### Package ‘shazam’

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**Baseline-class**

*S4 class defining a BASELINE (selection) object*

**Description**

Baseline defines a common data structure the results of selection analysis using the BASELINE method.
Usage

## S4 method for signature 'Baseline,character'
plot(x, y, ...)

## S4 method for signature 'Baseline'
summary(object, nproc = 1)

Arguments

- **x**: Baseline object.
- **y**: name of the column in the db slot of baseline containing primary identifiers.
- **...**: arguments to pass to plotBaselineDensity.
- **object**: Baseline object.
- **nproc**: number of cores to distribute the operation over.

Slots

- **description**: character providing general information regarding the sequences, selection analysis and/or object.
- **db**: data.frame containing annotation information about the sequences and selection results.
- **regionDefinition**: RegionDefinition object defining the regions and boundaries of the Ig sequences.
- **testStatistic**: character indicating the statistical framework used to test for selection. For example, "local" or "focused".
- **regions**: character vector defining the regions the BASELINE analysis was carried out on. For "cdr" and "fwr" or "cdr1", "cdr2", "cdr3", etc.
- **numbOfSeqs**: matrix of dimensions $r \times c$ containing the number of sequences or PDFs in each region, where:
  - $r$ = number of rows = number of groups or sequences.
  - $c$ = number of columns = number of regions.
- **binomK**: matrix of dimensions $r \times c$ containing the number of successes in the binomial trials in each region, where:
  - $r$ = number of rows = number of groups or sequences.
  - $c$ = number of columns = number of regions.
- **binomN**: matrix of dimensions $r \times c$ containing the total number of trials in the binomial in each region, where:
  - $r$ = number of rows = number of groups or sequences.
  - $c$ = number of columns = number of regions.
- **binomP**: matrix of dimensions $r \times c$ containing the probability of success in one binomial trial in each region, where:
  - $r$ = number of rows = number of groups or sequences.
  - $c$ = number of columns = number of regions.
- **pdfs**: list of matrices containing PDFs with one item for each defined region (e.g. cdr and fwr). Matrices have dimensions $r \times c$ demensions, where:
  - $r$ = number of rows = number of sequences or groups.
  - $c$ = number of columns = length of the PDF (default 4001).
stats data.frame of BASELINe statistics, including: mean selection strength (mean Sigma),
95% confidence intervals, and p-values with positive signs for the presence of positive selec-
tion and/or p-values with negative signs for the presence of negative selection.

See Also

See summarizeBaseline for more information on @stats.

calcBaseline  Calculate the BASELINe PDFs (including for regions that include
CDR3 and FWR4)

description
calcBaseline calculates the BASELINe posterior probability density functions (PDFs) for se-
quences in the given Change-O data.frame.

Usage

calcBaseline(
  db,
  sequenceColumn = "clonal_sequence",
  germlineColumn = "clonal_germline",
  testStatistic = c("local", "focused", "imbalanced"),
  regionDefinition = NULL,
  targetingModel = HH_S5F,
  mutationDefinition = NULL,
  calcStats = FALSE,
  nproc = 1,
  cloneColumn = NULL,
  juncLengthColumn = NULL
)

Arguments

db data.frame containing sequence data and annotations.
sequenceColumn character name of the column in db containing input sequences.
germlineColumn character name of the column in db containing germline sequences.
testStatistic character indicating the statistical framework used to test for selection. One
  of c("local", "focused", "imbalanced").
regionDefinition RegionDefinition object defining the regions and boundaries of the Ig sequences.
targetingModel TargetingModel object. Default is HH_S5F.
calcBaseline

mutationDefinition  
**MutationDefinition** object defining replacement and silent mutation criteria. If NULL then replacement and silent are determined by exact amino acid identity. Note, if the input data.frame already contains observed and expected mutation frequency columns then mutations will not be recalculated and this argument will be ignored.

calcStats  
logical indicating whether or not to calculate the summary statistics data.frame stored in the stats slot of a **Baseline** object.

nproc  
number of cores to distribute the operation over. If nproc=0 then the cluster has already been set and will not be reset.

cloneColumn  
character name of the column in db containing clonal identifiers. Relevant only for when regionDefinition includes CDR and FWR4 (else this value can be NULL)

juncLengthColumn  
character name of the column in db containing the junction length. Relevant only for when regionDefinition includes CDR and FWR4 (else this value can be NULL)

Details

Calculates the BASELINe posterior probability density function (PDF) for sequences in the provided db.

**Note:** Individual sequences within clonal groups are not, strictly speaking, independent events and it is generally appropriate to only analyze selection pressures on an effective sequence for each clonal group. For this reason, it is strongly recommended that the input db contains one effective sequence per clone. Effective clonal sequences can be obtained by calling the **collapseClones** function.

If the db does not contain the required columns to calculate the PDFs (namely mu_count & mu_expected) then the function will:

1. Calculate the numbers of observed mutations.
2. Calculate the expected frequencies of mutations and modify the provided db. The modified db will be included as part of the returned Baseline object.

The **testStatistic** indicates the statistical framework used to test for selection. E.g.

- **local** = CDR_R / (CDR_R + CDR_S).
- **focused** = CDR_R / (CDR_R + CDR_S + FWR_S).
- **imbalanced** = CDR_R + CDR_S / (CDR_R + CDR_S + FWR_S + FRW_R).

For focused the **regionDefinition** must only contain two regions. If more than two regions are defined the local test statistic will be used. For further information on the frame of these tests see **Uduman et al.** (2011).

**Value**

A **Baseline** object containing the modified db and BASELINe posterior probability density functions (PDF) for each of the sequences.
References


See Also

See Baseline for the return object. See groupBaseline and summarizeBaseline for further processing. See plotBaselineSummary and plotBaselineDensity for plotting results.

Examples

```r
# Load and subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHG" & sample_id == "+7d")

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
                      sequenceColumn="sequence_alignment",
                      germlineColumn="germline_alignment_d_mask",
                      method="thresholdedFreq", minimumFrequency=0.6,
                      includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINe
baseline <- calcBaseline(db,
                         sequenceColumn="clonal_sequence",
                         germlineColumn="clonal_germline",
                         testStatistic="focused",
                         regionDefinition=IMGT_V,
                         targetingModel=HH_S5F,
                         nproc=1)
```

---

**calcExpectedMutations**

*Calculate expected mutation frequencies of a sequence*

**Description**

calcExpectedMutations calculates the expected mutation frequencies of a given sequence. This is primarily a helper function for expectedMutations.
calcExpectedMutations

Usage

calcExpectedMutations(
  germlineSeq,
  inputSeq = NULL,
  targetingModel = HH_S5F,
  regionDefinition = NULL,
  mutationDefinition = NULL
)

Arguments

germlineSeq  germline (reference) sequence.
inputSeq  input (observed) sequence. If this is not NULL, then germlineSeq will be pro-
tessed to be the same same length as inputSeq and positions in germlineSeq corresponding to positions with Ns in inputSeq will also be assigned an N.
targetingModel  TargetingModel object. Default is HH_S5F.
regionDefinition  RegionDefinition object defining the regions and boundaries of the Ig sequences.
mutationDefinition  MutationDefinition object defining replacement and silent mutation criteria. If NULL then replacement and silent are determined by exact amino acid identity.

Details

calcExpectedMutations calculates the expected mutation frequencies of a given sequence and its germline.

Note, only the part of the sequences defined in regionDefinition are analyzed. For example, when using the default IMGT_V definition, mutations in positions beyond 312 will be ignored.

Value

A numeric vector of the expected frequencies of mutations in the regions in the regionDefinition. For example, when using the default IMGT_V definition, which defines positions for CDR and FWR, the following columns are calculated:

- mu_expected_cdr_r: number of replacement mutations in CDR1 and CDR2 of the V-segment.
- mu_expected_cdr_s: number of silent mutations in CDR1 and CDR2 of the V-segment.
- mu_expected_fwr_r: number of replacement mutations in FWR1, FWR2 and FWR3 of the V-segment.
- mu_expected_fwr_s: number of silent mutations in FWR1, FWR2 and FWR3 of the V-segment.

See Also

expectedMutations calls this function. To create a custom targetingModel see createTargetingModel. See calcObservedMutations for getting observed mutation counts.
Examples

# Load example data
data(ExampleDb, package="alakazam")

# Use first entry in the exampled data for input and germline sequence
in_seq <- ExampleDb["sequence_alignment"][1]
germline_seq <- ExampleDb["germline_alignment_d_mask"][1]

# Identify all mutations in the sequence
calcExpectedMutations(germ_seq, in_seq)

# Identify only mutations the V segment minus CDR3
calcExpectedMutations(germ_seq, in_seq, regionDefinition=IMGT_V)

# Define mutations based on hydropathy
calcExpectedMutations(germ_seq, in_seq, regionDefinition=IMGT_V, mutationDefinition=HYDROPATHY_MUTATIONS)

calcObservedMutations

Description

calcObservedMutations determines all the mutations in a given input sequence compared to its germline sequence.

Usage

calcObservedMutations(
  inputSeq,
  germlineSeq,
  regionDefinition = NULL,
  mutationDefinition = NULL,
  ambiguousMode = c("eitherOr", "and"),
  returnRaw = FALSE,
  frequency = FALSE
)

Arguments

inputSeq input sequence. IUPAC ambiguous characters for DNA are supported.
germlineSeq germline sequence. IUPAC ambiguous characters for DNA are supported.
regionDefinition RegionDefinition object defining the regions and boundaries of the Ig sequences. Note, only the part of sequences defined in regionDefinition are analyzed. If NULL, mutations are counted for entire sequence.
calcObservedMutations

**mutationDefinition**

*MutationDefinition* object defining replacement and silent mutation criteria. If NULL then replacement and silent are determined by exact amino acid identity.

**ambiguousMode**

whether to consider ambiguous characters as "either or" or "and" when determining and counting the type(s) of mutations. Applicable only if *inputSeq* and/or *germlineSeq* contain(s) ambiguous characters. One of c("eitherOr","and"). Default is "eitherOr".

**returnRaw**

return the positions of point mutations and their corresponding mutation types, as opposed to counts of mutations across positions. Also returns the number of bases used as the denominator when calculating frequency. Default is FALSE.

**frequency**

logical indicating whether or not to calculate mutation frequencies. The denominator used is the number of bases that are not one of "N", ".", or ",." in either the input or the germline sequences. If set, this overwrites *returnRaw*. Default is FALSE.

**Details**

**Each mutation is considered independently in the germline context.** For illustration, consider the case where the germline is TGG and the observed is TAC. When determining the mutation type at position 2, which sees a change from G to A, we compare the codon TGG (germline) to TAG (mutation at position 2 independent of other mutations in the germline context). Similarly, when determining the mutation type at position 3, which sees a change from G to C, we compare the codon TGG (germline) to TGC (mutation at position 3 independent of other mutations in the germline context).

If specified, only the part of *inputSeq* defined in *regionDefinition* is analyzed. For example, when using the default IMGT_V definition, then mutations in positions beyond 312 will be ignored. Additionally, non-triplet overhang at the sequence end is ignored.

Only replacement (R) and silent (S) mutations are included in the results. **Excluded** are:

- **Stop mutations**
  E.g.: the case where TAGTGG is observed for the germline TGGTGG.

- **Mutations occurring in codons where one or both of the observed and the germline involve(s) one or more of "N", ",", or ",."
  E.g.: the case where TTG is observed for the germline being any one of TNG, .TG, or -TG. Similarly, the case where any one of TTN, TT., or TT- is observed for the germline TTG.

In other words, a result that is NA or zero indicates absence of R and S mutations, not necessarily all types of mutations, such as the excluded ones mentioned above.

NA is also returned if *inputSeq* or *germlineSeq* is shorter than 3 nucleotides.

**Value**

For *returnRaw*=FALSE, an array with the numbers of replacement (R) and silent (S) mutations.

For *returnRaw*=TRUE, a list containing

- **$pos**: A data frame whose columns (position, r, s, and region) indicate, respectively, the nucleotide position, the number of R mutations at that position, the number of S mutations at that position, and the region in which that nucleotide is in.
calcObservedMutations

- $\text{nonN}$: A vector indicating the number of bases in regions defined by `regionDefinition` (excluding non-triplet overhang, if any) that are not one of "N", ",", or "." in either the `inputSeq` or `germlineSeq`.

For `frequency=TRUE`, regardless of `returnRaw`, an array with the frequencies of replacement (R) and silent (S) mutations.

**Ambiguous characters**

When there are ambiguous characters present, the user could choose how mutations involving ambiguous characters are counted through `ambiguousMode`. The two available modes are "eitherOr" and "and".

- With "eitherOr", ambiguous characters are each expanded but only 1 mutation is recorded. When determining the type of mutation, the priority for different types of mutations, in decreasing order, is as follows: no mutation, replacement mutation, silent mutation, and stop mutation.

  When counting the number of non-N, non-dash, and non-dot positions, each position is counted only once, regardless of the presence of ambiguous characters.

  As an example, consider the case where `germlineSeq` is "TST" and `inputSeq` is "THT". Expanding "H" at position 2 in `inputSeq` into "A", "C", and "T", as well as expanding "S" at position 2 in `germlineSeq` into "C" and "G", one gets:

  - "TCT" (germline) to "TAT" (observed): replacement
  - "TCT" (germline) to "TCT" (observed): no mutation
  - "TCT" (germline) to "TTT" (observed): replacement
  - "TGT" (germline) to "TAT" (observed): replacement
  - "TGT" (germline) to "TCT" (observed): replacement
  - "TGT" (germline) to "TTT" (observed): replacement

  Because "no mutation" takes priority over replacement mutation, the final mutation count returned for this example is NA (recall that only R and S mutations are returned). The number of non-N, non-dash, and non-dot positions is 3.

- With "and", ambiguous characters are each expanded and mutation(s) from all expansions are recorded.

  When counting the number of non-N, non-dash, and non-dot positions, if a position contains ambiguous character(s) in `inputSeq` and/or `germlineSeq`, the count at that position is taken to be the total number of combinations of germline and observed codons after expansion.

  Using the same example from above, the final result returned for this example is that there are 5 R mutations at position 2. The number of non-N, non-dash, and non-dot positions is 8, since there are 6 combinations stemming from position 2 after expanding the germline codon ("TST") and the observed codon ("THT").

**See Also**

See `observedMutations` for counting the number of observed mutations in a data.frame.
Examples

# Use an entry in the example data for input and germline sequence
data(ExampleDb, package="alakazam")
in_seq <- ExampleDb[["sequence_alignment"]][100]
germ_seq <- ExampleDb[["germline_alignment_d_mask"]][100]

# Identify all mutations in the sequence
ex1_raw <- calcObservedMutations(in_seq, germ_seq, returnRaw=TRUE)
# Count all mutations in the sequence
ex1_count <- calcObservedMutations(in_seq, germ_seq, returnRaw=FALSE)
ex1_freq <- calcObservedMutations(in_seq, germ_seq, returnRaw=FALSE, frequency=TRUE)
# Compare this with ex1_count
table(ex1_raw$pos$region, ex1_raw$pos$r)[, "1"]
table(ex1_raw$pos$region, ex1_raw$pos$s)[, "1"]
# Compare this with ex1_freq
(table(ex1_raw$pos$region, ex1_raw$pos$r)[, "1"]/ex1_raw$nonN)
(table(ex1_raw$pos$region, ex1_raw$pos$s)[, "1"]/ex1_raw$nonN)

# Identify only mutations the V segment minus CDR3
ex2_raw <- calcObservedMutations(in_seq, germ_seq,
       regionDefinition=IMGT_V, returnRaw=TRUE)
# Count only mutations the V segment minus CDR3
ex2_count <- calcObservedMutations(in_seq, germ_seq,
       regionDefinition=IMGT_V, returnRaw=FALSE)
ex2_freq <- calcObservedMutations(in_seq, germ_seq,
       regionDefinition=IMGT_V, returnRaw=FALSE, frequency=TRUE)
# Compare this with ex2_count
(table(ex2_raw$pos$region, ex2_raw$pos$r)[, "1"]
(table(ex2_raw$pos$region, ex2_raw$pos$s)[, "1"]
# Compare this with ex2_freq
(table(ex2_raw$pos$region, ex2_raw$pos$r)[, "1"]/ex2_raw$nonN)
(table(ex2_raw$pos$region, ex2_raw$pos$s)[, "1"]/ex2_raw$nonN)

# Identify mutations by change in hydropathy class
ex3_raw <- calcObservedMutations(in_seq, germ_seq, regionDefinition=IMGT_V,
       mutationDefinition=HYDROPATHY_MUTATIONS, returnRaw=TRUE)
# Count mutations by change in hydropathy class
ex3_count <- calcObservedMutations(in_seq, germ_seq, regionDefinition=IMGT_V,
       mutationDefinition=HYDROPATHY_MUTATIONS, returnRaw=FALSE)
ex3_freq <- calcObservedMutations(in_seq, germ_seq, regionDefinition=IMGT_V,
       mutationDefinition=HYDROPATHY_MUTATIONS, returnRaw=FALSE, frequency=TRUE)
# Compare this with ex3_count
(table(ex3_raw$pos$region, ex3_raw$pos$r)[, "1"]
(table(ex3_raw$pos$region, ex3_raw$pos$s)[, "1"]
# Compare this with ex3_freq
(table(ex3_raw$pos$region, ex3_raw$pos$r)[, "1"]/ex3_raw$nonN)
(table(ex3_raw$pos$region, ex3_raw$pos$s)[, "1"]/ex3_raw$nonN)
calcTargetingDistance  Calculates a 5-mer distance matrix from a TargetingModel object

Description

calcTargetingDistance converts either the targeting rates in a TargetingModel model to a matrix of 5-mer to single-nucleotide mutation distances, or the substitution rates in a 1-mer substitution model to a symmetric distance matrix.

