sk <- relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference")
names(sk) <- row.names(pData(es))
ct.inputCheck(sk, es)
```

---

ct.makeContrastReport	<i>Generate a Contrast report from a pooled CRISPR screen</i>
-----------------------	---

---

### Description

This is a function to generate an html Contrast report for a CRISPR screen, focusing on contrast-level analyses collected from other functions in gCrisprTools. It is designed to be used 'as-is', and analysts interested in using different functionalities of the various functions should do that outside of this wrapper script.

**Usage**

```
ct.makeContrastReport(
  eset,
  fit,
  sampleKey,
  results,
  annotation,
  comparison.id,
  identifier,
  contrast.subset = colnames(eset),
  outdir = NULL
)
```

**Arguments**

eset	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the <code>exprs()</code> function and some named phenodata extractable with <code>pData()</code> .
fit	A fit object for the contrast of interest, usually generated with <code>lmFit</code> .
sampleKey	A sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
results	A data.frame summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
annotation	An annotation object for the experiment. See the man page for <code>ct.prepareAnnotation()</code> for details and example format.
comparison.id	character with a name of the comparison.
identifier	A character string to name the report and corresponding subdirectories. If provided, the final report will be called <code>'identifier.html'</code> and will be located in a directory called <code>identifier</code> in the <code>outdir</code> . If NULL, a generic name
contrast.subset	character vector containing the sample labels to be used in the analysis; all elements must be contained in the <code>colnames</code> of the specified <code>eset</code> . including the timestamp will be generated. Default: <code>colnames(eset)</code> .
outdir	An optional character string indicating the directory in which to generate the report. If NULL, a temporary directory will be automatically generated.

**Value**

The path to the generated html report.

**Author(s)**

Russell Bainer, Dariusz Ratman

**Examples**

```
data('es')
data('fit')
data('ann')
data('resultsDF')
```



```
##' #Build the sample key
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))

path2report <- ct.makeContrastReport(es, fit, sk, resultsDF, ann, comparison.id = NULL, outdir = ".")
```

---

ct.makeQCReport      *Generate a QC report from a pooled CRISPR screen*

---

## Description

This is a function to generate an html QC report for a CRISPR screen, focusing on experiment-level and library-level analyses collected from other functions in `gCrisprTools`. It is designed to be used 'as-is', and analysts interested in using different functionalities of the various functions should do that outside of this wrapper script.

## Usage

```
ct.makeQCReport(
  eset,
  trim,
  log2.ratio,
  sampleKey,
  annotation,
  aln,
  identifier = NULL,
  lib.size,
  geneSymb = NULL,
  outdir = NULL
)
```

## Arguments

<code>eset</code>	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the <code>exprs()</code> function and some named phenodata extractable with <code>pData()</code> .
<code>trim</code>	The number of gRNAs to be trimmed from the top of the distribution before estimating the abundance range. Empirically, this usually should be equal to about 2 to 5 percent of the guides in the library.
<code>log2.ratio</code>	Maximum abundance of contaminant gRNAs, expressed on the log2 scale from the top of the trimmed range of each sample. That is, <code>log2.ratio = 4</code> means to discard all gRNAs whose abundance is $(1/2)^4$ of the trimmed maximum.
<code>sampleKey</code>	A sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
<code>annotation</code>	An annotation object for the experiment. See the man page for <code>ct.prepareAnnotation</code> for details and example format.

aln	A numeric alignment matrix, where rows correspond to "targets", "nomatch", "rejections", and "double_match", and where columns correspond to experimental samples.
identifier	A character string to name the report and corresponding subdirectories. If provided, the final report will be called 'identifier.html' and will be located in a directory called identifier. If NULL, a generic name including the timestamp will be generated.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <a href="#">calcNormFactors</a> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.
geneSymb	The geneSymbol identifier(s) in annotation that corresponds to gRNAs to be plotted on the curves. Passed through to <a href="#">ct.gRNARankByReplicate</a> , <a href="#">ct.viewControls</a> and <a href="#">ct.prepareAnnotation</a> (as controls argument if it's not NULL). Default NULL.
outdir	An optional character string indicating the directory in which to generate the report. If NULL, a temporary directory will be automatically generated.

**Value**

The path to the generated html report.

**Author(s)**

Russell Bainer, Dariusz Ratman

**Examples**

```
data('es')
data('ann')
data('aln')

##' #Build the sample key
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))

path2report <- ct.makeQCReport(es, trim = 1000, log2.ratio = 0.0625, sk, ann, aln, identifier = NULL, lib.size =
```

---

ct.makeReport

*Generate a full experimental report from a pooled CRISPR screen*

---

**Description**

This is a function to generate an html report for a CRISPR screen, incorporating information about a specified contrast. The report contains a combination of experiment-level and contrast-specific analyses, largely collected from other functions in `gCrisprTools`. It is designed to be used 'as-is', and analysts interested in using different functionalities of the various functions should do that outside of this wrapper script.

**Usage**

