DiffBind

March 24, 2012

```
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_REPLICATE DBA_CALLER DBA_CONSENSUS DBA_CONTROL DBA_GROUP DBA_OLAP_PEAKS DBA_OLAP_ALL DBA_OLAP_RATE DBA_SCORE_READS DBA_SCORE_RPKM DBA_SCORE_READS_FOLD DBA_SCORE_READS_MINUS DBA_SCORE_RPKM_FOLD DBA_EDGER DBA_DESEQ DBA_EDGER_BLOCK

Arguments

DBA_ID DBA peakset metadata: Peakset ID
DBA_FACTOR DBA peakset metadata: Factor

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DBA_TISSUE DBA peakset metadata: Tissue

DBA_CONDITION

DBA peakset metadata: Condition

DBA_REPLICATE

DBA peakset metadata: Replicate

DBA_CALLER DBA peakset metadata: Peak Caller

DBA_CONSENSUS

DBA peakset metadata: Is this a consensus peakset?

DBA_GROUP DBA peakset metadata: color PCA plot using contras groups

DBA_OLAP_PEAKS

dba.overlap mode: return overlapping/unique peaksets

 $\verb|DBA_OLAP_ALL| \ dba. overlap \ mode: \ return \ report \ of \ correlations/overlaps \ for \ each \ pair \ of \ samples$

DBA_OLAP_RATE

dba.overlap mode: return overlap rates

DBA_SCORE_READS

dba.count score should be number of reads

DBA_SCORE_RPKM

dba.count score should be RPKM

DBA_SCORE_READS_FOLD

dba.count score should be number of reads divided by number of reads in control

DBA_SCORE_READS_MINUS

dba.count score should be number of reads minus number of reads in control

DBA_SCORE_RPKM_FOLD

dba.count score should be RPKM divided by RPKM of control

DBA_EDGER differential analysis method: edgeR
DBA_DESEQ differential analysis method: DESeq

DBA_EDGER_BLOCK

differential analysis method: edgeR with blocking factor

Note

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

Author(s)

Rory Stark

DiffBind-package Differential Binding Analysis of ChIP-seq peaksets

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:

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dba: Construct a dba object

dba.peakset: Add a peakset to, or retrieve a peakset from, a dba object

dba.overlap: Compute binding site overlaps and/or correlations

dba.count: Count reads in binding sites

dba.contrast: Establish contrast(s) for analysis

dba.analyze: Execute affinity analysis

dba.report: Generate report for a contrast analysis

dba.plotHeatmap: Heatmap plot

dba.plotPCA: Principal Components plot

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

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

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

Author(s)

dba

Construct a DBA object

Description

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

Usage

```
dba(DBA,mask, minOverlap=2,
    sampleSheet="dba_samples.csv",
    config=data.frame(RunParallel=TRUE, reportInit="DBA"),
    caller='raw', skipLines=0, bAddCallerConsensus=FALSE,
    bRemoveM=TRUE, bRemoveRandom=TRUE,
    bCorPlot=FALSE, attributes)
```

Arguments

DBA existing DBA object - if present, will return a fully-constructed DBA object

based on the passed one, using criteria specified in the mask and/or minOverlap parameters. If missing, will create a new DBA object based on the sampleSheet.

mask logical or numerical vetcor indicating which peaksets to include in the resulting

model if basing DBA object on an existing one. See dba.mask.

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minOverlap

only include peaks in at least this many peaksets in the main binding matrix if basing DBA object on an existing one.

sampleSheet

data frame containing sample sheet, or file name of sample sheet to load (ignored if DBA is specified). Columns names in sample sheet should include:

- SampleID: Identifier string for sample
- Tissue: Identifier string for tissue type
- Factor: Identifier string for factor
- Condition: Identifier string for condition
- 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 (optional)
- 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
 - "macs": MACS .xls file
 - "swembl": SWEMBL .peaks file
 - "bayes": bayesPeak file
 - "peakset": peakset written out using pv.writepeakset
 - "fp4": FindPeaks v4

config

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

- RunParallel: logical indicating if counting and analysis operations should be run in parallel using multicore by default.
- RangedData: logical indicating if peaks should be RangedData objects by default.
- AnalysisMethod: either DBA_EDGER or DBA_DESEQ.

caller

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

skipLines

if a sampleSheet is specified, the number of lines (ie header lines) at the beginning 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 constructing 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.

bCorPlot

logical indicating that a correlation heatmap should be plotted before returning

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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

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)

Value

DBA object

Author(s)

Rory Stark and Gordon Brown

Examples

