

# Package ‘scoper’

July 23, 2025

**Type** Package

**Version** 1.3.0

**Date** 2023-10-05

**Title** Spectral Clustering-Based Method for Identifying B Cell Clones

**Description** Provides a computational framework for identification of B cell clones from Adaptive Immune Receptor Repertoire sequencing (AIRR-Seq) data. Three main functions are included (identicalClones, hierarchicalClones, and spectralClones) that perform clustering among sequences of BCRs/IGs (B cell receptors/immunoglobulins) which share the same V gene, J gene and junction length.  
Nouri N and Kleinstein SH (2018) <[doi:10.1093/bioinformatics/bty235](https://doi.org/10.1093/bioinformatics/bty235)>.  
Nouri N and Kleinstein SH (2019) <[doi:10.1101/788620](https://doi.org/10.1101/788620)>.  
Gupta NT, et al. (2017) <[doi:10.4049/jimmunol.1601850](https://doi.org/10.4049/jimmunol.1601850)>.

**License** AGPL-3

**URL** <https://scoper.readthedocs.io>

**BugReports** <https://bitbucket.org/kleinstein/scoper/issues>

**LazyData** true

**BuildVignettes** true

**VignetteBuilder** knitr

**Encoding** UTF-8

**LinkingTo** Rcpp

**Depends** R (>= 4.0), ggplot2 (>= 3.4.0)

**Imports** alakazam (>= 1.3.0), shazam (>= 1.2.0), data.table,  
doParallel, dplyr (>= 1.0), foreach, methods, Rcpp (>= 0.12.12), rlang, scales, stats, stringi, tidyr (>= 1.0)

**Suggests** knitr, rmarkdown, testthat

**RoxygenNote** 7.2.3

**Collate** 'Data.R' 'Scoper.R' 'Functions.R' 'RcppExports.R'

**NeedsCompilation** yes

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**Repository** CRAN  
**Date/Publication** 2023-10-06 09:30:02 UTC

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ExampleDb	<i>Example database</i>
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Description

A small example database subset from Laserson and Vigneault et al, 2014.

Usage

ExampleDb

Format

- A data.frame with the following columns:
- sequence\_id: Sequence identifier
  - sequence\_alignment: IMGT-gapped observed sequence.
  - germline\_alignment: IMGT-gapped germline sequence.
  - germline\_alignment\_d\_mask: IMGT-gapped germline sequence with N, P and D regions masked.
  - v\_call: V region allele assignments.
  - v\_call\_genotyped: TIGGER corrected V region allele assignment.
  - d\_call: D region allele assignments.

- j\_call: J region allele assignments.
- junction: Junction region sequence.
- junction\_length: Length of the junction region in nucleotides.
- np1\_length: Number of nucleotides between V and D segments
- np2\_length: Number of nucleotides between D and J segments
- sample\_id: Sample identifier
- c\_call: C region assignment.
- duplicate\_count: Copy number of the sequence
- locus: Locus of the receptor

## References

1. Laserson U and Vigneault F, et al. High-resolution antibody dynamics of vaccine-induced immune responses. Proc Natl Acad Sci USA. 2014 111:4928-33.

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hierarchicalClones	<i>Hierarchical clustering method for clonal partitioning</i>
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## Description

hierarchicalClones provides a hierarchical agglomerative clustering approach to infer clonal relationships in high-throughput Adaptive Immune Receptor Repertoire sequencing (AIRR-seq) data. This approach clusters B or T cell receptor sequences based on junction region sequence similarity within partitions that share the same V gene, J gene, and junction length, allowing for ambiguous V or J gene annotations.

