

Package ‘MetaScope’

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Type Package

Title Tools and functions for preprocessing 16S and metagenomic sequencing microbiome data

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Description This package contains tools and methods for preprocessing microbiome data. Functionality includes library generation, demultiplexing, alignment, and microbe identification. It is in part an R translation of the PathoScope 2.0 pipeline.

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URL <https://github.com/wejlab/metascope>
<https://wejlab.github.io/metascope-docs/>

BugReports <https://github.com/wejlab/MetaScope/issues>

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MetaScope-package	<i>MetaScope: Tools and functions for preprocessing 16S and metagenomic sequencing microbiome data</i>
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Description

This package contains tools and methods for preprocessing microbiome data. Functionality includes library generation, demultiplexing, alignment, and microbe identification. It is in part an R translation of the PathoScope 2.0 pipeline.

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See Also

Useful links:

- <https://github.com/wejlab/metascope><https://wejlab.github.io/metascope-docs/>
- Report bugs at <https://github.com/wejlab/MetaScope/issues>

add_in_taxa	<i>Adds in taxa if silva database Returns MetaScope Table with silva taxa in separate columns</i>
-------------	---

Description

Adds in taxa if silva database Returns MetaScope Table with silva taxa in separate columns

Usage

```
add_in_taxa(metascope_id_in, caching, path_to_write)
```

Arguments

metascope_id_in	MetaScope ID file with silva taxa
caching	Boolean for if all_silva_headers.rds is already downloaded
path_to_write	Path to save all_silva_headers.rds

Value

Data.frame of taxonomy information

add_in_taxa_ncbi	<i>Adds in taxa if input used NCBI database</i>
------------------	---

Description

Returns MetaScope Table with NCBI taxa in separate columns

Usage

```
add_in_taxa_ncbi(metascope_id_in, accession, BPPARAM)
```

Arguments

metascope_id_in	MetaScope ID file with NCBI taxa qnames
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation.

Value

data.frame or tibble of taxonomy information

align_details	<i>A universal parameter settings object for Rsubread alignment</i>
---------------	---

Description

This object is a named vector of multiple options that can be chosen for functions that involve alignment with Rsubread, namely `align_target()` and `filter_host()`. Both functions take an object for the parameter settings, which are provided by `align_details` by default, or may be given by a user-created object containing the same information.

Usage

```
data(align_details)
```

Format

list

Details

The default options included in `align_details` are `type = "dna"`, `maxMismatches = 3`, `nsubreads = 10`, `phredOffset = 33`, `unique = FALSE`, and `nBestLocations = 16`. Full descriptions of these parameters can be read by accessing `?Rsubread::align`.

Examples

```
data("align_details")
```

align_target

*Align microbiome reads to a set of reference libraries***Description**

This is the main MetaScope target library mapping function, using Rsubread and multiple libraries. Aligns to each library separately, filters unmapped reads from each file, and then merges and sorts the .bam files from each library into one output file. If desired, output can be passed to 'filter_host()' to remove reads that also map to filter library genomes.

Usage

```
align_target(
  read1,
  read2 = NULL,
  lib_dir = NULL,
  libs,
  threads = 1,
  align_file = tools::file_path_sans_ext(read1),
  subread_options = align_details,
  quiet = TRUE
)
```

Arguments

read1	Path to the .fastq file to align.
read2	Optional: Location of the mate pair .fastq file to align.
lib_dir	Path to the index files for all libraries.
libs	A vector of character strings giving the basenames of the Subread index files for alignment. If ALL indices to be used are located in the current working directory, set lib_dir = NULL. Default is lib_dir = NULL.
threads	The number of threads that can be utilized by the function. Default is 1 thread.
align_file	The basename of the output alignment file (without trailing .bam extension).
subread_options	A named list specifying alignment parameters for the Rsubread::align() function, which is called inside align_target(). Elements should include type, nthreads, maxMismatches, nsubreads, phredOffset, unique, and nBestLocations. Descriptions of these parameters are available under ?Rsubread::align. Defaults to the global align_details object.
quiet	Turns off most messages. Default is TRUE.

Value

This function writes a merged and sorted .bam file after aligning to all reference libraries given, along with a summary report file, to the user's working directory. The function also outputs the new .bam filename.

Examples

```
##### Align example reads to an example reference library using Rsubread

## Create temporary directory
target_ref_temp <- tempfile()
dir.create(target_ref_temp)

tax <- "Ovine atadenovirus D"

## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download genome
all_ref <- MetaScope::download_refseq(tax,
                                     reference = FALSE,
                                     representative = FALSE,
                                     compress = TRUE,
                                     out_dir = target_ref_temp,
                                     caching = TRUE,
                                     accession_path = tmp_accession)

## Create subread index
ind_out <- mk_subread_index(all_ref)

## Get path to example reads
readPath <- system.file("extdata", "reads.fastq",
                        package = "MetaScope")
## Copy the example reads to the temp directory
refPath <- file.path(target_ref_temp, "reads.fastq")
file.copy(from = readPath, to = refPath)

## Modify alignment parameters object
data("align_details")
align_details[["type"]] <- "rna"
align_details[["phredOffset"]] <- 50
# Just to get it to align - toy example!
align_details[["maxMismatches"]] <- 100

## Run alignment
target_map <- align_target(refPath,
                           libs = stringr::str_replace_all(tax, " ", "_"),
                           lib_dir = target_ref_temp,
                           subread_options = align_details)

## Remove temporary folder
unlink(target_ref_temp, recursive = TRUE)
```

align_targetBowtie *Align microbiome reads to set of indexed Bowtie2 libraries*

Description

This is the main MetaScope target library mapping function, using Rbowtie2 and multiple libraries. Aligns to each library separately, filters unmapped reads from each file, and then merges and

sorts the .bam files from each library into one output file. If desired, output can be passed to 'filter_host_bowtie()' to remove reads that also map to filter library genomes.

Usage

```
align_target_bowtie(
  read1,
  read2 = NULL,
  lib_dir,
  libs,
  align_dir,
  align_file,
  bowtie2_options = NULL,
  threads = 1,
  overwrite = FALSE,
  quiet = TRUE
)
```

Arguments

read1	Path to the .fastq file to align.
read2	Optional: Location of the mate pair .fastq file to align.
lib_dir	Path to the directory that contains the Bowtie2 indexes.
libs	The basename of the Bowtie2 indexes to align against (without trailing .bt2 or .bt2l extensions).
align_dir	Path to the directory where the output alignment file should be created.
align_file	The basename of the output alignment file (without trailing .bam extension).
bowtie2_options	Optional: Additional parameters that can be passed to the align_target_bowtie() function. To see all the available parameters use Rbowtie2::bowtie2_usage(). See Details for default parameters. NOTE: Users should pass all their parameters as one string and if optional parameters are given then the user is responsible for entering all the parameters to be used by Bowtie2. The only parameter that should NOT be specified here is the number of threads.
threads	The number of threads that can be utilized by the function. Default is 1 thread.
overwrite	Whether existing files should be overwritten. Default is FALSE.
quiet	Turns off most messages. Default is TRUE.

Details

The default parameters are the same that PathoScope 2.0 uses. "--very-sensitive-local -k 100 --score-min L,20,1.0"

If you experience any issues with reading the input files, make sure that the file(s) are not located in a read-only folder. This can be circumvented by copying files to a new location before running the function.

Value

Returns the path to where the output alignment file is stored.

Examples

```
##### Align example reads to an example reference library using Rbowtie2

## Create temporary directory to store file
target_ref_temp <- tempfile()
dir.create(target_ref_temp)

tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download reference genome
MetaScope::download_refseq("Morbillivirus hominis",
                           reference = FALSE,
                           representative = FALSE,
                           compress = TRUE,
                           out_dir = target_ref_temp,
                           caching = TRUE,
                           accession_path = tmp_accession
)

## Create temporary directory to store the indices
index_temp <- tempfile()
dir.create(index_temp)

## Create bowtie2 index
MetaScope::mk_bowtie_index(
  ref_dir = target_ref_temp,
  lib_dir = index_temp,
  lib_name = "target",
  overwrite = TRUE
)

## Create temporary directory for final file
output_temp <- tempfile()
dir.create(output_temp)

## Get path to example reads
readPath <- system.file("extdata", "virus_example.fastq",
                        package = "MetaScope")

## Align to target genomes
target_map <-
  MetaScope::align_target_bowtie(
    read1 = readPath,
    lib_dir = index_temp,
    libs = "target",
    align_dir = output_temp,
    align_file = "bowtie_target",
    overwrite = TRUE,
    bowtie2_options = "--very-sensitive-local"
  )

## Remove extra folders
unlink(target_ref_temp, recursive = TRUE)
unlink(index_temp, recursive = TRUE)
unlink(output_temp, recursive = TRUE)
```

bam_reheader_R	<i>Replace the header from a .bam file</i>
----------------	--

Description

This function replaces the header from one .bam file with a header from a different .sam file. This function mimics the function of the 'reheader' function in samtools. It is not intended for use by users.

