

methVisual

October 5, 2010

Cooccurrence

Visualization of binary methylation data

Description

Visualization of binary methylation data including neighbored cooccurrence

Usage

```
Cooccurrence (methData, file, real, lolli)
```

Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
file	String; path and file name for saving the result. Default format is .pdf
real	logical; real position (real=TRUE) or relative position (real=FALSE) according to the reference sequence
lolli	Integer; size of lollipops

Details

Visualization of methylation states using lollipop graphs, percentage of methylation across experiments and value of neighbored cooccurrence due to calculation of spearman correlation. Every single CpG site is marked with a circle with the following characteristic, a filled circle represents a methylated CpG and an empty circle a non methylated one. The modification in methVisual lollipop plot is the calculation and visualization of dependencies (also known as cooccurrence) between neighbored methylated CpG site and non methylated CpG site. That means, given a set of bisulfite sequenced clones one would like to detect subgroups where specific CpG sites always occur coordinately either methylated or non methylated.

Value

summary plot that will be saved by default as pdf file under the given path and name

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData, file is the path to R home directory.
## In order to save Cooccurrence.pdf, make sure that you have writing
## permission under R.home() directory. If you do not have permission
## choose your own path.
#dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
data(methData)
Cooccurrence(methData, file=paste(tempdir(), "/Cooccurrence.pdf", sep=""))
```

MethAlignNW

*Summary of methylation states***Description**

Summarize the methylation states after calculating pairwise alignments of each examined sequences and the genomic sequence

Usage

```
MethAlignNW(refSeq, QCdata, alignment)
```

Arguments

refSeq	String; Genomic sequence as String format
QCdata	Data frame; Names and paths of analysed sequences after quality control
alignment	If TRUE, alignments are included in summery, else not included

Details

Given aligned sequences after quality control, the function returns a list object with the following data: sequences name, methylation state on CpG position, start and end position of alignments and length of genomic sequence. The data includes the core information for the exploratory analysis and visualizations.

Value

Returns a summery on sequence alignments and methylation states

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## In order to use the following example
## make sure that you have writing permission under R.home()
## directory. If you do not have permission choose your own path.
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))
datameth <-MethDataInput(file.path(R.home(component="home"),
"/BiqAnalyzer/PathFileTab.txt"))
```

```
refseq <- selectRefSeq(file.path(R.home(component="home"),
"/BiqAnalyzer/Master_Sequence.txt"))
QCdata <- MethylQC(refseq, datameth)
methData <- MethAlignNW( refseq , QCdata)
```

MethDataInput *Sequences match control*

Description

Control function in order to check existence of sequences .fasta files

Usage

```
MethDataInput (sFileName)
```

Arguments

sFileName String; path and name of the Tab delimited text file which includes the names and paths of sequences under study

Details

This procedure controls whether the *.fasta files written in a tabdelimited file are exact matches to the *.fasta in given paths. It is useful if the tab delimited text file was created by hand. This can prevent non existing clone sequence files stopping the workflow in the later analysis steps. The input is a character string which is the path and name of the tab delimited text file which includes the names and paths of sequences under study. It returns a data frame object with names and paths of existing clone sequences.

Value

Returns a data frame object with names and paths of existing analysed sequences

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## This pipeline will save the package example data
## in tests directory under your R.home() directory
## make sure that you have writing
## permission under R.home() directory. If you do not have permission
## choose your own path.

dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))
datameth <-MethDataInput(file.path(R.home(component="home"),
"/BiqAnalyzer/PathFileTab.txt"))
```

MethLollipop *"Lollipops" Methylation plot*

Description

Visualization of methylation patterns in terms of "Lollipops" plot

Usage

```
MethLollipop (methData)
```

Arguments

methData List; contains information on the pairwise alignments, and methylated CpG motifs.

Details

The lollipop plot allows the user to study the genomic localization and states of CpG sites. Each circle marks a CpG site under study. Full circles display methylated CpGs and the non filled ones stand for non methylated CpG states. The examined sequences are aligned with respect to the genomic sequence in order to allow for an intuitive visualization of methylation states according to their genomic position.

Value

Returns a "lollipop" plot

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
data ("methData")
MethLollipop (methData)
```

