# Package 'DiffBind'

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Type Package

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Title Differential Binding Analysis of ChIP-Seq peak data

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**Description** Compute differentially bound sites from multiple ChIP-seq experiments using affinity (quantitative) data. Also enables occupancy (overlap) analysis and plotting functions.

biocViews Bioinformatics, HighThroughputSequencing, ChIPseq

License Artistic-2.0

LazyLoad yes

**Depends** R (>= 2.14.0), GenomicRanges

**Imports** 

RColorBrewer, amap, edgeR (>= 2.3.58), gplots, DESeq,grDevices, stats, utils, IRanges, zlibbioc

Suggests DESeq, Rsamtools

Enhances rgl, parallel

LinkingTo Rsamtools

Collate core.R parallel.R counts.R contrast.R analyze.R io.R helper.R utils.R overLapper.R DBA.R

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# Description

Differential binding analysis of ChIP-seq peaksets

# **Details**

Computes differentially bound sites from multiple ChIP-seq experiments using affinity (quantitative) data. Also enables occupancy (overlap) analysis and plotting functions.

**Entry Points:** 

dba: Construct a dba object
dba.peakset: Add a peakset to, or retrieve a peakset from, a dba object
dba.overlap: Compute binding site overlaps and/or correlations
dba.count: Count reads in binding sites

dba.contrast: Establish contrast(s) for analysis
dba.analyze: Execute affinity analysis
dba.report: Generate report for a contrast analysis

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dba.plotHeatmap: Heatmap plot

dba.plotPCA: Principal Components plot

dba.plotBox: Boxplots
dba.plotMA: MA/scatter plot
dba.plotVenn: Venn diagram plot

dba.show: Show dba metadata dba.mask: Mask samples or sites

dba.save: Save dba object dba.load: Load dba object

## Author(s)

dba Construct a DBA object

# **Description**

Constructs a new DBA object from a sample sheet, or based on an existing DBA object

# Usage

```
dba(DBA,mask, minOverlap=2,
    sampleSheet="dba_samples.csv",
    config=data.frame(RunParallel=TRUE,reportInit="DBA",DataType=DBA_DATA_GRANGES,AnalysisMethod=DBA_peakCaller='raw', peakFormat, scoreCol, bLowerScoreBetter, filter, skipLines=0,
    bAddCallerConsensus=FALSE, bRemoveM=TRUE, bRemoveRandom=TRUE, bSummarizedExperiment=FALSE,
    bCorPlot=FALSE, attributes)
```

# **Arguments**

DBA	existing DBA object – if present, will return a fully-constructed DBA object based on the passed one, using criteria specified in the mask and/or minOverlap parameters. If missing, will create a new DBA object based on the sampleSheet.
mask	logical or numerical vector indicating which peaksets to include in the resulting model if basing DBA object on an existing one. See dba.mask.
minOverlap	only include peaks in at least this many peaksets in the main binding matrix if basing DBA object on an existing one. If minOverlap is between zero and one, peak will be included from at least this proportion of peaksets.
sampleSheet	data frame containing sample sheet, or file name of sample sheet to load (ignored if DBA is specified). Columns names in sample sheet may include:

• SampleID: Identifier string for sample

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- Tissue: Identifier string for tissue type
- Factor: Identifier string for factor
- Condition: Identifier string for condition
- Treatment: Identifier string for treatment
- Replicate: Replicate number of sample
- bamReads: file path for bam file containing aligned reads for ChIP sample
- bamControl: file path for bam file containing aligned reads for control sample
- ControlID: Identifier string for control sample
- Peaks: path for file containing peaks for sample. format determined by PeakCaller field or caller parameter
- PeakCaller: Identifier string for peak caller used. If Peaks is not a bed file, this will determine how the Peaks file is parsed. If missing, will use default peak caller specified in caller parameter. Possible values:
  - "raw": text file file; peak score is in fourth column
  - "bed": .bed file; peak score is in fifth column
  - "narrow": default peak.format: narrowPeaks file
  - "macs": MACS .xls file
  - "swembl": SWEMBL .peaks file
  - "bayes": bayesPeak file
  - "peakset": peakset written out using pv.writepeakset
  - "fp4": FindPeaks v4
- PeakFormat: string indicating format for peak files; see PeakCaller and dba.peakset
- ScoreCol: column in peak files that contains peak scores
- LowerBetter: logical indicating that lower scores signify better peaks
- Counts: file path for externally computed read counts; see dba.peakset (counts parameter)

config

data frame containing configuration options, or file name of config file to load when constructing a new DBA object from a sample sheet. NULL indicates no config file. Relevant fields include:

- RunParallel: logical indicating if counting and analysis operations should be run in parallel using multicore by default.
- DataType: default class for peaks and reports (DBA\_DATA\_GRANGES, DBA\_DATA\_RANGEDDATA, or DBA\_DATA\_FRAME).
- AnalysisMethod: either DBA\_EDGER or DBA\_DESEQ.

peakCaller

if a sampleSheet is specified, the default peak caller that will be used if the fPeakCaller column is absent.

peakFormat

if a sampleSheet is specified, the default peak file format that will be used if the fPeakFormat column is absent.

scoreCol

if a sampleSheet is specified, the default column in the peak files that will be used for scoring if the ScoreCol column is absent.

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bLowerScoreBetter

if a sampleSheet is specified, the sort order for peak scores if the LowerBetter

column is absent.

filter if a sampleSheet is specified, a filter value if the Filter column is absent.

Peaks with scores lower than this value (or higher if bLowerScoreBetter or

LowerBetter is TRUE) will be removed.

if a sampleSheet is specified, the number of lines (ie header lines) at the beginskipLines

ning of each peak file to skip.

bAddCallerConsensus

add a consensus peakset for each sample with more than one peakset (i.e. different peak callers) when constructing a new DBA object from a sample sheet.

**bRemoveM** logical indicating whether to remove peaks on chrM (mitochondria) when con-

structing a new DBA object from a sample sheet.

bRemoveRandom logical indicating whether to remove peaks on chrN\_random when constructing

a new DBA object from a sample sheet.

bSummarizedExperiment

logical indicating whether to return resulting object as a SummarizedExperiment.

bCorPlot attributes logical indicating that a correlation heatmap should be plotted before returning vector of attributes to use subsequently as defaults when generating labels in

plotting functions:

- DBA\_ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA CONDITION
- DBA REPLICATE
- DBA CONSENSUS
- DBA\_CALLER
- DBA\_CONTROL

# **Details**

MODE: Construct a new DBA object from a samplesheet:

dba(sampleSheet, config, bAddCallerConsensus, bRemoveM, bRemoveRandom, attributes)

MODE: Construct a DBA object based on an existing one:

dba(DBA, mask, attributes)

MODE: Convert a DBA object to a SummarizedExperiment object:

dba(DBA, bSummarizedExperiment=TRUE)

# Value

DBA object

#### Author(s)

Rory Stark and Gordon Brown

DBA object methods

# See Also

```
dba.peakset, dba.show
```

## **Examples**

```
# Create DBA object from a samplesheet
setwd(system.file("extra", package="DiffBind"))
tamoxifen = dba(sampleSheet="tamoxifen.csv")
tamoxifen
tamoxifen = dba(sampleSheet="tamoxifen_allfields.csv")
tamoxifen
tamoxifen = dba(sampleSheet="tamoxifen_allfields.csv",config="config.csv")
tamoxifen
#Create a DBA object with a subset of samples
data(tamoxifen_peaks)
Responsive = dba(tamoxifen,tamoxifen$masks$Responsive)
Responsive
# change peak caller but leave peak format the same
setwd(system.file("extra", package="DiffBind"))
tamoxifen = dba(sampleSheet="tamoxifen.csv", peakCaller="macs", peakFormat="raw")
\label{local_dba_show} $$ \down{1.5cm} $$ dba.show(tamoxifen, attributes=c(DBA\_TISSUE,DBA\_CONDITION,DBA\_REPLICATE,DBA\_CALLER))$$ $$ \down{1.5cm} $$ down{1.5cm} $$ attributes=c(DBA\_TISSUE,DBA\_CONDITION,DBA\_REPLICATE,DBA\_CALLER))$$ $$ \down{1.5cm} $$ attributes=c(DBA\_TISSUE,DBA\_CONDITION,DBA\_REPLICATE,DBA\_CALLER)$$ $$ \down{1.5cm} $$ \down{
# Convert DBA object to SummarizedExperiment
data(tamoxifen_counts)
sset = dba(tamoxifen,bSummarizedExperiment=TRUE)
sset
```

DBA object methods

Standard S3 methods for DBA object

# **Description**

Standard S3 methods for DBA object.