## Usage

```
hierarchicalClones(
  db,
  threshold,
  method = c("nt", "aa"),
  linkage = c("single", "average", "complete"),
  normalize = c("len", "none"),
  junction = "junction",
  v_call = "v_call",
  j_call = "j_call",
  clone = "clone_id",
  fields = NULL,
  cell_id = NULL,
  locus = "locus",
  only_heavy = TRUE,
  split_light = TRUE,
  first = FALSE,
  cdr3 = FALSE,
```

```

    mod3 = FALSE,
    max_n = 0,
    nproc = 1,
    verbose = FALSE,
    log = NULL,
    summarize_clones = TRUE
)

```

## Arguments

db	data.frame containing sequence data.
threshold	numeric scalar where the tree should be cut (the distance threshold for clonal grouping).
method	one of the "nt" for nucleotide based clustering or "aa" for amino acid based clustering.
linkage	available linkage are "single", "average", and "complete".
normalize	method of normalization. The default is "len", which divides the distance by the length of the sequence group. If "none" then no normalization is performed.
junction	character name of the column containing junction sequences. Also used to determine sequence length for grouping.
v_call	name of the column containing the V-segment allele calls.
j_call	name of the column containing the J-segment allele calls.
clone	output column name containing the clonal cluster identifiers.
fields	character vector of additional columns to use for grouping. Sequences with disjoint values in the specified fields will be classified as separate clones.
cell_id	name of the column containing cell identifiers or barcodes. If specified, grouping will be performed in single-cell mode with the behavior governed by the locus and only_heavy arguments. If set to NULL then the bulk sequencing data is assumed.
locus	name of the column containing locus information. Only applicable to single-cell data. Ignored if cell_id=NULL.
only_heavy	use only the IGH (BCR) or TRB/TRD (TCR) sequences for grouping. Only applicable to single-cell data. Ignored if cell_id=NULL.
split_light	split clones by light chains. Ignored if cell_id=NULL.
first	specifies how to handle multiple V(D)J assignments for initial grouping. If TRUE only the first call of the gene assignments is used. If FALSE the union of ambiguous gene assignments is used to group all sequences with any overlapping gene calls.
cdr3	if TRUE removes 3 nucleotides from both ends of "junction" prior to clustering (converts IMGT junction to CDR3 region). If TRUE this will also remove records with a junction length less than 7 nucleotides.
mod3	if TRUE removes records with a junction length that is not divisible by 3 in nucleotide space.

max_n	The maximum number of degenerate characters to permit in the junction sequence before excluding the record from clonal assignment. Note, with linkage="single" non-informative positions can create artifactual links between unrelated sequences. Use with caution. Default is set to be zero. Set it as "NULL" for no action.
nproc	number of cores to distribute the function over.
verbose	if TRUE prints out a summary of each step cloning process. if FALSE (default) process cloning silently.
log	output path and filename to save the verbose log. The input file directory is used if path is not specified. The default is NULL for no action.
summarize_clones	if TRUE performs a series of analysis to assess the clonal landscape and returns a <a href="#">ScoperClones</a> object. If FALSE then a modified input db is returned. When grouping by fields, summarize_clones should be FALSE.

### Value

If summarize\_clones=TRUE (default) a [ScoperClones](#) object is returned that includes the clonal assignment summary information and a modified input db in the db slot that contains clonal identifiers in the specified clone column. If summarize\_clones=FALSE modified data.frame is returned with clone identifiers in the specified clone column.

### Single-cell data

To invoke single-cell mode the cell\_id argument must be specified and the locus column must be correct. Otherwise, clustering will be performed with bulk sequencing assumptions, using all input sequences regardless of the values in the locus column.

Values in the locus column must be one of c("IGH", "IGI", "IGK", "IGL") for BCR or c("TRA", "TRB", "TRD", "TRG") for TCR sequences. Otherwise, the operation will exit and return an error message.

Under single-cell mode with paired-chain sequences, there is a choice of whether grouping should be done by (a) using IGH (BCR) or TRB/TRD (TCR) sequences only or (b) using IGH plus IGK/IGL (BCR) or TRB/TRD plus TRA/TRG (TCR) sequences. This is governed by the only\_heavy argument. There is also choice as to whether inferred clones should be split by the light/short chain (IGK, IGL, TRA, TRG) following heavy/long chain clustering, which is governed by the split\_light argument.

In single-cell mode, clonal clustering will not be performed on data where cells are assigned multiple heavy/long chain sequences (IGH, TRB, TRD). If observed, the operation will exit and return an error message. Cells that lack a heavy/long chain sequence (i.e., cells with light/short chains only) will be assigned a clone\_id of NA.

