# Package 'DiffBind'

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Title Differential Binding Analysis of ChIP-Seq Peak Data
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<b>Description</b> Compute differentially bound sites from multiple ChIP-seq experiments using affinity (quantitative) data. Also enables occupancy (overlap) analysis and plotting functions.
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Suggests DESeq, BiocStyle, testthat
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DiffBind-package

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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 Add a peakset to, or retrieve a peakset from, a dba object dba.peakset: Compute binding site overlaps and/or correlations dba.overlap: 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 dba.plotHeatmap: Heatmap plot Principal Components plot dba.plotPCA: dba.plotBox: **Boxplots** MA/scatter plot dba.plotMA: 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)

Rory Stark <rur>Rory Stark ford Brown gdbzork @at@gmail.com>

dba

Construct a DBA object

#### **Description**

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

## Usage

## 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
- 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)

For sample sheets loaded from a file, the accepted formats are comma-separated values (column headers, followed by one line per sample), or Excel-formatted spreadsheets (.xls or .xlsx extension). Leading and trailing white space will be removed from all values, with a warning.

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:

- Relevant fields include:
  RunParallel: logical indicating if counting and analysis operations should
- DataType: default class for peaks and reports (DBA\_DATA\_GRANGES, DBA\_DATA\_RANGEDDATA, or DBA\_DATA\_FRAME).
- ReportInit: string to append to the beginning of saved report file names.
- AnalysisMethod: either DBA\_DESEQ2 or DBA\_EDGER.

be run in parallel using multicore by default.

- bCorPlot: logical indicating that a correlation heatmap should be plotted automatically
- th: default threshold for reporting and plotting analysis results.
- bUsePval: logical, default indicating whether to use FDR (FALSE) or p-values (TRUE).
- minQCth: numeric, for filtering reads based on mapping quality score; only reads with a mapping qulity score gretaer than or equal to this will be counted.
- fragmentSize: numeric with mean fragment size. Reads will be extended to this length before counting overlaps. May be a vector of legnths, one for each sample.

config

peakCaller if a sampleSheet is specified, the default peak caller that will be used if the

PeakCaller column is absent.

peakFormat if a sampleSheet is specified, the default peak file format that will be used if the

PeakFormat 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.

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.

skipLines if a sampleSheet is specified, the number of lines (ie header lines) at the begin-

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 logical indicating that a correlation heatmap should be plotted before returning.

If DBA is NULL (a new DBA object is being created), and bCorPlot is missing, then this will take the default value (FALSE). However if DBA is NULL (a new DBA object is being created), and bCorPlot is specified, then the specified value will

become the default value of bCorPlot for the resultant DBA object.

attributes 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

dir Directory path. If supplied, files referenced in the sampleSheet will have this path prepended. Applies to PeakFiles, bamReads, and bamControl, if present.

If sampleSheet is a filepath, this will prepended to that as well.

## **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

## See Also

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

#### **Examples**

```
# Create DBA object from a samplesheet
## Not run:
basedir <- system.file("extra", package="DiffBind")</pre>
tamoxifen <- dba(sampleSheet="tamoxifen.csv", dir=basedir)</pre>
tamoxifen
tamoxifen <- dba(sampleSheet="tamoxifen_allfields.csv")</pre>
tamoxifen
tamoxifen <- dba(sampleSheet="tamoxifen_allfields.csv",config="config.csv")</pre>
tamoxifen
## End(Not run)
#Create a DBA object with a subset of samples
data(tamoxifen_peaks)
Responsive <- dba(tamoxifen,tamoxifen$masks$Responsive)</pre>
Responsive
# change peak caller but leave peak format the same
basedir <- system.file("extra", package="DiffBind")</pre>
tamoxifen <- dba(sampleSheet="tamoxifen.csv", dir=basedir,</pre>
                  peakCaller="macs", peakFormat="raw", scoreCol=5 )
dba.show(tamoxifen, attributes=c(DBA_TISSUE,DBA_CONDITION,DBA_REPLICATE,DBA_CALLER))
# Convert DBA object to SummarizedExperiment
data(tamoxifen_counts)
sset <- dba(tamoxifen,bSummarizedExperiment=TRUE)</pre>
sset
```

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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 from the DiffBind package.

DBA objects are usually constructed using the dba function.

# 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

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#### 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

#### **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

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#### **Arguments**

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\_DESEQ2

also, for backward compatibility:

DBA\_DESEQ

Additionally, if this value is set to DBA\_ALL\_METHODS, this is equivalent to specifying c(DBA\_EDGER, DBA\_DESEQ2).

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 if

overall biding levels are expected to be similar between samples).

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.

filter value to use for filtering intervals with low read counts. Each contrast will be

 $filtered\ separately.\ The\ filter Fun\ will\ be\ applied\ t\ each\ interval,\ and\ any\ scores$ 

below the filter value will be removed prior to analysis.

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

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 analyzed data

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

default thresholds, no heatmap will be plotted.