MethylQC *Quality controle (QC) on Methylation Data*

Description

Processing a quality control (QC) procedure on bisulafite sequences

Usage

```
MethylQC (refSeq, methFileDataFrame, makeChange, identity, conversion)
```

Arguments

refSeq	String; genomic sequence, see selectRefSeq()
methFileDataFrame	Data frame; sequences names and their paths, see MethDataInput()
makeChange	Logical; if TRUE changes take place automatically, by default TRUE
identity	min. identity value , by default 80 percent
conversion	min conversion rate, by default 90 percent

Details

In order to avoid bad qualitative data entering this methylation analysis three measurements are made: 1) alignment check: if reverse, complement or reverse-complement 2) sequence identity between genomic sequence and every examined sequence 3) bisulfite conversion

Value

Returns data frame of sequences names after QC and their paths saves QCINFO.Rdata under sequence files directory

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## In order to use the following example
## make sure that you have writing permission under R.home()
## directory. If you do not have permission choose your own path.

dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))
datameth <-MethDataInput(file.path(R.home(component="home"),
"/BiqAnalyzer/PathFileTab.txt"))
refseq <- selectRefSeq(file.path(R.home(component="home"),
"/BiqAnalyzer/Master_Sequence.txt"))
QCdata <- MethylQC(refseq, datameth)
```

cgInAlign

Amount of CpGs

Description

Calculating amount of CpGs between alignments border

Usage

```
cgInAlign(methData)
```

Arguments

`methData` An object of type list that contains information on the pairwise alignments and methylation status of all CpG motifs under study. Created by applying `MethAlignNW()`

Details

This function computes the amount of CpGs on the positions corresponds to genomic sequence over all sequences under study between the alignment borders.

Value

Integer vector of CpG amount

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
data(methData)
cgInAlign(methData)
```

<code>cgMethFinder</code>	<i>Methylation status</i>
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Description

CpGs methylation status on clone sequence

Usage

```
cgMethFinder(ref, str)
```

Arguments

`ref` String, genomic sequence, see `selectRefSeq()`
`str` String, Single sequence under study after alignment to `ref`

Details

The function determines the methylation status of each CpG site by comparing TpG and CpG sites within the clone sequence to corresponding CpG sites in the reference sequence. The input values are the reference sequence and one of the clone sequences which is explored. It returns a (0,1) vector. 1 stands for methylated and 0 for non methylated state. This function is used in the `methVisual` package as internal function for the calculation of the methylation profiles.

Value

Returns a (0,1) vector. 1 stands for methylation and 0 for non methylation status.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, ,Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
ref <- "TTCGGGATCGTTTTTTTAGTAGGTCGGAAGTTTCGTTATGGATTCGTTTTTC"  
str <- "TTCGGGATCGTTTTTTTAGTAGGTTGGAAGTTTTGTTATGGATTCGTTTTTC"  
cgMethFinder(ref, str)
```

conversionGenom *Sequence conversion*

Description

Bisulfite conversion of genomic Sequence

Usage

```
conversionGenom(genomicSeq)
```

Arguments

genomicSeq String; genomic sequence

Details

This method simulates the bisulfite reaction by converting all Cs outside from CpG sites into Ts. In doing so, the percent of identity between genomic sequence and sequence under study can be determined. The input is the reference sequence that was selected by the function selectRefSeq(). The method returns the reference sequence as a string object with Cs converted into Ts.

Value

Returns String with C converted into T

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
genomicSeq <- "ACCGTTTGGCC"  
conversionGenom(genomicSeq)
```

`findNonAligned` *Aligned CpG positions*

Description

Determination of non-aligned CpG positions

Usage

```
findNonAligned(methData)
```

Arguments

`methData` List; contains information on the pairwise alignments, and methylated CpG motifs

Details

Determination of aligned and not-aligned positions of CpGs of examined sequences in relation to genomic sequence.