```
ct.makeReport(
  fit,
  eset,
  sampleKey,
  annotation,
  results,
  aln,
  outdir = NULL,
  contrast.term = NULL,
  identifier = NULL
)
```

**Arguments**

fit	An object of class MArrayLM containing, at minimum, a coefficients slot with coefficients from the comparison, and a stdev.unscaled slot with the corresponding standard deviation of the coefficient estimates. The row.names attribute should ideally match that which is found in annotation, but this will be checked internally.
eset	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the exprs() function and some named phenodata extractable with pData().
sampleKey	A sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in eset, and the control set is assumed to be the first level.
annotation	An annotation object for the experiment. See the man page for ct.prepareAnnotation() for details and example format.
results	A data.frame summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
aln	A numeric alignment matrix, where rows correspond to "targets", "nomatch", "rejections", and "double_match", and where columns correspond to experimental samples.
outdir	A directory in which to generate the report; if NULL, a temporary directory will be automatically generated. The report will be located in a subdirectory whose name is internally generated (see below). The path to the report itself is returned by the function.
contrast.term	A parameter passed to ct.preprocessFit in the event that the fit object contains data from multiple contrasts. See that man page for further details.
identifier	A character string to name the report and corresponding subdirectories. If provided, the final report will be called 'identifier.html' and will be located in a directory called identifier in the outdir. If NULL, a generic name including the timestamp will be generated.

**Value**

The path to the generated html report.

**Author(s)**

Russell Bainer

**Examples**

```

data('fit')
data('es')

##' #Build the sample key
library(Biobase)
sk <- relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference")
names(sk) <- row.names(pData(es))

data('ann')
data('resultsDF')
data('aln')
path2report <- ct.makeReport(fit, es, sk, ann, resultsDF, aln, outdir = ".")

```

---

ct.makeRhoNull	<i>Make null distribution for RRAalpha tests</i>
----------------	--

---

**Description**

Makes random distribution of Rho value by taking nperm random samples of n rank stats, p.

**Usage**

```
ct.makeRhoNull(n, p, nperm)
```

**Arguments**

n	single integer, number of guides per gene
p	numeric vector of rank statistics
nperm	single integer, how many random samples to take.

**Value**

numeric vector of Rho values

---

ct.multiGSEA	<i>Geneset Enrichment within a CRISPR screen using multiGSEA This function identifies differentially enriched/depleted ontological categories within the hits of a CRISPR screen given a provided 'GeneSetDb()' and a results 'data.frame' created by 'ct.generateResults()'. Testing is performed using a Hypergeometric test, and results are returned as a 'MultiGSEAResult' object defined in the 'multiGSEA' package. Note that the '@logFC' slot in the returned object will contain the median gRNA lfc across all associated guides, which in some cases may have dubious interpretive value. This method used over-representation analysis, derived from 'limma::kegga()', and incorporates the number of gRNAs associated with each Target (inferred from the 'geneSymbol' column of the 'resultsDF') as the bias vector (because standard aggregation methods should be underpowered for targets with few guides). Setting 'unbiased' = 'TRUE' suppresses this behavior, which is identical to a hypergeometric test.</i>
--------------	--

---

## Description

Geneset Enrichment within a CRISPR screen using multiGSEA

This function identifies differentially enriched/depleted ontological categories within the hits of a CRISPR screen given a provided 'GenseSetDb()' and a results 'data.frame' created by 'ct.generateResults()'. Testing is performed using a Hypergeometric test, and results are returned as a 'MultiGSEAResult' object defined in the 'multiGSEA' package. Note that the '@logFC' slot in the returned object will contain the median gRNA lfc across all associated guides, which in some cases may have dubious interpretive value.

This method used overrepresentation analysis, derived from 'limma::kegga()', and incorporates the number of gRNAs associated with each Target (inferred from the 'geneSymbol' column of the 'resultsDF') as the bias vector (because standard aggregation methods should be underpowered for targets with few guides). Setting 'unbiased' = 'TRUE' suppresses this behavior, which is identical to a hypergeometric test.

## Usage

```
ct.multiGSEA(  
  resultsDF,  
  gsdb,  
  cutoff = 0.1,  
  stat = c("q", "p", "rho"),  
  unbiased = FALSE  
)
```

## Arguments

resultsDF	'data.frame' returned by 'ct.generateResults()'.
gsdb	'GenseSetDb' object containing annotations.
cutoff	Q, P, or Rho statistic cutoff defining significant enrichment/depletion in the screen. Default is 0.1.
stat	Statistic to be used in calling enrichment/depletion in the screen. Must be one of 'q', 'p', or 'rho'.
unbiased	Logical indicating whether to estimate bias on the basis of the number of gRNAs associated with each target.

## Author(s)

Steve Lianoglou for multiGSEA; Russell Bainer for wrapping functions.

## Examples

```
data('ann')  
data('resultsDF')  
#gsd <- multiGSEA::getMSigGeneSetDb(c('h', 'c2'), 'mouse', id.type = 'entrez')
```

---

ct.normalizeBySlope     *Normalize sample abundance estimates by the slope of the values in the central range*

---

### Description

This function normalizes Crispr gRNA abundance estimates by equalizing the slopes of the middle (logged) values of the distribution across samples. Specifically, the algorithm ranks the gRNA abundance estimates within each sample and determines a relationship between rank change and gRNA within a trimmed region of the distribution via a linear fit. It then adjusts each sample such that the center of the logged abundance distribution is strictly horizontal and returns these values as median-scaled counts in the appropriate slot of the input ExpressionObject.

### Usage

```
ct.normalizeBySlope(ExpressionObject, trim = 0.25, lib.size = NULL, ...)
```

### Arguments

ExpressionObject	An ExpressionSet containing, at minimum, count data accessible by exprs, or an EList object with count data in the \$E slot (usually returned by <code>voom</code> ).
trim	The proportion to be trimmed from each end of the distribution before performing the linear fit; algorithm defaults to 25 fit is performed on the interquartile range.
lib.size	An optional vector of size factor adjusted library size. Default: NULL means to use sum of column counts as a lib.size.
...	Other arguments to be passed to <code>ct.normalizeMedians()</code> , if desired.

### Value

A renormalized object of the same type as the provided object.

### Author(s)

Russell Bainer

### Examples