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

save memory. If it is desired to use the DBA $\contrasts[[n]]$ edgeR and/or DBA $\contrasts[[n]]$ DESeq2 objects directly in the edgeR and/or DESeq2 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 DESeq2 analyses are carried out.

## Value

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

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#### 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
```

# **Examples**

```
data(tamoxifen_counts)

tamoxifen <- dba.analyze(tamoxifen)
tamoxifen

data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen,categories=DBA_CONDITION,block=tamoxifen$masks$MCF7)
tamoxifen <- dba.analyze(tamoxifen,method=DBA_ALL_METHODS)
tamoxifen</pre>
```

dba.contrast

Set up contrasts for differential binding affinity analysis

# Description

Sets up contrasts for differential binding affinity analysis

## Usage

## **Arguments**

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.

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bNot

include contrasts consisting of a group and all other samples not in that group (indicated by a! in the contrast name).

categories

when automatically generating contrasts, attribute or vector of attributes to base contrasts on:

- DBA\_ID
- DBA\_TISSUE
- DBA\_FACTOR
- DBA CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_CALLER

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
- 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

## **Examples**

```
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen, categories=DBA_CONDITION)</pre>
tamoxifen
# Another way to do the same thing
tamoxifen$contrasts=NULL
tamoxifen <- dba.contrast (tamoxifen, tamoxifen \$ masks \$ Responsive, tamoxifen \$ masks \$ Resistant, tamoxifen \$ Resistant, tamoxifen \$ Resistant, tamoxifen \$ Resistant, tamoxifen \$ Re
                                                                                                                                                                       "Responsive", "Resistant")
tamoxifen
# Add add default contrasts
tamoxifen$contrasts=NULL
 tamoxifen <- dba.contrast(tamoxifen)</pre>
 tamoxifen
# Specify a blocking factor
tamoxifen$contrasts=NULL
tamoxifen <- dba.contrast(tamoxifen, categories=DBA_CONDITION, block=DBA_TISSUE)</pre>
tamoxifen
tamoxifen$contrasts=NULL
tamoxifen <- dba.contrast(tamoxifen, categories=DBA_CONDITION, block=list(c(3,4,5,8,9),c(1,2,10,11)))</pre>
tamoxifen
tamoxifen$contrasts=NULL
tamoxifen <- dba.contrast(tamoxifen, categories=DBA_CONDITION, block=tamoxifen$masks$MCF7)</pre>
tamoxifen <- dba.analyze(tamoxifen)</pre>
tamoxifen
```

 ${\tt dba.count}$ 

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

filterFun=max,

bCorPlot=DBA\$config\$bCorPlot,

bUseSummarizeOverlaps=FALSE, readFormat=DBA\_READS\_DEFAULT,

bParallel=DBA\$config\$RunParallel)

#### **Arguments**

DBA DBA object

If GRanges, RangedData, dataframe, or matrix, this parameter contains the inpeaks

> tervals 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, the score, filter, and summits parameters are honored, updating the global binding matrix without recounting in the cases of score and filter, and only counting after re-centering

in the case of summits.

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.

which score to use in the binding affinity matrix. Note that all raw read counts score

are maintained for use by dba.analyze, regardless of how this is set. One of:

DBA\_SCORE\_READS

DBA\_SCORE\_READS\_FOLD

DBA SCORE READS MINUS

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

DBA\_SCORE\_TMM\_READS\_FULL\_CPM

DBA\_SCORE\_TMM\_READS\_EFFECTIVE\_CPM DBA\_SCORE\_TMM\_MINUS\_FULL\_CPM

DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE\_CPM

DBA\_SCORE\_SUMMIT

DBA\_SCORE\_SUMMIT\_ADJ

DBA\_SCORE\_SUMMIT\_POS

raw read count for interval using only reads from ChIP

raw read count for interval from ChIP divided by read count for int raw read count for interval from ChIP minus read count for interval

RPKM for interval using only reads from ChIP

RPKM for interval from ChIP divided by RPKM for interval from

TMM normalized (using edgeR), using ChIP read counts and Full

TMM normalized (using edgeR), using ChIP read counts and Effect

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

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

same as DBA\_SCORE\_TMM\_READS\_FULL, but reported in counts-per-

same as DBA\_SCORE\_TMM\_READS\_EFFECTIVE, but reported in count

same as DBA\_SCORE\_TMM\_MINUS\_FULL, but reported in counts-per-

same as DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE, but reported in count

summit height (maximum read pileup value)

summit height (maximum read pileup value), normalized to relative

summit position (location of maximum read pileup)

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

and DBA\_SCORE\_READS\_FOLD).

fragmentSize 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 set to zero, the read size indicated in the BAM/BED file will be used. fragmentSize may also be a vector of values, one for each ChIP sample plus one for each unique

Control library.

summits if present, summit heights (read pileup) and locations will be calculated for each

peak. The values can retrieved using dba.peakset. The summits can also be

used as a read score in the global binding matrix (see score).

If the value of summits is TRUE (or 0), the summits will be calculated but the

> peaksets will be unaffected. If the value is greater than zero, all consensus peaks will be re-centered around a consensus summit, with the value of summits indicating how many base pairs to include upstream and downstream of the summit (so all consensus peaks will be of the same width, namely 2 \* summits).

> Note that if summits is greater than zero, the counting procedure will take twice as long, and bUseSummarizeOverlaps must be FALSE.

filter

value to use for filtering intervals with low read counts. The filterFun will be applied to the scores for each interval, and if it returns a value below the filter value, the interval will be removed from further analysis. 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. The built-in counting code may not correctly handle certain cases when the bRemoveDuplicates parameter is set to TRUE. These cases include paired-end data and datasets where the read length may differ within a single BAM file. In these cases, see the bUseSummarizeOverlaps parameter.

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.

mapQCth

for filtering by mapping quality (mapqc). Only alignments with mapping scores of at least this value will be included. Only applicable for bam files when bUseSummarizeOverlaps=FALSE (setting DBA\$config\$scanbamparam appropriately to filter on quality scores when using summarizeOverlaps.)

filterFun

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

> logical indicating that summarizeOverlaps should be used for counting instead of the built-in counting code. This option is slower but uses the more standard counting function. If TRUE, all read files must be BAM (.bam extension), with associated index files (.bam.bai extension). The insertLength parameter must

See notes for when the bRemoveDuplicates parameter is set to TRUE, where the built-in counting code may not correctly handle certain cases and bUseSummarizeOverlaps should be set to TRUE.

Five additional parameters for summarizeOverlaps may be specified in DBA\$config:

DBA\$config\$yieldSize

yieldSize indicating how many reads to process at one time; default is 5000000. The lowe

DBA\$config\$intersectMode mode indicating which overlap algorithm to use; default is "IntersectionNotEmpty"

DBA\$config\$singleEnd logical indicating if reads are single end; default is TRUE

DBA\$config\$fragments logical indicating how unmatched reads are counted; default is FALSE

DBA\$config\$scanbamparam ScanBamParam object to pass to summarizeOverlaps. If present, bRemoveDuplicates is

readFormat Specify the file type of the read files, over-riding the file extension. Possible

values:

DBA\_READS\_DEFAULT use file extension (.bam, .bed, .bed.gz) to determine file type DBA\_READS\_BAM assume the file type is BAM, regardless of the file extension

DBA\_READS\_BED assume the file type is BED (or zipped BED), regardless of the file extension.

Note that if readFormat is anything other than DBA\_READS\_DEFAULT, all the

read files must be of the same file type.

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.

#### Author(s)

Rory Stark and Gordon Brown

#### See Also

dba.analyze

## **Examples**

