

Package ‘immGLIPH’

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Title Grouping of Lymphocyte Interactions by Paratope Hotspots

Version 0.99.5

Description An R implementation of the GLIPH and GLIPH2 algorithms for clustering T cell receptors (TCRs) predicted to bind the same HLA-restricted peptide antigen. Identifies specificity groups based on local (motif-based) and global (sequence-based) CDR3 similarities. Integrates with the scRepertoire ecosystem via immApex for single-cell immune repertoire analysis. Users should cite the original GLIPH algorithm papers: Glanville et al. (2017) <[doi:10.1038/nature22976](https://doi.org/10.1038/nature22976)> and Huang et al. (2020) <[doi:10.1038/s41587-020-0505-4](https://doi.org/10.1038/s41587-020-0505-4)>.

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<https://github.com/BorchLab/scRepertoire>,
<https://github.com/BorchLab/immApex>

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immGLIPH-package

*immGLIPH: Grouping of Lymphocyte Interactions by Paratope Hotspots***Description**

An R implementation of the GLIPH and GLIPH2 algorithms for clustering T cell receptors (TCRs) predicted to bind the same HLA-restricted peptide antigen. Identifies specificity groups based on local (motif-based) and global (sequence-based) CDR3 similarities. Integrates with the scRepertoire ecosystem via immApex for single-cell immune repertoire analysis. Users should cite the original GLIPH algorithm papers: Glanville et al. (2017) [doi:10.1038/nature22976](https://doi.org/10.1038/nature22976) and Huang et al. (2020) [doi:10.1038/s4158702005054](https://doi.org/10.1038/s4158702005054).

Author(s)

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References

Glanville, J. et al. (2017). Identifying specificity groups in the T cell receptor repertoire. *Nature*, 547, 94–98. [doi:10.1038/nature22976](https://doi.org/10.1038/nature22976)

Huang, H. et al. (2020). Analyzing the Mycobacterium tuberculosis immune response by T-cell receptor clustering with GLIPH2 and genome-wide antigen screening. *Nature Biotechnology*, 38, 1194–1202. [doi:10.1038/s4158702005054](https://doi.org/10.1038/s4158702005054)

See Also

Useful links:

- <https://github.com/BorchLab/immGLIPH>
- <https://github.com/BorchLab/scRepertoire>
- <https://github.com/BorchLab/immApex>
- Report bugs at <https://github.com/BorchLab/immGLIPH/issues>

`.check_existing_files` *Check for existing output files*

Description

Check for existing output files

Usage

```
.check_existing_files(result_folder, filenames)
```

Arguments

<code>result_folder</code>	Path to output folder
<code>filenames</code>	Character vector of filenames to check

Value

Logical indicating whether saving should proceed

.cluster_gliph1	<i>GLIPH1-style clustering via igraph connected components</i>
-----------------	--

Description

Builds a clone network from local and global edges, optionally filters by V-gene and donor constraints, constructs an igraph graph, and extracts connected components as convergence groups. Each component becomes one cluster named CRG-<first_CDR3b>.

Usage

```
.cluster_gliph1(
  clone_network,
  sequences,
  not_in_global_ids,
  seqs,
  vgene.info,
  patient.info,
  global_vgene,
  public_tcrs,
  cluster_min_size,
  verbose,
  BPPARAM
)
```

Arguments

clone_network	Data frame. Edge list with columns V1, V2, type (and optionally tag).
sequences	Data frame. Full sample sequences with at least CDR3b and optionally TRBV, patient.
not_in_global_ids	Integer vector. Indices of sequences without global neighbours (used to add singletons).
seqs	Character vector. Unique CDR3b sequences.
vgene.info	Logical. Whether V-gene info is available.
patient.info	Logical. Whether patient info is available.
global_vgene	Logical. Whether global edges require V-gene match.
public_tcrs	Logical. If FALSE, restrict edges to same donor.
cluster_min_size	Integer. Minimum cluster size to retain.
verbose	Logical. Print progress messages.
BPPARAM	A BiocParallelParam object for parallel evaluation.

Value

A list with:

cluster_properties Data frame with cluster_size, tag, members.

cluster_list Named list of data frames (member details).

clone_network Data frame of the final edge list.

save_cluster_list_df Data frame for saving cluster members.

.cluster_glip2	<i>GLIPH2-style clustering by individual motif/struct tags</i>
----------------	--

Description

Each local motif or global struct tag defines its own cluster. Edges are restricted by motif distance for local connections and by BLOSUM62 for global connections. Clusters are built as igraph components within each tag, and enrichment is reassessed per cluster.

Usage

```
.cluster_glip2(
  local_res,
  global_res,
  sequences,
  local_similarities,
  global_similarities,
  global_vgene,
  all_aa_interchangeable,
  structboundaries,
  boundary_size,
  motif_distance_cutoff,
  cluster_min_size,
  boost_local_significance,
  verbose,
  BPPARAM
)
```

Arguments

local_res	Data frame. Selected motifs from local enrichment (must have columns motif, start, stop, num_in_sample, num_in_ref, num_fold, fisher.score, members).
global_res	Data frame. Global cluster list from .global_fisher() (columns: cluster_tag, cluster_size, unique_CDR3b, num_in_ref, fisher.score, aa_at_position, TRBV, CDR3b).
sequences	Data frame. Full sample data.
local_similarities	Logical. Whether local similarities were run.
global_similarities	Logical. Whether global similarities were found.
global_vgene	Logical. Restrict global edges to matching V-gene.

all_aa_interchangeable Logical. BLOSUM62 filtering.
structboundaries Logical. Whether boundary trimming is active.
boundary_size Integer. Boundary trim size.
motif_distance_cutoff Integer. Max positional distance for local motifs.
cluster_min_size Integer. Minimum cluster size.
boost_local_significance Logical. Whether to boost local p-values using germline N-nucleotide information.
verbose Logical. Print progress messages.
BPPARAM A BiocParallelParam object for parallel evaluation.

Value

A list with:

merged_clusters Data frame of cluster properties (type, tag, cluster_size, unique_cdr3_sample, unique_cdr3_ref, OvE, fisher.score, members).
cluster_list Named list of data frames with member details.
clone_network Data frame of edges (V1, V2, type, cluster_tag).
save_cluster_list_df Data frame for saving cluster member details.

.coerce_numeric_cols *Coerce character columns to numeric where possible*

Description

Coerce character columns to numeric where possible

Usage

```
.coerce_numeric_cols(df)
```

Arguments

df A data frame

Value

Data frame with numeric columns where appropriate

<code>.extract_input</code>	<i>Extract TCR input data from various sources</i>
-----------------------------	--

Description

Handles Seurat objects, SingleCellExperiment objects, combineTCR/combineBCR list output, data frames, and character vectors. Uses `immApex::getIR()` when single-cell objects are detected.

Usage

```
.extract_input(input, chains = "TRB")
```

Arguments

<code>input</code>	Input data (data frame, vector, Seurat, SCE, or list)
<code>chains</code>	Chain type for getIR extraction. Default "TRB".

