Shazam: Mutation analysis

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Contents

Basic mutational load calculations are provided by the `observedMutations` function. `observedMutations` provides multiple options to control how mutations are calculated. Mutations can be calculated as either counts or frequencies, may be divided into replacement (R) and silent (S) mutations, and subset into FWR and CDR specific mutations. Additionally, alternative mutational definitions may be considered based on the physicochemical properties of translated codons.

Example data

A small example Change-O database is included in the `alakazam` package. Analyzing mutations requires the following fields (columns) to be present in the Change-O database:

- `SEQUENCE_IMGT`
- `GERMLINE_IMGT_D_MASK`

```
# Import required packages
library(alakazam)
library(shazam)
library(dplyr)
library(ggplot2)

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

Calculate the counts and frequencies of mutations over the entire sequence

When calling `observedMutations` with `regionDefinition=NULL`, the entire input sequence (`sequenceColumn`) is compared to the germline sequence (`germlineColumn`) to identify R and S mutations. If `frequency=TRUE`, the number of mutations is expressed as the frequency of mutations over the total number of positions that are non-N in both the input and the germline sequences.

In the example below, the counts (`frequency=FALSE`) and frequencies (`frequency=TRUE`) of R and S mutations are calculated separately. New columns containing mutation counts are appended to

the input data.frame with names in the form `MU_COUNT_<Region>_<R/S>`. Mutation frequencies appear in new columns named `MU_FREQ_<Region>_<R/S>`.

```
# Calculate R and S mutation counts
db_obs <- observedMutations(db, sequenceColumn="SEQUENCE_IMGT",
                            germlineColumn="GERMLINE_IMGT_D_MASK",
                            regionDefinition=NULL,
                            frequency=FALSE,
                            nproc=1)
# Show new mutation count columns
db_obs %>%
    select(SEQUENCE_ID, starts_with("MU_COUNT_")) %>%
    head(n=4)
```

```
##      SEQUENCE_ID MU_COUNT_SEQ_R MU_COUNT_SEQ_S
## 1 GN5SHBT07FUXY8              0              0
## 2 GN5SHBT05JMPI5              8              2
## 3 GN5SHBT08H4LPP              8              2
## 4 GN5SHBT05JGND3              0              0
```

```
# Calculate R and S mutation frequencies
db_obs <- observedMutations(db_obs, sequenceColumn="SEQUENCE_IMGT",
                            germlineColumn="GERMLINE_IMGT_D_MASK",
                            regionDefinition=NULL,
                            frequency=TRUE,
                            nproc=1)
# Show new mutation frequency columns
db_obs %>%
    select(SEQUENCE_ID, starts_with("MU_FREQ_")) %>%
    head(n=4)
```

