Package 'tigger'

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Title R Tools for Inferring New Immunoglobulin Alleles from Rep-Seq Data

Description Infers the V genotype of an individual from immunoglobulin (Ig) repertoire-sequencing (Rep-Seq) data, including detection of any novel alleles. This information is then used to correct existing V allele calls from among the sample sequences.

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URL http://tigger.readthedocs.io

BugReports https://bitbucket.org/kleinstein/tigger/issues

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BuildVignettes true

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2 cleanSeqs

R topics documented:

	cleanSeqs	2
	findNovelAlleles	3
	findUnmutatedCalls	5
	genotype	5
	genotypeFasta	
	germline_ighv	
	getMutatedPositions	
	getMutCount	
	getPopularMutationCount	
	inferGenotype	
	insertPolymorphisms	
	novel_df	
	plotGenotype	
	plotNovel	
	readIgFasta	
	reassignAlleles	15
	sample_db	16
	selectNovel	16
	sortAlleles	17
	tigger	18
	updateAlleleNames	
	writeFasta	
Index		21

 ${\tt cleanSeqs}$

Clean up nucleotide sequences

Description

cleanSeqs capitalizes nucleotides, replaces "." with "-", and then replaces all characters besides ACGT- with "N".

Usage

cleanSeqs(seqs)

Arguments

seqs

a vector of nucleotide sequences

Value

A vector of nucleotide sequences

findNovelAlleles 3

See Also

sortAlleles and updateAlleleNames can help format a list of allele names.

Examples

findNovelAlleles

Find novel alleles from repertoire sequencing data

Description

findNovelAlleles analyzes mutation patterns in sequences thought to align to each germline allele in order to determine which positions might be polymorphic.

Usage

```
findNovelAlleles(clip_db, germline_db, v_call = "V_CALL",
   germline_min = 200, min_seqs = 50, auto_mutrange = TRUE,
   mut_range = 1:10, pos_range = 1:312, y_intercept = 0.125,
   alpha = 0.05, j_max = 0.15, min_frac = 0.75, nproc = 1)
```

Arguments

clip_db	a data.frame in Change-O format. See details.
germline_db	a vector of named nucleotide germline sequences matching the V calls in $\ensuremath{\mathtt{clip_db}}$
v_call	name of the column in clip_db with V allele calls. Default is V_CALL.
germline_min	the minimum number of sequences that must have a particular germline allele call for the allele to be analyzed
min_seqs	the minimum number of total sequences (within the desired mutational range and nucleotide range) required for the samples to be considered
auto_mutrange	if TRUE, the algorithm will attempt to determine the appropriate mutation range automatically using the mutation count of the most common sequence assigned to each allele analyzed
mut_range	the range of mutations that samples may carry and be considered by the algorithm
pos_range	the range of IMGT-numbered positions that should be considered by the algorithm
y_intercept	the y-intercept above which positions should be considered potentially polymorphic

4 findNovelAlleles

alpha	the alpha cutoff to be used when constructing the confidence interval for the y-intercept
j_max	the maximum fraction of sequences perfectly aligning to a potential novel allele that are allowed to utilize to a particular combination of junction length and J gene
min_frac	the minimum fraction of sequences that must have usable nucleotides in a given position for that position to considered
nproc	the number of processors to use

Details

A data. frame in Change-O format contains the following columns:

- "SEQUENCE_IMGT" containing the IMGT-gapped nucleotide sequence
- "V_CALL" containing the IMGT/V-QUEST V allele call(s)
- "J_CALL" containing the IMGT/V-QUEST J allele call(s)
- "JUNCTION_LENGTH" containing the junction length

The TIgGER allele-finding algorithm, briefly, works as follows: Mutations are determined through comparison to the provided germline. Mutation frequency at each *position* is determined as a function of *sequence-wide* mutation counts. Polymorphic positions exhibit a high mutation frequency despite sequence-wide mutation count. False positive of potential novel alleles resulting from clonally-related sequences are guarded against by ensuring that sequences perfectly matching the potential novel allele utilize a wide range of combinations of J gene and junction length.

Value

a data. frame with a row for each known allele analyzed. Besides metadata on the parameters used in the search, each row will have either a note as to where the polymorphism-finding algorithm exited or a nucleotide sequence for the predicted novel allele.