```
# 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)</pre>
# 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)</pre>
## Not run: tamoxifen <- dba.count(tamoxifen,peaks=mcf7Common$inAll)</pre>
#First make consensus peaksets from each set of replicates,
#then derive master consensus set for counting from those
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)</pre>
## Not run: tamoxifen <- dba.count(tamoxifen, peaks=tamoxifen$masks$Consensus)</pre>
tamoxifen
# Change binding affinity scores
data(tamoxifen_counts)
tamoxifen <- dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_READS)</pre>
dba.peakset(tamoxifen, bRetrieve=TRUE)
tamoxifen <- dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_RPKM_FOLD)</pre>
dba.peakset(tamoxifen, bRetrieve=TRUE)
```

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```
tamoxifen <- dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_TMM_MINUS_FULL)
dba.peakset(tamoxifen, bRetrieve=TRUE)

# Plot effect of a range of filter values and then apply filter
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='l',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</pre>
```

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

# **Examples**

```
data(tamoxifen_peaks)
dba.save(tamoxifen, 'tamoxifenPeaks')
tamoxifen <- dba.load('tamoxifenPeaks')</pre>
```

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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 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 specifies the method for combining new mask with supplied mask:

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

bApply

when deriving a peakset/sample mask, a logical indicating that a new DBA object with the mask applied will be returned.

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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

#### **Examples**

```
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</pre>
```

dba.overlap 19

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,
            contrast, method=DBA$config$AnalysisMethod, th=DBA$config$th,
            bUsePval=DBA$config$bUsePval,
            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:

• DBA\_OLAP\_PEAKS

• DBA\_OLAP\_ALL

DBA\_OLAP\_RATE

contrast number to use. Only specified if contrast data is to be used when contrast

mode=DBA\_OLAP\_ALL. See dba.show(DBA,bContrast=T) to get contrast

numbers.

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

• DBA\_DESEQ2

DBA\_DESEQ2\_BLOCK

• DBA EDGER

• DBA EDGER 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.

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

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

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.

mode

th

report

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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

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

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

DataType

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, by Attribute, 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)

#### 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

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0250	peans anique to peanset o
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
CandD	peaks in peaksets C and D but not in peaksets A or B

peaks unique to peakset 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:

A	peakset number of first peakset in overlap
В	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)

onlyC

Rory Stark

## See Also

dba.plotVenn, dba.plotHeatmap

## **Examples**

```
data(tamoxifen_peaks)
# default mode: DBA_OLAP_PEAKS -- get overlapping/non overlapping peaksets
mcf7 <- dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
names(mcf7)
mcf7$inAll
# mode: DBA_OLAP_ALL -- get correlation record
mcf7 <- dba(tamoxifen,tamoxifen$masks$MCF7)
mcf7.corRec <- dba.overlap(mcf7,mode=DBA_OLAP_ALL,bCorOnly=FALSE)
mcf7.corRec</pre>
```

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 and empty peakset (zero peaks), set peaks=NA.

