Package 'TEQC'

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Type Package

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Title Quality control for target capture experiments

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|---|---|
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| Description Target capture experiments combine hybridization-based (in solution or on microarrays) capture and enrichment of genomic regions of interest (e.g. the exome) with high throughput sequencing of the captured DNA fragments. This package provides functionalities for assessing and visualizing the quality of the target enrichment process, like specificity and sensitivity of the capture, per-target read coverage and so on. | |
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Description

Barplot of numbers (or fractions) of reads (and targets) falling on each chromosome

Usage

chrom.barplot(reads, targets, plotchroms, col = c("darkgreen", "orange"), ylab, legendpos = "topri

Arguments

| reads | RangedData table containing read positions, i.e. output from get.reads. To ensure a useful ordering of the bars, the chromosome information ('spaces' of reads) should be given as "chr" plus a number/letter [plus further specification], e.g. "chr1", "chrX", "chr17_ctg5_hap1", "chrUn_gl000211". |
|------------|---|
| targets | Optional RangedData table containing positions of target regions, i.e. output from get.targets. The chromosome information should match the one of reads. If targets is missing, only numbers of reads will be displayed. |
| plotchroms | character vector specifying the chromosomes that shall be included in the plot (and their desired order) |
| col | color(s) of the bars |
| ylab | y-axis label |
| legendpos | Position of the legend. String from the list "bottomright", "bottom", "bottom-left", "left", "topleft", "top", "topright", "right" and "center". Ignored if targets is missing. |
| | graphical parameters passed to barplot |

Details

If targets is not specified, absolute read counts per chromosome are shown in the barplot. If targets is provided, fractions of reads and targets are shown. For reads, this is the fraction within the total *number* of reads (since reads are expected to have all the same length). In contrast, for the targets, the fraction of targeted bases on each chromosome is calculated. Since targets might vary in length it is reasonable to account for the actual target *sizes* instead of considering merely numbers of targets per chromosome.

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Value

Barplot of reads and optionally targets per chromosome.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.reads
```

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)
chrom.barplot(reads, targets)</pre>
```

coverage.correlation Coverage correlation plot

Description

Visualization of target coverage correlations between pairs of samples.

Usage

Arguments

| coveragelist | List where each element is the output of function coverage.target, where option perBase had to be set to TRUE. |
|----------------|---|
| normalized | if TRUE, correlation of normalized target coverages will be shown; original coverages otherwise |
| plotfrac | numeric value between 0 and 1. Coverages for a fraction of plotfrac of all target bases are shown. |
| seed | seed for random selection of plotfrac bases |
| labels | sample names that are written in the diagonal panels; if missing, names of coveragelist are taken; if those are NULL, "sample 'i'" is shown |
| main | main title |
| pch | plot symbol for the scatter plots |
| cex.labels, ce | ex.pch, cex.main |
| | sizes of sample labels, plot symbols, main title |

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```
cex.corr size of the correlation values; if missing, sizes are made proportionally to the values of (positive) correlation.

font.labels, font.main fonts for sample labels and main title

... further graphical parameters, e.g. limits and symbol color for the scatter plots
```

Details

If normalized = TRUE, the function calculates normalized coverages: per-base coverages divided by average coverage over all targeted bases. Normalized coverages are not dependent on the absolute quantity of reads and are hence better comparable between different samples or even different experiments.

Value

'pairs'-style plot where upper panels show scatter plot of (a randomly chosen fraction of) coverage values for pairs of samples. The lower panels show the respective Pearson correlation coefficients, calculated using all coverage values (even if not all of them are shown in the scatter plot).

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

coverage.target, covered.k, coverage.hist, coverage.density, coverage.uniformity, coverage.plot

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## simulate another sample
r <- sample(nrow(reads), 0.1 * nrow(reads))
reads2 <- reads[-r,,drop=TRUE]
Coverage2 <- coverage.target(reads2, targets, perBase=TRUE)

## coverage uniformity plot
covlist <- list(Coverage, Coverage2)
coverage.correlation(covlist, plotfrac=0.1)</pre>
```

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| coverage.density | Coverage density plot |
|------------------|-----------------------|
| | |

Description

Visualization of target coverage density for one or more samples.

Usage

```
coverage.density(coveragelist, normalized = TRUE, legend, main, xlab, col, lwd, lty, xlim, ylim, .
```

Arguments

| coveragelist | Output of function coverage.target, where option perBase had to be set to TRUE, i.e. a list with elements coverageTarget and avgTargetCoverage. Or, when density of several samples shall be visualized, a list with respective outputs of coverage.target. |
|--------------|---|
| normalized | if TRUE, densities of normalized coverages will be shown; original coverages otherwise |
| legend | legend text. If missing, names of coveragelist will be taken. If NULL, no legend will be drawn. |
| main | main title |
| xlab | x-axis label |
| col | line color(s) |
| lwd | line width(s) |
| lty | line style(s) |
| xlim, ylim | x- and y-axis coordinate ranges |
| | further graphical parameters passed to plot |

Details

If normalized = TRUE, the function calculates normalized coverages: per-base coverages divided by average coverage over all targeted bases. Normalized coverages are not dependent on the absolute quantity of reads and are hence better comparable between different samples or even different experiments.