Usage

calcTargetingDistance(model, places = 2)

Arguments

- **model**: TargetingModel object with mutation likelihood information, or a 4x4 1-mer substitution matrix normalized by row with rownames and colnames consisting of "A", "T", "G", and "C".
- **places**: decimal places to round distances to.

Details

The targeting model is transformed into a distance matrix by:

1. Converting the likelihood of being mutated $p = mutability \times substitution$ to distance $d = -\log_{10}(p)$.
2. Dividing this distance by the mean of the distances.
3. Converting all infinite, no change (e.g., A->A), and NA distances to zero.

The 1-mer substitution matrix is transformed into a distance matrix by:

1. Symmetrize the 1-mer substitution matrix.
2. Converting the rates to distance $d = -\log_{10}(p)$.
3. Dividing this distance by the mean of the distances.
4. Converting all infinite, no change (e.g., A -> A), and NA distances to zero.

Value

For input of TargetingModel, a matrix of distances for each 5-mer motif with rows names defining the center nucleotide and column names defining the 5-mer nucleotide sequence. For input of 1-mer substitution matrix, a 4x4 symmetric distance matrix.

See Also

See TargetingModel for this class of objects and createTargetingModel for building one.
calculateMutability

Examples

# Calculate targeting distance of HH_S5F
dist <- calcTargetingDistance(HH_S5F)

# Calculate targeting distance of HH_S1F
dist <- calcTargetingDistance(HH_S1F)

calculateMutability  Calculate total mutability

Description

calculateMutability calculates the total (summed) mutability for a set of sequences based on a
5-mer nucleotide mutability model.

Usage

calculateMutability(sequences, model = HH_S5F, progress = FALSE)

Arguments

sequences  character vector of sequences.
model       TargetingModel object with mutation likelihood information.
progress    if TRUE print a progress bar.

Value

Numeric vector with a total mutability score for each sequence.

Examples

# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Calculate mutability of germline sequences using \link{HH_S5F} model
mutability <- calculateMutability(sequences=db["germline_alignment_d_mask"], model=HH_S5F)
Description

collapseClones creates effective input and germline sequences for each clonal group and appends columns containing the consensus sequences to the input data.frame.

Usage

collapseClones(
  db,
  cloneColumn = "clone_id",
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  muFreqColumn = NULL,
  regionDefinition = NULL,
  method = c("mostCommon", "thresholdedFreq", "catchAll", "mostMutated",
            "leastMutated"),
  minimumFrequency = NULL,
  includeAmbiguous = FALSE,
  breakTiesStochastic = FALSE,
  breakTiesByColumns = NULL,
  expandedDb = FALSE,
  nproc = 1,
  juncLengthColumn = "junction_length",
  fields = NULL
)

Arguments

db: data.frame containing sequence data. Required.
cloneColumn: character name of the column containing clonal identifiers. Required.
sequenceColumn: character name of the column containing input sequences. Required. The length of each input sequence should match that of its corresponding germline sequence.
germlineColumn: character name of the column containing germline sequences. Required. The length of each germline sequence should match that of its corresponding input sequence.
muFreqColumn: character name of the column containing mutation frequency. Optional. Applicable to the "mostMutated" and "leastMutated" methods. If not supplied, mutation frequency is computed by calling observedMutations. Default is NULL. See Cautions for note on usage.
regionDefinition: RegionDefinition object defining the regions and boundaries of the Ig sequences. Optional. Default is NULL.
method method for calculating input consensus sequence. Required. One of "thresholdedFreq", "mostCommon", "catchAll", "mostMutated", or "leastMutated". See "Methods" for details.

minimumFrequency frequency threshold for calculating input consensus sequence. Applicable to and required for the "thresholdedFreq" method. A canonical choice is 0.6. Default is NULL.

includeAmbiguous whether to use ambiguous characters to represent positions at which there are multiple characters with frequencies that are at least minimumFrequency or that are maximal (i.e. ties). Applicable to and required for the "thresholdedFreq" and "mostCommon" methods. Default is FALSE. See "Choosing ambiguous characters" for rules on choosing ambiguous characters.

breakTiesStochastic In case of ties, whether to randomly pick a sequence from sequences that fulfill the criteria as consensus. Applicable to and required for all methods except for "catchAll". Default is FALSE. See "Methods" for details.

breakTiesByColumns A list of the form list(c(col_1,col_2,...),c(fun_1,fun_2,...)), where col_i is a character name of a column in db, and fun_i is a function to be applied on that column. Currently, only max and min are supported. Note that the two c()'s in list() are essential (i.e. if there is only 1 column, the list should be of the form list(c(col_1),c(func_1)). Applicable to and optional for the "mostMutated" and "leastMutated" methods. If supplied, fun_i's are applied on col_i's to help break ties. Default is NULL. See "Methods" for details.

expandedDb logical indicating whether or not to return the expanded db, containing all the sequences (as opposed to returning just one sequence per clone).

nproc Number of cores to distribute the operation over. If the cluster has already been set earlier, then pass the cluster. This will ensure that it is not reset.

juncLengthColumn character name of the column containing the junction length. Needed when regionDefinition includes CDR3 and FWR4.

fields additional fields used for grouping. Use sample_id, to avoid combining sequences with the same clone_id that belong to different sample_id.

Value

A modified db with the following additional columns:

- clonal_sequence: effective sequence for the clone.
- clonal_germline: germline sequence for the clone.
- clonal_sequence_mufreq: mutation frequency of clonal_sequence; only added for the "mostMutated" and "leastMutated" methods.

clonal_sequence is generated with the method of choice indicated by method, and clonal_germline is generated with the "mostCommon" method, along with, where applicable, user-defined parameters such as minimumFrequency, includeAmbiguous, breakTiesStochastic, and breakTiesByColumns.
Consensus lengths

For each clone, clonal_sequence and clonal_germline have the same length.

- For the "thresholdedFreq", "mostCommon", and "catchAll" methods:
  The length of the consensus sequences is determined by the longest possible consensus sequence (based on inputSeq and germlineSeq) and regionDefinition@seqLength (if supplied), whichever is shorter.
  Given a set of sequences of potentially varying lengths, the longest possible length of their consensus sequence is taken to be the longest length along which there is information contained at every nucleotide position across majority of the sequences. Majority is defined to be greater than \( \text{floor}(n/2) \), where \( n \) is the number of sequences. If the longest possible consensus length is 0, there will be a warning and an empty string ("") will be returned.
  If a length limit is defined by supplying a regionDefinition via regionDefinition@seqLength, the consensus length will be further restricted to the shorter of the longest possible length and regionDefinition@seqLength.

- For the "mostMutated" and "leastMutated" methods:
  The length of the consensus sequences depends on that of the most/least mutated input sequence, and, if supplied, the length limit defined by regionDefinition@seqLength, whichever is shorter. If the germline consensus computed using the "mostCommon" method is longer than the most/least mutated input sequence, the germline consensus is trimmed to be of the same length as the input consensus.

Methods

The descriptions below use "sequences" as a generalization of input sequences and germline sequences.

- method="thresholdedFreq"
  A threshold must be supplied to the argument minimumFrequency. At each position along the length of the consensus sequence, the frequency of each nucleotide/character across sequences is tabulated. The nucleotide/character whose frequency is at least (i.e. \( \geq \)) minimumFrequency becomes the consensus; if there is none, the consensus nucleotide will be "N".
  When there are ties (frequencies of multiple nucleotides/characters are at least minimumFrequency), this method can be deterministic or stochastic, depending on additional parameters.
  - With includeAmbiguous=TRUE, ties are resolved deterministically by representing ties using ambiguous characters. See "Choosing ambiguous characters" for how ambiguous characters are chosen.
  - With breakTiesStochastic=TRUE, ties are resolved stochastically by randomly picking a character amongst the ties.
  - When both TRUE, includeAmbiguous takes precedence over breakTiesStochastic.
  - When both FALSE, the first character from the ties is taken to be the consensus following the order of "A", "T", "G", "C", "N", ".", and "-".

Below are some examples looking at a single position based on 5 sequences with minimumFrequency=0.6, includeAmbiguous=FALSE, and breakTiesStochastic=FALSE:

- If the sequences have "A", "A", "A", "T", "C", the consensus will be "A", because "A" has frequency 0.6, which is at least minimumFrequency.
collapseClones

• method="mostCommon"
The most frequent nucleotide/character across sequences at each position along the length of the consensus sequence makes up the consensus.
When there are ties (multiple nucleotides/characters with equally maximal frequencies), this method can be deterministic or stochastic, depending on additional parameters. The same rules for breaking ties for method="thresholdedFreq" apply.
Below are some examples looking at a single position based on 5 sequences with includeAmbiguous=FALSE, and breakTiesStochastic=FALSE:
– If the sequences have "A", "A", "T", "T", "C", the consensus will be "A".
– If the sequences have "T", "T", "C", "C", "G", the consensus will be "T", because "T" is before "C" in the order of "A", "T", "G", "C", "N", ".", and ".-".

• method="catchAll"
This method returns a consensus sequence capturing most of the information contained in the sequences. Ambiguous characters are used where applicable. See "Choosing ambiguous characters" for how ambiguous characters are chosen. This method is deterministic and does not involve breaking ties.
Below are some examples for method="catchAll" looking at a single position based on 5 sequences:
– If the sequences have "N", "N", "N", "N", "N", the consensus will be "N".
– If the sequences have "N", "A", "A", "A", "A", the consensus will be "A".
– If the sequences have "N", "A", "G", "A", "A", the consensus will be "R".
– If the sequences have ".", ".", ".", ".", ".", the consensus will be ".".
– If the sequences have ".", ".", ".", ".", ".", the consensus will be ".".

• method="mostMutated" and method="leastMutated"
These methods return the most/least mutated sequence as the consensus sequence.
When there are ties (multiple sequences have the maximal/minimal mutation frequency), this method can be deterministic or stochastic, depending on additional parameters.
– With breakTiesStochastic=TRUE, ties are resolved stochastically by randomly picking a sequence out of sequences with the maximal/minimal mutation frequency.
– When breakTiesByColumns is supplied, ties are resolved deterministically. Column by column, a function is applied on the column and sequences with column value matching the functional value are retained, until ties are resolved or columns run out. In the latter case, the first remaining sequence is taken as the consensus.
– When breakTiesStochastic=TRUE and breakTiesByColumns is also supplied, breakTiesStochastic takes precedence over breakTiesByColumns.
– When breakTiesStochastic=FALSE and breakTiesByColumns is not supplied (i.e. NULL), the sequence that appears first amongst the ties is taken as the consensus.

Choosing ambiguous characters
Ambiguous characters may be present in the returned consensuses when using the "catchAll" method and when using the "thresholdedFreq" or "mostCommon" methods with includeAmbiguous=TRUE.
The rules on choosing ambiguous characters are as follows:
collapseClones

- If a position contains only "N" across sequences, the consensus at that position is "N".
- If a position contains one or more of "A", "T", "G", or "C", the consensus will be an IUPAC character representing all of the characters present, regardless of whether "N", "-", or "." is present.
- If a position contains only "-" and "." across sequences, the consensus at that position is taken to be "-".
- If a position contains only one of "-" or "." across sequences, the consensus at that position is taken to be the character present.

Cautions

- Note that this function does not perform multiple sequence alignment. As a prerequisite, it is assumed that the sequences in sequenceColumn and germlineColumn have been aligned somehow. In the case of immunoglobulin repertoire analysis, this usually means that the sequences are IMGT-gapped.
- When using the "mostMutated" and "leastMutated" methods, if you supply both muFreqColumn and regionDefinition, it is your responsibility to ensure that the mutation frequency in muFreqColumn was calculated with sequence lengths restricted to the same regionDefinition you are supplying. Otherwise, the "most/least mutated" sequence you obtain might not be the most/least mutated given the regionDefinition supplied, because your mutation frequency was based on a regionDefinition different from the one supplied.
- If you intend to run collapseClones before building a 5-mer targeting model, you must choose parameters such that your collapsed clonal consensuses do not include ambiguous characters. This is because the targeting model functions do NOT support ambiguous characters in their inputs.

See Also

See IMGT_SCHEMES for a set of predefined RegionDefinition objects.

Examples

```r
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHA", "IGHG") & sample_id == "+7d" &
clone_id %in% c("3100", "3141", "3184"))

# thresholdedFreq method, resolving ties deterministically without using ambiguous characters
clones <- collapseClones(db, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
germlineColumn="germline_alignment_d_mask", method="thresholdedFreq", minimumFrequency=0.6,
includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# mostCommon method, resolving ties deterministically using ambiguous characters
clones <- collapseClones(db, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
germlineColumn="germline_alignment_d_mask", method="mostCommon",
includeAmbiguous=TRUE, breakTiesStochastic=FALSE)
```
# Make a copy of db that has a mutation frequency column
db2 <- observedMutations(db, frequency=TRUE, combine=TRUE)

# mostMutated method, resolving ties stochastically
clones <- collapseClones(db2, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
                          germlineColumn="germline_alignment_d_mask",
                          method="mostMutated", muFreqColumn="mu_freq",
                          breakTiesStochastic=TRUE, breakTiesByColumns=NULL)

# mostMutated method, resolving ties deterministically using additional columns
clones <- collapseClones(db2, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
                          germlineColumn="germline_alignment_d_mask",
                          method="mostMutated", muFreqColumn="mu_freq",
                          breakTiesStochastic=FALSE,
                          breakTiesByColumns=list(c("duplicate_count"), c(max)))

# Build consensus for V segment only
# Capture all nucleotide variations using ambiguous characters
clones <- collapseClones(db, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
                          germlineColumn="germline_alignment_d_mask",
                          method="catchAll", regionDefinition=IMGT_V)

# Return the same number of rows as the input
clones <- collapseClones(db, cloneColumn="clone_id", sequenceColumn="sequence_alignment",
                          germlineColumn="germline_alignment_d_mask",
                          method="mostCommon", expandedDb=TRUE)

---

**consensusSequence**  
*Construct a consensus sequence*

**Description**

Construct a consensus sequence

**Usage**

```r
consensusSequence(
  sequences,
  db = NULL,
  method = c("mostCommon", "thresholdedFreq", "catchAll", "mostMutated",
             "leastMutated"),
  minFreq = NULL,
  muFreqColumn = NULL,
  lenLimit = NULL,
  includeAmbiguous = FALSE,
  breakTiesStochastic = FALSE,
  breakTiesByColumns = NULL
)
```
Arguments

sequences character vector of sequences.
db data.frame containing sequence data for a single clone. Applicable to and required for the "mostMutated" and "leastMutated" methods. Default is NULL.
method method to calculate consensus sequence. One of "thresholdedFreq", "mostCommon", "catchAll", "mostMutated", or "leastMutated". See "Methods" under collapseClones for details.
minFreq frequency threshold for calculating input consensus sequence. Applicable to and required for the "thresholdedFreq" method. A canonical choice is 0.6. Default is NULL.
muFreqColumn character name of the column in db containing mutation frequency. Applicable to and required for the "mostMutated" and "leastMutated" methods. Default is NULL.
lenLimit limit on consensus length. if NULL then no length limit is set.
includeAmbiguous whether to use ambiguous characters to represent positions at which there are multiple characters with frequencies that are at least minimumFrequency or that are maximal (i.e. ties). Applicable to and required for the "thresholdedFreq" and "mostCommon" methods. Default is FALSE. See "Choosing ambiguous characters" under collapseClones for rules on choosing ambiguous characters. Note: this argument refers to the use of ambiguous nucleotides in the output consensus sequence. Ambiguous nucleotides in the input sequences are allowed for methods catchAll, mostMutated and leastMutated.
breakTiesStochastic In case of ties, whether to randomly pick a sequence from sequences that fulfill the criteria as consensus. Applicable to and required for all methods except for "catchAll". Default is FALSE. See "Methods" under collapseClones for details.
breakTiesByColumns A list of the form list(c(col_1,col_2,...),c(fun_1,fun_2,...)), where col_i is a character name of a column in db, and fun_i is a function to be applied on that column. Currently, only max and min are supported. Note that the two c()'s in list() are essential (i.e. if there is only 1 column, the list should be of the form list(c(col_1),c(func_1)). Applicable to and optional for the "mostMutated" and "leastMutated" methods. If supplied, fun_i's are applied on col_i's to help break ties. Default is NULL. See "Methods" under collapseClones for details.

Details

See collapseClones for detailed documentation on methods and additional parameters.

