

Package ‘shazam’

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Title Immunoglobulin Somatic Hypermutation Analysis

Description Provides a computational framework for analyzing mutations in immunoglobulin (Ig) sequences. Includes methods for Bayesian estimation of antigen-driven selection pressure, mutational load quantification, building of somatic hypermutation (SHM) models, and model-dependent distance calculations. Also includes empirically derived models of SHM for both mice and humans. Citations:

Gupta and Vander Heiden, et al (2015) <doi:10.1093/bioinformatics/btv359>, Yaari, et al (2012) <doi:10.1093/nar/gks457>, Yaari, et al (2013) <doi:10.3389/fimmu.2013.00358>, Cui, et al (2016) <doi:10.4049/jimmunol.1502263>.

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URL <http://shazam.readthedocs.io>

BugReports <https://bitbucket.org/kleinstein/shazam/issues>

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Baseline-class	<i>S4 class defining a BASELINE (selection) object</i>
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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 $r \times c$ 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.

See Also

See [summarizeBaseline](#) for more information on @stats.

calcBaseline	<i>Calculate the BASELINE PDFs</i>
--------------	------------------------------------

Description

calcBaseline calculates the BASELINE posterior probability density functions (PDFs) for sequences 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)
```

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 .
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.

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

1. Hershberg U, et al. Improved methods for detecting selection by mutation analysis of Ig V region sequences. *Int Immunol.* 2008 20(5):683-94.
2. Uduman M, et al. Detecting selection in immunoglobulin sequences. *Nucleic Acids Res.* 2011 39(Web Server issue):W499-504.
3. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

See Also

See [Baseline](#) for the return object. See [groupBaseline](#) and [summarizeBaseline](#) for further processing. See [plotBaselineSummary](#) and [plotBaselineDensity](#) for plotting results.

Examples

```
# Load and subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE == "IgG" & SAMPLE == "+7d")

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                    germlineColumn="GERMLINE_IMGT_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](#).

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 processed 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 [createTargeting-Model](#). See [calcObservedMutations](#) for getting observed mutation counts.

Examples

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

# Use first entry in the example data for input and germline sequence
in_seq <- ExampleDb[["SEQUENCE_IMGT"]][1]
germ_seq <- ExampleDb[["GERMLINE_IMGT_D_MASK"]][1]

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

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

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

`calcObservedMutations` *Count the number of observed mutations in a sequence.*

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.
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

- `$pos`: A data frame whose columns (`position`, `R`, `S`, and `region`) indicate, respectively, the nucleotide position, the number of R mutations at that position, the number of S mutations at that position, and the region in which that nucleotide is in.
- `$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_IMGT"]][100]
germ_seq <- ExampleDb[["GERMLINE_IMGT_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 hydrophathy class
ex3_raw <- calcObservedMutations(in_seq, germ_seq, regionDefinition=IMGT_V,
                                mutationDefinition=HYDROPATHY_MUTATIONS,
                                returnRaw=TRUE)
# Count mutations by change in hydrophathy 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 $p = \text{mutability} * \text{substitution}$ 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.

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, ISOTYPE == "IgA" & SAMPLE == "-1h")

# Calculate mutability of germline sequences using \link{HH_S5F} model
mutability <- calculateMutability(sequences=db$GERMLINE_IMGT_D_MASK, model=HH_S5F)
```

collapseClones

Constructs effective clonal sequences for all clones

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",
  sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_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)
```

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

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.
- If the sequences have "A", "A", "T", "T", "C", the consensus will be "N", because none of "A", "T", or "C" has frequency that is at least minimumFrequency.