Value

Integer matrix, where columns=CpG positions, row=clone sequences. 0 = not methylated, 1 = methylated, 2 = not aligned

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
data(methData)
findNonAligned(methData)
```

`heatMapMeth` *HeatMap diagram over methylation data*

Description

HeatMap diagram of methylation classes over CpG position and sequence level

Usage

```
heatMapMeth(methData, file)
```


Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs
file	optionally, quoted character string for specification of path and file name for saving the result. By default, the result file's format is .pdf. If argument is omitted, screen output is provided only.

Details

Clustering is a prominent method for visualizing and studying groups of similar features, and is also widely used for the analysis of microarrays. In the case of analyzing DNA methylation datasets the matrix to be explored is a I*J binary matrix, where I are the clone sequences and J are the CpG positions. Every index in this matrix has the value 1 for methylated or 0 for non methylated CpG sites. Providing a hierarchical clustering option based on the quality checked methylation data can be useful in finding clusters in the explored data in two dimensions, of the methylation state of CpG sites (J) and for distribution of methylation state over explored clone sequences (I). Very importantly, one has to keep in mind, that this method does not take into account the genomic ordering of CpG sites. Clustering methods can be also useful in quality control check. Observing clones from the same PCR product in different clusters can point to bad quality clone sequences. The clustering method which was used in methVisual R package is the heatmap() function from stat package which is a color image with two dendrograms added to the sides of the columns and rows. Since the data is binary, the distances that are calculated among the columns and among the rows are computed with a binary distance function which is the asymmetric binary function. This method assumes that non zero elements are ON and zero elements are OFF. The distance is the proportion of bits in which only one is ON amongst those in which at least one is ON. Based on aligned sequences under study a heatmap is created that displays two way clustering of methylation status of all sequences and all aligned CpG positions.

Value

Heat-Map image is displayed and optionally saved as postscript in given path and name.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData, file is the path to R home directory.
## In order to save heatMapMeth.pdf, make sure that you have writing
## permission under R.home() directory. If you do not have permission
## choose your own path.
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
data(methData)
heatMapMeth(methData, file=file.path(R.home(component="home"),
"/BiqAnalyzer/heatMapMeth.pdf"))
```

makeDataMethGFF *GFF methylation files*

Description

Create methData object from processed .gff files

Usage

```
makeDataMethGFF (dir, chr, start, end, meth_value)
```

Arguments

dir	String; The local directory where the .gff files are located
chr	String; Chromosome under study
start	Integer; The start position of genomic region under study
end	Integer; The end position of the genomic region under study
meth_value	double; level of methylation on CpG

Details

This function reads and processes GFF files and creates a list object (like the one generated by MethAlignNW()) which can be later analyzed through the visualization, classification and clustering functions.

Value

methData object

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
methGFF <- makeDataMethGFF (dir=system.file (package="methVisual",  
"examples/GFF"), chr="7", start=0, end=70, 0.75)  
methGFF
```

makeLocalExpDir *Saving example data*

Description

Saving example data as provided by the package

Usage

```
makeLocalExpDir(dataPath, localDir)
```

Arguments

dataPath	String; path to the location of sequences under study and genomic reference sequence
localDir	string path to local directory for transferring the sequence files and Tab delimited text file

Details

Help function for saving data provided along with the package followed by creation of a tab delimited text file with information on PATHs and FILEs

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## saving example data under R.home() directory.
## make sure that you have writing permission under
## R.home() directory. If you do not have permission
## choose your own path (localDir=YOUR_OWN_PATH/).
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))
```

makeTabFilePath *Tab delimited text file*

Description

create tab delimited text file with PATH and FILE columns

Usage

```
makeTabFilePath(localDir)
```

Arguments

`localDir` String; path to local directory where sequence files and tab delimited text file are saved

Details

The function creates a tab delimited text file with information on path and file names for files which are stored in a given directory. This tab-delimited text file is the input file that contains the clone sequences. The `makeTabFilePath()` function was implemented in order to create a control step before starting the analysis. The best way of using the function is by collecting all clone sequence files which need to be analyzed into one empty directory and apply this function to the directory.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## on tests directory under R home directory
## make sure that you have writing permission under
## R.home() directory. If you do not have permission
## choose your own path (localDir=YOUR_OWN_PATH/).

makeTabFilePath(file.path(R.home(component="home"), "/BiqAnalyzer"))
```

matrixSNP

Correlation between methylation states

Description

Correlation between methylation states for each CpG position and each sequence under study

Usage