Value

A list containing cons, which is a character string that is the consensus sequence for sequences; and muFreq, which is the maximal/minimal mutation frequency of the consensus sequence for the "mostMutated" and "leastMutated" methods, or NULL for all other methods.
Examples

# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHA", "IGHG") & sample_id == "+7d")
clone <- subset(db, clone_id == "3192")

# First compute mutation frequency for most/leastMutated methods
clone <- observedMutations(clone, frequency=TRUE, combine=TRUE)

# Manually create a tie
clone <- rbind(clone, clone[which.max(clone$mu_freq), ])

# ThresholdedFreq method.
# Resolve ties deterministically without using ambiguous characters
cons1 <- consensusSequence(clone$sequence_alignment,
                           method="thresholdedFreq", minFreq=0.3,
                           includeAmbiguous=FALSE,
                           breakTiesStochastic=FALSE)

cons1$cons

createBaseline

createBaseline DESCRIPTION

createBaseline creates and initialize a Baseline object.

Usage

createBaseline(
  description = "",
  db = data.frame(),
  regionDefinition = createRegionDefinition(),
  testStatistic = "",
  regions = NULL,
  numbOfSeqs = matrix(),
  binomK = matrix(),
  binomN = matrix(),
  binomP = matrix(),
  pdfs = list(),
  stats = data.frame()
)

Arguments

description character providing general information regarding the sequences, selection analysis and/or object.
createBaseline

- **db**: data.frame containing annotation information about the sequences and selection results.
- **regionDefinition**: RegionDefinition object defining the regions and boundaries of the Ig sequences.
- **testStatistic**: character indicating the statistical framework used to test for selection. For example, "local" or "focused" or "imbalanced".
- **regions**: character vector defining the regions the BASELINe analysis was carried out on. For "cdr" and "fwr" or "cdr1", "cdr2", "cdr3", etc. If NULL then regions will be determined automatically from regionDefinition.
- **numbOfSeqs**: matrix of dimensions r x c containing the number of sequences or PDFs in each region, where:
  - r = number of rows = number of groups or sequences.
  - c = number of columns = number of regions.
- **binomK**: matrix of dimensions r x c containing the number of successes in the binomial trials in each region, where:
  - r = number of rows = number of groups or sequences.
  - c = number of columns = number of regions.
- **binomN**: matrix of dimensions r x c containing the total number of trials in the binomial in each region, where:
  - r = number of rows = number of groups or sequences.
  - c = number of columns = number of regions.
- **binomP**: matrix of dimensions r x c containing the probability of success in one binomial trial in each region, where:
  - r = number of rows = number of groups or sequences.
  - c = number of columns = number of regions.
- **pdfs**: list of matrices containing PDFs with one item for each defined region (e.g. cdr and fwr). Matrices have dimensions r x c dementions, where:
  - r = number of rows = number of sequences or groups.
  - c = number of columns = length of the PDF (default 4001).
- **stats**: data.frame of BASELINe statistics, including: mean selection strength (mean Sigma), 95% confidence intervals, and p-values with positive signs for the presence of positive selection and/or p-values with negative signs for the presence of negative selection.

**Details**

Create and initialize a Baseline object.

The testStatistic indicates the statistical framework used to test for selection. For example,

- **local** = CDR_R / (CDR_R + CDR_S).
- **focused** = CDR_R / (CDR_R + CDR_S + FWR_S).
- **imbalance** = CDR_R + CDR_S / (CDR_R + CDR_S + FWR_S + FWR_R)

For focused the regionDefinition must only contain two regions. If more than two regions are defined, then the local test statistic will be used. For further information on the frame of these tests see Uduman et al. (2011).
createMutabilityMatrix

Description

createMutabilityMatrix builds a 5-mer nucleotide mutability model by counting the number of mutations occurring in the center position for all 5-mer motifs.

Usage

createMutabilityMatrix(
  db,
  substitutionModel,  # e.g., c("s", "rs")
  model = c("s", "rs"),
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  vCallColumn = "v_call",
  multipleMutation = c("independent", "ignore"),
  minNumSeqMutations = 500,
  numSeqMutationsOnly = FALSE
)

Value

A Baseline object.

References


See Also

See Baseline for the return object.

Examples

# Creates an empty Baseline object
createBaseline()
createMutabilityMatrix

Arguments

db data.frame containing sequence data.
substitutionModel matrix of 5-mer substitution rates built by createSubstitutionMatrix. Note, this model will only impact mutability scores when model="s" (using only silent mutations).
model type of model to create. The default model, "s", builds a model by counting only silent mutations. model="s" should be used for data that includes functional sequences. Setting model="rs" creates a model by counting both replacement and silent mutations and may be used on fully non-functional sequence data sets.
sequenceColumn name of the column containing IMGT-gapped sample sequences.
germlineColumn name of the column containing IMGT-gapped germline sequences.
vCallColumn name of the column containing the V-segment allele call.
multipleMutation string specifying how to handle multiple mutations occurring within the same 5-mer. If "independent" then multiple mutations within the same 5-mer are counted independently. If "ignore" then 5-mers with multiple mutations are excluded from the total mutation tally.
minNumSeqMutations minimum number of mutations in sequences containing each 5-mer to compute the mutability rates. If the number is smaller than this threshold, the mutability for the 5-mer will be inferred. Default is 500. Not required if numSeqMutationsOnly=TRUE.
umSeqMutationsOnly when TRUE, return only a vector counting the number of observed mutations in sequences containing each 5-mer. This option can be used for parameter tuning for minNumSeqMutations during preliminary analysis using minNumSeqMutationsTune. Default is FALSE.

Details

Caution: The targeting model functions do NOT support ambiguous characters in their inputs. You MUST make sure that your input and germline sequences do NOT contain ambiguous characters (especially if they are clonal consensuses returned from collapseClones).

Value

When numSeqMutationsOnly is FALSE, a MutabilityModel containing a named numeric vector of 1024 normalized mutability rates for each 5-mer motif with names defining the 5-mer nucleotide sequence.

When numSeqMutationsOnly is TRUE, a named numeric vector of length 1024 counting the number of observed mutations in sequences containing each 5-mer.

References

createMutationDefinition

Creates a MutationDefinition

Description

createMutationDefinition creates a MutationDefinition.

Usage

createMutationDefinition(name, classes, description = "", citation = "")

See Also

MutabilityModel, extendMutabilityMatrix, createSubstitutionMatrix, createTargetingMatrix, createTargetingModel, minNumSeqMutationsTune

Examples

# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create model using only silent mutations
sub_model <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment",
                                       germlineColumn="germline_alignment_d_mask",
                                       vCallColumn="v_call", model="s")
mut_model <- createMutabilityMatrix(db, sub_model, model="s",
                                       sequenceColumn="sequence_alignment",
                                       germlineColumn="germline_alignment_d_mask",
                                       vCallColumn="v_call",
                                       minNumSeqMutations=200,
                                       numSeqMutationsOnly=FALSE)

# View top 5 mutability estimates
head(sort(mut_model, decreasing=TRUE), 5)

# View the number of S mutations used for estimating mutabilities
mut_model@numMutS

# Count the number of mutations in sequences containing each 5-mer
mut_count <- createMutabilityMatrix(db, sub_model, model="s",
                                     sequenceColumn="sequence_alignment",
                                     germlineColumn="germline_alignment_d_mask",
                                     vCallColumn="v_call",
                                     numSeqMutationsOnly=TRUE)
createRegionDefinition

createRegionDefinition

Description

createRegionDefinition creates a RegionDefinition.

Usage

createRegionDefinition(
    name = "",
    boundaries = factor(),
    description = "",
    citation = ""
)
createSubstitutionMatrix

Arguments

- **name**: name of the region definition.
- **boundaries**: factor defining the region boundaries of the sequence. The levels and values of boundaries determine the number of regions (e.g. CDR and FWR).
- **description**: description of the region definition and its source data.
- **citation**: publication source.

Value

A RegionDefinition object.

See Also

See RegionDefinition for the return object.

Examples

```r
# Creates an empty RegionDefinition object
createRegionDefinition()
```

---

createSubstitutionMatrix

*Builds a substitution model*

Description

createSubstitutionMatrix builds a 5-mer nucleotide substitution model by counting the number of substitution mutations occurring in the center position for all 5-mer motifs.

Usage

```r
createSubstitutionMatrix(
  db,
  model = c("s", "rs"),
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  vCallColumn = "v_call",
  multipleMutation = c("independent", "ignore"),
  returnModel = c("5mer", "1mer", "1mer_raw"),
  minNumMutations = 50,
  numMutationsOnly = FALSE
)
```
createSubstitutionMatrix

Arguments

db  data.frame containing sequence data.
model  type of model to create. The default model, "s", builds a model by counting only silent mutations. model="s" should be used for data that includes functional sequences. Setting model="rs" creates a model by counting both replacement and silent mutations and may be used on fully non-functional sequence data sets.
sequenceColumn  name of the column containing IMGT-gapped sample sequences.
germlineColumn  name of the column containing IMGT-gapped germline sequences.
vCallColumn  name of the column containing the V-segment allele call.
multipleMutation  string specifying how to handle multiple mutations occurring within the same 5-mer. If "independent" then multiple mutations within the same 5-mer are counted independently. If "ignore" then 5-mers with multiple mutations are excluded from the total mutation tally.
returnModel  string specifying what type of model to return; one of c("5mer","1mer","1mer_raw"). If "5mer" (the default) then a 5-mer nucleotide context model is returned. If "1mer" or "1mer_raw" then a single nucleotide substitution matrix (no context) is returned; where "1mer_raw" is the unnormalized version of the "1mer" model. Note, neither 1-mer model may be used as input to createMutabilityMatrix.
minNumMutations  minimum number of mutations required to compute the 5-mer substitution rates. If the number of mutations for a 5-mer is below this threshold, its substitution rates will be estimated from neighboring 5-mers. Default is 50. Not required if numMutationsOnly=TRUE.
numMutationsOnly  when TRUE, return counting information on the number of mutations for each 5-mer, instead of building a substitution matrix. This option can be used for parameter tuning for minNumMutations during preliminary analysis. Default is FALSE. Only applies when returnModel is set to "5mer". The data.frame returned when this argument is TRUE can serve as the input for minNumMutationsTune.

Details

Caution: The targeting model functions do NOT support ambiguous characters in their inputs. You MUST make sure that your input and germline sequences do NOT contain ambiguous characters (especially if they are clonal consensuses returned from collapseClones).

Value

For returnModel = "5mer":

When numMutationsOnly is FALSE, a 4x1024 matrix of column normalized substitution rates for each 5-mer motif with row names defining the center nucleotide, one of c("A","C","G","T"), and column names defining the 5-mer nucleotide sequence.
When `numMutationsOnly` is TRUE, a 1024x4 data frame with each row providing information on counting the number of mutations for a 5-mer. Columns are named `fivemer.total`, `fivemer.every`, `inner3.total`, and `inner3.every`, corresponding to, respectively, the total number of mutations when counted as a 5-mer, whether there is mutation to every other base when counted as a 5-mer, the total number of mutations when counted as an inner 3-mer, and whether there is mutation to every other base when counted as an inner 3-mer.

For `returnModel = "1mer"` or "1mer_raw": a 4x4 normalized or un-normalized 1-mer substitution matrix respectively.

References


See Also

`extendSubstitutionMatrix`, `createMutabilityMatrix`, `createTargetingMatrix`, `createTargetingModel`, `minNumMutationsTune`.

Examples

```r
# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Count the number of mutations per 5-mer
subCount <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment", germlineColumn="germline_alignment_d_mask", vCallColumn="v_call", model="s", multipleMutation="independent", returnModel="5mer", numMutationsOnly=TRUE)

# Create model using only silent mutations
sub <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment", germlineColumn="germline_alignment_d_mask", vCallColumn="v_call", model="s", multipleMutation="independent", returnModel="5mer", numMutationsOnly=FALSE, minNumMutations=20)
```

`createTargetingMatrix` *Calculates a targeting rate matrix*
**createTargetingMatrix**

**Description**

createTargetingMatrix calculates the targeting model matrix as the combined probability of mutability and substitution.

**Usage**

createTargetingMatrix(substitutionModel, mutabilityModel)

**Arguments**

- **substitutionModel**
  - matrix of 5-mers substitution rates built by `createSubstitutionMatrix` or `extendSubstitutionMatrix`.

- **mutabilityModel**
  - vector of 5-mers mutability rates built by `createMutabilityMatrix` or `extendMutabilityMatrix`.

**Details**

Targeting rates are calculated by multiplying the normalized mutability rate by the normalized substitution rates for each individual 5-mer.

**Value**

A `TargetingMatrix` with the same dimensions as the input `substitutionModel` containing normalized targeting probabilities for each 5-mer motif with row names defining the center nucleotide and column names defining the 5-mer nucleotide sequence.

If the input `mutabilityModel` is of class `MutabilityModel`, then the output `TargetingMatrix` will carry over the input `numMutS` and `numMutR` slots.

**References**


**See Also**

`createSubstitutionMatrix`, `extendSubstitutionMatrix`, `createMutabilityMatrix`, `extendMutabilityMatrix`, `TargetingMatrix`, `createTargetingModel`

**Examples**

```r
# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create 4x1024 models using only silent mutations
sub_model <- createSubstitutionMatrix(db, model="s", sequenceColumn="sequence_alignment",)
```
createTargetingModel

Description

createTargetingModel creates a 5-mer TargetingModel.

Usage

createTargetingModel(
  db,
  model = c("s", "rs"),
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  vCallColumn = "v_call",
  multipleMutation = c("independent", "ignore"),
  minNumMutations = 50,
  minNumSeqMutations = 500,
  modelName = "",
  modelDescription = "",
  modelSpecies = "",
  modelCitation = "",
  modelDate = NULL
)

Arguments

db     data.frame containing sequence data.
model  type of model to create. The default model, "s", builds a model by counting only silent mutations. model="s" should be used for data that includes functional sequences. Setting model="rs" creates a model by counting both replacement and silent mutations and may be used on fully non-functional sequence data sets.
**createTargetingModel**

sequenceColumn  name of the column containing IMGT-gapped sample sequences.

germlineColumn  name of the column containing IMGT-gapped germline sequences.

vCallColumn  name of the column containing the V-segment allele calls.

multipleMutation  string specifying how to handle multiple mutations occurring within the same 5-mer. If "independent" then multiple mutations within the same 5-mer are counted independently. If "ignore" then 5-mers with multiple mutations are excluded from the total mutation tally.

minNumMutations  minimum number of mutations required to compute the 5-mer substitution rates. If the number of mutations for a 5-mer is below this threshold, its substitution rates will be estimated from neighboring 5-mers. Default is 50.

minNumSeqMutations  minimum number of mutations in sequences containing each 5-mer to compute the mutability rates. If the number is smaller than this threshold, the mutability for the 5-mer will be inferred. Default is 500.

modelName  name of the model.

modelDescription  description of the model and its source data.

modelSpecies  genus and species of the source sequencing data.

modelCitation  publication source.

modelDate  date the model was built. If NULL the current date will be used.

**Details**

**Caution:** The targeting model functions do NOT support ambiguous characters in their inputs. You MUST make sure that your input and germline sequences do NOT contain ambiguous characters (especially if they are clonal consensuses returned from `collapseClones`).

**Value**

A `TargetingModel` object.

**References**


**See Also**

See `TargetingModel` for the return object. See `plotMutability` plotting a mutability model. See `createSubstitutionMatrix`, `extendSubstitutionMatrix`, `createMutabilityMatrix`, `extendMutabilityMatrix` and `createTargetingMatrix` for component steps in building a model.
Examples

```r
# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create model using only silent mutations and ignore multiple mutations
model <- createTargetingModel(db, model="s", sequenceColumn="sequence_alignment",
                              germlineColumn="germline_alignment_d_mask",
                              vCallColumn="v_call", multipleMutation="ignore")

# View top 5 mutability estimates
head(sort(model@mutability, decreasing=TRUE), 5)

# View number of silent mutations used for estimating mutability
model@numMutS
```

```
DensityThreshold-class

Output of the dens method of findThreshold

Description

DensityThreshold contains output from the dens method `findThreshold`.

Usage

```r
## S4 method for signature 'DensityThreshold'
print(x)

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

Arguments

- `x` DensityThreshold object
- `y` ignored.
- `...` arguments to pass to `plotDensityThreshold`.

Slots

- `x` input distance vector with NA or infinite values removed.
- `bandwidth` bandwidth value fit during density estimation.
- `xdens` x-axis (distance value) vector for smoothed density estimate.
- `ydens` y-axis (density) vector for smoothed density estimate.
- `threshold` distance threshold that separates two modes of the input distribution.
distToNearest

**Description**

Get non-zero distance of every heavy chain (IGH) sequence (as defined by `sequenceColumn`) to its nearest sequence in a partition of heavy chains sharing the same V gene, J gene, and junction length (V-J-length), or in a partition of single cells with heavy/long chains sharing the same heavy/long chain V-J-length combination, or of single cells with heavy/long and light/short chains sharing the same heavy/long chain V-J-length and light/short chain V-J-length combinations.

**Usage**

```r
distToNearest(
  db,
  sequenceColumn = "junction",
  vCallColumn = "v_call",
  jCallColumn = "j_call",
  model = c("ham", "aa", "hh_s1f", "hh_s5f", "mk_rs1nf", "mk_rs5nf", "m1n_compat",
            "hs1f_compat"),
  normalize = c("len", "none"),
  symmetry = c("avg", "min"),
  first = TRUE,
  VJthenLen = TRUE,
  nproc = 1,
  fields = NULL,
  cross = NULL,
  mst = FALSE,
  subsample = NULL,
  progress = FALSE,
  cellIdColumn = NULL,
  locusColumn = "locus",
  onlyHeavy = TRUE,
  keepVJLgroup = TRUE
)
```

**Arguments**

- `db` : data.frame containing sequence data.
- `sequenceColumn` : name of the column containing the junction for grouping and for calculating nearest neighbor distances. Note that while both heavy/long and light/short chain junctions may be used for V-J-length grouping, only the heavy/long chain (IGH, TRB, TRD) junction is used to calculate distances.

