Package ‘RFLPtools’

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Title Tools to Analyse RFLP Data
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Description Provides functions to analyse DNA fragment samples (i.e. derived from RFLP-
analysis) and standalone BLAST report files (i.e. DNA sequence analysis).
Depends R(>= 4.0.0), RColorBrewer
Imports stats, utils, graphics, grDevices
Suggests knitr, rmarkdown, lattice, MKomics
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R topics documented:

  - RFLPtools-package
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RFLPtools-package

Tools To Analyse RFLP-Data

Description

RFLPtools provides functions to analyse DNA fragment samples (i.e. derived from RFLP-analysis) and standalone BLAST report files (i.e. DNA sequence analysis).

Details

Package: RFLPtools
Version: 2.0
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Depends: R(>= 4.0.0)
Imports: stats, utils, graphics, grDevices, RColorBrewer
Suggests: knitr, rmarkdown, lattice, MKomics
License: LGPL-3

Author(s)

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References


Matsumoto, Masaru; Furuya, Naruto; Takanami, Yoichi; Matsuyama, Nobuaki. RFLP analysis of the PCR-amplified 28S rDNA in Rhizoctonia solani. Mycoscience 1996 37:351-356.


Poussier, Stephane; Trigalet-Demery, Danielle; Vandewalle, Peggy; Goffinet, Bruno; Luisetti, Jacques; Trigalet, Andre. Genetic diversity of Ralstonia solanacearum as assessed by PCR-RFLP of the hrp gene region, AFLP and 16S rRNA sequence analysis, and identification of an African subdivision. Microbiology 2000 146:1679-1692.


Examples

data(RFLPdata)
res <- RFLPdist(RFLPdata)
plot(hclust(res[[1]]), main = "Euclidean distance")

par(mfrow = c(1,2))
plot(hclust(RFLPdist(RFLPdata, nrBands = 3)), cex = 0.7)
RFLPplot(RFLPdata, nrBands = 3, mar.bottom = 6, cex.axis = 0.8)

data(RFLPref)
RFLPprefplot(RFLPdata, RFLPref, nrBands = 6, cex.axis = 0.8)

library(MKomics)
data(BLASTdata)
res <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 500)
myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)
simPlot(res, col = myCol, minVal = 0,
       labels = colnames(res), title = "(Dis-)Similarity Plot")
**Description**

This is an example data set for BLAST data generated with standalone BLAST from NCBI.

**Usage**

data(RFLPdata)

**Format**

A data frame with 737 observations on the following four variables

- **query.id**: character: sequence identifier.
- **subject.id**: character: subject identifier.
- **identity**: numeric: identity between sequences (in percent).
- **alignment.length**: integer: number of nucleotides.
- **mismatches**: integer: number of mismatches.
- **gap.opens**: integer: number of gaps.
- **q.start**: integer: query sequence start.
- **q.end**: integer: query sequence end.
- **s.start**: integer: subject sequence start.
- **s.end**: integer: subject sequence end.
- **evalue**: numeric: evalue.
- **bit.score**: numeric: score value.

**Details**

The data was generated with standalone BLAST from NCBI. Pairwise similarities of DNA sequences are calculated among all sequences to analyse applying Standalone Blast with the parameters `-m 8 -r 2 -G 5 -E 2`.

Alternatively data can be generated with "local BLAST" implemented in BioEdit v7.0.9 using the additional parameters `-m 8 -r 2 -G 5 -E 2` and by selecting "open output" and "tabular output".

**Source**

The data set was generated by F. Flessa.
diffDist

References
BioEdit: https://bioedit.software.informer.com/

Examples
data(BLASTdata)
str(BLASTdata)

diffDist

Distance Matrix Computation

Description
This function computes and returns the distance matrix computed by using the specified distance measure to compute the distances between the rows of a data matrix. Instead of the row values as in the case of dist, the successive differences of the row values are used.

Usage
diffDist(x, method = "euclidean", diag = FALSE, upper = FALSE, p = 2)

Arguments
  x      a numeric matrix, data frame or "dist" object.
  method the distance measure to be used. This must be one of "euclidean", "maximum", "manhattan", "canberra", "binary" or "minkowski". Any unambiguous substring can be given.
  diag   logical value indicating whether the diagonal of the distance matrix should be printed by print.dist.
  upper  logical value indicating whether the upper triangle of the distance matrix should be printed by print.dist.
  p      The power of the Minkowski distance.

Details
This function computes and returns the distance matrix computed by using the specified distance measure to compute the distances between the rows of a data matrix. Instead of the row values as in the case of dist, the successive differences of the row values are used.
It’s a simple wrapper function arround dist. For more details about the distances we refer to dist.
The function may be helpful, if there is a shift w.r.t. the measured bands; e.g. \( c(550, 500, 300, 250) \) vs. \( c(510, 460, 260, 210) \).
FragMatch

Value

diffDist returns an object of class "dist"; cf. dist.

Author(s)

Matthias Kohl <Matthias.Kohl@stamats.de>

References


Examples

## assume a shift in the measured bands
M <- rbind(c(550, 500, 300, 250), c(510, 460, 260, 210),
           c(550, 500, 300, 200))
dist(M)
diffDist(M)

FragMatch  Compute matches for RFLP data via FragMatch.

Description

Compute matches for RFLP data using FragMatch - a program for the analysis of DNA fragment data.