Usage

```
bam_reheader_R(  
  head,  
  old_bam,  
  new_bam = paste(tools::file_path_sans_ext(old_bam), "h.bam", sep = "")  
)
```

Arguments

head	A file name and location for the .sam file with the new header.
old_bam	A file name and location for the .bam file which you would
new_bam	A file name for the new .bam file with a replaced header. Defaults to the same base filename plus 'h.bam'. For example, 'example.bam' will be written as 'exampleh.bam'.

Value

This function will return a new .bam file with a replaced header. The function also outputs the new .bam filename.

blastn_results	<i>Reformat BLASTn results</i>
----------------	--------------------------------

Description

Reformat BLASTn results

Usage

```
blastn_results(  
  results_table,  
  bam_file,  
  num_results = 10,  
  num_reads_per_result = 100,  
  hit_list = 10,  
  num_threads = 1,  
  db_path,  
  out_path,  
  db = NULL,
```

```

    sample_name = NULL,
    quiet = quiet,
    accession_path,
    fasta_dir = NULL,
    BPPARAM
  )

```

Arguments

results_table	data.frame containing the MetaScope results.
bam_file	Rsamtools::bamFile instance for the given sample.
num_results	Integer; maximum number of Metascope results to BLAST. Default is 10.
num_reads_per_result	Integer; number of reads to BLAST per result. Default is 100.
hit_list	Integer; how many BLAST results to fetch for each read. Default is 10.
num_threads	Integer; how many threads to use if multithreading. Default is 1.
db_path	Character string; filepath for the location of the pre-installed BLAST database.
out_path	Character string; Output directory to save CSV output files, including base name of files. For example, given a sample "X78256", filepath would be file.path(directory_here, "X78256") with extension omitted.
db	Currently accepts one of c("ncbi", "silva", "other") Default is "ncbi", appropriate for samples aligned against indices compiled from NCBI whole genome databases. Alternatively, usage of an alternate database (like Green-genes2) should be specified with "other".
sample_name	Character string, sample name for output files.
quiet	Logical, whether to print out more informative messages. Default is FALSE.
accession_path	(character) Filepath to NCBI accessions SQL database. See taxonomizr::prepareDatabase().
fasta_dir	Character string; Directory where fastas from metascope_id are stored.
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation.

Value

Creates and exports num_results number of csv files with blast results from local blast

blastn_single_result	<i>blastn_single_result</i>
----------------------	-----------------------------

Description

blastn_single_result

Usage

```
blastn_single_result(
  results_table,
  bam_file,
  which_result,
  num_reads = 100,
  hit_list = 10,
  num_threads,
  db_path,
  quiet,
  accession_path,
  bam_seqs,
  out_path,
  sample_name,
  fasta_dir = NULL
)
```

Arguments

results_table	A dataframe of the Metascope results
bam_file	A sorted bam file and index file, loaded with Rsamtools::bamFile
which_result	Index in results_table for which result to Blast search
num_reads	Number of reads to blast per result
hit_list	Number of how many blast results to fetch per read
num_threads	Number of threads if multithreading
db_path	Blast database path
quiet	Logical, whether to print out more informative messages. Default is FALSE.
accession_path	(character) Filepath to NCBI accessions SQL database. See taxonomizr::prepareDatabase().
bam_seqs	A list of the sequence IDs from the bam file
out_path	Path to output results.
sample_name	Character string, sample name for output files.
fasta_dir	Path to where fasta files are stored.

Value

Returns a dataframe of blast results for a metascope result

blast_reassignment	<i>Reassign reads from MetaScope BLASTn alignment</i>
--------------------	---

Description

Using the output from metascope_blast(), the blast_reassignment() function takes the results and alters the original metascope_id() output to reassign reads that were invalidated by the BLAST findings. Currently, the implementation of this function only reassigns reads to a taxon that was already found in the sample at a higher abundance.

Usage

```
blast_reassignment(
  metascope_blast_path,
  species_threshold,
  num_hits,
  blast_tmp_dir,
  out_dir,
  sample_name,
  reassign_validated = FALSE,
  reassign_to_validated = TRUE
)
```

Arguments

metascope_blast_path	Character string. The filepath to a metascope_blast CSV output file.
species_threshold	Numeric. A number between 0 and 1 indicating the minimum proportion of reads needed for a taxon to be considered validated from the BLAST results. Default is 0.2, or 20%.
num_hits	Integer. The number of hits for which to assess validation. Default is 10, i.e., only the top 10 taxa will be assessed.
blast_tmp_dir	Character string. Filepath of the directory where BLAST results were output from the metascope_blast function. Referencing the arguments from metascope_blast, this would be file.path(tmp_dir, "blast")
out_dir	Character string, path to output directory.
sample_name	Character string, sample name for output files.
reassign_validated	Logical. Should reads from validated accessions be reassigned to other validated accessions. Defaults to FALSE
reassign_to_validated	Logical. Should reads only be re-assigned to validated accessions or to any accession with more reads than the current accession. Defaults to TRUE

Value

Returns a data.frame with the reassigned taxa and read counts.

blast_result_metrics	<i>Calculates result metrics from a blast results table</i>
----------------------	---

Description

This function calculates the best hit (genome with most blast read hits), uniqueness score (total number of genomes hit), species percentage hit (percentage of reads where MetaScope species also matched the blast hit species), genus percentage hit (percentage of reads where blast genus matched MetaScope aligned genus) and species contaminant score (percentage of reads that blasted to other species genomes) and genus contaminant score (percentage of reads that blasted to other genus genomes)

Usage

```
blast_result_metrics(blast_results_table_path, accession_path, db = NULL)
```

Arguments

blast_results_table_path
path for blast results csv file

accession_path (character) Filepath to NCBI accessions SQL database. See `taxonomizr::prepareDatabase()`.

db Currently accepts one of `c("ncbi", "silva", "other")` Default is "ncbi", appropriate for samples aligned against indices compiled from NCBI whole genome databases. Alternatively, usage of an alternate database (like Green-
genes2) should be specified with "other".

Value

a vector with `best_hit`, `uniqueness_score`, `species_percentage_hit`, `genus_percentage_hit`, `species_contaminant_score`, and `genus_contaminant_score`

bt2_16S_params	<i>A universal parameter object for Bowtie 2 16S alignment</i>
----------------	--

Description

This character string provides several Bowtie 2 options to provide an optimized alignment specifically optimized for 16S amplicon sequencing data. This object can be used with functions that use the Bowtie 2 aligner through the Rbowtie2 package, namely `align_target_bowtie()` and `filter_host_bowtie`. These settings can be substituted for default settings by passing to the `bowtie2_options` argument.

Usage

```
data(bt2_16S_params)
```

Format

list

Details

The default parameters listed in this object are `"-local -R 2 -N 0 -L 25 -i S,1,0.75 -k 5 --score-min L,0,1.88"`

Note that `k` is actually 10 and is doubled internally from 5. The `score-min` function was chosen such that the minimum alignment score allowed requires 98

Further delineation of Bowtie 2 parameters is provided in the [Bowtie 2 manual](#).

Examples

```
data("bt2_16S_params")
```

bt2_missing_params	<i>A universal parameter object for Bowtie 2 metagenomic alignment where the host genome is thought to be absent from the reference database</i>
--------------------	--

Description

This character string provides several Bowtie 2 options to conduct an alignment useful for metagenomes, especially in the case where a genome may not be present in the reference database. This object can be used with functions that use the Bowtie 2 aligner through the Rbowtie2 package, namely `align_target_bowtie()` and `filter_host_bowtie`. These settings can be substituted for default settings by passing to the `bowtie2_options` argument.