See Also

- `findThreshold`
distToNearest

vCallColumn  name of the column containing the V-segment allele calls.

jCallColumn  name of the column containing the J-segment allele calls.

model  underlying SHM model, which must be one of c("ham","aa","hh_s1f","hh_s5f","mk_rs1nf","hs1f"). See Details for further information.

normalize  method of normalization. The default is "len", which divides the distance by the length of the sequence group. If "none" then no normalization if performed.

symmetry  if model is hs5f, distance between seq1 and seq2 is either the average (avg) of seq1->seq2 and seq2->seq1 or the minimum (min).

first  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.

VJthenLen  logical value specifying whether to perform partitioning as a 2-stage process. If TRUE, partitions are made first based on V and J gene, and then further split based on junction lengths corresponding to sequenceColumn. If FALSE, perform partition as a 1-stage process during which V gene, J gene, and junction length are used to create partitions simultaneously. Defaults to TRUE.

nproc  number of cores to distribute the function over.

fields  additional fields to use for grouping.

cross  character vector of column names to use for grouping to calculate distances across groups. Meaning the columns that define self versus others.

mst  if TRUE, return comma-separated branch lengths from minimum spanning tree.

subsample  number of sequences to subsample for speeding up pairwise-distance-matrix calculation. Subsampling is performed without replacement in each V-J-length group of heavy chain sequences. If subsample is larger than the unique number of heavy chain sequences in each VJL group, then the subsampling process is ignored for that group. For each heavy chain sequence in db, the reported dist_nearest is the distance to the closest heavy chain sequence in the subsampled set for the V-J-length group. If NULL no subsampling is performed.

progress  if TRUE print a progress bar.

cellIdColumn  name of the character column containing cell identifiers or barcodes. If specified, grouping will be performed in single-cell mode with the behavior governed by the locusColumn and onlyHeavy arguments. If set to NULL then the bulk sequencing data is assumed.

locusColumn  name of the column containing locus information. Only applicable to single-cell data. Ignored if cellIdColumn=NULL. Valid loci values are "IGH", "IGI", "IGK", "IGL", "TRA", "TRB", "TRD", and "TRG".

onlyHeavy  use only the IGH (BCR) or TRB/TRD (TCR) sequences for grouping. Only applicable to single-cell data. Ignored if cellIdColumn=NULL. See groupGenes for further details.

keepVJLgroup  logical value specifying whether to keep in the output the the column column indicating grouping based on V-J-length combinations. Only applicable for 1-stage partitioning (i.e. VJthenLen=FALSE). Also see groupGenes.
distToNearest

Details

To invoke single-cell mode the cellIdColumn argument must be specified and locusColumn must be correct. Otherwise, distToNearest will be run with bulk sequencing assumptions, using all input sequences regardless of the values in the locusColumn column.

Under single-cell mode, only heavy/long chain (IGH, TRB, TRD) sequences will be used for calculating nearest neighbor distances. Under non-single-cell mode, all input sequences will be used for calculating nearest neighbor distances, regardless of the values in the locusColumn field (if present).

Values in the locusColumn must be one of c("IGH", "IGI", "IGK", "IGL") for BCR or c("TRA", "TRB", "TRD", "TRG") for TCR sequences. Otherwise, the function returns an error message and stops.

For single-cell mode, the input format is the same as that for groupGenes. Namely, each row represents a sequence/chain. Sequences/chains from the same cell are linked by a cell ID in the cellIdColumn field. In this mode, 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). This is governed by the onlyHeavy argument.

Note, distToNearest required that each cell (each unique value in cellIdColumn) correspond to only a single IGH (BCR) or TRB/TRD (TCR) sequence.

The distance to nearest neighbor can be used to estimate a threshold for assigning Ig sequences to clonal groups. A histogram of the resulting vector is often bimodal, with the ideal threshold being a value that separates the two modes.

The following distance measures are accepted by the model parameter.

- "ham": Single nucleotide Hamming distance matrix from getDNAMatrix with gaps assigned zero distance.
- "aa": Single amino acid Hamming distance matrix from getAAMatrix.
- "hh_s1f": Human single nucleotide distance matrix derived from HH_S1F with calcTargetingDistance.
- "hh_s5f": Human 5-mer nucleotide context distance matrix derived from HH_S5F with calcTargetingDistance.
- "mk_rs1nf": Mouse single nucleotide distance matrix derived from MK_RS1NF with calcTargetingDistance.
- "mk_rs5nf": Mouse 5-mer nucleotide context distance matrix derived from MK_RS1NF with calcTargetingDistance.
- "hs1f_compat": Backwards compatible human single nucleotide distance matrix used in SHazaM v0.1.4 and Change-O v0.3.3.
- "m1n_compat": Backwards compatible mouse single nucleotide distance matrix used in SHazaM v0.1.4 and Change-O v0.3.3.

Note on NAs: if, for a given combination of V gene, J gene, and junction length, there is only 1 heavy chain sequence (as defined by sequenceColumn), NA is returned instead of a distance (since it has no heavy/long chain neighbor). If for a given combination there are multiple heavy/long chain sequences but only 1 unique one, (in which case every heavy/long chain sequence in this group is the de facto nearest neighbor to each other, thus giving rise to distances of 0), NAs are returned instead of zero-distances.
Note on subsample: Subsampling is performed independently in each V-J-length group for heavy/long chain sequences. If subsample is larger than number of heavy/long chain sequences in the group, it is ignored. In other words, subsampling is performed only on groups in which the number of heavy/long chain sequences is equal to or greater than subsample. dist_nearest has values calculated using all heavy chain sequences in the group for groups with fewer than subsample heavy/long chain sequences, and values calculated using a subset of heavy/long chain sequences for the larger groups. To select a value of subsample, it can be useful to explore the group sizes in db (and the number of heavy/long chain sequences in those groups).

Value

Returns a modified db data.frame with nearest neighbor distances between heavy chain sequences in the dist_nearest column if cross=NULL. If cross was specified, distances will be added as the cross_dist_nearest column.

Note that distances between light/short (IGK, IGL, TRA, TRG) chain sequences are not calculated, even if light/short chains were used for V-J-length grouping via onlyHeavy=FALSE. Light/short chain sequences, if any, will have NA in the dist_nearest output column.

Note that the output vCallColumn and jCallColumn columns will be converted to type character if they were type factor in the input db.

References


See Also

See calcTargetingDistance for generating nucleotide distance matrices from a TargetingModel object. See HH_S5F, HH_S1F, MK_RS1NF, getDNAMatrix, and getAAMatrix for individual model details.

Examples

# Subset example data to one sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, sample_id == "-1h")

# Use genotyped V assignments, Hamming distance, and normalize by junction length
# First partition based on V and J assignments, then by junction length
# Take into consideration ambiguous V and J annotations
dist <- distToNearest(db, sequenceColumn="junction",
vCallColumn="v_call_genotyped", jCallColumn="j_call",
model="ham", first=FALSE, VJthenLen=TRUE, normalize="len")
# Plot histogram of non-NA distances
p1 <- ggplot(data=subset(dist, !is.na(dist_nearest))) +
  theme_bw() +
  ggtitle("Distance to nearest: Hamming") +
  xlab("distance") +
  geom_histogram(aes(x=dist_nearest), binwidth=0.025,
    fill="steelblue", color="white")
plot(p1)

## editBaseline

### Edit the Baseline object

editBaseline edits a field in a Baseline object.

#### Usage

```r
editBaseline(baseline, field, value)
```

#### Arguments

- `baseline` Baseline object to be edited.
- `field` name of the field in the Baseline object to be edited.
- `value` value to set the field.

#### Value

A Baseline object with the field of choice updated.

#### See Also

See Baseline for the input and return object.

#### Examples

```r
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHG" & sample_id == "+7d")

# Make Baseline object
baseline <- calcBaseline(db,
  sequenceColumn="sequence_alignment",
  germlineColumn="germline_alignment_d_mask",
  testStatistic="focused",
  regionDefinition=IMGT_V,
```
expectedMutations

Calculate expected mutation frequencies

Description

expectedMutations calculates the expected mutation frequencies for each sequence in the input data.frame.

Usage

expectedMutations(
  db,
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment",
  targetingModel = HH_S5F,
  regionDefinition = NULL,
  mutationDefinition = NULL,
  nproc = 1,
  cloneColumn = "clone_id",
  juncLengthColumn = "junction_length"
)

Arguments

db data.frame containing sequence data.
sequenceColumn character name of the column containing input sequences.
germlineColumn character name of the column containing the germline or reference sequence.
targetingModel TargetingModel object. Default is HH_S5F.
regionDefinition RegionDefinition object defining the regions and boundaries of the Ig sequences. To use regions definitions, sequences in sequenceColumn and germlineColumn must be aligned, following the IMGT schema.
mutationDefinition MutationDefinition object defining replacement and silent mutation criteria. If NULL then replacement and silent are determined by exact amino acid identity.
nproc numeric number of cores to distribute the operation over. If the cluster has already been set the call function with nproc = 0 to not reset or reinitialize. Default is nproc = 1.
expectedMutations

cloneColumn clone id column name in db
juncLengthColumn junction length column name in db

Details

Only the part of the sequences defined in regionDefinition are analyzed. For example, when using the IMGT_V definition, mutations in positions beyond 312 will be ignored.

Value

A modified db data.frame with expected mutation frequencies for each region defined in regionDefinition. The columns names are dynamically created based on the regions in regionDefinition. For example, when using the IMGT_V definition, which defines positions for CDR and FWR, the following columns are added:

- mu_expected_cdr_r: number of replacement mutations in CDR1 and CDR2 of the V-segment.
- mu_expected_cdr_s: number of silent mutations in CDR1 and CDR2 of the V-segment.
- mu_expected_fwr_r: number of replacement mutations in FWR1, FWR2 and FWR3 of the V-segment.
- mu_expected_fwr_s: number of silent mutations in FWR1, FWR2 and FWR3 of the V-segment.

See Also

calcExpectedMutations is called by this function to calculate the expected mutation frequencies. See observedMutations for getting observed mutation counts. See IMGT_SCHEMES for a set of predefined RegionDefinition objects.

Examples

# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHA", "IGHG") & sample_id == "+7d")

# Calculate expected mutations over V region
db_exp <- expectedMutations(db,
  sequenceColumn="sequence_alignment",
  germlineColumn="germline_alignment_d_mask",
  regionDefinition=IMGT_V,
  nproc=1)

# Calculate hydropathy expected mutations over V region
db_exp <- expectedMutations(db,
  sequenceColumn="sequence_alignment",
  germlineColumn="germline_alignment_d_mask",
  regionDefinition=IMGT_V,
  mutationDefinition=HYDROPATHY_MUTATIONS,
  nproc=1)
extendMutabilityMatrix

Description

extendMutabilityMatrix extends a 5-mer nucleotide mutability model with 5-mers that include Ns by averaging over all corresponding 5-mers without Ns.

Usage

`extendMutabilityMatrix(mutabilityModel)`

Arguments

- `mutabilityModel` vector of 5-mer mutability rates built by `createMutabilityMatrix`.

Value

A `MutabilityModel` containing a 3125 vector of normalized mutability rates for each 5-mer motif with names defining the 5-mer nucleotide sequence. Note that "normalized" means that the mutability rates for the 1024 5-mers that contain no "N" at any position sums up to 1 (as opposed to the entire vector summing up to 1).

If the input `mutabilityModel` is of class `MutabilityModel`, then the output `MutabilityModel` will carry over the input `numMutS` and `numMutR` slots.

See Also

- `createMutabilityMatrix`, `extendSubstitutionMatrix`, `MutabilityModel`

Examples

```r
# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create model using only silent mutations and ignore multiple mutations
sub_model <- createSubstitutionMatrix(db, model="s", sequenceColumn="sequence_alignment",
                                      germlineColumn="germline_alignment_d_mask",
                                      vCallColumn="v_call")
mut_model <- createMutabilityMatrix(db, sub_model, model="s",
                                     sequenceColumn="sequence_alignment",
                                     germlineColumn="germline_alignment_d_mask",
                                     vCallColumn="v_call")
ext_model <- extendMutabilityMatrix(mut_model)
```
extendSubstitutionMatrix

Extends a substitution model to include Ns.

Description

extendSubstitutionMatrix extends a 5-mer nucleotide substitution model with 5-mers that include Ns by averaging over all corresponding 5-mers without Ns.

Usage

extendSubstitutionMatrix(substitutionModel)

Arguments

substitutionModel

matrix of 5-mers substitution counts built by createSubstitutionMatrix.

Value

A 5x3125 matrix of normalized substitution rate for each 5-mer motif with rows names defining the center nucleotide, one of c("A","C","G","T","N"), and column names defining the 5-mer nucleotide sequence.

See Also

createSubstitutionMatrix, extendMutabilityMatrix

Examples

# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create model using only silent mutations
sub_model <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment",
  germlineColumn="germline_alignment_d_mask",
  vCallColumn="v_call", model="s")

ext_model <- extendSubstitutionMatrix(sub_model)
**findThreshold**

*Find distance threshold*

**Description**

`findThreshold` automatically determines an optimal threshold for clonal assignment of Ig sequences using a vector of nearest neighbor distances. It provides two alternative methods using either a Gamma/Gaussian Mixture Model fit (method="gmm") or kernel density fit (method="density").

**Usage**

```r
findThreshold(
  distances,
  method = c("density", "gmm"),
  edge = 0.9,
  cross = NULL,
  subsample = NULL,
  model = c("gamma-gamma", "gamma-norm", "norm-gamma", "norm-norm"),
  cutoff = c("optimal", "intersect", "user"),
  sen = NULL,
  spc = NULL,
  progress = FALSE
)
```

**Arguments**

- **distances**: numeric vector containing nearest neighbor distances.
- **method**: string defining the method to use for determining the optimal threshold. One of "gmm" or "density". See Details for methodological descriptions.
- **edge**: upper range as a fraction of the data density to rule initialization of Gaussian fit parameters. Default value is 90. Applies only when method="density".
- **cross**: supplementary nearest neighbor distance vector output from `distToNearest` for initialization of the Gaussian fit parameters. Applies only when method="gmm".
- **subsample**: maximum number of distances to subsample to before threshold detection.
- **model**: allows the user to choose among four possible combinations of fitting curves: "norm-norm", "norm-gamma", "gamma-norm", and "gamma-gamma". Applies only when method="gmm".
- **cutoff**: method to use for threshold selection: the optimal threshold "opt", the intersection point of the two fitted curves "intersect", or a value defined by user for one of the sensitivity or specificity "user". Applies only when method="gmm".
- **sen**: sensitivity required. Applies only when method="gmm" and cutoff="user".
- **spc**: specificity required. Applies only when method="gmm" and cutoff="user".
- **progress**: if TRUE print a progress bar.
Details

- "gmm": Performs a maximum-likelihood fitting procedure, for learning the parameters of two mixture univariate, either Gamma or Gaussian, distributions which fit the bimodal distribution entries. Retrieving the fit parameters, it then calculates the optimum threshold method="optimal", where the average of the sensitivity plus specificity reaches its maximum. In addition, the findThreshold function is also able to calculate the intersection point (method="intersect") of the two fitted curves and allows the user to invoke its value as the cut-off point, instead of optimal point.

- "density": Fits a binned approximation to the ordinary kernel density estimate to the nearest neighbor distances after determining the optimal bandwidth for the density estimate via least-squares cross-validation of the 4th derivative of the kernel density estimator. The optimal threshold is set as the minimum value in the valley in the density estimate between the two modes of the distribution.

Value

- "gmm" method: Returns a GmmThreshold object including the threshold and the function fit parameters, i.e. mixing weight, mean, and standard deviation of a Normal distribution, or mixing weight, shape and scale of a Gamma distribution.

- "density" method: Returns a DensityThreshold object including the optimum threshold and the density fit parameters.

Note

Visually inspecting the resulting distribution fits is strongly recommended when using either fitting method. Empirical observations imply that the bimodality of the distance-to-nearest distribution is detectable for a minimum of 1,000 distances. Larger numbers of distances will improve the fitting procedure, although this can come at the expense of higher computational demands.

See Also

See distToNearest for generating the nearest neighbor distance vectors. See plotGmmThreshold and plotDensityThreshold for plotting output.

Examples

# Subset example data to one sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, sample_id == "-1h")

# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, sequenceColumn="junction", vCallColumn="v_call",
                   jCallColumn="j_call", model="ham", normalize="len", nproc=1)

# Find threshold using the "gmm" method with optimal threshold
output <- findThreshold(db$dist_nearest, method="gmm", model="gamma-gamma", cutoff="opt")
plot(output, binwidth=0.02, title=paste0(output@model, " loglk=", output@loglk))
print(output)
# Find threshold using the "gmm" method with user defined specificity
output <- findThreshold(db$dist_nearest, method="gmm", model="gamma-gamma",
cutoff="user", spc=0.99)
plot(output, binwidth=0.02, title=paste0(output@model, " loglk=", output@loglk))
print(output)

# Find threshold using the "density" method and plot the results
output <- findThreshold(db$dist_nearest, method="density")
plot(output)
print(output)

---

**GmmThreshold-class**

*Output of the gmm method of findThreshold*

**Description**

GmmThreshold contains output from the gmm method `findThreshold`. It includes parameters of two Gaussian fits and threshold cut.