Usage

FragMatch(newData, refData, maxValue = 1000, errorBound = 25,
         weight = 1, na.rm = TRUE)

Arguments

newData  data.frame with new RFLP data; see newDataGerm.
refData  data.frame with reference RFLP data; see refDataGerm.
maxValue numeric: maximum value for which the error bound is applied. Can be a vector of length larger than 1.
errorBound numeric: error bound corresponding to maxValue. Can be a vector of length larger than 1.
weight    numeric: weight for weighting partial matches; see details section.
na.rm     logical: indicating whether NA values should be stripped before the computation proceeds.
germ

Details
A rather simple algorithm which consists of counting the number of matches where it is considered a match if the value is inside a range of +/- errorBound.

If there is more than one enzyme, one can use weights to give the partial perfect matches for a certain enzyme a higher (or also smaller) weight.

Value
A character matrix with entries of the form "a_b" which means that there were a out of b possible matches.

Author(s)
Mohammed Aslam Imtiaz, Matthias Kohl <Matthias.Kohl@stamats.de>

References

See Also
newDataGerm, refDataGerm

Examples
data(refDataGerm)
data(newDataGerm)
res <- FragMatch(newDataGerm, refDataGerm)

---

**germ**

*Compute matches for RFLP data via GERM.*

Description
Compute matches for RFLP data using the Good-Enough RFLP Matcher (GERM) program.

Usage
germ(newData, refData, parameters = list("Max forward error" = 25,  
"Max backward error" = 25,  
"Max sum error" = 100,  
"Lower measurement limit" = 100),  
method = "joint", na.rm = TRUE)
Arguments

newData   data.frame with new RFLP data; see newDataGerm.
refData   data.frame with reference RFLP data; see refDataGerm.
parameters list of the four program parameters of GERM; see details section.
method    matching and ranking method used for computation; see details section.
na.rm     logical: indicating whether NA values should be stripped before the computation proceeds.

Details

There are four matching and ranking methods which are "joint", "forward", "backward", and "sum". For more details see Dickie et al. (2003).

The parameters of the GERM software are: "Max forward error": Used if "matching and ranking method" is set to "forward" or "joint". "Max backward error": Used if "matching and ranking method" is set to "backward" or "joint". "Max sum error": Used for matching if "matching and ranking method" is set to "sum". "Lower measurement limit": The lower bound of measurements (often 100 or 50, depending on ladder used).

Value

A named list with the results.

Author(s)

Mohammed Aslam Imtiaz, Matthias Kohl <Matthias.Kohl@stamats.de>

References


See Also

newDataGerm, refDataGerm

Examples

data(refDataGerm)
data(newDataGerm)

## Example 1
res1 <- germ(newDataGerm[1:7,], refDataGerm)

## Example 2
res2 <- germ(newDataGerm[8:15,], refDataGerm)

## Example 3
res3 <- germ(newDataGerm[16:20,], refDataGerm)
## all three examples in one step
res.all <- germ(newDataGerm, refDataGerm)

---

**linCombDist**

*Linear Combination of Distances*

**Description**

This function computes linear combinations of distances.

**Usage**

```r
linCombDist(x, distfun1, w1, distfun2, w2, diag = FALSE, upper = FALSE)
```

**Arguments**

- `x`: object which is passed to `distfun1` and `distfun2`.
- `distfun1`: function used to compute an object of class "dist".
- `w1`: weight for result of `distfun1`.
- `distfun2`: function used to compute an object of class "dist".
- `w2`: weight for result of `distfun2`.
- `diag`: see `dist`.
- `upper`: see `dist`.

**Details**

This function computes and returns the distance matrix computed by a linear combination of two distance matrices.

**Value**

`linCombDist` returns an object of class "dist"; cf. `dist`.

**Author(s)**

Matthias Kohl <Matthias.Kohl@stamats.de>

**References**

Examples

```r
## assume a shift in the measured bands
M <- rbind(c(550, 500, 300, 250), c(510, 460, 260, 210),
           c(700, 650, 450, 400), c(550, 490, 310, 250))
dist(M)
diffDist(M)

## convex combination of dist and diffDist
linCombDist(M, distfun1 = dist, w1 = 0.5, distfun2 = diffDist, w2 = 0.5)

## linear combination
linCombDist(M, distfun1 = dist, w1 = 2, distfun2 = diffDist, w2 = 5)

## maximum distance
linCombDist(M, distfun1 = function(x) dist(x, method = "maximum"), w1 = 0.5,
            distfun2 = function(x) diffDist(x, method = "maximum"), w2 = 0.5)
```

```r
data(newDataGerm)
distfun <- function(x) linCombDist(x, distfun1 = dist, w1 = 0.1, distfun2 = diffDist, w2 = 0.9)
par(mfrow = c(2, 2))
plot(hclust(RFLPdist(RFLPdata, nrBands = 3, distfun = distfun)), cex = 0.7, cex.lab = 0.7)
RFLPplot(RFLPdata, nrBands = 3, distfun = distfun, mar.bottom = 6, cex.axis = 0.8)
plot(hclust(RFLPdist(RFLPdata, nrBands = 3)), cex = 0.7, cex.lab = 0.7)
RFLPplot(RFLPdata, nrBands = 3, mar.bottom = 6, cex.axis = 0.8)
```

Description

This is the reference data taken from the GERM software.