```
data('es')
data('ann')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeBySlope(es, lib.size= ls)
ct.gRNARankByReplicate(es, sk, lib.size= ls)
ct.gRNARankByReplicate(es.norm, sk, lib.size= ls)
```

---

`ct.normalizeFactoredQuantiles`*Apply Factored Quantile Normalization to gRNA counts*

---

## Description

This function normalizes Crispr gRNA abundance estimates by equalizing the median gRNA abundance values after correcting for library size. It does this by converting raw count values to log2 counts per million and optionally adjusting further in the usual way by dividing these values by user-specified library size factors. This method should be more stable than the endogenous scaling functions used in voom in the specific case of Crispr screens or other cases where the median number of observed counts may be low.

## Usage

```
ct.normalizeFactoredQuantiles(eset, lib.size = NULL)
```

## Arguments

<code>eset</code>	An ExpressionSet containing, at minimum, count data accessible by <code>exprs</code> .
<code>lib.size</code>	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <code>calcNormFactors</code> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the <code>exprs</code> slot of the <code>eset</code> .

## Value

A renormalized ExpressionSet object of the same type as the provided object.

## Author(s)

Russell Bainer

## Examples

```
data('es')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeMedians(es, lib.size= ls)
ct.gRNARankByReplicate(es, sampleKey = sk, lib.size= ls)
ct.gRNARankByReplicate(es.norm, sampleKey = sk, lib.size= ls)
```

ct.normalizeFQ

*Factored Quantile Normalization***Description**

This function applies quantile normalization to subsets of samples defined by a provided factor, correcting for library size. It does this by converting raw count values to log2 counts per million and optionally adjusting further in the usual way by dividing these values by user-specified library size factors; then this matrix is split into groups according to the provided factor that are quantile normalized, and then the groups are median scaled to each other before conversion back into raw counts. This method is best used in comparisons for long timecourse screens, where groupwise differences in growth rate cause uneven intrinsic dialation of construct distributions.

Note that this normalization strategy is not appropriate for experiments where significant distortion of the libraries is expected as a consequence of the screening strategy (e.g., strong selection screens).

**Usage**

```
ct.normalizeFQ(eset, sets, lib.size = NULL)
```

**Arguments**

eset	An ExpressionSet containing, at minimum, count data accessible by exprs.
sets	A character or factor object delineating which samples should be grouped together during the normalization step. Must be the same length as the number of columns in the provided eset, and cannot contain 'NA' or 'NULL' values.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <a href="#">calcNormFactors</a> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.

**Value**

A renormalized ExpressionSet object of the same type as the provided object.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeFQ(es, sets = gsub('(Death|Control)', '', pData(es)$TREATMENT_NAME), lib.size= ls)
ct.gRNARankByReplicate(es, sampleKey = sk, lib.size= ls)
ct.gRNARankByReplicate(es.norm, sampleKey = sk, lib.size= ls)
```



---

ct.normalizeGuides      *Normalize an ExpressionSet Containing a Crispr Screen*

---

## Description

This function normalizes Crispr gRNA abundance estimates contained in an `ExpressionSet` object. Currently four normalization methods are implemented: median scaling (via `normalizeMedianValues`), slope-based normalization (via `ct.normalizeBySlope()`), scaling to the median of the nontargeting control values (via `ct.normalizeNTC()`), and spline fitting to the distribution of the nontargeting gRNAs (via `ct.normalizeSpline()`). Because of the peculiarities of pooled Crispr screening data, these implementations may be more stable than the endogenous methods used downstream by [voom](#). See the respective man pages for further details about specific normalization approaches.

## Usage

```
ct.normalizeGuides(
  eset,
  method = c("scale", "FQ", "slope", "controlScale", "controlSpline"),
  annotation = NULL,
  sampleKey = NULL,
  lib.size = NULL,
  plot.it = FALSE,
  ...
)
```

## Arguments

<code>eset</code>	An <code>ExpressionSet</code> object with integer count data extractable with <code>exprs()</code> .
<code>method</code>	The normalization method to use.
<code>annotation</code>	The annotation object for the library, required for the methods employing nontargeting controls.
<code>sampleKey</code>	An (optional) sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level. If <code>method = 'FQ'</code> , the <code>sampleKey</code> is taken as the <code>'sets'</code> argument (and its format requirements are similarly relaxed; see <code>?ct.normalizeFC</code> ).
<code>lib.size</code>	An optional vector of <code>voom</code> -appropriate library size adjustment factors, usually calculated with <code>calcNormFactors</code> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the <code>exprs</code> slot of the <code>eset</code> .
<code>plot.it</code>	Logical indicating whether to plot the ranked $\log_2$ gRNA count distributions before and after normalization.
<code>...</code>	Other parameters to be passed to the individual normalization methods.

## Value

A renormalized `ExpressionSet`. If specified, the sample level counts will be scaled so as to maintain the validity of the specified `lib.size` values.

**Author(s)**

Russell Bainer

**See Also**[ct.normalizeMedians](#), [ct.normalizeBySlope](#), [ct.normalizeNTC](#), [ct.normalizeSpline](#)**Examples**

```

data('es')
data('ann')

#Build the sample key as needed
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))

es.norm <- ct.normalizeGuides(es, 'scale', annotation = ann, sampleKey = sk, plot.it = TRUE)
es.norm <- ct.normalizeGuides(es, 'slope', annotation = ann, sampleKey = sk, plot.it = TRUE)
es.norm <- ct.normalizeGuides(es, 'controlScale', annotation = ann, sampleKey = sk, plot.it = TRUE, geneSymb =
es.norm <- ct.normalizeGuides(es, 'controlSpline', annotation = ann, sampleKey = sk, plot.it = TRUE, geneSymb =

```

---

ct.normalizeMedians     *Normalize sample abundance estimates by median gRNA counts*

---

**Description**

This function normalizes Crispr gRNA abundance estimates by equalizing the median gRNA abundance values after correcting for library size. It does this by converting raw count values to log2 counts per million and optionally adjusting further in the usual way by dividing these values by user-specified library size factors. This method should be more stable than the endogenous scaling functions used in voom in the specific case of Crispr screens or other cases where the median number of observed counts may be low.