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

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

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

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

treatment

replicate

peak.caller

control

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

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

added.

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 in the first column and the counts in the 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.

## **Examples**

```
# create a new DBA object by adding three peaksets
mcf7 <- dba.peakset(NULL,</pre>
                  peaks=system.file("extra/peaks/MCF7_ER_1.bed.gz", package="DiffBind"),
                   peak.caller="bed", sampID="MCF7.1",tissue="MCF7",
                   factor="ER",condition="Responsive",replicate=1)
mcf7 <- dba.peakset(mcf7,</pre>
                  peaks=system.file("extra/peaks/MCF7_ER_2.bed.gz", package="DiffBind"),
                   peak.caller="bed", sampID="MCF7.2",tissue="MCF7",
                   factor="ER",condition="Responsive",replicate=2)
mcf7 <- dba.peakset(mcf7,</pre>
                  peaks=system.file("extra/peaks/MCF7_ER_3.bed.gz", package="DiffBind"),
                   peak.caller="bed", 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, min0verlap=3, bRetrieve=TRUE)</pre>
mcf7.consensus
#add a consensus peakset -- peaks in all three replicates
mcf7 <- dba.peakset(mcf7, 1:3, minOverlap=3, sampID="MCF7_3of3")</pre>
mcf7
```

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```
#add consensus peaksets for all sample types by combining replicates
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)</pre>
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))</pre>
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)</pre>
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,17:18)
#retrieve the consensus peakset as RangedData object
mcf7.consensus <- dba.peakset(mcf7,mcf7$masks$Consensus,bRetrieve=TRUE)</pre>
mcf7.consensus
```

dba.plotBox

**Boxplots** 

## **Description**

Boxplots for read count distributions within differentially bound sites

#### Usage

# Arguments

DBA object.

contrast number of contrast to use for boxplot.

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

• DBA DESEQ2

• DBA\_DESEQ2\_BLOCK

• DBA\_EDGER

DBA\_EDGER\_BLOCK

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

th

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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 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
- DBA\_REPLICATE
- DBA\_CONSENSUS
- DBA CALLER

bAll logical indicating if plot should include a set of boxplots using all counts, regardless of whether or not they pass the significance threshold.

bAllIncreased logical indicating if plot should include a set of boxplots using all counts that increase in affinity, regardless of whether or not they pass the significance threshold.

logical indicating if plot should include a set of boxplots using all counts that decrease in affinity, regardless of whether or not they pass the significance threshold.

logical indicating if plot should include a set of boxplots using all counts in significantly differentially bound sites (i.e. those that pass the significance threshold), regardless of whether they increase or decrease in affinity.

logical indicating if plot should include a set of boxplots using all counts in significantly differentially bound sites that increase in affinity.

logical indicating if plot should include a set of boxplots using all counts in significantly differentially bound sites that decrease in affinity.

method to use when computing matrix of p-values. If NULL, no matrix is computed, and NULL is returned; this may speed up processing if there are many boxplots.

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).

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

**bAllDecreased** 

bDBIncreased

**bDBDecreased** 

pvalMethod

bReversePos

attribOrder

bDB

... 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**

th

report

score

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

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

ing a correlation heatmap (correlations=FALSE)

minval Set all scores less than this to minval

maxval Set all scores greater than this to maxval

contrast number of contrast to report on; if present, draws a heatmap based on a dif-

ferential 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 proin binding matrix

in the main binding matrix.

method analysis method (used in conjunction with contrast):

• DBA\_DESEQ2

- DBA\_DESEQ2\_BLOCK
- DBA\_EDGER
- DBA EDGER 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.

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

olding. 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 conjunc-

tion 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

bLog Logical indicating that log2 values should be used. Only applicable to read

count scores (not peak scores).

mask 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.

sites logical vector indicating which sites to include; first maxSites of these. Only

relevant when using global binding matrix (contrast is missing).

sortFun function taking a vector of scores and returning a single value. Only relevant

when using global binding matrix (contrast is missing). If not equal to FALSE, 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.

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 <a href="mailto:dba.overlap(mode=DBA\_OLAP\_ALL">dba.overlap(mode=DBA\_OLAP\_ALL)</a>, 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. Can

also be a list of color vectors.

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

also be a list of color vectors.

margin size of plot

colScheme Color scheme; see colorRampPalette RColorBrewer

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\_DESEQ2, th=0.05, 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.

if correlations is FALSE, the sites used in the heatmap are returned in a GRanges object, in the row order they appear (top to bottom). The metadata contains a column for each sample (also in the order they are appear in the clustering plot), with the values being the actual plotted values.

#### Author(s)

Rory Stark

#### See Also

dba.overlap

## **Examples**