# Usage

```
## S3 method for class 'DBA'
print(x, ...)
## S3 method for class 'DBA'
summary(object, ...)
## S3 method for class 'DBA'
plot(x, ...)
```

# **Arguments**

x DBA objectobject DBA object

... Arguments passed on to parent methods

#### **Details**

S3 methods for DBA object

## Author(s)

Rory Stark

## **Examples**

```
data(tamoxifen_peaks)
tamoxifen
data(tamoxifen_counts)
tamoxifen
```

DBA tamoxifen resistance dataset

Tamoxifen resistance dataset used for DBA examples

# **Description**

Tamoxifen resistance dataset used for DBA examples

# Usage

```
data(tamoxifen_peaks)
data(tamoxifen_counts)
data(tamoxifen_analysis)
```

# **Arguments**

tamoxifen\_peaks

load tamoxifen resistance dataset DBA object with peak (occupancy) data

tamoxifen\_counts

load tamoxifen resistance dataset DBA object with count (affinity) data

tamoxifen\_analysis

load tamoxifen resistance dataset DBA object with count (affinity) data and edgeR-based differential binding analysis results

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## **Details**

The tamoxifen resistance dataset is used for the DBA vignette and man page examples.

#### Value

loads a DBA object named tamoxifen

## Author(s)

Rory Stark

## **Examples**

```
data(tamoxifen_peaks)
tamoxifen
data(tamoxifen_counts)
plot(tamoxifen)
data(tamoxifen_analysis)
dba.plotMA(tamoxifen)
```

dba.analyze

Perform differential binding affinity analysis

# Description

Performs differential binding affinity analysis

## Usage

## **Arguments**

DBA

DBA object. If no contrasts are specified (DBA\$contrast is NULL), default contrasts will be added via a call to dba.contrast.

method

method, or vector of methods, by which to analyze differential binding affinity. Supported methods:

- DBA\_EDGER
- DBA\_DESEQ
- DBA\_EDGER\_CLASSIC
- DBA\_DESEQ\_CLASSIC
- DBA\_EDGER\_GLM
- DBA\_DESEQ\_GLM

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bSubControl logical indicating whether Control read counts are subtracted for each site in each sample before performing analysis.

bFullLibrarySize

logical indicating if the full library size (total number of reads in BAM/SAM/BED file) for each sample is used for scaling normalization. If FALSE, the total number of reads present in the peaks for each sample is used (generally preferable).

bTagwise logical indicating if dispersion should be calculated on a tagwise (or per-condition)

basis. If there are only a very few members of each group in a contrast (e.g. no

replicates), this should be set to FALSE.

bCorPlot logical indicating whether to plot a correlation heatmap for the analyzed data

(first contrast only). If no sites are significantly differentially bound using the

default threholds, no heatmap will be plotted.

bReduceObjects logical indicating whether strip the analysis objects of unnecessary fields to

save memory. If it is desired to used the DBA\$contrasts[[n]]\$edgeR and/or DBA\$contrasts[[n]]\$DESeq objects directly in the edgeR and/or DESeq pack-

ages, this should be set to FALSE.

bParallel logical indicating that the analyses is to be done in parallel using multicore

(one process for each contrast for each method, plus an additional process per

method).

#### **Details**

See the DBA User Guide for more details on how the edgeR and DESeq analyses are carried out.

## Value

DBA object with results of analysis added to DBA\$contrasts.

#### Note

If there is a blocking factor for the contrast(s) specified using a previous call to dba.contrast, a multi-factor analysis will automatically be carried out in addition to a single factor analysis.

# Author(s)

Rory Stark

# See Also

```
dba.contrast, dba.report
```

```
data(tamoxifen_counts)

tamoxifen = dba.analyze(tamoxifen)

tamoxifen

tamoxifen = dba.analyze(tamoxifen,method=c(DBA_EDGER,DBA_DESEQ))
tamoxifen
```

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dba.contrast	Set up contrasts for differential binding affinity analysis

# Description

Sets up contrasts for differential binding affinity analysis

# Usage

```
dba.contrast(DBA, group1, group2=!group1, name1="group1", name2="group2",
            minMembers=3, block ,
             categories = c(DBA_TISSUE,DBA_FACTOR,DBA_CONDITION,DBA_TREATMENT))
```

# Arg

guments		
DBA	DBA object with count data	
group1	mask of samples in first group (when adding a specific contrast). See dba.mask.	
group2	mask of samples in second group (when adding a specific contrast). See dba.mask.	
name1	label for samples in first group (when adding a specific contrast).	
name2	label for samples in second group (when adding a specific contrast).	
minMembers	when automatically generating contrasts, minimum number of unique samples in a group. Must be at least 2, as replicates are strongly advised. If you wish to do an analysis with no replicates, you can set the group1 and group2 parameters explicitly.	
categories	when automatically generating contrasts, attribute or vector of attributes to base contrasts on:	
	<ul> <li>DBA_ID</li> <li>DBA_TISSUE</li> <li>DBA_FACTOR</li> <li>DBA_CONDITION</li> <li>DBA_TREATMENT</li> <li>DBA_REPLICATE</li> <li>DBA_CALLER</li> </ul>	
block	blocking attribute for multi-factor analysis. This may be specified as either a	

value, a vector, or a list.

If block is a value, the specified metadata field is used to derive the blocking factor. One of:

- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT

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- DBA\_REPLICATE
- DBA CALLER

If block is a vector, it can either be a mask (logical vector) or a vector of peakset numbers. In this case, the peaksets indicated in the blocking vector are all given the same value (true), while any peaksets not included in the vector take the alternative value (false).

If block is a list, it should be a list of vectors (either logical masks or vectors of peakset numbers), with each indicating a set of peaksets that should share the same value. Each peakset should appear at most once, and any peaksets not specified will be given an default value (other).

## **Details**

MODE: Set up all possible contrasts:

dba.contrast(DBA, minMembers, categories)

MODE: Set up a specific contrast:

dba.contrast(DBA, group1, group2, name1, name2, block)

#### Value

DBA object with contrast(s) set as DBA\$contrasts. Contrast list can be retrieved using dba.show(DBA, bContrasts=T).

## Note

Contrasts will only be set up for peaksets where DBA\_CALLER == "counts".

Contrasts can be cleared by DBA\$contrasts=NULL.

## Author(s)

Rory Stark

#### See Also

dba.analyze

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```
tamoxifen$contrasts=NULL
tamoxifen = dba.contrast(tamoxifen)
tamoxifen

# Specify a blocking factor
tamoxifen$contrasts=NULL
tamoxifen = dba.contrast(tamoxifen, categories=DBA_CONDITION, block=DBA_TISSUE)
tamoxifen

tamoxifen$contrasts=NULL
tamoxifen = dba.contrast(tamoxifen, categories=DBA_CONDITION, block=list(c(3,4,5,8,9),c(1,2,10,11)))
tamoxifen