Value

A data frame with standardized column names

<code>.get_blosum_vec</code>	<i>Get BLOSUM62-compatible amino acid pairs</i>
------------------------------	---

Description

Returns a character vector of two-letter amino acid pairs whose BLOSUM62 substitution score is ≥ 0 , derived from the full BLOSUM62 matrix in `immApex::immapex_blosum.pam.matrices`.

Usage

```
.get_blosum_vec()
```

Value

Character vector of AA pair strings (e.g. "AA", "AS").

.get_reference_list	<i>Load reference list (internal, with caching)</i>
---------------------	---

Description

Loads the reference list, using a package-level cache to avoid repeated disk reads or downloads within a single session.

Usage

```
.get_reference_list()
```

Value

The reference list.

.global_cutoff	<i>Global similarity search using Hamming distance cutoff (GLIPH1.0 method)</i>
----------------	---

Description

Global similarity search using Hamming distance cutoff (GLIPH1.0 method)

Usage

```
.global_cutoff(
  seqs,
  motif_region,
  sequences,
  gccutoff,
  global_vgene,
  BPPARAM,
  verbose
)
```

Arguments

seqs	character vector. Unique CDR3b sequences filtered by C/F start/end (if applicable) and minimum length.
motif_region	character vector. The motif region of each sequence in seqs (i.e. with boundaries stripped if structboundaries is active).
sequences	data.frame. The full input data frame containing at least columns CDR3b and optionally TRBV.
gccutoff	numeric. Maximum Hamming distance for two sequences to be considered globally similar.
global_vgene	logical. If TRUE, global connections are restricted to sequence pairs that share a V gene.
BPPARAM	A BiocParallelParam object controlling parallel evaluation (e.g. <code>BiocParallel::MulticoreParam</code>).
verbose	logical. If TRUE, progress messages are printed.

Value

A list with two elements:

edges A `data.frame` with columns V1, V2, and type (always "global"). Each row represents a pair of CDR3b sequences that are globally similar.

not_in_global_ids An integer vector of indices (into seqs) for sequences that have no global neighbour.

`.global_cutoff_immapex`*immApex-accelerated global cutoff via buildNetwork()*

Description

immApex-accelerated global cutoff via buildNetwork()

Usage

```
.global_cutoff_immapex(  
  seqs,  
  motif_region,  
  sequences,  
  gccutoff,  
  global_vgene,  
  verbose  
)
```

Value

A list with edge data and excluded sequence IDs.

`.global_cutoff_stringdist`*stringdist + BiocParallel fallback for global cutoff*

Description

stringdist + BiocParallel fallback for global cutoff

Usage

```
.global_cutoff_stringdist(  
  seqs,  
  motif_region,  
  sequences,  
  gccutoff,  
  global_vgene,  
  BPPARAM,  
  verbose  
)
```

Value

A list with edge data and excluded sequence IDs.

.global_fisher	<i>Global similarity search using structural matching and Fisher's test (GLIPH2)</i>
----------------	--

Description

Identifies globally similar CDR3b sequences by generating "struct" tags (motif region with one variable position replaced by "%"), then testing for enrichment of each struct in the sample set vs. a naive reference database using the hypergeometric distribution (one-sided Fisher's exact test). Optionally filters for BLOSUM62-compatible amino acid substitutions at the variable position.

Usage

```
.global_fisher(
  seqs,
  motif_region,
  sequences,
  refseqs,
  refseqs_motif_region,
  structboundaries,
  boundary_size,
  global_vgene,
  all_aa_interchangeable,
  BPPARAM,
  verbose
)
```

Arguments

seqs	Character vector. Unique CDR3b sequences from the sample.
motif_region	Character vector. Motif regions of the sample sequences (boundaries already stripped if applicable).
sequences	Data frame. Full sample data frame with at least columns CDR3b and (if applicable) TRBV.
refseqs	Data frame. Reference database with at least a CDR3b column.
refseqs_motif_region	Character vector. Motif regions of the reference sequences.
structboundaries	Logical. Whether structural boundaries are applied.
boundary_size	Integer. Number of AAs trimmed from each end.
global_vgene	Logical. If TRUE, restrict edges to pairs sharing a V-gene.
all_aa_interchangeable	Logical. If FALSE, only pairs whose variable-position amino acids have BLOSUM62 ≥ 0 are kept.
BPPARAM	A BiocParallelParam object controlling parallel evaluation.
verbose	Logical. Print progress messages.

Value

A list with elements:

cluster_list Data frame of struct clusters with columns `cluster_tag`, `cluster_size`, `unique_CDR3b`, `num_in_ref`, `fisher.score`, `aa_at_position`, `TRBV`, `CDR3b`.

global_similarities Logical indicating whether any global similarities were found.

<code>.load_reference</code>	<i>Load and prepare reference database</i>
------------------------------	--

Description

Handles both named reference databases and user-provided data frames.

Usage

```
.load_reference(
  refdb_beta,
  accept_CF = TRUE,
  min_seq_length = 8,
  global_vgene = FALSE,
  vgene_stratify = FALSE,
  structboundaries = TRUE,
  boundary_size = 3,
  verbose = TRUE
)
```

Arguments

<code>refdb_beta</code>	Character name or data frame
<code>accept_CF</code>	Filter for C/F start/end
<code>min_seq_length</code>	Minimum sequence length
<code>global_vgene</code>	Whether V-gene info is needed
<code>vgene_stratify</code>	Whether V-gene stratification is needed
<code>structboundaries</code>	Whether to use structural boundaries
<code>boundary_size</code>	Boundary size
<code>verbose</code>	Print messages

Value

A list with `refseqs` (character vector of CDR3b), `ref_vgenes`, `refseqs_motif_region`, and the full reference data frame

`.local_fisher`*Local similarity detection using Fisher's exact test*

Description

Implements the GLIPH2-style Fisher's exact test approach for detecting locally enriched CDR3 motifs in a sample set compared to a reference database.

Usage

```
.local_fisher(
  motif_region,
  refseqs_motif_region,
  seqs,
  refseqs,
  sequences,
  motif_length,
  kmer_mindepth,
  lcminp,
  lcminove,
  discontinuous_motifs,
  motif_distance_cutoff,
  BPPARAM,
  verbose
)
```

Arguments

<code>motif_region</code>	Character vector. Motif regions extracted from sample sequences (e.g. CDR3b with structural boundaries trimmed).
<code>refseqs_motif_region</code>	Character vector. Motif regions extracted from reference sequences.
<code>seqs</code>	Character vector. Unique sample CDR3b sequences.
<code>refseqs</code>	Data frame. Reference database containing at least a CDR3b column.
<code>sequences</code>	Data frame. Sample data containing at least a CDR3b column.
<code>motif_length</code>	Numeric vector. Lengths of motifs to search for (e.g. 2:4).
<code>kmer_mindepth</code>	Numeric. Minimum number of times a motif must be observed in the sample set to be considered.
<code>lcminp</code>	Numeric. Maximum p-value threshold for a motif to be considered significant.
<code>lcminove</code>	Numeric or numeric vector. Minimum fold change threshold(s) for enrichment filtering. If a vector, each element corresponds to the matching entry in <code>motif_length</code> .
<code>discontinuous_motifs</code>	Logical. Whether to include discontinuous motifs in the search.
<code>motif_distance_cutoff</code>	Numeric. Maximum positional distance for motifs to be grouped together. Not used directly in this function but kept for interface consistency.
<code>BPPARAM</code>	A BiocParallelParam object specifying the parallel backend. Defaults to <code>BiocParallel::SerialP</code> .
<code>verbose</code>	Logical. If TRUE, print status messages via <code>message()</code> .