```
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
sites <- dba.plotHeatmap(tamoxifen,contrast=1,th=1,</pre>
```

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```
correlations=FALSE, sortFun=var)
sites

data(tamoxifen_counts)
#Examples of heatmaps using DB sites with different subsets of samples
#exclude T47D
tamoxifen <- dba.contrast(tamoxifen,tamoxifen$masks$Resistant,c(3:5,10:11))
tamoxifer <- dba.analyze(tamoxifen,bCorPlot=FALSE)
# regular heatmaps with two contrast groups
dba.plotHeatmap(tamoxifen, contrast=1)
#also include the T47D samples
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$All)
#correlation heatmap without MCF7
plot(tamoxifen,contrast=1,mask=!tamoxifen$masks$MCF7)
# binding heatmap using only the MCF7 samples
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$MCF7,correlations=FALSE)
```

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=TRUE) to get

contrast numbers.

method method or vector of methods to plot results for:

• DBA\_DESEQ2

DBA\_DESEQ2\_BLOCK

DBA\_EDGER

• DBA EDGER BLOCK

th significance threshold; all sites with FDR (or p-values, see bUsePva1) 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).

dba.plotPCA 33

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.

bFlip logical indicating that order of groups in contrast should be "flipped", allowing

control of which sample group will have positive and which will have negative

fold changes.

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 over-

laid in red.

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

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

xrange vector of length 2 containing the desired minimum and maximum concentrations

to plot.

yrange vector of length 2 containing the desired minimum and maximum fold changes

to plot.

... passed to plot.

#### Author(s)

Rory Stark

#### 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,bSmooth=FALSE,bNormalized=FALSE)
dba.plotMA(tamoxifen,bXY=TRUE,bSmooth=FALSE,bNormalized=TRUE)
```

dba.plotPCA

PCA plot

## Description

Principal Component Analysis plot

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#### **Usage**

## **Arguments**

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

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

minval

Set all scores less than this to minval

maxval

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

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

- DBA\_DESEQ2
- DBA DESEQ2 BLOCK
- DBA\_EDGER
- 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 PCA, subject to maxVal. Used in conjunction with contrast.

bUsePval

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

report

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

score

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

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•	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

bLog Logical indicating that log2 values should be used. Only applicable to read

count scores (not peak scores).

mask 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.

sites logical vector indicating which sites to include in PCA. Only relevant when

using global binding matrix (contrast is missing).

label A metadata field to use as a label in 2D plots. The value for this field will be

written directly onthe plot near the dot for each sample. Values can be any of

those vlaid for the attributes parameter.

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

trix or the covariance matrix. Passed into princomp.

b3D 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.

labelSize Scaling factor for labels if present. Default is 0.8.

labelCols Vector of colors to use for labels. Default is "black".

components Number(s) of the components to plot. Can be a vector of two or three component

numbers, or a single integer. If an integer, that component, in addition to the

succeeding one (b3D=FALSE) or two (b3D=TRUE) will be plotted.

... arguments passed to plot or plot3d (rgl).

#### **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

trellis plot from lattice package; see xyplot

## Note

uses rgl package for 3D plots (if available)

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#### Author(s)

Rory Stark

#### See Also

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

### **Examples**

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

## **Arguments**

DBA	DBA object: if pre	esent, only the mask p	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 dba.overlap(Report=DBA\_OLAP\_PEAKS). Only

one of mask or overlaps is used.

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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

main main title for plot sub subtitle for plot

contrast number(s) to use for results-based plots. This can be a vector of contrast

numbers. See dba.show(DBA, bContrast=T) to get contrast numbers.

method if contrast is specified, include results from analyses using this method or

methods:

- DBA\_DESEQ2
- DBA\_DESEQ2\_BLOCK
- DBA EDGER
- DBA\_EDGER\_BLOCK
- DBA\_ALL\_METHODS
- DBA\_ALL\_BLOCK
- DBA ALL METHODS BLOCK

th if contrast is specified, use this significance threshold; all sites with FDR (or

p-values, see bUsePval) less than or equal to this value will be considered dif-

ferentially bound (DB).

bUsePval if contrast is specified, this logical indicates whether to use FDR (FALSE) or

p-value (TRUE) for thresholding.

bDB if contrast is specified, this logical indicates that peaksets should include Dif-

ferentially Bound (DB) sites (respecting the th, bUsePval, and fold parame-

ters).

bNotDB if contrast is specified, this logical indicates that peaksets should include non-

Differentially Bound (non-DB) sites (respecting the th, bUsePval, and fold

parameters).

bAll if contrast is specified, this logical indicates peaksets combining peaks with

both positive and negative fold changes should be included.

bGain if contrast is specified, this logical indicates that peaksets with only positive

fold changes should be included.

bLoss if contrast is specified, this logical indicates that peaksets with only negative

fold changes should be included.

### labelAttributes

is labels are not specified, use these attributes to create default labels:

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

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Only specified attributes that differ between peaksets will be used for labels; the ones that have the same value for all peaksets will be used as the default subtitle.

DataType

if bReturnPeaksets is set to TRUE, 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.

Alternatively, this can be set to:

DBA\_DATA\_DBAOBJECT

to return a results-based DBA object, if a contrast is specified.

### Value

Either a list of peaksets is returned invisibly (as decribed in dba.overlap), or, if DataType=DBA\_DATA\_DBAOBJECT, a results-based DBA object.

### Note

When working with results overlaps (a least one contrast is specified), and results-oriented DBA object is generated internally (as decribed in dba.report). In some cases, it may be better to generate the DBA object explicitly (using dba.report or setting bReturnPeaksets=TRUE and DataType=DBA\_DATA\_DBAOBJECT). This include the case where mseveral plots are being made of the same results set, and it takes a long time to generate the results-based DBA object, as well as the case where there are more than four results peaksets and a mask needs to be specified. I

This function relies on vennPlot in the systemPipeR package, written by Thomas Girke.

### Author(s)

Rory Stark

### See Also

