Package 'DiffBind'

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| 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:

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

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Author(s)

dba

Construct a DBA object

Description

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

Usage

```
dba(DBA,mask, minOverlap=2, sampleSheet="dba_samples.csv", config=data.frame(RunParallel=TRUE,reportInit="DBA",DataType=DBA_DATA_GRANGES,AnalysisIpeakCaller='raw', peakFormat, scoreCol, bLowerScoreBetter, 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 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

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- "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
- bLowerBetter: logical indicating that lower scores signify better peaks

config

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

- RunParallel: logical indicating if counting and analysis operations should be run in parallel using multicore by default.
- DataType: default class for peaks and reports (DBA_DATA_GRANGES, DBA_DATA_RANGEDDATA, or DBA_DATA_FRAME).
- AnalysisMethod: either DBA_EDGER or DBA_DESEQ.

peakCaller

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

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

- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA REPLICATE
- DBA CONSENSUS
- DBA_CALLER
- DBA_CONTROL

DBA object methods 5

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

See Also

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

Examples

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

tamoxifen = dba(sampleSheet="tamoxifen_allfields.csv")
tamoxifen

tamoxifen = dba(sampleSheet="tamoxifen_allfields.csv",config="config.csv")
tamoxifen

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

# change peak caller but leave peak format the same
setwd(system.file("extra", package="DiffBind"))
tamoxifen = dba(sampleSheet="tamoxifen.csv", peakCaller="macs", peakFormat="raw")
dba.show(tamoxifen, attributes=c(DBA_TISSUE,DBA_CONDITION,DBA_REPLICATE,DBA_CALLER))
```

DBA object methods

Standard S3 methods for DBA object

Description

Standard S3 methods for DBA object.

Usage

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

Arguments

x DBA objectobject DBA object... Arguments passed on to parent methods

Details

S3 methods for DBA object

Author(s)

Rory Stark

Examples

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

DBA tamoxifen resistance dataset

Tamoxifen resistance dataset used for DBA examples

Description

Tamoxifen resistance dataset used for DBA examples

Usage

```
\begin{tabular}{ll} $\operatorname{data}(\operatorname{tamoxifen\_peaks})$ \\ $\operatorname{data}(\operatorname{tamoxifen\_counts})$ \\ \\ $\operatorname{data}(\operatorname{tamoxifen\_analysis})$ \\ \end{tabular}
```

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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

```
dba.analyze(DBA, method=DBA$config$AnalysisMethod,
bSubControl=TRUE, bFullLibrarySize=FALSE, bTagwise=TRUE,
bCorPlot=TRUE, bReduceObjects=T, bParallel=DBA$config$RunParallel)
```

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_DESEQ

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DBA_EDGER_CLASSIC

• DBA_DESEQ_CLASSIC

• DBA_EDGER_GLM

DBA_DESEQ_GLM

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

each sample before performing analysis.

bFullLibrarySize logical indicating if the full library size (total number of reads in BAM/SAM/BED

file) for each sample is used for scaling normalization. If FALSE, the total number of reads present in the peaks for each sample is used (generally preferable).

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

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

replicates), this should be set to FALSE.

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

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

default threholds, no heatmap will be plotted.

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

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

ages, this should be set to FALSE.

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

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

method).

Details

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

Value

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

Note

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

Author(s)

Rory Stark

See Also

dba.contrast, dba.report

```
\label{eq:data} $\operatorname{data}(\operatorname{tamoxifen}_{\operatorname{counts}})$$ tamoxifen = dba.analyze(tamoxifen) $$ tamoxifen = dba.analyze(tamoxifen,method=c(DBA_EDGER,DBA_DESEQ))$$ tamoxifen
```

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| dba.contrast Set up contrasts for differential binding affinity analysis |
|--|
|--|

Description

Sets up contrasts for differential binding affinity analysis

Usage

```
\label{local_contrast} $$ dba.contrast(DBA, group1, group2=!group1, name1="group1", name2="group2", minMembers=3, block, categories = c(DBA\_TISSUE,DBA\_FACTOR,DBA\_CONDITION,DBA\_TREATMENT))
```

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 ${ m dba.mask.}$ |
| 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_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

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

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

Details

```
MODE: Set up all possible contrasts:
dba.contrast(DBA, minMembers, categories)
MODE: Set up a specific contrast:
dba.contrast(DBA, group1, group2, name1, name2, block)
```

