# Package: LocaTT (via r-universe)

August 21, 2024

Title Geographically-Conscious Taxonomic Assignment for Metabarcoding

Version 1.1.15

**Description** A bioinformatics pipeline for performing taxonomic assignment of DNA metabarcoding sequence data while considering geographic location. A detailed tutorial is available at <https://urodelan.github.io/Local\_Taxa\_Tool\_Tutorial/>. A manuscript describing these methods is in preparation.

License GPL (>= 3)

URL https://github.com/Urodelan/LocaTT

BugReports https://github.com/Urodelan/LocaTT/issues Encoding UTF-8 Roxygen list(markdown = TRUE) RoxygenNote 7.2.3 Imports utils, stats, parallel, taxize Repository https://urodelan.r-universe.dev RemoteUrl https://github.com/urodelan/locatt RemoteRef HEAD RemoteSha 90985b382b855450c6d68d7e1d8ab46d3864e5ff

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adjust\_taxonomies Adjust Taxonomies

## Description

Performs adjustments to a taxonomy system according to a taxonomy edits file.

# Usage

```
adjust_taxonomies(
   path_to_input_file,
   path_to_output_file,
   path_to_taxonomy_edits
)
```

## Arguments

```
path_to_input_file
```

String specifying path to list of species (in CSV format) whose taxonomies are to be adjusted. The file should contain the following fields: 'Common\_Name', 'Domain', 'Phylum', 'Class', 'Order', 'Family', 'Genus', 'Species'. There should be no NAs or blanks in the taxonomy fields, and the species field should contain the binomial name. Additional fields may be present in the input file, and fields can be in any order.

path\_to\_output\_file

String specifying path to output species list with adjusted taxonomies. The output file will be in CSV format.

## binomial\_test

## path\_to\_taxonomy\_edits

String specifying path to taxonomy edits file in CSV format. The file must contain the following fields: 'Old\_Taxonomy', 'New\_Taxonomy', 'Notes'. Old taxonomies are replaced with new taxonomies in the order the records appear in the file. The taxonomic levels in the 'Old\_Taxonomy' and 'New\_Taxonomy' fields should be delimited by a semi-colon.

# Value

No return value. Writes an output CSV file with adjusted taxonomies.

# See Also

get\_taxonomies.species\_binomials for remotely fetching NCBI taxonomies from species binomials.

get\_taxonomies.IUCN for formatting taxonomies from the IUCN Red List.

## Examples

```
print("Insert example here.")
```

binomial\_test Binomial Test

## Description

Performs binomial tests.

## Usage

binomial\_test(k, n, p, alternative = "greater")

## Arguments

k	A numeric vector of the number of successes.
n	A numeric vector of the number of trials.
р	A numeric vector of the hypothesized probabilities of success.
alternative	A string specifying the alternative hypothesis. Must be "less" or "greater" (the default).

# Details

Calls on the pbinom function in the stats package to perform vectorized binomial tests. Arguments are recycled as in pbinom. Only one-sided tests are supported, and only p-values are returned.

## Value

A numeric vector of p-values from the binomial tests.

# Examples

blast\_command\_found Check BLAST Installation

## Description

Checks whether a BLAST program can be found.

# Usage

blast\_command\_found(blast\_command)

## Arguments

blast\_command String specifying the path to a BLAST program.

# Value

Logical. Returns TRUE if the BLAST program could be found.

# Examples

blast\_command\_found(blast\_command="blastn")

blast\_version Get BLAST Version

# Description

Gets the version of a BLAST program.

# Usage

```
blast_version(blast_command = "blastn")
```

## Arguments

blast\_command String specifying the path to a BLAST program. The default ('blastn') should return the version of the blastn program for standard BLAST installations. The user can provide a path to a BLAST program for non-standard BLAST installations.

# Value

Returns a string of the version of the BLAST program.

# Examples

blast\_version()

contains\_wildcards Check Whether DNA Sequences Contain Wildcard Characters

## Description

Checks whether DNA sequences contain wildcard characters.

#### Usage

```
contains_wildcards(sequences)
```

## Arguments

sequences A character vector of DNA sequences.

# Value

A logical vector indicating whether each DNA sequence contains wildcard characters.

# Examples

contains\_wildcards(sequences=c("TKCTAGGTGW","CATAATTAGG","ATYGGCTATG"))

decode\_quality\_scores Decode DNA Sequence Quality Scores

## Description

Decodes Phred quality scores in Sanger format from symbols to numeric values.

# Usage

```
decode_quality_scores(symbols)
```

## Arguments

symbols A string containing quality scores encoded as symbols in Sanger format.

# Value

A numeric vector of Phred quality scores.

# Examples

decode\_quality\_scores(symbols="989!.C;F@\"")

expand\_taxonomies Expand Taxonomies

# Description

Extracts each taxonomic level from a vector of taxonomic strings.

## Usage

```
expand_taxonomies(
  taxonomies,
  levels = c("Domain", "Phylum", "Class", "Order", "Family", "Genus", "Species"),
  full_names = TRUE,
  delimiter = ";",
  ignore
)
```

# filter\_sequences

## Arguments

taxonomies	A character vector of taxonomic strings.
levels	A character vector of taxonomic level names. The length of levels determines the number of taxonomic levels to extract from the taxonomies, and the order of elements in levels is assumed to match the order of taxonomic levels in the taxonomies. levels is also used as the field names of the returned data frame (see return value section). The default vector includes: "Domain", "Phylum", "Class", "Order", "Family", "Genus", and "Species".
full_names	Logical. If TRUE (the default), then full taxonomies are returned down to the extracted taxonomic level. If FALSE, then only the extracted taxonomic level is returned.
delimiter	A character string of the delimiter between taxonomic levels in the input tax- onomies. The default is ";".
ignore	An optional character vector of taxonomic strings for which taxonomic expan- sion will be skipped. In the returned data frame (see return value section), the record for each skipped taxonomic string will be filled with NAs.

# Value

Returns a data frame of extracted taxonomic levels. One record for each element of taxonomies, and one field for each element of levels. Field names are inherited from levels. If a taxonomic level is not present in a taxonomic string, then the respective cell in the returned data frame will contain NA.

# See Also

get\_taxonomic\_level for extracting a taxonomic level from taxonomic strings.

get\_consensus\_taxonomy for generating a consensus taxonomy from taxonomic strings.