Usage

```
data(bt2_missing_params)
```

Format

list

Details

The default parameters listed in this object are "-local -R 2 -N 0 -L 25 -i S,1,0.75 -k 5 -score-min L,0,1.4".

Further delineation of Bowtie 2 parameters is provided in the [Bowtie 2 manual](#).

Examples

```
data("bt2_missing_params")
```

bt2_regular_params	<i>A universal parameter object for Bowtie 2 metagenomic or non-16S alignment</i>
--------------------	---

Description

This character string provides several Bowtie 2 options to provide a 95 alignment useful for metagenomes. This object can be used with functions that use the Bowtie 2 aligner through the Rbowtie2 package, namely `align_target_bowtie()` and `filter_host_bowtie`. These settings can be substituted for default settings by passing to the `bowtie2_options` argument.

Usage

```
data(bt2_regular_params)
```

Format

list

Details

The default parameters listed in this object are "-local -R 2 -N 0 -L 25 -i S,1,0.75 -k 5 -score-min L,0,1.7".

Further delineation of Bowtie 2 parameters is provided in the [Bowtie 2 manual](#).

Examples

```
data("bt2_regular_params")
```

check_blastn_exists	<i>Check if blastn exists on the system</i>
---------------------	---

Description

This is an internal function that is not meant to be used outside of the package. It checks whether blastn exists on the system.

Usage

```
check_blastn_exists()
```

Details

Checks if blastn is installed

Value

Returns TRUE if blastn exists on the system, else FALSE.

check_samtools_exists	<i>Check if samtools exists on the system</i>
-----------------------	---

Description

This is an internal function that is not meant to be used outside of the package. It checks whether samtools exists on the system.

Usage

```
check_samtools_exists()
```

Value

Returns TRUE if samtools exists on the system, else FALSE.

combined_header	<i>Create a combined .bam header</i>
-----------------	--------------------------------------

Description

This function generates a combined header from multiple .bam files from different reference libraries (e.g. a split bacterial library). It is not intended for use by users.

Usage

```
combined_header(bam_files, header_file = "header_tmp.sam")
```

Arguments

bam_files	A character vector of the locations/file names of .bam files from which to combine the headers.
header_file	A file name and location for the output file for the combined header. This will be a .sam format file without any reads. Defaults to 'header_tmp.sam'.

Value

This function will return a combined header from all the supplied .bam files.

convert_animalcules	<i>Create a multi-assay experiment from MetaScope output for usage with animalcules</i>
---------------------	---

Description

Upon completion of the MetaScope pipeline, users can analyze and visualize abundances in their samples using the animalcules package. This function allows interoperability of metascope_id output with both animalcules and QIIME. After running this function, the user should save the returned MAE to an RDS file using a function like saveRDS to upload the output into the animalcules package.

Usage

```
convert_animalcules(
  meta_counts,
  annot_path,
  which_annot_col,
  end_string = ".metascope_id.csv",
  qiime_biom_out = FALSE,
  path_to_write = ".",
  accession_path = NULL
)
```


Arguments

<code>meta_counts</code>	A vector of filepaths to the counts ID CSVs output by <code>metascope_id()</code> .
<code>annot_path</code>	The filepath to the CSV annotation file for the samples. This CSV metadata/annotation file should contain at least two columns, one with names of all samples WITH-OUT the extension listed in <code>end_string</code> , e.g. for output file "sample_x76.metascope_id.csv", the column specified in <code>which_annot_col</code> should contain the entry "sample_x76". Sample names containing characters "_", "-", and "." are fine, however sample names beginning with numbers should be renamed to have a prefix, e.g. "777897sample" should be renamed to "X777897sample" for both the output file name and the annotation name.
<code>which_annot_col</code>	The name of the column of the annotation file containing the sample IDs. These should be the same as the <code>meta_counts</code> root filenames.
<code>end_string</code>	The end string used at the end of the <code>metascope_id</code> files. Default is ".metascope_id.csv".
<code>qiime_biom_out</code>	Would you also like a qiime-compatible biom file output? If yes, two files will be saved: one is a biom file of the counts table, and the other is a specifically formatted mapping file of metadata information. Default is FALSE.
<code>path_to_write</code>	If <code>qiime_biom_out</code> = TRUE, where should output QIIME files be written? Should be a character string of the folder path. Default is '.', i.e. the current working directory.
<code>accession_path</code>	(character) Path to taxonomizr accessions. See <code>taxonomizr::prepareDatabase()</code> .

Value

Returns a MultiAssay Experiment file of combined sample counts data and/or biom file and mapping file for analysis with QIIME. The MultiAssay Experiment will have a counts assay ("MGX").

Examples

```
tempfolder <- tempfile()
dir.create(tempfolder)

# Create three different samples
samp_names <- c("X123", "X456", "X789")
all_files <- file.path(tempfolder,
  paste0(samp_names, ".csv"))

create_IDcsv <- function (out_file) {
  final_taxids <- c("273036", "418127", "11234")
  final_genomes <- c(
    "Staphylococcus aureus RF122, complete sequence",
    "Staphylococcus aureus subsp. aureus Mu3, complete sequence",
    "Measles virus, complete genome")
  best_hit <- sample(seq(100, 1050), 3)
  proportion <- best_hit/sum(best_hit) |> round(2)
  EMreads <- best_hit + round(runif(3), 1)
  EMprop <- proportion + 0.003
  dplyr::tibble(TaxonomyID = final_taxids,
    Genome = final_genomes,
    read_count = best_hit, Proportion = proportion,
    EMreads = EMreads, EMProportion = EMprop) |>
  dplyr::arrange(dplyr::desc(.data$read_count)) |>
```

```

    utils::write.csv(file = out_file, row.names = FALSE)
    message("Done!")
    return(out_file)
}
out_files <- vapply(all_files, create_IDcsv, FUN.VALUE = character(1))

# Create annotation data for samples
annot_dat <- file.path(tempfolder, "annot.csv")
dplyr::tibble(Sample = samp_names, RSV = c("pos", "neg", "pos"),
              month = c("March", "July", "Aug"),
              yrsold = c(0.5, 0.6, 0.2)) |>
  utils::write.csv(file = annot_dat,
                  row.names = FALSE)

# Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

# Convert samples to MAE
outMAE <- convert_animalcules(meta_counts = out_files,
                              annot_path = annot_dat,
                              which_annot_col = "Sample",
                              end_string = ".metascope_id.csv",
                              qiime_biom_out = FALSE,
                              accession_path = tmp_accession)

unlink(tempfolder, recursive = TRUE)

```

```
convert_animalcules_patho
```

Create a multi-assay experiment from PathoScope 2.0 output for usage with animalcules

Description

This function serves as a legacy integration method for usage with PathoScope 2.0 outputs. Upon completion of the PathoScope 2.0 pipeline, users can analyze and visualize abundances in their samples using the animalcules package. After running this function, the user should save the returned MAE to an RDS file using a function like saveRDS to upload the output into the animalcules package.

Usage

```

convert_animalcules_patho(
  patho_counts,
  annot_path,
  which_annot_col,
  end_string = "-sam-report.tsv"
)

```

Arguments

patho_counts	Character string, a directory filepath to the counts ID CSVs output by metascope_id().
annot_path	The filepath to the CSV annotation file for the samples.

which_annot_col	The name of the column of the annotation file containing the sample IDs. These should be the same as the meta_counts root filenames.
end_string	The end string used at the end of the metascope_id files. Default is "-sam-report.tsv".

Value

Returns a MultiAssay Experiment file of combined sample counts data. The MultiAssay Experiment will have a counts assay ("MGX").

convert_animalcules_silva

Create a multi-assay experiment from MetaScope output for usage with animalcules with the SILVA 13_8 database

Description

Upon completion of the MetaScope pipeline, users can analyze and visualize abundances in their samples using the animalcules package. This function allows interoperability of metascope_id output with both animalcules and QIIME. After running this function, the user should save the returned MAE to an RDS file using a function like saveRDS to upload the output into the animalcules package. NOTE: This function is for outputs that were generated with the SILVA 13_8 database.

