Package ‘primerTree’

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Title Visually Assessing the Specificity and Informativeness of Primer Pairs

Version 1.0.6

Description Identifies potential target sequences for a given set of primers and generates phylogenetic trees annotated with the taxonomies of the predicted amplification products.

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Depends R (>= 3.5.0), directlabels, gridExtra

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R topics documented:

accession2taxid .................................................. 2
bryophytes_trnL .................................................. 2
calc_rank_dist_ave ............................................. 3
clustalo .......................................................... 4
filter_seqs ....................................................... 4
get_sequence .................................................... 5
get_sequences ................................................... 6
get_taxonomy ..................................................... 7
identify.primerTree_plot ..................................... 7
accession2taxid

Maps a nucleotide database accession to a taxonomy database taxId

Usage

accession2taxid(accessions)

Arguments

accessions character vector to lookup.

Value

named vector of taxIds.

bryophytes_trnL

PrimerTree results for the bryophyte trnL primers

Description

PrimerTree results for the bryophyte trnL primers
calc_rank_dist_ave

Description

Summarize pairwise differences.

Usage

calc_rank_dist_ave(x, ranks = common_ranks)

Arguments

x  
a primerTree object
ranks  
ranks to show unique counts for, defaults to the common ranks

Details

The purpose of this function is to calculate the average number of nucleotide differences between species within each taxa of given taxonomic level.

For example, at the genus level, the function calculates the average number of nucleotide differences between all species within each genus and reports the mean of those values.

There are several key assumptions and calculations made in this function.

First, the function randomly selects one sequence from each species in the primerTree results. This is to keep any one species (e.g. human, cow, etc.) with many hits from skewing the results.

Second, for each taxonomic level tested, the function divides the sequences by each taxon at that level and calculates the mean number of nucleotide differences within that taxa, then returns the mean of those values.

Third, when calculating the average distance, any taxa for which there is only one species is omitted, as the number of nucleotide differences will always be 0.

Value

returns a data frame of results

Examples

## Not run:
calc_rank_dist_ave(mammals_16S)
calc_rank_dist_ave(bryophytes_trnL)

# Note that the differences between the results from these two primers
# the mean nucleotide differences is much higher for the mammal primers
# than the byrophyte primers. This suggests that the mammal primers have
# better resolution to distinguish individual species.

## End(Not run)
### clustalo

**Multiple sequence alignment with clustal omega**

**Description**

Calls clustal omega to align a set of sequences of class DNAbin. Run without any arguments to see all the options you can pass to the command line clustal omega.

**Usage**

```r
clustalo(x, exec = "clustalo", quiet = TRUE, original.ordering = TRUE, ...)
```

**Arguments**

- `x`: an object of class 'DNAbin'
- `exec`: a character string with the name or path to the program
- `quiet`: whether to suppress output to stderr or stdout
- `original.ordering`: use the original ordering of the sequences
- `...`: additional arguments passed to the command line clustalo

### filter_seqs

**Filter out sequences retrieved by search_primer_pair() that are either too short or too long. The alignment and tree will be recalculated after removing unwanted reads.**

**Description**

Filter out sequences retrieved by search_primer_pair() that are either too short or too long. The alignment and tree will be recalculated after removing unwanted reads.

**Usage**

```r
filter_seqs(x, ...)
```

#### S3 method for class 'primerTree'

```r
filter_seqs(x, min_length = 0, max_length = Inf, ...)
```

**Arguments**

- `x`: a primerTree object
- `...`: additional arguments passed to methods.
- `min_length`: the minimum sequence length to keep
- `max_length`: the maximum sequence length to keep
get_sequence

Value

a primerTree object

Methods (by class)

  • primerTree: Method for primerTree objects

Examples

```r
## Not run:
# filter out sequences longer or shorter than desired:
mammals_16S_filtered <- filter_seqs(mammals_16S, min_length=131, max_length=156)
## End(Not run)
```

get_sequence

Retrieves a fasta sequence from NCBI nucleotide database.

Description

Retrieves a fasta sequence from NCBI nucleotide database.

Usage

```r
get_sequence(
  accession,
  start = NULL,
  stop = NULL,
  api_key = Sys.getenv("NCBI_API_KEY")
)
```

Arguments

- **accession**: nucleotide accession to retrieve.
- **start**: start base to retrieve, numbered beginning at 1. If NULL the beginning of the sequence.
- **stop**: last base to retrieve, numbered beginning at 1. If NULL the end of the sequence.
- **api_key**: NCBI api-key to allow faster sequence retrieval.

Value

an DNAbin object.

See Also

DNAbin
get_sequences

Retrieves fasta sequences from NCBI nucleotide database.

Description

Retrieves fasta sequences from NCBI nucleotide database.

