# Package 'QoRTs'

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Title Quality of RNA-seq Tool

 makePlot.missingness.rate
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build.plotter

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build.plotter

Generating plotters

### Description

Generating QC\_Plotter objects, which can be used in many of the QoRT utilities to organize samples in various ways to allow for easy comparison and detection of consistent biases and artifacts.

### Usage

### **Arguments**

```
res A QORT_QC_Results object, created by read.qc.results.data.

curr.sample A character string. For the sample highlight summary plots, this should be the sample.ID of the sample that is to be highlighted.
```

build.plotter 3

merge.offset.outgroup

(For advanced users) A logical value. For the sample highlight plots, determines whether the all lanes that do not include the current sample should be treated as a single "outgroup".

plotter.params

(For advanced users) A named list. Allows you to specify colors, offsets, and other similar patterns. By default these will all be set to reasonable values, however, if you want more control over colors, line-transparency, point plotting characters, or similar, then you can specify a named list.

Any parameters that are not specified in the plotter.params list will be left as default.

### Legal parameters are:

- "contrasting.colors": colors to use for contrast. By default these are set to a series of reasonably-contrasting colors. However, if you have too many different categories then it may be hard to tell some colors apart.
- "contrasting.pch": point types to use for contrast (see pch in graphical parameters). By default this is set to the basic point types, then following through the upper and lower case letters.
- "std.lines.lty": Line type to use for the "highlighted" bams. (see lty in graphical parameters)
- "std.lines.lwd": Line width to use for the "highlighted" bams. (see lwd in graphical parameters)
- "std.lines.alpha": Alpha transparency value to use on lines for the "highlighted" bams. Numeric value between 0 and 255.
- "std.lines.color": Color to use for the "highlighted" bams.
- "std.points.pch": Character to use for points for the "highlighted" bams. (see pch in graphical parameters)
- "std.points.alpha": Alpha transparency value to use on points for the "highlighted" bams. Numeric value between 0 and 255.
- "std.points.color": Color to use for the "highlighted" bams.
- "std.NVC.colors": A named list with elements named "A", "T", "C", and "G", with each element specifying a color. The colors used to indicate each base for the "highlighted" bams in the nucleotide-rate-by-position plots.
- "alt.lines.lty": Line type to use for the "non-highlighted" bams. (see lty in graphical parameters)
- "alt.lines.lwd": Line width to use for the "non-highlighted" bams. (see lwd in graphical parameters)
- "alt.lines.alpha": Alpha transparency value to use on lines for the "non-highlighted" bams. Numeric value between 0 and 255.
- "alt.lines.color": Color to use for the "non-highlighted" bams.
- "alt.points.pch": Character to use for points for the "non-highlighted" bams. (see pch in graphical parameters)
- "alt.points.alpha": Alpha transparency value to use on points for the "non-highlighted" bams. Numeric value between 0 and 255.
- "alt.points.color": Color to use for the "non-highlighted" bams.
- "alt.NVC.colors": A named list with elements named "A", "T", "C", and "G", with each element specifying a color. The colors used to indicate each base for the "non-highlighted" bams in the nucleotide-rate-by-position plots.

4 get.summary.table

```
• "show.legend": DEPRECIATED. Currently nonfunctional.

color.by.name

(For advanced users) A character string. (TODO: document functionality)

color.by.title.name

(For advanced users) A character string. (TODO: document functionality)
```

#### Value

A QoRT\_Plotter reference object used to create QC summary plots. Depending on which plotter is used, samples/lane-bams can be organized by group, sample, lane, or any arbitrary variable found in the decoder.

#### See Also

```
read.qc.results.data
```

```
get.summary.table Get summary data table
```

### **Description**

Retrieves and compiles a summary data table.

### Usage

```
get.summary.table(res, outfile, debugMode)
```

### **Arguments**

res A QoRT\_QC\_Results object, created by read.qc.results.data.

outfile Optional. A file name where the table should be written.

debugMode Logical. If TRUE, debugging data will be printed to the console.

### **Details**

Returns summary data in table form.

### See Also

```
read.qc.results.data
```

makePlot.all.std 5

```
makePlot.all.std Generating all default plots
```

### **Description**

Saves MANY compiled QC plots for the given dataset.

### Usage

#### **Arguments**

res A QoRT\_QC\_Results object, created by read.qc.results.data.

outfile.dir A file prefix, used for all output files. Usually the directory to which you want all files to be written.

plotter.params

Additional parameters (advanced) used in creation of the Plotter objects. See build.plotter.

plot.device.name

The method to use to save plots. Can be one of:

- "png" for standard png compression,
- "CairoPNG" for png compression using package Cairo. Note that this requires the package Cairo.

plotting.device.params

A named list of parameters to be passed to the graphics device. For example:

• "width = 2000"

Reasonable values for height, width, and pointsize will be chosen by default.

debugMode

Logical. If TRUE, debugging data will be printed to the console.

rasterize.large.plots

Logical. If TRUE, then if the currently selected plotting device is a vector device (or the "curr" device), then certain large plots will have their plotting areas rasterized. The axis labels, titles, and text will all remain vectorized, only the plotting areas will be flattened. *Note that this requires the png package*.

By default, this parameter will be set to TRUE when a vector device is selected.

raster.height

Numeric. If rasterize plotting area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels. Double-pane plots will be twice this width.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

Saves MANY compiled QC plots for the given dataset, calling all standard variants of makePlot.summary.

#### See Also

```
read.qc.results.data, build.plotter, makePlot.summary
```

```
makePlot.chrom.type.rates

Chromosome type rate plot
```

#### **Description**

Plots the number or percent of read-pairs falling on each type of chromosome.

