Package ‘microclass’

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microclass-package .................................................. 2
blastClassify16S .................................................... 2
blastDbase16S ....................................................... 4
KmerCount ........................................................... 5
multinomClassify .................................................... 6
multinomTrain ....................................................... 7
rdpClassify ............................................................ 8
Methods for 16S based taxonomic classification of prokaryotes

Description
The package provides functions for assigning 16S sequence data to a taxonomic level in the tree-of-life for prokaryotes.

Usage
microclass()

Details

Package: microclass
Type: Package
Version: 1.0.1
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Author(s)
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Classifying using BLAST

Description
A 16S based classification based on BLAST.

Usage
blastClassify16S(sequence, bdb)
blastClassify16S

Arguments

- sequence: Character vector of 16S sequences to classify.
- bdb: Name of BLAST data base, see blastDbase16S.

Details

A vector of 16S sequences (DNA) are classified by first using BLAST blastn against a database of 16S DNA sequences, and then classify according to the nearest-neighbour principle. The nearest neighbour of a query sequence is the hit with the largest bitscore. The blast+ software [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download) must be installed on the system. Type `system("blastn -help")` in the Console window, and a sensible Help-text should appear.

The database must contain 16S sequences where the Header starts with a token specifying the taxon. More specifically, the tokens must look like:

- `<taxon>_1`
- `<taxon>_2`
- `...etc`

where `<taxon>` is some proper taxon name. Use blastDbase16S to make such databases.

The identity of each alignment is also computed. This should be close to 1.0 for a classification to be trusted. Identity values below 0.95 could indicate uncertain classifications, but this will vary between taxa.

Value

A data.frame with two columns: Taxon is the predicted taxon for each sequence and Identity is the corresponding identity-value. If no BLAST hit is seen, the sequence is "unclassified".

Author(s)

Lars Snipen.

See Also

- blastDbase16S.

Examples

```r
data("small.16S")
## Not run:
dbase <- blastDbase16S("test", small.16S$Sequence, word(small.16S$Header, 2, 2))
reads <- str_sub(small.16S$Sequence, 100, 550)
broadcastClassify16S(reads, dbase) %>%
  bind_cols(small.16S) -> tbl

## End(Not run)
```
blastDbase16S  

Building a BLAST database

Description

Building a BLAST database for 16S based classification.

Usage

```r
blastDbase16S(name, sequence, taxon)
```

Arguments

- `name`: The name of the database (text).
- `sequence`: A character vector with 16S sequence data.
- `taxon`: A character vector with taxon information.

Details

This function builds a database using the `makeblastdb` program of the BLAST+ software [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastDocs&DOC_TYPE=Download). Thus, this software must be available on the system when using this function. If you type `system("makeblastdb -help")` in the Console window some meaningful Help-text should be displayed.

This function is most typically used prior to `blastClassify16S` to set up the database before searching and classifying. It can be seen as the 'training step' of a BLAST-based classification procedure.

The sequence must be a vector of DNA-sequences (16S sequences). The taxon is a vector of the same length as sequence, containing the corresponding taxon information.

Value

The database files are created, and the name of the database (`name`) is returned.

Author(s)

Lars Snipen.

See Also

- `blastClassify16S`.

Examples

```r
# See examples for blastClassify16S.
```
**KmerCount**

**K-mer counting**

Description

Counting overlapping words of length K in DNA/RNA sequences.

Usage

KmerCount(sequences, K = 1, col.names = FALSE)

Arguments

- sequences: Vector of sequences (text).
- K: Word length (integer).
- col.names: Logical indicating if the words should be added as column names.

Details

For each input sequence, the frequency of every word of length K is counted. Counting is done with overlap. The counting itself is done by a C++ function.

With col.names=TRUE the K-mers are added as column names, but this makes the computations slower.

Value

A matrix with one row for each sequence in sequences and one column for each possible word of length K.

Author(s)

Kristian Hovde Liland and Lars Snipen.

See Also

multinomTrain, multinomClassify.

Examples

KmerCount("ATGCTGAAGTACCTGC", K=2)
**multinomClassify**  
*Classifying with a Multinomial model*

**Description**
Classifying sequences by a trained Multinomial model.

**Usage**
```r
multinomClassify(sequence, trained.model, post.prob = FALSE, prior = FALSE)
```

**Arguments**
- `sequence` Character vector of 16S sequences to classify.
- `trained.model` A list with a trained model, see `multinomTrain`.
- `post.prob` Logical indicating if posterior log-probabilities should be returned.
- `prior` Logical indicating if classification should be done by flat priors (default) or with empirical priors (prior=TRUE).

**Details**
The classification step of the Multinomial method (Vinje et al, 2015) means counting K-mers on all sequences, and computing the posterior probabilities for each taxon in the trained model. The predicted taxon for each input sequence is the one with the maximum posterior probability for that sequence.