```
dba.analyze, dba.overlap, dba.report, dba.plotPCA, vennPlot
```

### **Examples**

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```
Responsive <- dba.peakset(Responsive,1:3,sampID="MCF7")</pre>
Responsive <- dba.peakset(Responsive, 4:5, sampID="T47D")</pre>
Responsive <- dba.peakset(Responsive,6:7,sampID="ZR75")</pre>
par(mfrow=c(1,1))
dba.plotVenn(Responsive, Responsive$masks$Consensus)
#4-way overlap
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen, consensus=DBA_TISSUE)</pre>
par(mfrow=c(1,1))
dba.plotVenn(tamoxifen,tamoxifen$masks$Consensus,main="Tissue consensus overlaps")
#Venns of differentially bound sites
data(tamoxifen_analysis)
tamoxifen <- dba.contrast(tamoxifen,categories=DBA_CONDITION,block=tamoxifen$masks$MCF7)</pre>
tamoxifen <- dba.analyze(tamoxifen,method=c(DBA_EDGER,DBA_DESEQ2))</pre>
\verb|dba.plotVenn(tamoxifen,contrast=1,method=DBA\_ALL\_METHODS\_BLOCK)||
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_BLOCK,bAll=FALSE,bGain=TRUE,bLoss=TRUE)
par(mfrow=c(2,1))
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_BLOCK,bAll=FALSE,bGain=TRUE,bLoss=FALSE)
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_BLOCK,bAll=FALSE,bGain=FALSE,bLoss=TRUE)
```

dba.plotVolcano

Generate volcano plots of differential binding analysis results

### **Description**

Generates volcano 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=TRUE) to get

contrast numbers.

method or vector of methods to plot results for:

• DBA\_DESEQ2

• DBA\_DESEQ2\_BLOCK

• DBA\_EDGER

• DBA\_EDGER\_BLOCK

th significance threshold; 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).

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

bFlip logical indicating that order of groups in contrast should be "flipped", allowing

control of which sample group will have positive and which will have negative

fold changes.

bLabels logical indicating that labels should be drawn on the plot. The labels are the

site numbers, the row index in the (silently) returned set of significant sites. The

maximim number of sites can be specified using maxLabels.

maxLabels The maximum number of labels to use in the plot. Ignored if bLabels=FALSE.

dotSize size of points on plot.

### **Details**

Makes a volcal plot.

### Value

silently returns a GRanges object of the sites higlighted in red.

### Author(s)

Rory Stark

### See Also

```
dba.analyze, dba.plotMA
```

### **Examples**

```
data(tamoxifen_analysis)

# default volcano plot
dba.plotVolcano(tamoxifen)

# only highlight significant sites with at least 10x Fold Change
sigSites <- dba.plotVolcano(tamoxifen, fold=log2(10))

# use labels to find outlier sites
sigSites <- dba.plotVolcano(tamoxifen, fold=5,bLabels=TRUE)
sigSites</pre>
```

dba.report

Generate a report for a differential binding affinity analysis

### **Description**

Generates a report for a differential binding affinity analysis

### Usage

### **Arguments**

th

contrast

DBA object. A differential binding affinity analysis needs to have been previ-

ously carried out (see dba.analyze).

contrast number to report on. When generating a report-based DBA object, this can be a vector of contrast numbers. If missing, defaults to first contrast for reports, and all contrasts when generating a report-based DBA object. See

dba.show(DBA,bContrast=T) to get contrast numbers.

method method used for analysis:

DBA\_DESEQ2

DBA\_DESEQ2\_BLOCK

• DBA EDGER

DBA EDGER BLOCK

When generating a report-based DBA object (see bDB and bNotDB parameters below), a list of methods may be supplied, including the shortcuts

• DBA\_ALL\_METHODS

• DBA\_ALL\_BLOCK

• DBA\_ALL\_METHODS\_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. 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.

bFlip logical indicating that order of groups in contrast should be "flipped", allowing

control of which sample group will have positive and which will have negative

fold changes.

precision If present, alters the default precision for the Concentration, Fold, p-value, and

FDR values in the returned report. A value of 0 indicates maximum precision. Otherwise, it should be a 2-value vector. The first value controls how many digits to the right of the decimal to include for concentration and fold values. These second value control how many digits to the right of the decimal to include for the p-value and FDRs. Default is precision=2:3, unless DataType=DBA\_DATA\_SUMMARIZED\_EXPERIMENT, in which case the default is 0

(full precision).

bCalled logical indicating that peak caller status should be included. This will add a col-

umn for each group, each indicating the number of samples in the group identified as a peak in the original peaksets. Note that this option is only available if the consensus peakset was calculated by dba.count; if a consensus peakset was passed in explcictly using the peaks parameter, original peak origins are lost.