Value

A list with two elements:

selected_motifs Data frame of motifs passing significance and enrichment filters.

all_motifs Data frame of all motifs found in the sample set with associated statistics.

.local_rrs	<i>Local similarity detection via repeated random sampling (GLIPH1-style)</i>
------------	---

Description

Identifies enriched motifs in TCR CDR3b sequences by comparing motif frequencies in the sample set against repeated random subsamples drawn from a naive reference database.

Usage

```
.local_rrs(
  motif_region,
  refseqs_motif_region,
  seqs,
  sequences,
  motif_length,
  sim_depth,
  kmer_mindepth,
  lcminp,
  lcminove,
  discontinuous_motifs,
  cdr3_len_stratify,
  vgene_stratify,
  BPPARAM,
  verbose,
  motif_lengths_list,
  ref_motif_lengths_id_list,
  motif_region_vgenes_list,
  ref_motif_vgenes_id_list,
  lengths_vgenes_list,
  ref_lengths_vgenes_list
)
```

Arguments

<code>motif_region</code>	Character vector. Motif regions extracted from sample sequences.
<code>refseqs_motif_region</code>	Character vector. Motif regions extracted from reference sequences.
<code>seqs</code>	Character vector. Unique sample CDR3b sequences.
<code>sequences</code>	Data frame. Must contain columns CDR3b and TRBV.
<code>motif_length</code>	Numeric vector. Lengths of motifs to search for.
<code>sim_depth</code>	Numeric. Number of repeated random sampling iterations.

kmer_mindepth	Numeric. Minimum number of times a motif must appear in the sample set to be considered.
lcm inp	Numeric. Maximum p-value threshold for a motif to be selected.
lcm inove	Numeric vector. Minimum fold-change (observed / expected) threshold(s). If a single value, the same threshold is applied to all motif lengths.
discontinuous_motifs	Logical. Whether to include discontinuous motifs.
cdr3_len_stratify	Logical. Whether to stratify random subsamples by CDR3 length distribution.
vgene_stratify	Logical. Whether to stratify random subsamples by V-gene usage distribution.
BPPARAM	A BiocParallelParam object controlling parallel execution (default: <code>BiocParallel::bpparam()</code>).
verbose	Logical. If TRUE, emit progress messages.
motif_lengths_list	List. Pre-computed CDR3 length counts from the sample.
ref_motif_lengths_id_list	List. Pre-computed reference indices by CDR3 length.
motif_region_vgenes_list	List. Pre-computed V-gene counts from the sample.
ref_motif_vgenes_id_list	List. Pre-computed reference indices by V-gene.
lengths_vgenes_list	List. Pre-computed joint CDR3-length / V-gene counts from the sample.
ref_lengths_vgenes_list	List. Pre-computed reference indices by joint CDR3-length / V-gene.

Value

A list with three elements:

sample_log Data frame. Rows are "Discovery" followed by one row per simulation; columns are motifs. Values are motif counts.

selected_motifs Data frame of motifs passing enrichment filters with columns motif, counts, num_in_ref, avgRef, topRef, OvE, p.value.

all_motifs Data frame with the same columns as `selected_motifs` but for every motif found.

.parse_sequences	<i>Parse and filter sequences data frame</i>
------------------	--

Description

Consolidates the input preparation logic into a single function.

Usage

```
.parse_sequences(  
  cdr3_sequences,  
  accept_CF = TRUE,  
  min_seq_length = 8,  
  global_vgene = FALSE,  
  vgene_stratify = FALSE,  
  verbose = TRUE  
)
```

Arguments

<code>cdr3_sequences</code>	Data frame or vector of sequences
<code>accept_CF</code>	Accept only sequences starting with C and ending with F
<code>min_seq_length</code>	Minimum sequence length
<code>global_vgene</code>	Whether global vgene matching is required
<code>vgene_stratify</code>	Whether vgene stratification is required
<code>verbose</code>	Print notifications

Value

A list with sequences data frame and info flags

`.prepare_motif_region` *Prepare motif regions from sequences*

Description

Extracts the motif region by removing boundary amino acids.

Usage

```
.prepare_motif_region(seqs, structboundaries, boundary_size)
```

Arguments

<code>seqs</code>	Character vector of sequences
<code>structboundaries</code>	Whether to trim boundaries
<code>boundary_size</code>	Number of AAs to trim from each end

Value

Character vector of motif regions

`.prepare_result_folder`*Prepare result folder path*

Description

Prepare result folder path

Usage

```
.prepare_result_folder(result_folder)
```

Arguments

`result_folder` Path string

Value

Normalized path or "" if no saving

`.save_parameters`*Save parameter list to file*

Description

Save parameter list to file

Usage

```
.save_parameters(parameters, result_folder)
```

Arguments

`parameters` Named list of parameters

`result_folder` Path to output folder

Value

NULL (invisibly). Called for side effect of writing file.

<code>.setup_parallel</code>	Create a <i>BiocParallel</i> backend parameter object
------------------------------	---

Description

Returns a [BiocParallelParam](#) suitable for the current platform and requested number of cores. On Unix-like systems (macOS, Linux) with more than one core, a [MulticoreParam](#) is returned. On Windows or when only one core is requested, a [SerialParam](#) is used instead.

Usage

```
.setup_parallel(n_cores)
```

Arguments

<code>n_cores</code>	Number of cores. NULL auto-detects.
----------------------	-------------------------------------

Details

The core count is clamped to 2 when the `_R_CHECK_LIMIT_CORES_` environment variable is set (as during R CMD check).

Value

A `BiocParallelParam` object.

<code>.standardize_colnames</code>	Standardize column names from various input formats
------------------------------------	---

Description

Maps alternative column names to canonical GLIPH names. Supports `scRepertoire`, `immApex` `getIR`, and native GLIPH formats.

Usage

```
.standardize_colnames(df)
```

Arguments

<code>df</code>	A data frame
-----------------	--------------

Value

Data frame with standardized column names

.validate_params	<i>Validate parameters for runGLIPH</i>
------------------	---

Description

Consolidates all parameter validation into one function.

