

Package ‘rsahmi’

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Title Single-Cell Analysis of Host-Microbiome Interactions

Version 0.0.2

Description A computational resource designed to accurately detect microbial nucleic acids while filtering out contaminants and false-positive taxonomic assignments from standard transcriptomic sequencing of mammalian tissues. For more details, see Ghaddar (2023) <[doi:10.1038/s43588-023-00507-1](https://doi.org/10.1038/s43588-023-00507-1)>. This implementation leverages the 'polars' package for fast and systematic microbial signal recovery and denoising from host tissue genomic sequencing.

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Imports blit (>= 0.1.0), cli, rlang (>= 1.1.0), ShortRead, utils

Suggests polars (>= 0.17.0)

Additional_repositories <https://community.r-multiverse.org>

URL <https://github.com/Yunuuuu/rsahmi>

BugReports <https://github.com/Yunuuuu/rsahmi/issues>

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blsd	<i>Barcode level signal denoising</i>
------	---------------------------------------

Description

True taxa are detected on multiple barcodes and with a proportional number of total and unique k-mer sequences across barcodes, measured as a significant Spearman correlation between the number of total and unique k-mers across barcodes. ($\text{padj} < 0.05$)

Usage

```
blsd(
  kmer,
  method = "spearman",
  ...,
  p.adjust = "BH",
  min_kmer_len = 3L,
  min_number = 3L
)
```

Arguments

kmer	kmer data returned by prep_dataset() .
method	A character string indicating which correlation coefficient is to be used for the test. One of "pearson", "kendall", or "spearman", can be abbreviated.
...	Other arguments passed to cor.test .
p.adjust	Pvalue correction method, a character string. Can be abbreviated. Details see p.adjust .
min_kmer_len	An integer, the minimal number of kmer to filter taxa. SAHMI use 2.
min_number	An integer, the minimal number of cell per taxid. SAHMI use 4.

Value

A polars [DataFrame](#)

See Also

<https://github.com/sjdlabgroup/SAHMI>

Examples

```
## Not run:
# 1. `sahmi_datasets` should be the output of all samples from
#     `prep_dataset()`
# 2. `real_taxids_slsc` should be the output of `slsc()`
umi_list <- lapply(sahmi_datasets, function(dataset) {
  # Barcode level signal denoising (barcode k-mer correlation test)
  blsc <- blsc(dataset$kmers)
  real_taxids <- blsc$filter(pl$col("padj"))$get_column("taxid")
  # only keep taxids pass Sample level signal denoising
  real_taxids <- real_taxids$filter(real_taxids$is_in(real_taxids_slsc))
  # remove contaminants
  real_taxids <- real_taxids$filter(
    real_taxids$is_in(attr(truly_microbe, "truly"))
  )
  # filter UMI data
  dataset$umi$filter(pl$col("taxid")$is_in(real_taxids))
})

## End(Not run)
```

 extractor

Extract reads and output from Kraken

Description

Extract reads and output from Kraken

Usage

```
extract_taxids(
  kraken_report,
  taxon = c("d__Bacteria", "d__Fungi", "d__Viruses")
)

extract_kraken_output(
  kraken_out,
  taxids,
  odir,
  ofile = "kraken_microbiome_output.txt",
  ...
)

extract_kraken_reads(
```

```

    kraken_out,
    reads,
    ofile = NULL,
    odir = getwd(),
    threads = NULL,
    ...,
    envpath = NULL,
    seqkit = NULL
)

```

Arguments

kraken_report	The path to kraken report file.
taxon	An atomic character specify the taxa name wanted. Should follow the kraken style, connected by rank codes, two underscores, and the scientific name of the taxon (e.g., "d__Viruses")
kraken_out	The path to kraken output file.
taxids	A character specify NCBI taxonomy identifier to extract.
odir	A string of directory to save the ofile.
ofile	A string of file save the kraken output of specified taxids.
...	- extract_kraken_output: Additional arguments passed to <code>sink_csv()</code>. - extract_kraken_reads: Additional arguments passed to <code>cmd_run()</code> method.
reads	The original fastq files (input in kraken2). You can pass two paired-end files directly.
threads	Number of threads to use, see <code>blit::cmd_help(blit::seqkit("grep"))</code> .
envpath	A string of path to be added to the environment variable PATH.
seqkit	A string of path to seqkit command.