See Also

plotNovel to visualize the data supporting any novel alleles hypothesized to be present in the data and inferGenotype to determine if the novel alleles are frequent enought to be included in the subject's genotype

```
# Load example data and germlines
data(sample_db)
data(germline_ighv)

# Find novel alleles and return relevant data
## Not run: novel_df = findNovelAlleles(sample_db, germline_ighv)
```

findUnmutatedCalls 5

Description

findUnmutatedCalls determines which allele calls would represent a perfect match with the germline sequence, given a vector of allele calls and mutation counts. In the case of multiple alleles being assigned to a sequence, only the subset that would represent a perfect match is returned.

Usage

```
findUnmutatedCalls(allele_calls, sample_seqs, germline_db)
```

Arguments

allele_calls	a vector of strings respresenting Ig allele calls, where multiple calls are separated by a comma
sample_seqs	$V(D) \label{eq:proposed_prop} J-rearranged \ sample \ sequences \ matching \ the \ order \ of \ the \ given \ \verb allele_calls $
germline_db	a vector of named nucleotide germline sequences

Value

A vector of strings containing the members of allele_calls that represent unmutated sequences

Examples

genotype	Example of an Inferred Genotype	

Description

Example VDJ-rearranged immunoglobulin Rep-Seq sequences derived from a single individual (PGP1), sequenced on the Roche 454 platform, and thought by IMGT/V-QUEST to utilize IGHV1 family alleles, as processed by findNovelAlleles and inferGenotype

6 genotypeFasta

Format

A data. frame where rows correspond to genes carried by an individual and columns lists the alleles of those genes and their counts.

References

Gadala-Maria *et al.* (2015) Automated analysis of high-throughput B cell sequencing data reveals a high frequency of novel immunoglobulin V gene segment alleles. *PNAS*. 112(8):E862-70.

genotypeFasta

Return the nucleotide sequences of a genotype

Description

genotypeFasta converts a genotype table into a vector of nucleotide sequences.

Usage

```
genotypeFasta(genotype, germline_db, novel_df = NA)
```

Arguments

genotype a table of alleles denoting a genotype, as returned by inferGenotype

germline_db a vector of named nucleotide germline sequences matching the alleles detailed

in genotype

novel_df an optional data.frame containing putative novel alleeles of the type returned

by findNovelAlleles

Value

A named vector of strings containing the germline nucleotide sequences of the alleles in the provided genotype

See Also

inferGenotype

```
# Load example data
data(germline_ighv)
data(novel_df)
data(genotype)

# Find the sequences that correspond to the genotype
genotype_seqs = genotypeFasta(genotype, germline_ighv, novel_df)
```

germline_ighv 7

Description

A character vector of all 344 human IGHV germline gene segment alleles in IMGT Gene-db release 201408-4.

Format

Values correspond to IMGT-gaped nuceltoide sequences (with nucleotides capitalized and gaps represented by ".") while names correspond to stripped-down IMGT allele names (e.g. "IGHV1-18*01").

References

Xochelli *et al.* (2014) Immunoglobulin heavy variable (IGHV) genes and alleles: new entities, new names and implications for research and prognostication in chronic lymphocytic leukaemia. *Immunogenetics*. 67(1):61-6.

getMutatedPositions Find the location of mutations in a sequence

Description

getMutatedPositions takes two vectors of aligned sequences and compares pairs of sequences. It returns a list of the nucleotide positions of any differences.

Usage

```
getMutatedPositions(samples, germlines, ignored_regex = "[\\.N-]",
   match_instead = FALSE)
```

Arguments

samples	a vector of strings respresenting aligned sequences
germlines	a vector of strings respresenting aligned sequences to which samples will be compared. If only one string is submitted, it will be used for all samples.
ignored_regex	a regular expression indicating what characters should be ignored (such as gaps and N nucleotides).
match_instead	if TRUE, the function returns the positions that are the same instead of those that are different.

8 getMutCount

Value

A list of the nucleotide positions of any differences between the input vectors.