# Examples

```
print("Insert example here.")
```

filter\_sequences Filter DNA Sequences by PCR Replicates

# Description

Filters DNA sequences by minimum read count within a PCR replicate, minimum proportion within a PCR replicate, and number of detections across PCR replicates.

# Usage

```
filter_sequences(
    input_files,
    samples,
    PCR_replicates,
    output_file,
    minimum_reads.PCR_replicate = 1,
    minimum_reads.sequence = 1,
    minimum_proportion.sequence = 0.005,
    binomial_test.enabled = TRUE,
    binomial_test.p.adjust.method = "none",
    binomial_test.alpha_level = 0.05,
    minimum_PCR_replicates = 2,
    delimiter.read_counts = ": ",
    delimiter.PCR_replicates = ", "
)
```

# Arguments

input_files	A character vector of file paths to input FASTA files. DNA sequences in the input FASTA files are assumed to be summarized by frequency of occurrence, with each FASTA header line beginning with "Frequency: " and followed by the sequence's read count. Output FASTA files from truncate_and_merge_pairs have this format and can be used directly with this function. Each input FASTA file is assumed to contain the DNA sequence reads for a single PCR replicate for a single sample.	
samples	A character vector of sample identifiers, with one element for each element of input_files.	
PCR_replicates	A character vector of PCR replicate identifiers, with one element for each element of input_files.	
output_file	String specifying path to output file of filtered sequences in CSV format.	
minimum_reads.P	CR_replicate	
	Numeric. PCR replicates which contain fewer reads than this value are discarded and do not contribute detections to any sequence. The default is 1 ( <i>i.e.</i> , no PCR replicates discarded).	
minimum_reads.s	equence	
	Numeric. For a sequence to be considered detected within a PCR replicate, the sequence's read count within the PCR replicate must match or exceed this value. The default is 1 ( <i>i.e.</i> , no filtering by minimum read count within PCR replicates).	
minimum_proportion.sequence		
	Numeric. For a sequence to be considered detected within a PCR replicate, the proportion of reads in the PCR replicate comprised by the sequence must exceed this value. If binomial_test.enabled = TRUE, then this argument is used as the null hypothesis for a one-sided binomial test, and a significance test is	

used to determine whether the minimum proportion requirement for detection

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is satisfied instead. See the binomial\_test.enabled argument below. The default is 0.005 (*i.e.*, 0.5%). To disable sequence filtering by minimum proportion within PCR replicates, set to 0.

## binomial\_test.enabled

Logical. If TRUE (the default), then for a sequence to be considered detected within a PCR replicate, the proportion of reads in the PCR replicate comprised by the sequence must significantly exceed the value of the minimum\_proportion.sequence argument at the provided alpha level (binomial\_test.alpha\_level argument) based on a one-sided binomial test (*i.e.*, binomial\_test with alternative = "greater"). Optionally, p-values within a PCR replicate can be adjusted for multiple hypothesis testing by setting the binomial\_test.p.adjust.method argument below. To disable significance testing, set to FALSE (minimum proportion filtering will still occur if minimum\_proportion.sequence > 0, see above).

# binomial\_test.p.adjust.method

String specifying the p-value adjustment method for multiple hypothesis testing. p-value adjustments are performed within each PCR replicate for each sample. Passed to the method argument of p.adjust in the stats package. Available methods are contained within the stats::p.adjust.methods vector. If "none" (the default), then p-value adjustments are not performed. Ignored if binomial\_test.enabled = FALSE.

binomial\_test.alpha\_level

Numeric. The alpha level used in deciding whether the proportion of reads in a PCR replicate comprised by a sequence significantly exceeds a minimum threshold required for detection. See the binomial\_test.enabled argument. The default is 0.05. Ignored if binomial\_test.enabled = FALSE.

# minimum\_PCR\_replicates

Numeric. The minimum number of PCR replicates in which a sequence must be detected in order to be considered present (*i.e.*, not erroneous) in a sample. The default is 2.

# delimiter.read\_counts

String specifying the delimiter between PCR replicate identifiers and sequence read counts in the Read\_count\_by\_PCR\_replicate field of the output CSV file (see details section). The default is ": ".

# delimiter.PCR\_replicates

String specifying the delimiter between PCR replicates in the Read\_count\_by\_PCR\_replicate field of the output CSV file (see details section). The default is ", ".

## Details

For each set of input polymerase chain reaction (PCR) replicate FASTA files associated with a sample, writes out DNA sequences which are detected across a minimum number of PCR replicates (minimum\_PCR\_replicates argument). Detection within a PCR replicate is defined as a sequence having at least a minimum read count *and* exceeding a minimum proportion of reads (minimum\_reads.sequence and minimum\_proportion.sequence arguments, respectively). When binomial\_test.enabled = TRUE, a sequence must significantly exceed the minimum proportion within a PCR replicate at the provided alpha level (binomial\_test.alpha\_level argument) based on a one-sided binomial test (*i.e.*, binomial\_test with alternative = "greater"). Within a PCR replicate, p-values can be adjusted for multiple hypothesis testing by setting the binomial\_test.p.adjust.method

argument (see stats::p.adjust.methods and p.adjust in the stats package). PCR replicates which contain fewer than a minimum number of reads are discarded (minimum\_reads.PCR\_replicate argument) and do not contribute detections to any sequence.

DNA sequences in the input FASTA files are assumed to be summarized by frequency of occurrence, with each FASTA header line beginning with "Frequency: " and followed by the sequence's read count. Output FASTA files from truncate\_and\_merge\_pairs have this format and can be used directly with this function. Each input FASTA file is assumed to contain the DNA sequence reads for a single PCR replicate for a single sample.

For pipeline calibration purposes, a data frame containing unfiltered DNA sequences with their read counts, proportions, and p-values in each PCR replicate is invisibly returned (see return value section). While the primary output of this function is the written CSV file of filtered sequences (described below), the invisibly returned data frame of unfiltered sequences can be helpful when calibrating or troubleshooting filtering parameters. To aid in troubleshooting filtering parameters, the data frame is invisibly returned even if the error "Filtering removed all sequences" is received.