Value

- `extract_taxids`: An atomic character vector of taxon identifiers.
- `extract_kraken_output`: A polars [DataFrame](#).
- `extract_kraken_reads`: Exit status invisibly.

See Also

<https://github.com/DerrickWood/kraken2/blob/master/docs/MANUAL.markdown>

Examples

```

## Not run:
# For 10x Genomic data, `fq1` only contain barcode and umi, but the official
# didn't give any information for this. In this way, I prefer using
# `umi-tools` to transform the `umi` into fq2 and then run `rsahmi` with
# only fq2.

```

```

blit::kraken2(
  fq1 = fq1,
  fq2 = fq2,
  classified_out = "classified.fq",
  # Number of threads to use
  blit::arg("--threads", 10L, format = "%d"),
  # the kraken database
  blit::arg("--db", kraken_db),
  "--use-names", "--report-minimizer-data",
) |> blit::cmd_run()

# `kraken_report` should be the output of `blit::kraken2()`
taxids <- extract_taxids(kraken_report = "kraken_report.txt")

# 1. `kraken_out` should be the output of `blit::kraken2()`
# 2. `taxids` should be the output of `extract_taxids()`
# 3. `odir`: the output directory
extract_kraken_output(
  kraken_out = "kraken_output.txt",
  taxids = taxids,
  odir = # specify the output directory
)

# 1. `kraken_out` should be the output of `extract_kraken_output()`
# 2. `fq1` and `fq2` should be the same with `blit::kraken2()`
extract_kraken_reads(
  kraken_out = "kraken_microbiome_output.txt",
  reads = c(fq1, fq2),
  threads = 10L, # Number of threads to use
  # try to change `seqkit` argument into your seqkit path. If `NULL`, the
  # internal will detect it in your `PATH` environment variable
  seqkit = NULL
)

## End(Not run)

```

parse_kraken_report *Parse kraken report file*

Description

Parse kraken report file

Usage

```
parse_kraken_report(kraken_report, intermediate_ranks = TRUE, mpa = FALSE)
```

Arguments

kraken_report The path to kraken report file.

intermediate_ranks A bool indicates whether to include non-traditional taxonomic ranks in output.

mpa A bool indicates whether to use mpa-style.

Value

A polars [DataFrame](#).

See Also

<https://github.com/DerrickWood/kraken2/blob/master/docs/MANUAL.markdown>

prep_dataset	<i>Prepare kraken report, k-mer statistics, UMI data</i>
--------------	--

Description

Three elements returned by this function:

- kreport: Used by [slsd\(\)](#).
- kmer: Used by [blsd\(\)](#). The function count the number of k-mers and unique k-mers assigned to a taxon across barcodes. The cell barcode and unique molecular identifier (UMI) are used to identify unique barcodes and reads. Data is reported for taxa of pre-specified ranks (default genus + species) taking into account all subsequently higher resolution ranks. The output is a table of barcodes, taxonomic IDs, number of k-mers, and number of unique k-mers.
- umi: Used by [taxa_counts\(\)](#).

Usage

```
prep_dataset(
  fa1,
  kraken_report,
  kraken_out,
  fa2 = NULL,
  cb_and_umi = function(sequence_id, read1, read2) {
    list(substring(read1, 1L, 16L),
         substring(read1, 17L, 28L))
  },
  ranks = c("G", "S"),
  kmer_len = 35L,
  min_frac = 0.5,
  exclude = "9606",
  threads = 10L,
  overwrite = TRUE,
```

```

    odir = NULL
  )

  read_dataset(dir)

```

Arguments

fa1, fa2	The path to microbiome fasta 1 and 2 file (returned by extract_kraken_reads()).
kraken_report	The path to kraken report file.
kraken_out	The path of microbiome output file. Usually should be filtered with extract_kraken_output() .
cb_and_umi	A function takes sequence id, read1, read2 and return a list of 2 corresponding to cell barcode and UMI respectively., each should have the same length of the input.
ranks	Taxa ranks to analyze.
kmer_len	Kraken kmer length. Default: 35L, which is the default kmer size of kraken2.
min_frac	Minimum fraction of kmers directly assigned to taxid to use read. Reads with $\leq \text{min_frac}$ of the k-mers map inside the taxon's lineage are also discarded.
exclude	A character of taxid to exclude, for SAHMI, the host taxid. Reads with any k-mers mapped to the exclude are discarded.
threads	Number of threads to use.
overwrite	A bool indicates whether to overwrite the files in odir.
odir	A string of directory to save the results.
dir	A string of directory containing the files returned by prep_dataset.