### Usage

### **Arguments**

```
plotter A QORT_Plotter reference object. See build.plotter.

plot.rates A logical value indicating whether to plot percent of the total (for each bam file), rather than read-counts.

chromosome.name.style
```

A string value indicating the style of the chromosome names, and also how to split up the categories. There are 4 legal options:

- "UCSC": The default. Chromosomes are named: "chr1,chr2,...,chrX,chrY,chrXY,chrM". There are 6 categories: autosome, X, Y, XY, mitochondrial, and other.
- "ENSEMBL": Chromosomes are named: "1,2,...,X,Y,XY,MT". There are 6 categories: autosome, X, Y, XY, mitochondrial, and other.
- "UCSC\_WITH\_ERCC": As UCSC, but there is an additional category, which contains all chromosomes that begin with the text "ERCC".
- "ENSEMBL\_WITH\_ERCC": As ENSEMBL, but there is an additional category, which contains all chromosomes that begin with the text "ERCC".

A logical value indicating whether to exclude autosomes from the plot.

```
custom.chromosome.style.def.function
```

(For advanced users) If your chromosomes do not match any of the above styles, then you can set your own chromosome style by handing this option a function. The function must take one argument. When handed NULL, it must return a list of all legal categories. When handed a single chromosome name, it must return one of those categories.

return.table A Logical value. If TRUE, the function will return a table containing the plotted

data.

debugMode Logical. If TRUE, debugging data will be printed to the console.

.. Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

For each sample-run, this function plots the number of read-pairs mapping to each category of chromosome.

#### Value

By default, this function returns nothing. If the return.table parameter is TRUE, then it returns a data.frame with the data that was plotted.

#### See Also

```
build.plotter
```

```
makePlot.cigarLength.distribution
```

Plot the length distribution of a given cigar operation

### **Description**

Plots the length distribution of a given cigar operation

#### Usage

#### **Arguments**

 $\hbox{{\tt plotter}} \qquad \qquad A \, \hbox{{\tt QoRT\_Plotter}} \, \, \hbox{{\tt reference object.}} \, \, \hbox{{\tt See build.plotter.}}$ 

op A character string naming which cigar op to plot. Must be one of the following:

- "SoftClip" Soft Clip (Cigar Op "S")
- "HardClip" Hard Clip (Cigar Op "H")
- "Del" Deletion from reference (Cigar Op "D")

```
• "Ins" Insertion from reference (Cigar Op "I")
```

- "Pad" Padding (Cigar Op "P")
- "Splice" Splice Junction (Cigar Op "N")
- "Aln" Aligned to reference (Cigar Op "M")

Note that makePlot.cigarOp.byCycle(plotter,"SoftClip") gives identical results as makePlot.clipping, although the default value for the rate.per.million argument is different.

r2.buffer Buffer space to place between the plotting of read 1 and read 2. By default this

will choose a reasonable value.

perMillion A logical value indicating whether or not to scale the y axis to rate-per-million-

reads, rather than rate-per-read. Some people may find the results more readable

this way, even though the plots themselves will appear the same.

log.x A logical value indicating whether or not to log-scale the x axis.

log.y A logical value indicating whether or not to log-scale the y axis.

debugMode Logical. If TRUE, debugging data will be printed to the console.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

x-axis: Length of the cigar operation.

y-axis: Frequency of the given length.

### See Also

```
build.plotter
```

### **Description**

Plots the rate at which the given CIGAR operator occurs, by read cycle.

makePlot.clipping 9

#### **Arguments**

 $\hbox{{\tt plotter}} \qquad \quad A \, \hbox{{\tt QoRT\_Plotter}} \, \, \hbox{{\tt reference object.}} \, \, \hbox{{\tt See build.plotter.}}$ 

op A character string naming which cigar op to plot. Must be one of the following:

- "SoftClip" Soft Clip (Cigar Op "S")
- "HardClip" Hard Clip (Cigar Op "H")
- "Del" Deletion from reference (Cigar Op "D")
- "Ins" Insertion from reference (Cigar Op "I")
- "Pad" Padding (Cigar Op "P")
- "Splice" Splice Junction (Cigar Op "N")
- "Aln" Aligned to reference (Cigar Op "M")

Note that makePlot.cigarOp.byCycle(plotter, "SoftClip") gives identical results as makePlot.clipping, although the default value for the rate.per.million argument is different.

r2.buffer

Buffer space to place between the plotting of read 1 and read 2. By default this will choose a reasonable value.

rate.per.million

A logical value indicating whether or not to scale the y axis to rate-per-million-reads, rather than rate-per-read. Some people may find the results more readable this way, even though the plots themselves will appear the same.

•

debugMode Logical. If TRUE, debugging data will be printed to the console.

... Arguments to be passed to methods, such as graphical parameters (see par).

### **Details**

x-axis: The read cycle (ie. the base-pair position in the read).

y-axis: The rate at which the bases at the given read-cycle is clipped off.

### See Also

```
build.plotter
```

### **Description**

Plots the rate at which the aligner soft-clips off portions of aligned reads.

#### **Arguments**

plotter A QoRT\_Plotter reference object. See build.plotter.

rate.per.million

A logical value indicating whether or not to scale the y axis to rate-per-millionreads, rather than rate-per-read. Some people may find the results more readable
this way, even though the plots themselves will appear the same.

r2.buffer Buffer space to place between the plotting of read 1 and read 2. By default this
will choose a reasonable value.

debugMode Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

x-axis: The read cycle (ie. the base-pair position in the read). y-axis: The rate at which the bases at the given read-cycle is clipped off.