Usage

```
.validate_params(
  refdb_beta = "human_v2.0_CD48",
  v_usage_freq = NULL,
  cdr3_length_freq = NULL,
  ref_cluster_size = "original",
  sim_depth = 1000,
  lcminp = 0.01,
  lcminove = c(1000, 100, 10),
  kmer_mindepth = 3,
  accept_CF = TRUE,
  min_seq_length = 8,
  gccutoff = NULL,
  structboundaries = TRUE,
  boundary_size = 3,
  motif_length = c(2, 3, 4),
  local_similarities = TRUE,
  global_similarities = TRUE,
  cluster_min_size = 2,
  hla_cutoff = 0.1,
  n_cores = 1,
  motif_distance_cutoff = 3,
  discontinuous_motifs = FALSE,
  all_aa_interchangeable = FALSE,
  boost_local_significance = FALSE
)
```

Arguments

refdb_beta	Reference database
v_usage_freq	V-gene usage frequency data frame
cdr3_length_freq	CDR3 length frequency data frame
ref_cluster_size	Cluster size reference type
sim_depth	Simulation depth
lcminp	Local convergence min p-value
lcminove	Local convergence min OvE
kmer_mindepth	Minimum kmer observations
accept_CF	Accept C/F start/end only

min_seq_length	Minimum sequence length
gccutoff	Global convergence cutoff
structboundaries	Use structural boundaries
boundary_size	Boundary size in AAs
motif_length	Motif lengths to search
local_similarities	Search local similarities
global_similarities	Search global similarities
cluster_min_size	Minimum cluster size
hla_cutoff	HLA significance cutoff
n_cores	Number of cores
motif_distance_cutoff	Motif distance cutoff (GLIPH2)
discontinuous_motifs	Allow discontinuous motifs
all_aa_interchangeable	BLOSUM62 filtering
boost_local_significance	Germline boost

Value

List of validated (and possibly adjusted) parameter values

.valid_reference_names

Valid built-in reference database names

Description

Valid built-in reference database names

Usage

.valid_reference_names()

Value

Character vector of valid reference database names.

clusterScoring

*Score CDR3 clusters using the GLIPH or GLIPH2 algorithm***Description**

Calculates scores for CDR3 clusters following the GLIPH and GLIPH2 scoring procedures. Depending on the information provided, a final score is computed from up to five cluster properties: cluster size, enrichment of CDR3 lengths, enrichment of V genes, enrichment of clonal expansions, and enrichment of common HLA alleles.

Usage

```
clusterScoring(
  cluster_list,
  cdr3_sequences,
  refdb_beta = "human_v2.0_CD48",
  v_usage_freq = NULL,
  cdr3_length_freq = NULL,
  ref_cluster_size = "original",
  gliph_version = 1,
  sim_depth = 1000,
  hla_cutoff = 0.1,
  n_cores = 1
)
```

Arguments

- | | |
|----------------|--|
| cluster_list | A list where each element contains a data.frame of CDR3b sequences and additional information needed for scoring. Corresponds to the \$cluster_list element returned by runGLIPH . |
| cdr3_sequences | <p>A vector or data.frame of CDR3 sequences and optional metadata. The columns must be named as specified below in arbitrary order:</p> <p>"CDR3b" CDR3 sequences of beta chains.</p> <p>"TRBV" Optional. V-genes of beta chains.</p> <p>"patient" Optional. Donor index for the corresponding sequence, composed of a donor identifier and an optional experimental condition separated by a colon (e.g., 09/0410:MtbLys). Only the identifier before the colon is used for HLA scoring.</p> <p>"HLA" Optional. Comma-separated HLA alleles for the corresponding donor in standard notation (e.g., DPA1*01:03). Information after the colon in each allele is ignored during HLA scoring.</p> <p>"counts" Optional. Clone frequency.</p> |
| refdb_beta | <p>A character string or data.frame specifying the reference database. When a data.frame is supplied, CDR3b sequences must be in the first column and V-gene information (if available) in the second column. Built-in databases include "human_v1.0_CD4", "human_v1.0_CD8", "human_v1.0_CD48", "human_v2.0_CD4", "human_v2.0_CD8", "human_v2.0_CD48", "mouse_v1.0_CD4", "mouse_v1.0_CD8", "mouse_v1.0_CD48", and the legacy alias "gliph_reference" (= "human_v1.0_CD48"). See reference_list for details. Default: "human_v2.0_CD48"</p> |

v_usage_freq	A data.frame with V-gene alleles in the first column and their naive-repertoire frequencies in the second column. Default: NULL
cdr3_length_freq	A data.frame with CDR3 lengths in the first column and their naive-repertoire frequencies in the second column. Default: NULL
ref_cluster_size	A character string defining which cluster-size probabilities to use for scoring. "original" Standard probabilities from the original algorithm, constant across sample sizes. "simulated" Probabilities estimated for different sample sizes via a 500-step simulation using random sequences from the reference database. Default: "original"
gliph_version	A numeric value indicating the algorithm version. 1 GLIPH scoring (product of individual scores multiplied by 0.064). 2 GLIPH2 scoring (product of individual scores only). Default: 1
sim_depth	A numeric value for simulated resampling depth in non-parametric convergence significance tests. Higher values increase runtime but improve reproducibility. Default: 1000
hla_cutoff	A numeric threshold below which HLA probability scores are considered significant. Default: 0.1
n_cores	A numeric value for the number of cores to use. When NULL, it is set to the number of available cores minus two. Default: 1

Value

A data.frame of cluster scoring results. The first column contains the total score and additional columns contain up to five individual scores (cluster size, CDR3 length enrichment, V-gene enrichment, clonal expansion enrichment, and common HLA enrichment).

References

Glanville, Jacob, et al. "Identifying specificity groups in the T cell receptor repertoire." *Nature* 547.7661 (2017): 94.

<https://github.com/immunoengineer/gliph>

Examples

```
utils::data("gliph_input_data")
ref_df <- gliph_input_data[, c("CDR3b", "TRBV")]

res <- runGLIPH(cdr3_sequences = gliph_input_data[seq_len(200), ],
               refdb_beta = ref_df,
               sim_depth = 100,
               n_cores = 1)

scoring_results <- clusterScoring(
  cluster_list = res$cluster_list,
  cdr3_sequences = gliph_input_data[seq_len(200), ],
  refdb_beta = ref_df,
  gliph_version = 1,
```

```
sim_depth = 100,
n_cores = 1)
```

deNovoTCRs

Generate de novo TCR sequences

Description

De novo generation of CDR3 sequences based on GLIPH or GLIPH2 clustering results. Using the position-specific abundance of amino acids in the CDR3 region of sequences within a convergence group, artificial sequences are simulated following the approach established in Glanville et al. The generated sequences are scored by a positional weight matrix (PWM) derived from the convergence group, and optionally normalized against a reference database. The top-scoring sequences are returned.