Value

Line plot(s) showing densities.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
coverage. target, covered. k, coverage. hist, coverage. uniformity, coverage. correlation, \\coverage.plot
```

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Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## coverage density
coverage.density(Coverage)</pre>
```

coverage.GC

Bait coverage versus GC content plot

Description

Calculates and plots average normalized coverage per hybridization probe versus GC content of the respective probe. A smoothing spline is added to the scatter plot.

Usage

```
coverage.GC(coverageAll, baits, returnBaitValues = FALSE, linecol = "darkred", lwd, xlab, ylab, po
```

Arguments

coverageAll RleList containing Rle vectors of per-base coverages for each chromosome,

i.e. coverageAll output of coverage.target

baits A RangedData table holding the hybridization probe ("bait") positions and se-

quences, i.e. output ofget.baits

returnBaitValues

if TRUE, average coverage, average normalized coverage and GC content per bait

are returned

linecol, lwd color and width of spline curve

xlab, ylab x- and y-axis labels pch plotting character

col, cex color and size of plotting character

... further graphical parameters passed to plot

Details

The function calculates average normalized coverages for each bait: the average coverage over all bases within a bait is divided by the average coverage over all bait-covered bases. Normalized coverages are not dependent on the absolute quantity of reads and are hence better comparable between different samples or even different experiments.

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Value

A scatterplot with normalized per-bait coverages on the y-axis and GC content of respective baits on the x-axis. A smoothing spline is added to the plot.

If returnBaitValues = TRUE average coverage, average normalized coverage and GC content per bait are returned as 'values' columns of the baits input RangedData table

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

References

Tewhey R, Nakano M, Wang X, Pabon-Pena C, Novak B, Giuffre A, Lin E, Happe S, Roberts DN, LeProust EM, Topol EJ, Harismendy O, Frazer KA. Enrichment of sequencing targets from the human genome by solution hybridization. Genome Biol. 2009; 10(10): R116.

See Also

coverage.target, covered.k, coverage.hist, coverage.plot, coverage.uniformity, coverage.targetlength.

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## get bait positions and sequences
baitsfile <- file.path(exptPath, "ExampleSet_Baits.txt")
baits <- get.baits(baitsfile, chrcol=3, startcol=4, endcol=5, seqcol=2)

## do coverage vs GC plot
coverage.GC(Coverage$coverageAll, baits)</pre>
```

coverage.hist

Coverage histogram

Description

Histogram and cumulative density of target base coverages

Usage

```
coverage.hist(coverageTarget, col.hist = "lightblue", col.line = "orange", covthreshold, outline =
```

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Arguments

coverageTarget RleList containing Rle vectors of per-target-base coverages for each chromo-

some, i.e. coverageTarget output from coverage.target

col.hist histogram color

col.line color of the cumulative density line

covthreshold indicates with dashed vertical and horizontal lines, which fraction of bases has

a coverage of at least covthreshold; if missing, no dashed lines are drawn

outline if FALSE, outliers (according to boxplot.stats) are removed before plotting.

breaks number of cells for the histogram, or string naming an algorithm to compute the

number of cells, or function to compute the number of cells, or vector giving the breakpoints between histogram cells (see ?hist) but the latter option only with

equidistant breakpoints

xlab, ylab x- and y-axis labels

main plot title
lwd line width

... further graphical parameters, passed to plot(histogram)

Value

Histogram of read coverages for bases within the target. Additionally, a line and the right axis indicate the cumulative fraction of target bases with coverage of at least x. If option covthreshold is specified, red dashed lines highlight the cumulative fraction of target bases with at least the specified coverage.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

coverage.target, coverage.uniformity, coverage.density, coverage.plot, coverage.targetlength.plot

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## coverage histogram
coverage.hist(Coverage$coverageTarget, covthreshold=8)</pre>
```

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| coverage.plot | Coverage versus base position plot |
|---------------|------------------------------------|
| | |

Description

Line plot of per-base coverages along a genomic region. Position of target regions can be shown.

Usage

```
coverage.plot(coverageAll, targets, chr, Start, End, Offset = 0, add = FALSE, col.line = 1, col.tar
```

Arguments

| coverageAll | RleList containing Rle vectors of per-base coverages for each chromosome, i.e. coverageAll output from coverage.target |
|-------------|--|
| targets | optional; RangedData table containing positions of target regions, i.e. output from get.targets; if missing no genomic regions are highlighted |
| chr | on which chromosome the region to plot is located (string, e.g. "chr1") |
| Start | genomic position where to start the plot |
| End | genomic position where to end the plot |
| Offset | integer; highlight Offset bases on both sides of each targeted region; defaults to $\boldsymbol{0}$ |
| add | if TRUE, the coverage line of a new sample is added to an already existing plot |
| col.line | color of the coverage line |
| col.target | color of the bar indicating target regions |
| col.offset | color for highlighting Offset on the sides of target regions |
| xlab, ylab | x- and y-axis labels |
| ylim | y-axis coordinate ranges |
| | further graphical parameters, passed to plot |

Details

If coverage of a new sample is added to an existing plot with add = TRUE, parameters chr, Start, End still have to be specified and should be the same as in the previous call in order to make sense. Parameters targets and Offset can but do not have to be given again. They can also differ from the previous ones, if for the additional sample a different target was captured.