For the primary output, this function writes a CSV file of filtered DNA sequences with the following field definitions:

- Sample: The sample name.
- Sequence: The DNA sequence.
- Detections\_across\_PCR\_replicates: The number of PCR replicates the sequence was detected in.
- Read\_count\_by\_PCR\_replicate: The sequence's read count in each PCR replicate the sequence was detected in.
- Sequence\_read\_count: The sequence's total read count across the PCR replicates the sequence was detected in. Calculated as the sum of the read counts in the Read\_count\_by\_PCR\_replicate field.
- Sample\_read\_count: The sample's total read count across all sequences detected in the PCR replicates. Calculated as the sum of the read counts in Sequence\_read\_count field associated with the sample.
- Proportion\_of\_sample: The proportion of sample reads comprised by the sequence. Calculated by dividing the Sequence\_read\_count field by the Sample\_read\_count field. Equivalent to the weighted average of the sequence's proportion in each PCR replicate, with weights given by the proportion of the sample's total reads contained in each PCR replicate.

## Value

Invisibly returns a data frame containing unfiltered DNA sequences with their read counts, proportions, and p-values in each PCR replicate. While the primary output of this function is the written CSV file of filtered sequences described in the details section, the invisibly returned data frame of unfiltered sequences can be helpful when calibrating or troubleshooting filtering parameters. To aid in troubleshooting filtering parameters, the data frame is invisibly returned even if the error "Filtering removed all sequences" is received. Field definitions for the invisibly returned data frame of unfiltered sequences are:

• Sample: The sample name.

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- PCR\_replicate: The PCR replicate identifier.
- Sequence: The DNA sequence.
- Read\_count.sequence: The sequence's read count within the PCR replicate.
- Read\_count.PCR\_replicate: The number of reads in the PCR replicate.
- Proportion\_of\_PCR\_replicate.observed: The proportion of reads in the PCR replicate comprised by the sequence.
- Proportion\_of\_PCR\_replicate.null (Field only present if binomial\_test.enabled = TRUE): The null hypothesis for a one-sided binomial test (inherited from the minimum\_proportion.sequence argument). See the p.value field below.
- p.value (Field only present if binomial\_test.enabled = TRUE): The p-value from a onesided binomial test of whether the proportion of reads in the PCR replicate comprised by the sequence exceeds the null hypothesis (*i.e.*, binomial\_test with alternative = "greater").
- p.value.adjusted (Field only present if binomial\_test.enabled = TRUE): The p-value from the one-sided binomial test adjusted for multiple comparisons within each PCR replicate for each sample. See the p.value\_adjustment\_method field below.
- p.value\_adjustment\_method (Field only present if binomial\_test.enabled = TRUE): The p-value adjustment method (inherited from the binomial\_test.p.adjust.method argument).

#### References

A manuscript describing these methods is in preparation.

## See Also

binomial\_test for performing vectorized one-sided binomial tests.

truncate\_and\_merge\_pairs for truncating and merging read pairs prior to sequence filtering.

local\_taxa\_tool for performing geographically-conscious taxonomic assignment of filtered sequences.

## Examples

```
print("Insert example here.")
```

format\_reference\_database

Format Reference Databases

## Description

Formats reference databases from MIDORI or UNITE for use with the local\_taxa\_tool function.

# Usage

```
format_reference_database(
   path_to_input_database,
   path_to_output_database,
   input_database_source = "MIDORI",
   path_to_taxonomy_edits = NA,
   path_to_sequence_edits = NA,
   path_to_taxa_subset_list = NA,
   makeblastdb_command = "makeblastdb",
   ....
```

)

#### Arguments

path\_to\_input\_database

String specifying path to input reference database in FASTA format.

path\_to\_output\_database

String specifying path to output BLAST database in FASTA format. File path cannot contain spaces.

input\_database\_source

String specifying input reference database source ('MIDORI' or 'UNITE'). The default is 'MIDORI'.

path\_to\_taxonomy\_edits

String specifying path to taxonomy edits file in CSV format. The file must contain the following fields: 'Old\_Taxonomy', 'New\_Taxonomy', 'Notes'. Old taxonomies are replaced with new taxonomies in the order the records appear in the file. The taxonomic levels in the 'Old\_Taxonomy' and 'New\_Taxonomy' fields should be delimited by a semi-colon. If no taxonomy edits are desired, then set this variable to NA (the default).

path\_to\_sequence\_edits

String specifying path to sequence edits file in CSV format. The file must contain the following fields: 'Action', 'Common\_Name', 'Domain', 'Phylum', 'Class', 'Order', 'Family', 'Genus', 'Species', 'Sequence', 'Notes'. The values in the 'Action' field must be either 'Add' or 'Remove', which will add or remove the respective sequence from the reference database. Values in the 'Common\_Name' field are optional. Values should be supplied to all taxonomy fields. If using a reference database from MIDORI, then use NCBI superkingdom names (*e.g.*, 'Eukaryota') in the 'Domain' field. If using a reference database from UNITE, then use kingdom names (*e.g.*, 'Fungi') in the 'Domain' field. The 'Species' field should contain species binomials. Sequence edits are performed after taxonomy edits, if applied. If no sequence edits are desired, then set this variable to NA (the default).

path\_to\_taxa\_subset\_list

String specifying path to list of species (in CSV format) to subset the reference database to. This option is helpful if the user wants the reference database to include only the sequences of local species. The file should contain the following fields: 'Common\_Name', 'Domain', 'Phylum', 'Class', 'Order', 'Family', 'Genus', 'Species'. There should be no NAs or blanks in the taxonomy fields.

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The species field should contain the binomial name without subspecies or other information below the species level. There should be no duplicate species (*i.e.*, multiple records with the same species binomial and taxonomy) in the species list. Subsetting the reference database to the sequences of certain species is performed after taxonomy and sequence edits are applied to the reference database, and species must match at all taxonomic levels in order to be retained in the reference database. If subsetting the reference database to the sequences of certain species of certain species is not desired, set this variable to NA (the default).

makeblastdb\_command

String specifying path to the makeblastdb program, which is a part of BLAST. The default ('makeblastdb') should work for standard BLAST installations. The user can provide a path to the makeblastdb program for non-standard BLAST installations.

... Accepts former argument names for backwards compatibility.

#### Value

No return value. Writes formatted BLAST database files.

## See Also

local\_taxa\_tool for performing geographically-conscious taxonomic assignment.

adjust\_taxonomies for adjusting a taxonomy system.

## Examples

# Create a temporary file path for the output reference database FASTA file. path\_to\_output\_file<-tempfile(fileext=".fasta")</pre>

get\_consensus\_taxonomy

Get Consensus Taxonomy from Taxonomic Strings

## Description

Gets the consensus taxonomy from a vector of taxonomic strings.

