Package 'Scale4C'

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Type Package

Title Scale4C: an R/Bioconductor package for scale-space transformation of 4C-seq data

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Description Scale4C is an R/Bioconductor package for scale-space transformation and visualization of 4C-seq data. The scale-space transformation is a multi-scale visualization technique to transform a 2D signal (e.g. 4C-seq reads on a genomic interval of choice) into a tesselation in the scale space (2D, genomic position x scale factor) by applying different smoothing kernels (Gauss, with increasing sigma). This transformation allows for explorative analysis and comparisons of the data's structure with other samples.

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addPointsOfInterest Add points of interest to a Scale4C object

Description

The function addPointsOfInterest adds marker points to a Scale4C object, which are subsequently used to mark points of interest in created plots.

Usage

Index

addPointsOfInterest(data, poi)

Arguments

data Scale4C object the points are to be added to

poi Points of interest data, in a GRanges object. Important: column names must

be specified and include 'colour' and 'name' for each point of interest with

appropriate values

Details

The function addPointsOfInterest adds predefined points of interest to a Scale4C object. Each point of interest is defined by 'chr', 'start', 'end', 'colour', and 'name'. A bed file or text file can be used to store the information, however, column names have to be added before import. Other additional columns are ignored by the function. The function then converts the information to a GRanges object.

Value

A data frame that contains the data for all points of interest

Author(s)

Carolin Walter

Examples

calculateFingerprintMap

Calculate scale-space fingerprint map for given 4C-seq data

Description

calculateFingerprintMap uses the scale space map to calculate the inflection points of the smoothed signals.

Usage

```
calculateFingerprintMap(data, maxSQSigma = 5000, epsilon = 0.0000001)
```

Arguments

data Scale-space object for the 4C-seq data

maxSQSigma Maximum square sigma used to calculate the fingerprint map

epsilon Small numeric value (can also be zero); used to test for inflection points

Details

Scale4C uses Gauss kernels of increasing (square) sigma to smooth the original 4C-seq signal. The resulting inflection points for a chosen sigma are stored in the corresponding line of the fingerprint map, i.e. a 2D matrix (position x sigma).

Value

A Scale4C object containing the fingerprint map for a Scale4C object, i.e. a matrix with quite a lot of zeros and the occasional 2 or -1 as symbols for inflection points. The fingerprint map is included as second assay of the Scale4C object's scaleSpace SummarizedExperiment slot.

Author(s)

Carolin Walter

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Examples

```
# read prepared example data
data(liverData)
# use small maxSQSigma for a fast example
liverData<-calculateFingerprintMap(liverData, maxSQSigma = 50)
head(t(assay(scaleSpace(liverData), 2))[1:10,1:20])</pre>
```

calculateScaleSpace

Calculate scale-space representation for given 4C-seq data

Description

Scale4C uses Gauss kernels of increasing (square) sigma to smooth the original 4C-seq signal. The resulting data is stored in a 2D matrix (position x sigma).

Usage

```
calculateScaleSpace(data, maxSQSigma = 5000)
```

Arguments

data Scale-space object for the 4C-seq data

maxSQSigma Maximum square sigma used to calculate the scale space

Details

The central idea of the scale-space transformation is to smooth the original signal with increasing strength, identify inflection points, track those inflection points throughout the different smoothing layers, and find singularities in those inflection point 'lines'. In case of 4C-seq data, this corresponds to smoothing the signal gradually, while making notes when features such as 'peaks' or 'valleys' disappear by merging with other features. calculateScaleSpace smoothes the original signal up to a provided smoothing factor square sigma (Gauss kernel).

Value

A SummarizedExperiment that contains in its first assay the scale space representation for a Scale4C object

Author(s)

Carolin Walter

Examples

```
# read prepared example data
data(liverData)
# use small maxSQSigma for a fast example
scaleSpace(liverData)<-calculateScaleSpace(liverData, maxSQSigma = 10)
head(t(assay(scaleSpace(liverData), 1))[,1:20])</pre>
```

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Find singularities in a provided scale-space fingerprint map

Description

This function allows to identify singular points in a scale-space fingerprint map.