Usage

get_sequences(
  accession,
  start = NULL,
  stop = NULL,
  api_key = Sys.getenv("NCBI_API_KEY"),
  simplify = TRUE,
  .parallel = FALSE,
  .progress = "none"
)

Arguments

accession the accession number of the sequence to retrieve
start start bases to retrieve, numbered beginning at 1. If NULL the beginning of the sequence.
stop stop bases to retrieve, numbered beginning at 1. if NULL the stop of the sequence.
api_key NCBI api-key to allow faster sequence retrieval.
simplify simplify the FASTA headers to include only the genbank accession.
.parallel if 'TRUE', perform in parallel, using parallel backend provided by foreach
.progress name of the progress bar to use, see 'create_progress_bar'

Value

an DNAbin object.

See Also

DNAbin
get_taxonomy

Retrieve the taxonomy information from NCBI for a set of nucleotide gis.

Description
Retrieve the taxonomy information from NCBI for a set of nucleotide gis.

Usage
get_taxonomy(accessions)

Arguments
accessions a character vector of the accessions to retrieve

Value
data.frame of the 'accessions, taxIds, and taxonomy

identify.primerTree_plot

identify the point closest to the mouse click only works on single ranks

Description
identify the point closest to the mouse click only works on single ranks

Usage
## S3 method for class 'primerTree_plot'
identify(x, ...)

Arguments
x the plot to identify
... additional arguments passed to annotate
layout_tree_ape

layout a tree using ape, return an object to be plotted by plot_tree

Description
layout a tree using ape, return an object to be plotted by plot_tree

Usage
layout_tree_ape(tree, ...)

Arguments
- tree: The phylo tree to be plotted
- ...: additional arguments to plot.phylo

Value
- edge: list of x, y and xend, yend coordinates as well as ids for the edges
- tips: list of x, y, label and id for the tips
- nodes: list of x, y and id for the nodes

mammals_16S
PrimerTree results for the mammalian 16S primers

Description
PrimerTree results for the mammalian 16S primers

parse_primer_hits
Parse the primer hits

Description
Parse the primer hits

Usage
parse_primer_hits(response)

Arguments
- response: a httr response object obtained from primer_search
Description
plot function for a primerTree object, calls plot_tree_ranks

Usage
## S3 method for class 'primerTree'
plot(x, ranks = NULL, main = NULL, ...)

Arguments
x primerTree object to plot
ranks The ranks to include, defaults to all common ranks, if NULL print all ranks. If 'none' just print the layout.
main an optional title to display, if NULL displays the name as the title
... additional arguments passed to plot_tree_ranks

See Also
plot_tree_ranks, plot_tree

Examples
library(gridExtra)
library(directlabels)
#plot with all common ranks
plot(mammals_16S)

#plot only the class
plot(mammals_16S, 'class')

#plot the layout only
plot(mammals_16S, 'none')
Usage

plot_tree(
   tree,
   type = "unrooted",
   main = NULL,
   guide_size = NULL,
   rank = NULL,
   taxonomy = NULL,
   size = 2,
   legend_cutoff = 25,
   ...
)

Arguments

tree
   to be plotted, use layout_tree to layout tree.
type
   The type of tree to plot, default unrooted.
main
   An optional title for the plot
guide_size
   The size of the length guide. If NULL auto detects a reasonable size.
rank
   The rank to include, if null only the tree is plotted
taxonomy
   A data.frame with an accession field corresponding to the tree tip labels.
size
   The size of the colored points
legend_cutoff
   The number of different taxa names after which the names are no longer printed.
...
   additional arguments passed to layout_tree_ape

Value

plot to be printed.

plot_tree_ranks

plots a tree along with a series of taxonomic ranks

Description

plots a tree along with a series of taxonomic ranks

Usage

plot_tree_ranks(
   tree,
   taxonomy,
   main = NULL,
   type = "unrooted",
   ranks = common_ranks,
primerTree has two main commands: `search_primer_pair` which takes a primer pair and returns an primerTree object of the search results, and `plot.primerTree` a S3 method for plotting the primerTree object obtained using `search_primer_pair`.
primer_search  

Query a pair of primers using ncbi's Primer-BLAST, if primers contain iupac

Description

ambiguity codes, enumerate all possible combinations and combine the results.

Usage

primer_search(
  forward,
  reverse,
  num_aligns = 500,
  num_permutations = 25,
  ..., 
  .parallel = FALSE, 
  .progress = "none"
)

Arguments

forward forward primer to search by 5'-3' on plus strand
reverse reverse primer to search by 5'-3' on minus strand
num_aligns number of alignment results to keep
num_permutations the number of primer permutations to search, if the degenerate bases cause more than this number of permutations to exist, this number will be sampled from all possible permutations.