Value

Line plot showing per-base read coverages for a specified genomic region. When positions of target regions are provided, a bar on the bottom indicates their location such that coverage can be related to the captured targets.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

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See Also

coverage.target, make.wigfiles, covered.k, coverage.hist, coverage.uniformity, coverage.targetlength.

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## coverage plot
coverage.plot(Coverage$coverageAll, targets, Offset=100, chr="chr1", Start=11157524, End=11158764)</pre>
```

coverage.target

Calculates read coverage

Description

Calculates average coverage over all target bases, average coverage for each target separately, and per-base coverage for all and for targeted bases

Usage

```
coverage.target(reads, targets, Offset = 0, perTarget = TRUE, perBase = TRUE)
```

Arguments

reads RangedData table containing positions of sequenced reads, i.e. output from

get.reads

targets RangedData table containing positions of target regions, i.e. output from get.targets

Offset integer; add Offset bases on both sides to targeted regions and potentially col-

lapse resulting overlapping target regions

perTarget if TRUE, coverage average and standard deviation per target are calculated and

returned

perBase if TRUE, the per-base coverages i) only for targeted bases and ii) for all se-

quenced and/or targeted bases, are returned

Value

A list is returned with elements

avgTargetCoverage

average coverage over all target bases

targetCoverageSD

standard deviation of coverage of all target bases

targetCoverageQuantiles

0% (minium), 25%, 50% (median), 75% and 100% (maximum) quantiles of coverage of all target bases

targetCoverages

Input RangedData table targets with two additional 'values' columns avgCoverage and coverageSD. The former contains the average coverage for each target, the latter the respective coverage standard deviation. Only returned if perTarget equals TRUE.

coverageAll

RleList containing a Rle vector for each chromosome with coverages for all bases that are sequenced and/or within a targeted; only returned if perBase equals TRUE

coverageTarget RleList containing a Rle vector for each chromosome with coverages for target bases only; only returned if perBase equals TRUE

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

coverage.hist, coverage.uniformity, coverage.plot, coverage.targetlength.plot

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")</pre>
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")</pre>
reads <- get.reads(readsfile, idcol=4, skip=0)</pre>
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")</pre>
targets <- get.targets(targetsfile, skip=0)</pre>
## total average, per-base and per-target coverages
Coverage <- coverage.target(reads, targets)</pre>
```

coverage.targetlength.plot

Number of reads or average coverage versus target length scatter plot

Description

Plots either numbers of on-target reads or average per-target coverage (or potentially other per-target values) against respective target lengths. A smoothing spline is added to the scatter plot.

Usage

```
coverage.targetlength.plot(targets, plotcolumn, linecol = 2, xlab, ylab, lwd, pch, cex, ...)
```

Arguments

| targets | RangedData table containing positions of target regions and further 'values' columns that should be plotted, i.e. output from coverage.target or readsPerTarget |
|------------|---|
| plotcolumn | name or index of column to plot (of the 'values' DataFrame within targets) |
| linecol | color of spline curve |
| xlab, ylab | x- and y-axis labels |
| lwd | line width of spline curve |
| pch | plotting character |
| cex | size of plotting character |
| | further graphical parameters, passed to plot |

Details

coverage.target and readsPerTarget can be used to calculate average per-target coverages and numbers of reads overlapping each target. The values are added to the RangedData table containing the target positions. Such RangedData table can then be used for plotting the calculated values against the respective target lengths.

Value

A scatterplot with the given per-target values on the y-axis and corresponding target lengths on the x-axis. A smoothing spline is added to the plot.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

coverage.target, readsPerTarget, covered.k, coverage.hist, coverage.uniformity, coverage.plot

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## get average per-target coverage
Coverage <- coverage.target(reads, targets, perTarget=TRUE)
targets2 <- Coverage$targetCoverages