## Usage

get\_consensus\_taxonomy(taxonomies, full\_names = TRUE, delimiter = ";")

## Arguments

taxonomies	A character vector of taxonomic strings.
full_names	Logical. If TRUE (the default), then the full consensus taxonomy is returned. If FALSE, then only the lowest taxonomic level of the consensus taxonomy is returned.
delimiter	A character string of the delimiter between taxonomic levels in the input tax- onomies. The default is ";".

# Value

A character string containing the taxonomy agreed upon by all input taxonomies. If the input taxonomies are not the same at any taxonomic level, then NA is returned.

## See Also

get\_taxonomic\_level for extracting a taxonomic level from taxonomic strings.

expand\_taxonomies for extracting each taxonomic level from a vector of taxonomic strings.

## Examples

```
get_consensus_taxonomy(taxonomies=
    c("Eukaryota;Chordata;Amphibia;Caudata;Ambystomatidae;Ambystoma;Ambystoma_mavortium",
    "Eukaryota;Chordata;Amphibia;Anura;Bufonidae;Anaxyrus;Anaxyrus_boreas",
    "Eukaryota;Chordata;Amphibia;Anura;Ranidae;Rana;Rana_luteiventris"),
    full_names=TRUE,
    delimiter=";")
```

get\_taxonomic\_level Get Specified Taxonomic Level from Taxonomic Strings

# Description

Gets the specified taxonomic level from a vector of taxonomic strings.

## Usage

```
get_taxonomic_level(taxonomies, level, full_names = TRUE, delimiter = ";")
```

## Arguments

taxonomies	A character vector of taxonomic strings.
level	A numeric value representing the taxonomic level to be extracted. A value of 1 retrieves the highest taxonomic level ( <i>e.g.</i> , domain) from the input taxonomies, with each sequentially higher value retrieving sequentially lower taxonomic levels. $\emptyset$ is a special value which retrieves the lowest taxonomic level available in the input taxonomies.
full_names	Logical. If TRUE (the default), then full taxonomies are returned down to the requested taxonomic level. If FALSE, then only the requested taxonomic level is returned.
delimiter	A character string of the delimiter between taxonomic levels in the input tax- onomies. The default is ";".

# Value

A character vector containing the requested taxonomic level for each element of the input taxonomies.

# See Also

expand\_taxonomies for extracting each taxonomic level from a vector of taxonomic strings.

get\_consensus\_taxonomy for generating a consensus taxonomy from taxonomic strings.

# Examples

```
get_taxonomic_level(taxonomies=
    c("Eukaryota;Chordata;Amphibia;Caudata;Ambystomatidae;Ambystoma;Ambystoma_mavortium",
        "Eukaryota;Chordata;Amphibia;Anura;Bufonidae;Anaxyrus;Anaxyrus_boreas",
        "Eukaryota;Chordata;Amphibia;Anura;Ranidae;Rana;Rana_luteiventris"),
        level=5,
        full_names=TRUE,
        delimiter=";")
```

get\_taxonomies.IUCN Get Taxonomies from IUCN Red List Files

# Description

Formats taxonomies from IUCN Red List taxonomy.csv and common\_names.csv files for use with the local\_taxa\_tool function.

# Usage

```
get_taxonomies.IUCN(
   path_to_taxonomies,
   path_to_common_names,
   path_to_output_file,
   domain = "Eukaryota",
   path_to_taxonomy_edits = NA,
   ...
)
```

# Arguments

path_to_taxonomies		
	String specifying path to input IUCN Red List taxonomy.csv file.	
path_to_common	_names	
	String specifying path to input IUCN Red List common_names.csv file.	
path_to_output	_file	
	String specifying path to output species list (in CSV format) with formatted taxonomies.	
domain	String specifying the domain name to use for all species. The IUCN Red List files do not include domain information, so a domain name must be provided. If using a reference database from UNITE, provide a kingdom name here ( <i>e.g.</i> , 'Fungi'). The default is 'Eukaryota'.	
path_to_taxonomy_edits		
	String specifying path to taxonomy edits file in CSV format. The file must contain the following fields: 'Old_Taxonomy', 'New_Taxonomy', 'Notes'. Old taxonomies are replaced with new taxonomies in the order the records appear in the file. The taxonomic levels in the 'Old_Taxonomy' and 'New_Taxonomy' fields should be delimited by a semi-colon. If no taxonomy edits are desired, then set this variable to NA (the default).	
	Accepts former argument names for backwards compatibility.	

# Value

No return value. Writes an output CSV file with formatted taxonomies.

# See Also

get\_taxonomies.species\_binomials for remotely fetching NCBI taxonomies from species binomials.

adjust\_taxonomies for adjusting a taxonomy system.

# Examples

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get\_taxonomies.species\_binomials Get NCBI Taxonomies from Species Binomials

## Description

Remotely fetches taxonomies from the NCBI taxonomy database for a list of species binomials.

# Usage

```
get_taxonomies.species_binomials(
   path_to_species_binomials,
   path_to_output_file,
   path_to_taxonomy_edits = NA,
   print_queries = TRUE,
   ...
)
```

## Arguments

```
path_to_species_binomials
```

String specifying path to input species list with common and scientific names. The file should be in CSV format and contain the following fields: 'Common\_Name', 'Scientific\_Name'. Values in the 'Common\_Name' field are optional. Values in the 'Scientific\_Name' field are required.

```
path_to_output_file
```

String specifying path to output species list with added NCBI taxonomies. The output file will be in CSV format.

path\_to\_taxonomy\_edits

String specifying path to taxonomy edits file in CSV format. The file must contain the following fields: 'Old\_Taxonomy', 'New\_Taxonomy', 'Notes'. Old

	taxonomies are replaced with new taxonomies in the order the records appear in the file. The taxonomic levels in the 'Old_Taxonomy' and 'New_Taxonomy' fields should be delimited by a semi-colon. If no taxonomy edits are desired, then set this variable to NA (the default).
print_queries	Logical. Whether taxa queries should be printed. The default is TRUE.
	Accepts former argument names for backwards compatibility.

# Value

No return value. Writes an output CSV file with added taxonomies. Species which could not be found in the NCBI taxonomy database appear in the top records of the output file.

# See Also

get\_taxonomies.IUCN for formatting taxonomies from the IUCN Red List.

adjust\_taxonomies for adjusting a taxonomy system.