**Usage**

```r
## S4 method for signature 'GmmThreshold'
print(x)

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

**Arguments**

- `x` GmmThreshold object
- `y` ignored.
- `...` arguments to pass to `plotGmmThreshold`.

**Slots**

- `x` input distance vector with NA or infinite values removed.
- `model` first-second fit functions.
- `cutoff` type of threshold cut.
- `a1` mixing weight of the first curve.
- `b1` second parameter of the first curve. Either the mean of a Normal distribution or shape of a Gamma distribution.
- `c1` third parameter of the first curve. Either the standard deviation of a Normal distribution or scale of a Gamma distribution.
- `a2` mixing weight of the second curve.
b2 second parameter of the second curve. Either the mean of a Normal distribution or shape of a Gamma distribution.

c2 third parameter of the second curve. Either the standard deviation of a Normal distribution or scale of a Gamma distribution.

loglk log-likelihood of the fit.

threshold threshold.

sensitivity sensitivity.

specificity specificity.

pvalue p-value from Hartigans’ dip statistic (HDS) test. Values less than 0.05 indicate significant bimodality.

See Also

findThreshold

groupBaseline  Group BASELINe PDFs

Description

groupBaseline convolves groups of BASELINe posterior probability density functions (PDFs) to get combined PDFs for each group.

Usage

groupBaseline(baseline, groupBy, nproc = 1)

Arguments

baseline Baseline object containing the db and the BASELINe posterior probability density functions (PDF) for each of the sequences, as returned by calcBaseline.

groupBy The columns in the db slot of the Baseline object by which to group the sequence PDFs.

nproc number of cores to distribute the operation over. If nproc = 0 then the cluster has already been set and will not be reset.

Details

While the selection strengths predicted by BASELINe perform well on average, the estimates for individual sequences can be highly variable, especially when the number of mutations is small.

To overcome this, PDFs from sequences grouped by biological or experimental relevance, are convolved to from a single PDF for the selection strength. For example, sequences from each sample may be combined together, allowing you to compare selection across samples. This is accomplished through a fast numerical convolution technique.
groupBaseline

Value

A Baseline object, containing the modified db and the BASELINe posterior probability density functions (PDF) for each of the groups.

References


See Also

To generate the Baseline object see calcBaseline. To calculate BASELINe statistics, such as the mean selection strength and the 95% confidence interval, see summarizeBaseline.

Examples

```r
# Subset example data from alakazam
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHM", "IGHG"))

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
                      sequenceColumn="sequence_alignment",
                      germlineColumn="germline_alignment_d_mask",
                      method="thresholdedFreq", minimumFrequency=0.6,
                      includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINe
baseline <- calcBaseline(db,
                         sequenceColumn="clonal_sequence",
                         germlineColumn="clonal_germline",
                         testStatistic="focused",
                         regionDefinition=IMGT_V,
                         targetingModel=HH_S5F,
                         nproc=1)

# Group PDFs by sample
grouped1 <- groupBaseline(baseline, groupBy="sample_id")
sample_colors <- c("-1h"="steelblue", "+7d"="firebrick")
plotBaselineDensity(grouped1, idColumn="sample_id", colorValues=sample_colors,
                     sigmaLimits=c(-1, 1))

# Group PDFs by both sample (between variable) and isotype (within variable)
grouped2 <- groupBaseline(baseline, groupBy=c("sample_id", "c_call"))
isotype_colors <- c("IGHM"="darkorchid", "IGHD"="firebrick",
                   "IGHG"="seagreen", "IGHA"="steelblue")
plotBaselineDensity(grouped2, idColumn="sample_id", groupColumn="c_call",
                   colorElement="group", colorValues=isotype_colors,
                   sigmaLimits=c(-1, 1))
```
HH_S1F

Human heavy chain, silent, 1-mer, functional substitution model.

Description

1-mer substitution model of somatic hypermutation based on analysis of silent mutations in functional heavy chain Ig sequences from Homo sapiens.

Usage

HH_S1F

Format

A 4x4 matrix of nucleotide substitution rates. The rates are normalized, therefore each row sums up to 1.

Note

HH_S1F replaces HS1FDistance in versions of SHazaM prior to 0.1.5.

References


See Also

See HKL_S1F for the human light chain 1-mer substitution model and MK_RS1NF for the mouse light chain 1-mer substitution model.
HH_S5F

Human heavy chain, silent, 5-mer, functional targeting model.

Description

5-mer model of somatic hypermutation targeting based on analysis of silent mutations in functional heavy chain Ig sequences from Homo sapiens.

Usage

HH_S5F

Format

A TargetingModel object.

References


See Also

See HH_S1F for the 1-mer substitution matrix from the same publication; HKL_S5F for the human light chain 5-mer targeting model; MK_RS5NF for the mouse 5-mer targeting model; and U5N for the uniform 5-mer null targeting model.

HKL_S1F

Human kappa and lambda chain, silent, 1-mer, functional substitution model.

Description

1-mer substitution model of somatic hypermutation based on analysis of silent mutations in functional kappa and lambda light chain Ig sequences from Homo sapiens.

Usage

HKL_S1F

Format

A 4x4 matrix of nucleotide substitution rates. The rates are normalized, therefore each row sums up to 1.
Note

Reported in Table III in Cui et al, 2016.

References


See Also

See HH_S1F for the human heavy chain 1-mer substitution model and MK_RS1NF for the mouse light chain 1-mer substitution model.

---

**HKL_S5F**  
*Human kappa and lambda light chain, silent, 5-mer, functional targeting model.*

Description

5-mer model of somatic hypermutation targeting based on analysis of silent mutations in functional kappa and lambda light chain Ig sequences from Homo sapiens.

Usage

**HKL_S5F**

Format

A `TargetingModel` object.

References


See Also

See HH_S5F for the human heavy chain 5-mer targeting model; MK_RS5NF for the mouse kappa light chain 5-mer targeting model; and U5N for the uniform 5-mer null targeting model.
Description

Sequence region definitions according to the IMGT unique numbering scheme.

Format

A RegionDefinition object defining:

- **IMGT_V**: The IMGT numbered V segment up to position nucleotide 312. This definition combines the CDR1 and CDR2 into a single CDR region, and FWR1, FWR2 and FWR3 into a single FWR region. CDR3 and FWR4 are excluded as they are downstream of nucleotide 312.

- **IMGT_V_BY_CODONS**: The IMGT numbered V segment up to position nucleotide 312. This definition treats each codon, from codon 1 to codon 104, as a distinct region.

- **IMGT_V_BY_REGIONS**: The IMGT numbered V segment up to position nucleotide 312. This defines separate regions for each of CDR1, CDR2, FWR1, FWR2 and FWR3. CDR3 and FWR4 are excluded as they are downstream of nucleotide 312.

- **IMGT_V_BY_SEGMENTS**: The IMGT numbered V segment up to position nucleotide 312. This definition has no subdivisions and treats the entire V segment as a single region.

- **IMGT_VDJ**: IMGT numbered regions for CDR1-3 and FWR1-4 with combined CDR and FWR definitions spanning CDR1-3 and FWR1-4, respectively. Note, unless the definition object has been updated using setRegionBoundaries this schema will have a value of 0 for the seqLength slot and the boundaries slot will be empty. This is because these slots depend on the junction length which is unknown in the template scheme. After setRegionBoundaries has been run, these slots will be populated with the appropriate values for the specified sequence and junction length.

- **IMGT_VDJ_BY_REGIONS**: The IMGT numbered regions for FWR1-4 and CDR1-3 with separate region boundaries for each of CDR1, CDR2, CDR3, FWR1, FWR2, FWR3 and FWR4. Note, unless the definition object has been updated using setRegionBoundaries this schema will have a value of 0 for the seqLength slot and the boundaries slot will be empty. This is because these slots depend on the junction length which is unknown in the template scheme. After setRegionBoundaries has been run, these slots will be populated with the appropriate values for the specified sequence and junction length.

References

Description

makeAverage1merMut averages mutability rates in a 5-mer mutability model to derive a 1-mer mutability model.

Usage

makeAverage1merMut(mut5mer)

Arguments

mut5mer a named vector of length 1024 such as that returned by createMutabilityMatrix and that returned by makeDegenerate5merMut with extended=FALSE. Names should correspond to 5-mers made up of "A", "T", "G", and "C" (case-insensitive). NA values are allowed.

Details

For example, the mutability rate of "A" in the resultant 1-mer model is derived by averaging the mutability rates of all the 5-mers that have an "A" as their central 1-mer, followed by normalization.

Value

A named vector of length 4 containing normalized mutability rates.

See Also

See makeDegenerate5merMut for making a degenerate 5-mer mutability model based on a 1-mer mutability model.

Examples

# Make a degenerate 5-mer model (length of 1024) based on a 1-mer model
degenerate5merMut <- makeDegenerate5merMut(mut1mer = example1merMut)

# Now make a 1-mer model by averaging over the degenerate 5-mer model
# Expected to get back example1merMut
makeAverage1merMut(mut5mer = degenerate5merMut)
makeAverage1merSub

Make a 1-mer substitution model by averaging over a 5-mer substitution model

Description

makeAverage1merSub averages substitution rates in a 5-mer substitution model to derive a 1-mer substitution model.

Usage

makeAverage1merSub(sub5mer)

Arguments

sub5mer a 4x1024 matrix such as that returned by createSubstitutionMatrix and that returned by makeDegenerate5merSub with extended=FALSE. Column names should correspond to 5-mers containing the central 1-mer to mutate from. Row names should correspond to nucleotides to mutate into. Nucleotides should include "A", "T", "G", and "C" (case-insensitive).

Details

For example, the substitution rate from "A" to "T" in the resultant 1-mer model is derived by averaging the substitution rates into a "T" of all the 5-mers that have an "A" as their central 1-mer.

Value

A 4x4 matrix with row names representing nucleotides to mutate from and column names representing nucleotides to mutate into. Rates are normalized by row.

See Also

See makeDegenerate5merSub for making a degenerate 5-mer substitution model based on a 1-mer substitution model.

Examples

# Make a degenerate 5-mer model (4x1024) based on HKL_S1F (4x4)
degenerate5merSub <- makeDegenerate5merSub(sub1mer = HKL_S1F)

# Now make a 1-mer model by averaging over the degenerate 5-mer model
# Expected to get back HKL_S1F
makeAverage1merSub(sub5mer = degenerate5merSub)
makeDegenerate5merMut

Make a degenerate 5-mer mutability model based on a 1-mer mutability model

Description

makeDegenerate5merMut populates mutability rates from a 1-mer mutability model into 5-mers with corresponding central 1-mers.

Usage

makeDegenerate5merMut(mut1mer, extended = FALSE)

Arguments

- **mut1mer**: a named vector of length 4 containing (normalized) mutability rates. Names should correspond to nucleotides, which should include "A", "T", "G", and "C" (case-insensitive).
- **extended**: whether to return the unextended (extended=FALSE) or extended (extended=TRUE) 5-mer mutability model. Default is FALSE.

Details

As a concrete example, consider a 1-mer mutability model in which mutability rates of "A", "T", "G", and "C" are, respectively, 0.14, 0.23, 0.31, and 0.32. In the resultant degenerate 5-mer mutability model, all the 5-mers that have an "A" as their central 1-mer would have mutability rate of 0.14/256, where 256 is the number of such 5-mers.

When extended=TRUE, extendMutabilityMatrix is called to extend the mutability vector of length 1024 into a vector of length 3125.

Value

For extended=FALSE, a vector of length 1024. The vector returned is normalized. For extended=TRUE, a vector of length 3125.

See Also

See makeAverage1merMut for making a 1-mer mutability model by taking the average of a 5-mer mutability model. See extendMutabilityMatrix for extending the mutability vector.

Examples

# Make a degenerate 5-mer model (length of 1024) based on a 1-mer model
dgeneate5merMut <- makeDegenerate5merMut(mut1mer = example1merMut)

degenerate5merMut[c("AAAAT", "AACAT", "AAGAT", "AATAT")]

# Look at a few 5-mers
degenerate5merMut[c("AAAAT", "AACAT", "AAGAT", "AATAT")]
# Normalized
sum(degenerate5merMut)

## makeDegenerate5merSub

### Description

makeDegenerate5merSub populates substitution rates from a 1-mer substitution model into 5-mers with corresponding central 1-mers.

### Usage

```r
makeDegenerate5merSub(sub1mer, extended = FALSE)
```

### Arguments

- **sub1mer**: a 4x4 matrix containing (normalized) substitution rates. Row names should correspond to nucleotides to mutate from. Column names should correspond to nucleotides to mutate into. Nucleotides should include "A", "T", "G", and "C" (case-insensitive).
- **extended**: whether to return the unextended (extended=FALSE) or extended (extended=TRUE) 5-mer substitution model. Default is FALSE.

### Details

As a concrete example, consider a 1-mer substitution model in which substitution rates from "A" to "T", "G", and "C" are, respectively, 0.1, 0.6, and 0.3. In the resultant degenerate 5-mer substitution model, all the 5-mers (columns) that have an "A" as their central 1-mer would have substitution rates (rows) of 0.1, 0.6, and 0.3 to "T", "G", and "C" respectively.

When extended=TRUE, extendSubstitutionMatrix is called to extend the 4x1024 substitution matrix.

### Value

For extended=FALSE, a 4x1024 matrix. For extended=TRUE, a 5x3125 matrix.

### See Also

See `makeAverage1merSub` for making a 1-mer substitution model by taking the average of a 5-mer substitution model. See `extendSubstitutionMatrix` for extending the substitution matrix.
Examples

# Make a degenerate 5-mer model (4x1024) based on HKL_S1F (4x4)
# Note: not to be confused with HKL_S5F@substitution, which is non-degenerate
degenerate5merSub <- makeDegenerate5merSub(sub1mer = HKL_S1F)

# Look at a few 5-mers
degenerate5merSub[, c("AAAAT", "AACAT", "AAGAT", "AATAT")]

makeGraphDf

Build a data.frame from a ChangeoClone and an igraph object containing a clonal lineage

Description

makeGraphDf creates a data.frame from a ChangeoClone and an igraph graph object containing a B cell lineage tree and associated sequence data. The data.frame contains the original fields and additions such as each sequence's parent in the lineage tree, the lineage germline, and additional rows for inferred sequences.

Usage

makeGraphDf(
  curCloneGraph,
  curCloneObj,
  objSeqId = "sequence_id",
  objSeq = "sequence"
)

Arguments

curCloneGraph an igraph graph object for the lineage tree generated by buildPhylipLineage. Note that the field containing the nucleotide sequence in the object must be named sequence.
curCloneObj ChangeoClone object used to generate the lineage.
objSeqId name of the sequence identifier field in curCloneObj.
objSeq name of the nucleotide sequence field in curCloneObj.

Value

A data.frame with sequence and lineage information, including the the parent nucleotide sequence in the lineage tree(parent_sequence), an internal parent identifier (parent), and additional rows for germline sequence and inferred intermediate sequences.

Values in the sequence_id field are renamed to numeric values, prefixed with the clonal grouping identifier and labeled as either "Inferred" or "Germline" if they are not an observed sequence. For example, for a lineage with clone_id = 34 the new identifiers would be of the form: "34_Germline", "34_Inferred1", "34_1", "34_2", etc.
Note that the original sequence identifier is preserved in the orig_sequence_id field and the original parent sequence identifier is retained in orig_parent.

See Also

See observedMutations to calculate mutation frequencies using parent_sequence as the reference germline. See ChangeoClone, buildPhylipLineage, and graph for details on the input objects.

Examples

```r
# Load and subset example data
data(ExampleDb, package = "alakazam")
data(ExampleTrees, package = "alakazam")
graph <- ExampleTrees[[17]]
db <- subset(ExampleDb, clone_id == graph$clone)
clone <- alakazam::makeChangeoClone(db)

# Extend data with lineage information
df <- makeGraphDf(graph, clone)
```

---

### minNumMutationsTune

Parameter tuning for minNumMutations

**Description**

minNumMutationsTune helps with picking a threshold value for minNumMutations in createSubstitutionMatrix by tabulating the number of 5-mers for which substitution rates would be computed directly or inferred at various threshold values.

**Usage**