```
matrixSNP(methData, correlation)
```

Arguments

`methData` List; contains information on the pairwise alignments, and methylated CpG motifs.

`correlation` calculation of correlation on Matrix, if TRUE: calculation of spearman correlation matrix. if FALSE: computes a matrix with absolute numbers of methylation over all CpG position given methylation in a certain CpG sites.

Details

The function enable the user to explore cooccurrence between non neighbored CpG sites, which can be made by calculating all pairwise cooccurrences, due to correlation over all methylation CpG sites. Furthermore it is possible to display the computed distance cooccurrences in a plot containing all CpG sites and their correlation with other CpG sites. The correlation values are color coded (gray levels) and the color coding bar is given beside the graph. The red numbers in the diagonal give the genomic position of each displayed CpG site.

Value

Returns a correlation matrix displaying dependencies values for all CpG positions

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData
data(methData)
matrixSNP(methData)
```

methCA

CA on methylation

Description

Correspondence Analysis over methylation Data

Usage

```
methCA(methData, file)
```

Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
file	String; optionally, quoted character string for specification of path and file name for saving the result. By default, the result file's format is .pdf. If argument is omitted, screen output is provided only.

Details

Correspondence analysis (CA) is a multivariate statistical technique which is applicable to tables of categorical data. CA can be useful in understanding the data in an inter relationship manner, meaning the dependencies between categories. Unlike conventional statistical methods that try to prove a hypothesis, the CA is an exploratory technique that can reveal data content. With the help of a graphical application, which displays each category as a point in a two dimensional plot, it is easier to locate special characteristics in the numerical data. It is used in order to study the association between CpG methylation states and clone sequences based on the aligned clone sequences under study.

Value

CA diagram as postscript file saved in given path and name.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData, file is the path to R home directory.
## In order to save methCA.pdf, make sure that you have writing
## permission under R.home() directory. If you do not have permission
## choose your own path.
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
data(methData)
methCA(methData, file=file.path(R.home(component="home"),
"/BiqAnalyzer/methCA.pdf"))
```

methData	<i>BiQAnalyzer dataset</i>
----------	----------------------------

Description

Summary of BiQAnalyzer methylation Data created using the function MethAlignNW()

Usage

```
data(methData)
```

Format

A list

Details

methData is the summary of methylation data given in the software BiqAnalyzer. the list is created by applying MethAlignNW() on BiQAnalyzer example data.

References

<http://biq-analyzer.bioinf.mpi-inf.mpg.de/>

Examples

```
data(methData)
```

methFisherTest	<i>Fisher exact Test on methylation Data</i>
----------------	----------------------------------------------

Description

Fisher exact Test on two subsets of experiments over matched CpG sites

Usage

```
methFisherTest(methData, set1, set2)
```

Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
set1	First subset - Integer vector of experiments due to there order at methData
set2	Second subset - Integer vector of indexes of experiments due to there order at methData

Details

Given two clone sequences groups A and B for each CpG site the user can investigate whether there is a difference of methylation status between the two groups at each of the CpG sites. In order to calculate this difference at each CpG site, the two-tailed p-value of Fisher's exact test is calculated from the 2*2 tables at each CpG site. This p-value indicates the level of difference at every single CpG in those two groups of clone sequences. A p-value smaller than 0.05 is an indication for the independence of the methylation state in a certain CpG site when comparing two groups of clone sequences.

Value

P- Values vector and a Plot

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
data(methData)
methFisherTest(methData, c(1, 2, 3), c(4, 5, 6))
```

methWhitneyUTest *Mann Whitney U-Test on methylation data*

Description

Mann Whitney U-Test on entire sets of CpG sites

Usage

```
methWhitneyUTest(methData, set1, set2)
```

Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
set1	First subset - Integer vector of experiments due to there order at methData
set2	Second subset - Integer vector of indexes of experiments due to there order at methData

Details

Mann Whitney U-Test (known also as wilcoxon-rank-sum test), is a non parametric test for assessing whether two independent samples of observations come from the same distribution. The null hypothesis in the Mann-Whitney U test is that the two samples are from a single population, which means that their probability distributions are equal. In the methVisual package the Mann-Whitney U test is applied in order to test if the distribution of methylated and non methylated sites in the profile under study between the given experimental sub groups of clone sequences is different. In order to calculate it, the two-tailed p-value of the Mann-Whitney U test is computed by ranking the ratios of methylated CpG sites to all CpG sites in a given clone sequence. The p-value in this case indicates if the distribution of ratio over all methylation states differ between the two groups.