## get numbers of reads per target
targets2 <- readsPerTarget(reads, targets2)

## coverage vs target length
coverage.targetlength.plot(targets2, plotcolumn="avgCoverage", pch="o")

## coverage vs number of reads per target
coverage.targetlength.plot(targets2, plotcolumn="nReads", pch="o")</pre>
```

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coverage.uniformity Coverage uniformity plot

Description

Visualization of target coverage uniformity. A line shows the cumulative fraction of targeted bases that reach at least a certain normalized coverage.

Usage

coverage.uniformity(coveragelist, addlines = TRUE, add = FALSE, xlab, ylab, xlim, ylim, col, lwd,

Arguments

| coveragelist | output of function coverage.target, where option perBase had to be set to TRUE, i.e. a list with elements coverageTarget and avgTargetCoverage |
|--------------|---|
| addlines | if TRUE, dashed lines are added to the plot that indicate the fractions of bases achieving at least the average or at least half the average coverage |
| add | if TRUE, the coverage uniformity line of a new sample is added to an already existing plot |
| xlab, ylab | x- and y-axis labels |
| xlim, ylim | x- and y-axis coordinate ranges |
| col | line color |
| lwd | line width |
| | further graphical parameters passed to plot |

Details

The function calculates normalized coverages: per-base coverages divided by average coverage over all targeted bases. Normalized coverages are not dependent on the absolute quantity of reads and are hence better comparable between different samples or even different experiments.

Value

Line plot showing the fraction of targeted bases (y-axis) achieving a normalized coverage of at least x. The x-axis by default is truncated at 1, which corresponds to the average normalized coverage. The steeper the curve is falling, the less uniform is the coverage. If addlines = TRUE, dashed lines indicate the fractions of bases achieving at least the average (=1) or at least half (=0.5) the average coverage.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

References

Gnirke A, Melnikov A, Maguire J, Rogov P, LeProust EM, Brockman W, Fennell T, Giannoukos G, Fisher S, Russ C, Gabriel S, Jaffe DB, Lander ES, Nusbaum C. Solution hybrid selection with ultralong oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol. 2009; 27(2): 182-9.

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See Also

coverage. target, coverage. hist, coverage. density, coverage. plot, coverage. targetlength. plot and targetlength and targ

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## coverage uniformity plot
coverage.uniformity(Coverage)</pre>
```

covered.k

Target capture sensitivity

Description

Calculates fraction of target bases covered by at least k reads

Usage

```
covered.k(coverageTarget, k = c(1, 2, 3, 5, 10, 20))
```

Arguments

```
coverageTarget RleList containing Rle vectors of per-target-base coverages for each chromo-
some, i.e. coverageTarget output from coverage.target
k integer vector of k-values for which to show fraction of target bases with cover-
age >= k
```

Value

Named vector of same length as k giving the corresponding fractions of target bases achieving coverages >= k

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

coverage.target, coverage.hist, coverage.uniformity, coverage.plot, coverage.targetlength.plot

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Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)
covered.k(Coverage$coverageTarget, k=c(1,10,20))</pre>
```

duplicates.barplot

Read duplicates barplot

Description

Barplot showing fractions of reads / read pairs which are unique and for which there are two, three, ... copies. Separate bars are made for on- and off-target reads / read pairs

Usage

```
duplicates.barplot(reads, targets, returnDups=FALSE, truncateX, col=c("red","lightblue"), xlab, y
```

RangedData table containing positions of sequenced reads i.e. output from

Arguments

randa

| reaus | get.reads. Alternatively, for paired-end data, it can be the output of reads2pairs when multiplicities of read <i>pairs</i> instead of fraction of single reads shall be visualized. |
|------------|--|
| targets | RangedData table containing positions of target regions, i.e. output from get.targets |
| returnDups | if TRUE, on- and off-target read / read pair multiplicities are returned |
| truncateX | integer; show bars only up to a read / read pair multiplicity of truncateX (x-axis) |
| col | vector specifying the two colors of bars and legend for on- and off-target read multiplicities |
| xlab, ylab | x- and y-axis labels |
| ylim | y-axis coordinate ranges |
| | further graphical parameters passed to barplot |

Details

Single-end reads are considered as duplicates if they have same start end end position. Paired-end read pairs are considered as duplicates if start and end positions of both reads of the pairs are identical. Usually, duplicates are removed before further analyses (e.g. SNP detection), because they could represent PCR artefacts. However, in target capture experiments it is likely to have also many "real" duplicates (actual different molecules that happen to start at same position) due to the "enrichment" of the target regions. The separation in the barplot between on- and off-target reads / read pairs gives an impression on whether on-target there are more reads with higher multiplicites, which hence might indicate a reasonable amount of "real" duplication. A paired-end read pair is considered on-target if at least one of its reads overlaps with a target.

16 fraction.reads.target

Value

Barplot where the bar heights correspond to fractions of reads / read pairs which are present in the data with the respective number of copies (x-axis). Fractions are calculated separately for on- and off-target reads / read pairs. A read pair is considered on-target if at least one of its reads overlaps with a target. Absolute numbers (in millions) are additionally written on top of the bars.

If returnDups equals TRUE, a list with two elements absolute and relative is returned. The former is a matrix that contains the absolute numbers of reads / read pairs for each multiplicity (columns), for both on- and off-target reads / read pairs (rows). The latter gives row-based fractions which correspond to the bar heights.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.reads, reads2pairs, get.targets
```

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## duplicates barplot for single reads
duplicates.barplot(reads, targets, returnDups=TRUE)

## duplicates barplot for read pairs
readpairs <- reads2pairs(reads)
duplicates.barplot(readpairs, targets, returnDups=TRUE)</pre>
```

```
fraction.reads.target Target capture specificity
```

Description

Calculates the fraction of reads that align to target regions. Can also be used to retrieve those reads mapping to targets.

Usage