Usage

```
deNovoTCRs(
  convergence_group_tag,
  result_folder = "",
  clustering_output = NULL,
  refdb_beta = "gliph_reference",
  normalization = FALSE,
  accept_sequences_with_C_F_start_end = TRUE,
  sims = 1e+05,
  num_tops = 1000,
  min_length = 10,
  make_figure = FALSE,
  n_cores = 1
)
```

Arguments

- | | |
|-----------------------|---|
| convergence_group_tag | Character. Tag of the convergence group to use for prediction. |
| result_folder | Character. Path to the folder containing clustering output files and where results will be saved. If the value is "", results are not saved to disk and the clustering output must be provided via clustering_output. Default: "" |
| clustering_output | List. The output list from runGLIPH . Required when result_folder is "". Default: NULL |
| refdb_beta | Character or data.frame. Specifies the reference database to use. When a data.frame is provided, the first column should contain CDR3b sequences and the second column (optional) should contain V genes. The following keyword can be used to select a built-in database: <ul style="list-style-type: none"> • "gliph_reference": 162,165 CDR3b sequences of naive human CD4+ or CD8+ T cells from two individuals (GLIPH paper). Default: "gliph_reference" |

<code>normalization</code>	Logical. If TRUE, calculated scores are normalized to the reference database. The returned value represents the probability that a reference sequence has a score greater than or equal to the sample sequence score. When V gene information is available, only sequences with identical V genes are compared. Default: FALSE
<code>accept_sequences_with_C_F_start_end</code>	Logical. If TRUE, only sequences beginning with cysteine (C) and ending with phenylalanine (F) are accepted. Default: TRUE
<code>sims</code>	Numeric. Number of de novo CDR3 sequences to generate. Default: 100000
<code>num_tops</code>	Numeric. Number of top-scoring de novo sequences to return. Default: 1000
<code>min_length</code>	Numeric. Minimum CDR3 sequence length; also determines the number of N-terminal positions used for PWM scoring. Default: 10
<code>make_figure</code>	Logical. Whether to plot the num_tops best-scoring de novo sequences as a function of rank. Default: FALSE
<code>n_cores</code>	Numeric. Number of cores for parallel computation. If NULL, the number of available cores minus two is used. Default: 1

Value

A list with the following elements:

de_novo_sequences A data.frame of the num_tops best-scoring generated sequences and their corresponding scores.

sample_sequences_scores A data.frame of the convergence group sequences and their corresponding scores.

cdr3_length_probability A data.frame with each observed CDR3 length and its probability of occurrence in the convergence group. The length distribution of generated sequences mirrors this distribution.

PWM_Scoring A data.frame containing the positional weight matrix used for scoring. Columns represent amino acids and rows represent positions relative to the N-terminus.

PWM_Prediction A list of data.frames containing the positional weight matrices used for sequence generation, one per observed CDR3 length. Columns represent amino acids and rows represent positions relative to the N-terminus.

If `result_folder` is specified, a tab-delimited file named `<convergence_group_tag>_de_novo.txt` is also written to disk.

References

Glanville, Jacob, et al. "Identifying specificity groups in the T cell receptor repertoire." *Nature* 547.7661 (2017): 94.

<https://github.com/immunoengineer/glyph>

Examples

```
# Build a minimal clustering output to demonstrate deNovoTCRs
fake_cluster <- data.frame(
  CDR3b = c("CASSLAPGATNEKLFF", "CASSLAPGGTNEKLFF",
            "CASSLAPGDTNEKLFF", "CASSLAPGETNEKLFF",
            "CASSLAPGANEKLFF", "CASSLAPGVTNEKLFF"),
  TRBV = rep("TRBV5-1", 6),
  stringsAsFactors = FALSE
```

```

)
fake_output <- list(cluster_list = list("motif-LAP" = fake_cluster))
ref_seqs <- fake_cluster[, c("CDR3b", "TRBV")]
new_seqs <- deNovoTCRs(
  convergence_group_tag = "motif-LAP",
  clustering_output = fake_output,
  refdb_beta = ref_seqs,
  sims = 100,
  num_tops = 10,
  min_length = 8,
  make_figure = FALSE,
  n_cores = 1
)

```

findMotifs
Find continuous and discontinuous sequence motifs

Description

Searches a character vector of amino acid sequences for k-mer motifs and returns their frequencies. Both continuous and discontinuous (gapped) motifs are supported. When **immApex** ($\geq 2.0.0$) is installed, the C++-accelerated `immApex::calculateMotif()` backend is used automatically for improved performance; otherwise the function falls back to a pure-R implementation based on [qgrams](#).

Usage

```
findMotifs(seqs, q = 2:4, kmer_mindepth = NULL, discontinuous = FALSE)
```

Arguments

<code>seqs</code>	A character vector of amino acid sequences in which motifs will be identified and counted.
<code>q</code>	A numeric vector of motif lengths to search for. Default: 2:4.
<code>kmer_mindepth</code>	The minimum number of times a k-mer must be observed in <code>seqs</code> for it to be included in the output. Default: NULL (no filtering).
<code>discontinuous</code>	Whether to include discontinuous (gapped) motifs in the search. Default: FALSE.

Value

A `data.frame` with two columns: `motif` (the k-mer string) and `V1` (the observed frequency).

Examples

```

utils::data("gliph_input_data")
sample_seqs <- as.character(gliph_input_data$CDR3b)
res <- findMotifs(seqs = sample_seqs)

```

getGLIPHreference	<i>Get or download the immGLIPH reference list</i>
-------------------	--

Description

Downloads the reference repertoire data from Zenodo on first use and caches locally via **BiocFileCache**. Subsequent calls load from the cache without re-downloading.

Usage

```
getGLIPHreference(force_download = FALSE, verbose = TRUE)
```

Arguments

`force_download` Logical. If TRUE, re-download even if cached. **Default:** FALSE
`verbose` Logical. Print messages. **Default:** TRUE

Details

The cached file contains a named `list` with entries for each built-in reference database (see [.valid_reference_names](#)).

Value

A named `list` of reference databases. Each element is a `list` with `refseqs`, `vgene_frequencies`, and `cdr3_length_frequencies`.

Examples

```
# Available reference database names
c("human_v1.0_CD4", "human_v1.0_CD8", "human_v1.0_CD48",
  "human_v2.0_CD4", "human_v2.0_CD8", "human_v2.0_CD48",
  "mouse_v1.0_CD4", "mouse_v1.0_CD8", "mouse_v1.0_CD48",
  "gliph_reference")

ref <- getGLIPHreference()
names(ref)
head(ref[["human_v2.0_CD48"]]$refseqs)
```

getRandomSubsample	<i>Draw a stratified random subsample from the reference repertoire</i>
--------------------	---

Description

Draws a random subset of reference motif regions with the same size as the sample set. When `cdr3_len_stratify` and/or `vgene_stratify` are enabled, the function preserves the CDR3 length and/or V-gene distribution of the sample in the subsample. This is used internally by the repeated random sampling (RRS) local-similarity method in [runGLIPH](#).

