Package 'DiffBind'

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Type Package

Version 3.0.15

Title Differential Binding Analysis of ChIP-Seq Peak Data

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

License Artistic-2.0

LazyLoad yes

Depends R (>= 4.0), GenomicRanges, SummarizedExperiment

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GenomicAlignments, locfit, stats, utils, IRanges, lattice,
systemPipeR, tools, Rcpp, dplyr, ggplot2, BiocParallel,
parallel, S4Vectors, Rsamtools (>= 2.0), DESeq2, methods,
graphics, ggrepel, apeglm, ashr, GreyListChIP

Suggests BiocStyle, testthat, xtable

Enhances rgl, XLConnect, edgeR, csaw, BSgenome, GenomeInfoDb

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SystemRequirements GNU make

Collate core.R parallel.R model.R counts.R contrast.R normalize.R
analyze.R analyze_deseq2.R analyze_edgeR.R blacklist.R report.R
plots.R io.R helper.R utils.R RcppExports.R cpp_wrapper.R DBA.R

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Author Rory Stark [aut, cre],
Gord Brown [aut]

Maintainer Rory Stark <rory.stark@cruk.cam.ac.uk>

R topics documented:

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.plotVolcano:	Volcano plot
dba.show:	Show dba metadata
dba.mask:	Mask samples or sites
dba.save:	Save dba object
dba.load:	Load dba object

Author(s)

Rory Stark <rory.stark @at@ cruk.cam.ac.uk> and Gord Brown <gdbzork @at@ gmail.com>

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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(AnalysisMethod=DBA_DESEQ2,th=0.05,
                      DataType=DBA_DATA_GRANGES, RunParallel=TRUE,
                      minQCth=15, fragmentSize=125,
                      bCorPlot=FALSE, reportInit="DBA",
                      bUsePval=FALSE, design=TRUE,
                      doBlacklist=TRUE, doGreylist=TRUE),
    peakCaller="raw", peakFormat, scoreCol, bLowerScoreBetter,
    filter, skipLines=0,
    bAddCallerConsensus=FALSE,
    bRemoveM=TRUE, bRemoveRandom=TRUE,
    bSummarizedExperiment=FALSE,
    attributes, dir)
```

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

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

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:

- AnalysisMethod: either DBA_DESEQ2 or DBA_EDGER.
- th: default threshold for reporting and plotting analysis results.
- DataType: default class for peaks and reports (DBA_DATA_GRANGES,DBA_DATA_RANGEDDATA,or DBA_DATA_FRAME).

- RunParallel: logical indicating if counting and analysis operations should be run in parallel using multicore by default.
- minQCth: numeric, for filtering reads based on mapping quality score; only reads with a mapping quality score greater than or equal to this will be counted.
- fragmentSize: numeric with mean fragment size. Reads will be extended to this length before counting overlaps. May be a vector of lengths, one for each sample.
- bCorPlot: logical indicating that a correlation heatmap should be plotted automatically
- ReportInit: string to append to the beginning of saved report file names.
- bUsePval: logical, default indicating whether to use FDR (FALSE) or p-values (TRUE).
- doBlacklist: logical, whether to attempt to find and apply a blacklist if none is present when running dba.analyze.
- doBlacklist: logical, whether to attempt to generate and apply a greylist if none is present when running dba.analyze.

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

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

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

bLowerScoreBetter
                    if a sampleSheet is specified, the sort order for peak scores if the LowerBetter column is absent.

filter   if a sampleSheet is specified, a filter value if the Filter column is absent. Peaks with scores lower than this value (or higher if bLowerScoreBetter or LowerBetter is TRUE) will be removed.

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

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.

bSummarizedExperiment
                    logical indicating whether to return resulting object as a SummarizedExperiment.

bCorPlot   logical indicating that a correlation heatmap should be plotted before returning. If DBA is NULL (a new DBA object is being created), and bCorPlot is missing, then this will take the default value (FALSE). However if DBA is NULL (a new DBA object is being created), and bCorPlot is specified, then the specified value will become the default value of bCorPlot for the resultant DBA object.

attributes   vector of attributes to use subsequently as defaults when generating labels in plotting functions:

                    - DBA_ID

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

dir   Directory path. If supplied, files referenced in the sampleSheet will have this
                      path prepended. Applies to PeakFiles, bamReads, bamControl, and Spikein,
                      if present. If sampleSheet is a filepath, this will prepended to that as well.

Details

MODE: Construct a new DBA object from a samplesheet:

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

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

dba(DBA,mask,attributes)

MODE: Convert a DBA object to a SummarizedExperiment object:

dba(DBA,bSummarizedExperiment=TRUE)

Value

DBA object

Author(s)

Rory Stark and Gordon Brown

See Also

[dba.peakset](), [dba.show]()

Examples

```
# Create DBA object from a samplesheet
## Not run:
basedir <- system.file("extra", package="DiffBind")
tamoxifen <- dba(sampleSheet="tamoxifen.csv", dir=basedir)
tamoxifen

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

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

## End(Not run)

#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
basedir <- system.file("extra", package="DiffBind")
tamoxifen <- dba(sampleSheet="tamoxifen.csv", dir=basedir,
                 peakCaller="macs", peakFormat="raw", scoreCol=5 )
dba.show(tamoxifen, attributes=c(DBA_TISSUE,DBA_CONDITION,DBA_REPLICATE,DBA_CALLER))

# Convert DBA object to SummarizedExperiment
data(tamoxifen_counts)
sset <- dba(tamoxifen,bSummarizedExperiment=TRUE)
sset
```

DBA object methods   *Standard S3 methods for DBA object*

Description

Standard S3 methods for DBA object.

Usage

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

Arguments

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

Details

S3 methods for DBA object from the DiffBind package.

DBA objects are usually constructed using the dba function.

Author(s)

Rory Stark

Examples

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

DBA tamoxifen resistance dataset

Tamoxifen resistance dataset used for DBA examples

Description

Tamoxifen resistance dataset used for DBA examples

Usage

```
data(tamoxifen_peaks)

data(tamoxifen_counts)

data(tamoxifen_analysis)

data(tamoxifen_greylist)
```

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. Also includes background bins counts for background normalization.

`tamoxifen_analysis`

                load tamoxifen resistance dataset DBA object with count (affinity) data and `DESeq2`-based differential binding analysis results. This analysis uses a black-lists, computed greylists, background normalization, and a two-factor design.

`tamoxifen_greylist`

                load greylist for tamoxifen dataset. Generated as shown in `dba.blacklist` example: `dba.blacklist`.

Details

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

Data used to create these objects can be downloaded at '`http://DiffBind.starkhome.com/`'.

Value

loads a DBA object named `tamoxifen` (or `tamoxifen.greylist`).

Note

The script for generating these files (`GenerateDataFiles.R`) is included with the package in the `inst/extras` directory.

Author(s)

Rory Stark

Examples

```
data(tamoxifen_peaks)
tamoxifen
data(tamoxifen_counts)
plot(tamoxifen)
data(tamoxifen_analysis)
dba.plotMA(tamoxifen)
data(tamoxifen_greylist)
tamoxifen.greylist$master
```

---

dba.analyze   *Perform differential binding affinity analysis*

Description

Performs differential binding affinity analysis. Performs default generation of a consensus peakset, read counting, normalization, and setting up of contrasts if they have not been specified.

Usage

```
dba.analyze(DBA, method=DBA$config$AnalysisMethod, design,
            bBlacklist=DBA$config$doBlacklist,
            bGreylist=DBA$config$doGreylist,
            bRetrieveAnalysis=FALSE, bReduceObjects=TRUE,
            bParallel=DBA$config$RunParallel)
```

Arguments

DBA
Either a DBA object, or a sample sheet (either a character vector with the name of the sample sheet, or a data.frame containing the experimental metadata.

If no blacklist or greylists are included, a call will be made to dba.blacklist using defaults. This can be skipped by setting the bBlacklist and/or bGreylist parameters.

If no counts are included, a default consensus will be formed and read counts computed via a call to dba.count using defaults.

If no normalization has been specified, the reads will be normalized via a call to dba.normalize using defaults.

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

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

Supported methods:

- DBA_EDGER use edgeR package for analysis
- DBA_DESEQ2 use DESeq2 package for analysis
- DBA_ALL_METHODS perform two analyses, using both edgeR and DESeq2

design
If present and a character string, will be used as the design formula for the analysis, replacing any previously established design if present.

If FALSE, will complete analysis in pre-version 3 mode.

See link{dba.contrast}.

bBlacklist   If TRUE, and no blacklist has been applied to the DBA object (or when start-
                    ing from a samplesheet), the read bam files will be examined to determine
                    the reference genome, and an appropriate blacklist applied, if available. See
                    link{dba.blacklist}.

bGreylist   If TRUE, and no greylist has been applied to the DBA object (or when starting from
                    a samplesheet), the control bam files, if present, will be examined to determine
                    the reference genome, greylists will be computed for each, merged into a master
                    greylist, and applied to the peaksets. See link{dba.blacklist}.

bRetrieveAnalysis

                    If changed from FALSE, the underlying DE analysis object is returned instead of
                    running a new analysis. Possible values for bRetrieveAnalysis:

                    • DBA_DESEQ2 Returns the DESeq2 [DESeqDataSet](#).
                    • DBA_EDGER Returns the edgeR DGEList.
                    • TRUE Returns the DESeq2 [DESeqDataSet](#), if present. If not, returns the
                      edgeR DGEList, if present..

                    An analysis object will only be successfully returned if there is at least one
                    contrast utilizing an explicit design (see [dba.contrast](#)), and an analysis has
                    been carried out.

bReduceObjects   logical indicating whether strip the analysis objects of unnecessary fields to
                    save memory. If it is desired to use the DBA$contrasts[[n]]$edgeR and/or
                    DBA$contrasts[[n]]$DESeq2 objects directly in the edgeR and/or DESeq2 pack-
                    ages, this should be set to FALSE.

bParallel   logical indicating that the analyses is to be done in parallel using multicore
                    (one process for each contrast for each method, plus an additional process per
                    method).

Details

In general, prior to calling dba.analyze, [dba.count](#) should have been run. If no contrasts have
been established prior to invoking dba.analyze, then the default set of contrasts will be added
using ([dba.contrast](#)).

If no normalization parameters have been supplied by calling [dba.normalize](#), default normaliza-
tion parameters will be used.

See the DBA User Guide for more details on how the edgeR and DESeq2 analyses are carried out.

Value

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

Alternatively, an analysis object (either a [DESeqDataSet](#) or a DGEList) if bRetrieveAnalysis if
not FALSE.

Note

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

Author(s)

Rory Stark

See Also

dba.blacklist, dba.count, dba.contrast, dba.normalize, dba.report

Examples

```
data(tamoxifen_counts)
dba.analyze(tamoxifen)

tamoxifen <- dba.analyze(tamoxifen, method=DBA_ALL_METHODS,
                         design="~Tissue + Condition")
dba.show(tamoxifen, bContrasts=TRUE)

dba.analyze(tamoxifen, bRetrieveAnalysis=TRUE)
edger.object <- dba.analyze(tamoxifen, bRetrieveAnalysis=DBA_EDGER)
class(edger.object)
```

---

dba.blacklist   *Apply blacklists and/or greylists to peaks (and generate greylist)*

Description

Filters peak intervals that overlap a blacklist (from ENCODE or user supplied.) Filter peak intervals that overlap a greylist, either user supplied or generated based on aligned reads for control samples (e.g. Inputs).

Usage