#### Examples

```
# Get path to example input species binomials CSV file.
path_to_species_binomials<-system.file("extdata",</pre>
                                         "example_species_binomials.csv",
                                         package="LocaTT",
                                         mustWork=TRUE)
```

```
# Create a temporary file path for the output CSV file.
path_to_output_file<-tempfile(fileext=".csv")</pre>
```

```
# Fetch taxonomies from species binomials.
get_taxonomies.species_binomials(path_to_species_binomials=path_to_species_binomials,
                                 path_to_output_file=path_to_output_file,
                                 print_queries=FALSE)
```

isolate_amplicon	Trim DNA Sequences to an Amplicon Region Using Forward and Re-
	verse Primer Sequences

## Description

Trims DNA sequences to an amplicon region using forward and reverse primer sequences. Ambiguous nucleotides in forward and reverse primers are supported.

## Usage

```
isolate_amplicon(sequences, forward_primer, reverse_primer)
```

#### Arguments

sequences	A character vector of DNA sequences to trim to the amplicon region.
forward_primer	A string specifying the forward primer sequence. Can contain ambiguous nucleotides.
reverse_primer	A string specifying the reverse primer sequence. Can contain ambiguous nucle-todies.

## Details

For each DNA sequence, nucleotides matching and preceding the forward primer are removed, and nucleotides matching and following the reverse complement of the reverse primer are removed. The reverse complement of the reverse primer is internally derived from the reverse primer using the reverse\_complement function. Ambiguous nucleotides in primers (*i.e.*, the forward and reverse primer arguments) are supported through the internal use of the substitute\_wildcards function on the forward primer and the reverse complement of the reverse primer, and primer regions in DNA sequences are located using regular expressions. Trimming will fail for DNA sequences which contain ambiguous nucleotides in their primer regions (*e.g.*, Ns), resulting in NAs for those sequences.

# Value

A character vector of DNA sequences trimmed to the amplicon region. NAs are returned for DNA sequences which could not be trimmed, which occurs when either primer region is missing from the DNA sequence or when the forward primer region occurs after a region matching the reverse complement of the reverse primer.

## Examples

local\_taxa\_tool Perform Geographically-Conscious Taxonomic Assignment

## Description

Performs taxonomic assignment of DNA metabarcoding sequences while considering geographic location.

## Usage

```
local_taxa_tool(
   path_to_query_sequences,
   path_to_BLAST_database,
   path_to_output_file,
   path_to_local_taxa_list = NA,
   include_missing = FALSE,
   blast_e_value = 1e-05,
   blast_max_target_seqs = 2000,
   blast_task = "megablast",
   full_names = FALSE,
   underscores = FALSE,
   separator = ", ",
   blastn_command = "blastn",
   ...
)
```

## Arguments

path\_to\_query\_sequences

String specifying path to FASTA file containing sequences to classify. File path cannot contain spaces.

path\_to\_BLAST\_database

String specifying path to BLAST reference database in FASTA format. File path cannot contain spaces.

path\_to\_output\_file

String specifying path to output file of classified sequences in CSV format.

#### path\_to\_local\_taxa\_list

String specifying path to list of local species in CSV format. The file should contain the following fields: 'Common\_Name', 'Domain', 'Phylum', 'Class', 'Order', 'Family', 'Genus', 'Species'. There should be no 'NA's or blanks in the taxonomy fields. The species field should contain the binomial name without subspecies or other information below the species level. There should be no duplicate species (*i.e.*, multiple records with the same species binomial and taxonomy) in the local species list. If local taxa suggestions are not desired, set this variable to NA (the default).

#### include\_missing

Logical. If TRUE, then additional fields are included in the output CSV file in which local sister taxonomic groups without reference sequences are added to the local taxa suggestions. If FALSE (the default), then this is not performed.

blast\_e\_value Numeric. Maximum E-value of returned BLAST hits (lower E-values are associated with more 'significant' matches). The default is 1e-05.

## blast\_max\_target\_seqs

Numeric. Maximum number of BLAST target sequences returned per query sequence. Enough target sequences should be returned to ensure that all minimum E-value matches are returned for each query sequence. A warning will be produced if this value is not sufficient. The default is 2000.

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blast_task	String specifying BLAST task specification. Use 'megablast' (the default) to find very similar sequences ( <i>e.g.</i> , intraspecies or closely related species). Use 'blastn-short' for sequences shorter than 50 bases. See the blastn program help documentation for additional options and details.
full_names	Logical. If TRUE, then full taxonomies are returned in the output CSV file. If FALSE (the default), then only the lowest taxonomic levels ( <i>e.g.</i> , species binomials instead of the full species taxonomies) are returned in the output CSV file.
underscores	Logical. If TRUE, then taxa names in the output CSV file use underscores instead of spaces. If FALSE (the default), then taxa names in the output CSV file use spaces.
separator	String specifying the separator to use between taxa names in the output CSV file. The default is ', '.
blastn_command	String specifying path to the blastn program. The default ('blastn') should work for standard BLAST installations. The user can provide a path to the blastn program for non-standard BLAST installations.
	Accepts former argument names for backwards compatibility.

## Details

Sequences are BLASTed against a global reference database, and the tool suggests locally occurring species which are most closely related (by taxonomy) to any of the best-matching BLAST hits (by bit score). Optionally, local sister taxonomic groups without reference sequences can be added to the local taxa suggestions by setting the include\_missing argument to TRUE. If a local taxa list is not provided, then local taxa suggestions will be disabled, but all best-matching BLAST hits will still be returned. Alternatively, a reference database containing just the sequences of local species can be used, and local taxa suggestions can be disabled to return all best BLAST matches of local species. The reference database should be formatted with the format\_reference\_database function, and the local taxa lists can be prepared using the get\_taxonomies.species\_binomials and get\_taxonomies.IUCN functions. Output field definitions are:

- Sequence\_name: The query sequence name.
- Sequence: The query sequence.
- Best\_match\_references: Species binomials of all best-matching BLAST hits (by bit score) from the reference database.
- Best\_match\_E\_value: The E-value associated with the best-matching BLAST hits.
- Best\_match\_bit\_score: The bit score associated with the best-matching BLAST hits.
- Best\_match\_query\_cover.mean: The mean query cover of all best-matching BLAST hits.
- Best\_match\_query\_cover.SD: The standard deviation of query cover of all best-matching BLAST hits.
- Best\_match\_PID.mean: The mean percent identity of all best-matching BLAST hits.
- Best\_match\_PID.SD: The standard deviation of percent identity of all best-matching BLAST hits.