Usage

```
getRandomSubsample(
  cdr3_len_stratify = FALSE,
  vgene_stratify = FALSE,
  refseqs_motif_region,
  motif_region,
  motif_lengths_list,
  ref_motif_lengths_id_list,
  motif_region_vgenes_list,
  ref_motif_vgenes_id_list,
  ref_lengths_vgenes_list,
  lengths_vgenes_list
)
```

Arguments

cdr3_len_stratify Whether to preserve the CDR3 length distribution. **Default:** FALSE

vgene_stratify Whether to preserve the V-gene distribution. **Default:** FALSE

refseqs_motif_region Character vector of reference motif regions.

motif_region Character vector of sample motif regions.

motif_lengths_list Named list mapping CDR3 lengths to their frequency in **motif_region**. Required when **cdr3_len_stratify** = TRUE.

ref_motif_lengths_id_list Named list mapping CDR3 lengths to indices in **refseqs_motif_region**. Required when **cdr3_len_stratify** = TRUE.

motif_region_vgenes_list Named list mapping V-genes to their frequency in **motif_region**. Required when **vgene_stratify** = TRUE.

ref_motif_vgenes_id_list Named list mapping V-genes to indices in **refseqs_motif_region**. Required when **vgene_stratify** = TRUE.

ref_lengths_vgenes_list Nested list mapping CDR3 length x V-gene combinations to indices in **refseqs_motif_region**. Required when both stratification flags are TRUE.

lengths_vgenes_list Nested list mapping CDR3 length x V-gene combinations to their frequency in the sample. Required when both stratification flags are TRUE.

Value

A character vector of length `length(motif_region)` drawn from **refseqs_motif_region**.

Examples

```
ref_seqs <- c("ASSG", "ASSD", "ASSE", "ASSF", "ASSK", "ASSL")
sample_seqs <- c("ASSG", "ASSF", "ASSL")
sub <- getRandomSubsample(
  refseqs_motif_region = ref_seqs,
```

```
motif_region = sample_seqs
)
```

gliph_input_data	<i>Example TCR input data</i>
------------------	-------------------------------

Description

A `data.frame` of 365 TRB CDR3 sequences with V-gene and patient annotations, derived from the **scRepertoire** example dataset (Yost et al. 2021). The data were extracted from the [gliph_sce](#) `SingleCellExperiment` object using `immApex::getIR()`.

Usage

```
data(gliph_input_data)
```

Format

A `data.frame` with 365 rows and 3 columns (CDR3b, TRBV, patient).

Details

CDR3b Amino acid sequence of the TRB CDR3 region.

TRBV TRBV gene name (e.g. "TRBV9").

patient Patient/sample identifier (e.g. "P17B", "P19L").

Source

Yost, K. E. et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nature Medicine* 25, 1251–1259 (2019).

Built from **scRepertoire** example data; see `data-raw/build_example_data.R`.

See Also

[gliph_sce](#) for the parent `SingleCellExperiment` object, [runGLIPH](#) for the main analysis function.

gliph_sce	<i>Example SingleCellExperiment with TCR clonal information</i>
-----------	---

Description

A `SingleCellExperiment` object containing 2,000 genes across 500 cells, with T-cell receptor clonotype information stored in the `colData`. Built from the **scRepertoire** example dataset using `combineTCR()` and `combineExpression()`.

Usage

```
data(gliph_sce)
```

Format

A SingleCellExperiment with 2000 genes and 500 cells.

Details

The colData includes scRepertoire columns such as CTaa (amino acid clonotype), CTgene (gene-level clonotype), CTnt (nucleotide clonotype), CTstrict (strict clonotype), and clone frequency/proportion columns. These can be parsed by `immApex::getIR()` to extract chain-specific TCR data.

This object demonstrates how to pass a SingleCellExperiment directly to [runGLIPH](#).

Source

Yost, K. E. et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nature Medicine* 25, 1251–1259 (2019).

Built from **scRepertoire** example data; see `data-raw/build_example_data.R`.

See Also

[gliph_input_data](#) for a plain data.frame extracted from this object, [runGLIPH](#) for the main analysis function.

gTRB

Germline TCR-beta CDR3 fragments

Description

A list of three data.frames containing germline-encoded fragments of V (gTRV), D (gTRD), and J (gTRJ) gene segments that may appear in the CDR3 region. These fragments are used by the GLIPH2 algorithm to identify germline-encoded sequence segments.

Usage

```
data(gTRB)
```

Format

A list of 3 data.frames: gTRV, gTRD, and gTRJ.

Source

Lefranc, M.-P. IMGT, the international ImMunoGeneTics database. *Nucl. Acids Res.* 29, 207–209 (2001).

loadGLIPH

*Load saved GLIPH results from disk***Description**

Reads the tab-delimited output files produced by [runGLIPH](#) (when `result_folder` was specified) and reconstructs the same list structure that `runGLIPH()` returns.

Usage

```
loadGLIPH(result_folder = "")
```

Arguments

`result_folder` Path to the folder containing the saved GLIPH output files.

Value

A list with the same structure as the return value of [runGLIPH](#), including elements such as `cluster_list`, `cluster_properties`, `motif_enrichment`, `connections`, and `parameters`.

Examples

```
utils::data("gliph_input_data")
ref_df <- gliph_input_data[, c("CDR3b", "TRBV")]
tmp_dir <- tempfile("gliph_out_")
res <- runGLIPH(
  cdr3_sequences = gliph_input_data[seq_len(200), ],
  method = "gliph1",
  refdb_beta = ref_df,
  result_folder = tmp_dir,
  sim_depth = 50,
  n_cores = 1
)
reloaded <- loadGLIPH(result_folder = tmp_dir)
unlink(tmp_dir, recursive = TRUE)
```

plotNetwork

*Visualize TCR convergence group network***Description**

Uses the `visNetwork` package to build an interactive network graph from the clustering results produced by [runGLIPH](#). Nodes represent individual CDR3b sequences and edges encode local or global sequence similarities. The resulting visualization is fully interactive: scroll to zoom, hover over a node for details, and click a node to highlight its direct neighbors.

Usage

```
plotNetwork(
  clustering_output = NULL,
  result_folder = "",
  show_additional_columns = NULL,
  color_info = "total.score",
  color_palette = viridis::viridis,
  local_edge_color = "orange",
  global_edge_color = "#68bceb",
  size_info = NULL,
  absolute_size = FALSE,
  cluster_min_size = 3,
  n_cores = 1
)
```

Arguments

- | | |
|-------------------------|--|
| clustering_output | Output list returned by runGLIPH . Default: NULL |
| result_folder | Path to the folder containing saved GLIPH output files. When a non-empty path is supplied the results are loaded from disk and clustering_output is ignored. Default: "" |
| show_additional_columns | Character vector of extra column names whose values should be displayed in the node tooltips. Column names from the original cdr3_sequences data frame and from clustering_output\$cluster_properties are accepted. Default: NULL |
| color_info | Column name used to colour the nodes. Accepts any column from the input cdr3_sequences or clustering_output\$cluster_properties. Set to "none" to colour all nodes grey, or "color" to use pre-assigned colour values stored in that column. For numeric columns the viridis palette is applied automatically (purple = low, yellow = high). Default: "total.score" |
| color_palette | A function that accepts a single integer n and returns n colour values. Default: viridis::viridis |
| local_edge_color | Colour applied to edges representing local similarities. Default: "orange" |
| global_edge_color | Colour applied to edges representing global similarities. Default: "#68bceb" |
| size_info | Column name whose numeric values determine node sizes. Accepts columns from cdr3_sequences or clustering_output\$cluster_properties. Default: NULL |
| absolute_size | If TRUE the raw values from the size_info column are used as node sizes; otherwise the values are linearly scaled to the range 12–20. Default: FALSE |
| cluster_min_size | Minimum number of members a cluster must contain to be included in the plot. Default: 3 |
| n_cores | Number of cores for parallel processing. When NULL the number of available cores minus two is used. Default: 1 |

Value

A visNetwork object containing the interactive network graph.