**Usage**

```
ct.normalizeMedians(eset, lib.size = NULL)
```

**Arguments**

eset	An ExpressionSet containing, at minimum, count data accessible by exprs.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <a href="#">calcNormFactors</a> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.

**Value**

A renormalized ExpressionSet object of the same type as the provided object.

**Author(s)**

Russell Bainer

**Examples**

```

data('es')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeMedians(es, lib.size= ls)
ct.gRNARankByReplicate(es, sampleKey = sk, lib.size= ls)
ct.gRNARankByReplicate(es.norm, sampleKey = sk, lib.size= ls)

```

---

ct.normalizeNTC	<i>Normalize sample abundance estimates by the median values of nontargeting control guides</i>
-----------------	---

---

**Description**

This function normalizes Crispr gRNA abundance estimates by equalizing the median abundances of the nontargeting gRNAs within each sample. The normalized values are returned as normalized counts in the 'exprs' slot of the input eset. Note that this method may be unstable if the screening library contains relatively few nontargeting gRNAs.

**Usage**

```
ct.normalizeNTC(eset, annotation, lib.size = NULL, geneSymb = NULL)
```

**Arguments**

eset	An ExpressionSet object containing, at minimum, count data accessible by exprs.
annotation	An annotation dataframe indicating the nontargeting controls in the geneID column.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <code>calcNormFactors</code> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.
geneSymb	The geneSymbol identifier in annotation that corresponds to nontargeting gRNAs. If absent, <code>ct.gRNARankByReplicate</code> will attempt to infer nontargeting guides by searching for "no_gid" or NA in the appropriate columns via <code>ct.prepareAnnotation()</code> .

**Value**

A normalized eset.

**Author(s)**

Russell Bainer

**Examples**

```

data('es')
data('ann')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeNTC(es, ann, lib.size = ls, geneSymb = 'NoTarget')

ct.gRNARankByReplicate(es, sk, lib.size = ls)
ct.gRNARankByReplicate(es.norm, sk, lib.size = ls)

```

---

ct.normalizeSpline	<i>Normalize sample abundance estimates by a spline fit to the nontargeting controls</i>
--------------------	--

---

**Description**

This function normalizes Crispr gRNA abundance estimates by fitting a smoothed spline to the nontargeting gRNAs within each sample and then equalizing these curves across the experiment. Specifically, the algorithm ranks the gRNA abundance estimates within each sample and uses a smoothed spline to determine a relationship between the ranks of nontargeting guides and their abundance estimates. It then removes the spline trend from each sample, centering each experiment around the global median abundance; these values are returned as normalized counts in the 'exprs' slot of the input eset.

**Usage**

```
ct.normalizeSpline(eset, annotation, geneSymb = NULL, lib.size = NULL)
```

**Arguments**

eset	An ExpressionSet object containing, at minimum, count data accessible by exprs.
annotation	An annotation dataframe indicating the nontargeting controls in the geneID column.
geneSymb	The geneSymbol identifier in annotation that corresponds to nontargeting gRNAs. If absent, ct.gRNARankByReplicate will attempt to infer nontargeting guides by searching for "no_gid" or NA in the appropriate columns.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with <a href="#">calcNormFactors</a> and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.

**Value**

A normalized eset.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')
data('ann')

#Build the sample key and library sizes for visualization
library(Biobase)
sk <- (relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))
ls <- colSums(exprs(es))

es.norm <- ct.normalizeSpline(es, ann, 'NoTarget', lib.size = ls)
ct.gRNARankByReplicate(es, sk, lib.size = ls)
ct.gRNARankByReplicate(es.norm, sk, lib.size = ls)
```

---

ct.PantherPathwayEnrichment

*Run a (limited) Pathway Enrichment Analysis on the results of a Crispr experiment.*

---

**Description**

This function enables some limited geneset enrichment-type analysis of data derived from a pooled Crispr screen using the PANTHER pathway database. Specifically, it identifies the set of targets significantly enriched or depleted in a `summaryDF` object returned from `ct.generateResults` and compares that set to the remaining targets in the screening library using a hypergeometric test.

Note that many Crispr gRNA libraries specifically target biased sets of genes, often focusing on genes involved in a particular pathway or encoding proteins with a shared biological property. Consequently, the enrichment results returned by this function represent the pathways containing genes disproportionately targeted *\*within the context of the screen\**, and may or may not be informative of the underlying biology in question. This means that pathways not targeted by a Crispr library will obviously never be enriched within the positive target set regardless of their biological relevance, and pathways enriched within a focused library screen are similarly expected to partially reflect the composition of the library and other confounding issues (e.g., number of targets within a pathway). Analysts should therefore use this function with care. For example, it might be unsurprising to detect pathways related to histone modification within a screen employing a crispr library targeting epigenetic regulators.

**Usage**

```
ct.PantherPathwayEnrichment(
  summaryDF,
  pvalue.cutoff = 0.01,
  enrich = TRUE,
```