Usage

```r
data(newDataGerm)
```

Format

A data frame with 20 observations on the following six variables

- **Sample** character: sample identifier.
- **Enzyme** character: enzyme used.
- **Band** integer: band number.
- **MW** integer: molecular weight.
- **Genus** character: genus of sample.
- **Species** character: species of sample.
**nrBands**

*Details*

See GERM software.

*Source*

The data set was taken from the GERM software (table 'Example Unknowns').

*References*


*Examples*

```r
data(newDataGerm)
str(newDataGerm)
```

---

**nrBands**

*Function to compute number of bands.*

---

*Description*

Computes groups based on the number of bands per sample in a RFLP data set. Each group comprises RFLP-samples with equal number of bands.

*Usage*

```r
nrBands(x)
```

*Arguments*

- `x`  
  data.frame with RFLP data; see *RFLPdata*.

*Details*

The function computes groups based on the number of bands per sample in a RFLP data set. Each group comprises RFLP-samples with equal number of bands.

*Value*

Number of bands per RFLP-samples.

*Author(s)*

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>
References

See Also
RFLPdata, RFLPdist2, dist

Examples

data(RFLPdata)
nrBands(RFLPdata)

Description
Function to read BLAST data generated with standalone BLAST from NCBI.

Usage
read.blast(file, sep = "\t")

Arguments
  file character: BLAST file to read in.
  sep the field separator character. Values on each line of the file are separated by this character. Default "\t".

Details
The function reads data which was generated with standalone BLAST from NCBI; see ftp://ftp.ncbi.nih.gov/blast/executables/release/.

Possible steps:
1) Install NCBI BLAST
2) Generate and import database(s)
3) Apply BLAST with options outfmt and out; e.g.
   blastn -query Testquery -db Testdatabase -outfmt 6 -out out.txt
   or
   blastn -query Testquery -db Testdatabase -outfmt 10 -out out.csv
   One can also call BLAST from inside R by using function system
   system("blastn -query Testquery -db Testdatabase -outfmt 6 -out out.txt")
4) Read in the results
   test.res <- read.blast(file = "out.txt")
   or
   test.res <- read.blast(file = "out.csv", sep = ",")
**Value**

A data.frame with variables

- query.id character: sequence identifier.
- subject.id character: subject identifier.
- identity numeric: identity between sequences (in percent).
- alignment.length integer: number of nucleotides.
- mismatches integer: number of mismatches.
- gap.opens integer: number of gaps.
- q.start integer: query sequence start.
- q.end integer: query sequence end.
- s.start integer: subject sequence start.
- s.end integer: subject sequence end.
- evalue numeric: evalue.
- bit.score numeric: score value.

**Author(s)**

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

- `BLASTdata`, `simMatrix`

**Examples**

```r
Dir <- system.file("extdata", package = "RFLPtools") # input directoryilename <- file.path(Dir, "BLASTexample.txt")
BLAST1 <- read.blast(file = filename)
str(BLAST1)
```
read.rflp  

**Description**

Function to read RFLP data (e.g. generated with software package Gene Profiler 4.05 (Scanalytics Inc.)) for DNA fragment analysis and genotyping, and exported to a text file.

**Usage**

```
read.rflp(file)
```

**Arguments**

- `file` character: RFLP file to read in.

**Details**

The function reads data from a text file which was generated e.g. with the software package Gene Profiler 4.05 (Scanalytics Inc.) for DNA fragment analysis and genotyping. The data file contains sample identifier (`Sample`), band number (`Band`), molecular weight (`MW`) and gel identifier (`Gel`) (see `RFLPdata`).

If gel identifier `Gel` is missing it is extracted from the sample identifier `Sample`.

**Value**

A `data.frame` with variables

- `Sample` character: sample identifier.
- `Band` integer: band number.
- `MW` integer: molecular weight.
- `Gel` character: gel identifier.

**Author(s)**

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Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>,
Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

`RFLPdata`, `RFLPdist`
refDataGerm

Examples

Dir <- system.file("extdata", package = "RFLPtools") # input directory
filename <- file.path(Dir, "RFLPexample.txt")
RFLP1 <- read.rflp(file = filename)
str(RFLP1)

filename <- file.path(Dir, "AZ091016_report.txt")
RFLP2 <- read.rflp(file = filename)
str(RFLP2)

refDataGerm       Example data set from GERM software

Description

This is the reference data taken from the GERM software.

Usage

data(refDataGerm)

Format

A data frame with 250 observations on the following six variables

Sample  character: sample identifier.
Enzyme   character: enzyme used.
Band     integer: band number.
MW       integer: molecular weight.
Genus    character: genus of sample.
Species  character: species of sample.

Details

See GERM software.

Source

The data set was taken from the GERM software (table 'Example Data').

References


Examples

data(refDataGerm)
str(refDataGerm)
RFLPcombine

Combine RFLP data sets

Description
Function to combine an arbitrary number of RFLP data sets.

Usage
RFLPcombine(...)

Arguments
... two or more data.frames with RFLP data.

Details
The data sets are combined using `rbind`. If data sets with identical sample identifiers are given, the identifiers are made unique using `make.unique`.

Value
A `data.frame` with variables
- `Sample` character: sample identifier.
- `Band` integer: band number.
- `MW` integer: molecular weight.
- `Gel` character: gel identifier.