Examples

```
utils::data("gliph_input_data")
ref_df <- gliph_input_data[, c("CDR3b", "TRBV")]
res <- runGLIPH(cdr3_sequences = gliph_input_data[seq_len(200),],
               method = "gliph1",
               refdb_beta = ref_df,
               sim_depth = 100,
               n_cores = 1)

plotNetwork(clustering_output = res,
            n_cores = 1)
```

reference_list	<i>GLIPH reference repertoire list (external data)</i>
----------------	--

Description

A named list of naive TCR repertoire reference databases used for motif enrichment testing and cluster scoring. The data is **not** bundled with the package; it is downloaded on first use from Zenodo and cached locally via **BiocFileCache** (see [getGLIPHreference](#)).

Format

NULL. Data is downloaded on first use via [getGLIPHreference](#).

Details

Each element is itself a list with three components:

`refseqs` A data.frame with columns CDR3b (amino acid sequence) and TRBV (V-gene name).

`vgene_frequencies` A data.frame with columns vgene and freq giving the relative frequency of each V gene in the reference repertoire.

`cdr3_length_frequencies` A data.frame with columns len and freq giving the relative frequency of each CDR3 length in the reference repertoire.

The following named entries are available:

- "human_v1.0_CD4", "human_v1.0_CD8", "human_v1.0_CD48" – Glanville et al. (2017)
- "human_v2.0_CD4", "human_v2.0_CD8", "human_v2.0_CD48" – Huang et al. (2020)
- "mouse_v1.0_CD4", "mouse_v1.0_CD8", "mouse_v1.0_CD48" – Glanville et al. (2017)
- "gliph_reference" – Legacy alias for "human_v1.0_CD48"

Value

No return value. This documents the reference_list object which is downloaded at runtime by [getGLIPHreference](#).

Source

Glanville, J. et al. Identifying specificity groups in the T cell receptor repertoire. *Nature* 547, 94–98 (2017).

Huang, H. et al. Analyzing the *Mycobacterium tuberculosis* immune response by T-cell receptor clustering with GLIPH2 and genome-wide antigen screening. *Nature Biotechnology* 38, 1194–1202 (2020).

Raw data downloaded from <http://50.255.35.37:8080/tools>.

See Also

[getGLIPHreference](#) to download or load the data, [runGLIPH](#) and [clusterScoring](#) which use the reference internally via the `refdb_beta` parameter.

ref_cluster_sizes	<i>Cluster size probabilities in naive reference repertoire</i>
-------------------	---

Description

A list with two elements providing expected cluster-size probabilities under the null model (no true convergence):

`original` Probabilities from the original GLIPH algorithm, applied uniformly across all sample sizes.

`simulated` Probabilities estimated from 500-step simulations at sample sizes of 125, 250, 500, 1000, 2000, 4000, 6000, 8000, and 10000 random reference sequences. During scoring the row closest to the actual sample size is used.

Usage

```
data(ref_cluster_sizes)
```

Format

A list with 2 elements: `original` and `simulated`.

Source

Glanville, J. et al. Identifying specificity groups in the T cell receptor repertoire. *Nature* 547, 94–98 (2017).

runGLIPH

*Run the GLIPH or GLIPH2 TCR clustering algorithm***Description**

Unified entry point for the GLIPH/GLIPH2 algorithm for grouping T cell receptors by antigen specificity. The function identifies locally and globally similar CDR3b sequences, clusters them into convergence groups, and scores each group for biological relevance.

Usage

```
runGLIPH(
  cdr3_sequences,
  method = c("gliph2", "gliph1", "custom"),
  chains = "TRB",
  result_folder = "",
  refdb_beta = "human_v2.0_CD48",
  v_usage_freq = NULL,
  cdr3_length_freq = NULL,
  ref_cluster_size = "original",
  sim_depth = 1000,
  lcminp = 0.01,
  lcminove = c(1000, 100, 10),
  kmer_mindepth = 3,
  accept_CF = TRUE,
  min_seq_length = 8,
  gccutoff = NULL,
  structboundaries = TRUE,
  boundary_size = 3,
  motif_length = c(2, 3, 4),
  local_similarities = TRUE,
  global_similarities = TRUE,
  local_method = NULL,
  global_method = NULL,
  clustering_method = NULL,
  scoring_method = NULL,
  cluster_min_size = 2,
  hla_cutoff = 0.1,
  n_cores = 1,
  motif_distance_cutoff = 3,
  discontinuous_motifs = FALSE,
  all_aa_interchangeable = FALSE,
  boost_local_significance = FALSE,
  global_vgene = FALSE,
  cdr3_len_stratify = FALSE,
  vgene_stratify = FALSE,
  public_tcrs = TRUE,
  vgene_match = "none",
  scoring_sim_depth = 1000,
  verbose = TRUE
)
```