```

organism = "human",
db.cut = 10
)

```

### Arguments

summaryDF	A dataframe summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
pvalue.cutoff	A gene-level p-value cutoff defining targets of interest within the screen. Note that this is a nominal p-value cutoff to preserve end-user flexibility.
enrich	Logical indicating whether to consider guides that are enriched (default) or depleted within the screen.
organism	The species of the cell line used in the screen; currently only 'human' or 'mouse' are supported.
db.cut	Minimum number of genes annotated to a given to a pathway within the screen in order to consider it in the enrichment test.

### Value

A dataframe of enriched pathways.

### Author(s)

Russell Bainer, Steve Lianoglou

### Examples

```

data('resultsDF')
ct.PantherPathwayEnrichment(resultsDF, organism = 'mouse')

```

---

ct.PRC

*Generate a Precision-Recall Curve from a CRISPR screen*

---

### Description

Given a set of targets of interest, this function generates a Precision Recall curve from the results of a CRISPR screen. Specifically, it orders the target elements in the screen by the specified statistic, and then plots the recall rate (proportion of true targets identified) against the precision (proportion of identified targets that are true targets).

Note that ranking statistics in CRISPR screens are (usually) permutation-based, and so some granularity in the rankings is expected. This function does a little extra work to ensure that hits are counted as soon as the requisite value of the ranking statistic is reached regardless of where the gene is located within the block of equally-significant genes. Functionally, this means that the drawn curve is somewhat anticonservative in cases where the gene ranks are not well differentiated.

**Usage**

```
ct.PRC(
  summaryDF,
  target.list,
  stat = c("enrich.p", "deplete.p", "enrich.fc", "deplete.fc", "enrich.rho",
           "deplete.rho"),
  plot.it = TRUE
)
```

**Arguments**

summaryDF	A dataframe summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
target.list	A character vector containing the names of the targets to be tested. Only targets contained in the geneID column of the provided summaryDF are considered.
stat	The statistic to use when ordering the genes. Must be one of "enrich.p", "deplete.p", "enrich.fc", or "deplete.fc".
plot.it	Logical value indicating whether to plot the curves.

**Value**

A list containing the the x and y coordinates of the curve.

**Author(s)**

Russell Bainer

**Examples**

```
data('resultsDF')
data('essential.genes') #Note that this is an artificial example.
pr <- ct.PRC(resultsDF, essential.genes, 'enrich.p')
str(pr)
```

---

ct.prepareAnnotation    *Check and optionally subset an annotation file for use in a Crispr Screen*

---

**Description**

This function processes a supplied annotation object for use in a pooled screening experiment. Originally this was processed into something special, but now it essentially returns the original annotation object in which the geneSymbol column has been factorized. This is primarily used internally during a call to the `ct.generateResults()` function. Also performs some minor functionality checking.

Valid annotations contain both 'geneID' and 'geneSymbol' columns. This is because there is often a distinction between the official gene that is being targeted and a coherent set of gRNAs that make up a testing cohort. For example, multiple sets of guides may target distinct promoters, exons, or other entities that are expected to produce distinct biological phenomena related to the gene that should be interpreted separately. For this reason, the 'geneID' column encodes the official gene designation (typically an ensembl or entrez gene identifier) while the 'geneSymbol' column contains a human-readable descriptor of the gRNA target (such as a gene symbol or promoter name).

**Usage**

```
ct.prepareAnnotation(ann, object = NULL, controls = TRUE, throw.error = TRUE)
```

**Arguments**

ann	A data.frame containing an annotation object with gRNA-level information encoded as rows. The row.names attribute should correspond to the individual gRNAs, and it should at minimum contain columns named "geneID" and "geneSymbol" indicating the corresponding gRNA target gene ID and symbol, respectively.
object	If supplied, an object with row.names to be used to subset the supplied annotation frame for downstream analysis.
controls	The name of a value in the geneSymbol column of ann that corresponds to nontargeting control gRNAs. May also be supplied as a logical value, in which case the function will try to identify and format nontargeting guides.
throw.error	Logical indicating whether to throw an error when controls is TRUE but no nontargeting gRNAs are detected.

**Value**

A new annotation data frame, usually with nontargeting controls and NA values reformatted to NoTarget (and geneID set to 'no\_gid'), and the "geneSymbol" column of ann factorized. If supplied with an object, the gRNAs not present in the object will be omitted.

**Author(s)**

Russell Bainer

**Examples**

```
data('ann')
data('es')
es <- ct.filterReads(es)
newann <- ct.prepareAnnotation(ann, es)
```

---

ct.rawCountDensities *Visualization of Raw gRNA Count Densities*

---

**Description**

This function plots the per-sample densities of raw gRNA read counts on the log10 scale. The curve colors are assigned based on a user-specified sampleKey. This function is primarily useful to determine whether libraries are undersequenced (low mean raw gRNA counts), contaminated (many low-abundance gRNAs present), or if PCR artifacts may be present (subset of extremely abundant guides, multiple gRNA distribution modes). In most well-executed experiments the majority of gRNAs will form a tight distribution around some reasonably high average read count (hundreds of reads), at least among the control samples. Excessively low raw count values can compromise normalization steps and subsequent estimation of gRNA levels, especially in screens in which most gRNAs have minimal effects on cell viability.



**Usage**

```
ct.rawCountDensities(eset, sampleKey = NULL, lib.size = NULL)
```

**Arguments**

eset	An ExpressionSet object containing, at minimum, count data accessible by exprs() and some phenoData.
sampleKey	A sample key, supplied as a (possibly ordered) factor linking the samples to experimental variables. The names attribute should exactly match those present in eset, and the control set is assumed to be the first level.
lib.size	Optional named vector of library sizes (total reads within the library) to enable normalization

**Value**

A density plot as specified on the default device.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')

#Build the sample key
library(Biobase)
sk <- relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference")
names(sk) <- row.names(pData(es))

ct.rawCountDensities(es, sk)
```

---

ct.resultCheck	<i>Determine whether a supplied object contains the results of a Pooled Screen</i>
----------------	--

---

**Description**

Many gCrisprTools functions operate on a data.frame of results generated by a CRISPR screen. This function takes in a supplied object and returns a logical indicating whether the object can be treated as one of these data.frames for the purposes of downstream analyses. This is largely used internally, but can be useful if a user needs to build a result object for some reason.