tamoxifen$contrasts=NULL
tamoxifen = dba.contrast(tamoxifen, categories=DBA_CONDITION, block=tamoxifen$masks$MCF7)
tamoxifen = dba.analyze(tamoxifen)
tamoxifen
```

dba.count

Count reads in binding site intervals

# Description

Counts reads in binding site intervals. Files must be one of bam, bed and gzip-compressed bed. File suffixes must be ".bam", ".bed", or ".bed.gz" respectively.

## Usage

#### **Arguments**

DBA peaks	DBA object If GRanges, RangedData, dataframe, or matrix, this parameter contains the intervals to use for counting. If character string, it specifies a file containing the intervals to use (with the first three columns specifying chromosome, startpos, endpos). If missing or a mask, generates a consensus peakset using minOverlap parameter (after applying the mask if present). If NULL, changes the score used
	in the global binding matrix to the score type specified in the score parameter without re-counting.
minOverlap	only include peaks in at least this many peaksets when generating consensus peakset (i.e. when peaks parameter is missing). If minOverlap is between zero and one, peak will be included from at least this proportion of peaksets.
score	which score to use in the binding affinity matrix. Note that all raw read counts are maintained for use by dba.analyze, regardless of how this is set. One of:

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DBA\_SCORE\_READS raw read count for interval using only reads from ChIP

DBA\_SCORE\_READS\_FOLD raw read count for interval from ChIP divided by read count for interval from DBA SCORE READS MINUS raw read count for interval from ChIP minus read count for interval from cont

DBA\_SCORE\_RPKM RPKM for interval using only reads from ChIP

DBA\_SCORE\_RPKM\_FOLD RPKM for interval from ChIP divided by RPKM for interval from control DBA\_SCORE\_TMM\_READS\_FULL TMM normalized (using edgeR), using ChIP read counts and Full Library siz TMM normalized (using edgeR), using ChIP read counts and Effective Librar

DBA\_SCORE\_TMM\_READS\_EFFECTIVE

DBA SCORE TMM MINUS FULL DBA SCORE TMM MINUS EFFECTIVE

TMM normalized (using edgeR), using ChIP read counts minus Control read

TMM normalized (using edgeR), using ChIP read counts minus Control read

bLog logical indicating whether log2 of score should be used (only applies to DBA\_SCORE\_RPKM\_FOLD

and DBA\_SCORE\_READS\_FOLD).

insertLength if present, this value will be used as the length of the reads. Each read will be

extended from its endpoint along the appropriate strand by this many bases. If

missing, the read size indicated in the BAM/BED file will be used.

filter value to use for filtering intervals with low read counts. Only intervals where

> at least one sample has a score at least this high will be included. If peaks is NULL, will remove sites from existing DBA object without recounting. If filter is a vector of values, dba.count will return a vector of the same length, indicating how many intervals will be retained for each specified filter level.

bRemoveDuplicates

logical indicating if duplicate reads (ones that map to exactly the same genomic position) should be removed. If TRUE, any location where multiple reads map will be counted as a single read. Note that if bLowMem is set, duplicates needs to

have been already marked in all of the BAM files.

bScaleControl logical indicating if the Control reads should be scaled based on relative library

> sizes. If TRUE, and there are more reads in the Control library than in the ChIP library, the number of Control reads for each peak will be multiplied by a scaling factor determined by dividing the total number of reads in the ChIP library by the total number of reads in the Control library. If this value is not an integer,

the number of Control reads for each peak will be the next highest integer.

bCalledMasks logical indicating whether to compute site masks for each peakset indicating

which sites were originally identified as peaks (used by dba.report).

function that will be invoked for each interval with a vector of scores for each filterFun

> sample. Returns a score that will be evaluated against the filter value (only intervals with a score at least as high as filter will be kept). Default is max, indicating that at least one sample should have a score of at least filter; other useful values include sum (indicating that all the scores added together should be at least filter) and mean (setting a minimum mean normalized count level).

Users can supply their own function as well.

bCorPlot logical indicating whether to plot a correlation heatmap for the counted data

bLowMem logical indicating that the low-memory options should be used for counting (us-

ing summarizeOverlaps). This option is slower but memory use does not increase with the number of reads to count. If TRUE, all read files must be BAM (.bam extension), with associated index files (.bam.bai extension). insertLength 14 dba.count

must absent. Two additional parameters for summarizeOverlaps may be specified in DBA\$config:

DBA\$config\$yieldSize DBA\$config\$intersectMode DBA\$config\$singleEnd yieldSize indicating how many reads to process at one time; default is 5000000. The lower this mode indicating which overlap algorithm to use; default is "IntersectionNotEmpty"

logical indicating if reads are single end; default is TRUE

DBA\$config\$scanbamparam

ScanBamParam object to pass to summarizeOverlaps. If present, bRemoveDuplicates is ignored

bParallel if TRUE, use multicore to get counts for each read file in parallel

#### Value

DBA object with binding affinity matrix based on read count scores.

# Plot effect of a range of filter values and then apply filter

#### Author(s)

Rory Stark and Gordon Brown

head(tamoxifen\$vectors)

#### See Also

dba.analyze

```
# These won't run unless you have the reads available in a BAM or BED file
data(tamoxifen_peaks)
## Not run: tamoxifen = dba.count(tamoxifen)
# Count using a peakset made up of only peaks in all responsive MCF7 replicates
data(tamoxifen_peaks)
mcf7Common = dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
## Not run: tamoxifen = dba.count(tamoxifen,peaks=mcf7Common$inAll)
tamoxifen
#First make consensus peaksets from each set of replicates, then derive master consensus set for counting from those
data(tamoxifen_peaks)
tamoxifen = dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)
## Not run: tamoxifen = dba.count(tamoxifen, peaks=tamoxifen$masks$Consensus)
tamoxifen
# Change binding affinity scores
data(tamoxifen_counts)
tamoxifen = dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_READS)
head(tamoxifen$vectors)
tamoxifen = dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_RPKM_FOLD)
head(tamoxifen$vectors)
tamoxifen = dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_TMM_MINUS_FULL)
```

dba.load

```
data(tamoxifen_counts)
rate.max = dba.count(tamoxifen, peaks=NULL, filter=0:250)
rate.sum = dba.count(tamoxifen, peaks=NULL, filter=0:250,filterFun=sum)
plot(0:250,rate.max/rate.max[1],type='1',xlab="Filter Value",ylab="Proportion Retained Sites")
lines(0:250,rate.sum/rate.sum[1],col=2)
tamoxifen = dba.count(tamoxifen,peaks=NULL,filter=125,filterFun=sum)
tamoxifen
```

dba.load

load DBA object

# **Description**

Reads in saved DBA object

# Usage

```
dba.load(file='DBA', dir='.', pre='dba_', ext='RData')
```

# Arguments

file main filename

dir directory in which to save model pre string to pre-pend to filename

ext file extension to use

# Value

loaded DBA object

## Author(s)

Rory Stark

# See Also

dba.save

```
data(tamoxifen_peaks)
dba.save(tamoxifen,'tamoxifenPeaks')
tamoxifen = dba.load('tamoxifenPeaks')
```

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dba.mask

Derive a mask to define a subset of peaksets or sites for a DBA object

# **Description**

Derives a mask to define a subset of peaksets or sites for a DBA object.

## Usage

## **Arguments**

DBA

DBA object

attribute

when deriving a peakset mask, attribute to base mask on:

- DBA ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_KEFLICATE
- DBA\_CONSENSUS
- DBA\_CALLER
- DBA CONTROL

value

when deriving a peakset/sample mask, attribute value (or vector of attribute values) to match.

combine

when deriving a peakset/sample mask, if value is a vector, OR when deriving a site mask, and peaksets is a vector, this is method for combining result of each value:

- "or"
- "and"
- "nor"
- "nand"

mask

when deriving a peakset/sample mask, this specifies an existing mask to merge with; if missing, create new mask

merge

when deriving a peakset/sample mask, and an existing mask is supplied, this speficies the method for combining new mask with supplied mask:

- "or"
- · "and"
- "nor"
- "nand" note: if mask is missing, "nand" results in negative of mask

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bApply when deriving a peakset/sample mask, a logical indicating that a new DBA ob-

ject with the mask applied will be returned.

peakset when deriving a peak/site mask, this specifies a peakset number, or a vector of

peakset numbers. The resulting mask will indicate which of the overall sites were called as peaks in this peakset or set of peaksets. If a vector, the masks for each of the peaksets will be combined using the method specified in the combine

parameter.

minValue when deriving a peak/site mask, scores greater than this value will be considered

as indicating that the site corresponds to a called peakset.

## **Details**

MODE: Derive a a mask of peaksets/samples:

dba.mask(DBA, attribute, value, combine, mask, merge, bApply)

MODE: Derive a mask of peaks/sites:

dba.mask(DBA, combine, mask, merge,bApply, peakset, minValue)

## Value

either a logical mask, or new DBA object if bApply is TRUE.

#### Note

dba automatically generates masks for each unique value of DBA\_TISSUE, DBA\_FACTOR, DBA\_CONDITION, DBA\_TREATMENT, DBA\_CALLER, and DBA\_REPLICATE. These are accessible using masks field of the DBA object (DBA\$masks), and can be viewed using names(DBA\$masks).

## Author(s)

Rory Stark

#### See Also

dba.show

```
data(tamoxifen_peaks)

# Pre-made masks
names(tamoxifen$masks)
dba.show(tamoxifen,tamoxifen$masks$MCF7)

# New masks
mcf7Mask = dba.mask(tamoxifen,DBA_TISSUE, "MCF7")
mcf7DerivedMask = dba.mask(tamoxifen,DBA_TISSUE,"TAMR",mask=mcf7Mask)
mcf7Derived = dba(tamoxifen,mcf7DerivedMask)
mcf7Derived
```

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dba.overlap

Compute binding site overlaps (occupancy analysis)

# **Description**

Computes binding overlaps and co-occupancy statistics

# Usage