Examples

```
# Create strings to act as a sample sequences and a reference sequence
seqs = c("----GATA", "GAGAGAGA", "TANA")
ref = "GATAGATA"

# Find the differences between the two
getMutatedPositions(seqs, ref)
```

getMutCount

Determine the mutation counts from allele calls

Description

getMutCount takes a set of nucleotide sequences and their allele calls and determines the distance between that sequence and any germline alleles contained within the call

Usage

```
getMutCount(samples, allele_calls, germline_db)
```

Arguments

samples a vector of IMGT-gapped sample V sequences

allele_calls a vector of strings respresenting Ig allele calls for the sequences in samples, where multiple calls are separated by a comma

germline_db a vector of named nucleotide germline sequences matching the calls detailed in allele_calls

Value

A list equal in length to samples, containing the Hamming distance to each germline allele contained within each call within each element of samples

```
# Load germline database
data(germline_ighv)

# Use createGermlines to insert a mutation into a germline sequence
#sample_seqs = c(germline_ighv[2],
# createGermlines(germline_ighv[1], 103, "G"),
# createGermlines(germline_ighv[1], 107, "C"))
```

```
# Pretend that one sample sequence has received an ambiguous allele call
#sample_alleles = c(paste(names(germline_ighv[1:2]), collapse=","),
# names(germline_ighv[2]),
# compare each sequence to its assigned germline(s) to determine the distance
#getMutCount(sample_seqs, sample_alleles, germline_ighv)
```

getPopularMutationCount

Find Frequent Sequences' Mutation Counts

Description

getPopularMutationCount determines which sequences occur frequently for each V gene and returns the mutation count of those sequences.

Usage

```
getPopularMutationCount(sample_db, germline_db, gene_min = 0.001,
  seq_min = 50, seq_p_of_max = 1/8, full_return = FALSE)
```

Arguments

sample_db	A Change-O db data frame. See findNovelAlleles for a list of required columns.
germline_db	A named list of IMGT-gapped germline sequences.
gene_min	The portion of all unique sequences a gene must constitute to avoid exclusion.
seq_min	The number of copies of the V that must be present for to avoid exclusion.
seq_p_of_max	For each gene, fraction of the most common V sequence's count that a sequence must meet to avoid exclusion.
full_return	If true, will return all sample_db columns and will include sequences with mutation count < 1.

Value

A data frame of genes that have a frequent sequence mutation count above 1.

See Also

getMutatedPositions can be used to find which positions of a set of sequences are mutated.

```
data(sample_db, germline_ighv)
getPopularMutationCount(sample_db, germline_ighv)
```

10 inferGenotype

inferGenotype	Infer a subject-specific genotype	

Description

inferGenotype infers an subject's genotype by finding the minimum number set of alleles that can explain the majority of each gene's calls. The most common allele of each gene is included in the genotype first, and the next most common allele is added until the desired fraction of alleles can be explained. In this way, mistaken allele calls (resulting from sequences which by chance have been mutated to look like another allele) can be removed.

Usage

```
inferGenotype(clip_db, v_call = "V_CALL", fraction_to_explain = 0.875,
  gene_cutoff = 1e-04, find_unmutated = TRUE, germline_db = NA,
  novel_df = NA)
```

Arguments

clip_db	a data. frame containing V allele calls from a single subject. If find_unmutated is TRUE, then the sample IMGT-gapped $V(D)J$ sequence should
v_call	column in clip_db with V allele calls. Default is "V_CALL" be provided in a column "SEQUENCE_IMGT"
fraction_to_exp	plain
	the portion of each gene that must be explained by the alleles that will be included in the genotype
gene_cutoff	either a number of sequences or a fraction of the length of allele_calls denoting the minimum number of times a gene must be observed in allele_calls to be included in the genotype
find_unmutated	if TRUE, use germline_db to find which samples are unmutated. Not needed if allele_calls only represent unmutated samples.
germline_db	named vector of sequences containing the germline sequences named in allele_calls. Only required if find_unmutated is TRUE.
novel_df	an optional data.frame of the type novel returned by findNovelAlleles containing germline sequences that will be utilized if find_unmutated is TRUE. See details.

Details

Allele calls representing cases where multiple alleles have been assigned to a single sample sequence are rare among unmutated sequences but may result if nucleotides for certain positions are not available. Calls containing multiple alleles are treated as belonging to all groups. If novel_df is provided, all sequences that are assigned to the same starting allele as any novel germline allele will have the novel germline allele appended to their assignent prior to searching for unmutated sequences.

insertPolymorphisms 11

Value

A table of alleles denoting the genotype of the subject

Note

This method works best with data derived from blood, where a large portion of sequences are expected to be unmutated. Ideally, there should be hundreds of allele calls per gene in the input.

See Also

plotGenotype for a colorful visualization and genotypeFasta to convert the genotype to nucleotide sequences.