- `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", "A", "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 "-".
- 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 consensus 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:

- 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 consensus 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

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE %in% c("IgA", "IgG") & SAMPLE == "+7d" &
            CLONE %in% c("3100", "3141", "3184"))

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

# mostCommon method, resolving ties deterministically using ambiguous characters
clones <- collapseClones(db, 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, method="mostMutated", muFreqColumn="MU_FREQ",
                        breakTiesStochastic=TRUE, breakTiesByColumns=NULL)

# mostMutated method, resolving ties deterministically using additional columns
clones <- collapseClones(db2, method="mostMutated", muFreqColumn="MU_FREQ",
                        breakTiesStochastic=FALSE,
                        breakTiesByColumns=list(c("DUPCOUNT"), c(max)))

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

# Return the same number of rows as the input
clones <- collapseClones(db, method="mostCommon", expandedDb=TRUE)

```

createBaseline	<i>Creates a Baseline object</i>
----------------	----------------------------------

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.
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 $r \times c$ 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 $r \times c$ dimensions, 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).

Value

A Baseline object.

References

1. Hershberg U, et al. Improved methods for detecting selection by mutation analysis of Ig V region sequences. *Int Immunol.* 2008 20(5):683-94.
2. Uduman M, et al. Detecting selection in immunoglobulin sequences. *Nucleic Acids Res.* 2011 39(Web Server issue):W499-504.
3. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

See Also

See [Baseline](#) for the return object.

Examples

```
# Creates an empty Baseline object
createBaseline()
```

```
createMutabilityMatrix
```

Builds a mutability model

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, model = c("S", "RS"),
  sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_D_MASK", vCallColumn = "V_CALL",
  multipleMutation = c("independent", "ignore"),
  minNumSeqMutations = 500, numSeqMutationsOnly = FALSE,
  returnSource = FALSE)
```

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.


```
# Count the number of mutations in sequences containing each 5-mer
mut_count <- createMutabilityMatrix(db, sub_model, model="S",
                                   numSeqMutationsOnly=TRUE)
```

```
createMutationDefinition
```

Creates a MutationDefinition

Description

createMutationDefinition creates a MutationDefinition.

Usage

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

Arguments

name	name of the mutation definition.
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", "*", "-", "."). Replacement (R) is defined as a change in amino acid class and silent (S) as no change in class.
description	description of the mutation definition and its source data.
citation	publication source.

Value

A MutationDefinition object.

See Also

See [MutationDefinition](#) for the return object.

Examples

```
# Define hydropathy classes
library(alakazam)
hydropathy <- list(hydrophobic=c("A", "I", "L", "M", "F", "W", "V"),
                  hydrophilic=c("R", "N", "D", "C", "Q", "E", "K"),
                  neutral=c("G", "H", "P", "S", "T", "Y"))
chars <- unlist(hydropathy, use.names=FALSE)
classes <- setNames(translateStrings(chars, hydropathy), chars)

# Create hydropathy mutation definition
md <- createMutationDefinition("Hydropathy", classes)
```

`createRegionDefinition`*Creates a RegionDefinition*

Description

`createRegionDefinition` creates a `RegionDefinition`.

Usage

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

Arguments

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

Value

A `RegionDefinition` object.

See Also

See [RegionDefinition](#) for the return object.

Examples

```
# 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

```
createSubstitutionMatrix(db, model = c("S", "RS"),
  sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_D_MASK", vCallColumn = "V_CALL",
  multipleMutation = c("independent", "ignore"),
  returnModel = c("5mer", "1mer", "1mer_raw"), minNumMutations = 50,
  numMutationsOnly = FALSE)
```

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 consensus 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

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

See Also

[extendSubstitutionMatrix](#), [createMutabilityMatrix](#), [createTargetingMatrix](#), [createTargetingModel](#), [minNumMutationsTune](#).

Examples

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

# Count the number of mutations per 5-mer
subCount <- createSubstitutionMatrix(db, model="S", multipleMutation="independent",
                                     returnModel="5mer", numMutationsOnly=TRUE)

# Create model using only silent mutations
sub <- createSubstitutionMatrix(db, model="S", multipleMutation="independent",
                               returnModel="5mer", numMutationsOnly=FALSE,
                               minNumMutations=20)
```

createTargetingMatrix *Calculates a targeting rate matrix*

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 matrix 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.

References

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

See Also

[createSubstitutionMatrix](#), [extendSubstitutionMatrix](#), [createMutabilityMatrix](#), [extendMutabilityMatrix](#), [createTargetingModel](#)

Examples

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

# Create 4x1024 models using only silent mutations
sub_model <- createSubstitutionMatrix(db, model="S")
mut_model <- createMutabilityMatrix(db, sub_model, model="S")

# Extend substitution and mutability to including Ns (5x3125 model)
sub_model <- extendSubstitutionMatrix(sub_model)
mut_model <- extendMutabilityMatrix(mut_model)

# Create targeting model from substitution and mutability
tar_model <- createTargetingMatrix(sub_model, mut_model)
```

createTargetingModel *Creates a TargetingModel*

Description

createTargetingModel creates a 5-mer TargetingModel.

Usage

```
createTargetingModel(db, model = c("S", "RS"),
  sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_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.
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 consensus returned from collapseClones).