```
dba.overlap(DBA, mask, mode=DBA_OLAP_PEAKS, minVal=0,
```

contrast, method=DBA\$config\$AnalysisMethod, th=.1, bUsePval=FALSE, report, byAttribute, bCorOnly=TRUE, CorMethod="pearson", DataType=DBA\$config\$DataType)

#### **Arguments**

DBA DBA object

mask mask or vector of peakset numbers indicating a subset of peaksets to use (see

dba.mask). When generating overlapping/unique peaksets, either two, three, or four peaksets may be specified. If the mode type is DBA OLAP ALL, and a contrast is specified, a value of TRUE (mask=TRUE) indicates that all samples should be included (otherwise only those present in one of the contrast groups

will be included).

indicates which results should be returned (see MODES below). One of: mode

DBA\_OLAP\_PEAKS

• DBA\_OLAP\_ALL

DBA\_OLAP\_RATE

minVal minimum score value to be considered a "called" peak.

> contrast number to use. Only specified if contrast data is to be used when mode=DBA\_OLAP\_ALL. See dba.show(DBA, bContrast=T) to get contrast

numbers.

if contrast is specified and mode=DBA\_OLAP\_ALL, use data from method used

for analysis:

DBA\_EDGER

DBA\_DESEQ

DBA EDGER BLOCK

DBA\_DESEQ\_BLOCK

if contrast is specified and mode=DBA\_OLAP\_ALL, significance threshold; all

sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included. A value of 1 will include all binding sites, but only the samples

included in the contrast.

if contrast is specified and mode=DBA\_OLAP\_ALL, logical indicating whether

to use FDR (FALSE) or p-value (TRUE) for thresholding.

contrast

method

th

bUsePval

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report

if contrast is specified and mode=DBA\_OLAP\_ALL, a report (obtained from dba.report) specifying the data to be used. If counts are included in the report (and a contrast is specified), the count data from the report will be used to compute correlations, rather than the scores in the global binding affinity matrix. If report is present, the method, th, and bUsePval parameters are ignored.

byAttribute

when computing co-occupancy statistics (DBA\_OLAP\_ALL), limit comparisons to peaksets with the same value for a specific attribute, one of:

- DBA ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_CONSENSUS
- DBA\_CALLER
- DBA\_CONTROL

bCorOnly

when computing co-occupancy statistics (DBA\_OLAP\_ALL), logical indicating that only correlations, and not overlaps, should be computed. This is much faster if only correlations are desired (e.g. to plot the correlations using dba.plotHeatmap).

CorMethod

DataType

when computing co-occupancy statistics (DBA\_OLAP\_ALL), method to use when computing correlations.

if mode==DBA\_OLAP\_PEAKS, the class of object that peaksets should be returned as:

- DBA\_DATA\_GRANGES
- DBA\_DATA\_RANGEDDATA
- DBA\_DATA\_FRAME

Can be set as default behavior by setting DBA\$config\$DataType.

# Details

MODE: Generate overlapping/unique peaksets:

dba.overlap(DBA, mask, mode=DBA\_OLAP\_PEAKS, minVal)

MODE: Compute correlation and co-occupancy statistics (e.g. for dba.plotHeatmap):

dba.overlap(DBA, mask, mode=DBA\_OLAP\_ALL, byAttribute, minVal, attributes, bCorOnly, CorMethod)

MODE: Compute correlation and co-occupancy statistics using significantly differentially bound sites (e.g. for dba.plotHeatmap):

dba.overlap(DBA, mask, mode=DBA\_OLAP\_ALL, byAttribute, minVal, contrast, method, th=, bUsePval, attributes, bCorOnly, CorMethod)

Note that the scores from the global binding affinity matrix will be used for correlations unless a report containing count data is specified.

MODE: Compute overlap rates at different stringency thresholds:

dba.overlap(DBA, mask, mode=DBA\_OLAP\_RATE, minVal)

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## Value

Value depends on the mode specified in the mode parameter.

If mode = DBA\_OLAP\_PEAKS, Value is an overlap record: a list of three peaksets for an A-B overlap, seven peaksets for a A-B-C overlap, and fifteen peaksets for a A-B-C-D overlap:

inAll	peaks in all peaksets
onlyA	peaks unique to peakset A
onlyB	peaks unique to peakset B
onlyC	peaks unique to peakset C
onlyD	peaks unique to peakset D
notA	peaks in all peaksets except peakset A
notB	peaks in all peaksets except peakset B
notC	peaks in all peaksets except peakset C
notD	peaks in all peaksets except peakset D
AandB	peaks in peaksets A and B but not in peaksets C or D
AandC	peaks in peaksets A and C but not in peaksets B or D
AandD	peaks in peaksets A and D but not in peaksets B or C
BandC	peaks in peaksets B and C but not in peaksets A or D
BandD	peaks in peaksets B and D but not in peaksets A or C

If mode = DBA\_OLAP\_ALL, Value is a correlation record: a matrix with a row for each pair of peaksets and the following columns:

peaks in peaksets C and D but not in peaksets A or B

A peakset number of first peakset in overlap

B peakset number of second peakset in overlap

onlyA number of sites unique to peakset A onlyB number of sites unique to peakset B

inAll number of peaks in both peakset A and B (merged)

R2 correlation value A vs B

Overlap percentage overlap (number of overlapping sites divided by number of peaks

unique to smaller peakset

If mode = DBA\_OLAP\_RATE, Value is a vector whose length is the number of peaksets, containing the number of overlapping peaks at the corresponding minOverlaps threshold (i.e., Value[1] is the total number of unique sites, Value[2] is the number of unique sites appearing in at least two peaksets, Value[3] the number of sites overlapping in at least three peaksets, etc.).

## Author(s)

CandD

Rory Stark

# See Also

```
dba.plotVenn, dba.plotHeatmap
```

# **Examples**

dba.peakset

Add a peakset to, or retrieve a peakset from, a DBA object

## Description

Adds a peakset to, or retrieves a peakset from, a DBA object

# Usage

# Arguments

DBA

DBA object. Required unless creating a new DBA object by adding an initial peakset.

peaks

When adding a specified peakset: set of peaks, either a GRanges or RangedData object, or a peak dataframe or matrix (chr,start,end,score), or a filename where the peaks are stored.

When adding a consensus peakset: a sample mask or vector of peakset numbers to include in the consensus. If missing or NULL, a consensus is derived from all peaksets present in the model. See dba.mask, or dba.show to get peakset numbers.

When adding a set of consensus peaksets: a sample mask or vector of peakset numbers. Sample sets will be derived only from subsets of these peaksets.

When adding all the peaks from one DBA object to another: a DBA object. In this case, the only other parameter to have an effect is minOverlap.

When retrieving and/or writing a peakset: either a GRanges or RangedData object, or a peak dataframe or matrix (chr,start,end,score), or a peakset number; if NULL, retrieves/writes the full binding matrix.

sampID

ID string for the peakset being added; if missing, one is assigned (a serial number for a new peakset, or a concatenation of IDs for a consensus peakset).

tissue

tissue name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of tissues).

factor

factor name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of factors).

condition

condition name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of conditions).

treatment

treatment name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of treatment).

replicate

replicate number for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of replicate numbers).

control

control name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of control names).

peak.caller

peak caller name string. If peaks is specified as a file, and peak.format is missing, a default fie format for the caller will be used (see peak.format). Supported values:

- "raw": default peak.format: raw text file
- "bed": default peak.format: bed file
- "narrow": default peak.format: narrowPeaks file
- "macs": default peak.format: MACS .xls file
- "bayes": default peak.format: bayesPeak file
- "tpic": default peak.format: TPIC file
- "sicer": default peak.format: SICER file
- "fp4": default peak.format: FindPeaks v4 file
- "swembl": default peak.format: SWEMBL file
- "csv": default peak.format: comma separated value file
- "report": default peak.format: csv file saved via dba.report

When adding a consensus peakset, a default value (a concatenation of peak caller names) is assigned if this is missing.

peak.format

peak format string. If specified, overrides the default file format for the specified peak caller. Supported formats (with default score column):

- "raw": raw text file file; scoreCol=4
- "bed": bed file; scoreCol=5
- "narrow": narrowPeaks file; scoreCol=8
- "macs": MACS .xls file; scoreCol=7
- "bayes": bayesPeak file; scoreCol=4, filter=0.5
- "tpic": TPIC file; scoreCol=0 (all scores=1)
- "sicer": SICER file; scoreCol=7
- "fp4": FindPeaks v4 file; scoreCol=5
- "swembl": SWEMBL file; scoreCol=4
- "csv": csv file; scoreCol=4
- "report": report file; scoreCol=9, bLowerScoreBetter=T

reads

total number of ChIPed library reads for the peakset being added.

consensus

either the logical value of the consensus attribute when adding a specific peakset (set to TRUE for consensus peaksets generated by dba.peakset), or a metadata attribute or vector of attributes when generating a set of consensus peaksets. In the latter case, a consensus peakset will be added for each set of samples that have the same values for the specified attributes. Alternatively, attributes may be specified proceeded by a negative sign, in which case a consensus peakset will be added for each set of samples that differ only in their values for those attributes. See examples. Allowable attributes:

- DBA\_TISSUE; -DBA\_TISSUE
- DBA\_FACTOR; -DBA\_FACTOR
- DBA\_CONDITION; -DBA\_CONDITION
- DBA\_TREATMENT; -DBA\_TREATMENT
- DBA\_REPLICATE; -DBA\_REPLICATE
- DBA\_CALLER; -DBA\_CALLER

bamReads

file path of the BAM/BED file containing the aligned reads for the peakset being

bamControl

file path of the BAM/BED file containing the aligned reads for the control used for the peakset being added.

scoreCol

peak column to normalize to 0...1 scale when adding a peakset; 0 indicates no normalization

bLowerScoreBetter

Logical indicating that lower scores indicate higher confidence peaks; default is that higher scores indicate better peaks.

filter

Numeric indicating a filter value for peaks. If present, any peaks with a score less than this value (or higher if bLowerScoreBetter==TRUE) will be removed from the peakset.

counts

Used for adding externally computed peak counts. Can be a filename or a dataframe. Can consist of a single column (or vector) with the counts, or two columns, with an ID for each interval int he first column and the counts int he

second column, or four columns (chr, start, end, counts). When counts is specified, peaks and related parameters are ignored, and all peaksets in the DBA object must be specified in this way, all with exactly the same number of inter-

vals.

bRemoveM logical indicating whether to remove peaks on chrM when adding a peakset

bRemoveRandom logical indicating whether to remove peaks on chrN\_random when adding a

peakset

minOverlap the minimum number of peaksets a peak must be in to be included when adding

a consensus peakset. When retrieving, if the peaks parameter is a vector (logical mask or vector of peakset numbers), a binding matrix will be retrieved including all peaks in at least this many peaksets. If minOverlap is between zero and one,

peak will be included from at least this proportion of peaksets.

bMerge logical indicating whether global binding matrix should be compiled after adding

the peakset. When adding several peaksets via successive calls to dba.peakset, it may be more efficient to set this parameter to FALSE and call dba(DBA) after

all the peaksets have been added.

bRetrieve logical indicating that a peakset is being retrieved and/or written, not added.

writeFile file to write retrieved peakset.

numCols number of columns to include when writing out peakset. First four columns are

chr, start, end, score; the remainder are maintained from the original peakset.

Ignored when writing out complete binding matrix.

DataType The class of object for returned peaksets:

• DBA\_DATA\_GRANGES

• DBA\_DATA\_RANGEDDATA

DBA\_DATA\_FRAME

Can be set as default behavior by setting DBA\$config\$DataType.

## **Details**

MODE: Add a specified peakset:

dba.peakset(DBA=NULL, peaks, sampID, tissue, factor, condition, replicate, control, peak.caller, reads, consensus, bamReads, bamControl, normCol, bRemoveM, bRemoveRandom)

MODE: Add a consensus peakset (derived from overlapping peaks in peaksets already present):

dba.peakset(DBA, peaks, minOverlap)

MODE: Add a sets of consensus peaksets bases on sample sets that share or differ in specified

attributes

dba.peakset(DBA, peaks, consensus, minOverlap)

MODE: Retrieve a peakset:

dba.peakset(DBA, peaks, bRetrieve=T)

MODE: Write a peakset out to a file:

dba.peakset(DBA, peaks, bRetrieve=T, writeFile, numCols)

#### Value

DBA object when adding a peakset. Peakset matrix or RangedData object when retrieving and/or writing a peakset.

#### Author(s)

Rory Stark

#### See Also

to add peaksets using a sample sheet, see dba.