Examples

insertPolymorphisms

Insert polymorphisms into a nucleotide sequence

Description

insertPolymorphisms replaces nucleotides in the desired locations of a provided sequence.

Usage

```
insertPolymorphisms(sequence, positions, nucleotides)
```

Arguments

sequence the starting nucletide sequence

positions a vector of positions which to be changed

nucleotides a vector of nucletides to which to change the positions

Value

a sequence with the desired nucleotides in provided locations

```
insertPolymorphisms("hugged", c(1,6,2), c("t","r","i"))
```

12 plotGenotype

novel_df	Example of Analyzed Rep-Seq data	

Description

Example VDJ-rearranged immunoglobulin Rep-Seq sequences derived from a single individual (PGP1), sequenced on the Roche 454 platform, and thought by IMGT/V-QUEST to utilize IGHV1 family alleles, as processed by findNovelAlleles.

Format

A data.frame where rows correspond to alleles checked for polymorphisms and columns give results as well as paramaters used to run the test.

References

Gadala-Maria *et al.* (2015) Automated analysis of high-throughput B cell sequencing data reveals a high frequency of novel immunoglobulin V gene segment alleles. *PNAS.* 112(8):E862-70.

plotGenotype	Show a colorful representation of a genotype	
--------------	--	--

Description

plotGenotype plots a genotype table.

Usage

```
plotGenotype(genotype, facet_by = NULL, gene_sort = c("name", "position"),
  text_size = 12, silent = FALSE, ...)
```

Arguments

genotype	a table of alleles denoting a genotype, as returned by inferGenotype
facet_by	a column name in genotype to facet the plot by. If $\ensuremath{NULL},$ then do not facet the plot.
gene_sort	a string defining the method to use when sorting alleles. If "name" then sort in lexicographic order. If "position" then sort by position in the locus, as determined by the final two numbers in the gene name.
text_size	the point size of the plotted text
silent	if TRUE do not draw the plot and just return the ggplot object; if FALSE draw the plot.
	additional arguments to pass to ggplot2::theme.

plotNovel 13

Value

A ggplot object defining the plot.

See Also

inferGenotype

Examples

```
# Load example data
data(novel_df)
data(genotype)

# Plot genotype
plotGenotype(genotype)

# Facet by subject
genotypea = genotypeb = genotype
genotypea$SUBJECT = "A"
genotypeb$SUBJECT = "B"
geno_sub = rbind(genotypea, genotypeb)
plotGenotype(geno_sub, facet_by="SUBJECT", gene_sort="pos")
```

plotNovel

Visualize evidence of novel V alleles

Description

plotNovel is be used to visualize the evidence of any novel V alleles found using findNovelAlleles.

Usage

```
plotNovel(clip_db, novel_df_row, ncol = 1, v_call = "V_CALL")
```

Arguments

clip_db	a data.frame in Change-O format. See findNovelAlleles for details.
novel_df_row	a single row from a data frame as output by findNovelAlleles that contains a polymorphism-containing germline allele
ncol	number of columns to use when laying out the plots
v_call	name of the column in clip_db with V allele calls. Default is "V_CALL"

14 readIgFasta

Examples

```
# Load example data and germlines
data(sample_db)
data(germline_ighv)

# Find novel alleles and return relevant data
## Not run: novel_df = findNovelAlleles(sample_db, germline_ighv)
data(novel_df)
# Plot the evidence for the first (and only) novel allele in the example data
novel = selectNovel(novel_df)
plotNovel(sample_db, novel[1,])
```

readIgFasta

Read immunoglobulin sequences

Description

readIgFasta reads a fasta-formatted file of immunoglobulin (Ig) sequences and returns a named vector of those sequences.

Usage

```
readIgFasta(fasta_file, strip_down_name = TRUE, force_caps = TRUE)
```

Arguments

 $\begin{tabular}{ll} fasta-file & fasta-formatted file of immunoglobuling sequences \\ strip_down_name \\ \end{tabular}$

if TRUE, will extract only the allele name from the strings fasta file's sequence names

force_caps if TRUE, will force nucleotides to uppercase

Value

a named vector of strings respresenting Ig alleles

See Also

writeFasta to do the inverse.

reassignAlleles 15

r	eassignAlleles	Correct allele calls based on a personalized genotype

Description

reassignAlleles uses a subject-specific genotype to correct correct preliminary allele assignments of a set of sequences derived from a single subject.