```r
minNumMutationsTune(subCount, minNumMutationsRange)
```

**Arguments**

- `subCount`: data.frame returned by createSubstitutionMatrix with numMutationsOnly=TRUE.
- `minNumMutationsRange`: a number or a vector indicating the value or range of values of minNumMutations to try.

**Details**

At a given threshold value of minNumMutations, for a given 5-mer, if the total number of mutations is greater than the threshold and there are mutations to every other base, substitution rates are computed directly for the 5-mer using its mutations. Otherwise, mutations from 5-mers with the same inner 3-mer as the 5-mer of interest are aggregated. If the number of such mutations is greater than the threshold and there are mutations to every other base, these mutations are used for inferring
the substitution rates for the 5-mer of interest; if not, mutations from all 5-mers with the same center nucleotide are aggregated and used for inferring the substitution rates for the 5-mer of interest (i.e. the 1-mer model).

Value

A 3xn matrix, where n is the number of trial values of \( \text{minNumMutations} \) supplied in \( \text{minNumMutationsRange} \). Each column corresponds to a value in \( \text{minNumMutationsRange} \). The rows correspond to the number of 5-mers for which substitution rates would be computed directly using the 5-mer itself ("5mer"), using its inner 3-mer ("3mer"), and using the central 1-mer ("1mer"), respectively.

References


See Also

See argument `numMutationsOnly` in `createSubstitutionMatrix` for generating the required input data.frame `subCount`. See argument `minNumMutations` in `createSubstitutionMatrix` for what it does.

Examples

```r
# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Count the number of mutations per 5-mer
subCount <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment", 
                                        germlineColumn="germline_alignment_d_mask", 
                                        vCallColumn="v_call", 
                                        model="s", multipleMutation="independent", 
                                        returnModel="5mer", numMutationsOnly=TRUE)

# Tune minNumMutations
minNumMutationsTune(subCount, seq(from=10, to=100, by=10))
```

minNumSeqMutationsTune

Parameter tuning for minNumSeqMutations

Description

minNumSeqMutationsTune helps with picking a threshold value for minNumSeqMutations in `createMutabilityMatrix` by tabulating the number of 5-mers for which mutability would be computed directly or inferred at various threshold values.
minNumSeqMutationsTune

Usage

minNumSeqMutationsTune(mutCount, minNumSeqMutationsRange)

Arguments

mutCount
  a vector of length 1024 returned by createMutabilityMatrix with numSeqMutationsOnly=TRUE.

minNumSeqMutationsRange
  a number or a vector indicating the value or the range of values of minNumSeqMutations to try.

Details

At a given threshold value of minNumSeqMutations, for a given 5-mer, if the total number of mutations is greater than the threshold, mutability is computed directly. Otherwise, mutability is inferred.

Value

A 2xn matrix, where n is the number of trial values of minNumSeqMutations supplied in minNumSeqMutationsRange. Each column corresponds to a value in minNumSeqMutationsRange. The rows correspond to the number of 5-mers for which mutability would be computed directly ("measured") and inferred ("inferred"), respectively.

References


See Also

See argument numSeqMutationsOnly in createMutabilityMatrix for generating the required input vector mutCount. See argument minNumSeqMutations in createMutabilityMatrix for what it does.

Examples

# Subset example data to one isotype and sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA" & sample_id == "-1h")

# Create model using only silent mutations
sub <- createSubstitutionMatrix(db, sequenceColumn="sequence_alignment", germlineColumn="germline_alignment_d_mask", vCallColumn="v_call", model="s", multipleMutation="independent", returnModel="5mer", numMutationsOnly=FALSE, minNumMutations=20)

# Count the number of mutations in sequences containing each 5-mer
mutCount <- createMutabilityMatrix(db, substitutionModel = sub,
  sequenceColumn="sequence_alignment",
  germlineColumn="germline_alignment_d_mask",
  vCallColumn="v_call",
  model="s", multipleMutation="independent",
  numSeqMutationsOnly=TRUE)

# Tune minNumSeqMutations
minNumSeqMutationsTune(mutCount, seq(from=100, to=300, by=50))

MK_RS1NF

Mouse kappa chain, replacement and silent, 1-mer, non-functional substitution model.

Description

1-mer substitution model of somatic hypermutation based on analysis of replacement and silent mutations in non-functional kappa light chain Ig sequences from NP-immunized Mus musculus.

Usage

MK_RS1NF

Format

A 4x4 matrix of nucleotide substitution rates. The rates are normalized, therefore each row sums up to 1.

Note

MK_RS1NF replaces M1NDistance from versions of SHazaM prior to 0.1.5.

References


See Also

See HH_S1F for the human heavy chain 1-mer substitution model and HKL_S1F for the human light chain 1-mer substitution model.
MK_RS5NF  
Mouse kappa light chain, replacement and silent, 5-mer, non-functional targeting model.

Description

5-mer model of somatic hypermutation targeting based on analysis of replacement and silent mutations in non-functional kappa light chain Ig sequences from NP-immunized Mus musculus.

Usage

MK_RS5NF

Format

TargetingModel object.

References


See Also

See MK_RS1NF for the 1-mer substitution matrix from the same publication; HH_S5F for the human heavy chain silent 5-mer functional targeting model; HKL_S5F for the human light chain silent 5-mer functional targeting model; and U5N for the uniform 5-mer null targeting model.

MutabilityModel-class  
S4 class defining a mutability model

Description

MutabilityModel defines a data structure for the 5-mer motif-based SHM targeting mutability model.

Usage

## S4 method for signature 'MutabilityModel'
print(x)

## S4 method for signature 'MutabilityModel'
as.data.frame(x)
**MutationDefinition-class**

**Arguments**

- `x` MutabilityModel object.

**Slots**

- `.Data` numeric vector containing 5-mer mutability estimates
- `source` character vector annotating whether the mutability was inferred or directly measured.
- `numMutS` a number indicating the number of silent mutations used for estimating mutability
- `numMutR` a number indicating the number of replacement mutations used for estimating mutability

---

**MutationDefinition-class**

*S4 class defining replacement and silent mutation definitions*

---

**Description**

`MutationDefinition` defines a common data structure for defining whether a mutation is annotated as a replacement or silent mutation.

**Slots**

- `name` name of the `MutationDefinition`.
- `description` description of the model and its source.
- `classes` named character vectors with single-letter amino acid codes as names and amino acid classes as values, with `NA` assigned to set of characters c("X", "x", "-", "."). Replacement (R) is be defined as a change in amino acid class and silent (S) as no change in class.
- `codonTable` matrix of codons (columns) and substitutions (rows).
- `citation` publication source.

**See Also**

See `MUTATION_SCHEMES` for a set of predefined `MutationDefinition` objects.
MUTATION_SCHEMES  Amino acid mutation definitions

Description
Definitions of replacement (R) and silent (S) mutations for different amino acid physicochemical classes.

Format
A MutationDefinition object defining:

• CHARGE_MUTATIONS: Amino acid mutations are defined by changes in side chain charge class.
• HYDROPATHY_MUTATIONS: Amino acid mutations are defined by changes in side chain hydrophobicity class.
• POLARITY_MUTATIONS: Amino acid mutations are defined by changes in side chain polarity class.
• VOLUME_MUTATIONS: Amino acid mutations are defined by changes in side chain volume class.

References

observedMutations  Calculate observed numbers of mutations

Description
observedMutations calculates the observed number of mutations for each sequence in the input data.frame.

Usage
observedMutations(
  db,
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  regionDefinition = NULL,
  mutationDefinition = NULL,
  ambiguousMode = c("eitherOr", "and"),
  frequency = FALSE,
  combine = FALSE,
  nproc = 1,
  cloneColumn = "clone_id",
  juncLengthColumn = "junction_length"
)
Arguments

db data.frame containing sequence data.
sequenceColumn character name of the column containing input sequences. IUPAC ambiguous characters for DNA are supported.
germlineColumn character name of the column containing the germline or reference sequence. IUPAC ambiguous characters for DNA are supported.
regionDefinition RegionDefinition object defining the regions and boundaries of the Ig sequences. If NULL, mutations are counted for entire sequence. To use regions definitions, sequences in sequenceColumn and germlineColumn must be aligned, following the IMGT schema.
mutationDefinition MutationDefinition object defining replacement and silent mutation criteria. If NULL then replacement and silent are determined by exact amino acid identity.
ambiguousMode whether to consider ambiguous characters as "either or" or "and" when determining and counting the type(s) of mutations. Applicable only if sequenceColumn and/or germlineColumn contain(s) ambiguous characters. One of c("eitherOr", "and"). Default is "eitherOr".
frequency logical indicating whether or not to calculate mutation frequencies. Default is FALSE.
combine logical indicating whether for each sequence should the mutation counts for the different regions (CDR, FWR) and mutation types be combined and return one value of count/frequency per sequence instead of multiple values. Default is FALSE.
nproc number of cores to distribute the operation over. If the cluster has already been set the call function with nproc = 0 to not reset or reinitialize. Default is nproc = 1.
cloneColumn clone id column name in db
juncLengthColumn junction length column name in db

Details

Mutation counts are determined by comparing a reference sequence to the input sequences in the column specified by sequenceColumn. See calcObservedMutations for more technical details, including criteria for which sequence differences are included in the mutation counts and which are not.

The mutations are binned as either replacement (R) or silent (S) across the different regions of the sequences as defined by regionDefinition. Typically, this would be the framework (FWR) and complementarity determining (CDR) regions of IMGT-gapped nucleotide sequences. Mutation counts are appended to the input db as additional columns.

If db includes lineage information, such as the parent_sequence column created by makeGraphDf, the reference sequence can be set to use that field as reference sequence using the germlineColumn argument.
observedMutations

Value

A modified db data.frame with observed mutation counts for each sequence listed. The columns names are dynamically created based on the regions in the regionDefinition. For example, when using the IMGT_V definition, which defines positions for CDR and FWR, the following columns are added:

- `mu_count_cdr_r`: number of replacement mutations in CDR1 and CDR2 of the V-segment.
- `mu_count_cdr_s`: number of silent mutations in CDR1 and CDR2 of the V-segment.
- `mu_count_fwr_r`: number of replacement mutations in FWR1, FWR2 and FWR3 of the V-segment.
- `mu_count_fwr_s`: number of silent mutations in FWR1, FWR2 and FWR3 of the V-segment.

If `frequency=TRUE`, R and S mutation frequencies are calculated over the number of non-N positions in the specified regions.

- `mu_freq_cdr_r`: frequency of replacement mutations in CDR1 and CDR2 of the V-segment.
- `mu_freq_cdr_s`: frequency of silent mutations in CDR1 and CDR2 of the V-segment.
- `mu_freq_fwr_r`: frequency of replacement mutations in FWR1, FWR2 and FWR3 of the V-segment.
- `mu_freq_fwr_s`: frequency of silent mutations in FWR1, FWR2 and FWR3 of the V-segment.

If `frequency=TRUE` and `combine=TRUE`, the mutations and non-N positions are aggregated and a single `mu_freq` value is returned

- `mu_freq`: frequency of replacement and silent mutations in the specified region

See Also

calcObservedMutations is called by this function to get the number of mutations in each sequence grouped by the RegionDefinition. See IMGT_SCHEMES for a set of predefined RegionDefinition objects. See expectedMutations for calculating expected mutation frequencies. See makeGraphDf for creating the field parent_sequence.

Examples

# Subset example data
data(ExampleDb, package="alakazam")
db <- ExampleDb[1:10, ]

# Calculate mutation frequency over the entire sequence
db_obs <- observedMutations(db, sequenceColumn="sequence_alignment",
                           germlineColumn="germline_alignment_d_mask",
                           frequency=TRUE,
                           nproc=1)

# Count of V-region mutations split by FWR and CDR
# With mutations only considered replacement if charge changes
db_obs <- observedMutations(db, sequenceColumn="sequence_alignment",
                           germlineColumn="germline_alignment_d_mask",
# Count of VDJ-region mutations, split by FWR and CDR

db_obs <- observedMutations(db, sequenceColumn="sequence_alignment",
                           germlineColumn="germline_alignment_d_mask",
                           regionDefinition=IMGT_VDJ,
                           nproc=1)

data(ExampleTrees, package="alakazam")

# Extend data with lineage information
graph <- ExampleTrees[[17]]
clone <- alakazam::makeChangeoClone(subset(ExampleDb, clone_id == graph$clone))
gdf <- makeGraphDf(graph, clone)

# Count of mutations between observed sequence and immediate ancestor

db_obs <- observedMutations(gdf, sequenceColumn="sequence",
                           germlineColumn="parent_sequence",
                           regionDefinition=IMGT_VDJ,
                           nproc=1)

---

**plotBaselineDensity**  
*Plots BASELINe probability density functions*

**Description**

plotBaselineDensity plots the probability density functions resulting from selection analysis using the BASELINe method.

**Usage**

```r
plotBaselineDensity(  
  baseline,  
  idColumn,  
  groupColumn = NULL,  
  colorElement = c("id", "group"),  
  colorValues = NULL,  
  title = NULL,  
  subsetRegions = NULL,  
  sigmaLimits = c(-5, 5),  
  facetBy = c("region", "group"),  
  style = c("density"),  
  sizeElement = c("none", "id", "group"),  
  size = 1,  
  silent = FALSE,  
  ...)
```

Arguments

baseline Baseline object containing selection probability density functions.

idColumn name of the column in the db slot of baseline containing primary identifiers.

groupColumn name of the column in the db slot of baseline containing secondary grouping identifiers. If NULL, organize the plot only on values in idColumn.

colorElement one of c("id","group") specifying whether the idColumn or groupColumn will be used for color coding. The other entry, if present, will be coded by line style.

colorValues named vector of colors for entries in colorElement, with names defining unique values in the colorElement column and values being colors. Also controls the order in which values appear on the plot. If NULL alphabetical ordering and a default color palette will be used.

title string defining the plot title.

subsetRegions character vector defining a subset of regions to plot, corresponding to the regions for which the baseline data was calculated. If NULL all regions in baseline are plotted.

sigmaLimits numeric vector containing two values defining the c(lower, upper) bounds of the selection scores to plot.

facetBy one of c("region","group") specifying which category to facet the plot by, either values in groupColumn ("group") or regions defined in the regions slot of the baseline object ("region"). If this is set to "group", then the region will behave as the groupColumn for purposes of the colorElement argument.

style type of plot to draw. One of:
  * "density": plots a set of curves for each probability density function in baseline, with colors determined by values in the colorElement column. Faceting is determined by the facetBy argument.

sizeElement one of c("none","id","group") specifying whether the lines in the plot should be all of the same size (none) or have their sizes depend on the values in id or code.

size numeric scaling factor for lines, points and text 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

Takes as input a Baseline object returned from groupBaseline.
Examples

```r
# Subset example data
data(ExampleDb, package = "alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHM", "IGHG"))

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
                     sequenceColumn="sequence_alignment",
                     germlineColumn="germline_alignment_d_mask",
                     method="thresholdedFreq", minimumFrequency=0.6,
                     includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINe
baseline <- calcBaseline(db,
                         sequenceColumn="clonal_sequence",
                         germlineColumn="clonal_germline",
                         testStatistic="focused",
                         regionDefinition=IMGT_V,
                         targetingModel=HH_S5F,
                         nproc=1)

# Grouping the PDFs by the sample and isotype annotations
grouped <- groupBaseline(baseline, groupBy=c("sample_id", "c_call"))

# Plot density faceted by region with custom isotype colors
isotype_colors <- c("IGHM"="darkorchid", "IGHD"="firebrick",
                    "IGHG"="seagreen", "IGHA"="steelblue")
plotBaselineDensity(grouped, "sample_id", "c_call",
                    colorValues=isotype_colors,
                    colorElement="group", sigmaLimits=c(-1, 1))

# Facet by isotype instead of region
sample_colors <- c("-1h"="steelblue", "+7d"="firebrick")
plotBaselineDensity(grouped, "sample_id", "c_call",
                    facetBy="group",
                    colorValues=sample_colors, sigmaLimits=c(-1, 1))
```

---

**Description**

`plotBaselineSummary` plots a summary of the results of selection analysis using the BASELINe method.

**Usage**