### See Also

See [plotCloneSummary](#) for plotting summary results. See [groupGenes](#) for more details about grouping requirements.

## Examples

```
# Find clonal groups
results <- hierarchicalClones(ExampleDb, threshold=0.15)

# Retrieve modified input data with clonal clustering identifiers
df <- as.data.frame(results)

# Plot clonal summaries
plot(results, binwidth=0.02)
```

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identicalClones	<i>Sequence identity method for clonal partitioning</i>
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## Description

identicalClones provides a simple sequence identity based partitioning approach for inferring clonal relationships in high-throughput Adaptive Immune Receptor Repertoire sequencing (AIRR-seq) data. This approach partitions B or T cell receptor sequences into clonal groups based on junction region sequence identity within partitions that share the same V gene, J gene, and junction length, allowing for ambiguous V or J gene annotations.

## Usage

```
identicalClones(
  db,
  method = c("nt", "aa"),
  junction = "junction",
  v_call = "v_call",
  j_call = "j_call",
  clone = "clone_id",
  fields = NULL,
  cell_id = NULL,
  locus = "locus",
  only_heavy = TRUE,
  split_light = TRUE,
  first = FALSE,
  cdr3 = FALSE,
  mod3 = FALSE,
  max_n = 0,
  nproc = 1,
  verbose = FALSE,
  log = NULL,
  summarize_clones = TRUE
)
```

**Arguments**

db	data.frame containing sequence data.
method	one of the "nt" for nucleotide based clustering or "aa" for amino acid based clustering.
junction	character name of the column containing junction sequences. Also used to determine sequence length for grouping.
v_call	name of the column containing the V-segment allele calls.
j_call	name of the column containing the J-segment allele calls.
clone	output column name containing the clonal cluster identifiers.
fields	character vector of additional columns to use for grouping. Sequences with disjoint values in the specified fields will be classified as separate clones.
cell_id	name of the column containing cell identifiers or barcodes. If specified, grouping will be performed in single-cell mode with the behavior governed by the locus and only_heavy arguments. If set to NULL then the bulk sequencing data is assumed.
locus	name of the column containing locus information. Only applicable to single-cell data. Ignored if cell_id=NULL.
only_heavy	use only the IGH (BCR) or TRB/TRD (TCR) sequences for grouping. Only applicable to single-cell data. Ignored if cell_id=NULL.
split_light	split clones by light chains. Ignored if cell_id=NULL.
first	specifies how to handle multiple V(D)J assignments for initial grouping. If TRUE only the first call of the gene assignments is used. If FALSE the union of ambiguous gene assignments is used to group all sequences with any overlapping gene calls.
cdr3	if TRUE removes 3 nucleotides from both ends of "junction" prior to clustering (converts IMGT junction to CDR3 region). If TRUE this will also remove records with a junction length less than 7 nucleotides.
mod3	if TRUE removes records with a junction length that is not divisible by 3 in nucleotide space.
max_n	The maximum number of degenerate characters to permit in the junction sequence before excluding the record from clonal assignment. Default is set to be zero. Set it as "NULL" for no action.
nproc	number of cores to distribute the function over.
verbose	if TRUE prints out a summary of each step cloning process. if FALSE (default) process cloning silently.
log	output path and filename to save the verbose log. The input file directory is used if path is not specified. The default is NULL for no action.
summarize_clones	if TRUE performs a series of analysis to assess the clonal landscape and returns a <a href="#">ScoperClones</a> object. If FALSE then a modified input db is returned. When grouping by fields, summarize_clones should be FALSE.

**Value**

If `summarize_clones=TRUE` (default) a [ScoperClones](#) object is returned that includes the clonal assignment summary information and a modified input db in the db slot that contains clonal identifiers in the specified clone column. If `summarize_clones=FALSE` modified `data.frame` is returned with clone identifiers in the specified clone column.

**Single-cell data**

To invoke single-cell mode the `cell_id` argument must be specified and the locus column must be correct. Otherwise, clustering will be performed with bulk sequencing assumptions, using all input sequences regardless of the values in the locus column.