- Local\_taxa (Field only present if a path to a local taxa list is provided): The finest taxonomic unit(s) which include both any species of the best-matching BLAST hits and any local species. If the species of any of the best-matching BLAST hits are local, then the finest taxonomic unit(s) are at the species level.
- Local\_species (Field only present if a path to a local taxa list is provided): Species binomials of all local species which belong to the taxonomic unit(s) in the Local\_taxa field.
- Local\_taxa.include\_missing (Field only present if both a path to a local taxa list is provided and the include\_missing argument is set to TRUE): Local sister taxonomic groups without reference sequences are added to the local taxa suggestions from the Local\_taxa field.
- Local\_species.include\_missing (Field only present if both a path to a local taxa list is provided and include\_missing argument is set to TRUE): Species binomials of all local species which belong to the taxonomic unit(s) in the Local\_taxa.include\_missing field.

## Value

No return value. Writes an output CSV file with fields defined in the details section.

#### References

A manuscript describing this taxonomic assignment method is in preparation.

# See Also

format\_reference\_database for formatting reference databases.

get\_taxonomies.species\_binomials and get\_taxonomies.IUCN for creating local taxa lists.

adjust\_taxonomies for adjusting a taxonomy system.

#### Examples

# Create a temporary file path for the output CSV file.

#### merge\_pairs

merge\_pairs

```
Merge Forward and Reverse DNA Sequence Reads
```

## Description

Merges forward and reverse DNA sequence reads.

## Usage

```
merge_pairs(forward_reads, reverse_reads, minimum_overlap = 10)
```

#### Arguments

forward\_reads A character vector of forward DNA sequence reads. reverse\_reads A character vector of reverse DNA sequence reads. minimum\_overlap Numeric. The minimum length of an overlap that must be found between the end of the forward read and the start of the reverse complement of the reverse read in order for a read pair to be merged. The default is 10.

## Details

For each pair of forward and reverse DNA sequence reads, the reverse complement of the reverse read is internally derived using the reverse\_complement function, and the read pair is merged into a single sequence if an overlap of at least the minimum length is found between the end of the forward read and the start of the reverse complement of the reverse read. If an overlap of the minimum length is not found, then an NA is returned for the merged read pair.

# Value

A character vector of merged DNA sequence read pairs. NAs are returned for read pairs which could not be merged, which occurs when an overlap of at least the minimum length is not found between the end of the forward read and the start of the reverse complement of the reverse read.

## See Also

truncate\_and\_merge\_pairs for truncating and merging forward and reverse DNA sequence reads.

## Examples

read.fasta

Read FASTA Files

## Description

Reads FASTA files. Supports the reading of FASTA files with sequences wrapping multiple lines.

## Usage

```
read.fasta(file)
```

# Arguments

file A string specifying the path to a FASTA file to read.

# Value

A data frame with fields for sequence names and sequences.

## See Also

write.fasta for writing FASTA files.

read.fastq for reading FASTQ files.

write.fastq for writing FASTQ files.

# Examples

# Read the example FASTA file.
read.fasta(file=path\_to\_fasta\_file)

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read.fastq

## Description

Reads FASTQ files. Does not support the reading of FASTQ files with sequences or quality scores wrapping multiple lines.

#### Usage

```
read.fastq(file)
```

# Arguments

file A string specifying the path to a FASTQ file to read.

## Value

A data frame with fields for sequence names, sequences, comments, and quality scores.

# See Also

write.fastq for writing FASTQ files.

read.fasta for reading FASTA files.

write.fasta for writing FASTA files.

# Examples

# Read the example FASTQ file.
read.fastq(file=path\_to\_fastq\_file)

reverse\_complement Get the Reverse Complement of a DNA Sequence

### Description

Gets the reverse complement of a DNA sequence. Ambiguous nucleotides are supported.

## Usage

reverse\_complement(sequence)

## Arguments

sequence A string specifying the DNA sequence. Can contain ambiguous nucleotides.

# Value

A string of the reverse complement of the DNA sequence.

#### Examples

reverse\_complement(sequence="TTCTCCASCCGCGGATHTTG")

substitute\_wildcards Substitute Wildcard Characters in a DNA Sequence

## Description

Substitutes wildcard characters in a DNA sequence with their associated nucleotides surrounded by square brackets. The output is useful for matching in regular expressions.

#### Usage

```
substitute_wildcards(sequence)
```

#### Arguments

sequence A string specifying the DNA sequence containing wildcard characters.

## Value

A string of the DNA sequence in which wildcard characters are replaced with their associated nucleotides surrounded by square brackets.

## Examples

```
substitute_wildcards(sequence="CAADATCCGCGGSTGGAGAA")
```

summarize\_quality\_scores

Summarize Quality Scores

## Description

For each base pair position, summarizes Phred quality scores and the cumulative probability that all bases were called correctly.

## Usage

```
summarize_quality_scores(
  forward_files,
  reverse_files,
  n.total = 10000,
  n.each = ceiling(n.total/length(forward_files)),
  seed = NULL,
  FUN = mean,
  ...
)
```

## Arguments

forward_files	A character vector of file paths to FASTQ files containing forward DNA sequence reads.
reverse_files	A character vector of file paths to FASTQ files containing reverse DNA sequence reads.
n.total	Numeric. The number of read pairs to randomly sample from the input FASTQ files. Ignored if n. each is specified. The default is 10000.
n.each	Numeric. The number of read pairs to randomly sample from each pair of input FASTQ files. The default is ceiling(n.total/length(forward_files)).
seed	Numeric. The seed for randomly sampling read pairs. If NULL (the default), then a random seed is used.
FUN	A function to compute summary statistics of the quality scores. The default is mean.
	Additional arguments passed to FUN.

## Details

For each combination of base pair position and read direction, calculates summary statistics of Phred quality scores and the cumulative probability that all bases were called correctly. The cumulative probability is calculated from the first base pair up to the current position. Quality scores are assumed to be encoded in Sanger format. Read pairs are selected by randomly sampling up to n.each read pairs from each pair of input FASTQ files. By default, n.each is derived from n.total, and n.total will be ignored if n.each is provided. By default, mean is used to compute the summary

statistics, but the user may provide another summary function instead (*e.g.*, median). Functions which return multiple summary statistics are also supported (*e.g.*, summary and quantile). Arguments in . . . are passed to the summary function.