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.

bDB logical indicating that a report-based DBA object should be generated, and that

it should include Differentially Bound (DB) sites (respecting the th, bUsePval,

and fold parameters).

bNotDB logical indicating that a report-based DBA object should be generated, and that

it should include non-Differentially Bound (non-DB) sites (respecting the th,

bUsePval, and fold parameters).

bAll logical indicating that a report-based DBA object should be generated, and that

it should include peaksets combining peaks with both positive and negative fold

changes.

bGain logical indicating that a report-based DBA object should be generated, and that

it should include peaksets with only positive fold changes.

bLoss logical indicating that a report-based DBA object should be generated, and that

it should include peaksets with only negative fold changes.

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

if neither bDB or bNotDB is set to TRUE, a report dataframe, GRanges, or RangedData object is returned, with a row for each binding site within the thresholding parameters, and the following columns:

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 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.

If bDB or bNotDB is set to TRUE, a special DBA object is returned, containing peaksets based on sites determined to be differentially bound (or not) as specified using the bDB, bNotDB, bGain, bLoss, and bAll parameters. In this DBA object, the Tissue value will specify the direction of the change (Gain for positive fold changes, Loss for negative fold changes, and All for any fold change). The Factor value specifies if the peaks are differentially bound (DB) or not (!DB). The Condition value specifies the analysis method (e.g. edgeR), and the Treatment value is blank for unblocked analyses and set to block for blocked analyses.

### Author(s)

Rory Stark

### See Also

dba.analyze

### **Examples**

```
data(tamoxifen_analysis)

#Retrieve DB sites with FDR < 0.05
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
tamoxifen.DB <- dba.report(tamoxifen, th=1, bCalled=TRUE)
tamoxifen.DB</pre>
```

44 dba.save

```
#Retrieve all sites with confidence stats and normalized counts
tamoxifen.DB <- dba.report(tamoxifen, th=1, bCounts=TRUE)</pre>
tamoxifen.DB
#Retrieve all sites with confidence stats and raw counts
tamoxifen.DB <- dba.report(tamoxifen, th=1, bCounts=TRUE,bNormalized=FALSE)</pre>
tamoxifen.DB
#Retrieve report as a SummarizedObject
tamoxifen.sset <- dba.report(tamoxifen, DataType=DBA_DATA_SUMMARIZED_EXPERIMENT)</pre>
tamoxifen.sset
#Retrieve report-based DBA object
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen, categories=DBA_CONDITION, block=tamoxifen$masks$MCF7)</pre>
tamoxifen <- dba.analyze(tamoxifen,bCorPlot=FALSE)</pre>
tamoxifen.DB <- dba.report(tamoxifen,method=c(DBA_DESEQ2,DBA_DESEQ2_BLOCK),</pre>
                           bDB=TRUE, bGain=TRUE, bLoss=TRUE, bAll=FALSE)
dba.plotVenn(tamoxifen.DB,1:4,label1="Single Factor GAIN",label2="Single Factor LOSS",
                             label3="Blocking Factor GAIN",label4="Blocking Factor LOSS")
```

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
42	1

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

dba.show 45

### **Examples**

```
## Not run:
data(tamoxifen_peaks)
savefile <- dba.save(tamoxifen,'tamoxifenPeaks')
savefile
tamoxifen <- dba.load('tamoxifenPeaks')
unlink(savefile)
## End(Not run)</pre>
```

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

### **Arguments**

DBA

mask

mask of peaksets for which to get attributes (used when obtaining peakset attributes, i.e. bContrasts=FALSE).

attributes

attribute or vector of attributes to retrieve. Number of intervals is always shown. Used when obtaining peakset attributes, i.e. bContrasts=FALSE. Values:

• DBA\_ID

DBA object

- DBA\_TISSUE
- DBA\_FACTOR
- DBA\_CONDITION
- DBA\_TREATMENT
- DBA\_REPLICATE
- DBA\_CONSENSUS
- DBA CALLER
- DBA\_CONTROL
- DBA\_INTERVALS
- DBA\_FRIP

 ${\tt bContrasts}$ 

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.

th

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.

bUsePval

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.