Values in the locus column must be one of `c("IGH", "IGI", "IGK", "IGL")` for BCR or `c("TRA", "TRB", "TRD", "TRG")` for TCR sequences. Otherwise, the operation will exit and return an error message.

Under single-cell mode with paired-chain sequences, there is a choice of whether grouping should be done by (a) using IGH (BCR) or TRB/TRD (TCR) sequences only or (b) using IGH plus IGK/IGL (BCR) or TRB/TRD plus TRA/TRG (TCR) sequences. This is governed by the `only_heavy` argument. There is also choice as to whether inferred clones should be split by the light/short chain (IGK, IGL, TRA, TRG) following heavy/long chain clustering, which is governed by the `split_light` argument.

In single-cell mode, clonal clustering will not be performed on data where cells are assigned multiple heavy/long chain sequences (IGH, TRB, TRD). If observed, the operation will exit and return an error message. Cells that lack a heavy/long chain sequence (i.e., cells with light/short chains only) will be assigned a `clone_id` of NA.

**See Also**

See [plotCloneSummary](#) for plotting summary results. See [groupGenes](#) for more details about grouping requirements.

**Examples**

```
# Find clonal groups
results <- identicalClones(ExampleDb)

# Retrieve modified input data with clonal clustering identifiers
df <- as.data.frame(results)

# Plot clonal summaries
plot(results, binwidth=0.02)
```

---

plotCloneSummary	<i>Plot clonal clustering summary</i>
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## Description

plotCloneSummary plots the results in a ScoperClones object returned by spectralClones, identicalClones or hierarchicalClones. Includes the minimum inter (between) and maximum intra (within) clonal distances and the calculated effective threshold.

## Usage

```
plotCloneSummary(  
  data,  
  xmin = NULL,  
  xmax = NULL,  
  breaks = NULL,  
  binwidth = NULL,  
  title = NULL,  
  size = 0.75,  
  silent = FALSE,  
  ...  
)
```

## Arguments

data	<a href="#">ScoperClones</a> object output by the <a href="#">spectralClones</a> , <a href="#">identicalClones</a> or <a href="#">hierarchicalClones</a> .
xmin	minimum limit for plotting the x-axis. If NULL the limit will be set automatically.
xmax	maximum limit for plotting the x-axis. If NULL the limit will be set automatically.
breaks	number of breaks to show on the x-axis. If NULL the breaks will be set automatically.
binwidth	binwidth for the histogram. If NULL the binwidth will be set automatically.
title	string defining the plot title.
size	numeric value for lines in the plot.
silent	if TRUE do not draw the plot and just return the ggplot2 object; if FALSE draw the plot.
...	additional arguments to pass to ggplot2::theme.

## Value

A ggplot object defining the plot.

## See Also

See [ScoperClones](#) for the the input object definition. See [spectralClones](#), [identicalClones](#) and [hierarchicalClones](#) for generating the input object.

## Examples

```
# Find clones
results <- hierarchicalClones(ExampleDb, threshold=0.15)

# Plot clonal summaries
plot(results, binwidth=0.02)
```

---

scoper

*The SCOPer package*

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## Description

scoper is a member of the Immcantation framework and provides computational approaches for the identification of B cell clones from adaptive immune receptor repertoire sequencing (AIRR-Seq) datasets. It includes methods for assigning clonal identifiers using sequence identity, hierarchical clustering, and spectral clustering.

## Clonal clustering

- [identicalClones](#): Clonal assignment using sequence identity partitioning.
- [hierarchicalClones](#): Hierarchical clustering approach to clonal assignment.
- [spectralClones](#): Spectral clustering approach to clonal assignment.

## Visualization

- [plotCloneSummary](#): Visualize inter- and intra-clone distances.

## References

1. Nouri N and Kleinstein SH (2018). A spectral clustering-based method for identifying clones from high-throughput B cell repertoire sequencing data. *Bioinformatics*, 34(13):i341-i349.
2. Nouri N and Kleinstein SH (2019). Somatic hypermutation analysis for improved identification of B cell clonal families from next-generation sequencing data. *bioRxiv*, 10.1101/788620.
3. Gupta NT, et al. (2017). Hierarchical clustering can identify B cell clones with high confidence in Ig repertoire sequencing data. *The Journal of Immunology*, 198(6):2489-2499.

