Package 'NarrowPeaks'

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Type Package

Title Shape-based Analysis of Variation in ChIP-seq using Functional PCA

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Description The package applies a functional version of principal component analysis (FPCA) to: (1) Postprocess data in wiggle track format, commonly produced by generic ChIP-seq peak callers, by applying FPCA over a set of read-enriched regions (ChIP-seq peaks). This is done to study variability of the the peaks, or to shorten their genomic locations accounting for a given proportion of variation among the enrichment-score profiles. (2) Analyse differential variation between multiple ChIP-seq samples with replicates. The function 'narrowpeaksDiff' quantifies differences between the shapes, and uses Hotelling's T2 tests on the functional principal component scores to identify significant differences across conditions. An application of the package for Arabidopsis datasets is described in Mateos, Madrigal, et al. (2015) Genome Biology: 16:31.

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Maintainer Pedro Madrigal <pm12@sanger.ac.uk>

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`NarrowPeaks-package` *Shape-based Analysis of Variation in ChIP-seq using Functional PCA*

Description

The package applies a functional version of principal component analysis (FPCA) to: (1) Post-process data in wiggle track format, commonly produced by generic ChIP-seq peak callers, by applying FPCA over a set of read-enriched regions (ChIP-seq peaks). This is done to study variability of the the peaks, or to shorten their genomic locations accounting for a given proportion of variation among the enrichment-score profiles. (2) Analyse differential variation between multiple ChIP-seq samples with replicates. The function 'narrowpeaksDiff' quantifies differences between the shapes, and uses Hotelling's T2 tests on the functional principal component scores to identify significant differences across conditions. An application of the package for Arabidopsis datasets is described in Mateos, Madrigal, et al. (2015) Genome Biology: 16:31.

Details

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Author(s)

Pedro Madrigal, with contributions from Pawel Krajewski <pkra@igr.poznan.pl>

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References

Mateos JL, Madrigal P, et al. (2015) Combinatorial activities of SHORT VEGETATIVE PHASE and FLOWERING LOCUS C define distinct modes of flowering regulation in Arabidopsis. Genome Biology 16: 31.

Bailey T, Krajewski P, Ladunga I, Lefebvre C, Li Q, Liu T, Madrigal P, Taslim C, Zhang J (2013) Practical Guidelines for the Comprehensive Analysis of ChIP-seq data. PLOS Comput Biol. 9 (11): e1003326.

Examples

```
owd <- setwd(tempdir())

##For this example we will use a subset of the AP1 ChIP-seq data (Kaufmann et
##al., 2010)
##The data is obtained after analysis using the CSAR package available in
##Bioconductor
data("NarrowPeaks-dataset")
writeLines(wigfile_test, con="wigfile.wig")

##Write binary files with the WIG signal values for each chromosome
##independently and obtain regions of read-enrichment with score values greater
##than 't', allowing a gap of 'g'. Data correspond to enriched regions found up
##to 105Kb in the Arabidopsis thaliana genome
wigScores <- wig2CSARScore(wigfilename="wigfile.wig", nbchr = 1,
chrle=c(30427671))
gc(reset=TRUE)
library(CSAR)
candidates <- sigWin(experiment=wigScores$infoscores, t=1.0, g=30)

##Narrow down ChIPSeq enriched regions by functional PCA
shortpeaks <- narrowpeaks(inputReg=candidates,
scoresInfo=wigScores$infoscores, lmin=0, nbf=150, rpenalty=0,
nderiv=0, npcomp=2, pv=80, pmaxscor=3.0, ms=0)

###Export GRanges object with the peaks to annotation tracks in various
##formats. E.g.:
library(GenomicRanges)
names(elementMetadata(shortpeaks$broadPeaks))[3] <- "score"
names(elementMetadata(shortpeaks$narrowPeaks))[2] <- "score"
library(rtracklayer)
export.bedGraph(object=candidates, con="CSAR.bed")
export.bedGraph(object=shortpeaks$broadPeaks, con="broadPeaks.bed")
export.bedGraph(object=shortpeaks$narrowPeaks, con="narrowpeaks.bed")

setwd(owd)
```

---

narrowpeaks *Detect Narrow Peaks from Enrichment-Score Profiles*

Description

Detect narrow peaks from enrichment-score profiles (ChIP-seq peak regions).