```
dba.blacklist(DBA, blacklist=DBA$config$doBlacklist,
              greylist=DBA$config$doGreylist,
              Retrieve, cores=DBA$config$cores)
```

Arguments

DBA   DBA object

blacklist   If not equal to FALSE, specifies that a blacklist should be applied to the peak
                 intervals in the DBA object.

                 If equal to TRUE, the read bam files will be examined to determine an appropriate
                 reference genome. If successful, and a blacklist is available for that genome, it
                 will be applied.

                 A user specified blacklist can be specified by setting this parameter to a GRanges
                 object containing the blacklisted regions.

                 Otherwise, this parameter may be set to one of the following constants, indicat-
                 ing which of the ENCODE blacklists should be applied:

                 • DBA_BLACKLIST_HG19: Homo sapiens 19 (chromosomes have "chr")
                 • DBA_BLACKLIST_HG38: Homo sapiens 38 (chromosomes have "chr")
                 • DBA_BLACKLIST_GRCH37: Homo sapiens 37 (chromosomes are numbers)
                 • DBA_BLACKLIST_GRCH38: Homo sapiens 38 (chromosomes are numbers)
                 • DBA_BLACKLIST_MM9: Mus musculus 9
                 • DBA_BLACKLIST_MM10: Mus musculus 10
                 • DBA_BLACKLIST_CE10: C. elegans 10

- DBA_BLACKLIST_CE11: C. elegans 11
- DBA_BLACKLIST_DM3: Drosophila melanogaster 3
- DBA_BLACKLIST_DM6: Drosophila melanogaster 6

greylist   If not equal to FALSE, specifies that a greylist should be applied to the peak intervals in the DBA object.

If equal to TRUE, the control bam files (if present), will be examined to determine an appropriate reference genome. Genomes associated with a valid BSgenome can be detected. If successful, this genome will be used to generate greylists for each available control (eg specified as bamControls in the sample sheet.)).

The greylist parameter can also be set explicitly to either a valid BSgenome object, or a character string with the name of a valid BSgenome object.

The following constants map to a subset of possible BSgenome objects:

- DBA_BLACKLIST_HG19 : seqinfo from BSgenome.Hsapiens.UCSC.hg19
- DBA_BLACKLIST_HG38 : seqinfo from BSgenome.Hsapiens.UCSC.hg38
- DBA_BLACKLIST_GRCH38: seqinfo from BSgenome.Hsapiens.NCBI.GRCh38
- DBA_BLACKLIST_MM9 : seqinfo from BSgenome.Mmusculus.UCSC.mm9
- DBA_BLACKLIST_MM10: seqinfo from BSgenome.Mmusculus.UCSC.mm10
- DBA_BLACKLIST_CE10: seqinfo from BSgenome.Celegans.UCSC.ce10
- DBA_BLACKLIST_CE11: seqinfo from BSgenome.Celegans.UCSC.ce11
- DBA_BLACKLIST_DM3: seqinfo from BSgenome.Dmelanogaster.UCSC.dm3
- DBA_BLACKLIST_DM6: seqinfo from BSgenome.Dmelanogaster.UCSC.dm6

A user specified greylist can also be specified by setting this parameter to a GRanges object containing the greylisted regions. It can also be a list with an element named greylist$master, which is a GRanges object containing the greylist to be applied.

Retrieve   If present, some aspects of a previous run of the function is retrieved instead of returning a DBA object.

If Retrieve=DBA_BLACKLIST, the blacklist, if present, is returned as a GRanges object.

If Retrieve=DBA_GREYLIST, the greylist, if present, is returned. If it was generated from more than one control, it will be returned as a list object with the first element (named $master) a GRanges object containing the merged greylist, and the second element (named $controls) being a GRangesList with each element containing the greylist for one control

If Retrieve=DBA_BLACKLISTED_PEAKS, the excluded peaks for each sample will be returned in a GRangesList object (with each element containing the filtered peak intervals for each sample). If counts are available for the peaks, this will include the following metadata columns:

- cReads: Number of control reads overlapping this interval
- Reads: Number of primary (ChIP) reads overlapping this interval
- Score: Read score calculated by dba.count

Note that the if Retrieve is set, dba.blacklist must have been previously run, and all other parameters will be ignored.

cores   Parallel cores to use when running greylist generation.

Details

This function is intended to filter peak intervals that fall in regions of the genome that are known to be problematic for ChIP analysis. Blacklists, which are derived for a reference genome and should be applied for any experiments that use that reference, are distinguished from greylists, which are derived on a per-experiment basis using anomalous pileups in the control tracks (such as Inputs).

A core set of blacklists have been defined as part of the ENCODE project (see references).

Greylists can be generated using this function, which serves as a front-end to the `GreyListChIP` package. See the details of that package for more information on how it works. Note that the `GreyListChIP` package can be utilized separately to generate greylists with more fine-grained control, with the results passed back to `DiffBind` to filter peaks.

Value

DBA object, with peaks filtered (unless `Retrieve` is specified.)

Note

The p threshold can be altered by setting DBA$config$greylist.pval. The default is `0.999`. See `GreyListChIP::calcThreshold` for details.

Ideally, Blacklists and Greylists will be applied to the aligned reads prior to calling peaks, as removing reads in anomalous regions will yield better background noise models. Once greylists have been generated, peaks can be re-called and read into `DiffBind`.

Author(s)

Rory Stark with thanks to Gord Brown

References

- Amemiya HM, Kundaje A, Boyle AP. The ENCODE blacklist: identification of problematic regions of the genome. Sci Rep. 2019 Dec; 9(1) 9354 DOI: 10.1038/s41598-019-45839-z

- ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012 Sep 6;489(7414):57-74. doi: 10.1038/nature11247.

- Brown, Gord. Generating Grey Lists from Input Libraries. Bioconductor. https://bioconductor.org/packages/release

See Also

GreyListChIP (GreyList), BSgenome

Examples

```
data(tamoxifen_peaks)
## Not run: tamoxifen <- dba.blacklist(tamoxifen, blacklist=TRUE,
                                    greylist="BSgenome.Hsapiens.UCSC.hg19")
## End(Not run)
data(tamoxifen_greylist)
tamoxifen <- dba.blacklist(tamoxifen, blacklist=DBA_BLACKLIST_HG19,
                           greylist=tamoxifen.greylist$master)
dba.blacklist(tamoxifen,Retrieve=DBA_GREYLIST)

data(tamoxifen_counts)
tamoxifen <- dba.count(tamoxifen, peaks=NULL, score=DBA_SCORE_CONTROL_READS)
```

```
tamoxifen <- dba.blacklist(tamoxifen, blacklist=DBA_BLACKLIST_HG19,
                           greylist=tamoxifen.greylist$master)
blacklisted <- dba.blacklist(tamoxifen, Retrieve=DBA_BLACKLISTED_PEAKS)
mean(blacklisted[[1]]$cReads)
mean(dba.peakset(tamoxifen,peaks=1,bRetrieve=TRUE)$Score)
```

---

dba.contrast   *Set up contrasts for differential binding affinity analysis*

Description

Sets up contrasts for differential binding affinity analysis

Usage

```
dba.contrast(DBA, design=missing(group1), contrast,
             group1, group2=!group1, name1, name2,
             minMembers=3, block, bNot=FALSE, bComplex=FALSE,
             categories=c(DBA_TISSUE,DBA_FACTOR,DBA_CONDITION,DBA_TREATMENT),
             bGetCoefficients=FALSE, reorderMeta)
```

Arguments

DBA   DBA object with count data

design   Either a logical value, or a character string containing a valid design formula.

               If a logical value is specified, TRUE indicates that a design should automatically
               be generated. If contrast is missing, contrasts will automatically be added and
               an appropriate design computed. If a contrast is specified, it must consist of a
               character vector of length three, containing a factor and two factor values. No
               groups can be specified. If set to FALSE, the contrast will be added between the
               groups, if specified; otherwise, if group is missing, all possible contrasts will
               be added.

               If a design formula is specified, it must be composed from the following allow-
               able factors:

               • Tissue
               • Factor
               • Condition
               • Treatment
               • Replicate
               • Caller

               If design is not explictly specified, and no group is specified, then design will
               be set to the value of DBA$config$design, if present (see [DiffBind3](#)).

contrast   If a design has been specified (previously or in the current call), the following
               contrasts forms may be indicated:

               • Character vector of length three. The first element is a factor from the
                 design. The second and third elements are values for that factor associated
                 with sample groups.

- List of length 1, containing a design matrix column name (as obtained using bGetCoefficients).
- List of length 2, containing two design matrix column names (as obtained using bGetCoefficients), first the numerator and the second the denominator.
- Character vector of length one, containing a design matrix column name (as obtained using bGetCoefficients).
- Numeric vector of the same length as the list of design matrix column names (as obtained using bGetCoefficients), with a weighting for each column.

group1	mask of samples in first group (when adding a specific contrast). See [dba.mask](). Can not be used with an explicit design.
group2	mask of samples in second group (when adding a specific contrast). See [dba.mask](). Can not be used with an explicit design.
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.
bNot	include contrasts consisting of a group and all other samples not in that group (indicated by a ! in the contrast name).
bComplex	include complex contrasts where groups include samples with the same values for multiple factors.
categories	when automatically generating contrasts, attribute or vector of attributes to base contrasts on:

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

block	blocking attribute for multi-factor analysis. This may be specified as either a value, a vector, or a list.

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

- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_TREATMENT
- DBA_REPLICATE
- DBA_CALLER

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

bGetCoefficients

If TRUE, return the names of the columns (coefficients) associated with the design. These can be used to specify a contrast. If bGetCoefficients=TRUE, all other parameters (except DBA and design, if specified) will be ignored.

reorderMeta   By default, the metadata factor levels will be ordered in the order they appear in the sample sheet. They can be re-ordered using this parameter. reorderMeta is specified as a list, with each element being a vector of character strings corresponding to unique factor values in the desired order. Each element should be named for the appropriate metadata factor, one of:

- Tissue
- Factor
- Condition
- Treatment
- Replicate
- Caller

If the vector of factor values contains a subset of the possible values, the specified values will be set to be ordered first, with the remaining values following in their default order. If only one factor value is supplied, it will be set as the reference (or "control") value. Contrasts that are no longer valid will be removed (and a warning issued) if detected. These include contrasts specified as a numeric vector of coefficients, or contrasts specified using coefficient names that no longer exists after reordering the metadata factor levels. Any existing analysis will be removed when metadata factor levels are reordered, necessitating another call to dba.analyze

Details

MODE: Set up a specific contrast using a design:

dba.contrast(DBA,design,contrast)

MODE: Set up all possible contrasts:

dba.contrast(DBA,minMembers,categories)

MODE: Set up a specific contrast without an explicit design:

dba.contrast(DBA,design=FALSE,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=TRUE).

Note

Contrasts will only be set up for peaksets where DBA_CALLER == "counts".

Contrasts can be cleared by DBA$contrasts <-NULL.

Author(s)

Rory Stark

See Also

dba.analyze

Examples

```
# Set up an explicit contrast
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen, contrast=c("Condition","Responsive","Resistant"))
tamoxifen
tamoxifen <- dba.analyze(tamoxifen)
dba.show(tamoxifen,bContrasts=TRUE)

# Add another contrast
tamoxifen <- dba.contrast(tamoxifen, contrast=c("Tissue","MCF7","BT474"))
dba.show(tamoxifen,bDesign=TRUE)

# Change design
tamoxifen <- dba.contrast(tamoxifen,design="~Tissue + Condition")
tamoxifen <- dba.analyze(tamoxifen)
tamoxifen

# Automatically add all contrasts between sample groups
# where at least THREE samples have the same factor value
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen)
tamoxifen

# Automatically add all contrasts between sample groups
# where at least TWO samples have the same factor value
tamoxifen <- dba.contrast(tamoxifen, minMembers=2)
dba.show(tamoxifen,bContrasts=TRUE)

### Use of complex contrasts
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen, contrast=c("Tissue","BT474","MCF7"))
dba.contrast(tamoxifen, bGetCoefficients=TRUE)

#Change design and factor ordering
tamoxifen <- dba.contrast(tamoxifen,design="~Tissue + Condition",
                          reorderMeta=list(Condition="Responsive",
                          Tissue=c("MCF7","ZR75","T47D","BT474")))
dba.contrast(tamoxifen, bGetCoefficients=TRUE)
tamoxifen <- dba.contrast(tamoxifen,contrast="Tissue_BT474_vs_MCF7")
tamoxifen <- dba.contrast(tamoxifen,contrast=list("Tissue_BT474_vs_MCF7"))
tamoxifen <- dba.contrast(tamoxifen,contrast=c(0,0,0,1,0))
tamoxifen <- dba.contrast(tamoxifen,
                          contrast=list("Tissue_BT474_vs_MCF7","Tissue_T47D_vs_MCF7"))
tamoxifen <- dba.contrast(tamoxifen,contrast=c(0,0,-1,1,0))
tamoxifen <- dba.contrast(tamoxifen,contrast=c(0,0,0,0,1))
dba.show(tamoxifen,bContrasts=TRUE)
tamoxifen <- dba.analyze(tamoxifen)
tamoxifen
```