```
# Create DBA object from a samplesheet
setwd(system.file("extra", package="DiffBind"))
tamoxifen = dba(sampleSheet="tamoxifen.csv")
tamoxifen

#Create a DBA object with a subset of samples
data(tamoxifen_peaks)
Responsive = dba(tamoxifen,tamoxifen$masks$Responsive)
Responsive
```

dba.analyze

Perform differential binding affinity analysis

Description

Performs differential binding affinity analysis

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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(DBA).

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

Supported methods:

• DBA_EDGER

DBA_DESEQ

bSubControl logical indicating whether Control read counts are subtracted for each site in

each sample before performing analysis.

bFullLibrarySize

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

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

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

replicates), this should be set to FALSE.

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

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

default threholds, no heatmap will be plotted.

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

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

method).

Details

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

Value

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

Note

If the "edgeR" method is specified, and 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

Examples

```
data(tamoxifen_counts)

tamoxifen = dba.analyze(tamoxifen)

tamoxifen

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

tamoxifen
```

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dba.contrast Set up contrasts for differential binding affinity analysis	dba.contrast	Set up contrasts for differential binding affinity analysis	
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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.
categories	when automatically generating contrasts, attribute or vector of attributes to base contrasts on:
	 DBA_ID DBA_TISSUE DBA_FACTOR DBA_CONDITION DBA_REPLICATE DBA_CALLER
block	blocking attribute for multi-factor analysis. Only when adding a specific contrast, and will only work with edgeR analysis; see dba.analysis. There must be

blocking attribute for multi-factor analysis. Only when adding a specific contrast, and will only work with edgeR analysis; see dba.analysis. There must be at least one value for the specified attribute that is associated with samples in each of the two groups of the contrast. For example, if DBA_REPLICATE is specified, there must be at least one sample in each group with the same replicate number. Possible attribute values include:

- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_REPLICATE
- DBA_CALLER

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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

Examples

dba.count

tamoxifen

Count reads in binding site intervals

Description

Counts reads in binding site intervals

tamoxifen = dba.contrast(tamoxifen)

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Arguments

DBA object

peaks RangedData or matrix containing intervals to use. If missing, generates a con-

sensus peakset using minOverlap parameter.

minOverlap only include peaks in at least this many peaksets when generating consensus

peakset (i.e. when peaks parameter is missing).

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

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

DBA_SCORE_RPKM RPKM for interval using only reads from ChIP

DBA_SCORE_RPKM_FOLD RPKM for interval from ChIP divided by RPKM for interval from control

DBA_SCORE_READS raw read count for interval using only reads from ChIP

DBA_SCORE_READS_FOLD raw read count for interval from ChIP divided by read count for interval from control DBA_SCORE_READS_MINUS raw read count for interval from ChIP minus read count for interval from control

bLog logical indicating whether log2 of score should be used (only applies to DBA_SCORE_RPKM_FOLD

and DBA_SCORE_READS_FOLD).

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

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

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

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

at least one sample has at least minMaxval reads will be included. If missing, includes all intervals. If peaks is NULL, will remove sites from existing DBA

object without recounting.

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

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

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

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

Examples

```
\# These won't run unless you have the reads available in a BAM, SAM, or BED file data(tamoxifen_peaks)
```

```
## Not run: tamoxifen = dba.count(tamoxifen)
```

```
# Count using a peakset made up of only peaks in all MCF7 replicates
data(tamoxifen_peaks)
mcf7Common = dba.overlap(tamoxifen,tamoxifen$masks$MCF7)
```

Not run: tamoxifen = dba.count(tamoxifen,peaks=mcf7Common\$inAll)
tamoxifen

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```
# Change binding affinity scores
data(tamoxifen_counts)
tamoxifen = dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_READS)
head(tamoxifen$vectors)
tamoxifen = dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_RPKM_FOLD)
head(tamoxifen$vectors)
```