46 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

Members1 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
```

# **Examples**

```
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
tamoxifen$config$th <- .05
tamoxifen</pre>
```

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\_ALL\_ATTRIBUTES

DBA\_INTERVALS

DBA\_FRIP

DBA\_GROUP

DBA\_OLAP\_PEAKS

DBA\_OLAP\_ALL

DBA\_OLAP\_RATE

DBA\_COR

DBA\_OLAP

DBA\_INALL

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

DBA\_SCORE\_TMM\_READS\_FULL\_CPM

DBA\_SCORE\_TMM\_READS\_EFFECTIVE\_CPM

DBA\_SCORE\_TMM\_MINUS\_FULL\_CPM

DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE\_CPM

DBA\_SCORE\_SUMMIT

DBA\_SCORE\_SUMMIT\_ADJ

DBA\_SCORE\_SUMMIT\_POS

DBA\_READS\_DEFAULT DBA\_READS\_BAM DBA\_READS\_BED

DBA\_EDGER
DBA\_DESEQ
DBA\_DESEQ2
DBA\_EDGER\_BLOCK
DBA\_DESEQ\_BLOCK
DBA\_DESEQ2\_BLOCK
DBA\_EDGER\_CLASSIC
DBA\_DESEQ\_CLASSIC
DBA\_EDGER\_GLM
DBA\_DESEQ\_GLM
DBA\_ALL\_METHODS
DBA\_ALL\_BLOCK

DBA\_ALL\_METHODS\_BLOCK

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

DBA\_DATA\_SUMMARIZED\_EXPERIMENT

DBA\_DATA\_DBAOBJECT

### **Arguments**

DBA\_ID

DBA peakset metadata: Peakset ID

DBA\_FACTOR

DBA peakset metadata: 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\_CALLER

DBA peakset metadata: Peak Caller

DBA\_CONSENSUS DBA peakset metadata: Is this a consensus peakset?

DBA\_CONTROL DBA peakset metadata: ID of Control sample

DBA\_ALL\_ATTRIBUTES

DBA peakset metadata: all attributes that can be used in certain plot labels (cf

 $\label{locality} {\tt dba.plotVenn)}, equivalent to \verb|c(DBA_ID,DBA_TISSUE,DBA_FACTOR,DBA_CONDITION,DBA_TREATME|| \\$ 

DBA\_INTERVALS DBA peakset metadata: Number of intervals in peakset

DBA\_FRIP DBA peakset metadata: Fraction of Reads in Peaks (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\_COR When plotting a heatmap from an overlap record, use the correlation value.

DBA\_OLAP When plotting a heatmap from an overlap record, use the percentage overlap

value.

DBA\_INALL When plotting a heatmap from an overlap record, use the number of peaks in common to both samples.

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 Control

DBA\_SCORE\_READS\_MINUS

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 minus Control read counts and Effective Library size

DBA\_SCORE\_TMM\_READS\_FULL\_CPM

dba.count score is TMM normalized (using edgeR), using ChIP read counts and Full Library size, reported in counts-per-million.

DBA\_SCORE\_TMM\_READS\_EFFECTIVE\_CPM

dba.count score is TMM normalized (using edgeR), using ChIP read counts and Effective Library size, reported in counts-per-million.

DBA\_SCORE\_TMM\_MINUS\_FULL\_CPM

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

DBA\_SCORE\_TMM\_MINUS\_EFFECTIVE\_CPM

dba.count score is TMM normalized (using edgeR), using ChIP read counts minus Control read counts and Effective Library size, reported in counts-permillion.

DBA\_SCORE\_SUMMIT

dba.count score is summit height (highest pile-up).

DBA\_SCORE\_SUMMIT\_ADJ

dba.count score is summit height (highest pile-up), adjusted for library size.

DBA\_SCORE\_SUMMIT\_POS

dba.count score is summit location (position of highest pile-up).

DBA\_READS\_DEFAULT

When counting read files, use the file extension to determine the file type.

DBA\_READS\_BAM When counting read files, assume the file type is BAM, regardless of the file extension.

DBA\_READS\_BED When counting read files, assume the file type is BED (or zipped BED), regardless of the file extension.

DBA\_EDGER differential analysis method: edgeR (default: DBA\_EDGER\_GLM)
DBA\_DESEQ2 differential analysis method: DESeq2 (using a single-factor GLM)

DBA\_EDGER\_BLOCK

differential analysis method: edgeR with blocking factors (GLM)

DBA\_DESEQ2\_BLOCK

differential analysis method: DESeq2 with blocking factors (GLM)

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

DBA\_DESEQ\_BLOCK

differential analysis method: DESeq with blocking factors (GLM)

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\_ALL\_METHODS

use both analysis methods: c(DBA\_EDGER, DBA\_DESEQ2)

DBA\_ALL\_BLOCK report on block results for both analysis methods: c(DBA\_EDGER\_BLOCK, DBA\_DESEQ2\_BLOCK)
DBA\_ALL\_METHODS\_BLOCK

report on block results for all analysis methods, both blocked and unblocked: c(DBA\_ALL\_METHODS,DBA\_ALL\_BLOCK)

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.

DBA\_DATA\_DBAOBJECT

Return a result-based DBA object from dba.plotVenn.

### Note

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

### Author(s)

Rory Stark

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