Usage

```
reassignAlleles(clip_db, genotype_db, v_call = "V_CALL", method = "hamming",
   path = NA, keep_gene = TRUE)
```

Arguments

clip_db	a data.frame containing V allele calls from a single subject and the sample IMGT-gapped $V(D)J$ sequences under "SEQUENCE_IMGT"
genotype_db	a vector of named nucleotide germline sequences matching the calls detailed in allele_calls and personalized to the subject
v_call	name of the column in clip_db with V allele calls. Default is "V_CALL"
method	the method to be used when realigning sequences to the genotype_db sequences. Currently only "hammming" (for Hamming distance) is implemented.
path	directory containing the tool used in the realignment method, if needed. Hamming distance does not require a path to a tool.
keep_gene	logical indicating if gene assignments should be maintained when possible. Increases speed by minimizing required number of alignments. Currently only "TRUE" is implemented.

Details

In order to save time, initial gene assignments are preserved and the allele calls are chosen from among those provided in genotype_db, based on a simple alignment to the sample sequence.

Value

a single-column data.frame corresponding to clip.db and containing the best allele call from among the sequences listed in $genotype_db$

Examples

```
# Load example data
data(germline_ighv)
data(sample_db)
data(genotype)
data(novel_df)
```

Extract the database sequences that correspond to the genotype

16 selectNovel

```
genotype_seqs = genotypeFasta(genotype, germline_ighv, novel_df)

# Use the personlized genotype to determine corrected allele assignments
V_CALL_GENOTYPED = reassignAlleles(sample_db, genotype_seqs)
sample_db = cbind(sample_db, V_CALL_GENOTYPED)
```

sample_db

Example human Rep-Seq data

Description

Example VDJ-rearranged immunoglobulin Rep-Seq sequences derived from a single individual (PGP1), sequenced on the Roche 454 platform, and thought by IMGT/V-QUEST to utilize IGHV1 family alleles.

Format

A data.frame where rows correspond to unique VDJ sequences and columns include:

- IMGT-gapped nucleotide sequence ("SEQUENCE_IMGT")
- IMGT/V-QUEST allele calls ("V_CALL", "D_CALL", and "J_CALL")
- Junction length ("JUNCTION_LENGTH")

References

Gadala-Maria *et al.* (2015) Automated analysis of high-throughput B cell sequencing data reveals a high frequency of novel immunoglobulin V gene segment alleles. *PNAS.* 112(8):E862-70.

selectNovel

Select rows containing novel alleles

Description

selectNovel takes the result from findNovelAlleles and selects only the rows containing unique, novel alleles.

Usage

```
selectNovel(novel_df, keep_alleles = FALSE)
```

Arguments

novel_df A data.frame of the type returned by findNovelAlleles

keep_alleles A logical indicating if different alleles leading to the same novel sequence

should be kept. See details.

sortAlleles 17

Details

If, for instance, subject has in his genome IGHV1-2*02 and a novel allele equally close to IGHV1-2*02 and IGHV1-2*05, the novel allele may be detected by analyzing sequences that best align to either of these alleles. If keep_alleles is TRUE, both polymorphic allele calls will be retained. In the case that multiple mutation ranges are checked for the same allele, only one mutation range will be kept in the output.

Value

A data. frame containing only unique, novel alleles (if any) that were in the input.

Examples

```
data(novel_df)
novel = selectNovel(novel_df)
```

sortAlleles

Sort allele names

Description

sortAlleles returns a sorted vector of strings respresenting Ig allele names. Names are first sorted by gene family, then by gene, then by allele. Duplicated genes have their alleles are sorted as if they were part of their non-duplicated counterparts (e.g. IGHV1-69D*01 comes after IGHV1-69*01 but before IGHV1-69*02), and non-localized genes (e.g. IGHV1-NL1*01) come last within their gene family.

Usage

```
sortAlleles(allele_calls, method = c("name", "position"))
```

Arguments

allele_calls a

a vector of strings respresenting Ig allele names

method

a string defining the method to use when sorting alleles. If "name" then sort in lexicographic order. If "position" then sort by position in the locus, as

determined by the final two numbers in the gene name.

Value

A sorted vector of strings respresenting Ig allele names

See Also

Like sortAlleles, updateAlleleNames can help format a list of allele names.