Value

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

Note

```
Contrasts will only be set up for peaksets where DBA_CALLER == "counts". Contrasts can be cleared by DBA$contrasts=NULL.
```

Author(s)

Rory Stark

See Also

dba.analyze

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tamoxifen

tamoxifen\$contrasts=NULL

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

tamoxifen

tamoxifen\$contrasts=NULL

tamoxifen = dba.contrast(tamoxifen, categories=DBA CONDITION, block=tamoxifen\$masks\$MCF7)

tamoxifen = dba.analyze(tamoxifen)

tamoxifen

dba.count

Count reads in binding site intervals

Description

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

Usage

dba.count(DBA, peaks, minOverlap=2, score=DBA_SCORE_TMM_MINUS_EFFECTIVE, bLog=FALSE, insertLength, maxFilter, bRemoveDuplicates=FALSE, bScaleControl=TRUE, bCalledMasks=TRUE, bCorPlot=TRUE, bParallel=DBA\$config\$RunParallel)

Arguments

DBA DBA object

peaks If GRanges, RangedData, dataframe, or matrix, this parameter contains the in-

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, changes the score used in the global binding matrix to the score type specified in the score parameter

without re-counting.

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

peakset (i.e. when peaks parameter is missing). If minOverlap is between zero

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

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

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

DBA_SCORE_READS raw read count for interval using only reads from ChIP

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

DBA_SCORE_RPKM RPKM for interval using only reads from ChIP

DBA_SCORE_RPKM_FOLD RPKM for interval from ChIP divided by RPKM for interval from control DBA SCORE TMM READS FULL TMM normalized (using edgeR), using ChIP read counts and Full Library

DBA_SCORE_TMM_READS_EFFECTIVE TMM normalized (using edgeR), using ChIP read counts and Effective L

DBA_SCORE_TMM_MINUS_FULL

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

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

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

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bLog logical indicating whether log2 of score should be used (only applies to DBA SCORE RPKM FOLD

and DBA_SCORE_READS_FOLD).

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

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

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

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

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

object without recounting.

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.

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

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

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

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

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

See Also

dba.analyze

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

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

# Count using a peakset made up of only peaks in all responsive MCF7 replicates data(tamoxifen_peaks)

mcf7Common = dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)

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

tamoxifen

#First make consensus peaksets from each set of replicates, then derive master consensus set for counting from those data(tamoxifen_peaks)

tamoxifen = dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)

## Not run: tamoxifen = dba.count(tamoxifen, peaks=tamoxifen$masks$Consensus)
```

dba.load

```
tamoxifen
```

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

See Also

dba.save

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

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dba.mask

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

Description

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

Usage

```
dba.mask(DBA, attribute, value, combine='or', mask, merge='or', bApply=FALSE, peakset, minValue=-1)
```

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 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.

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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

```
data(tamoxifen_peaks)

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

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

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dba.overlap

Compute binding site overlaps (occupancy analysis)

Description

Computes binding overlaps and co-occupancy statistics

Usage

```
dba.overlap(DBA, mask, mode=DBA_OLAP_PEAKS, minVal=0,
       contrast, method=DBA$config$AnalysisMethod, th=.1, bUsePval=FALSE,
       report, by Attribute, bCorOnly=TRUE, CorMethod="pearson",
       DataType=DBA$config$DataType)
```

Arguments

DBA DBA object

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

> 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

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

numbers.

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

for analysis:

DBA_EDGER

• DBA_DESEQ

DBA_EDGER_BLOCK

DBA_DESEQ_BLOCK

if contrast is specified and mode=DBA_OLAP_ALL, significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included. A value of 1 will include all binding sites, but only the samples

included in the contrast.

if contrast is specified and mode=DBA_OLAP_ALL, logical indicating whether

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

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.

mode

method

th

bUsePval

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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
- 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.

DataType if mode==DBA_OLAP_PEAKS, the class of object that peaksets should be re-

turned as:

- DBA_DATA_GRANGES
- DBA_DATA_RANGEDDATA
- DBA_DATA_FRAME

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

Details

MODE: Generate overlapping/unique peaksets:

dba.overlap(DBA, mask, mode=DBA OLAP PEAKS, minVal)

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

dba.overlap(DBA, mask, mode=DBA_OLAP_ALL, byAttribute, minVal, attributes, bCorOnly, CorMethod)

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

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

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

MODE: Compute overlap rates at different stringency thresholds:

dba.overlap(DBA, mask, mode=DBA_OLAP_RATE, minVal)