... additional arguments passed to Primer-Blast
.parallel if 'TRUE', perform in parallel, using parallel backend provided by foreach
.progress name of the progress bar to use, see 'create_progress_bar'

Value

httr response object of the query, pass to parse_primer_hits to parse the results.
search_primer_pair

Automatic primer searching Search a given primer pair, retrieving the alignment results, their product sequences, the taxonomic information for the sequences, a multiple alignment of the products

Description

Automatic primer searching Search a given primer pair, retrieving the alignment results, their product sequences, the taxonomic information for the sequences, a multiple alignment of the products

Usage

search_primer_pair(
  forward,
  reverse,
  name = NULL,
  num_aligns = 500,
  num_permutations = 25,
  simplify = TRUE,
  clustal_options = list(),
  distance_options = list(model = "N", pairwise.deletion = T),
  api_key = Sys.getenv("NCBI_API_KEY"),
  ...
)

Arguments

forward  forward primer to search by 5'-3' on plus strand
reverse  reverse primer to search by 5'-3' on minus strand
name     name to give to the primer pair
num_aligns number of alignment results to keep
num_permutations the number of primer permutations to search, if the degenerate bases cause more than this number of permutations to exist, this number will be sampled from all possible permutations.
simplify use simple names for primer hit results or complex
clustal_options a list of options to pass to clustal omega, see link{clustalo} for a list of options
distance_options a list of options to pass to dist.dna, see link{dist.dna} for a list of options
api_key NCBI api-key to allow faster sequence retrieval
... additional arguments passed to Primer-Blast
seq_lengths

Value

A list with the following elements,

- name: name of the primer pair
- BLAST_result: html blast results from Primer-BLAST as a response object.
- taxonomy: taxonomy for the primer products from NCBI
- sequence: sequence of the primer products
- alignment: multiple alignment of the primer products
- tree: phylogenetic tree of the reconstructed from the multiple alignment

See Also

primer_search, clustalo

Examples

```r
## Not run:
# simple search
mammals_16S = search_primer_pair(name='Mammals 16S',
                               'CGGTTGGGACCTCGGA',
                               'GCTGTTATCCCTAGGGTAACT')
# returning 1000 alignments, allow up to 3 mismatches in primer
mammals_16S = search_primer_pair(name='Mammals 16S',
                               'CGGTTGGGACCTCGGA',
                               'GCTGTTATCCCTAGGGTAACT',
                               num_aligns=1000, total_primer_specificity_mismatch=3)
## End(Not run)
```

seq_lengths

*Get a summary of sequence lengths from a primerTree object*

Description

Get a summary of sequence lengths from a primerTree object

Usage

```r
seq_lengths(x, summarize = TRUE)
```

Arguments

- `x`: a primerTree object.
- `summarize`: a logical indicating if a summary should be displayed
**seq_lengths.primerTree**

**Value**

a table of sequence length frequencies

**Examples**

```r
# Show the counts for each length
seq_lengths(mammals_16S)

# Plot the distribution of lengths
seqLengths <- seq_lengths(mammals_16S)
barplot(seqLengths,
main = "Frequency of sequence lengths for 16S mammal primers",
 xlab="Amplicon length (in bp)",
ylab="Frequency")
```

**seq_lengths.primerTree**

*Method for primerTree objects*

**Description**

Method for primerTree objects

**Usage**

```r
## S3 method for class 'primerTree'
seq_lengths(x, summarize = TRUE)
```

**Arguments**

- `x` a primerTree object.
- `summarize` a logical indicating if a summary should be displayed

**summary.primerTree**

*Summarize a primerTree result, printing quantiles of sequence length and pairwise differences.*

**Description**

Summarize a primerTree result, printing quantiles of sequence length and pairwise differences.

**Usage**

```r
## S3 method for class 'primerTree'
summary(object, ..., probs = c(0, 0.05, 0.5, 0.95, 1), ranks = common_ranks)
```
tree_from_alignment

Arguments

object  the primerTree object to summarise
...
probs   quantile probabilities to compute, defaults to 0, 5, 50, 95, and 100 probabilities.
ranks   ranks to show unique counts for, defaults to the common ranks

Value

invisibly returns a list containing the printed results

Description

Construct a neighbor joining tree from a dna alignment

Usage

tree_from_alignment(dna, pairwise.deletion = TRUE, ...)

Arguments

dna      fasta dna object the tree is to be constructed from
pairwise.deletion  a logical indicating if the distance matrix should be constructed using pairwise deletion
...

See Also

dist.dna, nj
Index

accession2taxid, 2
bryophytes_trnL, 2
calc_rank_dist_ave, 3
clustalo, 4, 14
create_progress_bar, 14
dist.dna, 16
DNAbin, 5, 6
filter_seqs, 4
get_sequence, 5
get_sequences, 6
get_taxonomy, 7
identify.primerTree_plot, 7
layout_tree_ape, 8, 10, 11
mammals_16S, 8
nj, 16
parse_primer_hits, 8, 12
phylo, 8
plot.phylo, 8
plot.primerTree, 9, 11
plot_tree, 8, 9, 9, 11
plot_tree_ranks, 9, 10
primer_search, 8, 12, 14
primerTree, 11
response, 14
search_primer_pair, 11, 13
seq_lengths, 14
seq_lengths.primerTree, 15
summary.primerTree, 15
tree_from_alignment, 16