Value

A list of three polars [DataFrame](#):

- kreport: Used by [slsd\(\)](#).
- kmer: Used by [blsd\(\)](#).
- umi: Used by [taxa_counts\(\)](#).

See Also

<https://github.com/sjdlabgroup/SAHMI>

Examples

```

# for sequence from `umi-tools`, we can use following function
cb_and_umi <- function(sequence_id, read1, read2) {
  out <- lapply(
    strsplit(sequence_id, "_", fixed = TRUE),
    `[`, 2:3
  )
  lapply(1:2, function(i) {
    vapply(out, function(o) as.character(.subset2(o, i)), character(1L))
  })
}

```

```

    })
  }

## Not run:
# 1. `fa1` and `fa2` should be the output of `extract_kraken_reads()`
# 2. `kraken_report` should be the output of `blit::kraken2()`
# 3. `kraken_out` should be the output of `extract_kraken_output()`
# 4. `dir`: you may want to specify the output directory since this process
#     is time-consuming
sahmi_dataset <- prep_dataset(
  fa1 = "kraken_microbiome_reads.fa",
  # if you have paired sequence, please also specify `fa2`,
  # !!! Also pay attention to the file name of `fa1` (add suffix `_1`)
  # if you use paired reads.
  fa2 = "kraken_microbiome_reads_2.fa",
  kraken_report = "kraken_report.txt",
  kraken_out = "kraken_microbiome_output.txt",
  odir = NULL
)
# you may want to prepare all datasets for subsequent workflows.
# `paths` should be the output directory for each sample from
# `blit::kraken2()`, `extract_kraken_output()` and `extract_kraken_reads()`.
sahmi_datasets <- lapply(paths, function(dir) {
  prep_dataset(
    fa1 = file.path(dir, "kraken_microbiome_reads.fa"),
    # fa2 = file.path(dir, "kraken_microbiome_reads_2.fa"),
    kraken_report = file.path(dir, "kraken_report.txt"),
    kraken_out = file.path(dir, "kraken_microbiome_output.txt"),
    odir = dir
  )
})

## End(Not run)

```

remove_contaminants	<i>Identifying contaminants and false positives taxa (cell line quantile test)</i>
---------------------	--

Description

Identifying contaminants and false positives taxa (cell line quantile test)

Usage

```

remove_contaminants(
  kraken_reports,
  study = "current study",
  taxon = c("d__Bacteria", "d__Fungi", "d__Viruses"),
  quantile = 0.95,
  alpha = 0.05,

```

```

    alternative = "greater",
    exclusive = FALSE
  )

```

Arguments

kraken_reports	A character of path to all kraken report files.
study	A string of the study name, used to differentiate with cell line data.
taxon	An atomic character specify the taxa name wanted. Should follow the kraken style, connected by rank codes, two underscores, and the scientific name of the taxon (e.g., "d__Viruses")
quantile	Probabilities with values in $[0, 1]$ specifying the quantile to calculate.
alpha	Level of significance.
alternative	A string specifying the alternative hypothesis, must be one of "two.sided", "greater" (default) or "less". You can specify just the initial letter.
exclusive	A boolean value, indicates whether taxa not found in celllines data should be regarded as truly. Default: FALSE.

Value

A polars [DataFrame](#) with following attributes:

1. pvalues: Quantile test pvalue.
2. exclusive: taxids in current study but not found in cellline data.
3. significant: significant taxids with pvalues < alpha.
4. truly: truly taxids based on alpha and exclusive. If exclusive is TRUE, this should be the union of exclusive and significant, otherwise, this should be the same with significant.