```
tamoxifen <- dba.contrast(tamoxifen,
                          contrast=c("Condition","Responsive","Resistant"))
tamoxifen <- dba.analyze(tamoxifen)
dba.show(tamoxifen,bContrasts=TRUE)[7:8,]
dba.plotVenn(tamoxifen, contrast=7:8, bDB=TRUE,
             bAll=FALSE, bGain=TRUE, bLoss=TRUE)

## Explicit contrast, without design
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen, design=FALSE,
                          group1=tamoxifen$masks$Responsive, name1="Responsive",
                          group2=tamoxifen$masks$Resistant,  name2="Resistant",
                          block=DBA_TISSUE)
dba.show(tamoxifen, bContrasts=TRUE)
tamoxifen <- dba.analyze(tamoxifen)
dba.show(tamoxifen,bContrasts=TRUE)
dba.plotVenn(tamoxifen,contrast=1,method=c(DBA_DESEQ2,DBA_DESEQ2_BLOCK))
```

---

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_NORMALIZED,
          fragmentSize=DBA$config$fragmentSize,
          summits=200, filter=1, bRemoveDuplicates=FALSE, bScaleControl=TRUE,
          bSubControl = is.null(DBA$greylist),
          mapQCth=DBA$config$mapQCth, filterFun=max, minCount=0,
          bLog=FALSE, bUseSummarizeOverlaps=TRUE,
          readFormat=DBA_READS_DEFAULT, bParallel=DBA$config$RunParallel)
```

Arguments

DBA	DBA object
peaks	If GRanges, RangedData, dataframe, or matrix, this parameter contains the intervals to use for counting. If character string, it specifies a file containing the intervals to use (with the first three columns specifying chromosome, startpos, endpos).If missing or a mask, generates a consensus peakset using minOverlap parameter (after applying the mask if present). If NULL, the score, filter, and summits parameters are honored, updating the global binding matrix without re-counting in the cases of score and filter, and only counting after re-centering in the case of summits.
minOverlap	only include peaks in at least this many peaksets when generating consensus peakset (i.e. when peaks parameter is missing). If minOverlap is between zero and one, peak will be included from at least this proportion of peaksets.
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_NORMALIZED	normalized reads, as set by dba.normalize
DBA_SCORE_READS	raw read count for interval using only reads from ChIP
DBA_SCORE_CONTROL_READS	raw read count for interval using only reads from Control
DBA_SCORE_READS_FOLD	raw read count for interval from ChIP divided by read count for interval from cont
DBA_SCORE_READS_MINUS	raw read count for interval from ChIP minus read count for interval from control
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_RPKM_MINUS	RPKM for interval from ChIP minus RPKM for interval from control
DBA_SCORE_SUMMIT	summit height (maximum read pileup value)
DBA_SCORE_SUMMIT_ADJ	summit height (maximum read pileup value), normalized to relative library size
DBA_SCORE_SUMMIT_POS	summit position (location of maximum read pileup)

fragmentSize	This value will be used as the length of the reads. Each read will be extended from its endpoint along the appropriate strand by this many bases. If set to zero, the read size indicated in the BAM/BED file will be used. fragmentSize may also be a vector of values, one for each ChIP sample plus one for each unique Control library.
summits	unless set to FALSE, summit heights (read pileup) and locations will be calculated for each peak. The values can retrieved using dba.peakset. The summits can also be used as a read score in the global binding matrix (see score).
	If the value of summits is TRUE (or 0), the summits will be calculated but the peaksets will be unaffected. If the value is greater than zero, all consensus peaks will be re-centered around a consensus summit, with the value of summits indicating how many base pairs to include upstream and downstream of the summit (so all consensus peaks will be of the same width, namely 2 * summits + 1).
	Note that if summits is no FALSE on the first call, the counting procedure will take twice as long..
filter	value to use for filtering intervals with low read counts. The filterFun will be applied to the counts for each interval, and if it returns a value below the filter value, the interval will be removed from further analysis. If peaks is NULL, will remove sites from existing DBA object without recounting. If filter is a vector of values, dba.count will return a vector of the same length, indicating how many intervals will be retained for each specified filter level.
	NB: the filtering will be based on RPKM values. If bSubControl is FALSE, this is the RPKM value of the read counts (equivalent to score=DBA_SCORE_RPKM. If bSubControl is TRUE, this is the RPKM value of the control counts subtracted from the RPKM of the read counts (equivalent to score=DBA_SCORE_RPKM_MINUS).
bRemoveDuplicates	
	logical indicating if duplicate reads (ones that map to exactly the same genomic position) should be removed. If TRUE, any location where multiple reads map will be counted as a single read. Note that if bLowMem is set, duplicates needs to have been already marked in all of the BAM files. The built-in counting code may not correctly handle certain cases when the bRemoveDuplicates parameter is set to TRUE. These cases include paired-end data and datasets where the read length may differ within a single BAM file. In these cases, see the bUseSummarizeOverlaps parameter.
bScaleControl	logical indicating if the Control reads should be scaled based on relative library sizes. If TRUE, and there are more reads in the Control library than in the ChIP library, the number of Control reads for each peak will be multiplied by a scaling factor determined by dividing the total number of reads in the ChIP library by

	the total number of reads in the Control library. If this value is not an integer, the number of Control reads for each peak will be the next highest integer.
bSubControl	logical indicating whether Control read counts are subtracted for each site in each sample. If there are more overlapping control reads than ChIP reads, the count will be set to the minCount value specified when dba.count was called, or zero if no value is specified.
	If bSubControl is not explicitly specified, it will be set to TRUE unless a greylist has been applied (see dba.blacklist).
mapQCth	for filtering by mapping quality (mapqc). Only alignments with mapping scores of at least this value will be included. Only applicable for bam files when bUseSummarizeOverlaps=FALSE (setting DBA$config$scanbamparam appropriately to filter on quality scores when using summarizeOverlaps.)
filterFun	function that will be invoked for each interval with a vector of scores for each sample. Returns a score that will be evaluated against the filter value (only intervals with a score at least as high as filter will be kept). Default is max, indicating that at least one sample should have a score of at least filter; other useful values include sum (indicating that all the scores added together should be at least filter) and mean (setting a minimum mean normalized count level). Users can supply their own function as well.
minCount	minimum read count value. Any interval with fewer than this many overlapping reads will be set to have this count. Also applies to scores.
bLog	logical indicating whether log2 of score should be used (only applies to DBA_SCORE_RPKM_FOLD and DBA_SCORE_READS_FOLD).
bUseSummarizeOverlaps	
	logical indicating that summarizeOverlaps should be used for counting instead of the built-in counting code. This option is slower but uses the more standard counting function. If TRUE, all read files must be BAM (.bam extension), with associated index files (.bam.bai extension). The fragmentSize parameter must absent.
	See notes for when the bRemoveDuplicates parameter is set to TRUE, where the built-in counting code may not correctly handle certain cases and bUseSummarizeOverlaps should be set to TRUE.
	Five additional parameters for summarizeOverlaps may be specified in DBA$config:

DBA$config$yieldSize	yieldSize indicating how many reads to process at one time; default is 5000000. The lowe
DBA$config$intersectMode	mode indicating which overlap algorithm to use; default is "IntersectionNotEmpty"
DBA$config$singleEnd	logical indicating if reads are single end; if NULL, status will be automatically detected.
DBA$config$fragments	logical indicating how unmatched reads are counted; default is FALSE
DBA$config$scanbamparam	ScanBamParam object to pass to summarizeOverlaps. If present, bRemoveDuplicates is

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

DBA_READS_DEFAULT	use file extension (.bam, .bed, .bed.gz) to determine file type
DBA_READS_BAM	assume the file type is BAM, regardless of the file extension
DBA_READS_BED	assume the file type is BED (or zipped BED), regardless of the file extension.

Note that if readFormat is anything other than DBA_READS_DEFAULT, all the read files must be of the same file type.

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

Value

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

Author(s)

Rory Stark and Gordon Brown

See Also

dba.analyze

Examples

```
# These won't run unless you have the reads available in a BAM or BED file
data(tamoxifen_peaks)
## Not run: tamoxifen <- dba.count(tamoxifen)


# Count using a peakset made up of only peaks in all responsive MCF7 replicates
data(tamoxifen_peaks)
mcf7Common <- dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
## Not run: tamoxifen <- dba.count(tamoxifen,peaks=mcf7Common$inAll)
tamoxifen

#First make consensus peaksets from each set of replicates,
#then derive master consensus set for counting from those
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)
## Not run: tamoxifen <- dba.count(tamoxifen, peaks=tamoxifen$masks$Consensus)
tamoxifen

# Change binding affinity scores
data(tamoxifen_counts)
dba.peakset(tamoxifen, bRetrieve=TRUE) # default: DBA_SCORE_NORMALIZED
tamoxifen <- dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_READS)
dba.peakset(tamoxifen, bRetrieve=TRUE)
tamoxifen <- dba.count(tamoxifen,peaks=NULL,score=DBA_SCORE_RPKM_MINUS)
dba.peakset(tamoxifen, bRetrieve=TRUE)

# Plot effect of a range of filter values and then apply filter
data(tamoxifen_counts)
rate.max <- dba.count(tamoxifen, peaks=NULL, filter=0:250)
rate.sum <- dba.count(tamoxifen, peaks=NULL, filter=0:250,filterFun=sum)
plot(0:250,rate.max/rate.max[1],type='l',xlab="Filter Value",ylab="Proportion Retained Sites")
lines(0:250,rate.sum/rate.sum[1],col=2)
tamoxifen <- dba.count(tamoxifen,peaks=NULL,filter=125,filterFun=sum)
tamoxifen

# Calculate summits
data(tamoxifen_counts)
# pre-counted with summits=250 or 501bp intervals
as.numeric(dba.show(tamoxifen)$FRiP)
## Not run: tamoxifen <- dba.count(tamoxifen,peaks=NULL,summits=50)
# re-counted with summits=50 or 101bp intervals
```

```
as.numeric(dba.show(tamoxifen)$FRiP)
```

---

dba.load   *load DBA object*

Description

Reads in saved DBA object

Usage

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

Arguments

file	main filename
dir	directory in which to save model
pre	string to pre-pend to filename
ext	file extension to use

Value

loaded DBA object

Author(s)

Rory Stark

See Also

[dba.save](#)

Examples

```
data(tamoxifen_peaks)
savefile <- dba.save(tamoxifen,'tamoxifenPeaks')
savefile
rm(tamoxifen)
tamoxifen <- dba.load('tamoxifenPeaks')
tamoxifen
unlink(savefile)
```

---

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 specifies the method for combining new mask with supplied mask:

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

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

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

minValue   when deriving a peak/site mask, scores greater than this value will be considered
                as indicating that the site corresponds to a called peakset.

Details

MODE: Derive a a mask of peaksets/samples:

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

MODE: Derive a mask of peaks/sites:

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

Value

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

Note

dba automatically generates masks for each unique value of DBA_TISSUE, DBA_FACTOR, DBA_CONDITION,
DBA_TREATMENT, DBA_CALLER, and DBA_REPLICATE. These are accessible using masks field of the
DBA object (DBA$masks), and can be viewed using names(DBA$masks).