Author(s)
Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>,
Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>,
Matthias Kohl <Matthias.Kohl@stamats.de>

References

See Also
RFLPdata

Examples
data(RFLPdata)
res <- RFLPcombine(RFLPdata, RFLPdata, RFLPdata)
RFLPplot(res, nrBands = 4)
Description

This is an example data set for RFLP data.

Usage

data(RFLPdata)

Format

A data frame with 737 observations on the following four variables

Sample character: sample identifier.
Band integer: band number.
MW integer: molecular weight.
Gel character: gel identifier.

Details

The molecular weight was determined using the software package Gene Profiler 4.05 (Scanalytics Inc.) for DNA fragment analysis and genotyping, and exported to a text file.

Source

The data set was generated by F. Flessa.

References


Examples

data(RFLPdata)
str(RFLPdata)
RFLPdist

Compute distances for RFLP data.

Description

Within each group containing RFLP-samples exhibiting an equal number of bands, the distance between the molecular weights is computed.

Usage

RFLPdist(x, distfun = dist, nrBands, LOD = 0)

Arguments

x data.frame with RFLP data; see RFLPdata.
distfun function computing the distance with default dist; cf. dist.
.nrBands if not missing, then only samples with the specified number of bands are considered.
.LOD threshold for low-bp bands.

Details

For each number of bands the given distance between the molecular weights is computed. The result is a named list of distances where the names correspond to the number of bands which occur in each group.

If nrBands is specified only samples with this number of bands are considered.
If LOD > 0 is specified, all values below LOD are removed before the distances are calculated.

Value

A named list with the distances; see dist.
In case nrBands is not missing, an object of S3 class dist.

Author(s)

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Matthias Kohl <Matthias.Kohl@stamats.de>

References

Poussier, Stephane; Trigalet-Demery, Danielle; Vandewalle, Peggy; Goffinet, Bruno; Luisetti, Jacques; Trigalet, Andre. Genetic diversity of Ralstonia solanacearum as assessed by PCR-RFLP of the hrp

Matsumoto, Masaru; Furuya, Naruto; Takanami, Yoichi; Matsuyama, Nobuaki. RFLP analysis of the PCR-amplified 28S rDNA in Rhizoctonia solani. Mycoscience 1996 37:351 - 356

See Also

RFLPdata, dist

Examples

```r
## Euclidean distance
data(RFLPdata)
res <- RFLPdist(RFLPdata)
names(res) ## number of bands
res$"6"

RFLPdist(RFLPdata, nrBands = 6)

## Other distances
res1 <- RFLPdist(RFLPdata, distfun = function(x) dist(x, method = "manhattan"))
res2 <- RFLPdist(RFLPdata, distfun = function(x) dist(x, method = "maximum"))
res[[1]]
res1[[1]]
res2[[1]]

## cut dendrogram at height 50
clust4bd <- hclust(res[[2]])
cgroups50 <- cutree(clust4bd, h=50)
cgroups50

## or
library(MKomics)
res3 <- RFLPdist(RFLPdata, distfun = corDist)
res3$"9"

## hierarchical clustering
par(mfrow = c(2,2))
plot(hclust(res[[1]]), main = "Euclidean distance")
plot(hclust(res1[[1]]), main = "Manhattan distance")
plot(hclust(res2[[1]]), main = "Maximum distance")
plot(hclust(res3[[1]]), main = "Pearson correlation distance")

## Similarity matrix
library(MKomics)
myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)
ord <- order.dendrogram(as.dendrogram(hclust(res[[1]])))
temp <- as.matrix(res[[1]])
simPlot(temp[ord,ord], col = rev(myCol), minVal = 0,
       labels = colnames(temp), title = "(Dis-)Similarity Plot")
```
RFLPdist2

Compute distances for RFLP data.

Description

If gel image quality is low, faint bands may be disregarded and may lead to wrong conclusions. This function computes the distance between the molecular weights of RFLP samples, including samples containing one or more additional bands. Thus, failures during band detection could be identified. Visualisation of band patterns using this method can be done by RFLPplot using the argument nrMissing.

Usage

RFLPdist2(x, distfun = dist, nrBands, nrMissing, LOD = 0,
          diag = FALSE, upper = FALSE)

Arguments

x
  data.frame with RFLP data; see RFLPdata.
distfun
  function computing the distance with default dist; cf. dist.
nrBands
  samples with number of bands equal to nrBands are to be considered.
nrMissing
  number of bands that might be missing.
LOD
  threshold for low-bp bands.
diag
  see dist
upper
  see dist
Details

For a given number of bands the given distance between the molecular weights is computed. It is assumed that a number of bands might be missing. Hence all samples with number of bands in \( \text{nrBands}, \text{nrBands+1}, \ldots, \text{nrBands+nrMissing} \) are compared.

If \( \text{LOD} > 0 \) is specified, it is assumed that missing bands can only occur for molecular weights smaller than \( \text{LOD} \). As a consequence only samples which have \( \text{nrBands} \) bands with molecular weight larger or equal to \( \text{LOD} \) are selected.

For computing the distance between the molecular weight of a sample \( S_1 \) with \( x \) bands and a Sample \( S_2 \) with \( x+y \) bands the distances between the molecular weight of sample \( S_1 \) and the molecular weight of all possible subsets of \( S_2 \) with \( x \) bands are computed. The distance between \( S_1 \) and \( S_2 \) is then defined as the minimum of all these distances.