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

peaks unique to peakset A onlyA

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| onlyB | peaks unique to peakset B |
|--------|------------------------------------|
| onlyC | peaks unique to peakset C |
| only D | peaks unique to peakset D |
| notA | peaks in all peaksets except peaks |

set A notBpeaks in all peaksets except peakset B notC peaks in all peaksets except peakset C notDpeaks 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

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

Α 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

inAllnumber of peaks in both peakset A and B (merged)

R2correlation 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

See Also

dba.plotVenn, dba.plotHeatmap

```
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
# mode: DBA OLAP RATE -- get overlap rate vector
data(tamoxifen peaks)
rate = dba.overlap(tamoxifen, mode=DBA OLAP RATE)
plot(rate,type='b',xlab="# peaksets",ylab="# common peaks",
   main="Tamoxifen dataset overlap rate")
```

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

```
dba.peakset(DBA=NULL, peaks, sampID, tissue, factor, condition, treatment, replicate,
        control, peak.caller, peak.format, reads=0, consensus=FALSE,
        bamReads, bamControl,
        scoreCol, bLowerScoreBetter, bRemoveM=TRUE, bRemoveRandom=TRUE,
        minOverlap=2, bMerge=TRUE,
        bRetrieve=FALSE, writeFile, numCols=4,
        DataType=DBA$config$DataType)
```

Arguments

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 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 t include int he consensus. If missing or NULL, a consensus is derived from all peaksets present in the model. See dba.mask, or dba.show to get peakset numbers.

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

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

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

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 name for the peakset being added; if missing, one is assigned for a con-

sensus peakset (a concatenation of tissues).

peaks

sampID

tissue

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

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: SWEMBLfile

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

peak.format

condition

treatment

replicate

control

peak.caller

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
- "tpic": TPIC file; scoreCol=0 (all scores=1)
- "sicer": SICER file; scoreCol=7
- "fp4": FindPeaks v4 file; scoreCol=5
- "swembl": SWEMBLfile; scoreCol=4

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.

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

bRemoveRandom

logical indicating whether to remove peaks on chrN_random when adding a

peakset

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

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

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

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

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

all the peaksets have been added.

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

writeFile file to write retrieved peakset.

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

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

Ignored when writing out complete binding matrix.

DataType The class of object for returned peaksets:

• DBA_DATA_GRANGES

• DBA_DATA_RANGEDDATA

• DBA_DATA_FRAME

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

Details

MODE: Add a specified peakset:

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

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

dba.peakset(DBA, peaks, minOverlap)

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

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

MODE: Retrieve a peakset:
dba.peakset(DBA, peaks, bRetrieve=T)

MODE: Write a peakset out to a file:
dba.peakset(DBA, peaks, bRetrieve=T, writeFile, numCols)
```

Value

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

Author(s)

Rory Stark

See Also

to add peaksets using a sample sheet, see dba.

```
# create a new DBA object by adding three peaksets
mcf7 = dba.peakset(NULL,
             peaks=system.file("extra/peaks/MCF7_ER_1.bed.gz", package="DiffBind"),
         sampID="MCF7.1",tissue="MCF7",factor="ER",condition="Responsive",replicate=1)
mcf7 = dba.peakset(mcf7,
             peaks=system.file("extra/peaks/MCF7_ER_2.bed.gz", package="DiffBind"),
         sampID="MCF7.2",tissue="MCF7",factor="ER",condition="Responsive",replicate=2)
mcf7 = dba.peakset(mcf7,
             peaks=system.file("extra/peaks/MCF7 ER 3.bed.gz", package="DiffBind"),
         sampID="MCF7.3",tissue="MCF7",factor="ER",condition="Responsive",replicate=3)
mcf7
#retrieve peaks that are in all three peaksets
mcf7.consensus = dba.peakset(mcf7, 1:3, minOverlap=3, bRetrieve=TRUE)
mcf7.consensus
#add a consensus peakset -- peaks in all three replicates
mcf7 = dba.peakset(mcf7, 1:3, minOverlap=3,sampID="MCF7 3of3")
#add consensus peaksets for all sample types by combining replicates
data(tamoxifen peaks)
tamoxifen = dba.peakset(tamoxifen,consensus = -DBA REPLICATE)
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
#add consensus peaksets for all sample types by (same tissue and condition)
data(tamoxifen_peaks)
tamoxifen = dba.peakset(tamoxifen, consensus = c(DBA TISSUE, DBA CONDITION))
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,tamoxifen$masks$Responsive & tamoxifen$masks$Consensus)
#create consensus peaksets from sample type consensuses for Responsive and Resistant sample groups
tamoxifen = dba.peakset(tamoxifen,peaks = tamoxifen \$ masks \$ Consensus, consensus = DBA \quad CONDITION)
```

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```
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)
mcf7.consensus
```