Usage

```
convert_animalcules_silva(
  meta_counts,
  annot_path,
  which_annot_col,
  end_string = ".metascope_id.csv",
  qiime_biom_out = FALSE,
  path_to_write = ".",
  caching = TRUE
)
```

Arguments

meta_counts	A vector of filepaths to the counts ID CSVs output by metascope_id() created with the SILVA database.
annot_path	The filepath to the CSV annotation file for the samples. This CSV metadata/annotation file should contain at least two columns, one with names of all samples WITHOUT the extension listed in end_string, e.g. for output file "sample_x76.metascope_id.csv", the column specified in which_annot_col should contain the entry "sample_x76". Sample names containing characters "_", "-", and "." are fine, however sample names beginning with numbers should be renamed to have a prefix, e.g. "777897sample" should be renamed to "X777897sample" for both the output file name and the annotation name.
which_annot_col	The name of the column of the annotation file containing the sample IDs. These should be the same as the meta_counts root filenames.


```

        which_annot_col = "Sample",
        end_string = ".metascope_id.csv",
        qiime_biom_out = FALSE,
        caching = TRUE)

unlink(tempfolder, recursive = TRUE)

```

count_matches	<i>Count the number of base lengths in a CIGAR string for a given operation</i>
---------------	---

Description

The 'CIGAR' (Compact Idiosyncratic Gapped Alignment Report) string is how the SAM/BAM format represents spliced alignments. This function will accept a CIGAR string for a single read and a single character indicating the operation to be parsed in the string. An operation is a type of column that appears in the alignment, e.g. a match or gap. The integer following the operator specifies a number of consecutive operations. The count_matches() function will identify all occurrences of the operator in the string input, add them, and return an integer number representing the total number of operations for the read that was summarized by the input CIGAR string.

Usage

```
count_matches(x, char = "M")
```

Arguments

x	Character. A CIGAR string for a read to be parsed. Examples of possible operators include "M", "D", "I", "S", "H", "=", "P", and "X".
char	A single letter representing the operation to total for the given string.

Details

This function is best used on a vector of CIGAR strings using an apply function (see examples).

Value

an integer number representing the total number of alignment operations for the read that was summarized by the input CIGAR string.

Examples

```

# A single cigar string: 3M + 3M + 5M
cigar1 <- "3M1I3M1D5M"
count_matches(cigar1, char = "M")

# Parse with operator "P": 2P
cigar2 <- "4M1I2P9M"
count_matches(cigar2, char = "P")

# Apply to multiple strings: 1I + 1I + 5I
cigar3 <- c("3M1I3M1D5M", "4M1I1P9M", "76M13M5I")

```

```
vapply(cigar3, count_matches, char = "I",
      FUN.VALUE = numeric(1))
```

download_accessions *Download indexes required for MetaScope ID and MetaBlast modules*

Description

This is a necessary step for all samples utilizing NCBI and SILVA databases in the MetaScope pipeline. As specified by the user, download_accessions will automatically download the NCBI accessions database, the SILVA taxonomy database, and or the NCBI Blast 16S database and prepare consolidated databases for downstream use with the MetaID and MetaBLAST modules. This package relies on the taxonomizr package.

Usage

```
download_accessions(
  ind_dir,
  tmp_dir = file.path(ind_dir, "tmp"),
  remove_tmp_dir = TRUE,
  NCBI_accessions_database = TRUE,
  NCBI_accessions_name = "accessionTaxa",
  silva_taxonomy_database = TRUE,
  silva_taxonomy_name = "all_silva_headers",
  blast_16S_database = TRUE,
  blast_16S_name = "16S_ribosomal_RNA"
)
```

Arguments

ind_dir	Character string. Directory filepath where indices should be saved. Required.
tmp_dir	Character path to directory for storing temp files. (Useful to avoid redownload-ing) Defaults to file.path(ind_dir, "tmp")
remove_tmp_dir	Delete tmp_dir after downloads are complete? Defaults to TRUE
NCBI_accessions_database	Logical. Download taxonomizr NCBI accessions database? Defaults to TRUE.
NCBI_accessions_name	Character string. Filename (with or without extension) to save taxonomizr NCBI accessions database. Defaults to "accessionTaxa.sql".
silva_taxonomy_database	Logical. Download SILVA taxonomy database? Defaults to TRUE.
silva_taxonomy_name	Character string. Filename (with or without extension) to save SILVA taxonomy database. Defaults to the file supplied with the package, "all_silva_headers.rds".
blast_16S_database	Logical. Download BLAST 16S database? Defaults to TRUE.
blast_16S_name	Character string. Filename (without extension) to save \ BLAST 16S database. Defaults to the file supplied with the package, "16S_ribosomal_RNA".

Value

Exports database(s) with names and to location specified by the user.

Examples

```
## Not run:
download_accessions(
  ind_dir = "C:/Users/JohnSmith/Research",
  tmp_dir = file.path(ind_dir, "tmp"),
  remove_tmp_dir = TRUE,
  NCBI_accessions_database = TRUE,
  NCBI_accessions_name = "accessionTaxa.sql",
  silva_taxonomy_database = TRUE,
  silva_taxonomy_name = "all_silva_headers.rds")

## End(Not run)
```

download_refseq	<i>Download RefSeq genome libraries</i>
-----------------	---

Description

This function will automatically download RefSeq genome libraries in a fasta format from the specified taxon. The function will first download the summary report at: `ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/` and then use this file to download the genome(s) and combine them in a single compressed or uncompressed .fasta file.

Usage

```
download_refseq(
  taxon,
  reference = TRUE,
  representative = FALSE,
  compress = TRUE,
  patho_out = FALSE,
  out_dir = NULL,
  caching = FALSE,
  quiet = TRUE,
  accession_path = NULL
)
```

Arguments

taxon	Name of single taxon to download. The taxon name should be a recognized NCBI scientific or common name, with no grammatical or capitalization inconsistencies. All available taxonomies are visible by accessing the <code>MetaScope::taxonomy_table</code> object included in the package.
reference	Download only RefSeq reference genomes? Defaults to TRUE. Automatically set to TRUE if <code>representative = TRUE</code> .
representative	Download RefSeq representative and reference genomes? Defaults to FALSE. If TRUE, reference is automatically set at TRUE.

compress	Compress the output .fasta file? Defaults to TRUE.
patho_out	Create duplicate output files compatible with PathoScope? Defaults to FALSE.
out_dir	Character string giving the name of the directory to which libraries should be output. Defaults to creation of a new temporary directory.
caching	Whether to use BiocFileCache when downloading genomes. Default is FALSE.
quiet	Turns off most messages. Default is TRUE.
accession_path	(character) Filepath to NCBI accessions SQL database. See <code>taxonomizr::prepareDatabase()</code> .

Details

When selecting the taxon to be downloaded, if you receive an error saying Your input is not a valid taxon, please take a look at the `taxonomy_table` object, which can be accessed with the command `MetaScope::taxonomy_table`). Only taxa with exact spelling as they appear at any level of the table will be acknowledged.

Value

Returns a .fasta or .fasta.gz file of the desired RefSeq genomes. This file is named after the kingdom selected and saved to the current directory (e.g. 'bacteria.fasta.gz'). This function also has the option to return a .fasta file formatted for PathoScope as well (e.g. `bacteria.pathoscope.fasta.gz`) if `patho_out = TRUE`.

Examples

```
#### Download RefSeq genomes

## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download all RefSeq reference Bovismacovirus genus genomes
download_refseq('Bovismacovirus', reference = FALSE, representative = FALSE,
               out_dir = NULL, compress = TRUE, patho_out = FALSE,
               caching = TRUE, accession_path = tmp_accession)
```

download_refseq_16S	<i>Download RefSeq 16S rRNA Bacterial and Archaeal libraries</i>
---------------------	--

Description

This function will automatically download the 16S rRNA RefSeq libraries from <https://ftp.ncbi.nlm.nih.gov/refseq/> and combine them into a single .fna file

Usage

```
download_refseq_16S(out_dir, combined_name = "refseq_16S.fna")
```

Arguments

out_dir	Character string giving the name of the directory to which libraries should be output. **Required**
combined_name	Name of output combined file. Default is "refseq_16S.fna"

Value

Returns a character string of the path to the combined 16S rRNA .fna file

Note

This function requires the suggested packages **RCurl** and **R.utils**. If they are not installed, you will need to install them manually.

