Package 'MetaScope'

June 19, 2025

```
sequencing microbiome data
Version 1.9.3
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.
License GPL (>= 3)
URL https://github.com/wejlab/metascope
      https://wejlab.github.io/metascope-docs/
BugReports https://github.com/wejlab/MetaScope/issues
Depends R (>= 4.4.0)
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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/metascopehttps://wejlab.github.io/metascope-docs/
- Report bugs at https://github.com/wejlab/MetaScope/issues

align_details

A universal parameter settings object for Rsubread alignment

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.

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

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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	3
	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.

align_target_bowtie 5

Examples

```
#### Align example reads to an example reference library using Rsubread
## Create temporary directory
target_ref_temp <- tempfile()</pre>
dir.create(target_ref_temp)
tax <- "Ovine atadenovirus D"
## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")</pre>
## Download genome
all_ref <- MetaScope::download_refseq(tax,</pre>
                                        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)</pre>
## Get path to example reads
readPath <- system.file("extdata", "reads.fastq",</pre>
                         package = "MetaScope")
## Copy the example reads to the temp directory
refPath <- file.path(target_ref_temp, "reads.fastq")</pre>
file.copy(from = readPath, to = refPath)
## Modify alignment parameters object
data("align_details")
align_details[["type"]] <- "rna"</pre>
align_details[["phredOffset"]] <- 50</pre>
# Just to get it to align - toy example!
align_details[["maxMismatches"]] <- 100</pre>
## Run alignment
target_map <- align_target(refPath,</pre>
                            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_target_bowtie 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

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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.

1ibs 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 -scoremin 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.

align_target_bowtie 7

```
#### Align example reads to an example reference library using Rbowtie2
## Create temporary directory to store file
target_ref_temp <- tempfile()</pre>
dir.create(target_ref_temp)
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")</pre>
## Dowload 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()</pre>
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()</pre>
dir.create(output_temp)
## Get path to example reads
readPath <- system.file("extdata", "virus_example.fastq",</pre>
                        package = "MetaScope")
## Align to target genomes
target_map <-</pre>
 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)
```

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bam_reheader_R

Replace the header from a .bam file

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.

bt2_16S_params

A universal parameter object for Bowtie 2 16S alignment

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

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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

A universal parameter object for Bowtie 2 metagenomic alignment where the host genome is thought to be absent from the reference database

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.

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

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bt2_regular_params	A universal parameter object for Bowtie 2 metagenomic or non-16S alignment
--------------------	--

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")
```

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 11

|--|

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 com-

bine 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.

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
)
```

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Arguments

meta_counts A vector of filepaths to the counts ID CSVs output by metascope_id().

 $annot_path \qquad \qquad \text{The filepath to the CSV annotation file for the samples. This CSV metadata/annotation}$

file should contain at least two columns, one with names of all samples WITH-

OUT the extension listed in 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.

cope_id.csv".

qiime_biom_out 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.

 $\verb|path_to_write| If \verb|qiime_biom_out| = \verb|TRUE|, where should output| QIIME files be written? Should$

be a character string of the folder path. Default is '.', i.e. the current working

directory.

accession_path (character) Path to taxonomizr accessions. See taxonomizr::prepareDatabase().

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").

```
tempfolder <- tempfile()</pre>
dir.create(tempfolder)
# Create three different samples
samp_names <- c("X123", "X456", "X789")</pre>
all_files <- file.path(tempfolder,</pre>
                        paste0(samp_names, ".csv"))
create_IDcsv <- function (out_file) {</pre>
  final_taxids <- c("273036", "418127", "11234")
  final_genomes <- c(</pre>
    "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)</pre>
  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))</pre>
# Create annotation data for samples
annot_dat <- file.path(tempfolder, "annot.csv")</pre>
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")</pre>
# Convert samples to MAE
outMAE <- convert_animalcules(meta_counts = out_files,</pre>
                               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 WITH-OUT 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.

end_string The end string used at the end of the metascope id files. Default is ".metascope id.csv". qiime_biom_out 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. path_to_write If qiime_biom_out = 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. Whether to use BiocFileCache when downloading genomes. Default is FALSE.

caching

Value

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

```
tempfolder <- tempfile()</pre>
dir.create(tempfolder)
# Create three different samples
samp_names <- c("X123", "X456", "X789")</pre>
all_files <- file.path(tempfolder,</pre>
                        paste0(samp_names, ".csv"))
create_IDcsv <- function (out_file) {</pre>
  final_taxids <- c("AY846380.1.2583", "AY909584.1.2313", "HG531388.1.1375")
  final_genomes <- rep("Genome name", 3)</pre>
  best_hit <- sample(seq(100, 1050), 3)</pre>
  proportion <- best_hit/sum(best_hit) |> round(2)
  EMreads <- best_hit + round(runif(3), 1)</pre>
  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))</pre>
# Create annotation data for samples
annot_dat <- file.path(tempfolder, "annot.csv")</pre>
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)
# Convert samples to MAE
outMAE <- convert_animalcules_silva(meta_counts = out_files,</pre>
                                      annot_path = annot_dat,
```

16 count_matches

count_matches

Count the number of base lengths in a CIGAR string for a given operation

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.