Usage

```
narrowpeaks(inputReg, scoresInfo, lmin = 0, nbf = 50, rpenalty= 0,
nderiv= 0, npcomp = 5, pv = 80, pmaxscor = 0.0, ms = 0)
```

Arguments

inputReg	Output of the function sigWin in package CSAR.
scoresInfo	Output infoscores in the function wig2CSARScore, or the function ChIPseqScore after data analysis with package **CSAR**.
lmin	Minimum length of an enriched region from the WIG file to be processed. Integer value.
nbf	Number of order-4 B-spline basis functions that will represent the shape of each candidate transcription factor binding site. Integer value.
rpenalty	Smoothing parameter for derivative penalization. Positive numeric value.
nderiv	Order of derivative penalization, if rpenalty>0. Integer value.
npcomp	Number of functional principal components. Integer value greater than or equal to nbf.
pv	Minimum percentage of variation to take into account during the analysis. Numeric value in the range 0-100 (see the vignette and Mateos, Madrigal, et al. (2015)).
pmaxscor	Cutoff for trimming of scoring function. Numeric value in the range 0-100.
ms	Peaks closer to each other than ms nucleotides will be merged in the final list. Integer value.

Details

This function produces shortened sites from a list of candidate transcription factor binding sites of arbitrary extension and shape. First, the enrichment signal from each candidate site is represented by a smoothed function constructed using a linear combination of order-4 B-spline basis functions. The data values are fitted using either least squares (if $rpenalty = 0$), or penalized residuals sum of squares (spline smoothing if $rpenalty > 0$).

Then, a functional principal component analysis for npcomp eigenfunctions is performed (Ramsay and Silverman, 2005), giving as a result a set of probe scores (principal component scores) which sum of squares is reported in elementMetadata(broadPeaks)[,"fpcaScore"]. The higher the value of fpcaScore, the higher the variance that candidate peak accounts for within the original data. Details on the usage of semi-metrics in functional PCA is described in Ferraty and Vieu, 2006.

After that, we impose the condition that total scoring function for each reported narrow peak must be at least pmaxscor per cent of the maximum value. Max value is calculated from a set of scoring functions using only the eigenfunctions required to achieve pv percent of variance. A new set of scores is computed using trimmed versions of the eigenfunctions (see Vignette), and the root square is stored in elementMetadata(narrowPeaks)[,"trimmedScore"].

Value

A list containing the following elements:

`fdaprofiles`	A functional data object encapsulating the enrichment profiles (see **fda** package. To plot the data use `plot.fd(fdaprofiles)`).
`broadPeaks`	Description of the peaks prior to trimming. A `GRanges` object (see **GenomicRanges** package) with the information: `seqnames` (chromosome), `ranges` (start and end of the candidate site), `strand` (not used), `max` (maximum signal value for candidate site), `average` (mean signal value for candidate site), `fpcaScore` (sum of squares of the first `reqcomp` principal component scores for candidate site).
`narrowPeaks`	Description of the peaks after trimming. A `GRanges` object (see **GenomicRanges** package) with the information: `seqnames` (chromosome), `ranges` (start and end after trimming), `strand` (not used), `broadPeak.subpeak`, `trimmedScore` (see details), `narrowedDownTo` (length reduction relative to the candidate), `merged` (logical value).
`reqcomp`	Number of functional principal components used. Integer value.
`pvar`	Total proportion of variance accounted for by the `reqcomp` components used. Numeric value in the range 0-100 (always greater than or equal to argument `pv`).