```
# create a new DBA object by adding three peaksets
mcf7 = dba.peakset(NULL,
                  peaks=system.file("extra/peaks/MCF7_ER_1.bed.gz", package="DiffBind"),
             sampID="MCF7.1",tissue="MCF7",factor="ER",condition="Responsive",replicate=1)
mcf7 = dba.peakset(mcf7,
                  peaks=system.file("extra/peaks/MCF7_ER_2.bed.gz", package="DiffBind"),
             sampID="MCF7.2",tissue="MCF7",factor="ER",condition="Responsive",replicate=2)
mcf7 = dba.peakset(mcf7,
                  peaks=system.file("extra/peaks/MCF7_ER_3.bed.gz", package="DiffBind"),
             sampID="MCF7.3",tissue="MCF7",factor="ER",condition="Responsive",replicate=3)
mcf7
#retrieve peaks that are in all three peaksets
mcf7.consensus = dba.peakset(mcf7, 1:3, minOverlap=3, bRetrieve=TRUE)
mcf7.consensus
#add a consensus peakset -- peaks in all three replicates
mcf7 = dba.peakset(mcf7, 1:3, minOverlap=3,sampID="MCF7_3of3")
mcf7
#add consensus peaksets for all sample types by combining replicates
data(tamoxifen_peaks)
tamoxifen = dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
#add consensus peaksets for all sample types by (same tissue and condition)
data(tamoxifen_peaks)
tamoxifen = dba.peakset(tamoxifen,consensus = c(DBA_TISSUE,DBA_CONDITION))
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,tamoxifen$masks$Responsive & tamoxifen$masks$Consensus)
#create consensus peaksets from sample type consensuses for Responsive and Resistant sample groups
tamoxifen = dba.peakset(tamoxifen,peaks=tamoxifen$masks$Consensus,consensus=DBA_CONDITION)
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,17:18)
#retrieve the consensus peakset as RangedData object
```

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```
mcf7.consensus = dba.peakset(mcf7,mcf7$masks$Consensus,bRetrieve=TRUE)
mcf7.consensus
```

dba.plotBox

**Boxplots** 

# **Description**

Boxplots for read count distributions within differentially bound sites

# Usage

## **Arguments**

attribute

DBA DBA object. number of contrast to use for boxplot. contrast method method used for analysis (used in conjunction with contrast): • DBA EDGER DBA\_DESEQ • DBA\_EDGER\_BLOCK DBA DESEQ BLOCK th significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included in the boxplot. bUsePval logical indicating whether to use FDR (FALSE) or p-value (TRUE) for thresholding. bNormalized logical indicating that normalized data (using normalization factors computed

by differential analysis method) should be plotted. FALSE uses raw count data. attribute to use for determining groups of samples. Default (DBA\_GROUP) plots the two groups used in the contrast. Possible values:

- DBA\_GROUP
- DBA\_ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT

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DBA\_REPLICATEDBA\_CONSENSUSDBA\_CALLER

DBA CONTROL

bAll logical indicating if plot should include a set of boxplots using all counts, re-

gardless of whether or not they pass the significance threshold.

bAllIncreased logical indicating if plot should include a set of boxplots using all counts that in-

crease in affinity, regardless of whether or not they pass the significance thresh-

old.

bAllDecreased logical indicating if plot should include a set of boxplots using all counts that de-

crease in affinity, regardless of whether or not they pass the significance thresh-

old.

bDB logical indicating if plot should include a set of boxplots using all counts in sig-

nificantly differentially bound sites (i.e. those that pass the significance thresh-

old), regardless of whether they increase or decrease in affinity.

bDBIncreased logical indicating if plot should include a set of boxplots using all counts in

significantly differentially bound sites that increase in affinity.

bDBDecreased logical indicating if plot should include a set of boxplots using all counts in

significantly differentially bound sites that decrease in affinity.

pvalMethod method to use when computing matrix of p-values. If NULL, no matrix is com-

puted, and NULL is returned; this may speed up processing if there are many

boxplots.

bReversePos logical indicating if the default definition of positive affinity (higher affinity in

the second group of the contrast) should be reversed (i.e. positive affinity is

defined as being higher in the first group of the contrast).

attribOrder vector of group numbers used to change the order that groups are plotted. If

NULL, default order is used (group order for DBA\_GROUP, and the order the

attribute values appear for other values of attribute).

vColors vector of custom colors; if absent, default colors will be used.

varwidth passed to boxplot notch passed to boxplot

... other arguments passed to boxplot

## **Details**

Draws a boxplot showing distributions of read counts for various groups of samples under various conditions. In default mode, draws six boxes: one pair of boxes showing the distribution of read counts within all significantly differentially bound sites (one box for each sample group), one pair of boxes showing the distribution of read counts for significantly differentially bound sites that increase affinity in the second group, and a second pair of boxes showing the distribution of read counts for significantly differentially bound sites that have higher mean affinity in the first group.

#### Value

if pvalMethod is not NULL, returns a matrix of p-values indicating the significance of the difference between each pair of distributions.

## Author(s)

Rory Stark

## **Examples**

dba.plotHeatmap

Draw a binding site heatmap

# **Description**

Draws a binding site heatmap

## Usage

# **Arguments**

DBA DBA object.

attributes

attribute or vector of attributes to use for column labels:

- DBA\_ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_CONSENSUS
- DBA CALLER
- DBA\_CONTROL

maxSites maximum number of binding sites to use in heatmap. Only used when not draw-

ing a correlation heatmap (correlations=FALSE)

Set all scores less than this to minval

maxval Set all scores greater than this to maxval

number of contrast to report on; if present, draws a heatmap based on a differential binding affinity analysis (see dba.analyze). Only significantly differentially bound sites will be used (subject to the th and bUsePval parameters). If mask is unspecified, only the samples in the contrast will be included. See dba.show(DBA, bContrast=T) to get contrast numbers. If missing, uses scores

in the main binding matrix.

analysis method (used in conjunction with contrast):

- DBA\_EDGER
- DBA\_DESEQ
- DBA\_EDGER\_BLOCK
- DBA\_DESEQ\_BLOCK

significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included in the report (subject to maxSites). Used in conjunction with contrast.

logical indicating whether to use FDR (FALSE) or p-value (TRUE) for thresholding. Used in conjunction with contrast.

report (obtained from dba.report specifying the data to be used . If this is present, the method, th, and bUsePval parameters are ignored. Used in conjunction with contrast.