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ScoperClones-class	<i>S4 class containing clonal assignments and summary data</i>
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### Description

ScoperClones stores output from [identicalClones](#), [hierarchicalClones](#) and [spectralClones](#) functions.

### Usage

```
## S4 method for signature 'ScoperClones'
print(x)

## S4 method for signature 'ScoperClones'
summary(object)

## S4 method for signature 'ScoperClones,missing'
plot(x, y, ...)

## S4 method for signature 'ScoperClones'
as.data.frame(x)
```

### Arguments

x	ScoperClones object
object	ScoperClones object
y	ignored.
...	arguments to pass to <a href="#">plotCloneSummary</a> .

### Slots

db data.frame of repertoire data including with clonal identifiers in the column specified during processing.

vjl\_groups data.frame of clonal summary, including sequence count, V gene, J gene, junction length, and clone counts.

inter\_intra data.frame containing minimum inter (between) and maximum intra (within) clonal distances.

eff\_threshold effective cut-off separating the inter (between) and intra (within) clonal distances.

### See Also

[identicalClones](#), [hierarchicalClones](#) and [spectralClones](#)

spectralClones

*Spectral clustering method for clonal partitioning***Description**

spectralClones provides an unsupervised spectral clustering approach to infer clonal relationships in high-throughput Adaptive Immune Receptor Repertoire sequencing (AIRR-seq) data. This approach clusters B or T cell receptor sequences based on junction region sequence similarity and shared mutations within partitions that share the same V gene, J gene, and junction length, allowing for ambiguous V or J gene annotations.

**Usage**

```
spectralClones(
  db,
  method = c("novj", "vj"),
  germline = "germline_alignment",
  sequence = "sequence_alignment",
  junction = "junction",
  v_call = "v_call",
  j_call = "j_call",
  clone = "clone_id",
  fields = NULL,
  cell_id = NULL,
  locus = "locus",
  only_heavy = TRUE,
  split_light = TRUE,
  targeting_model = NULL,
  len_limit = NULL,
  first = FALSE,
  cdr3 = FALSE,
  mod3 = FALSE,
  max_n = 0,
  threshold = NULL,
  base_sim = 0.95,
  iter_max = 1000,
  nstart = 1000,
  nproc = 1,
  verbose = FALSE,
  log = NULL,
  summarize_clones = TRUE
)
```

**Arguments**

db	data.frame containing sequence data.
method	one of the "novj" or "vj". See Details for description.

germline	character name of the column containing the germline or reference sequence.
sequence	character name of the column containing input sequences.
junction	character name of the column containing junction sequences. Also used to determine sequence length for grouping.
v_call	name of the column containing the V-segment allele calls.
j_call	name of the column containing the J-segment allele calls.
clone	output column name containing the clonal cluster identifiers.
fields	character vector of additional columns to use for grouping. Sequences with disjoint values in the specified fields will be classified as separate clones.
cell_id	name of the column containing cell identifiers or barcodes. If specified, grouping will be performed in single-cell mode with the behavior governed by the locus and only_heavy arguments. If set to NULL then the bulk sequencing data is assumed.
locus	name of the column containing locus information. Only applicable to single-cell data. Ignored if cell_id=NULL.
only_heavy	use only the IGH (BCR) or TRB/TRD (TCR) sequences for grouping. Only applicable to single-cell data. Ignored if cell_id=NULL.
split_light	split clones by light chains. Ignored if cell_id=NULL.
targeting_model	<a href="#">TargetingModel</a> object. Only applicable if method="vj". See Details for description.
len_limit	<a href="#">IMGT_V</a> object defining the regions and boundaries of the Ig sequences. If NULL, mutations are counted for entire sequence. Only applicable if method = "vj".
first	specifies how to handle multiple V(D)J assignments for initial grouping. If TRUE only the first call of the gene assignments is used. If FALSE the union of ambiguous gene assignments is used to group all sequences with any overlapping gene calls.
cdr3	if TRUE removes 3 nucleotides from both ends of "junction" prior to clustering (converts IMGT junction to CDR3 region). If TRUE this will also remove records with a junction length less than 7 nucleotides.
mod3	if TRUE removes records with a junction length that is not divisible by 3 in nucleotide space.
max_n	the maximum number of degenerate characters to permit in the junction sequence before excluding the record from clonal assignment. Default is set to be zero. Set it as "NULL" for no action.
threshold	the supervising cut-off to enforce an upper-limit distance for clonal grouping. A numeric value between (0,1).
base_sim	required similarity cut-off for sequences in equal distances from each other.
iter_max	the maximum number of iterations allowed for kmean clustering step.
nstart	the number of random sets chosen for kmean clustering initialization.
nproc	number of cores to distribute the function over.