Usage

```
findSingularities(data, minSQSigma = 5, outputTrackingInfo = FALSE,
guessViewpoint = FALSE, useIndex = TRUE)
```

Arguments

data Scale-space object for the 4C-seq data

minSQSigma Minimum square sigma used to calculate singularities; for a square sigma of 1,

the data can be quite chaotic and identified singularities are less prone to error

when a minSQSigma of 2 or higher is used

outputTrackingInfo

If TRUE, notify the user that a certain position / singularity causes problems

during tracking in the fingerprint map

guessViewpoint If TRUE, add another 'peak' at the coordinates of the viewpoint, if provided.

Extra singularities can also be added manually. The idea is to decrease running speed significantly by not actually calculating the largest singularity for a typical 4C-seq experiment, i.e. the main viewpoint peak. Its inflection point contours should easily be visible in the fingerprint map, provided that the viewpoint position is actually included in the raw data, but calculating the full contours requires a very high sigma that should usually not be needed to identify other singularities in the area. Cave: Viewpoint contours don't have to start directly next to the

viewpoint coordinates.

useIndex If TRUE, use fragment index instead of genomic position data

Details

findSingularities identifies possible singular points in the fingerprint map's contours, i.e. points where a line of '2' and '-1' in the matrix meet. Starting from those points in scale-space, the contours are traced back down. This 'localization step' ensures that the coordinates for a feature ('peak' or 'valley') corresponding to a given singular point are as accurate as possible: Smoothing with a high-sigma Gauss kernel distorts the original signal somewhat, so that the inflection points identifying the start and the end of a certain feature 'move outwards'.

Value

A data frame that lists the position where a singular point occurs (genomic position and scale-space sigma), plus the size of the feature as given by its minimal / left and maximal / right position.

Author(s)

Carolin Walter

Examples

```
# read prepared example data
data(liverData)
singularities(liverData) = findSingularities(liverData, 5, useIndex = TRUE)
singularities(liverData)
```

importBasic4CseqData Import raw data from a provided Basic4Cseq output file

Description

A convenience function to easily include **Basic4Cseq** output data into **Scale4C**, importBasic4CseqData extracts valid fragments or valid fragment ends from a typical **Basic4Cseq** output table.

Usage

```
importBasic4CseqData(rawFile, viewpoint, viewpointChromosome,
    distance, useFragEnds = TRUE)
```

Arguments

rawFile Name for the raw file

viewpoint Viewpoint position: only fragments around a certain point of interest are im-

ported (doesn't have to be the actual viewpoint of the experiment, though)

viewpointChromosome

Viewpoint chromosome of the experiment

distance Distance from the viewpoint: ony fragments within a certain distance of the

viewpoint are imported

useFragEnds If TRUE, use full fragment end data, if FALSE, merge fragmentStart and frag-

mentEnd to a single item per fragment

Details

importBasic4CseqData is a convenience function to import data from **Basic4Cseq**. It can be ignored altogether if raw experimental data is imported from another source or with another function into R.

Value

A GRanges object that includes the experiment's raw data for further processing

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Author(s)

Carolin Walter

Examples

liverData

Example 4C-seq data set of fetal liver data

Description

This data set contains an instance of a Scale4C object.

The 4C-seq data was taken from Stadhouders et al's fetal liver data set.

Usage

```
data("liverData")
```

Format

Formal class 'Scale4C'

Value

A pre-computed instance of a Scale4C object with fingerprint map and singularities. Scale-space image is reduced to save space.

Source

Shortened version of Stadhouders et al's fetal liver data:

Stadhouders, R., Thongjuea, S., et al. (2012): Dynamic long-range chromatin interactions control Myb proto-oncogene transcription during erythroid development. EMBO, 31, 986-999.