Value

A [TargetingModel](#) object.

References

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

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

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

```
# Create model using only silent mutations and ignore multiple mutations
model <- createTargetingModel(db, model="S", multipleMutation="ignore")
```

DensityThreshold-class

Output of the dens method of findThreshold

Description

DensityThreshold contains output from the dens method [findThreshold](#).

Usage

```
## 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.

See Also

[findThreshold](#)

distToNearest	<i>Distance to nearest neighbor</i>
---------------	-------------------------------------

Description

Get non-zero distance of every sequence (as defined by sequenceColumn) to its nearest sequence sharing same V gene, J gene, and sequence length.

Usage

```
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, nproc = 1, fields = NULL, cross = NULL,
  mst = FALSE, subsample = NULL, progress = FALSE)
```

Arguments

db	data.frame containing sequence data.
sequenceColumn	name of the column containing nucleotide sequences to compare. Also used to determine sequence length for grouping.
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", "mk_rs5nf", "m1n_compat", "hs1f_compat"). 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 is 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.
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 group of sequences with the same vCallColumn, jCallColumn, and junction length. If subsample is larger than the unique number of sequences in each group, then the subsampling process is ignored for that group. For each sequence in db, the reported DIST_NEAREST is the distance to the closest sequence in the subsampled set for the group. If NULL no subsampling is performed.

progress if TRUE print a progress bar.

Details

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 sequence length, there is only 1 sequence (as defined by `sequenceColumn`), NA is returned instead of a distance (since it has no neighbor). If for a given combination there are multiple sequences but only 1 unique sequence, (in which case every 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 group of sequences sharing the same `vCallColumn`, `jCallColumn`, and junction length. If `subsample` is larger than number of sequences in the group, it is ignored. In other words, subsampling is performed only on groups of sequences of size equal to or greater than `subsample`. `DIST_NEAREST` has values calculated using all sequences in the group for groups of size smaller than `subsample` and values calculated using a subset of sequences for the larger groups. To select a value of `subsample`, it can be useful to explore the group sizes in db.

Value

Returns a modified db data.frame with nearest neighbor distances in the `DIST_NEAREST` column if `cross=NULL`. if `cross` was specified, distances will be added as the `CROSS_DIST_NEAREST` column

References

1. Smith DS, et al. Di- and trinucleotide target preferences of somatic mutagenesis in normal and autoreactive B cells. *J Immunol.* 1996 156:2642-52.
2. Glanville J, Kuo TC, von Budingen H-C, et al. Naive antibody gene-segment frequencies are heritable and unaltered by chronic lymphocyte ablation. *Proc Natl Acad Sci USA.* 2011 108(50):20066-71.
3. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4:358.

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 == "-1h")

# Use genotyped V assignments, Hamming distance, and normalize by junction length
dist <- distToNearest(db, vCallColumn="V_CALL_GENOTYPED", model="ham",
                     first=FALSE, 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

Description

editBaseline edits a field in a Baseline object.

Usage

```
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

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE == "IgG" & SAMPLE == "+7d")

# Make Baseline object
baseline <- calcBaseline(db,
  sequenceColumn="SEQUENCE_IMGT",
  germlineColumn="GERMLINE_IMGT_D_MASK",
  testStatistic="focused",
  regionDefinition=IMGT_V,
  targettingModel=HH_S5F,
  nproc=1)

# Edit the field "description"
baseline <- editBaseline(baseline, field="description",
  value="+7d IgG")
```

expectedMutations	<i>Calculate expected mutation frequencies</i>
-------------------	--

Description

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

Usage

```
expectedMutations(db, sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_D_MASK", targettingModel = HH_S5F,
  regionDefinition = NULL, mutationDefinition = NULL, nproc = 1)
```

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.
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.

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, ISOTYPE %in% c("IgA", "IgG") & SAMPLE == "+7d")

# Calculate expected mutations over V region
db_exp <- expectedMutations(db,
```

```

sequenceColumn="SEQUENCE_IMGT",
germlineColumn="GERMLINE_IMGT_D_MASK",
regionDefinition=IMGT_V,
nproc=1)

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

```

extendMutabilityMatrix

Extends a mutability model to include Ns.