If \( \text{LOD} > 0 \) is specified, only all combinations of values below \( \text{LOD} \) are considered.

This option may be useful, if gel image quality is low, and the detection of bands is doubtful.

Value

An object of class "dist" returned; cf. \texttt{dist}.

Author(s)

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Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>,
Matthias Kohl <Matthias.Kohl@stamats.de>

References


See Also

\texttt{RFLPdata}, \texttt{nrBands}, \texttt{RFLPdist}, \texttt{dist}

Examples

```r
## Euclidean distance
data(RFLPdata)
nrBands(RFLPdata)
res0 <- RFLPdist(RFLPdata, nrBands = 4)
res1 <- RFLPdist2(RFLPdata, nrBands = 4, nrMissing = 1)
res2 <- RFLPdist2(RFLPdata, nrBands = 4, nrMissing = 2)
res3 <- RFLPdist2(RFLPdata, nrBands = 4, nrMissing = 3)

## assume missing bands only below LOD
res1.lod <- RFLPdist2(RFLPdata, nrBands = 4, nrMissing = 1, LOD = 60)
```
## hierarchical clustering

```r
par(mfrow = c(2,2))
plot(hclust(res0), main = "0 bands missing")
plot(hclust(res1), main = "1 band missing")
plot(hclust(res2), main = "2 bands missing")
plot(hclust(res3), main = "3 bands missing")
```

## missing bands only below LOD

```r
par(mfrow = c(1,2))
plot(hclust(res0), main = "0 bands missing")
plot(hclust(res1.lod), main = "1 band missing below LOD")
```

## Similarity matrix

```r
library(MKomics)
myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)
ord <- order.dendrogram(as.dendrogram(hclust(res1)))
temp <- as.matrix(res1)
simPlot(temp[ord,ord], col = rev(myCol), minVal = 0,
       labels = colnames(temp), title = "(Dis-)Similarity Plot")
```

## missing bands only below LOD

```r
ord <- order.dendrogram(as.dendrogram(hclust(res1.lod)))
temp <- as.matrix(res1.lod)
simPlot(temp[ord,ord], col = rev(myCol), minVal = 0,
       labels = colnames(temp), title = "(Dis-)Similarity Plot\n1 band missing below LOD")
```

## or

```r
library(lattice)
levelplot(temp[ord,ord], col.regions = rev(myCol),
         at = do.breaks(c(0, max(temp)), 128),
         xlab = "", ylab = "",
         ## Rotate label of x axis
         scales = list(x = list(rot = 90)),
         main = "(Dis-)Similarity Plot")
```

### Other distances

```r
res11 <- RFLPdist2(RFLPdata, distfun = function(x) dist(x, method = "manhattan"),
                   nrBands = 4, nrMissing = 1)
res12 <- RFLPdist2(RFLPdata, distfun = corDist, nrBands = 4, nrMissing = 1)
res13 <- RFLPdist2(RFLPdata, distfun = corDist, nrBands = 4, nrMissing = 1, LOD = 60)
par(mfrow = c(2,2))
plot(hclust(res1), main = "Euclidean distance\n1 band missing")
plot(hclust(res11), main = "Manhattan distance\n1 band missing")
plot(hclust(res12), main = "Pearson correlation distance\n1 band missing")
plot(hclust(res13), main = "Pearson correlation distance\n1 band missing below LOD")
```

---

**RFLPdist2ref**

*Compute distance between RFLP data and RFLP reference data.*
**RFLPdist2ref**

**Description**

Function to compute distance between RFLP data and RFLP reference data.

**Usage**

`RFLPdist2ref(x, ref, distfun = dist, nrBands, LOD = 0)`

**Arguments**

- `x` data.frame with RFLP data; e.g. `RFLPdata`.
- `ref` data.frame with RFLP reference data; e.g. `RFLPref`.
- `distfun` function computing the distance with default `dist`; cf. `dist`.
- `nrBands` only samples and reference samples with this number of bands are considered.
- `LOD` threshold for low-bp bands.

**Details**

For each sample with `nrBands` bands the distance to each reference sample with `nrBands` bands is computed. The result is a matrix with the corresponding distances where rows represent the samples and columns the reference samples.

If `LOD > 0` is specified, all values below `LOD` are removed before the distances are calculated. This applies to `x` and `ref`.

**Value**

A matrix with distances.

**Author(s)**

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

`RFLPdata`, `dist`
Examples

```r
## Euclidean distance
data(RFLPdata)
data(RFLPref)
nrBands(RFLPref)
RFLPdist2ref(RFLPdata, RFLPref, nrBands = 4)
RFLPdist2ref(RFLPdata, RFLPref, nrBands = 6)

Dir <- system.file("extdata", package = "RFLPtools") # input directory
filename <- file.path(Dir, "AZ091016_report.txt")
RFLP1 <- read.rflp(file = filename)
RFLP2 <- RFLPqc(RFLP1)
nrBands(RFLP2)
RFLPdist2ref(RFLP1, RFLPref, nrBands = 4)
RFLPdist2ref(RFLP1, RFLPref, nrBands = 5)
```

---

**RFLP1od**  
*Remove bands below LOD*

**Description**

Function to exclude bands below a given LOD.

**Usage**

```
RFLP1od(x, LOD)
```

**Arguments**

- `x`  
data.frame with RFLP data.

- `LOD`  
threshold for low-bp bands.