Examples

```
data("liverData")
liverData
```

liverDataVP

Example 4C-seq data set of fetal liver data, with added VP

Description

This data set contains an instance of a Scale4C object.

The 4C-seq data was taken from Stadhouders et al's fetal liver data set. It contains a manually added viewpoint peak and a peak with a manual coordinate correction.

Usage

```
data("liverDataVP")
```

Format

Formal class 'Scale4C'

Value

A pre-computed instance of a Scale4C object with fingerprint map and singularities

Source

Shortened version of Stadhouders et al's fetal liver data:

Stadhouders, R., Thongjuea, S., et al. (2012): Dynamic long-range chromatin interactions control Myb proto-oncogene transcription during erythroid development. EMBO, 31, 986-999.

Examples

```
data("liverDataVP")
liverDataVP
```

outputScaleSpaceTree Output list of all features for a given scale-space map

Description

This function provides a list of features for a given fingerprint map in scale-space, with position and range of sigma for which the feature in question exists

Usage

```
outputScaleSpaceTree(data, outputPeaks = TRUE, useLog = TRUE,
   useIndex = TRUE)
```

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Arguments

data a Scale4C object with singularity data

outputPeaks If TRUE, output GRanges peak list only, if FALSE, also output valley data in a

larger table

useLog If TRUE, use a log2 transformation on the square sigma values (fewer changes

and fewer singularities for high sigma, in contrast to low sigma)

useIndex If TRUE, use fragment position

Details

Similar to plotTesselation, outputScaleSpaceTree analyzes a list of singular points and calculates corresponding features, i.e. 'peaks' and 'valleys'. Each singular point marks the disappearance (or occurrence, depending on the view) of a feature in scale space: With increasing square sigma as smoothing parameter for the Gauss kernel, smaller features are merged into larger features. In case of Gauss smoothing, one feature is always surrounded by two features of the opposite type, e.g. a 'peak' is surrounded by two 'valleys'. If a 'peak' is smoothed out, it is replaced by a new valley formed of the former peak's adjacent valleys. The singularity list contains only direct information on those 'central' features; outputScaleSpaceTree adds data on the direct neighbours / adjacent features and also provides the sigma ranges for the features as a measure of their stability throughout the smoothing process. Mean read counts for the identitied features are also provided ("signal"). If outputPeaks is true, a reduced list of peaks is printed, while omitting valleys or the central-left-right structural information.

Value

A GRanges object that includes all features as identified through singular points, plus 'neighbour features' at each side (each 'peak' is surrounded by two 'valleys' and vice versa for Gauss kernel smoothing), with positions and range of sigma for which the feature in question remains stable

Author(s)

Carolin Walter

Examples

```
# read prepared example data
data(liverDataVP)
output = outputScaleSpaceTree(liverDataVP, useLog = FALSE)
head(output)
```

plotInflectionPoints Draw a smoothed near-cis profile with marked inflection points for a 4C-seq signal

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Description

plotInflectionPoints plots the inflection points for a given square sigma (i.e. a row of the fingerprint map) onto a corresponding smoothed near-cis plot for the 4C-seq signal. This allows to check problematic parts of the fingerprint map in more detail (e.g. unclear tracking areas with close contours), and to improve possible corrections in the singularity list. Plotting the smoothed signal for a given square sigma before calculation of the fingerprint map is also possible.

Usage

```
plotInflectionPoints(data, sqsigma, fileName = "inflectionPlot.pdf",
width = 9, height = 5, maxVis = 5000, useIndex = TRUE, plotIP = TRUE)
```

Arguments

| data | Scale4C object with experimental 4C-seq data to be smoothed and processed |
|----------|---|
| sqsigma | Chosen square sigma, i.e. row of the fingerprint map to pick the inflection points from |
| fileName | Optional name for export file (pdf) |
| width | Width of the plot |
| height | Height of the plot |
| maxVis | Maximum y-axis value (read number, not sigma!) for visualization |
| useIndex | If TRUE, use fragment index for x-axis |
| plotIP | If TRUE, then mark chosen inflection points, if FALSE, simply plot smoothed data |

Value

A near-cis plot of the smoothed data with (optional) marked inflection points in darker or lighter grey, depending on their direction

Note

PDF export is supported. If no plot file name is provided, the result is plotted on screen.