Examples

```
## Not run:
#### Download 16S rRNA Genomes

download_refseq_16S(out_dir = "out_dir",
  combined_name = "refseq_16S.fna")

## End(Not run)
```

extract_reads

Helper function for demultiplexing

Description

Helper function for demultiplexing sequencing reads, designed in a way to allow for parallelization across barcodes (parallel extraction of reads by barcode). This function takes a specific barcode (numeric index) from lists of sample names/barcodes, a `Biostrings::DNAStringSet` of barcodes by sequence header, and a `Biostrings::QualityScaledXStringSet` of reads corresponding to the barcodes. Based on the barcode index given, it extracts all reads for the indexed barcode and writes all the reads from that barcode to a separate .fastq file.

Usage

```
extract_reads(
  barcodeIndex,
  barcodes,
  sampleNames,
  index,
  reads,
  location = "./demultiplex_fastq",
  rcBarcodes = TRUE,
  hDist = 0,
  quiet = TRUE
)
```

Arguments

barcodeIndex	Which barcode (integer number or index) in the barcodes or sample name to use for read extraction.
barcodes	A list of all barcodes in the sequencing dataset. Correlates and in same order as sampleNames.

sampleNames	A list of sample names or identifiers associated with each barcode in the barcodes list.
index	A <code>Biostrings::DNAStringSet</code> that contains the read headers and barcode sequence for each header in the sequence slot.
reads	A <code>Biostrings::QualityScaledXStringSet</code> that has the same headers and order as the index file, but contains the read sequences and their quality scores.
location	A directory location to store the demultiplexed read files. Defaults to generate a new subdirectory at <code>'./demultiplex_fastq'</code>
rcBarcodes	Should the barcode indices in the barcodes list be reverse complemented to match the sequences in the index <code>DNAStringSet</code> ? Defaults to <code>TRUE</code> .
hDist	Uses a Hamming Distance or number of base differences to allow for inexact matches for the barcodes/indexes. Defaults to 0. Warning: if the Hamming Distance is ≥ 1 and this leads to inexact index matches to more than one barcode, that read will be written to more than one demultiplexed read files.
quiet	Turns off most messages. Default is <code>TRUE</code> .

Value

Writes a single `.fastq` file that contains all reads whose index matches the barcode specified. This file will be written to the location directory, and will be named based on the specified `sampleName` and barcode, e.g. `'./demultiplex_fastq/SampleName1_GGAATTATCGGT.fastq.gz'`

Examples

```
## Create temporary directory
ref_temp <- tempfile()
dir.create(ref_temp)

## Load example barcode, index, and read data into R session
barcodePath <- system.file("extdata", "barcodes.txt", package = "MetaScope")
bcFile <- read.table(barcodePath, sep = "\t", header = TRUE)

indexPath <- system.file("extdata", "virus_example_index.fastq",
package = "MetaScope")
inds <- Biostrings::readDNAStringSet(indexPath, format = "fastq")

readPath <- system.file("extdata", "virus_example.fastq",
package = "MetaScope")
reads <- Biostrings::readQualityScaledDNAStringSet(readPath)

## Extract reads from the first barcode
results <- extract_reads(1, bcFile[, 2], bcFile[, 1], inds, reads,
rcBarcodes = FALSE, location = ref_temp)

## Extract reads from multiple barcodes
more_results <- lapply(1:6, extract_reads, bcFile[, 2], bcFile[, 1], inds,
reads, rcBarcodes = FALSE, location = ref_temp)

## Remove temporary directory
unlink(ref_temp, recursive = TRUE)
```

filter_host	<i>Use Rsubread to align reads against one or more filter libraries and subsequently remove mapped reads</i>
-------------	--

Description

After aligning your sample to a target library with `align_target()`, use `filter_host()` to remove unwelcome host contamination using filter reference libraries. This function takes as input the name of the .bam file produced via `align_target()`, and produces a sorted .bam file with any reads that match the filter libraries removed. This resulting .bam file may be used upstream for further analysis. This function uses Rsubread. For the Rbowtie2 equivalent of this function, see `filter_host_bowtie`.

Usage

```
filter_host(
  reads_bam,
  lib_dir = NULL,
  libs,
  make_bam = FALSE,
  output = paste(tools::file_path_sans_ext(reads_bam), "filtered", sep = "."),
  subread_options = align_details,
  YS = 1e+05,
  threads = 1,
  quiet = TRUE
)
```

Arguments

reads_bam	The name of a merged, sorted .bam file that has previously been aligned to a reference library. Likely, the output from running an instance of <code>align_target()</code> .
lib_dir	Path to the directory that contains the filter Subread index files.
libs	The basename of the filter libraries (without index extension).
make_bam	Logical, whether to also output a bam file with host reads filtered out. A .csv.gz file will be created instead if FALSE. Creating a bam file is costly on resources over creating a compressed csv file with only relevant information, so default is FALSE.
output	The desired name of the output .bam or .csv.gz file. Extension is automatically defined by whether <code>make_bam = TRUE</code> . Default is the basename of <code>unfiltered_bam</code> + <code>.filtered</code> + extension.
subread_options	A named list specifying alignment parameters for the <code>Rsubread::align()</code> function, which is called inside <code>align_target()</code> . Elements should include <code>type</code> , <code>nthreads</code> , <code>maxMismatches</code> , <code>nsubreads</code> , <code>phredOffset</code> , <code>unique</code> , and <code>nBestLocations</code> . Descriptions of these parameters are available under <code>?Rsubread::align</code> . Defaults to the global <code>align_details</code> object.
YS	<code>yieldSize</code> , an integer. The number of alignments to be read in from the bam file at once for chunked functions. Default is 100000.
threads	The amount of threads available for the function. Default is 1 thread.
quiet	Turns off most messages. Default is TRUE.

Details

A compressed .csv can be created to produce a smaller output file that is created more efficiently and is still compatible with `metascope_id()`.

Value

The name of a filtered, sorted .bam file written to the user's current working directory. Or, if `make_bam = FALSE`, a .csv.gz file containing a data frame of only requisite information to run `metascope_id()`.

Examples

```
#### Filter reads from bam file that align to any of the filter libraries

## Assuming a bam file has been created previously with align_target()

## Create temporary directory
filter_ref_temp <- tempfile()
dir.create(filter_ref_temp)

## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download filter genome
all_species <- c("Staphylococcus aureus subsp. aureus str. Newman")
all_ref <- vapply(all_species, MetaScope::download_refseq,
                 reference = FALSE, representative = FALSE, compress = TRUE,
                 out_dir = filter_ref_temp, caching = FALSE,
                 accession_path = tmp_accession,
                 FUN.VALUE = character(1))
ind_out <- vapply(all_ref, mk_subread_index, FUN.VALUE = character(1))

## Get path to example reads
readPath <- system.file("extdata", "subread_target.bam",
                        package = "MetaScope")

## Copy the example reads to the temp directory
refPath <- file.path(filter_ref_temp, "subread_target.bam")
file.copy(from = readPath, to = refPath)
utils::data("align_details")
align_details[["type"]] <- "rna"
align_details[["phredOffset"]] <- 10
# Just to get it to align - toy example!
align_details[["maxMismatches"]] <- 10

## Align and filter reads
filtered_map <- filter_host(
  refPath, lib_dir = filter_ref_temp,
  libs = stringr::str_replace_all(all_species, " ", "_"),
  threads = 1, subread_options = align_details)

## Remove temporary directory
unlink(filter_ref_temp, recursive = TRUE)
```

filter_host_bowtie	<i>Use Rbowtie2 to align reads against one or more filter libraries and subsequently remove mapped reads</i>
--------------------	--

Description

After a sample is aligned to a target library with `align_target_bowtie()`, we may use `filter_host_bowtie()` to remove unwelcome host contamination using filter reference libraries. This function takes as input the name of the .bam file produced via `align_target_bowtie()`, and produces a sorted .bam or .csv.gz file with any reads that match the filter libraries removed. This resulting .bam file may be used downstream for further analysis. This function uses Rbowtie2 For the Rsubread equivalent of this function, see `filter_host`.