```
# 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")</pre>
```

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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")
```

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".

Value

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

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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

Download RefSeq genome libraries

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 incon-

sistencies. All available taxonomies are visible by accessing the MetaScope:::taxonomy_table

object included in the package.

reference Download only RefSeq reference genomes? Defaults to TRUE. Automatically set

to TRUE if representative = TRUE.

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 outpute files compatible with PathoScope? Defaults to FALSE.

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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 taxonomzr::prepareDatabase().

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 path_out = TRUE.

Examples

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,
```

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```
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 bar-

codes list.

index A Biostrings::DNAStringSet that contains the read headers and barcode se-

quence for each header in the sequence slot.

reads A Biostrings::QualityScaledXStringSet that has the same headers and or-

der 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 './demultiplex_fastq'

rcBarcodes Should the barcode indices in the barcodes list be reverse complemented to

match the sequences in the index DNAStringSet? Defaults to TRUE.

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 TRUE.

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'

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filter_host

Use Rsubread to align reads against one or more filter libraries and subsequently remove mapped reads

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

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 align_target().

Path to the directory that contains the filter Subread index files.

The basename of the filter libraries (without index extension).

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.

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output The desired name of the output .bam or .csv.gz file. Extension is automatically

defined by whether make_bam = TRUE. Default is the basename of unfiltered_bam

+ .filtered + 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.

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 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().

```
#### 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()</pre>
dir.create(filter_ref_temp)
## Create temporary taxonomizr accession
tmp_accession <- system.file("extdata", "example_accessions.sql", package = "MetaScope")</pre>
## Download filter genome
all_species <- c("Staphylococcus aureus subsp. aureus str. Newman")</pre>
all_ref <- vapply(all_species, MetaScope::download_refseq,</pre>
                   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))</pre>
## Get path to example reads
readPath <- system.file("extdata", "subread_target.bam",</pre>
                         package = "MetaScope")
## Copy the example reads to the temp directory
refPath <- file.path(filter_ref_temp, "subread_target.bam")</pre>
file.copy(from = readPath, to = refPath)
utils::data("align_details")
```

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```
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)</pre>
```

filter_host_bowtie

Use Rbowtie2 to align reads against one or more filter libraries and subsequently remove mapped reads

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

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 align_target_bowtie().

1ib_dir Path to the directory that contains the filter Bowtie2 index files.

The basename of the filter libraries (without .bt2 or .bt2l extension).

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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 make_bam = TRUE. Default is the basename of unfiltered_bam

+ .filtered + extension.

bowtie2_options

Optional: Additional parameters that can be passed to the filter_host_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 parameters that

should NOT be specified here is the threads.

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 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 -scoremin 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().

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```
## Create temp directory to store the indices
index_temp <- tempfile()</pre>
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()</pre>
dir.create(output_temp)
## Get path to example bam
bamPath <- system.file("extdata", "bowtie_target.bam",</pre>
                        package = "MetaScope")
target_copied <- file.path(output_temp, "bowtie_target.bam")</pre>
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

 ${\tt bamfile}$

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

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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.

get_children

Get child nodes from NCBI taxonomy

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.

```
## 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')
```

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locations

Helper Function for MetaScope ID

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

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.

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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 destina-

tion. 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_id

Identify which genomes are represented in a processed sample

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,
  tmp_dir = dirname(input_file),
  out_dir = dirname(input_file),
  convEM = 1/10000,
  maxitsEM = 25,
  update_bam = FALSE,
  num_species_plot = NULL,
  out_fastas = FALSE,
  num\_genomes = 100,
  num_reads = 50,
  quiet = TRUE
)
```

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Arguments

input_file The .bam or .csv.gz file of sample reads to be identified. Extension of file input. Should be either "bam" or "csv.gz". Default is "csv.gz". input_type 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 Greengenes2) 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 taxonomzr::prepareDatabase(). tmp_dir Path to a directory to which bam and updated bam files can be saved. Required. The directory to which the .csv output file will be output. Defaults to dirname(input_file). out_dir 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 threshhold. Default set at 50. If set at 0, the algorithm skips the EM step and summarizes the .bam file 'as is'. Whether to update BAM file with new read assignments. Default is FALSE. If update_bam 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.

out_fastas Logical, whether or not to output fasta files of reads. Default is FALSE.

num_genomes Number of genomes to output fasta files for out_fastas. Default is 100.

Number of reads per genome per fasta file for out_fastas. Default is 50.

quiet Turns off most messages. Default is TRUE.

Value

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

```
#### 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")</pre>
```

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```
#### Subread aligned bam file
## Create object with path to filtered subread csv.gz file
filt_file <- "subread_target.filtered.csv.gz"</pre>
bamPath <- system.file("extdata", filt_file, package = "MetaScope")</pre>
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"</pre>
bamPath <- system.file("extdata", bowtie_file, package = "MetaScope")</pre>
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
)
```

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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

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.

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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.

1ib_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.

mk_subread_index 33

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

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

34 remove_matches

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 align_target().
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().

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