By setting `post.prob=TRUE` you will get the log-probability of the best and second best taxon for each sequence. This can be used for evaluating the certainty in the classifications, see `taxMachine`.

The classification is parallelized through RcppParallel employing Intel TBB and TinyThread. By default all available processing cores are used. This can be changed using the function `setParallel`.

**Value**
- If `post.prob=FALSE` a character vector of predicted taxa is returned.
- If `post.prob=TRUE` a data.frame with three columns is returned. Taxon is the vector of predicted taxa, one for each sequence in sequence. The Post.prob.1 and Post.prob.2 are vectors with the maximum and second largest posterior log-probabilities for each sequence.

**Author(s)**
Kristian Hovde Liland and Lars Snipen.

**References**
multinomTrain

Training multinomial model

Description
Training the multinomial K-mer method on sequence data.

Usage
multinomTrain(sequence, taxon, K = 8, col.names = FALSE, n.pseudo = 100)

Arguments
sequence Character vector of 16S sequences.
taxon Character vector of taxon labels for each sequence.
K Word length (integer).
col.names Logical indicating if column names should be added to the trained model matrix.
n.pseudo Number of pseudo-counts to use (positive numerics, need not be integer). Special case -1 will only return word counts, not log-probabilities.

Details
The training step of the multinomial method (Vinje et al, 2015) means counting K-mers on all sequences and compute the multinomial probabilities for each K-mer for each unique taxon. n.pseudo pseudo-counts are added, divided equally over all K-mers, before probabilities are estimated. The optimal choice of n.pseudo will depend on K and the training data set. The default value n.pseudo=100 has proven good for K=8 and the contax.trim data set (see the microcontax R-package).
Adding the actual K-mers as column names (col.names=TRUE) will slow down the computations.
The relative taxon sizes are also computed, and may be used as an empirical prior in the classification step (see "prior" below).

**Value**

A list with two elements. The first element is `Method`, which is the text "multinom" in this case. The second element is `Fitted`, which is a matrix of probabilities with one row for each unique taxon and one column for each possible word of length K. The sum of each row is 1.0. No probabilities are 0 if `n.pseudo>0.0`.

The matrix `Fitted` has an attribute `attr("prior", )`, that contains the relative taxon sizes.

**Author(s)**

Kristian Hovde Liland and Lars Snipen.

**References**


**See Also**

`KmerCount`, `multinomClassify`.

**Examples**

```r
# See examples for multinomClassify
```

---

### rdpClassify

*Classifying with the RDP classifier*

**Description**

Classifying sequences by a trained presence/absence K-mer model.

**Usage**

```r
dpClassify(sequence, trained.model, post.prob = FALSE, prior = FALSE)
```

**Arguments**

- `sequence`: Character vector of sequences to classify.
- `trained.model`: A list with a trained model, see `rdpTrain`.
- `post.prob`: Logical indicating if posterior log-probabilities should be returned.
- `prior`: Logical indicating if classification should be done by flat priors (default) or with empirical priors (prior=TRUE).
Details

The classification step of the presence/absence method known as the RDP classifier (Wang et al 2007) means looking for K-mers on all sequences, and computing the posterior probabilities for each taxon using a trained model and a naive Bayes assumption. The predicted taxon is the one producing the maximum posterior probability, for each sequence.

The classification is parallelized through RcppParallel employing Intel TBB and TinyThread. By default all available processing cores are used. This can be changed using the function setParallel.

Value

A character vector with the predicted taxa, one for each sequence.

Author(s)

Kristian Hovde Liland and Lars Snipen.

References


See Also

rdpTrain.

Examples

data("small.16S")
seq <- small.16S$Sequence
tax <- sapply(strsplit(small.16S$Header,split=" "),function(x){x[2]})
## Not run:
trn <- rdpTrain(seq,tax)
primer.515f <- "GTGYCAGCMGCCGCGGTAA"
primer.806rB <- "GGAAGTAAAAACGACAGGTTCTAAT"
reads <- amplicon(seq, primer.515f, primer.806rB)
predicted <- rdpClassify(unlist(reads[nchar(reads)>0]),trn)
print(predicted)
## End(Not run)
rdpTrain

Training the RDP classifier

Description
Training the RDP presence/absence K-mer method on sequence data.

Usage
rdpTrain(sequence, taxon, K = 8, cnames = FALSE)

Arguments
- sequence: Character vector of 16S sequences.
- taxon: Character vector of taxon labels for each sequence.
- K: Word length (integer).
- cnames: Logical indicating if column names should be added to the trained model matrix.

Details
The training step of the RDP method means looking for K-mers on all sequences, and computing the probability of each K-mer being present for each unique taxon. This is an attempt to re-implement the method described by Wang et al (2007), but without the bootstrapping. See that publications for all details.

The word-length K is by default 8, since this is the value used by Wang et al. Larger values may lead to memory-problems since the trained model is a matrix with \(4^K\) columns. Adding the K-mers as column names will slow down all computations.