### See Also

```
build.plotter
```

```
makePlot.dropped.rates

*Read Drop Plot*
```

### **Description**

Plots the rates at which reads are dropped from analysis for various causes.

### Usage

```
makePlot.dropped.rates(plotter, debugMode, ...)
```

### **Arguments**

```
plotter A QORT_Plotter reference object. See build.plotter.

debugMode Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).
```

#### **Details**

For each bam file, this function plots the rates and reasons for reads being dropped from QC analysis.

Note that in the example dataset reads were never dropped. This is a consequence of the preprocessing steps in the example pipeline.

### See Also

```
build.plotter
```

makePlot.gc 11

makePlot.gc I

Plot GC content

### **Description**

Plots GC content.

### Usage

### **Arguments**

plot.medians A logical value indicating whether or not to plot the median average GC content for each bam file at the bottom of the plot. Overrides plot.means.

plot.means A logical value indicating whether or not to plot the mean average GC content for each bam file at the bottom of the plot.

debugMode Logical. If TRUE, debugging data will be printed to the console.

paired Logical. If FALSE, GC content rates will be calculated for all reads individually. If TRUE, GC content rates will be calculated for all read-pairs. Note that when the insert size is small, the paired plot can sometimes appear jagged, as completely-overlapped read-pairs will always have an even number of G/C nucleotides.

Arguments to be passed to methods, such as graphical parameters (see par).

### **Details**

. . .

x-axis: Percent of the read-pairs that is made up of G's or C's. y-axis: Rate at which the given percent occurs.

### See Also

```
build.plotter
```

### **Description**

Plots the rate at which reads are assigned into various categories.

```
makePlot.gene.assignment.rates(plotter, debugMode, ...)
```

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#### **Arguments**

```
plotter A QoRT_Plotter reference object. See build.plotter.

debugMode Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).
```

#### **Details**

For each bam-file, this function plots the rate (y-axis) for which the bam-file's read-pairs are assigned to the given categories.

The categories are:

- *Unique Gene*: The read-pair overlaps with the exonic segments of one and only one gene. For many downstream analyses tools, such as DESeq, DESeq2, and EdgeR, only read-pairs in this category are used.
- Ambig Gene: The read-pair overlaps with the exons of more than one gene.
- No Gene: The read-pair does not overlap with the exons of any annotated gene.
- *No Gene, Intronic*: The read-pair does not overlap with the exons of any annotated gene, but appears in a region that is bridged by an annotated splice junction.
- *No Gene, 1kb from gene*: The read-pair does not overlap with the exons of any annotated gene, but is within 1 kilobase from the nearest annotated gene.
- *No Gene*, *10kb from gene*: The read-pair does not overlap with the exons of any annotated gene, but is within 10 kilobases from the nearest annotated gene.
- *No Gene, middle of nowhere*: The read-pair does not overlap with the exons of any annotated gene, and is *more* than 10 kilobases from the nearest annotated gene.

#### See Also

```
build.plotter
```

```
makePlot.gene.cdf Plot the cumulative gene diversity curve
```

### **Description**

Plots the cumulative gene diversity curve

### **Arguments**

plotter A QoRT\_Plotter reference object. See build.plotter.

sampleWise A logical value indicating whether to compile each sample together across all

runs.

plot.intercepts

A logical value indicating whether or not to plot the intercepts with the round

numbers on the x-axis, in dotted lines.

label.intercepts

A logical value indicating whether or not to label the intercepts. No effect if

plot.intercepts is FALSE.

debugMode Logical. If TRUE, debugging data will be printed to the console.

rasterize.plotting.area

Logical. If TRUE, the plotting area will be written to a temp png file then drawn to the current device as a raster image. This option is generally preferred for vector devices, because NVC plots can be very large when drawn in vector

format. Note: this requires the png package!

raster.height

Numeric. If rasterize plotting area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

For each bam-file, this function plots the cumulative gene diversity. For each bam-file, the genes are sorted by read-count. Then, a cumulative function is calculated for the percent of the total proportion of reads as a function of the number of genes. Intercepts are plotted as well, showing the cumulative percent for 1 gene, 10 genes, 100 genes, 1000 genes, and 10000 genes.

So, for example, across all the bam-files, around 50 to 55 percent of the read-pairs were found to map to the top 1000 genes. Around 20 percent of the reads were found in the top 100 genes. And so on.

This can be used as an indicator of whether a large proportion of the reads stem from of a small number of genes.

Note that this is restricted to only the reads that map to a single unique gene. Reads that map to more than one gene, or that map to intronic or intergenic areas are ignored.

#### See Also

build.plotter

makePlot.genebody.coverage

Plot Gene-Body coverage distribution

### **Description**

Plots Gene-Body coverage distribution

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#### **Usage**

### **Arguments**

```
plotter A QORT_Plotter reference object. See build.plotter.

plot.medians A logical value indicating whether or not to plot the median average for each bamfile at the bottom of the plot. Overrides plot.means.

plot.means A logical value indicating whether or not to plot the mean average for each bamfile at the bottom of the plot.

debugMode Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).
```

#### Details

x-axis: Gene-body quantile. By default this is broken up into 40 quantiles containing 2.5

y-axis: Rate at which reads falls into the given quantile of the genes' bodies.

makePlot.genebody.coverage plots the gene body coverage across all genes. makePlot.genebody.coverage plots the gene body coverage across the genes that fall in the upper-middle quartile of total expression for each sample-run (excluding genes with zero reads). makePlot.genebody.coverage.lowExpress plots the gene body coverage across the genes that fall in the lower two quartiles of total expression for each sample-run (excluding genes with zero reads).

