

# Package ‘MetaScope’

January 11, 2024

**Type** Package

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

**Version** 1.3.3

**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 (>= 4.3.0)

**Imports** BiocFileCache, Biostrings, data.table, dplyr, ggplot2, magrittr, Matrix, MultiAssayExperiment, qtlMatrix, Rbowtie2, readr, rlang, Rsamtools, S4Vectors, stringr, SummarizedExperiment, taxize, tidyr, tools

**Suggests** BiocStyle, biomformat, knitr, lintr, plyr, R.utils, rmarkdown, Rsubread, spelling, sys, testthat, usethis

**Enhances** BiocParallel

**VignetteBuilder** knitr

**BiocType** Software

**biocViews** MicrobiomeData, ReproducibleResearch, SequencingData

**Encoding** UTF-8

**Language** en-US

**LazyData** FALSE

**RoxygenNote** 7.2.3

**git\_url** <https://git.bioconductor.org/packages/MetaScope>

**git\_branch** devel

**git\_last\_commit** 15cc8fb

**git\_last\_commit\_date** 2024-01-10

**Repository** Bioconductor 3.19

**Date/Publication** 2024-01-11

**Author** Aubrey Odom [aut, cre] (<<https://orcid.org/0000-0001-7113-7598>>),  
 Rahul Varki [aut],  
 W. Evan Johnson [aut] (<<https://orcid.org/0000-0002-6247-6595>>),  
 Howard Fan [ctb]

**Maintainer** Aubrey Odom <aodom@bu.edu>

## Table of contents:

MetaScope-package . . . . .	3
align_details . . . . .	3
align_target . . . . .	4
align_target_bowtie . . . . .	6
bam_reheader_R . . . . .	8
bt2_16S_params . . . . .	9
bt2_missing_params . . . . .	10
bt2_regular_params . . . . .	10
check_samtools_exists . . . . .	11
combined_header . . . . .	11
convert_animalcules . . . . .	12
count_matches . . . . .	14
demultiplex . . . . .	15
download_refseq . . . . .	16
extract_reads . . . . .	18
filter_host . . . . .	19
filter_host_bowtie . . . . .	22
filter_unmapped_reads . . . . .	24
get_children . . . . .	25
locations . . . . .	26
merge_bam_files . . . . .	26
metascope_id . . . . .	27
mk_bowtie_index . . . . .	29
mk_subread_index . . . . .	30
remove_matches . . . . .	31
<b>Index</b>	<b>33</b>

---

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

---

## 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.

## Author(s)

**Maintainer:** Aubrey Odom <aodom@bu.edu> ([ORCID](#))

Authors:

- Rahul Varki <rvarki@bu.edu>
- W. Evan Johnson <wej@bu.edu> ([ORCID](#))

Other contributors:

- Howard Fan <hjfan@bu.edu> [contributor]

## See Also

Useful links:

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

---

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")
```

---

<code>align_target</code>	<i>Align microbiome reads to a set of reference libraries</i>
---------------------------	---------------------------------------------------------------

---

## 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

<code>read1</code>	Path to the <code>.fastq</code> file to align.
<code>read2</code>	Optional: Location of the mate pair <code>.fastq</code> file to align.
<code>lib_dir</code>	Path to the index files for all libraries.
<code>libs</code>	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 <code>lib_dir = NULL</code> . Default is <code>lib_dir = NULL</code> .
<code>threads</code>	The number of threads that can be utilized by the function. Default is 1 thread.
<code>align_file</code>	The basename of the output alignment file (without trailing <code>.bam</code> 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)

## Download genome
tax <- "Ovine atadenovirus D"
all_ref <- MetaScope::download_refseq(tax,
                                     reference = FALSE,
                                     representative = FALSE,
                                     compress = TRUE,
                                     out_dir = target_ref_temp,
                                     caching = TRUE)

## 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\_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 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)

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

## 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

*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	<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\_samtools\_exists *Check if samtools exists on the system*

---

**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 *Create a combined .bam header*

---

**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 = ".",
  NCBI_key = NULL,
  num_tries = 3
)
```

**Arguments**

meta_counts	A vector of filepaths 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 ".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.
NCBI_key	(character) NCBI Entrez API key. optional. See taxize::use_entrez(). Due to the high number of requests made to NCBI, this function will be less prone to errors if you obtain an NCBI key.
num_tries	(numeric) Number of attempts to get UID.

### Value

Returns a multi-assay experiment file of combined sample counts data and/or biom file and mapping file for analysis with QIIME. The multi-assay experiment will have assays for the counts ("MGX"), log counts, CPM, and log CPM.