18 tigger

Examples

tigger

tigger

Description

Here we provide a Tool for Immunoglobulin Genotype Elucidation via Rep-Seq (TIgGER). TIg-GER inferrs the set of Ig alleles carried by an individual (including any novel alleles) and then uses this set of alleles to correct the initial assignments given to sample sequences by existing tools.

Details

Immunoglobulin Repertoire-Sequencing (Rep-Seq) data is currently the subject of much study. A key step in analyzing these data involves assigning the closest known V(D)J germline alleles to the (often somatically mutated) sample sequences using a tool such as IMGT/HighV-QUEST. However, if the sample utilizes alleles not in the germline database used for alignment, this step will fail. Additionally, this alignment has an associated error rate of ~5 percent, notably among sequences carrying a large number of somatic mutations. The purpose of TIgGER is to address these issues.

Core tigger functions

- findNovelAlleles: Detect novel alleles
- plotNovel: Plot evidence of novel alleles
- inferGenotype: Infer an Ig genotype
- plotGenotype: A colorful genotype visualization
- genotypeFasta: Convert a genotype to sequences
- reassignAlleles: Correct allele calls

Mutation-related functions

- getMutatedPositions: Find mutation locations
- getMutCount: Find distance from germline
- findUnmutatedCalls: Subset unmutated sequences
- getPopularMutationCount: Find most common sequence's mutation count
- insertPolymorphisms: Insert SNPs into a sequence

updateAlleleNames 19

Input and formatting

- readIgFasta: Read a fasta file of Ig sequences
- updateAlleleNames: Correct outdated allele names
- sortAlleles: Sort allele names intelligently
- cleanSeqs: Standardize sequence format

References

Gadala-Maria *et al.* (2015) Automated analysis of high-throughput B cell sequencing data reveals a high frequency of novel immunoglobulin V gene segment alleles. *PNAS*. 112(8):E862-70.

updateAlleleNames

Update IGHV allele names

Description

updateAlleleNames takes a set of IGHV allele calls and replaces any outdated names (e.g. IGHV1-f) with the new IMGT names.

Usage

```
updateAlleleNames(allele_calls)
```

Arguments

allele_calls a vector of strings respresenting IGHV allele names

Details

The updated allele names are based on IMGT release 201408-4.

Value

vector of strings respresenting updated IGHV allele names

Note

IGMT has removed IGHV2-5*10 and IGHV2-5*07 as it has determined they are actually alleles *02 and *04, respectively.

References

Xochelli et al. (2014) Immunoglobulin heavy variable (IGHV) genes and alleles: new entities, new names and implications for research and prognostication in chronic lymphocytic leukaemia. Immunogenetics. 67(1):61-6

20 writeFasta

See Also

Like updateAlleleNames, sortAlleles can help format a list of allele names.

Examples

```
# Create a vector that uses old gene/allele names. alleles = c("IGHV1-c*01", "IGHV1-f*02", "IGHV2-5*07")
# Update the alleles to the new names updateAlleleNames(alleles)
```

writeFasta

Write to a fasta file

Description

writeFasta writes a named vector of sequences to a file in fasta format.

Usage

```
writeFasta(named_sequences, file, width = 60, append = FALSE)
```

Arguments

named_sequences

a vector of named string representing sequences

file the name of the output file

width the number of characters to be printed per line. If not between 1 and 255, width

with be infinite.

append logical indicating if the output should be appended to file instead of over-

writing it

Value

a named vector of strings respresenting Ig alleles

See Also

readIgFasta to do the inverse.

Index

```
*Topic data
    genotype, 5
    germline_ighv, 7
    novel_df, 12
    sample_db, 16
cleanSeqs, 2, 19
findNovelAlleles, 3, 5, 6, 9, 10, 12, 13, 16,
findUnmutatedCalls, 5, 18
genotype, 5
genotypeFasta, 6, 11, 18
germline_ighv, 7
getMutatedPositions, 7, 9, 18
getMutCount, 8, 18
{\tt getPopularMutationCount}, 9, 18
inferGenotype, 4-6, 10, 12, 13, 18
insertPolymorphisms, 11, 18
novel_df, 12
plotGenotype, 11, 12, 18
plotNovel, 4, 13, 18
readIgFasta, 14, 19, 20
reassignAlleles, 15, 18
sample_db, 16
selectNovel, 16
sortAlleles, 3, 17, 19, 20
tigger, 18
tigger-package (tigger), 18
updateAlleleNames, 3, 17, 19, 19
writeFasta, 14, 20
```