**Details**

Low-bp bands may be regarded as unreliable. Function RFLP1od can be used to exclude such bands, which are likely to be absent in some other samples, before further analyses.

**Value**

A data.frame with variables

- Sample: character: sample identifier.
- Band: integer: band number.
- MW: integer: molecular weight.
- Gel: character: gel identifier.
RFLPplot

Function to plot RFLP data.

Description

Given RFLP data is plotted where the samples are sorted according to the corresponding dendrogram.

Usage

RFLPplot(x, nrBands, nrMissing, distfun = dist, 
    hclust.method = "complete", mar.bottom = 5, 
    cex.axis = 0.5, colBands, xlab = ",
    ylab = "molecular weight", ylim, ...)

Arguments

x       data.frame with RFLP data; see RFLPdata.
nrBands if not missing, then only samples with the specified number of bands are considered.
nrMissing if not missing, then it is assumed that some bands may be missing. That is, all samples with number of bands in nrBands, nrBands+1, ..., nrBands+nrMissing are considered.
\texttt{distfun} \quad \text{function computing the distance with default dist; see \texttt{dist}.}

\texttt{hclust.method} \quad \text{method used for hierarchical clustering; see \texttt{hclust}.}

\texttt{mar.bottom} \quad \text{bottom margin of the plot; see \texttt{par}.}

\texttt{cex.axis} \quad \text{size of the x-axis annotation.}

\texttt{colBands} \quad \text{color for the bands. Has to be of length 1 or number of samples. If missing,}

\quad \text{"Set1" of \texttt{RColorBrewer} is used; see \texttt{ColorBrewer}.}

\texttt{xlab} \quad \text{passed to function \texttt{plot}.}

\texttt{ylab} \quad \text{passed to function \texttt{plot}.}

\texttt{ylim} \quad \text{passed to function \texttt{plot}. If missing an appropriate range of y-values is com-

\quad \text{puted.}

\texttt{...} \quad \text{additional arguments passed to function \texttt{plot} except \texttt{xlim} which is defined in-

\quad \text{side of RFLPplot.}

\textbf{Details}

RFLP data is plotted. The samples are sorted according to the corresponding dendrogram which is

\text{computed via function \texttt{hclust}.}

The option to specify \texttt{nrMissing} may be useful, if gel image quality is low, and the detection of

\text{bands is doubtful.}

\textbf{Value}

\text{invisible}

\textbf{Author(s)}

Fabienne Flessa \texttt{<Fabienne.Flessa@uni-bayreuth.de>},

Alexandra Kehl \texttt{<Alexandra.Kehl@uni-tuebingen.de>},

Matthias Kohl \texttt{<Matthias.Kohl@stamats.de>}

\textbf{References}

Flessa, F., Kehl, A., Kohl, M. Analysing diversity and community structures using PCR-RFLP: a

\text{new software application. Molecular Ecology Resources 2013 Jul; 13(4):726-33.}

\textbf{See Also}

\texttt{RFLPdata, dist}

\textbf{Examples}

data(RFLPdata)
par(mfrow = c(1,2))
plot(hclust(RFLPdist(RFLPdata, nrBands = 3)), cex = 0.7)
RFLPplot(RFLPdata, nrBands = 3, mar.bottom = 6, cex.axis = 0.8)

par(mfrow = c(1,2))
plot(hclust(RFLPdist2(RFLPdata, nrBands = 9, nrMissing = 1)), cex = 0.7)
RFLPqc

RFLPplot(RFLPdata, nrBands = 9, nrMissing = 1, mar.bottom = 6, cex.axis = 0.8)

distfun <- function(x) dist(x, method = "maximum")
par(mfrow = c(1,2))
plot(hclust(RFLPdist(RFLPdata, nrBands = 3, distfun = distfun),
method = "average"), cex = 0.7, cex.lab = 0.7)
RFLPplot(RFLPdata, nrBands = 3, distfun = distfun, hclust.method = "average",
mar.bottom = 6, cex.axis = 0.8)

RFLPqc

Quality control for RFLP data

Description

Function to perform quality control for RFLP data based on a comparison between the total length
of the digested PCR amplification product and the sum of the fragment lengths. If the sum is smaller
or larger than the PCR amplification product (within a certain range to define), the samples can be
excluded from further analyses. This function is helpful for data sets containing faint or uncertain
bands. It is necessary to include the total length of the PCR amplification product for each sample
as largest fragment in the data set, see RFLPdata.

Usage

RFLPqc(x, rm.band1 = TRUE, QC.lo = 0.8, QC.up = 1.07, QC.rm = FALSE)

Arguments

x data.frame with RFLP data.
rm.band1 logical: remove first band.
QC.lo numeric: a real number in (0,1).
QC.up numeric: a real number larger than 1.
QC.rm logical: remove samples with insufficient quality.

Details

In case the first band corresponds to the total length of the fragment one can perform a quality
control comparing the length of the first band with the sum of the lengths of the remaining bands
for each sample. If the sum is smaller than QC.lo times the length of the first band or larger than
QC.up times the length of the first band, respectively, a text message is printed.
If rm.band1 = TRUE band 1 of all samples is removed and the remaining band numbers are reduced
by 1.
If QC.rm = TRUE samples of insufficient quality are entirely removed from the given data and the
resulting data.frame is returned.
Value

A data.frame with variables

Sample character: sample identifier.
Band integer: band number.
MW integer: molecular weight.
Gel character: gel identifier.