```
fraction.reads.target(reads, targets, Offset = 0, mappingReads = FALSE)
```

Arguments

reads

RangedData table containing positions of sequenced reads, i.e. output of get.reads. Alternatively, for paired-end data, it can be the output of reads2pairs when fraction of on-target read *pairs* shall be calculated instead of fraction of single on-target reads.

fraction.reads.target 17

targets RangedData table containing positions of target regions, i.e. output from get.targets

Offset integer; add Offset bases on both sides to targeted regions and potentially col-

lapse resulting overlapping target regions

mappingReads if TRUE, reduced RangedData table with only those reads mapping to target re-

gions is returned. When reads is output of reads2pairs, mappingReads will

be the corresponding subset of on-target read pairs.

Value

If mappingReads equals FALSE, just the fraction of reads / read pairs mapping to targets is returned. When reads contains all single reads (i.e. is output of get.reads), this is the number of target-overlapping reads, divided by the number of all single reads. When reads contains read pairs (i.e. is output of reads2pairs), it is the number of read pairs with at least one target-overlapping read, divided by the number of read pairs (= half the number of reads). In case of small targets and large insert sizes the two reads of a pair could be located on both sides of the target without overlap, respectively. Still, the read pair will be counted as on-target, since the corresponding DNA molecule was covering the target.

If mappingReads equals TRUE, a list is returned with elements

onTargetFraction

fraction of reads / read pairs mapping to targets

mappingReads RangedData table containing positions of the reads

RangedData table containing positions of the reads / read pairs mapping to target regions

Note

With the output from fraction.target and fraction.reads.target the 'enrichment' of the target capture experiment can be calculated as 'fraction of on-target reads / fraction of target within genome'

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
fraction.target, get.reads, reads2pairs, get.targets
```

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## fraction of on-target reads
fraction.reads.target(reads, targets)</pre>
```

18 fraction.target

| fracti | on.target | Fraction of the target within the genome |
|--------|-----------|--|
| | | |

Description

Calculates the fraction of the reference genome that is targeted

Usage

```
fraction.target(targets, Offset = 0, genome = c(NA, "hg19", "hg18"), genomesize)
```

Arguments

| targets | ${\tt RangedData}\ table\ containing\ positions\ of\ target\ regions,\ i.e.\ output\ from\ {\tt get.targets}$ | |
|------------|---|--|
| Offset | integer; add Offset bases on both sides to targeted regions and potentially collapse resulting overlapping target regions | |
| genome | genome version targets were designed and reads aligned to. For the given options the total genome size is set automatically. For other genomes or versions, leave this option empty ('NA') and specify the genome size with option 'genomesize' | |
| genomesize | integer: specify the total genome size manually. If 'genomesize' is given, option 'genome' will be ignored. | |

Value

Returns the fraction of nucleotides within the genome that were targeted.

Note

With the output from fraction.target and fraction.reads.target the 'enrichment' of the target capture experiment can be calculated as 'fraction of on-target reads / fraction of target within genome'

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
fraction.reads.target, get.targets
```

```
exptPath <- system.file("extdata", package="TEQC")
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)
fraction.target(targets, genome="hg19")</pre>
```

get.baits 19

| get.baits | Read capture hybridization probe positions | |
|-----------|--|--|
| | | |

Description

Reads a file containing positions and sequences of the capture hybridization probes and creates a RangedData object.

Usage

```
get.baits(baitsfile, chrcol = 1, startcol = 2, endcol = 3, seqcol = 4, zerobased = TRUE, sep = "\t"
```

Arguments

| baitsfile | name of file giving the positions and sequences of each hybridization probe ("bait") |
|-----------|--|
| chrcol | in which column in baitsfile there is the chromosome information (chromosome information in the file should be in string format, e.g. " $chrX$ ") |
| startcol | in which column there are the starting positions of the baits |
| endcol | in which column there are the end positions of the baits |
| seqcol | in which column there are the sequences of the baits |
| zerobased | if TRUE, start coordinates in baitsfile are assumed to be 0-based and are then converted to 1-based system by adding 1. If FALSE, coordinates are not shifted. In this case they should already be 1-based in baitsfile. |
| sep | column separator character, defaults to tabs |
| header | a logical value indicating whether the file contains the names of the variables as its first line; defaults to FALSE |
| | further arguments passed to read.delim |

Details

The baitsfile containing positions and sequences of hybridization probes has to be created beforehand, in many cases manually. (The function was made like this in order to keep things as general and platform independent as possible.) E.g. with baits designed by Agilent's eArray tool, the baitsfile can be created by merging the files '..._D_BED_...bed' and '..._D_DNAFront_BCBottom_...txt'.

Value

A RangedData table holding the hybridization probe ("bait") positions and sequences. Overlapping or adjacent baits are not collapsed.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.reads, get.targets
```

20 get.reads

Examples

```
exptPath <- system.file("extdata", package="TEQC")</pre>
baitsfile <- file.path(exptPath, "ExampleSet_Baits.txt")</pre>
baits <- get.baits(baitsfile, chrcol=3, startcol=4, endcol=5, seqcol=2)</pre>
```

get.reads

Read genomic positions of sequencing data

Description

Reads a bedfile containing positions of sequenced read aligned to a reference genome and creates a RangedData object.

Usage