**Usage**

```
ct.resultCheck(summaryDF)
```

**Arguments**

summaryDF	A data.frame, usually returned by ct.generateResults. if you need to generate one of these by hand for some reason, see the example resultsDF object loaded in the example below.
-----------	---

**Value**

A logical indicating whether the object is of the appropriate format.

**Author(s)**

Russell Bainer

**Examples**

```
data('resultsDF')
ct.resultCheck(resultsDF)
```

---

ct.ROC	<i>Generate a Receiver-Operator Characteristic (ROC) Curve from a CRISPR screen</i>
--------	---

---

**Description**

Given a set of targets of interest, this function generates a ROC curve and associated statistics from the results of a CRISPR screen. Specifically, it orders the elements targeted in the screen by the specified statistic, and then plots the cumulative proportion of positive hits on the y-axis. The corresponding vectors and Area Under the Curve (AUC) statistic are returned as a list.

Note that ranking statistics in CRISPR screens are (usually) permutation-based, and so some granularity is expected. This function does a little extra work to ensure that hits are counted as soon as the requisite value of the ranking statistic is reached regardless of where the gene is located within the block of equally-significant genes. Functionally, this means that the drawn curve is somewhat anticonservative in cases where the gene ranks are not well differentiated.

**Usage**

```
ct.ROC(
  summaryDF,
  target.list,
  stat = c("enrich.p", "deplete.p", "enrich.fc", "deplete.fc", "enrich.rho",
           "deplete.rho"),
  condense = TRUE,
  plot.it = TRUE
)
```

**Arguments**

summaryDF	A dataframe summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
target.list	A character vector containing the names of the targets to be tested. Only targets contained in the geneID column of the provided summaryDF are considered.
stat	The statistic to use when ordering the genes. Must be one of "enrich.p", "deplete.p", "enrich.fc", "deplete.fc", "enrich.rho", or "deplete.rho".

condense	Logical indicating whether the returned x and y coordinates should be "condensed", returning only the points at which the detected proportion of <code>target.list</code> changes. If set to <code>FALSE</code> , the returned x and y vectors will explicitly indicate the curve value at every position (useful for performing curve arithmetic downstream).
plot.it	Logical value indicating whether to plot the curves.

**Value**

A list containing the the x and y coordinates of the curve, and the AUC statistic (invisibly).

**Author(s)**

Russell Bainer

**Examples**

```
data('resultsDF')
data('essential.genes') #Note that this is an artificial example.
roc <- ct.ROC(resultsDF, essential.genes, 'deplete.p')
str(roc)
```

---

ct.signalSummary	<i>Generate a Figure Summarizing Overall Signal for One or More Targets</i>
------------------	---

---

**Description**

Given one or more targets of interest, this function generates a summary image contextualizing the corresponding signals within the contest of the provided contrast. This takes the form of an annotated ranking curve of target-level signals, supplemented with horizontal Q-value cutoffs and an inset volcano plot of gRNA behavior.

Limited annotation is provided for the specified targets using the following logic:

- If a character vector is provided, up to five targets are annotated; longer lists are highlighted without specifying individual elements.
- If a list is provided, the 'names' element is used as the annotation. This is similarly constrained to a total of 5 annotated elements.

**Usage**

```
ct.signalSummary(
  summaryDF,
  targets,
  direction = c("enrich", "deplete"),
  callout = FALSE
)
```

**Arguments**

summaryDF	A dataframe summarizing the results of the screen, returned by the function <code>ct.generateResults</code> .
targets	A list or character vector containing the names of the targets to be displayed. Only targets contained in the <code>geneSymbol</code> column of the provided <code>summaryDF</code> are considered. Plotting priority (e.g., the points to plot last in the case of overlapping signals) is given to earlier elements in the list.
direction	Should enrichment or depletion be considered? Must be one of "enrich" or "deplete".
callout	Logical indicating whether lines should be plotted indicating individual gene sets to augment the point highlighting.

**Value**

A summary plot on the current device.

**Author(s)**

Russell Bainer

**Examples**

```
data('resultsDF')
ct.signalSummary(resultsDF, list('CandidateA' = 'Target229', 'Pathway3' = resultsDF$geneSymbol[c(42,116,1138)
data('es')
data('ann')
data('fit')

ct.GCbias(es, ann)
ct.GCbias(fit, ann)
```

---

ct.stackGuides	<i>View a stacked representation of the most variable targets or individual guides within an experiment, as a percentage of the total aligned reads</i>
----------------	---

---

**Description**

This function identifies the gRNAs or targets that change the most from sample to sample within an experiment as a percentage of the entire library. It then plots the abundance of the top guides as a stacked barplot for all samples in the experiment. The purpose of this algorithm is to detect potential distortions in the library composition that might not be properly controlled by sample normalization, and so the most variable entities are defined by calculating the percent of aligned reads that they contribute to each sample, and then ranking each entity by the range of these percentages across all samples. Consequently, gRNAs or Targets that are highly abundant in at least one condition will be more likely to be identified.