Author(s)

Pedro Madrigal, <pm12@sanger.ac.uk>

References

Mateos JL, Madrigal P, et al. (2015) Combinatorial activities of SHORT VEGETATIVE PHASE and FLOWERING LOCUS C define distinct modes of flowering regulation in Arabidopsis. Genome Biology 16: 31.
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Muino JM, Kaufmann K, van Ham RC, Angenent GC, Krajewski P (2011) ChIP-seq analysis in R (CSAR): An R package for the statistical detection of protein-bound genomic regions. Plant Methods 7:11.
Ramsay, J.O. and Silverman, B.W. (2005) Functional Data Analysis. New York: Springer.
Ferraty, F. and Vieu, P. (2006) Nonparametric Functional Data Analysis. New York: Springer.

See Also

`wig2CSARScore`, `NarrowPeaks-package`

Examples

```
owd <- setwd(tempdir())

##For this example we will use a subset of the AP1 ChIP-seq data (Kaufmann et
##al., 2010)
```

```
##The data is obtained after analysis using the CSAR package available in
##Bioconductor
data("NarrowPeaks-dataset")
writeLines(wigfile_test, con="wigfile.wig")

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##to 105Kb in the Arabidopsis thaliana genome
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candidates <- sigWin(experiment=wigScores$infoscores, t=1.0, g=30)

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names(elementMetadata(shortpeaks$broadPeaks))[3] <- "score"
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library(rtracklayer)
export.bedGraph(object=candidates, con="CSAR.bed")
export.bedGraph(object=shortpeaks$broadPeaks, con="broadPeaks.bed")
export.bedGraph(object=shortpeaks$narrowPeaks, con="narrowpeaks.bed")

setwd(owd)
```

`narrowpeaksDiff` *Differential Analysis of Transcription Factor Binding using FPCA*

Description

Shape-based differential binding analysis and hypothesis testing for ChIP-seq datasets using Functional Principal Component Analysis and Hotelling's T2 tests.

Usage

```
narrowpeaksDiff(bedFile,  headerBed= TRUE, flank=100, bigwigs , conditions ,
nbasis=50, pcs = 10, bigWigSummaryPath=getwd(), variation = 0.6)
```

Arguments

`bedFile`	Text file in BED format. It should contain at least 3 columns (chr, start, end), in which case the reference point for FDA is calculated as the central point. A 4-th column can be provided containing the reference point.

headerBed	'TRUE' if the first row in the BED file contain the name of the columns. 'FALSE' otherwise.
flank	Length (in bp.) that is considered upstream and downstream the reference point (or central point, if reference point is not given) for functional principal component analysis.
bigwigs	Vector contaning the name of the bigWig files to be used in the analysis.
conditions	Vector of characters with the LABELS for the bigWig files. Biological replicates must have the same label.
nbasis	Number of order-4 B-Spline basis functions for functional data analysis.
pcs	Number of principal components to be computed (default is 10).
bigWigSummaryPath	Path to the UCSC utility bigWigSummary (in case it is differente from the current directory). The tool can be downloaded for Linux and macOSX from the UCSC website: http://hgdownload.cse.ucsc.edu/admin/exe/
variation	Minimum proportion of variation that is considered to select the number of functional principal component scores used in the Hotelling's T2 tests (0-1, default is 0.6).

Details

Detailed information can be found in the vignette of the package.

Value

A list containing the following elements:

fdaprofiles	A list of matrices corresponding to the data of regions of interest (BED file) in the bigWig files.
p.values	A list of pairwise comparisons between experimental conditions (taking into account replicates) for each region in the BED file. P-values are computed using the Hotelling's T2 test.