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 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).

See Also

[createMutabilityMatrix](#), [extendSubstitutionMatrix](#)

Examples

```

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

```

```
# Create model using only silent mutations and ignore multiple mutations
sub_model <- createSubstitutionMatrix(db, model="S")
mut_model <- createMutabilityMatrix(db, sub_model, model="S")
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, ISOTYPE == "IgA" & SAMPLE == "-1h")

# Create model using only silent mutations
sub_model <- createSubstitutionMatrix(db, 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

```
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 == "-1h")

# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, 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

```
## 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	<i>Group BASELINE PDFs</i>
---------------	----------------------------

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 form 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.

Value

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

References

1. Yaari G, et al. Quantifying selection in high-throughput immunoglobulin sequencing data sets. *Nucleic Acids Res.* 2012 40(17):e134. (Corrections at <http://selection.med.yale.edu/baseline/correction/>)

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

```

# Subset example data from alakazam
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE %in% c("IgM", "IgG"))

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                     germlineColumn="GERMLINE_IMGT_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")
sample_colors <- c("-1h"="steelblue", "+7d"="firebrick")
plotBaselineDensity(grouped1, idColumn="SAMPLE", colorValues=sample_colors,
                    sigmaLimits=c(-1, 1))

# Group PDFs by both sample (between variable) and isotype (within variable)
grouped2 <- groupBaseline(baseline, groupBy=c("SAMPLE", "ISOTYPE"))
isotype_colors <- c("IgM"="darkorchid", "IgD"="firebrick",
                  "IgG"="seagreen", "IgA"="steelblue")
plotBaselineDensity(grouped2, idColumn="SAMPLE", groupColumn="ISOTYPE",
                    colorElement="group", colorValues=isotype_colors,
                    sigmaLimits=c(-1, 1))

# Collapse previous isotype (within variable) grouped PDFs into sample PDFs
grouped3 <- groupBaseline(grouped2, groupBy="SAMPLE")
sample_colors <- c("-1h"="steelblue", "+7d"="firebrick")
plotBaselineDensity(grouped3, idColumn="SAMPLE", colorValues=sample_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

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

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

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

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	<i>Human kappa and lambda chain, silent, 1-mer, functional substitution model.</i>
---------	--

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

1. Cui A, Di Niro R, Vander Heiden J, Briggs A, Adams K, Gilbert T, O'Connor K, Vigneault F, Shlomchik M and Kleinstein S (2016). A Model of Somatic Hypermutation Targeting in Mice Based on High-Throughput Ig Sequencing Data. *The Journal of Immunology*, 197(9), 3566-3574.

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	<i>Human kappa and lambda light chain, silent, 5-mer, functional targeting model.</i>
---------	---

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

1. Cui A, Di Niro R, Vander Heiden J, Briggs A, Adams K, Gilbert T, O'Connor K, Vigneault F, Shlomchik M and Kleinstein S (2016). A Model of Somatic Hypermutation Targeting in Mice Based on High-Throughput Ig Sequencing Data. *The Journal of Immunology*, 197(9), 3566-3574.

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.

IMGT_SCHEMES	<i>IMGT unique numbering schemes</i>
--------------	--------------------------------------

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.

References

1. Lefranc MP, et al. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. *Developmental and comparative immunology*. 2003 27:55-77.

makeAverage1merMut	<i>Make a 1-mer mutability model by averaging over a 5-mer mutability model</i>
--------------------	---

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
example1merMut <- c(A=0.2, T=0.1, C=0.4, G=0.3)
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	<i>Make a 1-mer substitution model by averaging over a 5-mer substitution model</i>
--------------------	---

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
example1merMut <- c(A=0.2, T=0.1, C=0.4, G=0.3)
degenerate5merMut <- makeDegenerate5merMut(mut1mer = example1merMut)

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

# Normalized
sum(degenerate5merMut)
```

makeDegenerate5merSub *Make a degenerate 5-mer substitution model based on a 1-mer substitution model*

Description

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

Usage

```
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")]
```

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

```
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 minNumMutations supplied in minNumMutationsRange. Each column corresponds to a value in 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

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

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

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

# Count the number of mutations per 5-mer
subCount <- createSubstitutionMatrix(db, 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.