```r
plotBaselineSummary()
```
baseline,  
idColumn,  
groupColumn = NULL,  
groupColors = NULL,  
subsetRegions = NULL,  
facetBy = c("region", "group"),  
title = NULL,  
style = c("summary"),  
size = 1,  
silent = FALSE,  
...  
)

Arguments

baseline  either a data.frame returned from `summarizeBaseline` or a Baseline object returned from `groupBaseline` containing selection probability density functions and summary statistics.

idColumn  name of the column in `baseline` containing primary identifiers. If the input is a Baseline object, then this will be a column in the `stats` slot of `baseline`.

groupColumn  name of the column in `baseline` containing secondary grouping identifiers. If the input is a Baseline object, then this will be a column in the `stats` slot of `baseline`.

groupColors  named vector of colors for entries in `groupColumn`, with names defining unique values in the `groupColumn` and values being colors. Also controls the order in which groups appear on the plot. If `NULL` alphabetical ordering and a default color palette will be used. Has no effect if `facetBy="group"`.

subsetRegions  character vector defining a subset of regions to plot, corresponding to the regions for which the `baseline` data was calculated. If `NULL` all regions in `baseline` are plotted.

facetBy  one of c("group", "region") specifying which category to facet the plot by, either values in `groupColumn` ("group") or regions defined in `baseline` ("region"). The data that is not used for faceting will be color coded.

title  string defining the plot title.

style  type of plot to draw. One of:
  - "summary": plots the mean and confidence interval for the selection scores of each value in `idColumn`. Faceting and coloring are determined by values in `groupColumn` and regions defined in `baseline`, depending upon the `facetBy` argument.

size  numeric scaling factor for lines, points and text 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.
plotDensityThreshold

See Also

Takes as input either a Baseline object returned by groupBaseline or a data.frame returned from summarizeBaseline.

Examples

# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHM", "IGHG"))

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
                     sequenceColumn="sequence_alignment",
                     germlineColumn="germline_alignment_d_mask",
                     method="thresholdedFreq", minimumFrequency=0.6,
                     includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINE
baseline <- calcBaseline(db,
                         sequenceColumn="clonal_sequence",
                         germlineColumn="clonal_germline",
                         testStatistic="focused",
                         regionDefinition=IMGT_V,
                         targetingModel=HH_S5F,
                         nproc=1)

# Grouping the PDFs by sample and isotype annotations
grouped <- groupBaseline(baseline, groupBy=c("sample_id", "c_call"))

# Plot mean and confidence interval by region with custom group colors
isotype_colors <- c("IGHM"="darkorchid", "IGHD"="firebrick",
                    "IGHG"="seagreen", "IGHA"="steelblue")
plotBaselineSummary(grouped, "sample_id", "c_call",
                    groupColors=isotype_colors)

# Facet by group instead of region
plotBaselineSummary(grouped, "sample_id", "c_call", facetBy="group")

plotDensityThreshold  Plot findThreshold results for the density method

Description

plotDensityThreshold plots the results from "density" method of findThreshold, including the smoothed density estimate, input nearest neighbor distance histogram, and threshold selected.
plotDensityThreshold

Usage

plotDensityThreshold(
  data,
  cross = NULL,
  xmin = NULL,
  xmax = NULL,
  breaks = NULL,
  binwidth = NULL,
  title = NULL,
  size = 1,
  silent = FALSE,
  ...
)

Arguments

data | DensityThreshold object output by the "density" method of findThreshold.
cross | numeric vector of distances from distToNearest to draw as a histogram below the data histogram for comparison purposes.
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 to the bandwidth parameter determined by findThreshold.
title | string defining the plot title.
size | numeric value for the plot line sizes.
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 DensityThreshold for the the input object definition and findThreshold for generating the input object. See distToNearest calculating nearest neighbor distances.

Examples

# Subset example data to one sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, sample_id == "-1h")
# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, sequenceColumn="junction", vCallColumn="v_call_genotyped",
jCallColumn="j_call", model="ham", normalize="len", nproc=1)

# To find the threshold cut, call findThreshold function for "gmm" method.
output <- findThreshold(db$dist_nearest, method="density")
print(output)

# Plot
plotDensityThreshold(output)

---

plotGmmThreshold  Plot findThreshold results for the gmm method

Description

plotGmmThreshold plots the results from "gmm" method of findThreshold, including the Gaussian distributions, input nearest neighbor distance histogram, and threshold selected.

Usage

plotGmmThreshold(
  data,  
cross = NULL,  
xmin = NULL,  
xmax = NULL,  
breaks = NULL,  
binwidth = NULL,  
title = NULL,  
size = 1,  
silent = FALSE,  
...
)

Arguments

data  
GmmThreshold object output by the "gmm" method of findThreshold.
cross  
numeric vector of distances from distToNearest to draw as a histogram below the data histogram for comparison purposes.
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.
plotMutability

Plot mutability probabilities

Description

plotMutability plots the mutability rates of a TargetingModel.

Usage

plotMutability(
  model,
  nucleotides = c("A", "C", "G", "T"),
  mark = NULL,
  style = c("hedgehog", "bar"),
  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 GmmThreshold for the the input object definition and findThreshold for generating the input object. See distToNearest calculating nearest neighbor distances.

Examples

# Subset example data to one sample as a demo
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, sample_id == "-1h")

# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, sequenceColumn="junction", vCallColumn="v_call_genotyped",
  jCallColumn="j_call", model="ham", normalize="len", nproc=1)

# To find the threshold cut, call findThreshold function for "gmm" method.
output <- findThreshold(db$dist_nearest, method="gmm", model="norm-norm", cutoff="opt")
print(output)

# Plot results
plotGmmThreshold(output, binwidth=0.02)
Arguments

- **model**: TargetingModel object or vector containing normalized mutability rates.
- **nucleotides**: vector of center nucleotide characters to plot.
- **mark**: vector of 5-mer motifs to highlight in the plot. If NULL only highlight classical hot and cold spot motifs.
- **style**: type of plot to draw. One of:
  - "hedgehog": circular plot showing higher mutability scores further from the circle. The 5-mer is denoted by the values of the inner circle. The 5-mer is read from the most interior position of the 5-mer (5') to most exterior position (3'), with the center nucleotide in the center ring. Note, the order in which the 5-mers are plotted is different for nucleotides c("A", "C") and c("G", "T").
  - "bar": bar plot of mutability similar to the hedgehog style with the most 5' positions of each 5-mer at the base of the plot.
- **size**: numeric scaling factor for lines and text in the plot.
- **silent**: if TRUE do not draw the plot and just return the ggplot2 objects; if FALSE draw the plot.
- **...**: additional arguments to pass to ggplot2::theme.

Value

A named list of ggplot objects defining the plots, with names defined by the center nucleotide for the plot object.

See Also

Takes as input a TargetingModel object. See createTargetingModel for model building.

Examples

```r
# Plot one nucleotide in circular style
plotMutability(HH_S5F, "C")

# Plot two nucleotides in bar chart style
plotMutability(HH_S5F, c("G", "T"), style="bar")
```
plotTune

Visualize parameter tuning for minNumMutations and minNumSeqMutations

Description

Visualize results from minNumMutationsTune and minNumSeqMutationsTune

Usage

plotTune(
  tuneMtx, thresh, criterion = c("5mer", "3mer", "1mer", "3mer+1mer", "measured", "inferred"), pchs = 1, ltys = 2, cols = 1, plotLegend = TRUE, legendPos = "topright", legendHoriz = FALSE, legendCex = 1)

Arguments

tuneMtx  a matrix or a list of matrices produced by either minNumMutationsTune or minNumSeqMutationsTune. In the case of a list, it is assumed that each matrix corresponds to a sample and that all matrices in the list were produced using the same set of trial values of minNumMutations or minNumSeqMutations.

thresh  a number or a vector of indicating the value or the range of values of minNumMutations or minNumSeqMutations to plot. Should correspond to the columns of tuneMtx.

criterion  one of "5mer", "3mer", "1mer", or "3mer+1mer" (for tuneMtx produced by minNumMutationsTune), or either "measured" or "inferred" (for tuneMtx produced by minNumSeqMutationsTune).

pchs  point types to pass on to plot.

ltys  line types to pass on to plot.

cols  colors to pass on to plot.

plotLegend  whether to plot legend. Default is TRUE. Only applicable if tuneMtx is a named list with names of the matrices corresponding to the names of the samples.

legendPos  position of legend to pass on to legend. Can be either a numeric vector specifying x-y coordinates, or one of "topright", "center", etc. Default is "topright".

legendHoriz  whether to make legend horizontal. Default is FALSE.

legendCex  numeric values by which legend should be magnified relative to 1.
Details

For `tuneMtx` produced by `minNumMutationsTune`, for each sample, depending on criterion, the numbers of 5-mers for which substitution rates are directly computed ("5mer"), inferred based on inner 3-mers ("3mer"), inferred based on central 1-mers ("1mer"), or inferred based on inner 3-mers and central 1-mers ("3mer+1mer") are plotted on the y-axis against values of `minNumMutations` on the x-axis.

For `tuneMtx` produced by `minNumSeqMutationsTune`, for each sample, depending on criterion, the numbers of 5-mers for which mutability rates are directly measured ("measured") or inferred ("inferred") are plotted on the y-axis against values of `minNumSeqMutations` on the x-axis.

Note that legends will be plotted only if `tuneMtx` is supplied as a named list of matrices, ideally with names of each matrix corresponding to those of the samples based on which the matrices were produced, even if `plotLegend=TRUE`.

See Also

See `minNumMutationsTune` and `minNumSeqMutationsTune` for generating `tuneMtx`.

Examples

```r
# Subset example data to one isotype and sample as demos
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call == "IGHA")

tuneMtx = list()
for (i in 1:length(unique(db$sample_id))) {
  # Get data corresponding to current sample
  curDb = db[db["sample_id"] == unique(db["sample_id"])[i], ]

  # Count the number of mutations per 5-mer
  subCount = createSubstitutionMatrix(db=curDb, model="s",
    sequenceColumn="sequence_alignment",
    germlineColumn="germline_alignment_d_mask",
    vCallColumn="v_call",
    multipleMutation="independent",
    returnModel="5mer", numMutationsOnly=TRUE)

  # Tune over minNumMutations = 5..50
  subTune = minNumMutationsTune(subCount, seq(from=5, to=50, by=5))

  tuneMtx = c(tuneMtx, list(subTune))
}

# Name tuneMtx after sample names
names(tuneMtx) = unique(db["sample_id"])

# plot with legend for both samples for a subset of minNumMutations values
plotTune(tuneMtx, thresh=c(5, 15, 25, 40), criterion="3mer",
  pchs=16:17, lty=1:2, cols=2:3,
  plotLegend=TRUE, legendPos=c(5, 100))
```
# plot for only 1 sample for all the minNumMutations values (no legend)
plotTune(tuneMtx[[1]], thresh=seq(from=5, to=50, by=5), criterion="3mer")

## RegionDefinition-class

S4 class defining a region definition

### Description

RegionDefinition defines a common data structure for defining the region boundaries of an Ig sequence.

### Slots

- **name** name of the RegionDefinition.
- **description** description of the model and its source.
- **boundaries** factor defining the region boundaries of the sequence. The levels and values of boundaries determine the number of regions.
- **seqLength** length of the sequence.
- **regions** levels of the boundaries; e.g., c("cdr","fwr").
- **labels** labels for the boundary and mutations combinations; e.g., c("cdr_r","cdr_s","fwr_r","fwr_s").
- **citation** publication source.

### See Also

See IMGT_SCHEMES for a set of predefined RegionDefinition objects.

## setRegionBoundaries

Build a RegionDefinition object that includes CDR3 and FWR4.

### Description

setRegionBoundaries takes as input a junction length and an IMGT-numbered sequence and outputs a custom RegionDefinition object that includes the boundary definitions of CDR1-3 and FWR1-4 for that sequence. In contrast to the universal RegionDefinition object that end with FWR3, the returned definition is per-sequence due to variable junction lengths.

### Usage

setRegionBoundaries(juncLength, sequenceImgt, regionDefinition = NULL)
Arguments

juncLength    junction length of the sequence.
sequenceImgt  IMGT-numbered sequence.
regionDefinition  RegionDefinition type to calculate the region definition for. Can be one of
                  IMGT_VDJ_BY_REGIONS or IMGT_VDJ, which are template definitions that
                  include CDR1-3 and FWR1-4. Only these two regions include all CDR1-3 and
                  FWR1-4 regions. If this argument is set to NULL, then an empty RegionDefinition
                  will be returned.

Value

A RegionDefinition object that includes CDR1-3 and FWR1-4 for the sequenceImgt, juncLength, and
regionDefinition specified.

For regionDefinition=IMGT_VDJ_BY_REGIONS, the returned RegionDefinition includes:

• fwr1: Positions 1 to 78.
• cdr1: Positions 79 to 114.
• fwr2: Positions 115 to 165.
• cdr2: Positions 166 to 195.
• fwr3: Positions 196 to 312.
• cdr3: Positions 313 to (313 + juncLength - 6) since the junction sequence includes (on the
        left) the last codon from FWR3 and (on the right) the first codon from FWR4.
• fwr4: Positions (313 + juncLength - 6 + 1) to the end of the sequence.

For regionDefinition=IMGT_VDJ, the returned RegionDefinition includes:

• fwr: Positions belonging to a FWR.
• cdr: Positions belonging to a CDR.

In the case that the regionDefinition argument is not one of the extended regions (IMGT_VDJ_BY_REGIONS
or IMGT_VDJ), the input regionDefinition is returned as is.

See Also

See RegionDefinition for the return object. See IMGT_SCHEMES for a set of predefined RegionDefinition
objects.

Examples

# Load and subset example data
data(ExampleDb, package = "alakazam")
len <- ExampleDb$junction_length[1]
sequence <- ExampleDb$sequence_alignment[1]
region <- setRegionBoundaries(len, sequence, regionDefinition = IMGT_VDJ)
The shazam package

Description

Dramatic improvements in high-throughput sequencing technologies now enable large-scale char-
acterization of Ig repertoires, defined as the collection of transmembrane antigen-receptor proteins
located on the surface of T and B lymphocytes. The shazam package provides tools for advanced
analysis of somatic hypermutation (SHM) in immunoglobulin (Ig) sequences. The key functions in
shazam, broken down topic, are described below.

Mutational profiling

shazam provides tools to quantify the extent and nature of SHM within full length V(D)J sequences
as well as sub-regions (e.g., FWR and CDR). Quantification of expected mutational loaded, under
specific SHM targeting models, can also be performed along with model driven simulations of
SHM.

- collapseClones: Build clonal consensus sequences.
- consensusSequence: Build a single consensus sequence.
- observedMutations: Compute observed mutation counts and frequencies.
- expectedMutations: Compute expected mutation frequencies.
- shmulateSeq: Simulate mutations in a single sequence.
- shmulateTree: Simulate mutations over a lineage tree.
- setRegionBoundaries: Extends a region definition to include CDR3 and FWR4.

SHM targeting models

Computational models and analyses of SHM have separated the process into two independent com-
ponents:

1. A mutability model that defines where mutations occur.
2. A nucleotide substitution model that defines the resulting mutation.

Collectively these are what form the targeting model of SHM. shazam provides empirically de-
derived targeting models for both humans and mice, along with tools to build these mutability and
substitution models from data.

- createTargetingModel: Build a 5-mer targeting model.
- plotMutability: Plot 5-mer mutability rates.
- HH_S5F: Human 5-mer SHM targeting model.
- MK_RS5NF: Mouse 5-mer SHM targeting model.
Quantification of selection pressure

Bayesian Estimation of Antigen-driven Selection in Ig Sequences is a novel method for quantifying antigen-driven selection in high-throughput Ig sequence data. Targeting models created using shazam can be used to estimate the null distribution of expected mutation frequencies used by BASELINe, providing measures of selection pressure informed by known AID targeting biases.

- calcBaseline: Calculate the BASELINe probability density functions (PDFs).
- groupBaseline: Combine PDFs from sequences grouped by biological or experimental relevance.
- summarizeBaseline: Compute summary statistics from BASELINe PDFs.
- testBaseline: Perform significance testing for the difference between BASELINe PDFs.
- plotBaselineDensity: Plot the probability density functions resulting from selection analysis.
- plotBaselineSummary: Plot summary statistics resulting from selection analysis.

Mutational distance calculation

shazam provides tools to compute evolutionary distances between sequences or groups of sequences, which can leverage SHM targeting models. This information is particularly useful in understanding and defining clonal relationships.

- findThreshold: Identify clonal assignment threshold based on distances to nearest neighbors.
- distToNearest: Tune clonal assignment thresholds by calculating distances to nearest neighbors.
- calcTargetingDistance: Construct a nucleotide distance matrix from a 5-mer targeting model.

References

**shmulateSeq**  
Simulate mutations in a single sequence

**Description**

Generates random mutations in a sequence iteratively using a targeting model. Targeting probabilities at each position are updated after each iteration.

**Usage**

```r
shmulateSeq(
  sequence,
  numMutations,
  targetingModel = HH_S5F,
  start = 1,
  end = nchar(sequence),
  frequency = FALSE
)
```

**Arguments**

- `sequence`  
  sequence string in which mutations are to be introduced. Accepted alphabet: {A,T,G,C,N,.}. Note that - is not accepted.

- `numMutations`  
  a whole number indicating the number of mutations to be introduced into `sequence`, if `frequency=FALSE`. A fraction between 0 and 1 indicating the mutation frequency if `frequency=TRUE`.

- `targetingModel`  
  5-mer `TargetingModel` object to be used for computing probabilities of mutations at each position. Defaults to `HH_S5F`.

- `start`  
  Initial position in `sequence` where mutations can be introduced. Default: 1

- `end`  
  Last position in `sequence` where mutations can be introduced. Default: last position (sequence length).

- `frequency`  
  If TRUE, treat `numMutations` as a frequency.

**Details**

If the input `sequence` has a non-triplet overhang at the end, it will be trimmed to the last codon. For example, ATGCATGC will be trimmed to ATGCAT.

Mutations are not introduced to positions in the input `sequence` that contain . or N.

With `frequency=TRUE`, the number of mutations introduced is the floor of the length of the `sequence` multiplied by the mutation frequency specified via `numMutations`.

**Value**

A string defining the mutated sequence.
**shmulateTree**

Simulate mutations in a lineage tree

### Description

shmulateTree returns a set of simulated sequences generated from an input sequence and a lineage tree. The input sequence is used to replace the most recent common ancestor (MRCA) node of the igraph object defining the lineage tree. Sequences are then simulated with mutations corresponding to edge weights in the tree. Sequences will not be generated for groups of nodes that are specified to be excluded.

### Usage

```r
shmulateTree(
    sequence,
    graph,
    targetingModel = HH_S5F,
    field = NULL,
    exclude = NULL,
    junctionWeight = NULL,
    start = 1,
    end = nchar(sequence)
)
```

### Arguments

- **sequence**: string defining the MRCA sequence to seed mutations from.
- **graph**: igraph object defining the seed lineage tree, with vertex annotations, whose edges are to be recreated.

### See Also

See `shmulateTree` for imposing mutations on a lineage tree. See HH_S5F and MK_RS5NF for predefined TargetingModel objects.

### Examples