dba.plotBox

Boxplots

Description

Boxplots for read count distributions within differentially bound sites

Usage

```
dba.plotBox(DBA, contrast=1, method=DBA$config$AnalysisMethod, th=0.1, bUsePval=FALSE, bNormalized=TRUE, attribute=DBA_GROUP, bAll=FALSE, bAllIncreased=FALSE, bAllDecreased=FALSE, bDB=TRUE, bDBIncreased=TRUE, bDBDecreased=TRUE, pvalMethod=wilcox.test, bReversePos=FALSE, attribOrder, vColors, varwidth=TRUE, notch=TRUE, ...)
```

Arguments

DBA DBA object.

contrast number of contrast to use for boxplot.

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

• DBA EDGER

• DBA_DESEQ

DBA_EDGER_BLOCK

DBA_DESEQ_BLOCK

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

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

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

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DBA_REPLICATEDBA_CONSENSUSDBA_CALLER

• DBA_CONTROL

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

gardless of whether or not they pass the significance threshold.

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

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

old.

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

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

old.

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

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

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

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

significantly differentially bound sites that increase in affinity.

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

significantly differentially bound sites that decrease in affinity.

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

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

boxplots.

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

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

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

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

NULL, default order is used (group order for DBA_GROUP, and the order the

attribute values appear for other values of attribute).

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

varwidth passed to boxplot notch passed to boxplot

... other arguments passed to boxplot

Details

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

Value

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

Author(s)

Rory Stark

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Examples

```
data(tamoxifen_analysis)

#default boxplot includes all DB sites, then divided into those increasing
# affinity in each group
dba.plotBox(tamoxifen)

# plot non-normalized data for DB sites by tissue
# (changing order to place Resistant samples last)
dba.plotBox(tamoxifen, attribute=DBA_CONDITION, bDBIncreased=FALSE,
bDBDecreased=FALSE, attribOrder=c(2,1), bNormalized=FALSE)
```

dba.plotHeatmap

Draw a binding site heatmap

Description

Draws a binding site heatmap

Usage

```
dba.plotHeatmap(DBA, attributes=DBA$attributes, maxSites=1000, minval, maxval, contrast, method=DBA$config$AnalysisMethod, th=.1, bUsePval=FALSE, report, score, mask, sites, sortFun, correlations=TRUE, olPlot=DBA_COR, ColAttributes,RowAttributes, colSideCols, rowSideCols = comargin=10, colScheme="Greens", distMethod="pearson", ...)
```

Arguments

DBA DBA object.

attributes

attribute or vector of attributes to use for column labels:

- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_TREATMENT
- DBA_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER
- DBA_CONTROL

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

ing a correlation heatmap (correlations=FALSE)

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 differential binding affinity analysis (see dba.analyze). Only significantly differentially bound sites will be used (subject to the th and bUsePval parameters). If mask is unspecified, only the samples in the contrast will be included. See dba.show(DBA, bContrast=T) to get contrast numbers. If missing, uses scores in the main binding matrix.

method

analysis method (used in conjunction with contrast):

- DBA EDGER
- DBA_DESEQ
- DBA_EDGER_BLOCK
- DBA_DESEQ_BLOCK

th

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

bUsePval

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

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
- DBA_SCORE_READS_FOLD
- DBA_SCORE_RPKM
- DBA_SCORE_RPKM_FOLD
- DBA_SCORE_TMM_READS_FULL
- DBA_SCORE_TMM_READS_EFFECTIVE
- DBA_SCORE_TMM_MINUS_FULL
- DBA_SCORE_TMM_MINUS_EFFECTIVE

mask

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 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:

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- DBA COR Correlation
- DBA_OLAP Percentage overlap
- DBA_INALL number of peaks common to both samples

ColAttributes

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

- DBA GROUP
- DBA_TISSUE
- DBA_FACTOR
- DBA CONDITION
- DBA TREATMENT
- DBA_REPLICATE
- DBA_CALLER

RowAttributes

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

the plotted attribute is DBA_GROUP.