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

1. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol.* 2013 4(November):358.

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, ISOTYPE == "IgA" & SAMPLE == "-1h")

# Create model using only silent mutations
sub <- createSubstitutionMatrix(db, 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,
                                  model="S", multipleMutation="independent",
                                  numSeqMutationsOnly=TRUE)

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

MK_RS1NF	<i>Mouse kappa chain, replacement and silent, 1-mer, non-functional substitution model.</i>
----------	---

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

1. Cui A, Di Niro R, Vander Heiden J, Briggs A, Adams K, Gilbert T, O'Connor K, Vigneault F, Shlomchik M and Kleinstein S (2016). A Model of Somatic Hypermutation Targeting in Mice Based on High-Throughput Ig Sequencing Data. *The Journal of Immunology*, 197(9), 3566-3574.

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	<i>Mouse kappa light chain, replacement and silent, 5-mer, non-functional targeting model.</i>
----------	--

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

1. Cui A, Di Niro R, Vander Heiden J, Briggs A, Adams K, Gilbert T, O'Connor K, Vigneault F, Shlomchik M and Kleinstein S (2016). A Model of Somatic Hypermutation Targeting in Mice Based on High-Throughput Ig Sequencing Data. *The Journal of Immunology*, 197(9), 3566-3574.

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.

MutationDefinition-class

S4 class defining replacement and silent mutation definitions

Description

MutationDefinition defines a common data structure for defining the 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", "*", "-", "."). 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

1. http://www.imgt.org/IMGTeducation/Aide-memoire/_UK/aminoacids/IMGTclasses.html

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_IMGT",
  germlineColumn = "GERMLINE_IMGT_D_MASK", regionDefinition = NULL,
  mutationDefinition = NULL, ambiguousMode = c("eitherOr", "and"),
  frequency = FALSE, combine = FALSE, nproc = 1)
```

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.
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.

Details

Mutation counts are determined by comparing the input sequences (in the column specified by sequenceColumn) to the germline sequence (in the column specified by germlineColumn). 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.

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.

Examples

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE == "IgG" & SAMPLE == "+7d")

# Calculate mutation frequency over the entire sequence
db_obs <- observedMutations(db, sequenceColumn="SEQUENCE_IMGT",
                           germlineColumn="GERMLINE_IMGT_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_IMGT",
                           germlineColumn="GERMLINE_IMGT_D_MASK",
                           regionDefinition=IMGT_V,
                           mutationDefinition=CHARGE_MUTATIONS,
                           nproc=1)
```

plotBaselineDensity *Plots BASELINE probability density functions*

Description

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

Usage

```
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: <ul style="list-style-type: none"> • "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

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE %in% c("IgM", "IgG"))

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                     germlineColumn="GERMLINE_IMGT_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,
                        targettingModel=HH_S5F,
                        nproc=1)

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

# Plot density faceted by region with custom isotype colors
isotype_colors <- c("IgM"="darkorchid", "IgD"="firebrick",
                  "IgG"="seagreen", "IgA"="steelblue")
plotBaselineDensity(grouped, "SAMPLE", "ISOTYPE", 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", "ISOTYPE", facetBy="group",
                  colorValues=sample_colors, sigmaLimits=c(-1, 1))
```

plotBaselineSummary *Plots BASELINE summary statistics*

Description

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

Usage

```
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: <ul style="list-style-type: none"> • "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.

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, ISOTYPE %in% c("IgM", "IgG"))

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                     germlineColumn="GERMLINE_IMGT_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", "ISOTYPE"))

# Plot mean and confidence interval by region with custom group colors
isotype_colors <- c("IgM"="darkorchid", "IgD"="firebrick",
                  "IgG"="seagreen", "IgA"="steelblue")
plotBaselineSummary(grouped, "SAMPLE", "ISOTYPE",
                   groupColors=isotype_colors)

# Facet by group instead of region
plotBaselineSummary(grouped, "SAMPLE", "ISOTYPE", 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.

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 == "-1h")

# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, 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 Guassian 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.
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 == "-1h")

# Use nucleotide Hamming distance and normalize by junction length
db <- distToNearest(db, 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)
```