```
get.reads(readsfile, filetype = c("bed", "bam"), chrcol = 1, startcol = 2, endcol = 3, idcol, zerok
```

Arguments

| readsfile | name of bedfile giving the positions of aligned reads |
|-----------|--|
| filetype | Input file type. If "bam", the .bam file is read using scanBam, where flag option isUnmappedQuery=FALSE is used. Defaults to "bed" |
| chrcol | In which column in the reads bedfile there is the chromosome information (chromosome information in the file should be in string format, e.g. "chrX"). Ignored if filetype = "bam". |
| startcol | In which column there are the starting positions of the reads. Ignored if filetype = "bam". |
| endcol | In which column there are the end positions of the reads. Ignored if filetype = "bam". |
| idcol | In which column there are read identifiers. For single-end data it is optionally. For paired-end data it is required for some functionalities. The two reads of one pair need to have the same ID. Ignored if filetype = "bam" (the ID column is automatically included then). If read IDs include "#0/1" and "#0/2" in the end (indicating read 1 and read 2 of a pair), those characters will be removed from the IDs. |
| zerobased | if TRUE, start coordinates in readsfile are assumed to be 0-based and are then converted to 1-based system by adding 1. If FALSE, coordinates are not shifted. In this case they should already be 1-based in readsfile. Ignored if filetype = "bam", since scanBam converts 0-based to 1-based coordinates. |
| sep | Column separator character, defaults to tabs. Ignored if filetype = "bam". |
| skip | Number of lines of the bedfile to skip before beginning to read data; defaults to 1. Ignored if filetype = "bam". |
| header | A logical value indicating whether the file contains the names of the variables as its first line; defaults to FALSE. Ignored if filetype = "bam". |
| | Further arguments passed to read.delim. Ignored if filetype = "bam". |

Value

A RangedData table holding the read positions

get.targets 21

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.targets
```

Examples

```
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)</pre>
```

get.targets

Read capture target positions

Description

Reads a bedfile containing positions of the capture targets and creates a RangedData object.

Usage

```
get.targets(targetsfile, chrcol = 1, startcol = 2, endcol = 3, zerobased = TRUE, sep = "\t", skip =
```

Arguments

| targetsfile | name of bedfile giving the positions of each target region |
|-------------|--|
| chrcol | in which column in the targets bedfile there is the chromosome information (chromosome information in the file should be in string format, e.g. " $chrX$ ") |
| startcol | in which column there are the starting positions of the targeted regions |
| endcol | in which column there are the end positions of the targeted regions |
| zerobased | if TRUE, start coordinates in targetsfile are assumed to be 0-based and are then converted to 1-based system by adding 1. If FALSE, coordinates are not shifted. In this case they should already be 1-based in targetsfile. |
| sep | column separator character, defaults to tabs |
| skip | number of lines of the bedfile to skip before beginning to read data; defaults to 1 |
| header | a logical value indicating whether the file contains the names of the variables as its first line; defaults to FALSE |
| | further arguments passed to read.delim |

Value

A RangedData table holding the target region positions. Note that overlapping or adjacent regions are collapsed to one region.

Note

Since overlapping regions are collapsed, the input bedfile can also contain positions of the (in most cases overlapping) hybridization probes used for the target capture.

22 insert.size.hist

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.reads
```

Examples

```
exptPath <- system.file("extdata", package="TEQC")
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)</pre>
```

insert.size.hist

Insert sizes histogram

Description

Computes read pair insert sizes, i.e. distance from first base of first read to last base of second read of a read pair, and plots a histogram for all insert sizes.

Usage

insert.size.hist(readpairs, returnInserts = FALSE, legendpos="topleft", outline=FALSE, main, xlab

Arguments

readpairs RangedData table containing positions of read pairs, i.e. output of reads2pairs

(or the element readpairs from the reads2pairs output in case single reads

without matching pair were found).

returnInserts if TRUE, the vector of read pair insert sizes is returned legendpos position of the legend, e.g. 'topleft' or 'topright'

outline if FALSE, outliers (according to boxplot.stats) are removed before plotting. If

returnInserts=TRUE, those outliers are still included in the output.

main plot title

xlab, ylab x- and y-axis labels

breaks e.g. integer specifying the number of cells for the histogram, see ?hist

col histogram color

... further graphical parameters passed to hist

Value

Histogram of read pair insert sizes. Average, standard deviation and median insert size are given in the legend and indicated by lines.

If returnInserts = TRUE, a named vector of insert sizes is returned.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

make.wigfiles 23

See Also

```
get.reads, reads2pairs, duplicates.barplot
```

Examples

```
## get reads
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)

## merge to read pairs
readpairs <- reads2pairs(reads)

## insert size histogram
insert.size.hist(readpairs, breaks=10)</pre>
```

make.wigfiles

Creates wiggle files with per-base coverages

Description

Prepares wiggle files with (non-zero) per-base coverages for the upload and visualization with genome browsers

Usage