```
##      SEQUENCE_ID MU_FREQ_SEQ_R MU_FREQ_SEQ_S
## 1 GN5SHBT07FUXY8    0.00000000   0.000000000
## 2 GN5SHBT05JMPI5    0.02366864   0.005917160
## 3 GN5SHBT08H4LPP    0.02359882   0.005899705
## 4 GN5SHBT05JGND3    0.00000000   0.000000000
```

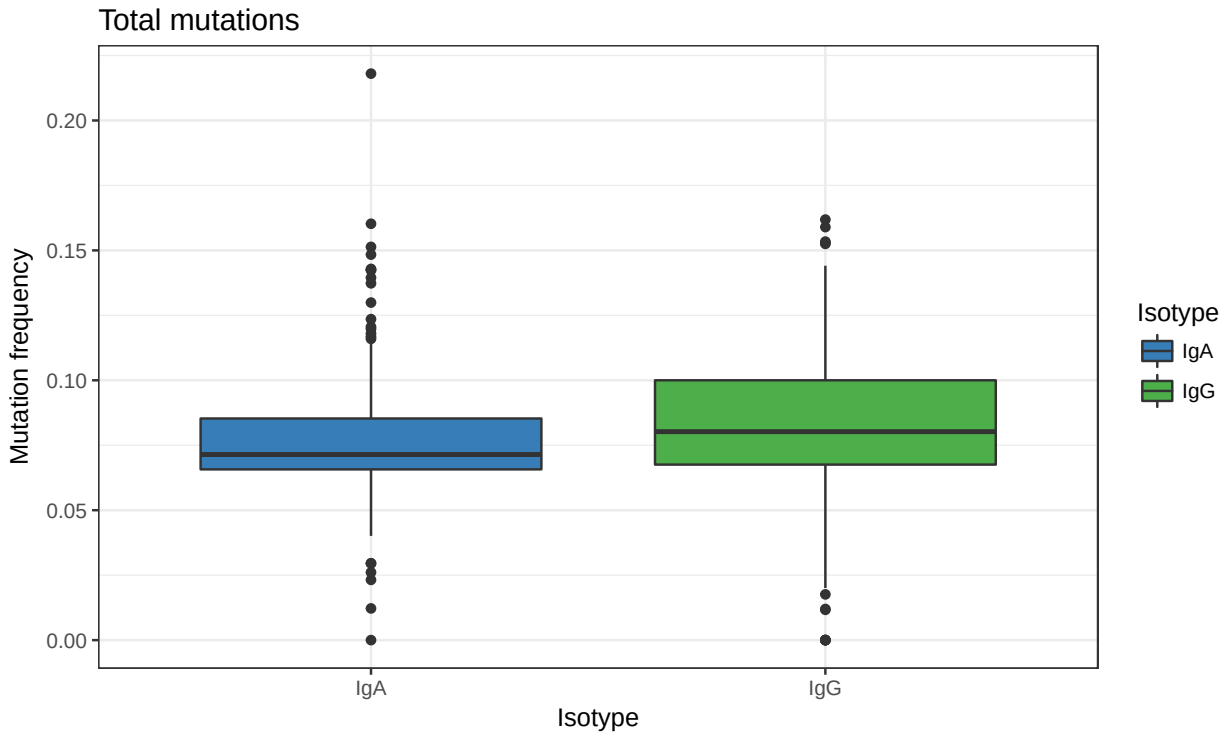
Specifying the `combine=TRUE` argument will aggregate all mutation columns into a single value.

```
# Calculate combined R and S mutation frequencies
db_obs <- observedMutations(db, sequenceColumn="SEQUENCE_IMGT",
                            germlineColumn="GERMLINE_IMGT_D_MASK",
                            regionDefinition=NULL,
                            frequency=TRUE,
                            combine=TRUE,
                            nproc=1)
# Show new mutation frequency columns
db_obs %>%
    select(SEQUENCE_ID, starts_with("MU_FREQ_")) %>%
    head(n=4)
```

```
## data frame with 0 columns and 4 rows
```

We can plot the mutation frequencies a explore differences between samples or isotypes.

```
g1 <- ggplot(db_obs, aes(x=ISOTYPE, y=MU_FREQ, fill=ISOTYPE)) +
    theme_bw() + ggtitle("Total mutations") +
    xlab("Isotype") + ylab("Mutation frequency") +
    scale_fill_manual(name="Isotype", values=IG_COLORS) +
    geom_boxplot()
plot(g1)
```



Calculate mutations within subregions of the V-segment

To restrict the mutational analysis to a particular area in the sequence, the `regionDefinition` argument needs to be assigned a `RegionDefinition` object, which simply defines the subregion boundaries of the Ig sequence. For convenience, `shazam` provides a set of such objects.

In the following example, we will explore the mutation frequency in the V-segment. The `IMGT_V RegionDefinition` defines the limits of the CDR and FWR in the V-segment, excluding the CDR3, according to the IMGT unique numbering scheme.