Score to use for count data. Only used when plotting the global binding matrix (no contrast specified). One of:

- DBA\_SCORE\_READS
- DBA\_SCORE\_READS\_MINUS
- DBA SCORE READS FOLD
- DBA SCORE RPKM
- DBA SCORE RPKM FOLD
- DBA SCORE TMM READS FULL
- DBA\_SCORE\_TMM\_READS\_EFFECTIVE
- DBA SCORE TMM MINUS FULL
- DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE

mask indicating a subset of peaksets to use when using global binding matrix scores. If a contrast is specified, these peaksets will be included, but only the significantly differentially bound sites (using th, bUsePval, and/or report) will be included.

logical vector indicating which sites to include; first maxSites of these. Only relevant when using global binding matrix (contrast is missing).

function taking a vector of scores and returning a single value. Only relevant when using global binding matrix (contrast is missing). If present, the global binding matrix will be sorted (descending) on the results, and the first maxSites used in the heatmap. Recommended sort function options include sd, mean, median, min.

method

minval

contrast

th

bUsePval

report

score

mask

sites

sortFun

correlations

logical indicating that a correlation heatmap should be plotted (TRUE). If FALSE, a binding heatmap of scores/reads is plotted. This parameter can also be set to a correlation record; see dba.overlap(mode=DBA\_OLAP\_ALL), in which case a correlation heatmap is plotted based on the specified correlation record, using the statistic specified in olPlot.

olPlot

if correlations is specified as a dataframe returned by dba.overlap, indicates which statistic to plot. One of:

- DBA\_COR Correlation
- DBA\_OLAP Percentage overlap
- DBA INALL number of peaks common to both samples

ColAttributes

Attribute or vector of attributes to plot for column color bars. If missing, all attributes with two or more unique non-NA values will be plotted. (For correlation heatmaps, DBA\_GROUP will be plotted in the column color bar by default when a contrast is specified). A value of NULL indicates that no column color bar should be drawn. Allowable attribute values include:

- DBA GROUP
- DBA\_TISSUE
- DBA FACTOR
- DBA\_CONDITION
- DBA TREATMENT
- DBA\_REPLICATE
- DBA\_CALLER

RowAttributes

Attribute or vector of attributes for row color bars. Row color bars are only allowed for correlation heatmaps. Same values as for ColAttributes parameter. Default is to draw a row color bar only if a contrast is specified, in which case the plotted attribute is DBA\_GROUP.

rowSideCols Vector of colors to use in row color bars. Uses default colors if missing. colSideCols Vector of colors to use in column color bars. Uses default colors if missing.

margin margin size of plot

Color scheme; see colorRampPalette RColorBrewer co1Scheme

distMethod distance method for clustering; see Dist

passed on to heatmap.2 (gplots), e.g. scale etc. . . .

## **Details**

MODE: Correlation Heatmap plot using statistics for global binding matrix:

dba.plotHeatmap(DBA, attributes=DBA\$attributes, minval, maxval, correlations, olPlot, colScheme="Greens", distMethod="pearson", ...)

MODE: Correlation Heatmap plot using statistics for significantly differentially bound sites:

dba.plotHeatmap(DBA, attributes=DBA\$attributes, minval, maxval, contrast, method=DBA\_EDGER, th=.1, bUsePval=F, mask, overlaps, olPlot=DBA\_COR, colScheme="Greens", distMethod="pearson", ...)

MODE: Binding heatmap plot using significantly differentially bound sites:

dba.plotHeatmap(DBA, attributes, maxSites, minval, maxval, contrast, method, th, bUsePval, correlations=FALSE, colScheme, distMethod, ...)

MODE: Binding heatmap plot using the global binding matrix:

dba.plotHeatmap(DBA, attributes, maxSites, minval, maxval, mask, sites, correlations=FALSE, sortFun, colScheme, distMethod, ...)

#### Value

if correlations is not FALSE, the overlap/correlation matrix is returned.

#### Author(s)

Rory Stark

#### See Also

dba.overlap

```
data(tamoxifen_peaks)
# peak overlap correlation heatmap
dba.plotHeatmap(tamoxifen)
data(tamoxifen_counts)
# counts correlation heatmap
dba.plotHeatmap(tamoxifen)
data(tamoxifen_analysis)
#correlation heatmap based on all normalized data
dba.plotHeatmap(tamoxifen,contrast=1,th=1)
#correlation heatmap based on DB sites only
dba.plotHeatmap(tamoxifen,contrast=1)
#binding heatmap based on DB sites
dba.plotHeatmap(tamoxifen,contrast=1,correlations=FALSE)
#binding heatmap based on 1,000 sites with highest variance
dba.plotHeatmap(tamoxifen,contrast=1,th=1,correlations=FALSE,sortFun=var)
data(tamoxifen_counts)
#Examples of heatmaps using DB sites with different subsets of samples
tamoxifen = dba.contrast(tamoxifen,tamoxifen$masks$Resistant,c(3:5,10:11)) #exclude T47D
tamoxifen = dba.analyze(tamoxifen,bCorPlot=FALSE)
dba.plotHeatmap(tamoxifen, contrast=1) # regular heatmaps with two contrast groups
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$All) #also include the T47D samples
plot(tamoxifen,contrast=1,mask=!tamoxifen$masks$MCF7) # correlation heatmap without MCF7 with with T47D
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$T47D,correlations=FALSE) # binding heatmaps using only
```

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dba.plotMA

Generate MA and scatter plots of differential binding analysis results

# **Description**

Generates MA and scatter plots of differential binding analysis results.

# Usage

# **Arguments**

DBA object, on which dba. analyze should have been successfully run.

contrast number of contrast to report on. See dba.show(DBA, bContrast=T) to get

contrast numbers.

method method or vector of methods to plot results for:

DBA\_EDGERDBA\_DESEQ

• DBA\_EDGER\_BLOCK

th significance threshold; all sites with FDR (or p-values, see bUsePval) less than

or equal to this value will be colored red in the plot

bUsePval logical indicating whether to use FDR (FALSE) or p-value (TRUE) for thresh-

olding.

fold will only include sites with fold change greater than this as significant (colored

red).

bNormalized logical indicating whether to plot normalized data using normalization factors

computed by differential analysis method (TRUE) or raw read counts (FALSE).

factor string to be prepended to plot main title; e.g. factor name.

bXY logical indicating whether to draw MA plot (FALSE) or XY scatter plot (TRUE).

dotSize size of points on plot (cex).

bSignificant Logical indicating if points corresponding to significantly differentially bound

sites (based on contrast, th, bUsePval, and fold parameters) should be overlaid

in red.

bSmooth logical indicating that basic plot should be plotted using smoothScatter. Note

that overlaid significant sites will be not plotted using a smoothing function.

... passed to plot.

#### Author(s)

Rory Stark

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## See Also

```
dba.analyze
```

# **Examples**

```
data(tamoxifen_analysis)

# default MA plot
dba.plotMA(tamoxifen)

#XY plots (with raw and normalized data)
par(mfrow=c(1,2))
dba.plotMA(tamoxifen,bXY=TRUE,bNormalized=FALSE)
dba.plotMA(tamoxifen,bXY=TRUE,bNormalized=TRUE)
```

dba.plotPCA

PCA plot

# **Description**

Principal Component Analysis plot

# Usage

# Arguments

 $\mathsf{DBA}$ 

DBA object.

attributes

attribute or vector of attributes to use to color plotted points. Each unique combination of attribute values will be assigned a color. Chosen from:

- DBA\_GROUP
- DBA\_ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_CONSENSUS
- DBA\_CALLER
- DBA\_CONTROL

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> Note that DBA\_GROUP is a special attribute which will result in samples from each group in a contrast being colored separately.

Set all scores less than this to minval minval Set all scores greater than this to maxval

contrast number of contrast to use for PCA; if present, plots a PCA based on a differential

binding affinity analysis (see dba.analyze). If mask is unspecified, only the samples in the contrast will be included. See dba.show(DBA, bContrast=T) to get contrast numbers. If missing, uses scores in the main binding matrix.

method used for analysis (used in conjunction with contrast): method

- DBA\_EDGER
- DBA\_DESEQ
- DBA\_EDGER\_BLOCK
- DBA\_DESEQ\_BLOCK

significance threshold; all sites with FDR (or p-values, see bUsePval) less than th or equal to this value will be included in the PCA, subject to maxVal. Used in conjunction with contrast.

if TRUE, uses p-value instead of FDR for thresholding. Used in conjunction

report (obtained from dba.report) specifying the data to be used. If this is present, the method, th, and bUsePval parameters are ignored.