# Value

Returns a data frame containing summary statistics of quality scores at each base pair position. The returned data frame contains the following fields:

- Direction: The read direction (i.e., "Forward" or "Reverse").
- Position: The base pair position.
- Score: The summary statistic(s) of Phred quality scores. If FUN returns multiple summary statistics, then a matrix of the summary statistics will be stored in this field, which can be accessed with \$Score.
- Probability: The summary statistic(s) of the cumulative probability that all bases were called correctly. If FUN returns multiple summary statistics, then a matrix of the summary statistics will be stored in this field, which can be accessed with \$Probability.

# See Also

decode\_quality\_scores for decoding quality scores.

#### Examples

```
print("Insert example here.")
```

trim\_sequences Trim Target Nucleotide Sequence from DNA Sequences

## Description

Trims a target nucleotide sequence from the front or back of DNA sequences. Ambiguous nucleotides in the target nucleotide sequence are supported.

## Usage

```
trim_sequences(
   sequences,
   target,
   anchor = "start",
   fixed = TRUE,
   required = TRUE,
   quality_scores
)
```

#### trim\_sequences

#### Arguments

sequences	A character vector of DNA sequences to trim.
target	A string specifying the target nucleotide sequence.
anchor	A string specifying whether the target nucleotide sequence should be trimmed from the start or end of the DNA sequences. Allowable values are "start" (the default) and "end".
fixed	A logical value specifying whether the position of the target nucleotide sequence should be fixed at the ends of the DNA sequences. If TRUE (the default), then the position of the target nucleotide sequence is fixed at either the start or end of the DNA sequences, depending on the value of the anchor argument. If FALSE, then the target nucleotide sequence is searched for anywhere in the DNA sequences.
required	A logical value specifying whether trimming is required. If TRUE (the default), then sequences which could not be trimmed are returned as NAs. If FALSE, then untrimmed sequences are returned along with DNA sequences for which trimming was successful.
quality_scores	An optional character vector of DNA sequence quality scores. If supplied, these will be trimmed to their corresponding trimmed DNA sequences.

## Details

For each DNA sequence, the target nucleotide sequence is searched for at either the front or back of the DNA sequence, depending on the value of the anchor argument. If the target nucleotide sequence is found, then it is removed from the DNA sequence. If the required argument is set to TRUE, then DNA sequences in which the target nucleotide sequence was not found will be returned as NAs. If the required argument is set to FALSE, then untrimmed DNA sequences will be returned along with DNA sequences for which trimming was successful. Ambiguous nucleotides in the target nucleotide sequence are supported through the internal use of the substitute\_wildcards function on the target nucleotide sequence in the DNA sequences. If the fixed argument is set to FALSE, then any number of characters are allowed between the start or end of the DNA sequences and the target nucleotide sequence. Trimming will fail for DNA sequences which contain ambiguous nucleotides (*e.g.*, Ns) in their target nucleotide sequence region, resulting in NAs for those sequences if the required argument is set to TRUE.

### Value

If quality scores are not provided, then a character vector of trimmed DNA sequences is returned. If quality scores are provided, then a list containing two elements is returned. The first element is a character vector of trimmed DNA sequences, and the second element is a character vector of quality scores which have been trimmed to their corresponding trimmed DNA sequences.

# Examples

```
trim_sequences(sequences=c("ATATAGCGCG", "TGCATATACG", "ATCTATCACCGC"),
    target="ATMTA",
    anchor="start",
    fixed=TRUE,
```

```
required=TRUE,
quality_scores=c("989!.C;F@\"","A((#-#;,2F","HD8I/+67=1>?"))
```

truncate\_and\_merge\_pairs

Truncate and Merge Forward and Reverse DNA Sequence Reads

# Description

Removes DNA read pairs containing ambiguous nucleotides, truncates reads by length and quality score, and merges forward and reverse reads.

# Usage

```
truncate_and_merge_pairs(
  forward_files,
  reverse_files,
  output_files,
  truncation_length = NA,
  threshold.quality_score = 3,
  threshold.probability = 0.5,
  minimum_overlap = 10,
  cores = 1,
  progress = FALSE
)
```

## Arguments

forward_files	A character vector of file paths to FASTQ files containing forward DNA sequence reads.
reverse_files	A character vector of file paths to FASTQ files containing reverse DNA sequence reads.
output_files	A character vector of file paths to output FASTA files.
truncation_leng	th
	Numeric. The length to truncate DNA sequences to (passed to the length ar- gument of truncate_sequences.length). If NA (the default), then DNA se- quences are not truncated by length. If a single value is supplied, then both forward and reverse reads are truncated to the same length. If two values are supplied in a numeric vector, then the first value is used to truncate the forward reads, and the second value is used to truncate the reverse reads. NA can also be supplied as either the first or second element of the numeric vector to prevent length truncation of the respective read direction while allowing the other read direction to be length truncated.
threshold.quali	ty_score
	Numeric. The Phred quality score threshold used for truncation (passed to the threshold argument of truncate_sequences.quality_score). The default

is 3 (*i.e.*, each base in a truncated sequence has a greater than 50% probability of having been called correctly). If NA, then DNA sequences are not truncated by quality score threshold. If a single value is supplied, then both forward and reverse reads are truncated by the same quality score threshold. If two values are supplied in a numeric vector, then the first value is used to truncate the forward reads, and the second value is used to truncate the reverse reads. NA can also be supplied as either the first or second element of the numeric vector to prevent quality-score-threshold truncation of the respective read direction while allowing the other read direction to be quality-score-threshold truncated.

#### threshold.probability

Numeric. The probability threshold used for truncation (passed to the threshold argument of truncate\_sequences.probability). The default is 0.5 (*i.e.*, each truncated sequence has a greater than 50% probability that all bases were called correctly). If NA, then DNA sequences are not truncated by probability threshold. If a single value is supplied, then both forward and reverse reads are truncated by the same probability threshold. If two values are supplied in a numeric vector, then the first value is used to truncate the forward reads, and the second value is used to truncate the reverse reads. NA can also be supplied as either the first or second element of the numeric vector to prevent probability-threshold truncation of the respective read direction while allowing the other read direction to be probability-threshold truncated.

<pre>minimum_overl</pre>	ap
	Numeric. The minimum length of an overlap that must be found between the end of the forward read and the start of the reverse complement of the reverse read in order for a read pair to be merged (passed to merge_pairs). The default is 10.
cores	Numeric. If 1 (the default), then FASTQ file pairs are processed sequentially on a single core. If greater than 1, then FASTQ file pairs are processed in parallel across the specified number of cores. Parallel processing is not supported on Windows.
progress	Logical. If TRUE, then a progress indicator is printed to the console. Ignored if cores > 1. If FALSE (the default), then no progress indicator is displayed.