### See Also

```
build.plotter
```

```
makePlot.insert.size
```

Plot Insert Size Distribution.

#### **Description**

Plots the distribution of the size of the region covered by the two paired reads.

makePlot.legend.box 15

### **Arguments**

plotter	A QoRT_Plotter reference object. See build.plotter.
calc.rate	A logical value indicating whether or not to calculate and plot the rate at which each insert size occurs as the y-axis. If this is set to false, it will instead plot the total number of times each insert size occurs.
pct.cutoff	A numeric value between 0 and 1, indicating the quantile within which to limit the x-axis. Generally the default value of 0.98 is perfectly usable.
plot.means	A logical value indicating whether or not to plot the mean average for each bamfile at the bottom of the plot.
plot.medians	A logical value indicating whether or not to plot the median average for each bamfile at the bottom of the plot. Overrides $plot.means$ .
debugMode	Logical. If TRUE, debugging data will be printed to the console.
	Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

x-axis: The insert size. This is the genomic distance from the start of one read to the end of the other. In other words, the size of the full region covered by the paired reads.

y-axis: The rate at which the given insert size occurs, for each bamfile.

Note: The insert size is calculated by using the alignment as well as the provided gene/splice-junction annotation.

- If the paired reads align to overlapping regions of the reference genome, then the insert size
  can be calculated exactly. This is NOT dependant on the annotation. If the two reads align
  inconsistantly (for example, one read showing a splice junction and the other not), then the
  read is ignored.
- 2. If the paired reads do NOT overlap, then the annotation information is required. Using the full set of all known splice junctions that lie between the inner alignment endpoints for the two reads, the shortest possible path from the two endpoints is found. In some rare cases this may underestimate the insert size, if the actual insert does not take the minimal path, but this is rare. In other (somewhat more common) cases this may overestimate the insert size, when the region between the paired reads bridges an unannotated splice junction.

#### See Also

```
build.plotter
```

```
makePlot.legend.box
```

Plot legend

#### **Description**

Plots the universal legend for a given plotter object.

```
makePlot.legend.box(plotter, debugMode, ...)
makePlot.legend.over(position, plotter, debugMode, ...)
```

#### **Arguments**

plotter	A QoRT_Plotter reference object. See build.plotter.
position	For makePlot.legend.over, a character string indicating the location where you want the legend to appear. This is passed to legend, and can be any keyword allowed by xy.coords. For example: "top", "topleft", "bottomright", etc.
debugMode	Logical. If TRUE, debugging data will be printed to the console.
	Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

makePlot.legend.box creates a new plot (opening the next graphics frame), and writes a small description of the given plotter type along with plotting a color-coded legend (if applicable). makePlot.legend.over adds a legend to the current plotting frame.

#### See Also

```
build.plotter
```

```
makePlot.mapping.rates

Plot mapping rates
```

### **Description**

Plots the rates at which reads map to the genome.

### Usage

### **Arguments**

### Details

For each sample-run, this function plots the number of mapped reads and the rate at which reads map (if the total reads is provided).

### See Also

```
build.plotter read.qc.results.data
```

```
{\it makePlot.missingness.rate} \\ {\it Plot N-Rate by read cycle}
```

### **Description**

Plots rate by which the sequencer cannot determine the base, by read cycle.

### Usage

### **Arguments**

```
plotter A QoRT_Plotter reference object. See build.plotter.

r2.buffer Buffer space to place between the plotting of read 1 and read 2. By default this will choose a reasonable value.

debugMode Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).
```

#### **Details**

```
x-axis: Read Cycle
y-axis: Rate at which the sequencer assigns an 'N' base.
```

### See Also

```
build.plotter
```

```
makePlot.norm.factors

Plot Alignment Clipping
```

### Description

Plots the rate at which the aligner soft-clips off portions of aligned reads.

### **Arguments**

plotter	A QoRT_Plotter reference object. See build.plotter.
by.sample	Logical. Whether to combine all lanebams for each sample before calculating normalization factors. By default, normalization factors for each lanebam will be calculated seperately.
return.table	Logical. Whether to return a data table containing the data that is plotted.
debugMode	Logical. If TRUE, debugging data will be printed to the console.
	Arguments to be passed to methods, such as graphical parameters (see par).

### **Details**

makePlot.norm.factors plots the normalization factors for each sample/lanebam. Note that unless DESeq2 and edgeR are installed, it will only plot total-count normalization by default. Also note that unless calc.DESeq2 = TRUE and/or calc.edgeR = TRUE in the execution of the read.qc.results.data that produced the QC results, only the total counts normalization factors will be calculated.

makePlot.norm.factors.vs.TC plots the ratio of alternative normalization factors against the total count normalization.

#### Value

Usually returns nothing, unless return.table is TRUE, in which case it returns a data.frame containing the plotted data for each sample.

#### See Also

```
build.plotter
```

```
makePlot.NVC.clip.matchByClipPosition

Plot clipped nucleotide rates, aligned by the distance from the
```

Plot clipped nucleotide rates, aligned by the distance from the point of clipping.

### **Description**

WARNING: THESE FUNCTIONS ARE BETA, AND ARE NOT FULLY TESTED OR READY FOR GENERAL USE.

### **Arguments**

rasterize.plotting.area

Logical. If TRUE, the plotting area will be written to a temp png file then drawn to the current device as a raster image. This option is generally preferred for vector devices, because NVC plots can be very large when drawn in vector format. *Note: this requires the* png *package!* 

raster.height

Numeric. If rasterize plotting area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels.

load.results UNDOCUMENTED debugMode UNDOCUMENTED

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

This function is currently BETA, and is not intended for general use. Documentation and testing is still pending.