Author(s)

Carolin Walter

Examples

```
data(liverData)
plotInflectionPoints(liverData, 50)
```

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| plotTesselation | Draw the final scale space tesselation |
|-----------------|--|
| | |

Description

This method draws the final scale space tesselation, as specified by the list of singularities identified for a Scale4C object. Features are marked with different colours; for the default colour scheme, brown corresponds to 'peaks' and blue to 'valleys', while slightly darker colours mark features originating from singularities ('central' features in a set of three features, e.g. 'valley-peak-valley' or 'peak-valley-peak') and lighter colours the two adjacent features. Different colours for 'central' and 'adjacent' features allow for optical quality control of the tesselation: a 'central' / dark feature's direct predecessor or successor (y-axis) can't be of the same colour (i.e. a 'peak' that passes through a singularity is smoothed out into a 'valley'), and neighbouring intervals have to be of the opposing (but lighter) colour (i.e. each 'peak' is surrounded by two 'valleys' for Gauss kernel smoothing). The same is not necessarily true for an 'adjacent' / light feature, however.

Usage

```
plotTesselation(data, minSQSigma = 5, maxSQSigma = -1, maxVis = -1,
fileName = "tesselationPlot.pdf", width = 5, height = 5, xInterval = 100,
yInterval = 50, chosenColour = c("grey50", "moccasin", "lightskyblue1",
"beige", "azure"), useIndex = TRUE)
```

Arguments

| data | Scale4C object with singularity data |
|--------------|---|
| minSQSigma | Minimum square sigma to consider |
| maxSQSigma | Maximum square sigma to consider; if -1 then the number of rows in the finger-print map is used |
| maxVis | Maximum y value for visualization (doesn't have to be maxSQSigma); if -1 also defaults to number of rows in the fingerprint map |
| fileName | Optional name for export file (pdf) |
| width | Width of the plot |
| height | Height of the plot |
| xInterval | Interval length for x-axis |
| yInterval | Interval length for y-axis |
| chosenColour | Chosen colours for the tesselation plot, five in total. Colour 1 is used for the actual lines of the plot, colour 2 for 'central peaks', colour 3 for 'central valleys', colour 4 for 'adjacent peaks', and colour 5 for 'adjacent valleys' |
| useIndex | If TRUE, use fragment index for x-axis |

Value

A tesselation plot, showing different features of the scale space with their range of existance (square sigma) and position)

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Note

PDF export is supported. If no plot file name is provided, the result is plotted on screen.

Author(s)

Carolin Walter

Examples

```
if(interactive()) {
    data(liverData)
    plotTesselation(liverData)
}
```

plotTraceback

Draw the traceback results for a list of singular points on a fingerprint map

Description

This method plots the traceback results together with fingerprint data, allowing to check for possible errors during tracking. Problems during tracking can occur if contours are very close, have holes, or if the singularity in question is not recognized at all due to holes at the meeting point of both contours that form a singular point. Each singular point is marked with a grey triangle, and the traced left and right end of the corresponding feature are connected with grey lines. If a contour's end doesn't match the traceback line, manual correction is possible in the singularity list.

Usage

```
plotTraceback(data, maxSQSigma = -1, fileName = "tracebackPlot.pdf",
width = 15, height = 15, useIndex = TRUE)
```

Arguments

data Scale4C object with singularity data

maxSQSigma Maximum square sigma (i.e. maximum y value) to be drawn; if -1 then all

available rows in the fingerprint map are used

fileName Optional name for export file (pdf)

width Width of the plot height Height of the plot

useIndex If TRUE, use fragment index for x-axis

Value

A traceback plot, showing the traced singular points with their points of origin throughout different smoothing layers)

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Note

PDF export is supported. If no plot file name is provided, the result is plotted on screen.