```
make.wigfiles(coverageAll, chroms, trackname = "Coverage", filename = "Coverage")
```

Arguments

coverageAll RleList containing Rle vectors of per-base coverages for each chromosome,

i.e. coverageAll output of coverage.target

chroms vector of chromosome names for which to produce wiggle files; if missing wig-

gle files will be produced for all chromosomes on which there are reads

trackname for wiggle file header

filename part of output wiggle file name. Respective chromosome number and '.wig' will

be added

Details

Only non-zero coverages will be listed

Value

One or more wiggle files listing per-base (non-zero) read coverages

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

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See Also

coverage.target, coverage.plot, covered.k, coverage.hist, coverage.uniformity, coverage.targetlength.

Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## calculate per-base coverages
Coverage <- coverage.target(reads, targets, perBase=TRUE)

## create wiggle files for read coverages on chromsomes 13 and 17
make.wigfiles(Coverage$coverageAll, chroms=c("chr13", "chr17"))</pre>
```

multiTEQCreport

Creates an html report for multiple samples

Description

Creates an automated html report comparing TEQC analysis results of several samples

Usage

Arguments

singleReportDirs

string of directory names; output directories of function TEQCreport(), launched

beforehand for each single sample

samplenames names of the samples that will be used in tables and figures

projectName descriptive name for the project / collection of samples; will be written on top

of the html report

targetsName descriptive name of the captured target; will be written on top of the html report

referenceName descriptive name of the reference genome the reads were aligned against; will

be written on top of the html report

destDir directory where results and html documents shall be saved

k integer vector of k-values for which to show fraction of target bases with cover-

age >= k in section 'Sensitivity'

figureFormat format of the figures produced for the html report (besides pdf graphs)

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Details

Before creating the html report for multiple samples, TEQCreport has to be run for each of the samples separately. The output directories of those analyses are the input for multiTEQCreport. While the creation of single-sample reports is time and memory intensive, multiTEQCreport finishes quickly, since it just collects and summarizes the results from the single analyses. The files for the multiple sample html report are created in destDir. The report can be viewed by opening destDir/index.html in a web browser. Images are saved in destDir/image.

Value

The function is invoked for its side effect

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

References

Hummel M, Bonnin S, Lowy E, Roma G. TEQC: an R-package for quality control in target capture experiments. Bioinformatics 2011; 27(9):1316-7.

See Also

TEQCreport

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")</pre>
reads <- get.reads(readsfile, skip=0, idcol=4)</pre>
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")</pre>
targets <- get.targets(targetsfile, skip=0)</pre>
## simulated 2nd sample
r <- sample(nrow(reads), 0.1 * nrow(reads))</pre>
reads2 <- reads[-r,,drop=TRUE]</pre>
## Not run:
## create single-sample reports
TEQCreport(sampleName="Test Sample A", targetsName="Human Exome", referenceName="Human Genome",
           destDir="./reportA", reads=reads, targets=targets, genome="hg19")
TEQCreport(sampleName="Test Sample B", targetsName="Human Exome", referenceName="Human Genome",
           destDir="./reportB", reads=reads2, targets=targets, genome="hg19")
## create multi-sample report
multiTEQCreport(singleReportDirs=c("./reportA", "./reportB"), samplenames=c("Sample A", "Sample B"),
        projectName="Test Project", targetsName="Human Exome", referenceName="Human Genome",
           destDir="./multiTEQCreport")
## End(Not run)
```

26 reads2pairs

| reads2pairs | Merges reads to read pairs |
|-------------|----------------------------|
|-------------|----------------------------|

Description

Combines the two reads of a read pair (in case of paired-end data) to a new 'range' starting at the first reads's start position and ending at the second read's end position.

Usage

```
reads2pairs(reads, max.distance)
```

Arguments

reads RangedData table containing positions of sequenced reads, i.e. output of get.reads.

The first 'values' column has to contain read pair identifiers, i.e. when reads was created by get.reads, the option idcol had to be specified. The input can also contain single reads without 'read mate' (e.g. when the first read of a pair did not align to the reference genome, however the second one did align and was still kept). Those single reads will be returned in a separate table singleReads. When the two reads in a pair align to different chromosomes, they will also be

returned in table singleReads.

max.distance Integer value defining the maximum allowed distance between two reads of a

pair (from start position of first read to end position of second read). Reads exceeding this distance will be returned in the separate table singleReads. If max.distance is not specified, reads will be joined to pairs regardless of their distance. Only when the two reads in a pair align to different chromosomes,

they will be removed in any case and added to table singleReads.

Details

The function puts together the two reads of each pair and creates new ranges spanning both reads and everything in between. Those ranges correspond to the extent of the actual DNA molecules for which both ends were sequenced. The output of the function can be used by several other functions, whenever calculations should be based on read pairs rather than on single reads, e.g. fraction.reads.target, readsPerTarget, duplicates.barplot

Value

If reads only contains complete read pairs and for all pairs the respective reads align to the same chromosome and their distances do not exceed max.distance (if specified), a RangedData object is returned containing positions of the merged reads per pair, ranging from start position of the first read to end position of the second read.

If reads also contains single reads, or if reads within a pair are further apart than max.distance (if specified) or align to different chromosome, a list is returned with elements

singleReads RangedData object containing original positions of single reads without 'read

mates' and/or read pairs aligning too far apart or on different chromosomes

readpairs RangedData object containing positions of the merged reads per pair, ranging

from start position of the first read to end position of the second read

readsPerTarget 27

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
get.reads, fraction.reads.target, readsPerTarget, duplicates.barplot, insert.size.hist
```

