Package ‘ClassifyITS’

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Title Fungal Assignment Pipeline

Version 0.1.0

Description Fungi are ubiquitous in Earth's wonderfully diverse ecosystems. The 'ClassifyITS' package aids in the taxonomic classification of environmental internal transcribed spacer (ITS) short-read barcoding data. Unlike previous methods, it employs taxon-specific e-value and percent identity cutoffs at each taxonomic rank from kingdom to species. The package takes a conservative approach and outputs both graphics and user-friendly files to help users manually inspect fungal operational taxonomic units (OTUs) that fail classification at relevant levels (e.g., Phylum). 'ClassifyITS' is based on taxonomic cutoff criteria from ``The Global Soil Mycobiome consortium dataset for boosting fungal diversity research'' (Fungal Diversity, Tedersoo, 2021, <doi:10.1007/s13225-021-00493-7>) and ``Best practices in metabarcoding of fungi: From experimental design to results'' (Molecular Ecology, Tedersoo, 2022, <doi:10.1111/mec.16460>).

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Imports ggplot2, dplyr, gridExtra, grid, reshape2, data.table, seqinr

Suggests formatR, knitr, rmarkdown

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Author Quinn Moon [aut, cre]

Maintainer Quinn Moon <`qmoon@umich.edu`>

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Contents

`best_hit_taxonomy_assignment`

Hierarchical best-hit taxonomy assignment with per-rank fallback rule

Description

Pass ONLY those OTUs that haven't been assigned already! For each rank, if the best e-value hit is undefined and the second-best hit is defined and at least 60

Usage

```
best_hit_taxonomy_assignment(blast_qc, cutoffs_long, defaults)
```

Arguments

`blast_qc`	A data.frame of BLAST results for query sequences, must include columns for taxonomic ranks and alignment statistics.
`cutoffs_long`	A data.frame specifying per-rank cutoffs for assignment. Must include columns 'rank', 'cutoff_type', and 'cutoff_value'.
`defaults`	A named list of default cutoff values for each rank, used as fallback if no matching cutoff found.

Value

A data.frame containing hierarchical taxonomy assignment for each query sequence.

`check_N` *Check proportion of N bases in each sequence.*

Description

Calculates the proportion of "N" bases (ambiguous bases) in each sequence and flags if above the given threshold.

Usage

```
check_N(rep_seqs, cutoff = 1)
```

Arguments

`rep_seqs`	Character vector, list (e.g., from seqinr::read.fasta(as.string=TRUE)), or (optionally) a DNAStringSet.
`cutoff`	Numeric, percent threshold (default 1).

Value

Data frame with columns: qseqid, N_percent, N_flag.

Examples

```
seqs <- c(seq1 = "ATGCNNNN", seq2 = "NNNNATGC")
check_N(seqs)
check_N(seqs, cutoff = 10)
```

`consensus_taxonomy_assignment` *Per-rank consensus filter for taxonomy assignment*

Description

Only confirms or demotes, never promotes Unclassified.

Usage

```
consensus_taxonomy_assignment(final_table, blast_qc)
```

Arguments

`final_table`	Data frame of taxonomic assignments.
`blast_qc`	Data frame of filtered BLAST hits for each OTU.

Value

Data frame of consensus assignments (same structure as input).

`easy_assignments` *Easy taxonomy assignment for OTUs using BLAST QC output & phylum-specific thresholds.*

Description

Easy taxonomy assignment for OTUs using BLAST QC output & phylum-specific thresholds.

Usage

```
easy_assignments(blast_filtered, cutoffs_file = NULL, default_cutoff = 98)
```

Arguments

`blast_filtered`	QC-filtered BLAST dataframe (with parsed taxonomy columns!)
`cutoffs_file`	Path to taxonomy cutoffs CSV file. If not supplied or invalid, attempts to locate the default file in the package.
`default_cutoff`	Default percent identity cutoff for species assignment (default: 98)

Value

List with assigned_otus_df and remaining_otus_df

`ensure_cols` *Ensure data frame has all required columns (as character)*

Description

Ensure data frame has all required columns (as character)

Usage

```
ensure_cols(df, all_cols)
```

Arguments

`df`	Data frame to fix
`all_cols`	Vector of required columns

Value

Fixed data frame (in correct order, with all columns present)

`ITS_assignment` *Complete Fungal Assignment Pipeline*

Description

Runs all steps: QC, filtering, plotting, assignments; optionally writes outputs.