Usage

```
filter_host_bowtie(
  reads_bam,
  lib_dir,
  libs,
  make_bam = FALSE,
  output = paste(tools::file_path_sans_ext(reads_bam), "filtered", sep = "."),
  bowtie2_options = NULL,
  YS = 1e+05,
  threads = 1,
  overwrite = FALSE,
  quiet = TRUE
)
```

Arguments

<code>reads_bam</code>	The name of a merged, sorted .bam file that has previously been aligned to a reference library. Likely, the output from running an instance of <code>align_target_bowtie()</code> .
<code>lib_dir</code>	Path to the directory that contains the filter Bowtie2 index files.
<code>libs</code>	The basename of the filter libraries (without .bt2 or .bt2l extension).
<code>make_bam</code>	Logical, whether to also output a bam file with host reads filtered out. A .csv.gz file will be created instead if FALSE. Creating a bam file is costly on resources over creating a compressed csv file with only relevant information, so default is FALSE.
<code>output</code>	The desired name of the output .bam or .csv.gz file. Extension is automatically defined by whether <code>make_bam = TRUE</code> . Default is the basename of <code>unfiltered_bam</code> + <code>.filtered</code> + extension.
<code>bowtie2_options</code>	Optional: Additional parameters that can be passed to the <code>filter_host_bowtie()</code> function. To see all the available parameters use <code>Rbowtie2::bowtie2_usage()</code> . See Details for default parameters. NOTE: Users should pass all their parameters as one string and if optional parameters are given then the user is responsible for entering all the parameters to be used by Bowtie2. The only parameters that should NOT be specified here is the threads.
<code>YS</code>	<code>yieldSize</code> , an integer. The number of alignments to be read in from the bam file at once for chunked functions. Default is 100000.

threads	The amount of threads available for the function. Default is 1 thread.
overwrite	Whether existing files should be overwritten. Default is FALSE.
quiet	Turns off most messages. Default is TRUE.

Details

A compressed .csv can be created to produce a smaller output file that is created more efficiently and is still compatible with `metascope_id()`.

The default parameters are the same that PathoScope 2.0 uses. `"-very-sensitive-local -k 100 -score-min L,20,1.0"`

Value

The name of a filtered, sorted .bam file written to the user's current working directory. Or, if `make_bam = FALSE`, a .csv.gz file containing a data frame of only requisite information to run `metascope_id()`.

Examples

```
#### Filter reads from bam file that align to any of the filter libraries

## Assuming a bam file has already been created with align_target_bowtie()
# Create temporary filter library
filter_ref_temp <- tempfile()
dir.create(filter_ref_temp)

## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download reference genome
MetaScope::download_refseq("Orthoebolavirus zairense",
                           reference = FALSE,
                           representative = FALSE,
                           compress = TRUE,
                           out_dir = filter_ref_temp,
                           caching = TRUE,
                           accession_path = tmp_accession)

## Create temp directory to store the indices
index_temp <- tempfile()
dir.create(index_temp)

## Create filter index
MetaScope::mk_bowtie_index(
  ref_dir = filter_ref_temp,
  lib_dir = index_temp,
  lib_name = "filter",
  overwrite = TRUE
)

## Create temporary folder to hold final output file
output_temp <- tempfile()
dir.create(output_temp)

## Get path to example bam
bamPath <- system.file("extdata", "bowtie_target.bam",
```

```
                                package = "MetaScope")
target_copied <- file.path(output_temp, "bowtie_target.bam")
file.copy(bamPath, target_copied)

## Align and filter reads
filter_out <-
  filter_host_bowtie(
    reads_bam = target_copied,
    lib_dir = index_temp,
    libs = "filter",
    threads = 1
  )

## Remove temporary directories
unlink(filter_ref_temp, recursive = TRUE)
unlink(index_temp, recursive = TRUE)
unlink(output_temp, recursive = TRUE)
```

filter_unmapped_reads *Filter unmapped reads*

Description

This function will remove all unmapped reads or lines in a .bam file (warning: overwrites the original file!). This function is needed because combining multiple .bam files from different microbial libraries may lead to some reads that mapped to one library and have unmapped entries from another library. This will remove any unmapped entries and leave all reference mapped lines in the .bam file.

Usage

```
filter_unmapped_reads(bamfile)
```

Arguments

bamfile	Location for the .bam file to filter & remove all unmapped reads
---------	--

Details

It is not intended for direct use.

Value

This function will overwrite the existing .bam file with a new .bam file in the same location that has only mapped lines. The function itself returns the output .bam file name.

find_taxids	<i>Gets Taxonomy IDS from accessions names Returns a dataframe with the original accession, the associated taxonomy ID and associated taxonomy species</i>
-------------	--

Description

Gets Taxonomy IDS from accessions names Returns a dataframe with the original accession, the associated taxonomy ID and associated taxonomy species

Usage

```
find_taxids(all_fastas, accession_path)
```

Arguments

all_fastas Biostrings loaded object of all fastas
 accession_path Path to taxonomizr accessions database

Value

Data.frame of taxonomy Ids and Species name

get_children	<i>Get child nodes from NCBI taxonomy</i>
--------------	---

Description

This function will utilize an organism classification table to obtain all children species and/or strains with available NCBI reference sequences given a parent taxon and its rank.

Usage

```
get_children(input_taxon, input_rank, tax_dat = NULL)
```

Arguments

input_taxon The parent taxon.
 input_rank The taxonomic rank of the input taxon.
 tax_dat A dataframe of organism classification information. At minimum, should have a column indicating "strain", and and all others should be taxonomic ranks. Each row should be a taxonomic relationship. This defaults to NULL, which calls the 'taxonomy_table' object.

Value

Returns a vector of all the child species and/or strains of the input taxon.

Examples

```
## Get all child species and strains in bacteria superkingdom
get_children('Bacteria','superkingdom')

## Get all child species and strains in fungi kingdom
get_children('Fungi', 'kingdom')

## Get all child species in primate order
get_children('Primates', 'order')
```

get_multi_seqs	<i>Gets multiple sequences from different accessions in a bam file</i>
----------------	--

Description

Returns fasta sequences from a bam file with given taxonomy IDs

Usage

```
get_multi_seqs(ids_n, bam_file, seq_info_df, metascope_id_tax, sorted_bam_file)
```

Arguments

ids_n	List of vectors with Taxonomy IDs and the number of sequences to get from each
bam_file	A sorted bam file and index file, loaded with Rsamtools::bamFile
seq_info_df	Dataframe of sequence information from metascope_blast()
metascope_id_tax	Data.frame of taxonomy information
sorted_bam_file	Filepath to sorted bam file

Value

Biostrings format sequences

get_seqs	<i>Gets sequences from bam file</i>
----------	-------------------------------------

Description

Returns fasta sequences from a bam file with a given taxonomy ID

Usage

```
get_seqs(id, bam_file, n = 10, bam_seqs)
```

Arguments

id	Taxonomy ID of genome to get sequences from
bam_file	A sorted bam file and index file, loaded with Rsamtools::bamFile
n	Number of sequences to retrieve
bam_seqs	A list of the sequence IDs from the bam file

Value

Biostrings format sequences

locations	<i>Helper Function for MetaScope ID</i>
-----------	---

Description

Used to create plots of genome coverage for any number of accession numbers

Usage

```
locations(
  which_taxid,
  which_genome,
  accessions,
  taxids,
  reads,
  out_base,
  out_dir
)
```

Arguments

which_taxid	Which taxid to plot
which_genome	Which genome to plot
accessions	List of accessions from metascope_id()
taxids	List of accessions from metascope_id()
reads	List of reads from input file
out_base	The basename of the input file
out_dir	The path to the input file

Value

A plot of the read coverage for a given genome

merge_bam_files	<i>Merge multiple .bam files</i>
-----------------	----------------------------------

Description

This function merges .bam files. It first used the combined_header function to generate a combined header for all the files, reheaders the files, and then merges and sorts the .bam files. It is similar to the 'samtools merge' function, but it allows the .bam files to have different headers. It is not intended for direct use.

Usage

```
merge_bam_files(
  bam_files,
  destination,
  head_file = paste(destination, "_header.sam", sep = ""),
  quiet = TRUE
)
```

Arguments

bam_files	A list of file names for the .bam files to be merged.
destination	A file name and location for the merged .bam file.
head_file	A file name and location for the combined header file. Defaults to the destination. For example, 'example.bam' will be written as 'example.bam'.
quiet	Turns off most messages. Default is TRUE.