Arguments

cdr3_sequences	Input data containing CDR3b amino acid sequences. Accepts a character vector, a <code>data.frame</code> with columns described below, a Seurat object, a <code>SingleCellExperiment</code> object, or a list returned by <code>scRepertoire::combineTCR()/combineBCR()</code>. When a <code>data.frame</code> is supplied, the following column names are recognized (alternative names in parentheses are mapped automatically): CDR3b (cdr3, cdr3_aa, CDR3.beta, junction_aa) Required. CDR3 beta-chain amino acid sequences. TRBV (v_gene, v.gene, Vgene, v_call) Optional. V-gene usage. patient (sample, donor, sample_id) Optional. Donor index. HLA (hla, HLA_alleles) Optional. HLA alleles, comma-separated. counts (frequency, clone_count, cloneCount) Optional. Clone frequency.
method	Character. Algorithm preset to use. "gliph2" Fisher-based local and global similarity, GLIPH2-style isolated clustering and scoring. "gliph1" Repeated random sampling for local similarity, Hamming distance cutoff for global similarity, GLIPH1-style connected-component clustering. "custom" All parameters can be set independently. Default: "gliph2"
chains	Character. Chain type for extraction from Seurat or <code>SingleCellExperiment</code> objects via <code>immApex::getIR()</code>. Default: "TRB"
result_folder	Character. Path to output folder. If "", results are not saved to disk. Default: ""
refdb_beta	Character or <code>data.frame</code>. Reference database for motif enrichment testing. Built-in databases include "human_v1.0_CD4", "human_v1.0_CD8", "human_v1.0_CD48", "human_v2.0_CD4", "human_v2.0_CD8", "human_v2.0_CD48", "mouse_v1.0_CD4", "mouse_v1.0_CD8", "mouse_v1.0_CD48", and the legacy alias "gliph_reference" (= "human_v1.0_CD48"). Alternatively, supply a <code>data.frame</code> with CDR3b in the first column and optional V-gene in the second. See reference_list for details. Default: "human_v2.0_CD48"
v_usage_freq	<code>data.frame</code> or NULL. V-gene frequencies for scoring. If NULL, built-in defaults are used. Default: NULL
cdr3_length_freq	<code>data.frame</code> or NULL. CDR3 length frequencies for scoring. If NULL, built-in defaults are used. Default: NULL
ref_cluster_size	Character. Reference cluster size strategy. "original" Use the original sample size. "simulated" Use simulated cluster sizes. Default: "original"
sim_depth	Integer. Simulation depth for repeated random sampling (local method "rrs") or cluster scoring. Default: 1000
lcmip	Numeric. Local convergence maximum p-value threshold. Default: 0.01
lcmiave	Numeric vector. Local convergence minimum fold-change per motif length (lengths 2, 3, and 4 respectively). Default: c(1000, 100, 10)
kmer_mindepth	Integer. Minimum number of kmer observations required to consider a motif. Default: 3

accept_CF	Logical. If TRUE, accept only sequences starting with C and ending with F. Default: TRUE
min_seq_length	Integer. Minimum CDR3b sequence length to retain. Default: 8
gccutoff	Numeric or NULL. Global convergence Hamming distance cutoff (used when global_method = "cutoff"). If NULL, the cutoff is auto-selected based on sample size. Default: NULL
structboundaries	Logical. If TRUE, trim structural boundaries from CDR3b sequences before motif search. Default: TRUE
boundary_size	Integer. Number of positions to trim from each end when structboundaries = TRUE. Default: 3
motif_length	Numeric vector. Motif lengths to search. Default: c(2, 3, 4)
local_similarities	Logical. If TRUE, search for locally similar CDR3b sequences. Default: TRUE
global_similarities	Logical. If TRUE, search for globally similar CDR3b sequences. Default: TRUE
local_method	Character or NULL. Method for local similarity detection. If NULL, set by the method preset. "fisher" Fisher exact test for motif enrichment. "rrs" Repeated random sampling. Default: NULL
global_method	Character or NULL. Method for global similarity detection. If NULL, set by the method preset. "fisher" Fisher exact test for struct enrichment. "cutoff" Hamming distance cutoff. Default: NULL
clustering_method	Character or NULL. Clustering strategy. If NULL, set by the method preset. "GLIPH1.0" Connected-component clustering. "GLIPH2.0" Isolated clustering with merging. Default: NULL
scoring_method	Character or NULL. Scoring strategy. If NULL, set by the method preset. "GLIPH1.0" GLIPH1-style scoring. "GLIPH2.0" GLIPH2-style scoring. Default: NULL
cluster_min_size	Integer. Minimum number of unique CDR3b sequences required to retain a convergence group. Default: 2
hla_cutoff	Numeric. Significance cutoff for HLA enrichment testing. Default: 0.1
n_cores	Integer or NULL. Number of cores for parallel processing. If NULL, the number of available cores is auto-detected. Default: 1
motif_distance_cutoff	Integer. Maximum positional distance between shared motifs for two CDR3b sequences to be linked (GLIPH2). Default: 3
discontinuous_motifs	Logical. If TRUE, allow discontinuous motif patterns during local similarity search. Default: FALSE

<code>all_aa_interchangeable</code>	Logical. If FALSE, BLOSUM62 filtering is applied to global similarities, restricting substitutions to biochemically similar amino acids. Default: FALSE
<code>boost_local_significance</code>	Logical. If TRUE, boost local p-values using germline N-nucleotide insertion information. Default: FALSE
<code>global_vgene</code>	Logical. If TRUE, restrict global similarity edges to pairs sharing the same V-gene. Default: FALSE
<code>cdr3_len_stratify</code>	Logical. If TRUE, stratify random subsamples by CDR3 length (used with <code>local_method = "rrs"</code>). Default: FALSE
<code>vgene_stratify</code>	Logical. If TRUE, stratify random subsamples by V-gene usage (used with <code>local_method = "rrs"</code>). Default: FALSE
<code>public_tcrs</code>	Logical or character. Controls cross-donor edge filtering. For <code>method = "gliph1"</code> or <code>"gliph2"</code> : if FALSE, restrict edges to same donor. For <code>method = "custom"</code> : <code>"all"</code> Allow cross-donor edges for all similarity types. <code>"local"</code> Allow cross-donor edges for local only. <code>"global"</code> Allow cross-donor edges for global only. <code>"none"</code> Restrict all edges to same donor. Default: TRUE
<code>vgene_match</code>	Character. V-gene matching requirement for custom clustering. <code>"none"</code> No V-gene matching required. <code>"local"</code> Require V-gene match for local edges. <code>"global"</code> Require V-gene match for global edges. <code>"all"</code> Require V-gene match for all edges. Default: "none"
<code>scoring_sim_depth</code>	Integer. Simulation depth used specifically for convergence group scoring. Default: 1000
<code>verbose</code>	Logical. If TRUE, print progress messages to the console. Default: TRUE

Value

A list with the following elements:

`sample_log` `data.frame`. Motif counts per simulation iteration (only present when `local_method = "rrs"`).

`motif_enrichment` list with two elements:

`selected_motifs` `data.frame` of significantly enriched motifs passing all thresholds.

`all_motifs` `data.frame` of all evaluated motifs with enrichment statistics.

`global_enrichment` list. Global struct enrichment results (GLIPH2 only; NULL otherwise).

`connections` `data.frame`. Edge list representing the clone network.

`cluster_properties` `data.frame`. Convergence group properties and scores.

`cluster_list` Named list of `data.frame` objects with per-cluster member details.

`parameters` list. All input parameters used for the run.

References

- Glanville, J. et al. (2017). Identifying specificity groups in the T cell receptor repertoire. *Nature*, 547, 94–98. doi:[10.1038/nature22976](https://doi.org/10.1038/nature22976)
- Huang, H. et al. (2020). Analyzing the Mycobacterium tuberculosis immune response by T-cell receptor clustering with GLIPH2 and genome-wide antigen screening. *Nature Biotechnology*, 38, 1194–1202. doi:[10.1038/s4158702005054](https://doi.org/10.1038/s4158702005054)

Examples

```
utils::data("gliph_input_data")
ref_df <- gliph_input_data[, c("CDR3b", "TRBV")]
res <- runGLIPH(
  cdr3_sequences = gliph_input_data[seq_len(200), ],
  method = "gliph2",
  refdb_beta = ref_df,
  sim_depth = 50,
  n_cores = 1
)
```

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