Usage

```
ITS_assignment(
  blast_file,
  rep_fasta,
  cutoffs_file = NULL,
  cutoff_fraction = 0.6,
  n_cutoff = 1,
  outdir = NULL,
  verbose = FALSE
)
```

Arguments

`blast_file`	Path to BLAST results TSV file
`rep_fasta`	Path to representative sequences FASTA file
`cutoffs_file`	Path to taxonomy cutoffs CSV file (optional; defaults to package example if omitted)
`cutoff_fraction`	Numeric, fraction of median rep-seq length for BLAST filtering (default: 0.6)
`n_cutoff`	Numeric, N base percentage cutoff (default: 1)
`outdir`	Output directory for results. If NULL (default), nothing is written.
`verbose`	Logical; if TRUE emit progress messages. Default FALSE.

Value

Named list of results and (if written) output file paths

`load_and_check` *Load and check BLAST results and rep-seq FASTA*

Description

Load and check BLAST results and rep-seq FASTA

Usage

```
load_and_check(blast_file, rep_fasta, taxonomy_col = "stitle", verbose = FALSE)
```

Arguments

`blast_file`	Path to BLAST results TSV file.
`rep_fasta`	Path to representative sequences FASTA file.
`taxonomy_col`	The column in BLAST file containing taxonomy strings (default "stitle").
`verbose`	Logical; if TRUE, emit progress messages. Default FALSE.

Value

List with BLAST dataframe (kingdom-filtered) and rep_seqs as a named list of DNA strings.

`parse_taxonomy_cutoffs` *Parse taxonomy cutoffs file*

Description

Reads and processes a taxonomy cutoffs CSV for assignment thresholds at various ranks.

Usage

```
parse_taxonomy_cutoffs(cutoffs_file = NULL)
```

Arguments

`cutoffs_file`	Path to a taxonomy cutoffs CSV file. If not supplied or invalid, attempts to locate the default file in the package.

Value

A list with two elements: `long`, a data frame of parsed cutoffs, and `ranks`, the vector of taxonomic ranks.

`plot_alignment_hist` *Create and return alignment length histogram (ggplot object)*

Description

Create and return alignment length histogram (ggplot object)

Usage

```
plot_alignment_hist(blast, rep_seqs, cutoff_fraction = 0.6)
```

Arguments

`blast`	BLAST data frame.
`rep_seqs`	Named list/character vector of DNA strings (from seqinr::read.fasta(as.string = TRUE)).
`cutoff_fraction`	Numeric; fraction of median alignment length for cutoff line. Default 0.6.

Value

A ggplot object.

`safe_rbind_list` *Safely rbinds list of data frames, ensuring columns match*

Description

Safely rbinds list of data frames, ensuring columns match

Usage

```
safe_rbind_list(dfs, all_cols = NULL)
```

Arguments

`dfs`	List of data frames
`all_cols`	Vector of required columns

Value

Combined data frame

`save_taxonomy_graphics` *Save taxonomy summary charts and tables to multi-page PDF*

Description

Save taxonomy summary charts and tables to multi-page PDF

Usage

```
save_taxonomy_graphics(
  all_results,
  hist_plot,
  pdf_file = NULL,
  caption_texts = NULL,
  rank_names = c("Phylum", "Class", "Order", "Family", "Genus", "Species"),
  verbose = FALSE
)
```

Arguments

`all_results`	Combined assignments table from write_initial_assignments
`hist_plot`	ggplot2 object for histogram
`pdf_file`	Output path for multi-page PDF. If NULL (default), no file is written.
`caption_texts`	Vector of captions for PDF pages (optional)
`rank_names`	Vector of rank names (default: c("Phylum",...))
`verbose`	Logical; if TRUE, emit a message when a PDF is written. Default FALSE.

Value

List with plots/tables; includes pdf_file when written.

`trim_alignments` *Trim BLAST alignments by minimum length*

Description

Trim BLAST alignments by minimum length

Usage

```
trim_alignments(blast, rep_seqs, fraction = 0.6)
```

Arguments

`blast`	BLAST data frame.
`rep_seqs`	Named list/character vector of DNA strings (from seqinr::read.fasta(as.string = TRUE)).
`fraction`	Numeric; fraction of the median rep-seq length used as the cutoff. Default 0.6.

Value

Filtered BLAST data frame.

`write_initial_assignments` *Create and write the initial assignments table including drops at all steps*

Description

Create and write the initial assignments table including drops at all steps

Usage

```
write_initial_assignments(
  easy_df,
  consensus_df,
  rep_seqs,
  blast,
  blast_filtered,
  file = NULL,
  verbose = FALSE
)
```

Arguments

`easy_df`	Data frame of easy-assigned OTUs
`consensus_df`	Data frame of consensus-assigned OTUs (hard ones)
`rep_seqs`	DNAStringSet or named character vector of rep seqs
`blast`	Data frame of all BLAST results
`blast_filtered`	Data frame of filtered BLAST results
`file`	Path for output CSV. If NULL (default), no file is written.
`verbose`	Logical; if TRUE emit a message when a file is written. Default FALSE.

Value

Data frame of assignments (written if file is not NULL)

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