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"), size = 1, silent = FALSE, ...)
```

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

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

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

plotTune	<i>Visualize parameter tuning for minNumMutations and minNumSeqMutations</i>
----------	--

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 a 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

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

tuneMtx = list()
```

```

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

  # Count the number of mutations per 5-mer
  subCount = createSubstitutionMatrix(db=curDb, model="S", 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)

# 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.

shazam

The shazam package

Description

Dramatic improvements in high-throughput sequencing technologies now enable large-scale characterization 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 (eg, 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 sequence.
- [observedMutations](#): Compute observed mutation counts and frequencies.
- [expectedMutations](#): Compute expected mutation frequencies.
- [shmutateSeq](#): Simulate mutations in a single sequence.
- [shmutateTree](#): Simulate mutations over a lineage tree.

SHM targeting models

Computational models and analyses of SHM have separated the process into two independent components:

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 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

1. Hershberg U, et al. Improved methods for detecting selection by mutation analysis of Ig V region sequences. *Int Immunol*. 2008 20(5):683-94.
2. Uduman M, et al. Detecting selection in immunoglobulin sequences. *Nucleic Acids Res*. 2011 39(Web Server issue):W499-504. (Corrections at <http://selection.med.yale.edu/baseline/correction/>)
3. Yaari G, et al. Quantifying selection in high-throughput immunoglobulin sequencing data sets. *Nucleic Acids Res*. 2012 40(17):e134.
4. Yaari G, et al. Models of somatic hypermutation targeting and substitution based on synonymous mutations from high-throughput immunoglobulin sequencing data. *Front Immunol*. 2013 4:358.
5. Cui A, Di Niro R, Vander Heiden J, Briggs A, Adams K, Gilbert T, O'Connor K, Vigneault F, Shlomchik M and Kleinstein S (2016). A Model of Somatic Hypermutation Targeting in Mice Based on High-Throughput Ig Sequencing Data. *The Journal of Immunology*, 197(9), 3566-3574.

shmutateSeq	<i>Simulate mutations in a single sequence</i>
-------------	--

Description

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

Usage

```
shmutateSeq(sequence, numMutations, targetingModel = HH_S5F)
```

Arguments

sequence	sequence string in which mutations are to be introduced. Accepted alphabet: {A, T, G, C, N, .}. Note that - is not accepted.
numMutations	number of mutations to be introduced into sequence.
targetingModel	5-mer TargetingModel object to be used for computing probabilities of mutations at each position. Defaults to HH_S5F .

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.

Value

A string defining the mutated sequence.

See Also

See [shmutateTree](#) for imposing mutations on a lineage tree. See [HH_S5F](#) and [MK_RS5NF](#) for predefined [TargetingModel](#) objects.

Examples

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

# Simulate using the default human 5-mer targeting model
shmutateSeq(sequence, numMutations=6)
```

shmutateTree	<i>Simulate mutations in a lineage tree</i>
--------------	---

Description

shmutateTree 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

```
shmutateTree(sequence, graph, targetingModel = HH_S5F, field = NULL,
             exclude = NULL, junctionWeight = NULL)
```

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.
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 off-spring nodes of the MRCA. Requires a value between 0 and 1. If NULL then edge weights are unmodified from the input graph.