Author(s)

Rory Stark

See Also

dba.show

Examples

```
data(tamoxifen_peaks)

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

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

---

dba.normalize   *Specify parameters for normalizing a dataset; calculate library sizes and normalization factors.*

Description

Enables normalization of datasets using a variety of methods, including background, spike-in, and parallel factor normalization. Alternatively, allows a user to specify library sizes and normalization factors directly, or retrieve computed ones.

Usage

```
dba.normalize(DBA, method = DBA$config$AnalysisMethod,
              normalize = DBA_NORM_DEFAULT, library = DBA_LIBSIZE_DEFAULT,
              background = FALSE, spikein = FALSE, offsets = FALSE,
              libFun=mean, bRetrieve=FALSE, ...)
```

Arguments

DBA   DBA object that includes count data for a consensus peakset.

method   Underlying method, or vector of methods, for which to normalize.

                Supported methods:

                - [DBA_EDGER](#) use edgeR package for analysis
                - [DBA_DESEQ2](#) use DESeq2 package for analysis
                - [DBA_ALL_METHODS](#) normalize for both both edgeR and DESeq2

normalize   Either user-supplied normalization factors in a numeric vector, or a specification of a method to use to calculate normalization factors.

                Methods can be specified using one of the following:

                - DBA_NORM_RLE ("RLE") RLE normalization (native to DBA_DESEQ2, and available for DBA_EDGER).
                - DBA_NORM_TMM ("TMM") TMM normalization (native to DBA_EDGER, and available for DBA_DESEQ2).
                - DBA_NORM_NATIVE ("native") Use native method based on method: DBA_NORM_RLE for DBA_DESEQ2 or DBA_NORM_TMM for DBA_EDGER.
                - DBA_NORM_LIB ("lib") Normalize by library size only. Library sizes can be specified using the library parameter. Normalization factors will be calculated to give each equal weight in a manner appropriate for the analysis method. See also the libFun parameter, which can be used to scale the normalization factors for DESeq2.
                - DBA_NORM_DEFAULT ("default") Default method: The "preferred" normalization approach depending on method and whether an explicit design is present. See Details below.
                - DBA_NORM_OFFSETS ("offsets") Indicates that offsets have been specified using the offsets parameter, and they should be used without alteration.
                - DBA_NORM_OFFSETS_ADJUST ("adjust offsets") Indicates that offsets have been specified using the offsets parameter, and they should be adjusted for library size and mean centering before being used in a DBA_DESEQ2 analysis.

library   Either user-supplied library sizes in a numeric vector, or a specification of a
                   method to use to calculate library sizes.

                   Library sizes can be based on one of the following:

                   - DBA_LIBSIZE_FULL ("full") Use the full library size (total number of reads
                     in BAM/SAM/BED file)
                   - DBA_LIBSIZE_PEAKREADS ("RiP") Use the number of reads that overlap
                     consensus peaks.
                   - DBA_LIBSIZE_BACKGROUND ("background") Use the total number of reads
                     aligned to the chromosomes for which there is at least one peak. This re-
                     quired a background bin calculation (see parameter background). These
                     values are usually the same or similar to DBA_LIBSIZE_FULL.
                   - DBA_LIBSIZE_DEFAULT ("default") Default method: The "preferred" library
                     size depending on method, background, and whether an explicit design is
                     present. See Details below.

background   This parameter controls the option to use "background" bins, which should not
                   have differential enrichment between samples, as the basis for normalizing (in-
                   stead of using reads counts overlapping consensus peaks). When enabled, the
                   chromosomes for which there are peaks in the consensus peakset are tiled into
                   large bins and reads overlapping these bins are counted.

                   If present, background can either be a logical value, a numeric value, or a pre-
                   viously computed $background object.

                   If background is a logical value and set to TRUE, background bins will be com-
                   puted using the default bin size of 15000bp. Setting this value to FALSE will
                   prevent background mode from being used in any default settings.

                   If background is a numeric value, it will be used as the bin size.

                   If background is a previously computed $background object, these counts will
                   be used as the background. A $background object can be obtained by calling
                   [dba.normalize](#) with bRetrieve=TRUE and method=DBA_ALL_METHODS.

                   After counting (or setting) background bins, both the normalize and library
                   parameters will be used to determine how the final normalization factors are
                   calculated.

                   If background is missing, it will be set to TRUE if library=DBA_LIBSIZE_BACKGROUND,
                   or if library=DBA_LIBSIZE_DEFAULT and certain conditions are met (see Details
                   below).

                   If background is not FALSE, then the library size will be set to library=DBA_LIBSIZE_BACKGROUND

spikein   Either a logical value, a character vector of chromosome names, a [GRanges](#)
                   object containing peaks for a parallel factor, or a $background object containing
                   previously computed spike-in read counts.

                   If spikein is a logical value set to FALSE, no spike-in normalization is per-
                   formed.

                   If spikein is a logical value set to TRUE, background normalization is performed
                   using spike-in tracks. There must be a spike-in track for each sample. see [dba](#)
                   and/or [dba.peakset](#) for details on how to include a spike-in track with a sample
                   (eg. by including a Spikein column in the sample sheet.) All chromosomes in
                   the spike-in bam files will be used.

                   If spikein is a character vector of one or more chromosome names, only reads
                   on the named chromosome(s) will be used for background normalization. If
                   spike-in tracks are available, reads on chromosomes with these names in the
                   spike-in track will be counted. If no spike-in tracks are available, reads on chro-
                   mosomes with these names in the main bamReads bam files will be counted.

If spikein is a [GRanges](#) object containing peaks for a parallel factor, then background normalization is performed counting reads in the spike-in tracks overlapping peaks in this object.

If spikein is a previously computed $background object, these counts will be used as the spikein background. A $background object can be obtained by calling [dba.normalize](#) with bRetrieve=TRUE and method=DBA_ALL_METHODS.

Note that if spikein is not FALSE, then the library size will be set to library=DBA_LIBSIZE_BACKGRO

offsets
This parameter controls the use of offsets (matrix of normalization factors) instead of a single normalization factor for each sample. It can either be a logical value, a matrix, or a [SummarizedExperiment](#).

If it is a logical value and set to FALSE, no offsets will be computed or used. A value of TRUE indicates that an offset matrix should be computed using a loess fit.

Alternatively, user-calculated normalization offsets can be supplied as a matrix or as a [SummarizedExperiment](#) (containing an assay named "offsets"). In this case, the user may also set the normalize parameter to indicate whether the offsets should be applied as-is to a DESeq2 analysis (DBA_NORM_OFFSETS, default), or if they should be adjusted for library size and mean centering (DBA_NORM_OFFSETS_ADJUST).

libFun
When normalize=DBA_NORM_LIB, normalization factors are calculated by dividing the library sizes for each sample by a common denominator, obtained by applying libFun to the vector of library sizes.

For method=DBA_EDGER, the normalization factors are further adjusted so as to make all the effective library sizes (library sizes multiplied by normalization factors) the same, and adjusted to multiply to 1.

bRetrieve
If set to TRUE, information about the current normalization will be returned. The only other relevant parameter in this case is the method.

If method=DBA_DESEQ2 or method=DBA_EDGER, a record will be returned including normalization values for the appropriate analysis method. This record is a list consists of the following elements:

- $norm.method A character string corresponding to the normalization method, generally one of the values that can be supplied as a value to normalize.
- $norm.factors A vector containing the computed normalization factors.
- $lib.method A character string corresponding to the value of the method used to calculate the library size, generally one of the values that can be supplied as a value to library.
- $lib.sizes A vector containing the computed library sizes.
- $background If the normalization if based on binned background reads, this field will be TRUE.
- $control.subtract If control reads were subtracted from the read counts, this field will be TRUE.

If method=DBA_ALL_METHODS, the record be a list with one of the above records for each method for which normalization factors have been computed ($DESeq2 and edgeR).

If background bins have been calculated, this will include an element called $background. This element can be passed in as the value to background or spikein to re-use a previously computed set of reads. It contains three subfields:

- $background$binned a SummarizedExperiment object containing the binned counts.
- $background$bin.size a numeric value with the bin size used.

- $background$back.calc character string indicating how the background was calculated (bins, spike-ins, or parallel factor).

  If offsets are available, this will include an element called $offsets with two subfields:

  - $offsets$offsets a matrix or a SummarizedExperiment object containing the offsets.
  - offsets$offset.method a character string indicating the source of the offsets, either "loess" or "user".

...   Extra parameters to be passed to limma::loessFit when computing offsets.

Details

The default normalization parameters are as follows:

- normalize=DBA_NORM_LIB
- library=DBA_LIBSIZE_FULL
- background=FALSE

If background=TRUE, then the default becomes library=DBA_LIBSIZE_BACKGROUND.

If dba.contrast has been used to set up contrasts with design=FALSE (pre-3.0 mode), then the defaults are:

- normalize=DBA_NORM_DEFAULT
- library=DBA_LIBSIZE_FULL
- background=FALSE

In this case, normalize=DBA_NORM_LIB will be set for method=DBA_DESEQ2 for backwards compatibility.

Value

Either a DBA object with normalization terms added, or (if bRetrieve=TRUE), a record or normalization details.

Note

The csaw package is used to compute background bins and offsets based on limma::loessFit.

See the DiffBind vignette for technical details of how this is done, and the csaw vignette for details on background bins and loess offsets can be used to address different biases in ChIP-seq data.

Author(s)

Rory Stark

See Also

dba.count, dba.analyze, dba.save

Examples

```
# load DBA object with counts
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen,design="~Tissue + Condition")

# default normalization: Full library sizes
tamoxifen <- dba.normalize(tamoxifen)
dba.normalize(tamoxifen, bRetrieve=TRUE)
dba.analyze(tamoxifen)

# RLE/TMM using Reads in Peaks
tamoxifen <- dba.normalize(tamoxifen, method=DBA_ALL_METHODS,
                           normalize=DBA_NORM_NATIVE,
                           library=DBA_LIBSIZE_PEAKREADS)
dba.normalize(tamoxifen, method=DBA_DESEQ2, bRetrieve=TRUE)
dba.normalize(tamoxifen, method=DBA_EDGER, bRetrieve=TRUE)
tamoxifen <- dba.analyze(tamoxifen, method=DBA_ALL_METHODS)
dba.show(tamoxifen,bContrasts=TRUE)
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_METHODS,bDB=TRUE)

# TMM in Background using precomputed background
norm <- dba.normalize(tamoxifen,method=DBA_ALL_METHODS,bRetrieve=TRUE)
tamoxifen <- dba.normalize(tamoxifen, background=norm$background,
                           normalize="TMM", method=DBA_ALL_METHODS)
tamoxifen <- dba.analyze(tamoxifen)
dba.show(tamoxifen,bContrasts=TRUE)
dba.plotMA(tamoxifen)

# LOESS offsets
tamoxifen <- dba.normalize(tamoxifen, method=DBA_ALL_METHODS, offsets=TRUE)
tamoxifen <- dba.analyze(tamoxifen, method=DBA_ALL_METHODS)
dba.show(tamoxifen,bContrasts=TRUE)

par(mfrow=c(3,1))
dba.plotMA(tamoxifen,th=0,bNormalized=FALSE)
dba.plotMA(tamoxifen,method=DBA_DESEQ2)
dba.plotMA(tamoxifen,method=DBA_EDGER)
```

---

dba.overlap   *Compute binding site overlaps (occupancy analysis)*

Description

Computes binding overlaps and co-occupancy statistics

Usage