Examples

```

## Not run:
# `paths` should be the output directory for each sample from
# `blit::kraken2()`
truly_microbe <- remove_contaminants(
  kraken_reports = file.path(paths, "kraken_report.txt"),
  quantile = 0.99, exclusive = FALSE
)
microbe_for_plot <- attr(truly_microbe, "truly")[
  order(attr(truly_microbe, "pvalue")[attr(truly_microbe, "truly")])
]
microbe_for_plot <- microbe_for_plot[
  !microbe_for_plot %in% attr(truly_microbe, "exclusive")
]
ggplot(
  truly_microbe$filter(pl$col("taxid")$is_in(microbe_for_plot))$
    to_data_frame(),
  aes(rpmm),
) +

```

```

geom_density(aes(fill = study), alpha = 0.5) +
scale_x_log10() +
facet_wrap(facets = vars(taxa), scales = "free") +
theme(
  strip.clip = "off",
  axis.text = element_blank(),
  axis.ticks = element_blank(),
  legend.position = "inside",
  legend.position.inside = c(1, 0),
  legend.justification.inside = c(1, 0)
)

## End(Not run)

```

slds

Sample level signal denoising

Description

In the low-microbiome biomass setting, real microbes also exhibit a proportional number of total k-mers, number of unique k-mers, as well as number of total assigned sequencing reads across samples; i.e. the following three Spearman correlations are significant when tested using sample-level data provided in Kraken reports: `cor(minimizer_len, minimizer_n_unique)`, `cor(minimizer_len, total_reads)` and `cor(total_reads, minimizer_n_unique)`. ($r1 > 0$ & $r2 > 0$ & $r3 > 0$ & $p1 < 0.05$ & $p2 < 0.05$ & $p3 < 0.05$).

Usage

```

slds(
  kreports,
  method = "spearman",
  ...,
  min_reads = 3L,
  min_minimizer_n_unique = 3L,
  min_number = 3L
)

```

Arguments

<code>kreports</code>	kreports data returned by <code>prep_dataset()</code> for all samples.
<code>method</code>	A character string indicating which correlation coefficient is to be used for the test. One of "pearson", "kendall", or "spearman", can be abbreviated.
<code>...</code>	Other arguments passed to <code>cor.test</code> .
<code>min_reads</code>	An integer, the minimal number of the total reads to filter taxa. SAHMI use 2.
<code>min_minimizer_n_unique</code>	An integer, the minimal number of the unique number of minimizer to filter taxa. SAHMI use 2.
<code>min_number</code>	An integer, the minimal number of samples per taxid. SAHMI use 4.

Value

A polars [DataFrame](#) of correlation coefficient and pvalue for `cor(minimizer_len, minimizer_n_unique)` (r1 and p1), `cor(minimizer_len, total_reads)` (r2 and p2) and `cor(total_reads, minimizer_n_unique)` (r3 and p3).

Examples

```
## Not run:
# `sahmi_datasets` should be the output of all samples from `prep_dataset()`
slsd <- slsd(lapply(sahmi_datasets, `[`, "kreport"))
real_taxids_slsd <- slsd$filter(
  pl$col("r1")$gt(0),
  pl$col("r2")$gt(0),
  pl$col("r3")$gt(0),
  pl$col("p1")$lt(0.05),
  pl$col("p2")$lt(0.05),
  pl$col("p3")$lt(0.05)
)$get_column("taxid")

## End(Not run)
```

taxa_counts

*Quantitation of microbes***Description**

After identifying true taxa, reads assigned to those taxa are extracted and then undergo a series of filters. The cell barcode and UMI are used to demultiplex the reads and create a barcode x taxa counts matrix. The full taxonomic classification of all resulting barcodes and the number of counts assigned to each clade are tabulated.

Usage

```
taxa_counts(umi_list, samples = NULL)
```

Arguments

`umi_list` A list of polars [DataFrame](#) for UMI data returned by [prep_dataset](#).

`samples` A character of sample identifier for each element in `umi_list`.

Value

A polars [DataFrame](#).

See Also

<https://github.com/sjdlabgroup/SAHMI>

Examples

```
## Not run:  
# `umi_list` should be the output of all samples from `prep_dataset()`, and  
# filtered by `sld()` and `blsd()`  
taxa_counts(umi_list, basename(names(umi_list)))  
  
## End(Not run)
```

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