### See Also

```
build.plotter, makePlot.NVC
```

```
makePlot.NVC.lead.clip
```

Clipped sequence nucleotide-by-position plot

### Description

Plots the nucleotide rates for clipped segments

### Usage

```
makePlot.NVC.lead.clip(plotter, clip.amt, r2.buffer,
                   points.highlighted = TRUE,
                   label.majority.bases = TRUE,
                   label.majority.bases.threshold = 0.5,
                   label.majority.bases.cex = 1,
                   rasterize.plotting.area = FALSE,
                   raster.height = 1000,
                   raster.width = 1000,
                   show.base.legend = TRUE,
                   debugMode,
                    ...)
makePlot.NVC.tail.clip(plotter, clip.amt, r2.buffer,
                       points.highlighted = TRUE,
                       label.majority.bases = TRUE,
                        label.majority.bases.threshold = 0.5,
                        label.majority.bases.cex = 1,
                        rasterize.plotting.area = FALSE,
                        raster.height = 1000,
                        raster.width = 1000,
                        show.base.legend = TRUE,
                        debugMode, ...)
```

### **Arguments**

plotter A QORT\_Plotter reference object. See build.plotter.

Clip.amt Numeric value. The number of bases clipped. These methods only plot the sequence for a single specific clip.amt.

r2.buffer Buffer space to place between the plotting of read 1 and read 2. By default this will choose a reasonable value.

points.highlighted

A logical value. Determines whether to ever plot points on top of the lines. This can be useful for identifying outliers. If TRUE, then all highlighted lane-bams will be overlaid with points using their designated pch symbol. If the plotter does not highlight any lanebams, then points will be overlaid on ALL lines.

label.majority.bases

A logical value indicating whether to label the genotypes of read cycles in which the most common base has a frequency exceeding label.majority.bases.threshold (see below).

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```
label.majority.bases.threshold
```

A numeric value indicating the cutoff above which the most common base will be labelled, if label.majority.bases is set TRUE (see above).

```
label.majority.bases.cex
```

The cex value fed to text() that is used to determine how big the labels are to be, if label.majority.bases is TRUE. (see par for information on cex).

```
rasterize.plotting.area
```

Logical. If TRUE, the plotting area will be written to a temp png file then drawn to the current device as a raster image. This option is generally preferred for vector devices, because NVC plots can be very large when drawn in vector format. *Note: this requires the png package!* 

raster.height

Numeric. If rasterize plotting area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels.

show.base.legend

A logical value indicating whether to print the base-color legend along the right edge of the plot.

debugMode

Logical. If TRUE, debugging data will be printed to the console.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

For general information on reading NVC plots, see makePlot.NVC.

This function plots the nucleotide rates for the sections of reads that were soft-clipped by the aligner. Note that these will not function on reads that have been aligned using an aligner that does not practice soft clipping (such as TopHat2).

### See Also

```
build.plotter, makePlot.NVC
```

```
makePlot.qual.pair Plot quality score by read cycle
```

### **Description**

Plots the Phred quality score as a function of the read cycle for both reads.

22 makePlot.raw.NVC

#### **Arguments**

A QORT\_Plotter reference object. See build.plotter.

y.name
The name of the quality score metric to plot. Must be one of:

 "min"
 "lowerQuartile"
 "median"
 "upperQuartile"
 "max"

r2.buffer
Buffer space to place between the plotting of read 1 and read 2. By default this will choose a reasonable value.

debugMode
Logical. If TRUE, debugging data will be printed to the console.

Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

These plots display information about the phred quality score (y-axis) as a function of the position in the read (x-axis). Five statistics can be plotted: minimum, maximum, upper and lower quartiles, and median. These statistics are calculated individually for each bam file and each read position (ie, each plotted line corresponds to a bam file).

Note that the Phred score is always an integer, and as such these plots would normally be very difficult to read because lines would be plotted directly on top of one another. To reduce this problem, the lines are vertically offset from one another. Most plotters offset each line by lane.ID.

### See Also

```
build.plotter
```

```
makePlot.raw.NVC Nucleotide-rate by read cycle plot
```

#### **Description**

Plots the nucleotide rate by read cycle

makePlot.raw.NVC 23

### **Arguments**

plotter A QoRT\_Plotter reference object. See build.plotter.

Buffer space to place between the plotting of read 1 and read 2. By default this will choose a reasonable value.

points.highlighted

A logical value. Determines whether to ever plot points on top of the lines. This can be useful for identifying outliers. If TRUE, then all highlighted lane-bams will be overlaid with points using their designated pch symbol. If the plotter does not highlight any lanebams, then points will be overlaid on ALL lines.

label.majority.bases

A logical value indicating whether to label the genotypes of read cycles in which the most common base has a frequency exceeding label.majority.bases.threshold (see below).

label.majority.bases.threshold

A numeric value indicating the cutoff above which the most common base will be labelled, if label.majority.bases is set TRUE (see above).

label.majority.bases.cex

The cex value fed to text() that is used to determine how big the labels are to be, if label.majority.bases is TRUE. (see par for information on cex).

rasterize.plotting.area

Logical. If TRUE, the plotting area will be written to a temp png file then drawn to the current device as a raster image. This option is generally preferred for vector devices, because NVC plots can be very large when drawn in vector format. *Note: this requires the* png *package!* 

raster.height

Numeric. If rasterize.plotting.area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels.

show.base.legend

A logical value indicating whether to print the base-color legend along the right edge of the plot.

debugMode Logical. If TRUE, debugging data will be printed to the console.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

This plot displays the nucleotide rates by read cycle. The color scheme for NVC plots is different from all other plots. Rather than being used for emphasis or to allow cross-comparisons by sample, biological-condition, or lane, the colors are used to indicate the four nucleotides: A (green), T (red), G (orange), or C (blue). Depending on the type of QoRT\_Plotter being used, sample-runs will be marked and differentiated by marking the lines with shapes (R points). In many cases the points will be unreadable due to overplotting, but clear outliers that stray from the general trends can be readily identified.

When used with a "sample.highlight" type QoRT\_Plotter (see build.plotter), "highlighted" samples will be drawn with a deeper shade of the given color.

makePlot.raw.NVC plots the nucleotides of all cycles of all aligned reads. makePlot.minus.clipping.NVC plots the nucleotides for all cycles that are NOT soft-clipped by the aligner. Note that for reads aligned with an aligner that does not perform soft-clipping (such as TopHat2), the two plots will be identical.

#### See Also