### 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)

# 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,
                              NCBI_key = NULL)

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))
```

---

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

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

## Arguments

barcodeFile	File name for a file containing a .tsv matrix with a header row, and then sample names (column 1) and barcodes (column 2).
indexFile	Location 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	Location 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 <- demultiplex(barcodePath, indexPath, readPath, rcBarcodes = FALSE,
                    hammingDist = 2)

demult
```

---

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/\\*\\*ki](ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/**ki) 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
)
```

**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, <code>reference</code> 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 <code>BiocFileCache</code> when downloading genomes. Default is FALSE.
quiet	Turns off most messages. Default is TRUE.

**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
```

```
## Download all RefSeq reference Shotokuvirae kingdom genomes
download_refseq('Shotokuvirae', reference = TRUE, representative = FALSE,
               out_dir = NULL, compress = TRUE, patho_out = FALSE,
               caching = TRUE)
```

---

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 './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 $\geq 1$ 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'

### 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

*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

<code>reads_bam</code>	The name of a merged, sorted <code>.bam</code> file that has previously been aligned to a reference library. Likely, the output from running an instance of <code>align_target()</code> .
<code>lib_dir</code>	Path to the directory that contains the filter <code>Subread</code> index files.
<code>libs</code>	The basename of the filter libraries (without index extension).
<code>make_bam</code>	Logical, whether to also output a <code>bam</code> file with host reads filtered out. A <code>.csv.gz</code> file will be created instead if <code>FALSE</code> . Creating a <code>bam</code> file is costly on resources over creating a compressed <code>csv</code> file with only relevant information, so default is <code>FALSE</code> .
<code>output</code>	The desired name of the output <code>.bam</code> or <code>.csv.gz</code> 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>subread_options</code>	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.
<code>YS</code>	<code>yieldSize</code> , an integer. The number of alignments to be read in from the <code>bam</code> file at once for chunked functions. Default is 100000.
<code>threads</code>	The amount of threads available for the function. Default is 1 thread.
<code>quiet</code>	Turns off most messages. Default is <code>TRUE</code> .

## 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)

## 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,
                 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      *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

<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.
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 --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)

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

## 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.

---

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 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')
```

---

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**

<code>bam_files</code>	A list of file names for the .bam files to be merged.
<code>destination</code>	A file name and location for the merged .bam file.
<code>head_file</code>	A file name and location for the combined header file. Defaults to the destination. For example, 'example.bam' will be written as 'example.bam'.
<code>quiet</code>	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.

---

<code>metascope_id</code>	<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,
  NCBI_key = NULL,
  out_dir = dirname(input_file),
  convEM = 1/10000,
  maxitsEM = 25,
  num_species_plot = NULL,
  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 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.
NCBI_key	(character) NCBI Entrez API key. optional. See taxize::use_entrez(). Due to the high number of requests made to NCBI, this function will be less prone to errors if you obtain an NCBI key. You may enter the string as an input or set it as ENTREZ_KEY in .Renviron.
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'
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.
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.

**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)

#### 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 metaspice id with the aligner option set to subread
metaspice_id(input_file = file.path(file_temp, filt_file),
             aligner = "subread", num_species_plot = 0,
             input_type = "csv.gz")

#### 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 metaspice id with the aligner option set to bowtie2
metaspice_id(file.path(file_temp, bowtie_file), aligner = "bowtie2",
             num_species_plot = 0, input_type = "csv.gz")

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

```

---

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)

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

## 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**

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.

**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)

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

## 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 <code>reads_bam</code> 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	<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 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()`.



# Index

## \* datasets

- [align\\_details](#), 3
- [bt2\\_16S\\_params](#), 9
- [bt2\\_missing\\_params](#), 10
- [bt2\\_regular\\_params](#), 10

## \* internal

- [MetaScope-package](#), 3

- [align\\_details](#), 3
- [align\\_target](#), 4
- [align\\_target\\_bowtie](#), 6

- [bam\\_reheader\\_R](#), 8
- [bt2\\_16S\\_params](#), 9
- [bt2\\_missing\\_params](#), 10
- [bt2\\_regular\\_params](#), 10

- [check\\_samtools\\_exists](#), 11
- [combined\\_header](#), 11
- [convert\\_animalcules](#), 12
- [count\\_matches](#), 14

- [demultiplex](#), 15
- [download\\_refseq](#), 16

- [extract\\_reads](#), 18

- [filter\\_host](#), 19
- [filter\\_host\\_bowtie](#), 22
- [filter\\_unmapped\\_reads](#), 24

- [get\\_children](#), 25

- [locations](#), 26

- [merge\\_bam\\_files](#), 26
- [MetaScope \(MetaScope-package\)](#), 3
- [MetaScope-package](#), 3
- [metascope\\_id](#), 27
- [mk\\_bowtie\\_index](#), 29
- [mk\\_subread\\_index](#), 30

- [remove\\_matches](#), 31