**Usage**

```
ct.stackGuides(
  eset,
  sampleKey = NULL,
  nguides = 20,
  plotType = "gRNA",
  annotation = NULL,
  ylimit = NULL,
  subset = NULL
)
```

**Arguments**

eset	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the <code>exprs()</code> function, and a metadata object containing a column named <code>SAMPLE_LABEL</code> containing unique identifiers for each sample. The <code>colnames</code> should be syntactically
sampleKey	An optional sample key, supplied as an ordered factor linking the samples to experimental variables. The <code>names</code> attribute should exactly match those present in <code>eset</code> , and the control set is assumed to be the first level.
nguides	The number of guides (or targets) to display.
plotType	A string indicating whether the individual guides should be displayed ("gRNA"), or if they should be aggregated into target-level estimates ("Target") according to the <code>geneSymbol</code> column in the annotation object.
annotation	An optional data.frame containing an annotation object to be used to aggregate the guides into targets. gRNAs are annotated by row, and must minimally contain a column <code>geneSymbol</code> indicating the target elements.
ylimit	An optional numeric vector of length 2 specifying the y limits for the plot, useful in comparin across studies.
subset	An optional character vector containing the sample labels to be used in the analysis; all elements must be contained in the <code>colnames</code> of the specified <code>eset</code> .

**Value**

A stacked barplot displaying the appropriate entities on the default device.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')
data('ann')
ct.stackGuides(es, nguides = 20, plotType = "Target", annotation = ann, ylimit = NULL, subset = NULL)
```

---

`ct.targetSetEnrichment`*Test Whether a Specified Target Set is Enriched Within a Pooled Screen*

---

### Description

This function takes in a `resultsDF` and a vector of targets (contained in the `geneID` column of `resultsDF`) and determines whether the specified targets are enriched within the set of all significantly altered targets. It does this by iteratively testing whether targets are more likely to be among the set of enriched or depleted targets at various significance thresholds using a hypergeometric test. Note that the returned Hypergeometric P-values are not corrected for multiple testing.

Returns a list detailing the targets used in the tests, and tables indicating the results of the hypergeometric test at various significance thresholds.

### Usage

```
ct.targetSetEnrichment(summaryDF, targets, enrich = TRUE, ignore = NULL)
```

### Arguments

<code>summaryDF</code>	A dataframe summarizing the results of the screen, returned by the function <code>ct.generateResults</code> .
<code>targets</code>	A character vector containing the names of the targets to be tested. Only targets contained in the <code>geneID</code> column of the provided <code>summaryDF</code> are considered.
<code>enrich</code>	Logical indicating whether to consider guides that are enriched (default) or depleted within the screen.
<code>ignore</code>	Optionally, a character vector containing elements of the <code>geneID</code> column of the provided <code>summaryDF</code> that should be ignored in the analysis (e.g., unassignable or nonfunctional targets, such as nontargeting controls). By default, this function omits targets with <code>geneSymbol</code> 'NoTarget'.

### Value

A named list containing the tested target set and tables detailing the hypergeometric test results using various P-value and Q-value thresholds.

### Author(s)

Russell Bainer

### Examples

```
data(resultsDF)
tar <- sample(unique(resultsDF$geneID), 20)
res <- ct.targetSetEnrichment(resultsDF, tar)
```

---

ct.topTargets	<i>Display the log<sub>2</sub> fold change estimates and associated standard deviations of the guides targeting the top candidates in a crispr screen</i>
---------------	---

---

### Description

This is a function for displaying candidates from a crispr screen, using the information summarized in the corresponding `fit` and the output from `ct.generateResults()`. The fold change and standard deviation estimates for each gRNA associated with each target (extracted from the `coefficients` and `stdev.unscaled` slot of `fit`) are plotted on the y axis. Targets are selected on the basis of their gene-level enrichment or depletion P-values; in the case of ties, they are ranked on the basis of their corresponding Rho statistics.

### Usage

```
ct.topTargets(
  fit,
  summaryDF,
  annotation,
  targets = 10,
  enrich = TRUE,
  contrast.term = NULL
)
```

### Arguments

<code>fit</code>	An object of class <code>MArrayLM</code> containing, at minimum, a <code>coefficients</code> slot with coefficients from the comparison, and a <code>stdev.unscaled</code> slot with the corresponding standard deviation of the coefficient estimates. The <code>row.names</code> attribute should ideally match that which is found in <code>annotation</code> .
<code>summaryDF</code>	A <code>data.frame</code> summarizing the results of the screen, returned by the function <a href="#">ct.generateResults</a> .
<code>annotation</code>	An annotation object for the experiment. gRNAs are annotated by row, and must minimally contain a <code>geneSymbol</code> column.
<code>targets</code>	Either the number of top targets to display, or a list of <code>geneSymbols</code> contained in the <code>geneSymbol</code> slot of the <code>annotation</code> object.
<code>enrich</code>	Logical indicating whether to display guides that are enriched (default) or depleted within the screen. If a vector of <code>geneSymbols</code> is specified, this controls the left-to-right ordering of the corresponding gRNAs.
<code>contrast.term</code>	If a <code>fit</code> object with multiple coefficients is passed in, a string indicating the coefficient of interest.

### Value

An image on the default device indicating each gRNA's log<sub>2</sub> fold change and the unscaled standard deviation of the effect estimate, derived from the `MArrayLM` object.

### Author(s)

Russell Bainer

**Examples**