```
dba.overlap(DBA, mask, mode=DBA_OLAP_PEAKS,
            contrast, method=DBA$config$AnalysisMethod, th=DBA$config$th,
            bUsePval=DBA$config$bUsePval,
            report, byAttribute, bCorOnly=TRUE, CorMethod="pearson",
            DataType=DBA$config$DataType)
```

Arguments

DBA	DBA object
mask	mask or vector of peakset numbers indicating a subset of peaksets to use (see [dba.mask](). When generating overlapping/unique peaksets, either two, three, or four peaksets may be specified. If the mode type is DBA_OLAP_ALL, and a contrast is specified, a value of TRUE (mask=TRUE) indicates that all samples should be included (otherwise only those present in one of the contrast groups will be included).
mode	indicates which results should be returned (see MODES below). One of:

- DBA_OLAP_PEAKS
- DBA_OLAP_ALL
- DBA_OLAP_RATE

contrast	contrast number to use. Only specified if contrast data is to be used when mode=DBA_OLAP_ALL. See [dba.show](DBA,bContrast=T) to get contrast numbers.
method	if contrast is specified and mode=DBA_OLAP_ALL, use data from method used for analysis:

- DBA_DESEQ2
- DBA_DESEQ2_BLOCK
- DBA_EDGER
- DBA_EDGER_BLOCK

th	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.
report	if contrast is specified and mode=DBA_OLAP_ALL, a report (obtained from [dba.report]()) specifying the data to be used. If counts are included in the report (and a contrast is specified), the count data from the report will be used to compute correlations, rather than the scores in the global binding affinity matrix. If report is present, the method, th, and bUsePval parameters are ignored.
byAttribute	when computing co-occupancy statistics (DBA_OLAP_ALL), limit comparisons to peaksets with the same value for a specific attribute, one of:

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

bCorOnly	when computing co-occupancy statistics (DBA_OLAP_ALL), logical indicating that only correlations, and not overlaps, should be computed. This is much faster if only correlations are desired (e.g. to plot the correlations using [dba.plotHeatmap]()).
CorMethod	when computing co-occupancy statistics (DBA_OLAP_ALL), method to use when computing correlations.

DataType   if mode==DBA_OLAP_PEAKS, the class of object that peaksets should be returned
                as:

- DBA_DATA_GRANGES
- DBA_DATA_RANGEDDATA
- DBA_DATA_FRAME

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

Details

MODE: Generate overlapping/unique peaksets:

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

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

dba.overlap(DBA,mask,mode=DBA_OLAP_ALL,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,attribute

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

MODE: Compute overlap rates at different stringency thresholds:

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

Value

Value depends on the mode specified in the mode parameter.

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

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

A	peakset number of first peakset in overlap
B	peakset number of second peakset in overlap
onlyA	number of sites unique to peakset A
onlyB	number of sites unique to peakset B
inAll	number of peaks in both peakset A and B (merged)
R2	correlation value A vs B
Overlap	percentage overlap (number of overlapping sites divided by number of peaks unique to smaller peakset

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

Author(s)

Rory Stark

See Also

[dba.plotVenn](), [dba.plotHeatmap]()

Examples

```
data(tamoxifen_peaks)
# default mode: DBA_OLAP_PEAKS -- get overlapping/non overlapping peaksets
mcf7 <- dba.overlap(tamoxifen,tamoxifen$masks$MCF7&tamoxifen$masks$Responsive)
names(mcf7)
mcf7$inAll

# mode:  DBA_OLAP_ALL -- get correlation record
mcf7 <- dba(tamoxifen,tamoxifen$masks$MCF7)
mcf7.corRec <- dba.overlap(mcf7,mode=DBA_OLAP_ALL,bCorOnly=FALSE)
mcf7.corRec

# mode: DBA_OLAP_RATE -- get overlap rate vector
data(tamoxifen_peaks)
rate <- dba.overlap(tamoxifen, mode=DBA_OLAP_RATE)
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, spikein,
            scoreCol, bLowerScoreBetter, filter, counts,
            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.
peaks	When adding a specified peakset: set of peaks, either a GRanges object, or a peak dataframe or matrix (chr,start,end,score), or a filename where the peaks are stored.
	When adding a consensus peakset: a sample mask or vector of peakset numbers to include in the consensus. If missing or NULL, a consensus is derived from all peaksets present in the model. See dba.mask, or dba.show to get peakset numbers.
	When adding and empty peakset (zero peaks), set peaks=NA.
	When adding a set of consensus peaksets: a sample mask or vector of peakset numbers. Sample sets will be derived only from subsets of these peaksets.
	When adding all the peaks from one DBA object to another: a DBA object. In this case, the only other parameter to have an effect is minOverlap.
	When retrieving and/or writing a peakset: either a GRanges, or a peak dataframe or matrix (chr,start,end,score), or a peakset number; if NULL, retrieves/writes the full binding matrix.
sampID	ID string for the peakset being added; if missing, one is assigned (a serial number for a new peakset, or a concatenation of IDs for a consensus peakset). Must be unique for each sample.
tissue	tissue name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of tissues).
factor	factor name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of factors).
condition	condition name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of conditions).
treatment	treatment name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of treatment).
replicate	replicate number for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of replicate numbers).
control	control name for the peakset being added; if missing, one is assigned for a consensus peakset (a concatenation of control names).
peak.caller	peak caller name string. If peaks is specified as a file, and peak.format is missing, a default fie format for the caller will be used (see peak.format). Supported values:

- "raw": default peak.format: raw text file

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

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

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

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

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

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

- DBA_TISSUE; -DBA_TISSUE
- DBA_FACTOR; -DBA_FACTOR
- DBA_CONDITION; -DBA_CONDITION
- DBA_TREATMENT; -DBA_TREATMENT
- DBA_REPLICATE; -DBA_REPLICATE
- DBA_CALLER; -DBA_CALLER

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

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

spikein   file path of the BAM/BED file containing the aligned reads for the spike-ins used for the peakset being added.

scoreCol	peak column to normalize to 0...1 scale when adding a peakset; 0 indicates no normalization
bLowerScoreBetter	
	Logical indicating that lower scores indicate higher confidence peaks; default is that higher scores indicate better peaks.
filter	Numeric indicating a filter value for peaks. If present, any peaks with a score less than this value (or higher if bLowerScoreBetter==TRUE) will be removed from the peakset.
counts	Used for adding externally computed peak counts. Can be a filename or a dataframe. Can consist of a single column (or vector) with the counts, or two columns, with an ID for each interval in the first column and the counts in the second column, or four columns (chr, start, end, counts). When counts is specified, peaks and related parameters are ignored, and all peaksets in the DBA object must be specified in this way, all with exactly the same number of intervals.
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 of 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_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,co

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 [GRanges](#) object when retrieving and/or writing a peakset.

Author(s)

Rory Stark

See Also

to add peaksets using a sample sheet, see [dba](#).

Examples

```
# create a new DBA object by adding three peaksets
mcf7 <- dba.peakset(NULL,
                  peaks=system.file("extra/peaks/MCF7_ER_1.bed.gz", package="DiffBind"),
                   peak.caller="bed", sampID="MCF7.1",tissue="MCF7",
                   factor="ER",condition="Responsive",replicate=1)
mcf7 <- dba.peakset(mcf7,
                  peaks=system.file("extra/peaks/MCF7_ER_2.bed.gz", package="DiffBind"),
                   peak.caller="bed", sampID="MCF7.2",tissue="MCF7",
                   factor="ER",condition="Responsive",replicate=2)
mcf7 <- dba.peakset(mcf7,
                  peaks=system.file("extra/peaks/MCF7_ER_3.bed.gz", package="DiffBind"),
                   peak.caller="bed", sampID="MCF7.3",tissue="MCF7",
                   factor="ER",condition="Responsive",replicate=3)
mcf7

#retrieve peaks that are in all three peaksets
mcf7.consensus <- dba.peakset(mcf7, 1:3, minOverlap=3, bRetrieve=TRUE)
mcf7.consensus

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

#add consensus peaksets for all sample types by combining replicates
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen,consensus = -DBA_REPLICATE)
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)

#add consensus peaksets for all sample types by (same tissue and condition)
data(tamoxifen_peaks)
tamoxifen <- dba.peakset(tamoxifen,consensus = c(DBA_TISSUE,DBA_CONDITION))
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,tamoxifen$masks$Responsive & tamoxifen$masks$Consensus)

#create consensus peaksets from sample type consensuses for Responsive and Resistant sample groups
tamoxifen <- dba.peakset(tamoxifen,peaks=tamoxifen$masks$Consensus,consensus=DBA_CONDITION)
```

```
dba.show(tamoxifen,mask=tamoxifen$masks$Consensus)
dba.plotVenn(tamoxifen,17:18)

#retrieve the consensus peakset as GRanges 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=DBA$config$th, bUsePval=DBA$config$bUsePval,
            bNormalized=TRUE, attribute=DBA_GROUP, mask,
            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_DESEQ2
- DBA_DESEQ2_BLOCK
- DBA_EDGER
- DBA_EDGER_BLOCK

th	significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included in the boxplot.
bUsePval	logical indicating whether to use FDR (FALSE) or p-value (TRUE) for thresholding.
bNormalized	logical indicating that normalized data (using normalization factors computed by differential analysis method) should be plotted. FALSE uses raw count data.
attribute	attribute to use for determining groups of samples. Default (DBA_GROUP) plots the two groups used in the contrast, if available. Possible values:

- DBA_GROUP
- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_TREATMENT

- DBA_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER

mask   logical mask of samples to include when no groups are present.

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 at-
                tribute values appear for other values of attribute).

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

varwidth   passed to boxplot

notch   passed to boxplot

...   other arguments passed to boxplot

Details

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

Value

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

Author(s)

Rory Stark

Examples

```
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$AnalysisMethod,
                th=DBA$config$th, bUsePval=DBA$config$bUsePval,
                report, score, bLog=TRUE, mask, sites, sortFun=sd,
                correlations=TRUE, olPlot=DBA_COR,
             ColAttributes,RowAttributes, colSideCols, rowSideCols=colSideCols,
                margin=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

maxSites   maximum number of binding sites to use in heatmap. Only used when not drawing a correlation heatmap (correlations=FALSE)

minval   Set all scores less than this to minval

maxval   Set all scores greater than this to maxval

contrast	number of contrast to report on; if present, draws a heatmap based on a 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=TRUE) to get contrast numbers. If missing, uses scores in the main binding matrix.
method	analysis method (used in conjunction with contrast): • [DBA_DESEQ2](#) • [DBA_DESEQ2_BLOCK](#) • [DBA_EDGER](#) • [DBA_EDGER_BLOCK](#)
th	significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be included in the 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_NORMALIZED](#) • [DBA_SCORE_READS](#) • [DBA_SCORE_CONTROL_READS](#) • [DBA_SCORE_READS_MINUS](#) • [DBA_SCORE_READS_FOLD](#) • [DBA_SCORE_RPKM](#) • [DBA_SCORE_RPKM_FOLD](#) • [DBA_SCORE_RPKM_MINUS](#)
bLog	Logical indicating that log2 values should be used. Only applicable with read count scores (not peak scores).
mask	mask indicating a subset of peaksets to use when using global binding matrix scores. If a contrast is specified, these peaksets will be included, but only the significantly differentially bound sites (using th, bUsePval, and/or report) will be included.
sites	logical vector indicating which sites to include; first maxSites of these. Only relevant when using global binding matrix (contrast is missing).
sortFun	function taking a vector of scores and returning a single value. Only relevant when using global binding matrix (contrast is missing). If not equal to FALSE, the global binding matrix will be sorted (descending) on the results, and the first maxSites used in the heatmap. Recommended sort function options include [sd](#), [mean](#), [median](#), [min](#).
correlations	logical indicating that a correlation heatmap should be plotted (TRUE). If FALSE, a binding heatmap of scores/reads is plotted. This parameter can also be set to a correlation record; see [dba.overlap](#)(mode=DBA_OLAP_ALL), in which case a correlation heatmap is plotted based on the specified correlation record, using the statistic specified in olPlot.

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

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

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

- DBA_GROUP
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_TREATMENT
- DBA_REPLICATE
- DBA_CALLER

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

rowSideCols   Vector of colors to use in row color bars. Uses default colors if missing. Can also be a list of color vectors.

colSideCols   Vector of colors to use in column color bars. Uses default colors if missing. Can also be a list of color vectors.

margin   margin size of plot

colScheme   Color scheme; see colorRampPalette

distMethod   distance method for clustering; see Dist

...   passed on to heatmap.2, 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="Green

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

dba.plotHeatmap(DBA,attributes=DBA$attributes,minval,maxval,contrast,method=DBA_DESEQ2,th=0.05,b

MODE: Binding heatmap plot using significantly differentially bound sites:

dba.plotHeatmap(DBA,attributes,maxSites,minval,maxval,contrast,method,th,bUsePval,correlations=F

MODE: Binding heatmap plot using the global binding matrix:

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

Value

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

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

Author(s)

Rory Stark

See Also

[dba.overlap](#)

Examples

```
data(tamoxifen_peaks)
# peak overlap correlation heatmap
dba.plotHeatmap(tamoxifen)

data(tamoxifen_counts)
# counts correlation heatmap
dba.plotHeatmap(tamoxifen)

data(tamoxifen_analysis)
#correlation heatmap based on all normalized data
dba.plotHeatmap(tamoxifen,contrast=1,th=1)

#correlation heatmap based on DB sites only
dba.plotHeatmap(tamoxifen,contrast=1)

#binding heatmap based on DB sites
dba.plotHeatmap(tamoxifen,contrast=1,correlations=FALSE)

#binding heatmap based on 1,000 sites with highest variance
sites <- dba.plotHeatmap(tamoxifen,contrast=1,th=1,
                         correlations=FALSE,sortFun=var)
sites

data(tamoxifen_counts)
#Examples of  heatmaps using DB sites with different subsets of samples
#exclude T47D
tamoxifen <- dba.contrast(tamoxifen,design=FALSE,
                          group1=tamoxifen$masks$Resistant,
                          group2=c(3:5,10:11))
tamoxifen <- dba.analyze(tamoxifen)

# regular heatmaps with samples from two contrast groups only
dba.plotHeatmap(tamoxifen, contrast=1)
#also include the T47D samples
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$All)

#correlation heatmap without MCF7
plot(tamoxifen,contrast=1,mask=!tamoxifen$masks$MCF7)

# binding heatmap using only the MCF7 samples
dba.plotHeatmap(tamoxifen,contrast=1,mask=tamoxifen$masks$MCF7,correlations=FALSE)
```

---

dba.plotMA   *Generate MA and scatter plots of differential binding analysis results*

Description

Generates MA and scatter plots of differential binding analysis results.