Score to use for count data. Only used when plotting the global binding matrix (no contrast specified). One of:

- DBA\_SCORE\_READS
- DBA\_SCORE\_READS\_MINUS
- DBA\_SCORE\_READS\_FOLD
- DBA SCORE RPKM
- DBA\_SCORE\_RPKM\_FOLD
- DBA\_SCORE\_TMM\_READS\_FULL
- DBA\_SCORE\_TMM\_READS\_EFFECTIVE
- DBA\_SCORE\_TMM\_MINUS\_FULL
- DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE

mask indicating a subset of peaksets to use when using global binding matrix scores. If a contrast is specified, these peaksets will be included, but only the significantly differentially bound sites (using th, bUsePval, and/or report) will be included. See dba.mask.

logical vector indicating which sites to include in PCA. Only relevant when using global binding matrix (contrast is missing).

a logical value indicating whether the calculation should use the correlation ma-

trix or the covariance matrix. Passed into princomp.

logical indicating that three principal components should be plotted (requires

package{rgl}). If FALSE, the first two principal components are plotted.

vColors vector of custom colors; is absent, default colors will be used. dotSize size of dots to plot; is absent, a default will be calculated.

arguments passed to plot or plot3d (rgl).

maxval

bUsePva1

report

score

mask

sites cor

b3D

dba.plotPCA 35

# **Details**

```
MODE: PCA plot using significantly differentially bound sites:

dba.plotPCA(DBA, attributes, minval, maxval, contrast, method, th, bUsePval, b3D=F, vColors, dotSize, ...)

MODE: PCA plot using global binding matrix:

dba.plotPCA(DBA, attributes, minval, maxval, mask, sites, b3D=F, vColors, dotSize, ...)
```

## Value

matrix with color legend

## Note

uses rgl package for 3D plots (if available)

# Author(s)

Rory Stark

## See Also

```
dba.analyze, dba.plotHeatmap
```

```
data(tamoxifen_peaks)

# peakcaller scores PCA
dba.plotPCA(tamoxifen)

# raw count correlation PCA
data(tamoxifen_analysis)
dba.plotPCA(tamoxifen)

#PCA based on normalized data for all sites
dba.plotPCA(tamoxifen,contrast=1,th=1)

#PCA based on DB sites only
par(mfrow=c(1,2))
dba.plotPCA(tamoxifen,contrast=1)
dba.plotPCA(tamoxifen,contrast=1,attributes=DBA_TISSUE)
```

36 dba.plotVenn

dba.plotVenn	Draw 2-way, 3-way, or 4-way Venn diagrams of overlaps

# Description

Draws 2-way, 3-way, or 4-way Venn diagrams of overlaps

# Usage

```
dba.plotVenn(DBA, mask, overlaps, label1, label2, label3, label4, main="", sub="")
```

# Arguments

DBA	DBA object; if present, only the mask parameter will apply.
mask	mask or vector of peakset numbers indicating which peaksets to include in Venn diagram. Only 2 or 3 peaksets should be included. See dba.mask. Only one of mask or overlaps is used.
overlaps	overlap record, as computed by $\mbox{dba.overlap}(\mbox{Report=DBA\_OLAP\_PEAKS}).$ Only one of mask or overlaps is used.
label1	label for first peakset in diagram
label2	label for second peakset in diagram
label3	label for third peakset in diagram
label4	label for fourth peakset in diagram
main	main title for plot
sub	subtitle for plot

# Note

Venn plotting code written by Thomas Girke as part of overLapper code: http://manuals.bioinformatics.ucr.edu/home/R\_BioCondManual#R\_graphics\_venn

# Author(s)

Rory Stark and Thomas Girke

## See Also

```
dba.analyze, dba.overlap, dba.plotPCA
```

dba.report 37

## **Examples**

```
data(tamoxifen_peaks)
par(mfrow=c(2,2))
# 2-way Venn
dba.plotVenn(tamoxifen,6:7)
dba.plotVenn(tamoxifen,tamoxifen$masks$ZR75)
# 3-way Venn (done two different ways)
dba.plotVenn(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
olaps = dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
dba.plotVenn(tamoxifen,overlaps=olaps,
        label1="Rep 1",label2="Rep 2",label3="Rep 3",main="MCF7 (Responsive) Replicates")
#Venn of overlaps
Responsive=dba(tamoxifen,tamoxifen$masks$Responsive)
Responsive = dba.peakset(Responsive,1:3,sampID="MCF7")
Responsive = dba.peakset(Responsive, 4:5, sampID="T47D")
Responsive = dba.peakset(Responsive,6:7,sampID="ZR75")
par(mfrow=c(1,1))
dba.plotVenn(Responsive, Responsive$masks$Consensus)
#4-way overlap
data(tamoxifen_peaks)
tamoxifen = dba.peakset(tamoxifen, consensus=DBA_TISSUE)
par(mfrow=c(1,1))
dba.plotVenn(tamoxifen,tamoxifen$masks$Consensus,main="Tissue consensus overlaps")
```

dba.report

Generate a report for a differential binding affinity analysis

## **Description**

Generates a report for a differential binding affinity analysis

## Usage

## **Arguments**

DBA

DBA object. A differential binding affinity analysis needs to have been previously carried out (see dba.analyze).

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contrast contrast number to report on. See dba.show(DBA, bContrast=T) to get con-

trast numbers.

method method used for analysis:

• DBA\_EDGER

DBA\_DESEQ

DBA\_EDGER\_BLOCK

th significance threshold; all sites with FDR (or p-values, see bUsePval) less than

or equal to this value will be included in the report. A value of 1 will include all

binding sites in the report.

bUsePval logical indicating whether to use FDR (FALSE) or p-value (TRUE) for thresh-

olding.

fold only sites with an absolute Fold value greater than equal to this will be included

in the report.

bNormalized logical indicating that normalized data (using normalization factors computed

by differential analysis method) should be reported. FALSE uses raw count

data.

bCalled logical indicating that peak caller status should be included (if available from a

previous call to dba.count(bCalledMasks=TRUE)). This will add a column for each group, each indicating the number of samples in the group identified as a

peak in the original peaksets.

bCounts logical indicating that count data for individual samples should be reported as

well as group statistics. Columns are added for each sample in the first group,

followed by columns for each sample in the second group.

bCalledDetail logical indicating that peak caller status should be included for each sample (if

available). Columns are added for each sample in the first group, followed by

columns for each sample in the second group.

file if present, also save the report to a comma separated value (csv) file, using this

filename.

initString if saving to a file, pre-pend this string to the filename.

ext if saving to a file, append this extension to the filename.

DataType The class of object for returned report:

DBA\_DATA\_GRANGES

DBA DATA RANGEDDATA

• DBA\_DATA\_FRAME

If set to DBA\_DATA\_SUMMARIZED\_EXPERIMENT, the result will be a SummarizedExperiment

object, with all the count data and sample metadata for the experiment. The contrast statistics will be included as metadata columns in the rowData of the object.

Can be set as default behavior by setting DBA\$config\$DataType.

## Value

A report dataframe, GRanges, or RangedData object, with a row for each binding site within the thresholding parameters, and the following columns:

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Chr Chromosome of binding site

Start Starting base position of binding site
End End base position of binding site

Conc Concentration – mean (log) reads across all samples in both groups

Conc\_group1 Group 1 Concentration – mean (log) reads across all samples first group

Conc\_group2 Group 2 Concentration – mean (log) reads across all samples in second group

Fold Fold difference – mean fold difference of binding affinity of group 1 over group 2 (Conc1 - Conc2). Absolute value indicates magnitude of the difference, and

sign indicates which one is bound with higher affinity, with a positive value

indicating higher affinity in the first group

p-value p-value calculation – statistic indicating significance of difference (likelihood

difference is not attributable to chance)

FDR adjusted p-value calculation – p-value subjected to multiple-testing correction

If bCalled is TRUE and caller status is available, two more columns will follow:

Called1 Number of samples in group 1 that identified this binding site as a peak
Called2 Number of samples in group 2 that identified this binding site as a peak

If bCounts is TRUE, a column will be present for each sample in group 1, followed by each sample in group 2. The Sample ID will be used as the column header. This column contains the read counts for the sample.

If bCalledDetail is TRUE, a column will be present for each sample in group 1, followed by each sample in group 2. The Sample ID will be used as the column header. This column contains a "+" to indicate for which sites the sample was called as a peak, and a "-" if it was not so identified.

## Author(s)

Rory Stark

#### See Also

dba.analyze

```
data(tamoxifen_analysis)

#Retrieve DB sites with FDR < 0.1
tamoxifen.DB = dba.report(tamoxifen)
tamoxifen.DB

#Retrieve DB sites with p-value < 0.05 and Fold > 2
tamoxifen.DB = dba.report(tamoxifen, th=.05, bUsePval=TRUE, fold=2)
tamoxifen.DB

#Retrieve all sites with confidence stats
# and how many times each site was identified as a peak
```

40 dba.save

```
tamoxifen.DB = dba.report(tamoxifen, th=1, bCalled=TRUE)
tamoxifen.DB

#Retrieve all sites with confidence stats and normalized counts
tamoxifen.DB = dba.report(tamoxifen, th=1, bCounts=TRUE)
tamoxifen.DB

#Retrieve all sites with confidence stats and raw counts
tamoxifen.DB = dba.report(tamoxifen, th=1, bCounts=TRUE,bNormalized=FALSE)
tamoxifen.DB

#Retrieve report as a SummarizedObject
tamoxifen.sset = dba.report(tamoxifen, DataType=DBA_DATA_SUMMARIZED_EXPERIMENT)
tamoxifen.sset
```

dba.save

save DBA object

# **Description**

Writes out DBA object

# Usage

```
dba.save(DBA, file='DBA', dir='.', pre='dba_', ext='RData', bMinimize=FALSE)
```

# **Arguments**

DBA	DBA object
file	main filename
dir	directory to save model in
pre	string to pre-pend to filename
ext	extensions to use
bMinimize	logical indicating saved DBA object should be compressed as much as possible.