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

Examples

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

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dba.mask

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

Description

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

Usage

Arguments

DBA

DBA object

attribute

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

- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_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 speficies the method for combining new mask with supplied mask:

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

bApply

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

dba.overlap

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_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

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
```

dba.overlap

Compute binding site overlaps (occupancy analysis)

Description

Computes binding overlaps and co-occupancy statistics

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Usage

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

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

Arguments

DRA 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 or three peaksets must be specified. If the mode type is DBA_OLAP_ALL, and a contrast is specified, a value of TRUE (mask=TRUE) indicates that all samples should be included (otherwise only those present in one of the contrast groups

will be included).

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

DBA_OLAP_PEAKS

• DBA_OLAP_ALL

• DBA_OLAP_RATE

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

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

mode=DBA_OLAP_ALL. See dba.show(DBA, bContrast=T) to get contrast num-

if contrast is specified and mode=DBA_OLAP_ALL, use data from method used met hod

for analysis:

DBA_EDGER

DBA DESEQ

• 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 report

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.

when computing co-occupancy statistics (DBA_OLAP_ALL), limit comparbyAttribute isons to peaksets with the same value for a specific attribute, one of:

DBA_ID

DBA_TISSUE

DBA FACTOR

DBA_CONDITION

th

DBA_REPLICATE

DBA_CONSENSUS

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• DBA_CALLER

• DBA_CONTROL

bCorOnly when computing co-occupancy statistics (DBA_OLAP_ALL), logical indicat-

ing 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.

bRangedData logical indicating whether, if mode==DBA_OLAP_PEAKS, the peaksets should

be returned as RangedData objects. Can be set as default behavior by setting

DBA\$config\$RangedData=TRUE.

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, and seven peaksets for a A-B-C overlap:

inAll	peaks in all peaksets
onlyA	peaks unique to peakset A
onlyB	peaks unique to peakset B
onlyC	peaks unique to peakset C
notA	peaks in both peaksets B and C but not peakset A
notB	peaks in both peaksets A and C but not peakset B
notC	peaks in both peaksets A and B but not 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 number of sites unique to peakset B

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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)

Rory Stark

Examples

dba.peakset

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

Description

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

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Arguments

normCol

bRemoveM

normalization

DBA DBA object. Required unless creating a new DBA object by adding an initial peakset. When adding a specified peakset: set of peaks, either a RangedData object, a peaks peak 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. 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 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: a peak matrix or RangedData object, or a peakset number; if NULL, retrieves/writes the full binding matrix. ID string for the peakset being added; if missing, one is assigned (a serial numsampID ber 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). replicate number for the peakset being added; if missing, one is assigned for a replicate consensus peakset (a concatenation of replicate numbers). control control name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of control names). peak caller name string. If peaks is specified as a file, this will control how it is peak.caller interpreted. Supported values: • "raw": text file file; peak score is in fourth column • "bed": .bed file; peak score is in fifth column • "macs": MACS .xls file • "swembl": SWEMBL .peaks file • "bayes": bayesPeak file • "peakset": peakset written out using pv.writepeakset • "fp4": FindPeaks v4 if missing, a name is assigned for a consensus peakset (a concatenation of peak caller names). total number of ChIPed library reads for the peakset being added. reads consensus TRUE if peakset being added is made from overlap of other peaksets (set automatically when adding a consensus peakset). file path of the BAM/SAM/BED file containing the aligned reads for the peakset bamReads being added. bamControl file path of the BAM/SAM/BED file containing the aligned reads for the control used for the peakset being added.

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

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

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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.

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.

bRangedData logical indicating whether, if bRetrieve is TRUE, the peakset should be returned

as a RangedData object. Can be set as default behavior by setting DBA\$config\$RangedData=TRUE.

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: 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

Examples

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```
sampID="MCF7.3",tissue="MCF7",factor="ER",condition="Responsive",repli
mcf7

#add a consensus peakset -- peaks in all three replicates
mcf7 = dba.peakset(mcf7, 1:3, minOverlap=3,sampID="MCF7_3of3")
mcf7

#retrieve the consensus peakset as RangedData object
mcf7.consensus = dba.peakset(mcf7,mcf7$masks$Consensus,bRetrieve=TRUE)
mcf7.consensus
```

dba.plotBox

Boxplots

Description

Boxplots for read count distributions within differentially bound sites

Usage

Arguments

DBA object.

contrast number of contrast to use for boxplot.