Usage

```
dba.plotMA(DBA, contrast=1, method=DBA$config$AnalysisMethod,
           th=DBA$config$th, bUsePval=DBA$config$bUsePval,
           fold=0, bNormalized=TRUE,
           factor="", bFlip=FALSE, bXY=FALSE, dotSize=.45,
           bSignificant=TRUE, bSmooth=TRUE, bLoess=TRUE,
           xrange, yrange, ...)
```

Arguments

DBA   DBA object, on which [dba.analyze](#) should have been successfully run.

contrast   number of contrast to report on. See [dba.show](#)(DBA,bContrast=TRUE) to get
                contrast numbers.

                Alternatively, an MA plot can be generated without a specific contrast, plotting
                one set of samples against another. In this case, contrast should be a list on
                length one or two. Each element of the list should be either a logical sample
                mask, or a vector of sample numbers. If the second set of samples is jot specified
                (list is length one), all the samples other than those specified will be used for the
                second group. The list elements should be named; these names will be used as
                labels for the sample groups in the plot.

method   method or vector of methods to plot results for:

                - [DBA_DESEQ2](#)
                - [DBA_DESEQ2_BLOCK](#)
                - [DBA_EDGER](#)
                - [DBA_EDGER_BLOCK](#)

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

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.

bFlip   logical indicating that order of groups in contrast should be "flipped", allowing
                control of which sample group will have positive and which will have negative
                fold changes.

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

dotSize   size of points on plot (cex).

bSignificant   Logical indicating if points corresponding to significantly differentially bound
                   sites (based on contrast, th, bUsePval, and fold parameters) should be over-
                   laid in red.

bSmooth   logical indicating that basic plot should be plotted using [smoothScatter](). Note
                   that overlaid significant sites will be not plotted using a smoothing function.

bLoess   logical indicating that a MA plot should include a fitted loess curve.

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

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

...   passed to underlying plotting functions.

Author(s)

Rory Stark

See Also

[dba.analyze]()

Examples

```
data(tamoxifen_analysis)

# default MA plot
dba.plotMA(tamoxifen)

# Show different normalizations
tamoxifen <- dba.normalize(tamoxifen,method=DBA_ALL_METHODS,
                           library=DBA_LIBSIZE_PEAKREADS, background=FALSE)
tamoxifen <- dba.analyze(tamoxifen, method=DBA_ALL_METHODS)

par(mfrow=c(3,2))
dba.plotMA(tamoxifen,th=0,bNormalized=FALSE,sub="NON-NORMALIZED")
dba.plotMA(tamoxifen,th=0,bNormalized=FALSE,sub="NON-NORMALIZED")

dba.plotMA(tamoxifen,method=DBA_DESEQ2,bNormalized=TRUE,
           sub="DESeq2_RLE-RiP")
dba.plotMA(tamoxifen,method=DBA_EDGER,bNormalized=TRUE,
           sub="edgeR_TMM-RiP")

tamoxifen <- dba.normalize(tamoxifen, method=DBA_ALL_METHODS,
                           normalize=DBA_NORM_LIB, background=FALSE)
tamoxifen <- dba.analyze(tamoxifen,method=DBA_ALL_METHODS)

dba.plotMA(tamoxifen,method=DBA_DESEQ2,bNormalized=TRUE,
           sub="DESeq2_LIB-FULL")
dba.plotMA(tamoxifen,method=DBA_EDGER,bNormalized=TRUE,
           sub="edgeR_LIB-FULL")

# MA plots of samples without a contrast
data(tamoxifen_counts)
par(mfrow=c(2,2))
dba.plotMA(tamoxifen,list(Resistant=tamoxifen$masks$Resistant,
```

```
                           Responsive=tamoxifen$masks$Responsive),
                           bNormalized=FALSE)
dba.plotMA(tamoxifen,list(MCF7=tamoxifen$masks$MCF7),
                           bNormalized=FALSE)
dba.plotMA(tamoxifen, list(Sample1=1), bNormalized=FALSE)
dba.plotMA(tamoxifen, list(Random=sample(1:11,5)), bNormalized=FALSE)

#XY plots (with raw and normalized data)
data(tamoxifen_analysis)
par(mfrow=c(1,2))
dba.plotMA(tamoxifen,bXY=TRUE,bSmooth=FALSE,bNormalized=FALSE,
            sub="NON_NORMALIZED")
dba.plotMA(tamoxifen,bXY=TRUE,bSmooth=FALSE,bNormalized=TRUE,
            sub="DESeq2-RLE-Background")
```

---

dba.plotPCA   *PCA plot*

Description

Principal Component Analysis plot

Usage

```
dba.plotPCA(DBA, attributes, minval, maxval,
            contrast, method=DBA$config$AnalysisMethod,
            th=DBA$config$th, bUsePval=DBA$config$bUsePval,
            report, score, bLog=TRUE, mask, sites, label, cor=FALSE,
            b3D=FALSE, vColors, dotSize, labelSize, labelCols,
            components=1:3, ...)
```

Arguments

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

> - DBA_GROUP
> - DBA_ID
> - DBA_TISSUE
> - DBA_FACTOR
> - DBA_CONDITION
> - DBA_TREATMENT
> - DBA_REPLICATE
> - DBA_CONSENSUS
> - DBA_CALLER

> Note that DBA_GROUP is a special attribute which will result in samples from each group in a contrast (if present) being colored separately.

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

contrast   number of contrast to use for PCA; if present, plots a PCA based on a differential
                 binding affinity analysis (see dba.analyze). If mask is unspecified, only the
                 samples in the contrast will be included. See dba.show(DBA,bContrast=T) to
                 get contrast numbers. If missing, uses scores in the main binding matrix.

method   method used for analysis (used in conjunction with contrast):
                   • DBA_DESEQ2
                   • DBA_DESEQ2_BLOCK
                   • DBA_EDGER
                   • DBA_EDGER_BLOCK

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

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

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

score   Score to use for count data. Only used when plotting the global binding matrix
                 (no contrast specified). One of:
                       • DBA_SCORE_READS
                       • DBA_SCORE_NORMALIZED
                       • DBA_SCORE_CONTROL_READS
                       • DBA_SCORE_READS_MINUS
                       • DBA_SCORE_READS_FOLD
                       • DBA_SCORE_RPKM
                       • DBA_SCORE_RPKM_FOLD
                       • DBA_SCORE_RPKM_MINUS

bLog   Logical indicating that log2 values should be used. Only applicable to read
                 count scores (not peak scores).

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

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

label   A metadata field to use as a label in 2D plots. The value for this field will be
                 written directly on the plot near the dot for each sample. Values can be any of
                 those valid for the attributes parameter.

cor   a logical value indicating whether the calculation should use the correlation ma-
                 trix or the covariance matrix. Passed into princomp.

b3D   logical indicating that three principal components should be plotted (requires
                 package rgl). If FALSE, the first two principal components are plotted.

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

dotSize   size of dots to plot; is absent, a default will be calculated.

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

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

components
Number(s) of the components to plot. Can be a vector of two or three component numbers, or a single integer. If an integer, that component, in addition to the succeeding one (b3D=FALSE) or two (b3D=TRUE) will be plotted.

...
arguments passed to [plot](#) or plot3d (rgl).

Details

MODE: PCA plot using significantly differentially bound sites:

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

MODE: PCA plot using global binding matrix:

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

Value

trellis plot from [lattice](#) package; see [xyplot](#)

Note

uses rgl package for 3D plots (if available)

Author(s)

Rory Stark

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
p <- dba.plotPCA(tamoxifen,contrast=1)
p <- dba.plotPCA(tamoxifen,contrast=1,attributes=DBA_TISSUE)
p <- dba.plotPCA(tamoxifen,contrast=1,attributes=DBA_TISSUE,label=DBA_CONDITION)
p <- dba.plotPCA(tamoxifen,contrast=1,attributes=DBA_CONDITION,label=DBA_TISSUE)
p <- dba.plotPCA(tamoxifen,contrast=1,attributes=c(DBA_TISSUE,DBA_CONDITION),
                 label=DBA_REPLICATE)
```

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,
             contrast, method=DBA$config$AnalysisMethod,
             th=DBA$config$th, bUsePval=DBA$config$bUsePval,
             bDB=TRUE, bNotDB, bAll=TRUE, bGain=FALSE, bLoss=FALSE,
             labelAttributes, DataType=DBA$config$DataType)
```

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
contrast	contrast number(s) to use for results-based plots. This can be a vector of contrast numbers. See [dba.show](DBA,bContrast=T) to get contrast numbers.
method	if contrast is specified, include results from analyses using this method or methods:

- [DBA_DESEQ2]()
- [DBA_DESEQ2_BLOCK]()
- [DBA_EDGER]()
- [DBA_EDGER_BLOCK]()
- [DBA_ALL_METHODS]()
- [DBA_ALL_BLOCK]()
- [DBA_ALL_METHODS_BLOCK]()

th	if contrast is specified, use this significance threshold; all sites with FDR (or p-values, see bUsePval) less than or equal to this value will be considered differentially bound (DB).
bUsePval	if contrast is specified, this logical indicates whether to use FDR (FALSE) or p-value (TRUE) for thresholding.

bDB	if `contrast` is specified, this logical indicates that peaksets should include Differentially Bound (DB) sites (respecting the `th`, `bUsePval`, and `fold` parameters).
bNotDB	if `contrast` is specified, this logical indicates that peaksets should include non-Differentially Bound (non-DB) sites (respecting the `th`, `bUsePval`, and `fold` parameters).
bAll	if `contrast` is specified, this logical indicates peaksets combining peaks with both positive and negative fold changes should be included.
bGain	if `contrast` is specified, this logical indicates that peaksets with only positive fold changes should be included.
bLoss	if `contrast` is specified, this logical indicates that peaksets with only negative fold changes should be included.

labelAttributes

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

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

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

DataType

if bReturnPeaksets is set to `TRUE`, the class of object that peaksets should be returned as:

- DBA_DATA_GRANGES
- DBA_DATA_RANGEDDATA
- DBA_DATA_FRAME

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

Alternatively, this can be set to:

- DBA_DATA_DBAOBJECT

to return a results-based DBA object, if a `contrast` is specified (see `dba.report`).

Value

Either a list of peaksets is returned invisibly (as described in `dba.overlap`), or, if DataType=DBA_DATA_DBAOBJECT, a results-based DBA object.