```

data('fit')
data('resultsDF')
data('ann')

ct.topTargets(fit, resultsDF, ann)

```

---

ct.viewControls

*View nontargeting guides within an experiment*


---

**Description**

This function tries to identify, and then plot the abundance of, the full set of non-targeting controls from an ExpressionSet object. Ideally, the user will supply a geneSymbol present in the appropriate annotation file that uniquely identifies the nontargeting gRNAs. Absent this, the the function will search for common identifier used by nontargeting controls (geneID "no\_gid", or geneSymbol NA).

**Usage**

```

ct.viewControls(
  eset,
  annotation,
  sampleKey,
  geneSymb = NULL,
  normalize = TRUE,
  lib.size = NULL
)

```

**Arguments**

eset	An ExpressionSet object containing, at minimum, a matrix of gRNA abundances extractable with the exprs function.
annotation	An annotation data.frame for the experiment. gRNAs are annotated by row, and must minimally contain columns geneSymbol and geneID.
sampleKey	A sample key, supplied as an ordered factor linking the samples to experimental variables. The names attribute should exactly match those present in eset, and the control condition is assumed to be the first level.
geneSymb	The geneSymbol identifier in annotation that corresponds to nontargeting gRNAs. If absent, ct.ViewControls will attempt to infer nontargeting guides by searching for "no_gid" or NA in the appropriate columns.
normalize	Logical indicating whether to attempt to normalize the data in the eset by DE-Seq size factors present in the metadata. If TRUE, then the metadata must contain a column containing these factors, named sizeFactor.crispr-gRNA.
lib.size	An optional vector of voom-appropriate library size adjustment factors, usually calculated with calcNormFactors and transformed to reflect the appropriate library size. These adjustment factors are interpreted as the total library sizes for each sample, and if absent will be extrapolated from the columnwise count sums of the exprs slot of the eset.



**Value**

An image of nontargeting control gRNA abundances on the default device.

**Author(s)**

Russell Bainer

**Examples**

```
data('es')
data('ann')

#Build the sample key
library(Biobase)
sk <- ordered(relevel(as.factor(pData(es)$TREATMENT_NAME), "ControlReference"))
names(sk) <- row.names(pData(es))

ct.viewControls(es, ann, sk, geneSymb = NULL, normalize = FALSE)
ct.viewControls(es, ann, sk, geneSymb = NULL, normalize = TRUE)
```

---

ct.viewGuides

*Generate a Plot of individual gRNA Pair Data in a Crispr Screen*


---

**Description**

This function generates a visualization of the effect estimates from a MArrayLM model result for all of the individual guides targeting a particular element, specified somewhere in the library annotation file. The estimated effect size and variance is plotted relative to zero for the specified contrast, with the color of the dot indicating the relative scale of the of the guide intercept within the model framework, with warmer colors indicating lowly expressed guides. For comparison, the density of gRNA fold change estimates is provided in a pane on the right, with white lines indicating the exact levels of the individual guides.

**Usage**

```
ct.viewGuides(
  gene,
  fit,
  ann,
  type = "geneSymbol",
  contrast.term = NULL,
  ylims = NULL
)
```

**Arguments**

gene	the name of the target element of interest, contained within the "type" column of the annotation file.
fit	An object of class MArrayLM containing, at minimum, an "Amean" slot containing the guide level abundances, a "coefficients" slot containing the effect estimates for each guide, and an "stdev.unscaled" slot giving the coefficient standard Deviations.

<code>ann</code>	A <code>data.frame</code> object containing the gRNA annotations. At minimum, it should have a column with the name specified by the <code>type</code> argument, containing the element targeted by each guide.
<code>type</code>	A character string indicating the column in <code>ann</code> containing the target of interest.
<code>contrast.term</code>	If a fit object with multiple coefficients is passed in, a string indicating the coefficient of interest.
<code>y.lims</code>	An optional numeric vector of length 2 indicating the extremes of the y-axis scale.

**Value**

An image summarizing gRNA behavior within the specified gene on the default device.

**Author(s)**

Russell Bainer

**Examples**

```
data('fit')
data('ann')
ct.viewGuides('Target1633', fit, ann)
```

---

es

*ExpressionSet of count data from a Crispr screen with strong selection*

---

**Description**

Expressionset of raw counts from a screen in mouse cells performed at Genentech, Inc. All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Genentech, Inc.

**See Also**

Please see ‘`vignettes/Crispr_example_workflow.R`’ for details.

**Examples**

```
data("es")
print(es)
```

---

essential.genes	<i>Artificial list of 'essential' genes in the example Crispr screen included for plotting purposes</i>
-----------------	---

---

**Description**

Example gene list, designed to demonstrate ROC and PRC functions All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Russell Bainer

**See Also**

Please see 'vignettes/Crispr\_example\_workflow.R' for details.

**Examples**

```
data("essential.genes")
essential.genes
```

---

fit	<i>Precalculated contrast fit from a Crispr screen</i>
-----	--

---

**Description**

A precalculated fit object (class MArrayLM) comparing the death and control expansion arms of a crispr screen performed at Genentech, Inc. All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Genentech, Inc.

**See Also**

Please see 'vignettes/Crispr\_example\_workflow.R' for model details.

**Examples**

```
data("fit")
show(fit)
```

---

`resultsDF`*Precalculated gene-level summary of a crispr screen*

---

**Description**

A precalculated summary Dataframe comparing the death and control expansion arms of the provided example Crispr screen (using 8 cores, seed = 2). All sample, gRNA, and Gene information has been anonymized and randomized.

**Source**

Genentech, Inc.

**See Also**

Please see ‘vignettes/Crispr\_example\_workflow.R’ for model details.

**Examples**

```
data("resultsDF")
head(resultsDF)
```

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