#### Value

string containing full path and filename.

# Author(s)

Rory Stark

# See Also

dba.load

41 dba.show

## **Examples**

```
data(tamoxifen_peaks)
savefile = dba.save(tamoxifen, 'tamoxifenPeaks')
tamoxifen = dba.load('tamoxifenPeaks')
unlink(savefile)
```

dba.show

List attributes of peaksets of contrasts associated with a DBA object

# **Description**

Returns attributes of peaksets and/or contrasts associated with a DBA object.

## Usage

```
dba.show(DBA, mask, attributes, bContrasts=FALSE, th=0.1, bUsePval=FALSE)
```

## **Arguments**

DBA DBA object

mask of peaksets for which to get attributes (used when obtaining peakset atmask

tributes, i.e. bContrasts=FALSE).

attribute or vector of attributes to retrieve. Number of intervals is always shown. attributes

Used when obtaining peakset attributes, i.e. bContrasts=FALSE. Values:

DBA ID

• DBA\_TISSUE

DBA\_FACTOR

DBA\_CONDITION

DBA\_CONDITION

DBA\_REPLICATE

DBA\_CONSENSUS

DBA\_CALLER

• DBA\_CONTROL

• DBA\_INTERVALS

• DBA\_SN\_RATIO

logical indicating whether peaksets or contrast attributes are to be retrieved.

TRUE retrieves a dataframe of contrast information instead of peakset attributes.

If no contrasts are set, returns possible contrasts. See dba.contrast.

if bContrasts is TRUE, then th is used as the threshold for determining how

many significant sites there are for each contrast. Only relevant when obtaining

contrast attributes (bContrasts=TRUE) and dba. analyze has been run.

logical indicating that p-values will be used (along with th) to determine how

many significant sites there are for each contrast; if FALSE, adjusted p-values (FDR) are used. Only relevant when obtaining contrast attributes (bContrasts=TRUE)

and dba.analyze has been run.

bContrasts

th

bUsePval

42 dba.show

#### **Details**

MODE: Return attributes of peaksets associated with a DBA object:

dba.show(DBA, mask, attributes)

MODE: Return contrasts associated with a DBA object:

dba.show(DBA,bContrasts=T, th, bUsePval)

#### Value

dataframe with peakset attributes.

If bContrasts == FALSE, each row represents a peakset, and each column is an attributes, with the final column, Intervals, indicating how many sites there are in the peakset.

If bContrasts == TRUE, each row represent a contrast, with the following columns:

Group1 Label for first group of contrast

Members 1 Number of samples in first group of contrast

Group2 Label for first group of contrast

Members 3 Number of samples in first group of contrast

if dba.analyze has been successfully run, there there will be up to four more columns showing the number of significant differentially bound (DB) sites identified for

DB. edgeR Number of significantly differentially bound sites identified using edgeR
DB. DESeq Number of significantly differentially bound sites identified using DESeq

DB.edgeR.block Number of significantly differentially bound sites identified for blocking analy-

sis using edgeR

DB.DESeq.block Number of significantly differentially bound sites identified for blocking analy-

sis using DESeq

## Author(s)

Rory Stark

# See Also

```
dba, dba.peakset, dba.contrast.dba.analyze
```

```
data(tamoxifen_peaks)
dba.show(tamoxifen)
dba.show(tamoxifen,tamoxifen$masks$Responsive)
dba.show(tamoxifen,attributes=c(DBA_TISSUE,DBA_REPLICATE,DBA_CONDITION))
data(tamoxifen_counts)
tamoxifen = dba.contrast(tamoxifen)
dba.show(tamoxifen,bContrasts=TRUE)
#alternatively:
tamoxifen
```

DiffBind -- DBA global constant variables

Constant variables used in DiffBind package

# Description

Constant variables used in DiffBind package

# Usage

DBA\_ID DBA\_FACTOR DBA\_TISSUE DBA\_CONDITION DBA\_TREATMENT DBA\_REPLICATE DBA\_CALLER DBA\_CONSENSUS DBA\_CONTROL DBA\_INTERVALS DBA\_SN\_RATIO DBA\_GROUP DBA\_OLAP\_PEAKS DBA\_OLAP\_ALL DBA\_OLAP\_RATE DBA\_SCORE\_READS DBA\_SCORE\_READS\_MINUS DBA\_SCORE\_READS\_FOLD DBA\_SCORE\_RPKM

DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE

DBA\_EDGER

DBA\_DESEQ

DBA\_EDGER\_BLOCK

DBA\_DESEQ\_BLOCK

DBA\_EDGER\_CLASSIC

DBA\_DESEQ\_CLASSIC

DBA\_EDGER\_GLM

DBA\_SCORE\_RPKM\_FOLD
DBA\_SCORE\_TMM\_READS\_FULL
DBA\_SCORE\_TMM\_READS\_EFFECTIVE
DBA\_SCORE\_TMM\_MINUS\_FULL

DBA\_DESEQ\_GLM

DBA\_DATA\_FRAME
DBA\_DATA\_GRANGES
DBA\_DATA\_RANGEDDATA
DBA\_DATA\_SUMMARIZED\_EXPERIMENT

#### **Arguments**

DBA\_ID DBA peakset metadata: Peakset ID DBA peakset metadata: Factor DBA\_FACTOR DBA\_TISSUE DBA peakset metadata: Tissue DBA\_CONDITION DBA peakset metadata: Condition DBA\_TREATMENT DBA peakset metadata: Treatment DBA\_REPLICATE DBA peakset metadata: Replicate DBA peakset metadata: Peak Caller DBA\_CALLER DBA\_CONSENSUS DBA peakset metadata: Is this a consensus peakset? DBA\_CONTROL DBA peakset metadata: ID of Control sample DBA\_INTERVALS DBA peakset metadata: Number of intervals in peakset DBA\_SN\_RATIO DBA peakset metadata: Signal to Noise ratio (number of reads in intervals divided by total number of reads in library) DBA\_GROUP DBA peakset metadata: color PCA plot using contras groups DBA\_OLAP\_PEAKS dba.overlap mode: return overlapping/unique peaksets DBA\_OLAP\_ALL dba.overlap mode: return report of correlations/overlaps for each pair of samples DBA\_OLAP\_RATE dba.overlap mode: return overlap rates DBA\_SCORE\_READS dba.count score is number of reads in ChIP DBA\_SCORE\_READS\_FOLD

dba.count score is number of reads in ChIP divided by number of reads in Con-

DBA\_SCORE\_READS\_MINUS

\_NEADS\_HINOS

dba.count score is number of reads in ChIP minus number of reads in Control

DBA\_SCORE\_RPKM dba.count score is RPKM of ChIP

DBA\_SCORE\_RPKM\_FOLD

dba.count score is RPKM of ChIP divided by RPKM of Control

DBA\_SCORE\_TMM\_READS\_FULL

dba.count score is TMM normalized (using edgeR), using ChIP read counts and Full Library size

DBA\_SCORE\_TMM\_READS\_EFFECTIVE

dba.count score is TMM normalized (using edgeR), using ChIP read counts and Effective Library size

DBA\_SCORE\_TMM\_MINUS\_FULL

dba.count score is TMM normalized (using edgeR), using ChIP read counts minus Control read counts and Full Library size

DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE

dba.count score is TMM normalized (using edgeR), using ChIP read counts mi-

nus Control read counts and Effective Library size

DBA\_EDGER differential analysis method: edgeR (default: DBA\_EDGER\_GLM)

DBA\_DESEQ differential analysis method: DESeq (default: DBA\_DESEQ\_CLASSIC)

DBA\_EDGER\_CLASSIC

differential analysis method: "classic" edgeR for two-group comparisons

DBA\_DESEQ\_CLASSIC

differential analysis method: "classic" DESeq for two-group comparisons

DBA\_EDGER\_GLM differential analysis method: use GLM in edgeR for two-group comparisons

DBA\_DESEQ\_GLM differential analysis method: use GLM in DESeq for two-group comparisons

DBA\_EDGER\_BLOCK

differential analysis method: edgeR with blocking factors (GLM)

DBA\_DESEQ\_BLOCK

differential analysis method: DESeq with blocking factors (GLM)

DBA\_DATA\_GRANGES

Use GRanges class for peaksets and reports. This is the default (DBA\$config\$DataType = DBA\_DATA\_GRANGES).

DBA\_DATA\_RANGEDDATA

Use RangedData class for peaksets and reports. Can be set as default (DBA\$config\$DataType = DBA DATA RANGEDDATA).

DBA\_DATA\_FRAME Use data.frame class for peaksets and reports. Can be set as default (DBA\$config\$DataType = DBA\_DATA\_FRAME).

DBA\_DATA\_SUMMARIZED\_EXPERIMENT

Return report as a SummarizedExperiment.

## Note

Variables with ALL CAP names are used as constants within DiffBind.

# Author(s)

Rory Stark

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