```r
# Define example input sequence
sequence <- "NGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATA.TTTA"

# Simulate using the default human 5-mer targeting model
# Introduce 6 mutations
shmulateSeq(sequence, numMutations=6, frequency=FALSE)

# Introduction 5% mutations
shmulateSeq(sequence, numMutations=0.05, frequency=TRUE)
```
targetingModel 5-mer TargetingModel object to be used for computing probabilities of mutations at each position. Defaults to HH_S5F.

field annotation to use for both unweighted path length exclusion and consideration as the MRCA node. If NULL do not exclude any nodes.

exclude vector of annotation values in field to exclude from potential MRCA set. If NULL do not exclude any nodes. Has no effect if field=NULL.

junctionWeight fraction of the nucleotide sequence that is within the junction region. When specified this adds a proportional number of mutations to the immediate offspring nodes of the MRCA. Requires a value between 0 and 1. If NULL then edge weights are unmodified from the input graph.

start Initial position in sequence where mutations can be introduced. Default: 1

end Last position in sequence where mutations can be introduced. Default: last position (sequence length).

Value

A data.frame of simulated sequences with columns:

- name: name of the corresponding node in the input graph.
- sequence: mutated sequence.
- distance: Hamming distance of the mutated sequence from the seed sequence.

See Also

See shmulateSeq for imposing mutations on a single sequence. See HH_S5F and MK_RS5NF for predefined TargetingModel objects.

Examples

# Load example lineage and define example MRCA
data(ExampleTrees, package="alakazam")
graph <- ExampleTrees[[17]]
sequence <- "NGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATAGTTTA"

# Simulate using the default human 5-mer targeting model
shmulateTree(sequence, graph)

# Simulate using the mouse 5-mer targeting model
# Exclude nodes without a sample identifier
# Add 20% mutation rate to the immediate offsprings of the MRCA
shmulateTree(sequence, graph, targetingModel=MK_RS5NF,
field="sample_id", exclude=NA, junctionWeight=0.2)
slideWindowDb

_Sliding window approach towards filtering sequences in a data.frame_

**Description**

slideWindowDb determines whether each input sequence in a data.frame contains equal to or more than a given number of mutations in a given length of consecutive nucleotides (a "window") when compared to their respective germline sequence.

**Usage**

```r
slideWindowDb(
  db,
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  mutThresh,
  windowSize
)
```

**Arguments**

- `db` _data.frame_ containing sequence data.
- `sequenceColumn` name of the column containing IMGT-gapped sample sequences.
- `germlineColumn` name of the column containing IMGT-gapped germline sequences.
- `mutThresh` threshold on the number of mutations in `windowSize` consecutive nucleotides. Must be between 1 and `windowSize` inclusive.
- `windowSize` length of consecutive nucleotides. Must be at least 2.

**Value**

a logical vector. The length of the vector matches the number of input sequences in `db`. Each entry in the vector indicates whether the corresponding input sequence should be filtered based on the given parameters.

**See Also**

See `slideWindowSeq` for applying the sliding window approach on a single sequence. See `slideWindowTune` for parameter tuning for `mutThresh` and `windowSize`.

**Examples**

```r
# Use an entry in the example data for input and germline sequence
data(ExampleDb, package="alakazam")

# Apply the sliding window approach on a subset of ExampleDb
slideWindowDb(db=ExampleDb[1:10, ], sequenceColumn="sequence_alignment",
```
slideWindowSeq

**Description**

`slideWindowSeq` determines whether an input sequence contains equal to or more than a given number of mutations in a given length of consecutive nucleotides (a "window") when compared to a germline sequence.

**Usage**

```
slideWindowSeq(inputSeq, germlineSeq, mutThresh, windowSize)
```

**Arguments**

- `inputSeq` input sequence.
- `germlineSeq` germline sequence.
- `mutThresh` threshold on the number of mutations in `windowSize` consecutive nucleotides. Must be between 1 and `windowSize` inclusive.
- `windowSize` length of consecutive nucleotides. Must be at least 2.

**Value**

TRUE if there are equal to or more than `mutThresh` number of mutations in any window of `windowSize` consecutive nucleotides (i.e. the sequence should be filtered); FALSE if otherwise.

**See Also**

calcObservedMutations is called by slideWindowSeq to identify observed mutations. See slideWindowDb for applying the sliding window approach on a data.frame. See slideWindowTune for parameter tuning for `mutThresh` and `windowSize`.

**Examples**

```r
data(ExampleDb, package="alakazam")
in_seq <- ExampleDb["sequence_alignment"][[100]]
germ_seq <- ExampleDb["germline_alignment_d_mask"][[100]]

# Determine if in_seq has 6 or more mutations in 10 consecutive nucleotides
slideWindowSeq(inputSeq=in_seq, germlineSeq=germ_seq, mutThresh=6, windowSize=10)
```
Parameter tuning for sliding window approach

Description

Apply `slideWindowDb` over a search grid made of combinations of `mutThresh` and `windowSize` to help with picking a pair of values for these parameters. Parameter tuning can be performed by choosing a combination that gives a reasonable number of filtered/remaining sequences.

Usage

```r
slideWindowTune(
  db,
  sequenceColumn = "sequence_alignment",
  germlineColumn = "germline_alignment_d_mask",
  dbMutList = NULL,
  mutThreshRange,
  windowSizeRange,
  verbose = TRUE
)
```

Arguments

- `db` data.frame containing sequence data.
- `sequenceColumn` name of the column containing IMGT-gapped sample sequences.
- `germlineColumn` name of the column containing IMGT-gapped germline sequences.
- `dbMutList` if supplied, this should be a list consisting of data.frames returned as `$pos` in the nested list produced by `calcObservedMutations` with `returnRaw=TRUE`; otherwise, `calcObservedMutations` is called on columns `sequenceColumn` and `germlineColumn` of `db`. Default is `NULL`.
- `mutThreshRange` range of threshold on the number of mutations in `windowSize` consecutive nucleotides to try. Must be between 1 and maximum `windowSizeRange` inclusive.
- `windowSizeRange` range of length of consecutive nucleotides to try. The lower end must be at least 2.
- `verbose` whether to print out messages indicating current progress. Default is `TRUE`.

Details

If, in a given combination of `mutThresh` and `windowSize`, `mutThresh` is greater than `windowSize`, `NA`s will be returned for that particular combination. A message indicating that the combination has been "skipped" will be printed if `verbose=TRUE`.

If `calcObservedMutations` was previously run on `db` and saved, supplying `$pos` from the saved result as `dbMutList` could save time by skipping a second call of `calcObservedMutations`. This could be helpful especially when `db` is large.
Value

- a list of logical matrices. Each matrix corresponds to a `WindowSize` in `WindowSizeRange`. Each column in a matrix corresponds to a `MutThresh` in `MutThreshRange`.

See Also

- `slideWindowDb` is called on `db` for tuning. See `slideWindowTunePlot` for visualization. See `calcObservedMutations` for generating `dbMutList`.

Examples

```r
# Load and subset example data
data(ExampleDb, package="alakazam")
db <- ExampleDb[1:5,]

# Try out thresholds of 2-4 mutations in window sizes of 7-9 nucleotides.
# In this case, all combinations are legal.
slideWindowTune(db, mutThreshRange=2:4, windowSizeRange=7:9)

# Illegal combinations are skipped, returning NAs.
slideWindowTune(db, mutThreshRange=2:4, windowSizeRange=2:4, verbose=FALSE)

# Run calcObservedMutations separately
exDbMutList <- sapply(1:5, function(i) {
  calcObservedMutations(inputSeq=db["sequence_alignment"][i],
                         germlineSeq=db["germline_alignment_d_mask"][i],
                         returnRaw=TRUE)$pos
})
slideWindowTune(db, dbMutList=exDbMutList, mutThreshRange=2:4, windowSizeRange=2:4)
```

---

`slideWindowTunePlot` Visualize parameter tuning for sliding window approach

Description

- Visualize results from `slideWindowTune`

Usage

```r
slideWindowTunePlot(tuneList, plotFiltered = TRUE, percentage = FALSE, jitter.x = FALSE, jitter.x.amt = 0.1, jitter.y = FALSE, jitter.y.amt = 0.1,)
```
*slideWindowTunePlot*

```r
pchs = 1,
lty = 2,
col = 1,
plotLegend = TRUE,
legendPos = "topright",
legendHoriz = FALSE,
legendCex = 1,
title = NULL
)
```

**Arguments**

- **tuneList**
  - a list of logical matrices returned by `slideWindowTune`.

- **plotFiltered**
  - whether to plot the number of filtered sequences (as opposed to the number of remaining sequences). Default is `TRUE`.

- **percentage**
  - whether to plot on the y-axis the percentage of filtered sequences (as opposed to the absolute number). Default is `FALSE`.

- **jitter.x**
  - whether to jitter x-axis values. Default is `FALSE`.

- **jitter.x.amt**
  - amount of jittering to be applied on x-axis values if `jitter.x=TRUE`. Default is `0.1`.

- **jitter.y**
  - whether to jitter y-axis values. Default is `FALSE`.

- **jitter.y.amt**
  - amount of jittering to be applied on y-axis values if `jitter.y=TRUE`. Default is `0.1`.

- **pchs**
  - point types to pass on to `plot`.

- **lty**
  - line types to pass on to `plot`.

- **col**
  - colors to pass on to `plot`.

- **plotLegend**
  - whether to plot legend. Default is `TRUE`.

- **legendPos**
  - position of legend to pass on to `legend`. Can be either a numeric vector specifying x-y coordinates, or one of "topright", "center", etc. Default is "topright".

- **legendHoriz**
  - whether to make legend horizontal. Default is `FALSE`.

- **legendCex**
  - numeric values by which legend should be magnified relative to 1.

- **title**
  - plot main title. Default is `NULL` (no title)

**Details**

For each `windowSize`, the numbers of sequences filtered or remaining after applying the sliding window approach are plotted on the y-axis against thresholds on the number of mutations in a window on the x-axis.

When plotting, a user-defined amount of jittering can be applied on values plotted on either axis or both axes via adjusting `jitter.x`, `jitter.y`, `jitter.x.amt` and `jitter.y.amt`. This may be helpful with visually distinguishing lines for different window sizes in case they are very close or identical to each other. If plotting percentages (`percentage=TRUE`) and using jittering on the y-axis values (`jitter.y=TRUE`), it is strongly recommended that `jitter.y.amt` be set very small (e.g. 0.01).

NA for a combination of `mutThresh` and `windowSize` where `mutThresh` is greater than `windowSize` will not be plotted.
summarizeBaseline  

Calculate BASELINe summary statistics

Description

summarizeBaseline calculates BASELINe statistics such as the mean selection strength (mean Sigma), the 95% confidence intervals and p-values for the presence of selection.

Usage

summarizeBaseline(baseline, returnType = c("baseline", "df"), nproc = 1)
summarizeBaseline

Arguments

baseline Baseline object returned by `calcBaseline` containing annotations and BASELINe posterior probability density functions (PDFs) for each sequence.

returnType One of c("baseline", "df") defining whether to return a Baseline object ("baseline") with an updated stats slot or a data.frame ("df") of summary statistics.

nproc number of cores to distribute the operation over. If nproc = 0 then the cluster has already been set and will not be reset.

Details

The returned p-value can be either positive or negative. Its magnitude (without the sign) should be interpreted as per normal. Its sign indicates the direction of the selection detected. A positive p-value indicates positive selection, whereas a negative p-value indicates negative selection.

Value

Either a modified Baseline object or data.frame containing the mean BASELINe selection strength, its 95% confidence intervals, and a p-value for the presence of selection.

References


See Also

See `calcBaseline` for generating Baseline objects and `groupBaseline` for convolving groups of BASELINe PDFs.

Examples

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

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
sequenceColumn="sequence_alignment",
germlineColumn="germline_alignment_d_mask",
method="thresholdedFreq", minimumFrequency=0.6,
includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINe
baseline <- calcBaseline(db,
sequenceColumn="clonal_sequence",
germlineColumn="clonal_germline",
testStatistic="focused",
regionDefinition=IMGT_V,
)
```
```r
# Grouping the PDFs by the sample annotation
grouped <- groupBaseline(baseline, groupBy="sample_id")

# Get a data.frame of the summary statistics
stats <- summarizeBaseline(grouped, returnType="df")
```

---

### TargetingMatrix-class

**S4 class defining a targeting matrix**

#### Description

TargetingMatrix defines a data structure for just the targeting matrix (as opposed to the entire TargetingModel).

#### Slots

- `.Data` matrix.
- `numMutS` number indicating the number of silent mutations used for estimating mutability.
- `numMutR` number indicating the number of replacement mutations used for estimating mutability.

---

### TargetingModel-class

**S4 class defining a targeting model**

#### Description

TargetingModel defines a common data structure for mutability, substitution and targeting of immunoglobulin (Ig) sequencing data in a 5-mer microsequence context.

#### Usage

```r
## S4 method for signature 'TargetingModel,missing'
plot(x, y, ...)
```

#### Arguments

- `x`  TargetingModel object.
- `y`  ignored.
- `...` arguments to pass to `plotMutability`.

---
testBaseline

Slots

name Name of the model.
description Description of the model and its source data.
species Genus and species of the source sequencing data.
date Date the model was built.
citation Publication source.

substitution Normalized rates of the center nucleotide of a given 5-mer mutating to a different nucleotide. The substitution model is stored as a 5x3125 matrix of rates. Rows define the mutated nucleotide at the center of each 5-mer, one of c("A","C","G","T","N"), and columns define the complete 5-mer of the unmutated nucleotide sequence.

mutability Normalized rates of a given 5-mer being mutated. The mutability model is stored as a numeric vector of length 3125 with mutability rates for each 5-mer. Note that "normalized" means that the mutability rates for the 1024 5-mers that contain no "N" at any position sums up to 1 (as opposed to the entire vector summing up to 1).

targeting Rate matrix of a given mutation occurring, defined as mutability * substitution. The targeting model is stored as a 5x3125 matrix. Rows define the mutated nucleotide at the center of each 5-mer, one of c("A","C","G","T","N"), and columns define the complete 5-mer of the unmutated nucleotide sequence.

numMutS number indicating the number of silent mutations used for estimating mutability.
numMutR number indicating the number of replacement mutations used for estimating mutability.

See Also

See createTargetingModel building models from sequencing data.

testBaseline Two-sided test of BASELINe PDFs

Description

testBaseline performs a two-sample significance test of BASELINe posterior probability density functions (PDFs).

Usage

testBaseline(baseline, groupBy)

Arguments

baseline Baseline object containing the db and grouped BASELINe PDFs returned by groupBaseline.
groupBy string defining the column in the db slot of the Baseline containing sequence or group identifiers.
Value

A data.frame with test results containing the following columns:

• region: sequence region, such as cdr and fwr.
• test: string defining the groups be compared. The string is formatted as the conclusion associated with the p-value in the form GROUP1 != GROUP2. Meaning, the p-value for rejection of the null hypothesis that GROUP1 and GROUP2 have equivalent distributions.
• pvalue: two-sided p-value for the comparison.
• fdr: FDR corrected pvalue.

References


See Also

To generate the Baseline input object see groupBaseline.

Examples

# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, c_call %in% c("IGHM", "IGHG", "IGHA"))

# Collapse clones
db <- collapseClones(db, cloneColumn="clone_id",
sequenceColumn="sequence_alignment",
germlineColumn="germline_alignment_d_mask",
method="thresholdedFreq", minimumFrequency=0.6,
includeAmbiguous=FALSE, breakTiesStochastic=FALSE)

# Calculate BASELINe
baseline <- calcBaseline(db,
sequenceColumn="clonal_sequence",
germlineColumn="clonal_germline",
testStatistic="focused",
regionDefinition=IMGT_V,
targetingModel=HH_SS5,
nproc=1)

# Group PDFs by the isotype
grouped <- groupBaseline(baseline, groupBy="c_call")

# Visualize isotype PDFs
plot(grouped, "c_call")

# Perform test on isotype PDFs
testBaseline(grouped, groupBy="c_call")
Uniform 5-mer null targeting model.

Description
A null 5-mer model of somatic hypermutation targeting where all substitution, mutability and targeting rates are uniformly distributed.

Usage
U5N

Format
A TargetingModel object.

See Also
See HH_S5F and HKL_S5F for the human 5-mer targeting models; and MK_RS5NF for the mouse 5-mer targeting model.

writeTargetingDistance
Write targeting model distances to a file

Description
writeTargetingDistance writes a 5-mer targeting distance matrix to a tab-delimited file.

Usage
writeTargetingDistance(model, file)

Arguments
model TargetingModel object with mutation likelihood information.
file name of file to write.

Details
The targeting distance write as a tab-delimited 5x3125 matrix. Rows define the mutated nucleotide at the center of each 5-mer, one of c("A", "C", "G", "T", "N"), and columns define the complete 5-mer of the unmutated nucleotide sequence. NA values in the distance matrix are replaced with distance 0.
See Also

Takes as input a TargetingModel object and calculates distances using calcTargetingDistance.

Examples

```r
## Not run:
# Write HS5F targeting model to working directory as hs5f.tab
writeTargetingDistance(HH_SS5F, "hh_s5f.tsv")

## End(Not run)
```
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