```
# Calculate R and S mutation counts for individual CDRs and FWRs
db_obs_v <- observedMutations(db, sequenceColumn="SEQUENCE_IMGT",
                              germlineColumn="GERMLINE_IMGT_D_MASK",
                              regionDefinition=IMGT_V_BY_REGIONS,
                              frequency=FALSE,
                              nproc=1)
```

```
# Show new FWR mutation columns
db_obs_v %>%
    select(SEQUENCE_ID, starts_with("MU_COUNT_FWR")) %>%
    head(n=4)
```

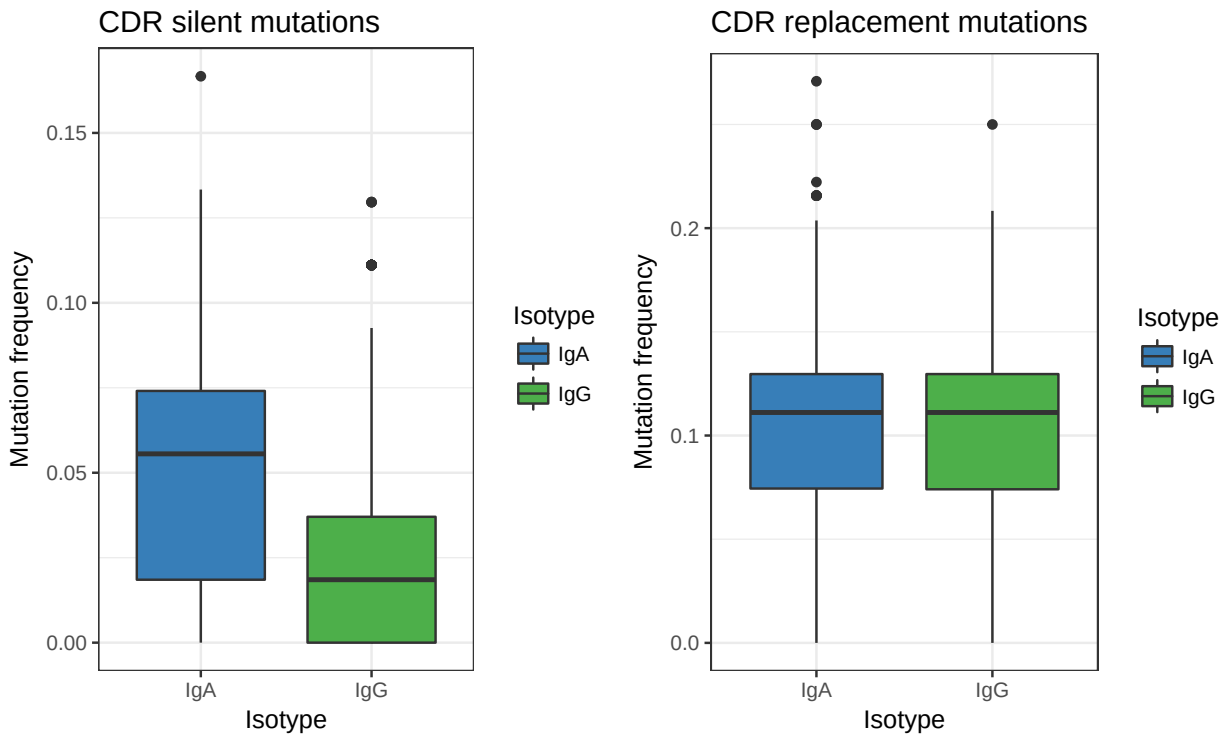
```
##      SEQUENCE_ID MU_COUNT_FWR1_R MU_COUNT_FWR1_S MU_COUNT_FWR2_R
## 1 GN5SHBT07FUXY8               0               0               0
## 2 GN5SHBT05JMPI5               1               0               0
## 3 GN5SHBT08H4LPP               1               0               0
## 4 GN5SHBT05JGND3               0               0               0
##   MU_COUNT_FWR2_S MU_COUNT_FWR3_R MU_COUNT_FWR3_S
## 1               0               0               0
## 2               0               5               1
## 3               0               5               1
## 4               0               0               0
```