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

DBA_EDGERDBA_DESEQ

• DBA_EDGER_BLOCK

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

or equal to this value will be included in the boxplot.

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

olding.

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.plotBox

- DBA CONDITION
- DBA_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER
- DBA_CONTROL

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

bAllDecreased

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 thresh-

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

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

significantly differentially bound sites that increase in affinity.

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

significantly differentially bound sites that decrease in affinity.

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

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

boxplots.

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

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

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

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

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

attribute values appear for other values of attribute).

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

varwidth passed to boxplot notch passed to boxplot

... other arguments passed to boxplot

Details

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

Value

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

20 dba.plotHeatmap

Author(s)

Rory Stark

Examples

dba.plotHeatmap

Draw a binding site heatmap

Description

Draws a binding site heatmap

Usage

Arguments

DBA

DBA object.

attributes

attribute or vector of attributes to use for column labels:

- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER
- DBA CONTROL

maxSites

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

ing a correlation heatmap (correlations=FALSE)

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

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

ential binding affinity analysis (see dba.analyze). See dba.show(DBA, bCon-

trast=T) to get contrast numbers.

method analysis method (used in conjunction with contrast):

DBA_EDGERDBA_DESEQ

• DBA EDGER BLOCK

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

or equal to this value will be 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 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 con-

junction with contrast.

mask mask indicating a subset of peaksets to use when using global binding matrix

(contrast is missing). See dba.mask.

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 present, the global binding matrix will be sorted (descending) on the results, and the first maxSites used in the heatmap. Recommended sort function options include sd, mean,

median, min.

correlations logical indicating that a correlation heatmap should be plotted (TRUE). If FALSE,

a binding heatmap of scores/reads is plotted. This parameter can also be set to a correlation record; see dba.overlap(mode=DBA_OLAP_ALL), in which case a correlation heatmap is plotted based on the specified correlation record, using

the statistic specified in olPlot.

olPlot if correlations is specified as a dataframe returned by dba.overlap, indicates

which statistic to plot. One of:

• DBA_COR Correlation

• DBA OLAP Percentage overlap

• DBA_INALL number of peaks common to both samples

margin margin size of plot

colScheme Color scheme; see colorRampPalette RColorBrewer

distMethod distance method for clustering; see Dist amap.

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

Details

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

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

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

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

22 dba.plotMA

MODE: Binding heatmap plot using significantly differentially bound sites:

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

MODE: Binding heatmap plot using the global binding matrix:

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

Value

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

Author(s)

Rory Stark

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
dba.plotHeatmap(tamoxifen,contrast=1,th=1,correlations=FALSE,sortFun=var)
```

dba.plotMA

Generate MA and scatter plots of differential binding analysis results

Description

Generates MA and scatter plots of differential binding analysis results.

dba.plotPCA 23

Arguments

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

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

trast numbers.

method or vector of methods to plot results for:

DBA_EDGERDBA_DESEQ

DBA_EDGER_BLOCK

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

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

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

olding.

bNormalized logical indicating whether to plot normalized data using normalization factors

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

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

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

dotSize size of points on plot (cex).

... passed to plot.

Author(s)

Rory Stark

Examples

```
data(tamoxifen_analysis)

# default MA plot
dba.plotMA(tamoxifen)

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

dba.plotPCA

PCA plot

Description

Principal Component Analysis plot

24 dba.plotPCA

Arguments

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_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER
- DBA_CONTROL

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). 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_EDGER

DBA_DESEQ

DBA_EDGER_BLOCK

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

or equal to this value will be included in the 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.

mask mask indicating a subset of peaksets to use when using global binding matrix

(contrast is missing). See dba.mask.

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

using global binding matrix (contrast is missing).

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

trix or the covariance matrix. Passed into princomp.

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.