Value

Returns a p-value

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData
data(methData)
methWhitneyUTest(methData, c(1, 2, 3), c(4, 5, 6))
```

plotAbsMethyl

Plot number mathylation on CpGs positions

Description

Plot of absolute/relative number of mathylation on aligned CpGs positions

Usage

```
plotAbsMethyl(methData, real)
```

Arguments

methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
real	real position (real=TRUE) or relative position (real=FLASE) on reference sequence

Details

This function generates a plot of the absolute number of methylation in all CpG sites over all examined sequences. It returns a vector with the absolute number of methylation sites of all examined clone sequences. The user supplies a list object containing information about the pairwise alignments, and methylated CpG sites as calculated by MethAlignNW() or makeDataMethGFF(). The user can display the absolute number of methylation over the CpG sites according to their relative position on the reference sequence.

Value

A vector with the absolute/relative number of methylation on genomic sequence CpG positions over all examined sequences

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData
data(methData)
plotAbsMethyl(methData, real=FALSE)
```

plotMatrixSNP *Plot of methylation states dependencies*

Description

Visualisation of dependencies between methylation states over explored bisulfite sequences

Usage

```
plotMatrixSNP(summeryMatrix, methData, file)
```

Arguments

summeryMatrix	see matrixSNP()
methData	List; contains information on the pairwise alignments, and methylated CpG motifs.
file	String; quoted character string for specification of path and file name for saving the result. The result file is in .pdf format

Details

The SNP Plot produce a visualisation of the cooccurrence between methylation states on CpGs over all explored sequences. The index of the CpGs is based on there position on genomic sequence.

Value

SNP Plot as pdf file saved in given path and name.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## using methData

data(methData)
summeryMatrix <- matrixSNP(methData)

## using methData, file is the path to R home directory.
## In order to save plotMatrixSNP.pdf, make sure that you have writing
## permission under R.home() directory. If you do not have permission
## choose your own path.
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
data(methData)
summary <- matrixSNP(methData)
plotMatrixSNP(summary, methData, file=file.path(R.home(component="home"),
"/BiqAnalyzer/", "plotMatrixSNP.pdf"))
```

```
readBisulfFASTA      Read multiple FASTA file
```

Description

Read multiple FASTA file and write bisulfite sequences in separate FASTA files for every bisulfite sequence

Usage

```
readBisulfFASTA(sFileName, sDirName)
```

Arguments

```
sFileName      String; path to multiple FASTA file
sDirName      String; path to directory of created separated FASTA files
```

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## In order to use the following example
## make sure that you have writing permission under R.home()
## directory. If you do not have permission choose your own path.
dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))

readBisulfFASTA(sFileName=file.path(R.home(component="home"), "/BiqAnalyzer/multiFASTA.fasta
```

selectRefSeq	<i>Uploading genomic sequence</i>
--------------	-----------------------------------

Description

Uploading genomic sequence

Usage

```
selectRefSeq(sFileName)
```

Arguments

sFileName path and name of genomic sequence to be uploaded

Details

Uploading genomic sequence

Value

This function is used in order to read the reference sequence into the R environment. The reference sequences must be in fasta format. The user must give the path and name of the reference sequence in a character string.

Author(s)

Arie Zackay <zackay@molgen.mpg.de>, Christine Steinhoff <steinhof@molgen.mpg.de>

Examples

```
## make sure that you have reading
## permission under R.home() directory. If you do not have permission
## choose your own path.

dir.create(file.path(R.home(component="home"), "/BiqAnalyzer"))
makeLocalExpDir(dataPath="/examples/BiqAnalyzer",
localDir=file.path(R.home(component="home"), "/BiqAnalyzer"))
refseq <- selectRefSeq(file.path(R.home(component="home"),
"/BiqAnalyzer/Master_Sequence.txt"))
```

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