```
build.plotter
```

### **Description**

Plots the rates at which splice junctions occur.

#### **Arguments**

#### **Details**

These functions plot various metrics for the rate at which splice junction "events" occur. A splice junction "event" is an occurrance of a mapped read-pair bridging a splice junction. Some read-pairs may contain multiple splice junction events, and some read-pairs may contain none.

Splice junctions are characterized into six categories:

- Known: The splice junction locus is found in the supplied transcript annotation gtf file.
- Novel: The splice junction locus is NOT found in the supplied transcript annotation gtf file.
- Known, 1-3 reads: The locus is known, and is only covered by 1-3 read-pairs.
- Known, 4+ reads: The locus is known, and is covered by 4 or more read-pairs.
- Novel, 1-3 reads: The locus is novel, and is only covered by 1-3 read-pairs.
- Novel, 4+ reads: The locus is novel, and is covered by 4 or more read-pairs.

makePlot.splice.junction.event.counts plots the number (y-axis) of all splice junction events falling into each of six categories. Note that because different samples/runs may have different total read counts and/or library sizes, this function is generally not the best for comparing between samples. For most purposes, makePlot.splice.junction.event.ratesPerRead will be preferable.

makePlot.splice.junction.event.ratesPerRead plots the rate (y-axis) at which each type of splice junction events appear, per read-pair.

makePlot.splice.junction.event.proportions plots the proportion (y-axis) of all splice junction events falling into the six categories.

makePlot.splice.junction.event.proportionsByType plots the proportion (y-axis) at which splice junction events appear on known vs novel splice junction loci, the proportion of known splice junction events that occur on low-coverage junctions (1-3 read-pairs) vs high-coverage junctions (4 or more read-pairs), and the proportion of novel splice junction events that occur on low vs high coverage junctions.

All of these plots are generally used to detect whether sample-specific or batch effects have a substantial or biased effect on splice junction appearance, either due to differences in the original RNA, or due to artifacts that alter the rate at which the aligner maps across splice junctions.

### See Also

```
build.plotter, makePlot.splice.junction.loci.counts
```

26 makePlot.strandedness.test

### Description

Plots the rate at which splice junction loci fall into various categories.

### Usage

### **Arguments**

plotter A QORT\_Plotter reference object. See build.plotter.

calc.rate Logical. If TRUE, the proportion of loci in each category will be calculated and plotted, rather than the raw number.

high.low.cutoff

(For advanced users only!) The cutoff between "high" and "low" expression junction loci. Note that this must match the cutoff used by the jarfile QC execution.

debugMode Logical. If TRUE, debugging data will be printed to the console.

... Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

This function plots the number (y-axis) of splice junction *loci* of each type that appear in the bamfile's reads. Splice junctions are split into 4 groups, first by whether the splice junction appears in the transcript annotation gtf ("known" vs "novel"), and then by whether the splice junction has 4 or more reads covering it, or 1-3 reads ("Hi" vs "Lo").

#### See Also

```
build.plotter, makePlot.splice.junction.event
```

### Description

Plots the apparent strandedness of the reads.

### Usage

### **Arguments**

#### **Details**

TODO!

#### See Also

```
build.plotter
```

makePlot.summary Generating summary plots

### Description

Creates a large multi-frame summary plot, or a report PDF file.

```
makePlot.summary.basic(res, outfile,
                    outfile.dir = "./",
                    outfile.prefix = "plot.summary.basic",
                    outfile.ext,
                    plotter.params = list(),
                    plot.device.name = "curr",
                    plotting.device.params = list(),
                    verbose = TRUE,
                    debugMode,
                    rasterize.large.plots,
                    raster.height = 1000,
                    raster.width = 1000,
                    separatePlots = FALSE,
makePlot.summary.colorByGroup(res, outfile,
                    outfile.dir = "./",
                    outfile.prefix = "plot.summary.colorByGroup",
                    outfile.ext,
```