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

dba.plotVenn 25

Details

```
MODE: PCA plot using significantly differentially bound sites: dba.plotPCA(DBA, attributes, minval, maxval, contrast, method, th, bUsePval, b3D=F, vColors, dotSize, ...)

MODE: PCA plot using global binding matrix: dba.plotPCA(DBA, attributes, minval, maxval, mask, sites, b3D=F, vColors, dotSize, ...)
```

Value

matrix with color legend

Note

uses rgl package for 3D plots (if available)

Author(s)

Rory Stark

Examples

```
data(tamoxifen_peaks)
# peakcaller scores PCA
dba.plotPCA(tamoxifen)
# raw count correlation PCA
data(tamoxifen_analysis)
dba.plotPCA(tamoxifen)
#PCA based on normalized data for all sites
dba.plotPCA(tamoxifen,contrast=1,th=1)
#PCA based on DB sites only
par(mfrow=c(1,2))
dba.plotPCA(tamoxifen,contrast=1)
dba.plotPCA(tamoxifen,contrast=1,attributes=DBA_TISSUE)
```

dba.plotVenn

Draw 2-way or 3-way Venn diagrams of overlaps

Description

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

```
dba.plotVenn(DBA, mask, overlaps, label1, label2, label3, ...)
```

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Arguments

DBA	DBA object; if present, only the mask parameter will apply.
mask	mask or vector of peakset numbers indicating which peaksets to include in Venn diagram. Only 2 or 3 peaksets should be included. See dba.mask. Only one of mask or overlaps is used.
overlaps	overlap record, as computed by dba.overlap(Report=DBA_OLAP_PEAKS). Only one of mask or overlaps is used.
label1	label for first peakset in diagram
label2	label for second peakset in diagram
label3	label for third peakset in diagram
	arguments passed on to vennDiagram{limma}

Author(s)

Rory Stark

Examples

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

dba.report

Generate a report for a differential binding affinity analysis

Description

Generates a report for a differential binding affinity analysis

dba.report 27

Usage

Arguments

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

ously carried out (see dba.analyze).

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

numbers.

method used for analysis:

• DBA_EDGER

• DBA_DESEQ

• DBA EDGER BLOCK

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

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

binding sites in the report.

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

olding.

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

in the report.

bNormalized logical indicating that normalized data (using normalization factors computed

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

data.

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

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

peak in the original peaksets.

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

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

followed by columns for each sample in the second group.

bCalledDetail

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

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

columns for each sample in the second group.

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

filename.

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

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

bRangedData logical indicating whether the report should be returned as a RangedData object.

Can be set as default behavior by setting DBA\$config\$RangedData=TRUE.

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Value

A report dataframe or RangedData object, 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

 ${\tt Conc_group2} \quad Group \ 2 \ Concentration - mean \ (log) \ reads \ across \ all \ samples \ in \ second \ group$

Fold Fold difference – mean fold difference of binding affinity of group 1 over group

2 (Conc1 - Conc2). Absolute value indicates magnitude of the difference, and sign indicates which one is bound with higher affinity, with a positive value

indicating higher affinity in the first group

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

difference is not attributable to chance)

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

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

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

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

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

Author(s)

Rory Stark

Examples

```
data(tamoxifen_analysis)

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

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

#Retrieve all sites with confidence stats
# and how many times each site was identified as a peak
tamoxifen.DB = dba.report(tamoxifen, th=1, bCalled=TRUE)
tamoxifen.DB

#Retrieve all sites with confidence stats and normalized counts
```

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

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

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

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

Value

string containing full path and filename.

DBA object

Author(s)

Rory Stark

Examples

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

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dba.show

List attributes of peaksets of contrasts associated with a DBA object

Description

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

Usage

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

Arguments

DBA object

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

tributes, i.e. bContrasts=FALSE).

attributes attributes 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_TISSUE

• DBA_FACTOR

• DBA_CONDITION

• DBA_REPLICATE

• DBA CONSENSUS

• DBA_CALLER

DBA_CONTROL

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.

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)

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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 two 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

Author(s)

Rory Stark

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
```

DBA object methods Standard S3 methods for DBA object

Description

Standard S3 methods for DBA object.

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

Arguments

x DBA object
object DBA object

... Arguments passed on to parent methods

Details

S3 methods for DBA object

Author(s)

Rory Stark

Examples

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

DBA tamoxifen resistance dataset

Tamoxifen resistance dataset used for DBA examples

Description

Tamoxifen resistance dataset used for DBA examples

Usage

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

Arguments

```
tamoxifen_peaks
```

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

tamoxifen_counts

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

tamoxifen_analysis

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

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)
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