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

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

margin margin size of plot

colScheme Color scheme; see colorRampPalette RColorBrewer

 ${\rm dist} {\rm Method} \qquad \quad {\rm dist} {\rm amap.}$

... 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, 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.

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

dba.plotMA

Generate MA and scatter plots of differential binding analysis results

Description

Generates MA and scatter plots of differential binding analysis results.

Usage

```
dba.plotMA(DBA, contrast=1, method=DBA$config$AnalysisMethod, th=.1, bUsePval=FALSE, fold=0, bNormalized=TRUE, factor="", bXY=FALSE, dotSize=.33, bSignificant=TRUE, bSmooth=TRUE, ...)
```

dba.plotMA

Arguments

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

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

contrast numbers.

method method or vector of methods to plot results for:

DBA_EDGERDBA_DESEQ

• DBA_EDGER_BLOCK

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

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

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

olding.

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

red).

bNormalized logical indicating whether to plot normalized data using normalization factors

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

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

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

dotSize size of points on plot (cex).

bSignificant Logical indicating if points corresponding to significantly differentially bound

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

in red.

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

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

... passed to plot.

Author(s)

Rory Stark

See Also

dba.analyze

```
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)
```

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dba.plotPCA

PCA plot

Description

Principal Component Analysis plot

Usage

```
dba.plotPCA(DBA, attributes, minval, maxval, contrast, method=DBA$config$AnalysisMethod, th=.1, bUsePval=FALSE, report, score, mask, sites, cor=FALSE, b3D=FALSE, vColors, dotSize, ...)
```

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
- 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). 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_EDGER
- DBA_DESEQ
- DBA_EDGER_BLOCK
- DBA_DESEQ_BLOCK

 $^{\mathrm{th}}$

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.

dba.plotPCA 31

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

• DBA_SCORE_READS_FOLD

DBA_SCORE_RPKM

• DBA_SCORE_RPKM_FOLD

• DBA_SCORE_TMM_READS_FULL

• DBA_SCORE_TMM_READS_EFFECTIVE

• DBA_SCORE_TMM_MINUS_FULL

• DBA_SCORE_TMM_MINUS_EFFECTIVE

mask 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).

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.

... 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

matrix with color legend

Note

uses rgl package for 3D plots (if available)

Author(s)

Rory Stark

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See Also

dba.analyze, dba.plotHeatmap

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)
```

 ${\bf dba.plotVenn}$

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

Description

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

Usage

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

Arguments

| DBA | DBA object; if present, only the mask parameter will apply. |
|----------|--|
| mask | mask or vector of peakset numbers indicating which peaksets to include in Venn diagram. Only 2 or 3 peaksets should be included. See dba.mask. Only one of mask or overlaps is used. |
| overlaps | overlap record, as computed by 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 |
| label4 | label for fourth peakset in diagram |
| main | main title for plot |
| sub | subtitle for plot |

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Note

Venn plotting code written by Thomas Girke as part of overLapper code: http://manuals.bioinformatics.ucr.edu/home/R_BioCondManual#R_graphics_venn

Author(s)

Rory Stark and Thomas Girke

See Also

dba.analyze, dba.overlap, dba.plotPCA

Examples

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

dba.report

Generate a report for a differential binding affinity analysis

Description

Generates a report for a differential binding affinity analysis

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Usage

$$\label{lem:bushes} \begin{split} & dba.report(DBA,\,contrast=1,\,method=DBA\$config\$AnalysisMethod,\\ & th=.1,\,bUsePval=FALSE,\,fold=0,\,\,bNormalized=TRUE,\\ & bCalled=FALSE,\,bCounts=FALSE,\,bCalledDetail=FALSE,\\ & file,initString=DBA\$config\$reportInit,ext='csv',\\ & DataType=DBA\$config\$DataType) \end{split}$$

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 con-

trast numbers.

method method used for analysis:

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. A value of 1 will include all

binding sites in the report.

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

olding.

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

in the report.

bNormalized logical indicating that normalized data (using normalization factors computed

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

data.

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

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

a peak in the original peaksets.

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

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

followed by columns for each sample in the second group.

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

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

columns for each sample in the second group.

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

filename.

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

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

DataType The class of object for returned report:

• DBA_DATA_GRANGES

• DBA_DATA_RANGEDDATA

• DBA DATA FRAME

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

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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

Conc_group2 Group 2 Concentration – mean (log) reads across all samples in second group

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

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

indicating higher affinity in the first group

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

difference is not attributable to chance)

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

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

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

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

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

Author(s)

Rory Stark

See Also

dba.analyze