The relative taxon sizes are also computed, and returned as an attribute to the model matrix. They may be used as empirical priors in the classification step.

Value
A list with two elements. The first element is Method, which is the text "RDPclassifier" in this case. The second element is Fitted, which is a matrix with one row for each unique taxon and one column for each possible word of length K. The value in row i and column j is the probability that word j is present in taxon i.

Author(s)
Kristian Hovde Liland and Lars Snipen.

References
**setParallel**

See Also

* rdpClassify.

Examples

```r
# See examples for rdpClassify.
```

---

**setParallel**  
*Set number of parallel threads*

Description

Simple function to set the number of threads to use in parallel computations. The default equals all available logical cores. An integer is interpreted as the number of threads. A numeric < 1 is interpreted as a proportion of the available logical cores.

Usage

```r
setParallel(C = NULL)
```

Arguments

- `C`  
a scalar indicating the number of threads, default = NULL (#available logical cores)

Value

NULL, returned silently.

Examples

```r
## Not run:
setParallel() # Use all available logical cores.

## End(Not run)
```
small.16S A small example data set

Description
A tibble object (data.frame) with some 16S sequences with taxon information.

Usage
data(small.16S)

Details
This is a tibble object (data.frame) with 71 sequences used in some examples. The taxonomy information for each sequence follows the ConTax format, see the microcontax package for more details.

Author(s)
Hilde Vinje, Kristian Hovde Liland, Lars Snipen.

Examples
data(small.16S)
str(small.16S)

---
taxMachine Classifying 16S sequences

Description
Optimized classification of 16S sequence data.

Usage
taxMachine(
  sequence,
  model.in.memory = TRUE,
  model.on.disk = FALSE,
  verbose = TRUE,
  chunk.size = 10000
)
taxMachine

Arguments

- **sequence**: Character vector with DNA sequences.
- **model.in.memory**: Logical indicating if model should be cached in memory (default=TRUE).
- **model.on.disk**: Logical or text, for reading/saving models, see Details below (default=FALSE).
- **verbose**: Logical, if TRUE progress is reported during computations (default=TRUE).
- **chunk.size**: The number of sequence to classify in each iteration of the loop (default=10000).

Details

This function provides optimized taxonomy classifications from 16S sequence data. All sequences are classified to the genus level based on a Multinomial model (see `multinomTrain`) trained on the designed consensus taxonomy data set `contax.trim` found in the R-package `microcontax`. The word length K=8 has been used in the model.

To avoid saving fitted models in the package, a model is trained the first time you run `taxMachine` in an R session. This takes only a few seconds, and the result is cached for latter use if `model.in.memory` is TRUE.

If a path to an existing file with a trained model is supplied in `model.on.disk`, this Multinomial model is read from the file and used. If a path to a new file is supplied, the trained Multinomial model will be saved to that file. The default (model.on.disk=FALSE), means no files are read/saved, while model.on.disk=TRUE will attempt to load/save models from the microclass/extdata directory.

Both `verbose` and `chunk.size` are used to monitor the progress, which is nice when classifying huge data sets, since this will take some time.

Value

A `data.frame` with one row for each sequence. The columns are Genus, D.score, R.score and P.recognize.

Genus is the predicted genus for each sequence. Note that all sequences get a prediction, but may still be more or less reliable.

The D.score is a measure of how the predicted genus wins over all other genera in the race for being the chosen one. A large D.score means the winner stands out clearly, and we can be confident it is the correct genus. A D.score close to 0 means we have an uncertain classification. Only D.scores below 1.0, should be of any concern, see Liland et al (2016) for details.

The R.score is a measure of the models ability to recognize the sequence. The more negative the R.score gets, the more unusual the sequence is compared to the training set (the `contax.trim` data set). The P.recognize is a rough probability of seeing an R.score this small, or smaller, given the training data. Thus, a very small P.recognize means the sequence is not really recognized, and the classification is worthless. A very negative R.score indicates either not 16S at all, many sequencing errors that has destroyed the read, or a completely new taxon never seen before. See Liland et al (2016) for details.

Author(s)

Lars Snipen and Kristian Hovde Liland
References


See Also

KmerCount, multinomClassify.

Examples

```r
## Not run:
data(small.16S)
tax.tab <- taxMachine(small.16S$Sequence)

## End(Not run)
```
Index

* package
  microclass-package, 2

blastClassify16S, 2, 4
blastDbase16S, 3, 4

contax.trim, 7, 13
KmerCount, 5, 7, 8, 14

microclass (microclass-package), 2
microclass-package, 2
microcontax, 12
multinomClassify, 5, 6, 8, 14
multinomTrain, 5–7, 7, 13

rdpClassify, 8, 11
rdpTrain, 8, 9, 10

setParallel, 6, 9, 11
small.16S, 12

taxMachine, 6, 12