Value

This function merges .bam files and combines them into a single file. The function also outputs the new .bam filename.

metascope_blast	<i>Blast reads from MetaScope aligned files</i>
-----------------	---

Description

This function allows the user to check a subset of identified reads against NCBI BLAST and the nucleotide database to confirm or contradict results provided by MetaScope. It aligns the top 'metascope_id()' results to NCBI BLAST database. It **REQUIRES** that command-line BLAST and a separate nucleotide database have already been installed on the host machine. It returns a csv file updated with BLAST result metrics.

Usage

```
metascope_blast(
  metascope_id_path,
  bam_file_path = list.files(tmp_dir, ".updated.bam$", full.names = TRUE)[1],
  tmp_dir,
  out_dir,
  sample_name,
  fasta_dir = NULL,
  num_results = 10,
  num_reads = 100,
  hit_list = 10,
  num_threads = 1,
  db_path,
  quiet = FALSE,
  db = NULL,
  accession_path = NULL
)
```

Arguments

<code>metascope_id_path</code>	Character string; path to a csv file output by 'metascope_id()'.
<code>bam_file_path</code>	Character string; full path to bam file for the same sample processed by 'metascope_id'. Note that the 'filter_bam' function must have output a bam file, and not a .csv.gz file. See '?filter_bam_bowtie' for more details. Defaults to <code>list.files(file_temp, ".updated.bam\$")[1]</code> .
<code>tmp_dir</code>	Character string, a temporary directory in which to host files.
<code>out_dir</code>	Character string, path to output directory.
<code>sample_name</code>	Character string, sample name for output files.
<code>fasta_dir</code>	Directory where fasta files for blast will be stored.
<code>num_results</code>	Integer, the maximum number of taxa from the metascope_id output to check reads. Takes the top n taxa, i.e. those with largest abundance. Default is 10.
<code>num_reads</code>	Integer, the maximum number of reads to blast per microbe. If the true number of reads assigned to a given taxon is smaller, then the smaller number will be chosen. Default is 100. Too many reads will involve more processing time.
<code>hit_list</code>	Integer, number of blast hit results to keep. Default is 10.
<code>num_threads</code>	Integer, number of threads if running in parallel (recommended). Default is 1.
<code>db_path</code>	Character string. The database file to be searched (including basename, but without file extension). For example, if the nt database is in the nt folder, this would be /filepath/nt/nt assuming that the database files have the nt basename. Check this path if you get an error message stating "No alias or index file found".
<code>quiet</code>	Logical, whether to print out more informative messages. Default is FALSE.
<code>db</code>	Currently accepts one of <code>c("ncbi", "silva", "other")</code> . Default is "ncbi", appropriate for samples aligned against indices compiled from NCBI whole genome databases. Alternatively, usage of an alternate database (like GreenGenes2) should be specified with "other".
<code>accession_path</code>	(character) Filepath to NCBI accessions SQL database. See <code>taxonomizr::prepareDatabase()</code> .

Details

This function assumes that you used the NCBI nucleotide database to process samples, with a download date of 2021 or later. This is necessary for compatibility with the bam file headers.

This is highly computationally intensive and should be ran with multiple cores, submitted as a multi-threaded computing job if possible.

Note, if `best_hit_strain` is `FALSE`, then no strain was observed more often among the BLAST results.

Value

This function writes an updated csv file with metrics.

Examples

```
## Not run:
### Create temporary directory
file_temp <- tempfile()
dir.create(file_temp)

bamPath <- system.file("extdata", "bowtie_target.bam",
                       package = "MetaScope")
file.copy(bamPath, file_temp)

metascope_id(file.path(file_temp, "bowtie_target.bam"), aligner = "bowtie2",
             input_type = "bam", out_dir = file_temp, num_species_plot = 0,
             update_bam = TRUE)

## Run metascope blast
### Get export name and metascope id results
out_base <- bamPath |> basename() |> tools::file_path_sans_ext() |>
  tools::file_path_sans_ext()
metascope_id_path <- file.path(file_temp, paste0(out_base, ".metascope_id.csv"))

# NOTE: change db_path to the location where your BLAST database is stored!
db <- "/restricted/projectnb/pathoscope/data/blastdb/nt/nt"

tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

metascope_blast(metascope_id_path,
               bam_file_path = file.path(file_temp, "bowtie_target.bam"),
               tmp_dir = file_temp,
               out_dir = file_temp,
               sample_name = out_base,
               db_path = db,
               num_results = 10,
               num_reads = 5,
               hit_list = 10,
               num_threads = 3,
               db = "ncbi",
               quiet = FALSE,
               fasta_dir = NULL,
               accession_path = tmp_accession)

## Remove temporary directory
unlink(file_temp, recursive = TRUE)
```

```
## End(Not run)
```

metascope_id	<i>Identify which genomes are represented in a processed sample</i>
--------------	---

Description

This function will read in a .bam or .csv.gz file, annotate the taxonomy and genome names, reduce the mapping ambiguity using a mixture model, and output a .csv file with the results. Currently, it assumes that the genome library/.bam files use NCBI accession names for reference names (rnames in .bam file).

Usage

```
metascope_id(
  input_file,
  input_type = "csv.gz",
  aligner = "bowtie2",
  db = "ncbi",
  db_feature_table = NULL,
  accession_path = NULL,
  priors_df = NULL,
  tmp_dir = dirname(input_file),
  out_dir = dirname(input_file),
  convEM = 1/10000,
  maxitsEM = 25,
  update_bam = FALSE,
  num_species_plot = NULL,
  group_by_taxa = "species",
  quiet = TRUE
)
```

Arguments

input_file	The .bam or .csv.gz file of sample reads to be identified.
input_type	Extension of file input. Should be either "bam" or "csv.gz". Default is "csv.gz".
aligner	The aligner which was used to create the bam file. Default is "bowtie2" but can also be set to "subread" or "other".
db	Currently accepts one of c("ncbi", "silva", "other") Default is "ncbi", appropriate for samples aligned against indices compiled from NCBI whole genome databases. Alternatively, usage of an alternate database (like Green-genes2) should be specified with "other".
db_feature_table	If db = "other", a data.frame must be supplied with two columns, "Feature ID" matching the names of the alignment indices, and a second character column supplying the taxon identifying information.
accession_path	(character) Filepath to NCBI accessions SQL database. See taxonomizr::prepareDatabase().

priors_df	data.frame containing priors data. The data.frame consists of two columns, 'species' containing species name, and 'prior_weights' containing the prior weights (as a percent; integer).
tmp_dir	Path to a directory to which bam and updated bam files can be saved. Required.
out_dir	The directory to which the .csv output file will be output. Defaults to dirname(input_file).
convEM	The convergence parameter of the EM algorithm. Default set at 1/10000.
maxitsEM	The maximum number of EM iterations, regardless of whether the convEM is below the threshold. Default set at 50. If set at 0, the algorithm skips the EM step and summarizes the .bam file 'as is'.
update_bam	Whether to update BAM file with new read assignments. Default is FALSE. If TRUE, requires input_type = "bam" such that a BAM file is the input to the function.
num_species_plot	The number of genome coverage plots to be saved. Default is NULL, which saves coverage plots for the ten most highly abundant species.
group_by_taxa	Character. Taxonomy level at which accessions should be grouped. Defaults to "species"
quiet	Turns off most messages. Default is TRUE.

Value

This function exports a .csv file with annotated read counts to genomes with mapped reads to the location returned by the function. Depending on the parameters specified, can also output an updated BAM file, and fasta files for additional analysis downstream.

Examples

```
#### Align reads to reference library and then apply metascope_id()
## Assuming filtered bam files already exist

## Create temporary directory
file_temp <- tempfile()
dir.create(file_temp)

## Get temporary accessions database
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

#### Subread aligned bam file

## Create object with path to filtered subread csv.gz file
filt_file <- "subread_target.filtered.csv.gz"
bamPath <- system.file("extdata", filt_file, package = "MetaScope")
file.copy(bamPath, file_temp)

## Run metascope id with the aligner option set to subread
metascope_id(input_file = file.path(file_temp, filt_file),
             aligner = "subread", num_species_plot = 0,
             input_type = "csv.gz", accession_path = tmp_accession)

#### Bowtie 2 aligned .csv.gz file

## Create object with path to filtered bowtie2 bam file
bowtie_file <- "bowtie_target.filtered.csv.gz"
```

```

bamPath <- system.file("extdata", bowtie_file, package = "MetaScope")
file.copy(bamPath, file_temp)

## Run metascope id with the aligner option set to bowtie2
metascope_id(file.path(file_temp, bowtie_file), aligner = "bowtie2",
              num_species_plot = 0, input_type = "csv.gz",
              accession_path = tmp_accession)

## Remove temporary directory
unlink(file_temp, recursive = TRUE)

```

meta_demultiplex

Demultiplexing sequencing reads

Description

Function for demultiplexing sequencing reads arranged in a common format provided by sequencers (such as Illumina) generally for 16S data. This function takes a matrix of sample names/barcodes, a .fastq file of barcodes by sequence header, and a .fastq file of reads corresponding to the barcodes. Based on the barcodes given, the function extracts all reads for the indexed barcode and writes all the reads from that barcode to separate .fastq files.