Note

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

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

Author(s)

Rory Stark

See Also

[dba.analyze](), [dba.overlap](), [dba.report](), [dba.plotPCA](), [vennPlot]()

Examples

```
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")

#Venns of differentially bound sites
data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen,design="~Tissue+Condition")
tamoxifen <- dba.analyze(tamoxifen,method=c(DBA_EDGER,DBA_DESEQ2))
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_METHODS,
             bAll=FALSE,bGain=TRUE,bLoss=TRUE)
par(mfrow=c(2,1))
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_METHODS,
             bAll=FALSE,bGain=TRUE,bLoss=FALSE)
dba.plotVenn(tamoxifen,contrast=1,method=DBA_ALL_METHODS,
             bAll=FALSE,bGain=FALSE,bLoss=TRUE)

data(tamoxifen_counts)
tamoxifen <- dba.contrast(tamoxifen,design=FALSE,block=DBA_TISSUE)
tamoxifen <- dba.contrast(tamoxifen,design="~Tissue + Condition",
                          contrast=c("Condition","Responsive","Resistant"))
tamoxifen <- dba.analyze(tamoxifen,method=DBA_ALL_METHODS)
```

```
dba.plotVenn(tamoxifen,contrast=1:2,method=c(DBA_DESEQ2,DBA_DESEQ2_BLOCK))
tamoxifen.db <- dba.report(tamoxifen,contrast=1:2,method=DBA_ALL_METHODS_BLOCK,
                            bDB=TRUE)
dba.plotVenn(tamoxifen.db,mask=1:2)
dba.plotVenn(tamoxifen.db,mask=3:6)
```

---

dba.plotVolcano   *Generate volcano plots of differential binding analysis results*

Description

Generates volcano plots of differential binding analysis results.

Usage

```
dba.plotVolcano(DBA, contrast=1, method=DBA$config$AnalysisMethod,
                        th=DBA$config$th, bUsePval=DBA$config$bUsePval,
                        fold=0, factor="", bFlip=FALSE,
                        bLabels=FALSE, maxLabels=50, dotSize=1)
```

Arguments

DBA	DBA object, on which dba.analyze should have been successfully run.
contrast	number of contrast to report on. See dba.show(DBA,bContrast=TRUE) to get contrast numbers.
method	method or vector of methods to plot results for:

- DBA_DESEQ2
- DBA_DESEQ2_BLOCK
- DBA_EDGER
- DBA_EDGER_BLOCK

th	significance threshold; 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 thresholding.
fold	will only include sites with fold change greater than this as significant (colored red).
factor	string to be prepended to plot main title; e.g. factor name.
bFlip	logical indicating that order of groups in contrast should be "flipped", allowing control of which sample group will have positive and which will have negative fold changes.
bLabels	logical indicating that labels should be drawn on the plot. The labels are the site numbers, the row index in the (silently) returned set of significant sites. The maximum number of sites can be specified using maxLabels.
maxLabels	The maximum number of labels to use in the plot. Ignored if bLabels=FALSE.
dotSize	size of points on plot.

Details

Makes a volcano plot.

Value

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

Author(s)

Rory Stark

See Also

[dba.analyze](), [dba.plotMA]()

Examples

```
data(tamoxifen_analysis)

# default volcano plot
dba.plotVolcano(tamoxifen)

# only highlight significant sites with at least 5x Fold Change
sigSites <- dba.plotVolcano(tamoxifen, fold=log2(5))

# use labels to find outlier sites
sigSites <- dba.plotVolcano(tamoxifen, fold=log2(10),bLabels=TRUE)
sigSites
```

---

dba.report   *Generate a report for a differential binding affinity analysis*

Description

Generates a report for a differential binding affinity analysis

Usage

```
dba.report(DBA, contrast, method=DBA$config$AnalysisMethod,
           th=DBA$config$th, bUsePval=DBA$config$bUsePval,
           fold=0, bNormalized=TRUE, bFlip=FALSE, precision,
           bCalled=FALSE, bCounts=FALSE, bCalledDetail=FALSE,
           bDB, bNotDB, bAll=TRUE, bGain=FALSE, bLoss=FALSE,
      file,initString=DBA$config$reportInit,ext='csv',DataType=DBA$config$DataType)
```

Arguments

DBA   DBA object. A differential binding affinity analysis needs to have been previ-
               ously carried out (see [dba.analyze]()).

contrast   contrast number to report on. When generating a report-based DBA object,
               this can be a vector of contrast numbers. If missing, defaults to first contrast
               for reports, and all contrasts when generating a report-based DBA object. See
               [dba.show](DBA,bContrast=T) to get contrast numbers.

method   method used for analysis:

                  • [DBA_DESEQ2]()

- DBA_DESEQ2_BLOCK
- DBA_EDGER
- DBA_EDGER_BLOCK

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

- DBA_ALL_METHODS
- DBA_ALL_BLOCK
- DBA_ALL_METHODS_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 thresholding.
fold	only sites with an absolute log Fold value greater than equal to this will be included in the report.
bNormalized	logical indicating that normalized data (using normalization factors computed b y differential analysis method) should be reported. FALSE uses raw count data.
bFlip	logical indicating that order of groups in contrast should be "flipped", allowing control of which sample group will have positive and which will have negative fold changes.
precision	If present, alters the default precision for the Concentration, Fold, p-value, and FDR values in the returned report. A value of 0 indicates maximum precision. Otherwise, it should be a 2-value vector. The first value controls how many digits to the right of the decimal to include for concentration and fold values. These second value control how many digits to the right of the decimal to include for the p-value and FDRs. Default is precision=2:3, unless DataType=DBA_DATA_SUMMARIZED_EXPERIMENT, in which case the default is 0 (full precision).
bCalled	logical indicating that peak caller status should be included. This will add a column for each group, each indicating the number of samples in the group identified as a peak in the original peaksets. Note that this option is only available if the consensus peakset was calculated by dba.count; if a consensus peakset was passed in explicitly using the peaks parameter, original peak origins are lost.
bCounts	logical indicating that count data for individual samples should be reported as well as group statistics. Columns are added for each sample in the first group, followed by columns for each sample in the second group.
bCalledDetail	logical indicating that peak caller status should be included for each sample (if available). Columns are added for each sample in the first group, followed by columns for each sample in the second group.
bDB	logical indicating that a report-based DBA object should be generated, and that it should include Differentially Bound (DB) sites (respecting the th, bUsePval, and fold parameters).
bNotDB	logical indicating that a report-based DBA object should be generated, and that it should include non-Differentially Bound (non-DB) sites (respecting the th, bUsePval, and fold parameters).
bAll	logical indicating that a report-based DBA object should be generated, and that it should include peaksets combining peaks with both positive and negative fold changes.

bGain	logical indicating that a report-based DBA object should be generated, and that it should include peaksets with only positive fold changes.
bLoss	logical indicating that a report-based DBA object should be generated, and that it should include peaksets with only negative fold changes.
file	if present, also save the report to a comma separated value (csv) file, using this filename.
initString	if saving to a file, pre-pend this string to the filename.
ext	if saving to a file, append this extension to the filename.
DataType	The class of object for returned report:

  - DBA_DATA_GRANGES
  - DBA_DATA_RANGEDDATA
  - DBA_DATA_FRAME

If set to DBA_DATA_SUMMARIZED_EXPERIMENT, the result will be a SummarizedExperiment object, with all the count data and sample metadata for the experiment. The contrast statistics will be included as metadata columns in the rowData of the object.

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

Value

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

Chr	Chromosome of binding site
Start	Starting base position of binding site
End	End base position of binding site
Conc	Concentration – mean (log) reads across all samples in both groups
Conc_group1	Group 1 Concentration – mean (log) reads across all samples first group
Conc_group2	Group 2 Concentration – mean (log) reads across all samples in second group
Fold	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, if present. The SampleID 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, if present. The SampleID will be used as the column header. This column contains a "+" to indicate for which sites the sample was called as a peak, and a "-" if it was not so identified.

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

Author(s)

Rory Stark

See Also

dba.analyze

Examples

```
data(tamoxifen_analysis)

#Retrieve DB sites with FDR <  0.05
tamoxifen.DB <- dba.report(tamoxifen)
tamoxifen.DB

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

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

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

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

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

#Retrieve report-based DBA object
data(tamoxifen_analysis)
tamoxifen <- dba.analyze(tamoxifen,method=DBA_ALL_METHODS)
tamoxifen.DB <- dba.report(tamoxifen,method=c(DBA_EDGER,DBA_DESEQ2),
                           bDB=TRUE)
tamoxifen.DB
dba.plotVenn(tamoxifen.DB,1:2)
```

---

dba.save   *save DBA object*

Description

Writes out DBA object

Usage

```
dba.save(DBA, file='DBA', dir='.', pre='dba_', ext='RData',
         bRemoveAnalysis=FALSE, bRemoveBackground=FALSE,
         bCompress=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

bRemoveAnalysis
                   if TRUE, will remove the global DESeq2 and/or edgeR analysis objects. The anal-
                   ysis results will be retained. If the analysis objects are required after re-loading,
                   they will be automatically re-generated.

bRemoveBackground
                   if TRUE, will remove the global binned background counts used for normaliza-
                   tion. Any normalization factors calculated using these counts will be retained.
                   If the the normalization factors need to be re-re-calculated after re-loading, the
                   binned background counts will be automatically re-generated.

bCompress   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](#)

Examples

```
## Not run:
data(tamoxifen_peaks)
savefile <- dba.save(tamoxifen,'tamoxifenPeaks')
savefile
rm(tamoxifen)
```

```
tamoxifen <- dba.load('tamoxifenPeaks')
unlink(savefile)

## End(Not run)
```

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, bDesign=FALSE,
         th=DBA$config$th)
```

Arguments

DBA	DBA object
mask	mask of peaksets for which to get attributes (used when obtaining peakset attributes, i.e. bContrasts=FALSE).
attributes	attribute or vector of attributes to retrieve. Number of intervals is always shown. Used when obtaining peakset attributes, i.e. bContrasts=FALSE. Values:

- DBA_ID
- DBA_TISSUE
- DBA_FACTOR
- DBA_CONDITION
- DBA_TREATMENT
- DBA_REPLICATE
- DBA_CONSENSUS
- DBA_CALLER
- DBA_CONTROL
- DBA_READS
- DBA_INTERVALS
- DBA_FRIP

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.
bDesign	logical indicating whether the model design should be returned, if present. bContrasts must be FALSE for this parameter to be used.
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.

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=TRUE,th)

MODE: Return design associated with a DBA object:

dba.show(DBA,bDesign=TRUE)

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
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 analysis using edgeR
DB.DESeq.block	Number of significantly differentially bound sites identified for blocking analysis using DESeq

Author(s)

Rory Stark

See Also

[dba](#), [dba.peakset](#), [dba.contrast](#). [dba.analyze](#)

Examples

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

data(tamoxifen_analysis)
dba.show(tamoxifen,bContrasts=TRUE)

#alternatively:
data(tamoxifen_analysis)
tamoxifen
```

```
    tamoxifen$config$th <- .01
    tamoxifen
```

DiffBind -- DBA global constant variables
Constant variables used in DiffBind package

Description

Constant variables used in DiffBind package

Usage

```
    DBA_ID
    DBA_FACTOR
    DBA_TISSUE
    DBA_CONDITION
    DBA_TREATMENT
    DBA_REPLICATE
    DBA_CALLER
    DBA_CONSENSUS
    DBA_CONTROL
    DBA_READS
    DBA_ALL_ATTRIBUTES

    DBA_INTERVALS
    DBA_FRIP

    DBA_GROUP

    DBA_OLAP_PEAKS
    DBA_OLAP_ALL
    DBA_OLAP_RATE

    DBA_COR
    DBA_OLAP
    DBA_INALL

    DBA_SCORE_READS
    DBA_SCORE_NORMALIZED
    DBA_SCORE_CONTROL_READS
    DBA_SCORE_READS_MINUS
    DBA_SCORE_READS_FULL
    DBA_SCORE_READS_MINUS_FULL
    DBA_SCORE_READS_EFFECTIVE
    DBA_SCORE_READS_MINUS_EFFECTIVE
    DBA_SCORE_READS_FOLD
    DBA_SCORE_RPKM
    DBA_SCORE_RPKM_FOLD
    DBA_SCORE_RPKM_MINUS
    DBA_SCORE_TMM_READS_FULL
```

```
DBA_SCORE_TMM_READS_EFFECTIVE
DBA_SCORE_TMM_MINUS_FULL
DBA_SCORE_TMM_MINUS_EFFECTIVE
DBA_SCORE_TMM_READS_FULL_CPM
DBA_SCORE_TMM_READS_EFFECTIVE_CPM
DBA_SCORE_TMM_MINUS_FULL_CPM
DBA_SCORE_TMM_MINUS_EFFECTIVE_CPM
DBA_SCORE_SUMMIT
DBA_SCORE_SUMMIT_ADJ
DBA_SCORE_SUMMIT_POS