Author(s)

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>,
Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>,
Matthias Kohl <Matthias.Kohl@stamats.de>

References

Flessa, F., Kehl, A., Kohl, M. Analysing diversity and community structures using PCR-RFLP: a

See Also

RFLPdata, RFLPdist

Examples

Dir <- system.file("extdata", package = "RFLPtools") # input directory
filename <- file.path(Dir, "AZ091016_report.txt")
RFLP1 <- read.rflp(file = filename)
str(RFLP1)

RFLP2 <- RFLPqc(RFLP1, rm.band1 = FALSE) # identical to RFLP1
identical(RFLP1, RFLP2)

RFLP3 <- RFLPqc(RFLP1)
str(RFLP3)

RFLP4 <- RFLPqc(RFLP1, rm.band1 = TRUE, QC.rm = TRUE)
str(RFLP4)

RFLPref

Example data set for RFLP reference

Description

This is an example data set for RFLP reference.
Usage

data(RFLPref)

Format

A data frame with 35 observations on the following five variables

**Sample** character: sample identifier.

**Band** integer: band number.

**MW** integer: molecular weight.

**Taxonname** character: taxon name.

**Accession** character: accession number.

Details


Source

The data set was generated by F. Flessa.

References


Examples

data(RFLPref)
str(RFLPref)

---

**RFLPrefplot**

*Function for a visual comparison of RFLP samples with reference samples.*

Description

Given RFLP samples are plotted together with reference samples and sorted by their distance to the reference sample.

Usage

RFLPrefplot(x, ref, distfun = dist, nrBands, mar.bottom = 5, cex.main = 1.2, cex.axis = 0.5, devNew = FALSE, colBands, xlab = "", ylab = "molecular weight", ylim, ...)
Arguments

- `x` data.frame with RFLP data; e.g. `RFLPdata`.
- `ref` data.frame with RFLP reference data; e.g. `RFLPref`.
- `distfun` function computing the distance with default `dist`; see `dist`.
- `nrBands` if not missing, then only samples with the specified number of bands are considered.
- `mar.bottom` bottom margin of the plot; see `par`.
- `cex.main` size of the plot title.
- `cex.axis` size of the x-axis annotation.
- `devNew` logical. Open new graphics device for each plot.
- `colBands` color for the bands. Has to be of length 1 or number of samples. If missing, "Set1" of `RColorBrewer` is used; see `ColorBrewer`.
- `xlab` passed to function `plot`.
- `ylab` passed to function `plot`.
- `ylim` passed to function `plot`. If missing an appropriate range of y-values is computed.
- `...` additional arguments passed to function `plot` except `xlim` which is defined inside of RFLPplot.

Details

Given RFLP samples are plotted together with reference samples and sorted by their distance to the reference sample.

Value

`invisible`

Author(s)

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>

References


See Also

`RFLPplot`
**Examples**

```r
data(RFLPdata)
data(RFLPref)
dev.new(width = 12)
RFLPrefplot(RFLPdata, RFLPref, nrBands = 4, cex.axis = 0.5)

dev.new()
RFLPrefplot(RFLPdata, RFLPref, nrBands = 6, cex.axis = 0.8)
RFLPrefplot(RFLPdata, RFLPref, nrBands = 9, cex.axis = 0.8)

RFLPrefplot(RFLPdata, RFLPref[RFLPref$Sample == "Ni_29_A3",], nrBands = 4, cex.axis = 0.7)

Dir <- system.file("extdata", package = "RFLPtools") # input directory
filename <- file.path(Dir, "AZ091016_report.txt")
RFLP1 <- read.rflp(file = filename)
RFLP2 <- RFLPqc(RFLP1)

dev.new(width = 12)
RFLPrefplot(RFLP1, RFLPref, nrBands = 4, cex.axis = 0.8)

dev.new()
RFLPrefplot(RFLP1, RFLPref, nrBands = 5, cex.axis = 0.8)
```

---

**sim2dist**

*Convert similarity matrix to dist object.*

**Description**

Function to convert similarity matrix to object of S3 class "dist".

**Usage**

```r
sim2dist(x, maxSim = 1)
```

**Arguments**

- `x` symmetric matrix: similarity matrix.
- `maxSim` maximum similarity possible.

**Details**

Similarity is converted to distance by `maxSim - x`. The resulting matrix is converted to an object of S3 class "dist" by `as.dist`.

**Value**

Object of S3 class "dist" is returned; see `dist`.
**Author(s)**

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

BLASTdata, simMatrix

**Examples**

data(BLASTdata)

```r
## without sequence range
## Not run:
res <- simMatrix(BLASTdata)

## End(Not run)

## with sequence range
range(BLASTdata$alignment.length)
res1 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 100, Max = 450)
res2 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 500)

## visualize similarity matrix
library(MKomics)
simPlot(res2, minVal = 0,
       labels = colnames(res2), title = "(Dis-)Similarity Plot")

## or
library(lattice)
myCol <- colorRampPalette(brewer.pal(8, "RdYlGn"))(128)
levelplot(res2, col.regions = myCol,
          at = do.breaks(c(0, max(res2)), 128),
          xlab = "", ylab = "",
          ## Rotate label of x axis
          scales = list(x = list(rot = 90)),
          main = "(Dis-)Similarity Plot")

## convert to distance
res.d <- sim2dist(res2)

## hierarchical clustering
plot(hclust(res.d))
```
**simMatrix**

Similarity matrix for BLAST data.