Usage

```

meta_demultiplex(
  barcodeFile,
  indexFile,
  readFile,
  rcBarcodes = TRUE,
  location = NULL,
  threads = 1,
  hammingDist = 0,
  quiet = TRUE
)

```

Arguments

barcodeFile	Path to a file containing a .tsv matrix with a header row, and then sample names (column 1) and barcodes (column 2).
indexFile	Path to a .fastq file that contains the barcodes for each read. The headers should be the same (and in the same order) as readFile, and the sequence in the indexFile should be the corresponding barcode for each read. Quality scores are not considered.
readFile	Path to the sequencing read .fastq file that corresponds to the indexFile.
rcBarcodes	Should the barcode indexes in the barcodeFile be reverse complemented to match the sequences in the indexFile? Defaults to TRUE.
location	A directory location to store the demultiplexed read files. Defaults to generate a new temporary directory.

threads	The number of threads to use for parallelization (BiocParallel). This function will parallelize over the barcodes and extract reads for each barcode separately and write them to separate demultiplexed files.
hammingDist	Uses a Hamming Distance or number of base differences to allow for inexact matches for the barcodes/indexes. Defaults to 0. Warning: if the Hamming Distance is ≥ 1 and this leads to inexact index matches to more than one barcode, that read will be written to more than one demultiplexed read files.
quiet	Turns off most messages. Default is TRUE.

Value

Returns multiple .fastq files that contain all reads whose index matches the barcodes given. These files will be written to the location directory, and will be named based on the given sampleNames and barcodes, e.g. './demultiplex_fastq/SampleName1_GGAATTATCGGT.fastq.gz'

Examples

```
## Get barcode, index, and read data locations
barcodePath <- system.file("extdata", "barcodes.txt", package = "MetaScope")
indexPath <- system.file("extdata", "virus_example_index.fastq",
                        package = "MetaScope")
readPath <- system.file("extdata", "virus_example.fastq",
                      package = "MetaScope")

## Demultiplex
demult <- meta_demultiplex(barcodePath, indexPath, readPath, rcBarcodes = FALSE,
                          hammingDist = 2)

demult
```

mk_bowtie_index

Make a Bowtie2 index

Description

This function is a wrapper for the `Rbowtie2::bowtie2_build` function. It will create either small (.bt2) or large Bowtie2 indexes (.bt2l) depending on the combined size of the reference fasta files.

Usage

```
mk_bowtie_index(
  ref_dir,
  lib_dir,
  lib_name,
  bowtie2_build_options,
  threads = 1,
  overwrite = FALSE
)
```

Arguments

ref_dir	The path to the directory that contains the reference files either uncompressed or compressed (.gz). NOTE: This directory should contain only the reference fasta files to be indexed.
lib_dir	The path to the directory where Bowtie2 index files should be created.
lib_name	The basename of the index file to be created (without the .bt2 or .bt2l extension)
bowtie2_build_options	Optional: Options that can be passed to the mk_bowtie_index() function. All options should be passed as one string. To see all the available options that can be passed to the function use Rbowtie2::bowtie2_build_usage(). NOTE: Do not specify threads here.
threads	The number of threads available to the function. Default is 1 thread.
overwrite	Whether existing files should be overwritten. Default is FALSE.

Value

Creates the Bowtie2 indexes of the supplied reference .fasta files. Returns the path to the directory containing these files.

Examples

```
#### Create a bowtie index from the example reference library

## Create a temporary directory to store the reference library
ref_temp <- tempfile()
dir.create(ref_temp)

tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download reference genome
download_refseq('Bovismacovirus', reference = FALSE, representative = FALSE,
               out_dir = ref_temp, compress = TRUE, patho_out = FALSE,
               caching = TRUE, accession_path = tmp_accession)

## Create the reference library index files in the current directory
mk_bowtie_index(ref_dir = ref_temp, lib_dir = ref_temp,
               lib_name = "target", threads = 1, overwrite = FALSE)

## Remove temporary directory
unlink(ref_temp, recursive = TRUE)
```

mk_subread_index

*Make a Subread index***Description**

This function is a wrapper for the Rsubread::buildindex function. It will generate one or more Subread indexes from a .fasta file. If the library is too large (default >4GB) it will automatically be split into multiple indexes, with _1, _2, etc at the end of the ref_lib basename.

Usage

```
mk_subread_index(ref_lib, split = 4, mem = 8000, quiet = TRUE)
```

Arguments

<code>ref_lib</code>	The name/location of the reference library file, in (uncompressed) .fasta format.
<code>split</code>	The maximum allowed size of the genome file (in GB). If the <code>ref_lib</code> file is larger than this, the function will split the library into multiple parts.
<code>mem</code>	The maximum amount of memory (in MB) that can be used by the index generation process (used by the <code>Rsubread::buildindex</code> function).
<code>quiet</code>	Turns off most messages. Default is TRUE.

Value

Creates one or more Subread indexes for the supplied reference .fasta file. If multiple indexes are created, the libraries will be named the `ref_lib` basename + "_1", "_2", etc. The function returns the names of the folders holding these files.

Examples

```
#### Create a subread index from the example reference library

## Create a temporary directory to store the reference library
ref_temp <- tempfile()
dir.create(ref_temp)
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")

## Download reference genome
out_fasta <- download_refseq('Orthoebolavirus zairense', reference = FALSE,
                             representative = FALSE, out_dir = ref_temp,
                             compress = TRUE, patho_out = FALSE,
                             caching = TRUE, accession_path = tmp_accession)

## Make subread index of reference library
mk_subread_index(out_fasta)
unlink(ref_temp)
```

remove_matches

Helper function to remove reads matched to filter libraries

Description

Using the `filter_host()` function, we align our sequencing sample to all filter libraries of interest. The `remove_matches()` function allows for removal of any target reads that are also aligned to filter libraries.

Usage

```
remove_matches(
  reads_bam,
  read_names,
  output,
  YS,
  threads,
  aligner,
  make_bam,
  quiet
)
```

Arguments

reads_bam	The name of a merged, sorted .bam file that has previously been aligned to a reference library. Likely, the output from running an instance of <code>align_target()</code> .
read_names	A list of target query names from reads_bam that have also aligned to a filter reference library. Each list element should be a vector of read names.
output	The name of the .bam or .csv.gz file that to which the filtered alignments will be written.
YS	yieldSize, an integer. The number of alignments to be read in from the bam file at once for chunked functions. Default is 100000.
threads	The number of threads to be used in filtering the bam file. Default is 1.
aligner	The aligner which was used to create the bam file.
make_bam	Logical, whether to also output a bam file with host reads filtered out. A .csv.gz file will be created instead if FALSE. Creating a bam file is costly on resources over creating a compressed csv file with only relevant information, so default is FALSE.
quiet	Turns off most messages. Default is TRUE.

Details

This function is not intended for direct use.

Value

Depending on input make_bam, either the name of a filtered, sorted .bam file written to the user's current working directory, or an RDS file containing a data frame of only requisite information to run `metascope_id()`.

taxid_to_name	<i>Converts NCBI taxonomy ID to scientific name</i>
---------------	---

Description

Converts NCBI taxonomy ID to scientific name

Usage

```
taxid_to_name(taxids, accession_path)
```

Arguments

`taxids` List of NCBI taxids to convert to scientific name

`accession_path` (character) Filepath to NCBI accessions SQL database. See `taxonomizr::prepareDatabase()`.

Value

Returns a dataframe of blast results for a metascope result

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