```
plotter.params = list(),
                    plot.device.name = "curr",
                    plotting.device.params = list(),
                    verbose = TRUE,
                    debugMode,
                    rasterize.large.plots,
                    raster.height = 1000,
                    raster.width = 1000,
                    separatePlots = FALSE,
                    ...)
makePlot.summary.colorByLane(res, outfile,
                    outfile.dir = "./",
                    outfile.prefix = "plot.summary.colorByLane",
                    outfile.ext,
                    plotter.params = list(),
                    plot.device.name = "curr",
                    plotting.device.params = list(),
                    verbose = TRUE,
                    debugMode,
                    rasterize.large.plots,
                    raster.height = 1000,
                    raster.width = 1000,
                    separatePlots = FALSE,
makePlot.summary.sample.highlight(res,
                    curr.sample,
                    outfile,
                    outfile.dir = "./",
                    outfile.prefix,
                    outfile.ext,
                    plotter.params = list(),
                    plot.device.name = "curr",
                    plotting.device.params = list(),
                    verbose = TRUE,
                    debugMode,
                    rasterize.large.plots,
                    raster.height = 1000,
                    raster.width = 1000,
                    separatePlots = FALSE,
                    ...)
makePlot.summary.sample.highlight.andColorByLane(res,
                    curr.sample,
                    outfile,
                    outfile.dir = "./",
                    outfile.prefix,
                    outfile.ext,
                    plotter.params = list(),
                    plot.device.name = "curr",
                    plotting.device.params = list(),
```

```
verbose = TRUE,
debugMode,
rasterize.large.plots,
raster.height = 1000,
raster.width = 1000,
separatePlots = FALSE,
...)
```

#### **Arguments**

res A QoRT\_QC\_Results object, created by read.qc.results.data.

curr.sample A character string. For the sample highlight summary plots, this should be the sample.ID of the sample that is to be highlighted.

outfile The output file can be specified in two ways: first, by setting the outfile parameter, which should be the full path for the output file. Alternatively, the file path will be the concatenation of the three parameters: outfile.dir, outfile.prefix, and outfile.ext.

If plot.device.name == "curr", then all the outfile parameters will be nonfunctional.

outfile.dir A prefix to be appended to the output filename. Usually the path to the destination directory. By default, this will be the current working directory.

outfile.prefix

A second prefix to be appended to the output filename, after outfile.dir. This is usually the name of the file, without the file extension. By default, a reasonable file name will be selected.

outfile.ext The file extension. By default, this will be set to match the selected graphics device.

plotter.params

Additional parameters (advanced) used in creation of the Plotter objects. See build.plotter.

plot.device.name

The method to use to save plots. Several formats and devices are supported:

- "curr" (default) Do not create output files. Plot directly to the current or default device.
- "png" for standard png compression.
- "CairoPNG" for png compression using package Cairo. Note that this requires the package Cairo.
- "tiff" for the tiff device.
- "'jpeq" for the jpeg device (not recommended!)
- "svg" for the vector svg device. Note that for all vector devices, by default, certain large plots will be rasterized if the png package is found. If not, the function will still work, but output files may be very large and may have trouble rendering and printing.
- "pdf" for a multi-page pdf report.
- "CairoPDF" for a multi-page pdf report using package Cairo. Note that this requires the package Cairo.

plotting.device.params

A named list of parameters to be passed to the graphics device. For example:

```
• width = 2000
```

- height = 2000
- units = "px"

Reasonable values for height, width, and pointsize will be chosen by default. Generally all raster plots will be set to 1000 by 1000 pixels, and all vector plots will be set to 7 inches by 7 inches.

verbose

Logical. If TRUE, more information will be printed to the console.

debugMode

Logical. If TRUE, debugging data will be printed to the console.

rasterize.large.plots

Logical. If TRUE, then if the currently selected plotting device is a vector device (or the "curr" device), then certain large plots will have their plotting areas rasterized. The axis labels, titles, and text will all remain vectorized, only the plotting areas will be flattened. Note that this requires the png package.

By default, this parameter will be set to TRUE when a vector device is selected.

raster.height

Numeric. If rasterize plotting area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize plotting area is TRUE, then this is the width of the plotting area raster image, in pixels. Double-pane plots will be twice this width.

separatePlots

Logical. If TRUE, then separate image files will be saved for each individual plot, rather than saving one large multi-pane plot. Note that this is not compatible with the "curr" device. Also note: if this parameter is set to TRUE, then the output files will be saved using the outfile.dir, outfile.prefix and outfile.ext parameters. The "outfile" parameter cannot be set. The files will be saved to the contatenation of outfile.dir, outfile.prefix, the name of the plot type, and then outfile.ext.

Arguments to be passed to methods, such as graphical parameters (see par).

### **Details**

Produces large, multi-frame summary plots, with a large number of different plots and graphs.

For devices pdf and CairoPDF, this function will produce a multi-page document suitable for printing, with 6 panels on each page.

Note that for all vector devices (svg, pdf, and CairoPDF), by default, QoRTs will attempt to load the png package. If this package is found, then certain large plots (the NVC plots and the cumulative diversity plots) will be rasterized within the plotting area. The labels and text will still be vectorized. If the png package is not installed, the function will still work, but output files may be very large and may have trouble rendering and printing.

Rasteration of large plots can be turned off via the rasterize.large.plots option.

### See Also

build.plotter

### Description

Generates multiple sample highlight summary plots, one for every sample.

### Usage