---

**Description**

Function to compute similarity matrix for all-vs-all BLAST results of rDNA sequences generated with standalone BLAST from NCBI or local BLAST implemented in BioEdit.

**Usage**

```r
simMatrix(x, sequence.range = FALSE, Min, Max)
```

**Arguments**

- `x`  
  data.frame with BLAST data; see `BLASTdata`.

- `sequence.range`  
  logical: use sequence range.

- `Min`  
  minimum sequence length.

- `Max`  
  maximum sequence length.

**Details**

The given BLAST data is used to compute a similarity matrix using the following algorithm: First, the length of each sequence (LS) comprised in the input data file is extracted. If there is more than one comparison for one sequence including different parts of the respective sequence, that one with maximum base length is chosen. Subsequently, the number of matching bases (mB) is calculated by multiplying two variables comprised in the BLAST output: the identity between sequences (%) and the number of nucleotides divided by 100. The, resulting value is rounded to integer. Furthermore, the similarity is calculated by dividing mB by LS. Finally, the similarity matrix including all sequences is built. If the similarity of a combination is not shown in the BLAST report file (because the similarity was lower than 70%), this comparison is included in the similarity matrix with the result zero.

**Value**

Similarity matrix.

**Author(s)**

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>,  
Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>,  
Matthias Kohl <Matthias.Kohl@stamats.de>
 simulateRFLPdata

References

BioEdit: https://bioedit.software.informer.com/


See Also

BLASTdata, sim2dist

Examples

data(BLASTdata)

## without sequence range
## code takes some time
## Not run:
res <- simMatrix(BLASTdata)

## End(Not run)

## with sequence range
range(BLASTdata$alignment.length)
res1 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 100, Max = 450)
res2 <- simMatrix(BLASTdata, sequence.range = TRUE, Min = 500)

simulateRFLPdata Simulate RFLP data.

Description

Simulates RFLP data for comparisons of algorithms.

Usage

simulateRFLPdata(N = 10, nrBands = 3:12, bandCenters = seq(100, 800, by = 100),
                   delta = 50, refData = FALSE)
**simulateRFLPdata**

**Arguments**

- `N` integer: number samples which shall be simulated per number of bands.
- `nrBands` integer: vector of number of bands.
- `bandCenters` numeric: vector of band centers.
- `delta` numeric: uniform distribution with `min = bandCenter - delta` and `max = bandCenter + delta` is used.
- `refData` logical: if TRUE, additional columns `Taxonname` and `Accession` are generated.

**Details**

The function can be used to simulate RFLP data. For every number of band specified in `nrBands` a total number of `N` samples are generated.

First the band centers are randomly selected (with replacement) from `bandCenter` which form the centers of intervals of length `2*delta`. From these intervals uniform random numbers are drawn leading to randomly generated RFLP data.

**Value**

A data frame with `N*length(nrBands)` observations on the following four variables

- `Sample` character: sample identifier.
- `Band` integer: band number.
- `MW` integer: molecular weight.
- `Enzyme` character: enzyme name.

is generated. If `refData = TRUE` then the following two additional variables are added.

- `Taxonname` character: taxon name.
- `Accession` character: accession number.

**Author(s)**

Mohammed Aslam Imtiaz, Matthias Kohl <Matthias.Kohl@stamats.de>

**See Also**

- `RFLPdata`, `RFLPref`

**Examples**

```r
simData <- simulateRFLPdata()
```
write.hclust

Description

The tree obtained by a hierarchical cluster analysis is cut into groups by using `cutree` and the results are exported to a text file.

Usage

```r
write.hclust(x, file, prefix, h = NULL, k = NULL, append = FALSE, dec = ",")
```

Arguments

- `x` object of class `hclust`: result of hierarchical cluster analysis computed via function `hclust`.
- `file` either a character string naming a file or a connection open for writing. "" indicates output to the console.
- `prefix` character. Information about the cluster analysis.
- `h` numeric scalar or vector with heights where the tree should be cut.
- `k` an integer scalar or vector with the desired number of groups.
- `append` logical. Only relevant if `file` is a character string. If TRUE, the output is appended to the file. If FALSE, any existing file of the name is destroyed.
- `dec` the string to use for decimal points in numeric or complex columns: must be a single character.

Details

The results are written to file by a call to `write.table` where the columns in the resulting file are separated by tabulators (i.e. `sep="\t"`) and no row names are exported (i.e. `row.names = FALSE`).

Author(s)

Fabienne Flessa <Fabienne.Flessa@uni-bayreuth.de>, Alexandra Kehl <Alexandra.Kehl@uni-tuebingen.de>, Matthias Kohl <Matthias.Kohl@stamats.de>

References


See Also

`write.table`, `cutree`
write.hclust

Examples

data(RFLPdata)
res <- RFLPdist(RFLPdata, nrBands = 4)
cl <- hclust(res)
## Not run:
write.hclust(cl, file = "Test.txt", prefix = "Bd4", h = 50)
## End(Not run)

res <- RFLPdist2(RFLPdata, nrBands = 4, nrMissing = 1)
cl <- hclust(res)
## Not run:
write.hclust(cl, file = "Test.txt", append = TRUE, prefix = "Bd4_Mis1", h = 60)
## End(Not run)
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