```
# Calculate aggregate CDR and FWR V-segment R and S mutation frequencies
db_obs_v <- observedMutations(db_obs_v, sequenceColumn="SEQUENCE_IMGT",
                              germlineColumn="GERMLINE_IMGT_D_MASK",
                              regionDefinition=IMGT_V,
                              frequency=TRUE,
                              nproc=1)
# Show new CDR and FWR mutation frequency columns
db_obs_v %>%
    select(SEQUENCE_ID, starts_with("MU_FREQ_")) %>%
    head(n=4)
```

```
##      SEQUENCE_ID MU_FREQ_CDR_R MU_FREQ_CDR_S MU_FREQ_FWR_R MU_FREQ_FWR_S
## 1 GN5SHBT07FUXY8             0             0     0.0000000   0.000000000
## 2 GN5SHBT05JMPI5             0             0     0.0251046   0.004184100
## 3 GN5SHBT08H4LPP             0             0     0.0250000   0.004166667
## 4 GN5SHBT05JGND3             0             0     0.0000000   0.000000000
```

Plot a comparison between CDR silent and replacement mutations.

```
g2 <- ggplot(db_obs_v, aes(x=ISOTYPE, y=MU_FREQ_CDR_S, fill=ISOTYPE)) +
    theme_bw() + ggtitle("CDR silent mutations") +
    xlab("Isotype") + ylab("Mutation frequency") +
    scale_fill_manual(name="Isotype", values=IG_COLORS) +
    geom_boxplot()
g3 <- ggplot(db_obs_v, aes(x=ISOTYPE, y=MU_FREQ_CDR_R, fill=ISOTYPE)) +
    theme_bw() + ggtitle("CDR replacement mutations") +
    xlab("Isotype") + ylab("Mutation frequency") +
    scale_fill_manual(name="Isotype", values=IG_COLORS) +
    geom_boxplot()
alakazam::gridPlot(g2, g3, ncol=2)
```



Use amino acid physicochemical properties to define mutations

By default, replacement and silent are determined by exact amino acid identity. But this can be changed by setting the `mutationDefinition` argument. For convenience, `shazam` provides a set of `MutationDefinition` objects defining changes in amino acid charge, hydrophobicity, polarity and volume.

In the following example, replacement mutation are defined as amino acid changes that lead to a change in charge (`mutationDefinition=CHARGE_MUTATIONS`). Mutations that do not alter the charge classification of a translated codon will be considered silent mutations.

```
# Calculate charge mutation frequency for the full sequence
db_obs_ch <- observedMutations(db, sequenceColumn="SEQUENCE_IMGT",
                               germlineColumn="GERMLINE_IMGT_D_MASK",
                               regionDefinition=NULL,
                               mutationDefinition=CHARGE_MUTATIONS,
                               frequency=TRUE,
                               nproc=1)
# Show new charge mutation frequency columns
db_obs_ch %>%
    select(SEQUENCE_ID, starts_with("MU_FREQ_")) %>%
    head(n=4)
```

```
##      SEQUENCE_ID MU_FREQ_SEQ_R MU_FREQ_SEQ_S
## 1 GN5SHBT07FUXY8   0.000000000    0.00000000
## 2 GN5SHBT05JMPI5   0.002958580    0.02662722
```

```
## 3 GN5SHBT08H4LPP   0.002949853    0.02654867
## 4 GN5SHBT05JGND3   0.000000000    0.00000000
```

We can make a plot to visualize if mutations that change the sequence charge are more frequent in one isotype.

```
g4 <- ggplot(db_obs_ch, aes(x=ISOTYPE, y=MU_FREQ_SEQ_R, fill=ISOTYPE)) +
    theme_bw() + ggtitle("Charge replacement mutations") +
    xlab("Isotype") + ylab("Mutation frequency") +
    scale_fill_manual(name="Isotype", values=IG_COLORS) +
    geom_boxplot()
plot(g4)
```