verbose	if TRUE prints out a summary of each step cloning process. if FALSE (default) process cloning silently.
log	output path and filename to save the verbose log. The input file directory is used if path is not specified. The default is NULL for no action.
summarize_clones	if TRUE performs a series of analysis to assess the clonal landscape and returns a <a href="#">ScoperClones</a> object. If FALSE then a modified input db is returned. When grouping by fields, summarize_clones should be FALSE.

## Details

If method="novj", then clonal relationships are inferred using an adaptive threshold that indicates the level of similarity among junction sequences in a local neighborhood.

If method="vj", then clonal relationships are inferred not only on junction region homology, but also taking into account the mutation profiles in the V and J segments. Mutation counts are determined by comparing the input sequences (in the column specified by sequence) to the effective germline sequence (IUPAC representation of sequences in the column specified by germline).

While not mandatory, the influence of SHM hot-/cold-spot biases in the clonal inference process will be noted if a SHM targeting model is provided through the targeting\_model argument. See [TargetingModel](#) for more technical details.

If the threshold argument is specified, then an upper limit for clonal grouping will be imposed to prevent sequences with dissimilarity above the threshold from grouping together. Any sequence with a distance greater than the threshold value from the other sequences, will be assigned to a singleton group.

## Value

If summarize\_clones=TRUE (default) a [ScoperClones](#) object is returned that includes the clonal assignment summary information and a modified input db in the db slot that contains clonal identifiers in the specified clone column. If summarize\_clones=FALSE modified data.frame is returned with clone identifiers in the specified clone column.

## Single-cell data

To invoke single-cell mode the cell\_id argument must be specified and the locus column must be correct. Otherwise, clustering will be performed with bulk sequencing assumptions, using all input sequences regardless of the values in the locus column.

Values in the locus column must be one of c("IGH", "IGI", "IGK", "IGL") for BCR or c("TRA", "TRB", "TRD", "TRG") for TCR sequences. Otherwise, the operation will exit and return an error message.

Under single-cell mode with paired-chain sequences, there is a choice of whether grouping should be done by (a) using IGH (BCR) or TRB/TRD (TCR) sequences only or (b) using IGH plus IGK/IGL (BCR) or TRB/TRD plus TRA/TRG (TCR) sequences. This is governed by the only\_heavy argument. There is also choice as to whether inferred clones should be split by the light/short chain (IGK, IGL, TRA, TRG) following heavy/long chain clustering, which is governed by the split\_light argument.

In single-cell mode, clonal clustering will not be performed on data where cells are assigned multiple heavy/long chain sequences (IGH, TRB, TRD). If observed, the operation will exit and return an error message. Cells that lack a heavy/long chain sequence (i.e., cells with light/short chains only) will be assigned a clone\_id of NA.

### See Also

See [plotCloneSummary](#) for plotting summary results. See [groupGenes](#) for more details about grouping requirements.

### Examples

```
# Subset example data
db <- subset(ExampleDb, c_call == "IGHG")

# Find clonal groups
results <- spectralClones(db, method="novj", germline="germline_alignment_d_mask")

# Retrieve modified input data with clonal clustering identifiers
df <- as.data.frame(results)

# Plot clonal summaries
plot(results, binwidth=0.02)
```

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