```
data(tamoxifen_analysis)

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

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

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

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```
\label{eq:tamoxifen.DB} $$ tamoxifen.DB = dba.report(tamoxifen, th=1, bCalled=TRUE)$ tamoxifen.DB $$ \#Retrieve all sites with confidence stats and normalized counts $$ tamoxifen.DB = dba.report(tamoxifen,th=1,bCounts=TRUE)$ tamoxifen.DB $$ \#Retrieve all sites with confidence stats and raw counts $$ tamoxifen.DB = dba.report(tamoxifen,th=1,bCounts=TRUE,bNormalized=FALSE)$ tamoxifen.DB $$
```

dba.save

save DBA object

Description

Writes out DBA object

Usage

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

Arguments

| DBA | DBA object |
|------|---------------|
| file | main filename |

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

ext extensions to use

bMinimize logical indicating saved DBA object should be compressed as much as possible.

Value

string containing full path and filename.

Author(s)

Rory Stark

See Also

dba.load

```
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 DBA object

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

tributes, 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_TISSUE

• DBA_FACTOR

• DBA_CONDITION

DBA_CONDITION

DBA_REPLICATE

• DBA_CONSENSUS

• DBA_CALLER

• DBA_CONTROL

• DBA_INTERVALS

• DBA_SN_RATIO

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

Group 2 Label for first group of contrast

Members3 Number of samples in first group of contrast

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

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

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

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

sis using edgeR

DB.DESeq.block

Number of significantly differentially bound sites identified for blocking analy-

sis using DESeq

Author(s)

Rory Stark

See Also

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

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

#alternatively:
tamoxifen
```

DiffBind – DBA global constant variables

Constant variables used in DiffBind package

Description

Constant variables used in DiffBind package

Usage

```
DBA ID
DBA FACTOR
DBA_TISSUE
DBA_CONDITION
DBA TREATMENT
DBA REPLICATE
DBA CALLER
DBA CONSENSUS
{\tt DBA\_CONTROL}
DBA INTERVALS
DBA_SN_RATIO
DBA GROUP
DBA OLAP PEAKS
DBA_OLAP_ALL
DBA_OLAP_RATE
DBA\_SCORE\_READS
DBA SCORE READS MINUS
DBA SCORE READS FOLD
DBA SCORE RPKM
DBA SCORE RPKM FOLD
DBA_SCORE_TMM_READS_FULL
DBA_SCORE_TMM_READS_EFFECTIVE
DBA_SCORE_TMM_MINUS_FULL
DBA SCORE TMM MINUS EFFECTIVE
DBA EDGER
DBA DESEQ
{\tt DBA\_EDGER\_BLOCK}
DBA DESEQ BLOCK
DBA_EDGER_CLASSIC
DBA_DESEQ_CLASSIC
DBA EDGER GLM
DBA DESEQ GLM
DBA DATA FRAME
DBA DATA GRANGES
DBA DATA RANGEDDATA
```

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 INTERVALS

DBA peakset metadata: Number of intervals in peakset

DBA SN RATIO

DBA peakset metadata: Signal to Noise ratio (number of reads in intervals divided by total number of reads in library)

DBA GROUP DBA peakset metadata: color PCA plot using contras groups

DBA OLAP PEAKS

dba.overlap mode: return overlapping/unique peaksets

DBA_OLAP_ALL

dba.overlap mode: return report of correlations/overlaps for each pair of samples

DBA OLAP RATE

dba.overlap mode: return overlap rates

DBA_SCORE READS

dba.count score is number of reads in ChIP

DBA SCORE READS FOLD

dba.count score is number of reads in ChIP divided by number of reads in 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 EDGER differential analysis method: edgeR (default: DBA_EDGER_GLM)

DBA DESEQ differential analysis method: DESeq (default: DBA DESEO CLASSIC)

DBA EDGER CLASSIC

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

DBA_DESEQ_CLASSIC

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

DBA EDGER GLM

differential analysis method: use GLM in edgeR for two-group comparisons

DBA DESEQ GLM

differential analysis method: use GLM in DESeq for two-group comparisons

DBA EDGER BLOCK

differential analysis method: edgeR with blocking factors (GLM)

DBA DESEQ BLOCK

differential analysis method: DESeq with blocking factors (GLM)

DBA DATA GRANGES

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

DBA_DATA RANGEDDATA

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

DBA_DATA_FRAME

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

Note

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

Author(s)

Rory Stark

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