DBA_READS_DEFAULT
DBA_READS_BAM
DBA_READS_BED

DBA_EDGER
DBA_DESEQ2
DBA_EDGER_BLOCK
DBA_DESEQ2_BLOCK
DBA_EDGER_GLM
DBA_ALL_METHODS
DBA_ALL_BLOCK
DBA_ALL_METHODS_BLOCK

DBA_DATA_FRAME
DBA_DATA_GRANGES
DBA_DATA_RANGEDDATA
DBA_DATA_SUMMARIZED_EXPERIMENT
DBA_DATA_DBAOBJECT

DBA_BLACKLIST_CE10
DBA_BLACKLIST_CE11
DBA_BLACKLIST_DM3
DBA_BLACKLIST_DM6
DBA_BLACKLIST_GRCH37
DBA_BLACKLIST_GRCH38
DBA_BLACKLIST_HG19
DBA_BLACKLIST_HG38
DBA_BLACKLIST_MM9
DBA_BLACKLIST_MM10
DBA_BLACKLIST
DBA_GREYLIST
DBA_BLACKLISTED_PEAKS

DBA_LIBSIZE_DEFAULT
DBA_LIBSIZE_FULL
DBA_LIBSIZE_PEAKREADS
DBA_LIBSIZE_BACKGROUND
DBA_LIBSIZE_USER
DBA_NORM_DEFAULT
DBA_NORM_NATIVE
DBA_NORM_LIB
```

```
DBA_NORM_TMM
DBA_NORM_RLE
DBA_NORM_SPIKEIN
DBA_NORM_USER
DBA_NORM_OFFSETS
DBA_NORM_OFFSETS_ADJUST
DBA_OFFSETS_LOESS
DBA_OFFSETS_USER
```

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_READS	Number of reads counted in BAM file.
DBA_ALL_ATTRIBUTES	
	DBA peakset metadata: all attributes that can be used in certain plot labels (cf [dba.plotVenn](#)), equivalent to c(DBA_ID,DBA_TISSUE,DBA_FACTOR,DBA_CONDITION,DBA_TREATMENT)
DBA_INTERVALS	DBA peakset metadata: Number of intervals in peakset
DBA_FRIP	DBA peakset metadata: Fraction of Reads in Peaks (number of reads in intervals divided by total number of reads in library)
DBA_GROUP	DBA peakset metadata: color PCA plot using contras groups
DBA_OLAP_PEAKS	dba.overlap mode: return overlapping/unique peaksets
DBA_OLAP_ALL	dba.overlap mode: return report of correlations/overlaps for each pair of samples
DBA_OLAP_RATE	dba.overlap mode: return overlap rates
DBA_COR	When plotting a heatmap from an overlap record, use the correlation value.
DBA_OLAP	When plotting a heatmap from an overlap record, use the percentage overlap value.
DBA_INALL	When plotting a heatmap from an overlap record, use the number of peaks in common to both samples.
DBA_SCORE_READS	
	dba.count score is number of reads in ChIP
DBA_SCORE_CONTROL_READS	
	dba.count score is number of reads in Control
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_READS_FULL`
>   dba.count score is normalized ChIP read counts, using Full Library size

`DBA_SCORE_READS_MINUS_FULL`
>   dba.count score is normalized ChIP read counts minus Control read counts, using Full Library size

`DBA_SCORE_READS_EFFECTIVE`
>   dba.count score is normalized ChIP read counts, using Effective Library size

`DBA_SCORE_READS_MINUS_EFFECTIVE`
>   dba.count score is normalized ChIP read counts minus Control read counts, using Effective Library size

`DBA_SCORE_NORMALIZED`
>   dba.count score is normalized reads

`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_RPKM_MINUS`
>   dba.count score is RPKM of ChIP minus RPKM of Control

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

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

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

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

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

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

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

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

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

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

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

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

DBA_READS_BAM	When counting read files, assume the file type is BAM, regardless of the file extension.
DBA_READS_BED	When counting read files, assume the file type is BED (or zipped BED), regardless of the file extension.
DBA_EDGER	differential analysis method: edgeR (default: DBA_EDGER_GLM)
DBA_DESEQ2	differential analysis method: DESeq2 (using a single-factor GLM)
DBA_EDGER_BLOCK	
	differential analysis method: edgeR with blocking factors (GLM)
DBA_DESEQ2_BLOCK	
	differential analysis method: DESeq2 with blocking factors (GLM)
DBA_EDGER_GLM	differential analysis method: use GLM in edgeR for two-group comparisons
DBA_ALL_METHODS	
	use both analysis methods: c(DBA_EDGER,DBA_DESEQ2)
DBA_ALL_BLOCK	report on block results for both analysis methods: c(DBA_EDGER_BLOCK,DBA_DESEQ2_BLOCK)
DBA_ALL_METHODS_BLOCK	
	report on block results for all analysis methods, both blocked and unblocked: c(DBA_ALL_METHODS,DBA_ALL_BLOCK)
DBA_DATA_GRANGES	
	Use GRanges class for peaksets and reports. This is the default (DBA$config$DataType = DBA_DATA_GRANGES).
DBA_DATA_RANGEDDATA	
	Use RangedData class for peaksets and reports. Can be set as default (DBA$config$DataType = DBA_DATA_RANGEDDATA).
DBA_DATA_FRAME	Use data.frame class for peaksets and reports. Can be set as default (DBA$config$DataType = DBA_DATA_FRAME).
DBA_DATA_SUMMARIZED_EXPERIMENT	
	Return report as a [SummarizedExperiment]().
DBA_DATA_DBAOBJECT	
	Return a result-based DBA object from [dba.plotVenn]().
DBA_BLACKLIST_HG19	
	Homo sapiens 19 (chromosomes have "chr")
DBA_BLACKLIST_HG38	
	Homo sapiens 38 (chromosomes have "chr")
DBA_BLACKLIST_GRCH37	
	Homo sapiens 37 (chromosomes are numbers)
DBA_BLACKLIST_GRCH38	
	Homo sapiens 38 (chromosomes are numbers)
DBA_BLACKLIST_MM9	
	Mus musculus 9
DBA_BLACKLIST_MM10	
	Mus musculus 10
DBA_BLACKLIST_CE10	
	C. elegans 10
DBA_BLACKLIST_CE11	
	C. elegans 11
DBA_BLACKLIST_DM3	
	Drosophila melanogaster 3

DBA_BLACKLIST_DM6
                    Drosophila melanogaster 6

DBA_BLACKLIST   Retrieve blacklist

DBA_GREYLIST   Retrieve greylist

DBA_BLACKLISTED_PEAKS
                    Retrieve blacklisted peaks

DBA_LIBSIZE_DEFAULT
                    Default library size (`DBA_LIBSIZE_FULL` if no background, and `DBA_LIBSIZE_CHR`
                    if background present)

DBA_LIBSIZE_FULL
                    Full library size (all reads in library)

DBA_LIBSIZE_PEAKREADS
                    Library size is Reads in Peaks

DBA_LIBSIZE_BACKGROUND
                    Library size is Reads in Background

DBA_LIBSIZE_USER
                    User supplied library sizes

DBA_NORM_DEFAULT
                    Default normalization method

DBA_NORM_NATIVE
                    "Native"" normalization method (TMM for `DBA_EDGER` and RLE for `DBA_DESEQ2`)

DBA_NORM_LIB   Normalize by library size only

DBA_NORM_TMM   Normalize using TMM method (edgeR)

DBA_NORM_RLE   Normalize using RLE method (DESeq2)

DBA_NORM_SPIKEIN
                    Normalize based on spike-ins

DBA_NORM_USER   User supplied normalization factors

DBA_NORM_OFFSETS
                    Use offsets instead of normalization factors

DBA_NORM_OFFSETS_ADJUST
                    Use offsets instead of normalization factors; adjust based on library size (DE-
                    Seq)

DBA_OFFSETS_LOESS
                    Compute offsets using loess fit

DBA_OFFSETS_USER
                    Use offsetrs supplied by user

Note

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

Author(s)

Rory Stark

DiffBind3   *Differences between DiffBind 3.0 and earlier versions*

Description

Notes on the differences between DiffBind 3.0 and previous versions, and how run in a "backward compatible" manner.

Overview

Beginning with version 3.0, `DiffBind` introduces substantial updates and new features that may cause scripts written for earlier versions to function differently (or not at all), as well as altering the results. This page givens details on these changes, and how to approximate results computed with earlier version if desired.

The major change in version 3.0 is in how the data are modeled. In previous versions, a separate model was derived for each contrast, including data only for those samples present in the contrast. Model design options were implicit and limited to either a single factor, or a subset of two-factor "blocked" designs. Starting in version 3.0, the default mode is to include all the data in a single model, allowing for any allowable design formula and any set of allowable contrasts.

Another change starting from version 3.0 is in how normalization is done. There are more normalization options, and more explicit control over them. The default normalization options have also changed, so reproducing a pre-3.0 analysis requires that normalization parameters to be specified.

It is recommended that existing analyses be re-run with the current software. Existing scripts should execute (with the exception of two normalization parameters which have been moved from `dba.analyze` to the new interface function `dba.normalize`.)

See the `DiffBind` vignette for more information on processing and analyzing ChIP-seq (and ATAC-seq) experiments.

Changes to Defaults

- **blacklist** is applied by default, if available, using automatic detection of reference genome.

- **greylists** are generated from controls and applied by default.

- **minimum read counts** are now 0 instead of being rounded up to 1 (this is now controllable).

- **centering peaks around summits** is now done by default using 401-bp wide peaks (recommend to use 'summits=100' for ATAC-seq).

- **read counting** is now performed by 'summarizeOverlaps()' by default, with single-end/paired-end counting automatically detected.

- **filtering** is performed by default; consensus peaks where no peak has and RPKM value of at least 1 in any sample are filtered.

- **control read subtraction** is now turned off by default if a greylist is present

- **normalization** is based on full library sizes by default for both 'edgeR' and 'DESeq2'analyses.

- **score** is set to normalized values by default.

Backward compatibility

Most existing [DiffBind](#) scripts and saved objects will run correctly using version 3.0, but there may be differences in the results.

This section describes how to approximate earlier results for existing scripts and objects.

Running with saved DBA objects::   If a DBA object was created with an earlier version of [DiffBind](#), and saved using the [dba.save](#) function, and loaded using the [dba.load](#) function, all settings should be preserved, such that running the analysis anew will yield the same results.

In order to re-run the analysis using the post-version 3.0 settings, the original script should be used to re-create the DBA object.

Re-running DiffBind scripts::   By default, if you re-run a [DiffBind](#) script, it will use the new defaults from version 3.0 and beyond. In order to re-analyze an experiment in the pre-version 3.0 mode, a number of defaults need to be changed.

When calling [dba.count](#), the following defaults are changed:

- summits: This parameter is now set by default. Setting summits=FALSE will preempt re-centering each peak interval around its point of highest pileup.
- filter: The new default for this parameter is 1 and is based on RPKM values; previously it was set to filter=0 and was based on read counts.
- minCount: This is a new parameter representing a minimum read count value. It now default to 0; to get the previous behavior, set minCount=1.

The easiest way to perform subsequent processing in a pre-version 3.0 manner is to set a configuration option:

DBA$config$design <-FALSE

This will result in the appropriate defaults being set for the new interface function, [dba.normalize](#) (which does not need to be invoked explicitly.) The pre-version 3.0 settings for [dba.normalize](#) parameters are as follows:

- normalize: DBA_NORM_DEFAULT
- library: DBA_LIBSIZE_FULL
- background: FALSE

Note that two parameters that used to be available when calling [dba.analyze](#) have been moved:

- bSubControl: now integrated into [dba.count](#). FALSE by default (unless a greylist has been added using [dba.blacklist](#)).
- bFullLibrarySize: now integrated into [dba.normalize](#) as an option for the library parameter. library=DBA_LIBSIZE_FULL is equivalent to bFullLibrarySize=TRUE, and library=DBA_LIBSIZE_PE is equivalent to bFullLibrarySize=FALSE.

Author(s)

Rory Stark

See Also

The [DiffBind](#) vignette has been updated to show how to analyze experiments using version 3.0.

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