#### Details

For each pair of input FASTQ files, removes DNA read pairs containing ambiguous nucleotides, truncates reads by length, quality score threshold, and probability threshold (in that order), and then merges forward and reverse reads. Merged reads are summarized by frequency of occurrence and written to a FASTA file. See contains\_wildcards, truncate\_sequences.length, truncate\_sequences.quality\_score, truncate\_sequences.probability, and merge\_pairs for methods. Quality scores are assumed to be encoded in Sanger format. Forward and reverse reads can be truncated by different thresholds (see truncation\_length, threshold.quality\_score, and threshold.probability arguments).

Multicore parallel processing is supported on Mac and Linux operating systems (not available on Windows). When cores > 1 (parallel processing enabled), warnings and errors are printed to the console in addition to being invisibly returned as a list (see the return value section), and errors produced while processing a pair of FASTQ files will not interrupt the processing of other FASTQ

file pairs. When cores = 1, FASTQ file pairs are processed sequentially on a single core, and errors will prevent the processing of subsequent FASTQ file pairs (but warnings will not).

#### Value

If cores = 1, then no return value. Writes a FASTA file for each pair of input FASTQ files with DNA sequence counts stored in the header lines. If cores > 1, then also invisibly returns a list where each element contains warning or error messages associated with processing each pair of input FASTQ files. A NULL value in the returned list means that no warnings or errors were generated from processing the respective pair of FASTQ files.

## References

A manuscript describing these methods is in preparation.

## See Also

contains\_wildcards for detecting ambiguous nucleotides in DNA sequences.

truncate\_sequences.length for truncating DNA sequences to a specified length.

truncate\_sequences.quality\_score for truncating DNA sequences by Phred quality score.

truncate\_sequences.probability for truncating DNA sequences by cumulative probability that all bases were called correctly.

merge\_pairs for merging forward and reverse DNA sequence reads.

filter\_sequences for filtering merged read pairs by PCR replicate.

# Examples

```
print("Insert example here.")
```

truncate\_sequences.length *Truncate DNA Sequences to Specified Length* 

## Description

Truncates DNA sequences to a specified length.

# Usage

truncate\_sequences.length(sequences, length, quality\_scores)

#### Arguments

sequences	A character vector of DNA sequences to truncate.
length	Numeric. The length to truncate DNA sequences to.
quality_scores	An optional character vector of DNA sequence quality scores. If supplied, these will be truncated to their corresponding truncated DNA sequences.

# Value

If quality scores are not provided, then a character vector of truncated DNA sequences is returned. If quality scores are provided, then a list containing two elements is returned. The first element is a character vector of truncated DNA sequences, and the second element is a character vector of quality scores which have been truncated to their corresponding truncated DNA sequences.

# See Also

truncate\_sequences.quality\_score for truncating DNA sequences by Phred quality score.

truncate\_sequences.probability for truncating DNA sequences by cumulative probability that all bases were called correctly.

truncate\_and\_merge\_pairs for truncating and merging forward and reverse DNA sequence reads.

## Examples

truncate\_sequences.probability

*Truncate DNA Sequences at Specified Probability that All Bases were Called Correctly* 

## Description

Calculates the cumulative probability that all bases were called correctly along each DNA sequence and truncates the DNA sequence immediately prior to the first occurrence of a probability being equal to or less than a specified value.

## Usage

```
truncate_sequences.probability(sequences, quality_scores, threshold = 0.5)
```

## Arguments

sequences	A character vector of DNA sequences to truncate.	
quality_scores	A character vector of DNA sequence quality scores encoded in Sanger format.	
threshold	Numeric. The probability threshold used for truncation. The default is $0.5$ ( <i>i.e.</i> , each truncated sequence has a greater than 50% probability that all bases were called correctly).	

# Value

A list containing two elements. The first element is a character vector of truncated DNA sequences, and the second element is a character vector of quality scores which have been truncated to their corresponding truncated DNA sequences.

#### See Also

truncate\_sequences.length for truncating DNA sequences to a specified length.

truncate\_sequences.quality\_score for truncating DNA sequences by Phred quality score.

truncate\_and\_merge\_pairs for truncating and merging forward and reverse DNA sequence reads.

## Examples

truncate\_sequences.quality\_score
 Truncate DNA Sequences at Specified Quality Score

## Description

Truncates DNA sequences immediately prior to the first occurrence of a Phred quality score being equal to or less than a specified value.

## Usage

```
truncate_sequences.quality_score(sequences, quality_scores, threshold = 3)
```

## Arguments

sequences	A character vector of DNA sequences to truncate.
quality_scores	A character vector of DNA sequence quality scores encoded in Sanger format.
threshold	Numeric. The Phred quality score threshold used for truncation. The default is
	3 ( <i>i.e.</i> , each base in a truncated sequence has a greater than 50% probability of
	having been called correctly).

# write.fasta

# Value

A list containing two elements. The first element is a character vector of truncated DNA sequences, and the second element is a character vector of quality scores which have been truncated to their corresponding truncated DNA sequences.

#### See Also

truncate\_sequences.length for truncating DNA sequences to a specified length.

truncate\_sequences.probability for truncating DNA sequences by cumulative probability that all bases were called correctly.

truncate\_and\_merge\_pairs for truncating and merging forward and reverse DNA sequence reads.

## Examples

write.fasta	Write FASTA Files	

## Description

Writes FASTA files.

# Usage

```
write.fasta(names, sequences, file)
```

## Arguments

names	A character vector of sequence names.
sequences	A character vector of sequences.
file	A string specifying the path to a FASTA file to write.

## Value

No return value. Writes a FASTA file.

# See Also

read.fasta for reading FASTA files.

write.fastq for writing FASTQ files.

read.fastq for reading FASTQ files.

# Examples

write.fastq

# Write FASTQ Files

# Description

Writes FASTQ files.

#### Usage

```
write.fastq(names, sequences, quality_scores, file, comments)
```

#### Arguments

names	A character vector of sequence names.
sequences	A character vector of sequences.
quality_scores	A character vector of quality scores.
file	A string specifying the path to a FASTQ file to write.
comments	An optional character vector of sequence comments.

#### Value

No return value. Writes a FASTQ file.

# See Also

read.fastq for reading FASTQ files.

write.fasta for writing FASTA files.

read.fasta for reading FASTA files.

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# write.fastq

# Examples

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