Author(s)

Carolin Walter

Examples

```
if(interactive()) {
    data(liverData)
    plotTraceback(liverData)
}
```

Scale4C

Creating a Scale4C object

Description

This function creates a Scale4C object. Data on the 4C-seq experiment, i.e. read counts per fragment and viewpoint coordinates, are stored and checked for plausibility.

Usage

```
Scale4C(viewpoint, viewpointChromosome, rawData)
```

Arguments

viewpoint The experiment's viewpoint (start, single coordinate)

viewpointChromosome

The experiment's viewpoint Chromosome

rawData Reads of the 4C-seq experiment per fragment on an interval of interest (GRanges

object with position and read data)

Details

A Scale4C object contains the basic information on a 4C-seq experiment for a certain interval of interest, i.e. read counts at given positions. See Scale4C-class for details. Scale-space features such as fingerprint maps or tesselation are calculated during further steps of the analysis by the appropriate functions.

Scale4C expects the raw data to be in a simple data frame consisting of 'position' and 'reads'. importBasic4CseqData allows to import fragment data from Basic4Cseq for convenience, however, preparing and importing a simple table with two columns into R is sufficient.

Value

An instance of the Scale4C class.

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Author(s)

Carolin Walter

See Also

```
Scale4C-class
```

Examples

```
# create a Scale4C object from a Basic4Cseq export table with added
# viewpoint data
csvFile <- system.file("extdata", "liverData.csv", package="Scale4C")
liverReads <- importBasic4CseqData(csvFile, viewpoint = 21160072,
    viewpointChromosome = "chr10", distance = 1000000)
liverData = Scale4C(rawData = liverReads, viewpoint = 21160072,
    viewpointChromosome = "chr10")
liverData</pre>
```

Scale4C-class

Class "Scale4C"

Description

This class is a container for information on a specific 4C-seq scale-space transformation. Stored information includes raw read data, the experiment's viewpoint location (optional), possible points of interest, the scale-space fingerprint map, and a list of identified singularities in scale-space.

Objects from the Class

Objects can be created by calls of the form new("Scale4C", ...).

Slots

viewpoint: Object of class "numeric" representing the viewpoint's location

viewpointChromosome: Object of class "character" representing the viewpoint's chromosome

pointsOfInterest: Object of class "GRanges" representing any points of interest to be marked in the visualizations (usually near-cis based, i.e. close to the viewpoint)

rawData: Object of class "GRanges" representing the 4C-seq reads (or signal strength) of the experiment at given genomic positions

scaleSpace: Object of class "SummarizedExperiment" representing the gradually smoothed 4C-seq signal ('scale space') in its first assay and the corresponding fingerprint map in its second assay.

singularities: Object of class "GRanges" representing singularities in the fingerprint map for the given 4C-seq signal

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Methods

viewpoint<- signature(object = "Scale4C", value = "numeric"): Setter-method for the viewpoint slot.

viewpoint signature(object = "Scale4C"): Getter-method for the viewpoint slot.

viewpointChromosome<- signature(object = "Scale4C", value = "character"): Setter-method
for the viewpointChromosome slot.</pre>

viewpointChromosome signature(object = "Scale4C"): Getter-method for the viewpointChromosome slot.

pointsOfInterest<- signature(object = "Scale4C", value = "GRanges"): Setter-method for the
pointsOfInterest slot.</pre>

pointsOfInterest signature(object = "Scale4C"): Getter-method for the pointsOfInterest slot.

rawData<- signature(object = "Scale4C", value = "GRanges"): Setter-method for the raw-Data slot.

rawData signature(object = "Scale4C"): Getter-method for the rawData slot.

scaleSpace signature(object = "Scale4C"): Getter-method for the scaleSpace slot.

singularities<- signature(object = "Scale4C", value = "GRanges"): Setter-method for the singularities slot.

singularities signature(object = "Scale4C"): Getter-method for the singularities slot.

Author(s)

Carolin Walter

Examples

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