Author(s)

Pedro Madrigal, <pm12@sanger.ac.uk>

References

Mateos JL, Madrigal P, et al. (2015) Combinatorial activities of SHORT VEGETATIVE PHASE and FLOWERING LOCUS C define distinct modes of flowering regulation in Arabidopsis. Genome Biology 16: 31.
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Ramsay, J.O. and Silverman, B.W. (2005) Functional Data Analysis. New York: Springer.

See Also

narrowpeaks, NarrowPeaks-package

Examples

```
##Example code:
##library(NarrowPeaks)
##bigwigs <- c("SVP_WT_rep1.bw","SVP_WT_rep2.bw","SVP_WT_rep3.bw",
## "SVP_mt_rep1.bw","SVP_mt_rep2.bw","SVP_mt_rep3.bw")
##conds   <- c("SVP_WT","SVP_WT","SVP_WT","SVP_mt","SVP_mt","SVP_mt")
##x <- narrowpeaksDiff(bedFile="regions.bed", bigwigs=bigwigs, conditions=conds, variation = 0.8)
##x$p.values
```

---

wig2CSARScore *Convert Data from a Wiggle Track (WIG) File to CSAR Binary Format*

Description

Convert data from a wiggle track (WIG) file to CSAR binary format and extract read-enriched regions.

Usage

```
wig2CSARScore(wigfilename, nbchr, chrle)
```

Arguments

wigfilename	WIG file containing the enrichment-score signal of a transcription factor binding experiment.
nbchr	Number of chromosomes.
chrle	Vector of lengths of the chromosomes (in base pairs).

Details

The Wiggle format (WIG/bigWig) is described on the UCSC Genome Bioinformatics web site: http://genome.ucsc.edu/FAQ/FAQformat. It allows the display of continuous value data in the genome browser. Although specifically designed for post-processing of WIG files, resulting from the analysis of ChIP-seq experiments (with Bioconductor packages **BayesPeak**, **CSAR**, **PICS**, or other tools such as MACS, F-seq, etc.), **NarrowPeaks** can process other type of sequencing data encoded in WIG format in order to locate regions of high variability in the data.

Value

A list of two elements:

`infoscores` A list with the same elements as reported by the function `ChIPseqScore` in the **CSAR** Bionductor package: chr (Chromosome names), chrL (Chromosome length (bp).), filenames (Name of the files where the score values are stored.), digits (Score values stored on the files need to be divided by 10^`digits`).

Author(s)

Pedro Madrigal, <pm12@sanger.ac.uk>

References

Mateos JL, Madrigal P, et al. (2015) Combinatorial activities of SHORT VEGETATIVE PHASE and FLOWERING LOCUS C define distinct modes of flowering regulation in Arabidopsis. Genome Biology 16: 31.
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See Also

narrowpeaks, NarrowPeaks-package

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##al., 2010)
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##than 't', allowing a gap of 'g'. Data correspond to enriched regions found up
##to 105Kb in the Arabidopsis thaliana genome
wigScores <- wig2CSARScore(wigfilename="wigfile.wig", nbchr = 1,
chrle=c(30427671))

setwd(owd)
```

wigfile_test *Example Wiggle Track Produced After ChIP-seq Data Analysis*

Description

Example of wiggle track produced after ChIP-seq data analysis. The data represents a small subset of a WIG file storing continuous-valued scores based on a Poisson test for the chromosome 1 of *Arabidopsis thaliana* (Kaufmann et al., 2010). It contains first 49515 lines of the WIG file for the complete experiment.

Format

Wiggle track format (WIG) data in a character vector.

Source

Gene Expression Omnibus GSE20176 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20176). Record from chromatin immunoprecipitation experiments wit AP1-specific antibodies followed by deep-sequencing in order to determine AP1 binding sites on a genome-wide scale in *Arabidopsis thaliana*.

References

Kaufmann et al. (2010) Orchestration of Floral Initiation by APETALA1. Science 328:85-89.

See Also

NarrowPeaks-package

Examples

```
data(NarrowPeaks-dataset)
```

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