```
makePlot.summary.sample.highlight.all(res,
                   outfile.dir = "./",
                   plotter.params = list(),
                   plot.device.name = "png",
                   plotting.device.params = list(),
                   verbose = TRUE, debugMode,
                    rasterize.large.plots,
                    raster.height = 1000,
                    raster.width = 1000,
                     ...)
makePlot.summary.sample.highlight.andColorByLane.all(res,
                   outfile.dir = "./",
                   plotter.params = list(),
                   plot.device.name = "png",
                   plotting.device.params = list(),
                   verbose = TRUE, debugMode,
                     rasterize.large.plots,
                     raster.height = 1000,
                    raster.width = 1000,
                    ...)
```

#### **Arguments**

res A QoRT\_QC\_Results object, created by read.qc.results.data.

outfile.dir A file prefix, used for all output files. Usually the directory to which you want all files to be written.

plotter.params

Additional parameters (advanced) used in creation of the Plotter objects. See build.plotter.

plot.device.name

The method to use to save plots. Can be one of:

- "png" for standard png compression,
- "CairoPNG" for png compression using package Cairo. Note that this requires the package Cairo.

plotting.device.params

A named list of parameters to be passed to the graphics device. For example:

• "width = 2000"

Reasonable values for height, width, and pointsize will be chosen by default.

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```
verbose Logical. If TRUE, more info will be printed to the console.

debugMode Logical. If TRUE, debugging data will be printed to the console.

rasterize.large.plots
```

Logical. If TRUE, then if the currently selected plotting device is a vector device (or the "curr" device), then certain large plots will have their plotting areas rasterized. The axis labels, titles, and text will all remain vectorized, only the plotting areas will be flattened. *Note that this requires the png package*.

By default, this parameter will be set to TRUE when a vector device is selected.

raster.height

Numeric. If rasterize.plotting.area is TRUE, then this is the height of the plotting area raster image, in pixels.

raster.width Numeric. If rasterize.plotting.area is TRUE, then this is the width of the plotting area raster image, in pixels. Double-pane plots will be twice this width.

.. Arguments to be passed to methods, such as graphical parameters (see par).

#### **Details**

Generates a sample highlight plot for each sample in the dataset.

#### See Also

```
build.plotter, makePlot.summary
```

```
read.qc.results.data
```

Reading QC results data

### **Description**

Creates a QoRT\_QC\_Results object using a set of QC result data files.

#### Usage

### **Arguments**

```
infile.dir REQUIRED. The base file directory where all the QC results data is stored. decoder.files
```

Either a character string or a character vector, containing the decoder.

Either decoder.files OR decoder.data.frame must be set. Not both.

decoder.data.frame

A data frame containing the decoder information.

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calc.DESeq2 Logical. If TRUE, this function will attempt to load the DESeq2 package. If the DESeq2 package is found, it will then calculate DESeq2's geometric normalization factors (also known as "size factors") for each replicate and for each sample.
 calc.edgeR Logical. If TRUE, this function will attempt to load the edgeR package. If

Logical. If TRUE, this function will attempt to load the edgeR package. If the edgeR package is found, it will then calculate all of edgeR's normalization factors (also known as "size factors") for each replicate and for each sample.

debugMode Logical. If TRUE, debugging data will be printed to the console.

#### **Details**

Single Decoder: The "decoder" is a file or data frame that describes each replicate. It must have one row per replicate, with the following columns:

- lanebam.ID: The base identifier for the individual replicate. Must be unique!
- lane.ID: The identifier for the lane, run, or batch.
- group.ID: The identifier for the biological condition for the given replicate.
- sample.ID: The identifier for the specific biological replicate from which the replicate belongs. Note that this is distinct from "lanebam.ID" because in many RNA-Seq studies each "sample" can have multiple technical replicates, as multiple sequencing runs may be needed to acquire sufficient reads for analysis.
- qc.data.dir: (OPTIONAL) This column indicates the subdirectory in which the replicate's QC data was written. If this column is missing, it is assumed to be equal to the lanebam.ID.
- input.read.pair.count: (OPTIONAL) This column contains the number of input reads, before alignment. This is used later to calculate mapping rate.
- multi.mapped.read.pair.count: (OPTIONAL) This column contains the number of reads that were multi-mapped. This must be included for multi-mapping rate to be calculated.
- cycle.CT: (OPTIONAL) This column contains the length of the reads for the replicate, in base-pairs. By default this is calculated automatically, so this column never needs to be set.

Dual Decoder: Alternatively, two decoders can be set. In this case the first decoder should be the lanebam decoder, and the second should be the sample decoder. The lanebam decoder should have one row per replicate, with the following columns:

- lanebam.ID: The base identifier for the individual replicate. Must be unique!
- lane.ID: The identifier for the lane, run, or batch.
- sample.ID: The identifier for the specific biological replicate from which the replicate belongs.
  Note that this is distinct from "lanebam.ID" because in many RNA-Seq studies each "sample"
  can have multiple technical replicates, as multiple sequencing runs may be needed to acquire
  sufficient reads for analysis.
- qc.data.dir: (OPTIONAL) This column indicates the subdirectory in which the replicate's QC data was written. If this column is missing, it is assumed to be equal to the lanebam.ID. Must be unique!
- input.read.pair.count: (OPTIONAL) This column contains the number of input reads, before alignment. This is used later to calculate mapping rate.
- multi.mapped.read.pair.count: (OPTIONAL) This column contains the number of reads that were multi-mapped. This must be included for multi-mapping rate to be calculated.
- cycle.CT: (OPTIONAL) This column contains the length of the reads for the replicate, in base-pairs. By default this is calculated automatically, so this column never needs to be set.

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The sample decoder should have one row per replicate, with the following columns:

- group.ID: The identifier for the biological condition for the given replicate.
- sample.ID: The identifier for the specific biological replicate from which the replicate belongs.
  Note that this is distinct from "lanebam.ID" because in many RNA-Seq studies each "sample"
  can have multiple technical replicates, as multiple sequencing runs may be needed to acquire
  sufficient reads for analysis. Must be unique!

All decoders are allowed to contain other columns in addition to the ones listed here, so long as their names are distinct. Columns do not need to appear in any particular order, so long as they are named according to the specifications above.

### See Also

build.plotter

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