Examples

```
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
readpairs <- reads2pairs(reads)</pre>
```

readsPerTarget

Numbers of reads per target

Description

Counts the numbers of reads overlapping each target region

Usage

```
readsPerTarget(reads, targets, Offset = 0)
```

Arguments

reads RangedData table containing positions of sequenced reads, i.e. output from

get.reads

targets RangedData table containing positions of target regions, i.e. output from get.targets

Offset integer; add Offset bases on both sides to targeted regions and potentially col-

lapse resulting overlapping target regions

Value

The input RangedData table targets with an additional 'values' column containing numbers of reads overlapping each target

Note

As reads input also the mappingReads output of function fraction.reads.target can be used to speed up calculation. In this case, make sure that targets and Offset parameters were the same in fraction.reads.target as then specified in readsPerTarget.

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

See Also

```
coverage.target, fraction.reads.target, covered.k, coverage.hist, coverage.uniformity,
coverage.plot, coverage.targetlength.plot
```

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Examples

```
## get reads and targets
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
reads <- get.reads(readsfile, idcol=4, skip=0)
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")
targets <- get.targets(targetsfile, skip=0)

## number of reads per target
readsPerTarget(reads, targets)</pre>
```

TEQCreport

Creates an html report

Description

Creates an automated html report for the complete TEQC analysis of one sample

Usage

Arguments

| sampleName | descriptive sample name; will be written on top of the html report |
|---------------|--|
| targetsName | descriptive name of the captured target; will be written on top of the html report |
| referenceName | descriptive name of the reference genome the reads were aligned against; will be written on top of the html report |
| destDir | directory where results and html documents shall be saved |
| reads | RangedData table containing positions of sequenced reads, or call to get.reads to read in positions from a bed or BAM file |
| targets | RangedData table containing positions of target regions, or call to get.targets to read in positions from a bed file |
| Offset | integer; add Offset bases on both sides to targeted regions and potentially collapse resulting overlapping target regions |
| pairedend | if TRUE, data will be considered to be paired-end data, i.e. reads will be "merged" to read pairs, and chromosome bar plot, specificity, enrichment and duplicate analysis (if selected) will be based on read pairs rather than on single reads |
| genome | genome version targets were designed and reads aligned to. For the given options the total genome size is set automatically. For other genomes or versions, leave this option empty ('NA') and specify the genome size with option 'genomesize' |
| genomesize | integer: specify the total genome size manually. If 'genomesize' is given, option 'genome' will be ignored. |
| k | integer vector of k-values for which to show fraction of target bases with coverage >= k in 'Sensitivity' table. Passed to covered.k |

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covthreshold integer indicating which coverage value shall be highlighted by dashed lines in

the coverage histogram. Passed to coverage.hist

CovUniformityPlot

if TRUE, a coverage uniformity plot is created, see coverage.uniformity

CovTargetLengthPlot

 $if \ \mathsf{TRUE}, coverage \ vs \ target \ \mathsf{length} \ \mathsf{plots} \ are \ \mathsf{created}, see \ \mathsf{coverage} \ . \ \mathsf{targetlength} . \ \mathsf{plot}$

CovGCPlot if TRUE, a coverage vs GC content plot is created, see coverage .GC duplicatesPlot if TRUE, a duplicates barplot is created, see duplicates .barplot

baits A RangedData table holding the hybridization probe ("bait") positions and se-

quences, or call to get. baits to read in positions from a bed file. Only needed

if CovGCPlot = TRUE.

WigFiles if TRUE, wiggle files with per-base coverage are created for each chromosome

saveWorkspace if TRUE, an R workspace with objects reads, targets and output of coverage. target

and reads2pairs (in case pairedend = TRUE) are saved in destDir to be avail-

able for further analyses

figureFormat format of the figures produced for the html report (besides pdf graphs)

Details

TEQC analysis is performed and files for an html report are created in destDir. The report can be viewed by opening destDir/index.html in a web browser. Images are saved in destDir/image. Wiggle files (in case WigFiles = TRUE) are saved in destDir/wiggle. A table with general target coverage statistics, a table with average coverage values per target, a table with cumulative fractions of targeted bases with certain coverage and the R workspace containing R objects for potential further analysis (in case saveWorkspace = TRUE) are saved in destDir.

Value

The function is invoked for its side effect

Author(s)

Manuela Hummel <m.hummel@dkfz.de>

References

Hummel M, Bonnin S, Lowy E, Roma G. TEQC: an R-package for quality control in target capture experiments. Bioinformatics 2011; 27(9):1316-1317

See Also

```
get.reads, get.targets, fraction.target, fraction.reads.target, coverage.target, readsPerTarget,
reads2pairs, covered.k, coverage.hist, coverage.uniformity, coverage.targetlength.plot,
coverage.GC, get.baits, make.wigfiles
```

```
## get reads and targets files
exptPath <- system.file("extdata", package="TEQC")
readsfile <- file.path(exptPath, "ExampleSet_Reads.bed")
targetsfile <- file.path(exptPath, "ExampleSet_Targets.bed")</pre>
```

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