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 [shmutateSeq](#) 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
shmutateTree(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
shmutateTree(sequence, graph, targetingModel=MK_RS5NF,
              field="SAMPLE", exclude=NA, junctionWeight=0.2)
```

slideWindowDb	<i>Sliding window approach towards filtering sequences in a data.frame</i>
---------------	--

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

```
slideWindowDb(db, sequenceColumn = "SEQUENCE_IMGT",
              germlineColumn = "GERMLINE_IMGT_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

```
# 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, ], mutThresh=6, windowSize=10)
```

slideWindowSeq

Sliding window approach towards filtering a single sequence

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

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

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

slideWindowTune	<i>Parameter tuning for sliding window approach</i>
-----------------	---

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

```
slideWindowTune(db, sequenceColumn = "SEQUENCE_IMGT",
  germlineColumn = "GERMLINE_IMGT_D_MASK", dbMutList = NULL,
  mutThreshRange, windowSizeRange, verbose = TRUE)
```

Arguments

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

Details

If, in a given combination of `mutThresh` and `windowSize`, `mutThresh` is greater than `windowSize`, NAs 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

```
# 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[i, "SEQUENCE_IMGT"],
                        germlineSeq=db[i, "GERMLINE_IMGT_D_MASK"],
                        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

```
slideWindowTunePlot(tuneList, plotFiltered = TRUE, percentage = FALSE,
  jitter.x = FALSE, jitter.x.amt = 0.1, jitter.y = FALSE,
  jitter.y.amt = 0.1, pchs = 1, ltys = 2, cols = 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 .
ltys	line types to pass on to plot .
cols	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 help 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.

See Also

See [slideWindowTune](#) for how to get tuneList. See [jitter](#) for use of amount of jittering.

Examples

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

# Try out thresholds of 2-4 mutations in window sizes of 3-5 nucleotides
# on a subset of ExampleDb
tuneList <- slideWindowTune(db = ExampleDb[1:10, ],
                           mutThreshRange = 2:4, windowSizeRange = 3:5,
                           verbose = FALSE)

# Visualize
# Plot numbers of sequences filtered without jittering y-axis values
slideWindowTunePlot(tuneList, pchs=1:3, ltys=1:3, cols=1:3,
                    plotFiltered=TRUE, jitter.y=FALSE)

# Notice that some of the lines overlap
# Jittering could help
slideWindowTunePlot(tuneList, pchs=1:3, ltys=1:3, cols=1:3,
                    plotFiltered=TRUE, jitter.y=TRUE)

# Plot numbers of sequences remaining instead of filtered
slideWindowTunePlot(tuneList, pchs=1:3, ltys=1:3, cols=1:3,
                    plotFiltered=FALSE, jitter.y=TRUE,
                    legendPos="bottomright")

# Plot percentages of sequences filtered with a tiny amount of jittering
slideWindowTunePlot(tuneList, pchs=1:3, ltys=1:3, cols=1:3,
                    plotFiltered=TRUE, percentage=TRUE,
                    jitter.y=TRUE, jitter.y.amt=0.01)
```

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)
```

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

1. Uduman M, et al. Detecting selection in immunoglobulin sequences. *Nucleic Acids Res.* 2011 39(Web Server issue):W499-504.

See Also

See [calcBaseline](#) for generating Baseline objects and [groupBaseline](#) for convolving groups of BASELINE PDFs.

Examples

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE == "IgG")

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                     germlineColumn="GERMLINE_IMGT_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 annotation
grouped <- groupBaseline(baseline, groupBy="SAMPLE")

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

```

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

```

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

```

Arguments

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

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.

See Also

See [createTargetingModel](#) building models from sequencing data.

testBaseline	<i>Two-sided test of BASELINE PDFs</i>
--------------	--

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

1. Yaari G, et al. Quantifying selection in high-throughput immunoglobulin sequencing data sets. Nucleic Acids Res. 2012 40(17):e134. (Corrections at <http://selection.med.yale.edu/baseline/correction/>)

See Also

To generate the [Baseline](#) input object see [groupBaseline](#).

Examples

```
# Subset example data
data(ExampleDb, package="alakazam")
db <- subset(ExampleDb, ISOTYPE %in% c("IgM", "IgG", "IgA"))

# Collapse clones
db <- collapseClones(db, sequenceColumn="SEQUENCE_IMGT",
                     germlineColumn="GERMLINE_IMGT_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 the isotype
grouped <- groupBaseline(baseline, groupBy="ISOTYPE")

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

# Perform test on isotype PDFs
testBaseline(grouped, groupBy="ISOTYPE")
```

U5N

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

```
## Not run:  
# Write HS5F targeting model to working directory as hs5f.tab  
writeTargetingDistance(HH_S5F, "hh_s5f.tsv")  
  
## End(Not run)
```

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