

# Alakazam: Analysis of clonal abundance and diversity

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The clonal diversity of the repertoire can be analyzed using the general form of the diversity index, as proposed by Hill in:

Hill, M. Diversity and evenness: a unifying notation and its consequences.  
Ecology 54, 427-432 (1973).

Coupled with resampling strategies to correct for variations in sequencing depth, as well as inference of complete clonal abundance distributions as described in:

Chao A, et al. Rarefaction and extrapolation with Hill numbers:  
A framework for sampling and estimation in species diversity studies.  
Ecol Monogr. 2014 84:45-67.

Chao A, et al. Unveiling the species-rank abundance distribution by  
generalizing the Good-Turing sample coverage theory.  
Ecology. 2015 96, 11891201.

This package provides methods for the inference of a complete clonal abundance distribution (using the `estimateAbundance` function) along with two approaches to assess the diversity of these distributions:

1. Generation of a smooth diversity (D) curve over a range of diversity orders (q) using `alphaDiversity`, and
2. A significance test of the diversity (D) at a fixed diversity order (q).

## Example data

A small example AIRR database, `ExampleDb`, is included in the `alakazam` package. Diversity calculation requires the `clone` field (column) to be present in the AIRR file, as well as an additional grouping column. In this example we will use the grouping columns `sample_id` and `c_call`.

```
# Load required packages  
library(alakazam)
```

```
# Load example data
data(ExampleDb)
```

For details about the AIRR format, visit the AIRR Community documentation site.

## Generate a clonal abundance curve

A simple table of the observed clonal abundance counts and frequencies may be generated using the `countClones` function either with or without copy numbers, where the size of each clone is determined by the number of sequence members:

```
# Partitions the data based on the sample column
clones <- countClones(ExampleDb, group="sample_id")
head(clones, 5)
```

```
## # A tibble: 5 x 4
## # Groups:   sample_id [1]
##   sample_id clone_id seq_count seq_freq
##   <chr>      <dbl>    <int>    <dbl>
## 1 +7d        3128      100    0.100
## 2 +7d        3100       50    0.0501
## 3 +7d        3141       44    0.0440
## 4 +7d        3177       30    0.0300
## 5 +7d        3170       28    0.0280
```

You may also specify a column containing the abundance count of each sequence (usually copy numbers), that will include weighting of each clone size by the corresponding abundance count. Furthermore, multiple grouping columns may be specified such that `seq_freq` (unweighted clone size as a fraction of total sequences in the group) and `copy_freq` (weighted fraction) are normalized to within multiple group data partitions.

```
# Partitions the data based on both the sample_id and c_call columns
# Weights the clone sizes by the duplicate_count column
clones <- countClones(ExampleDb, group=c("sample_id", "c_call"), copy="duplicate_count", clones=
head(clones, 5)
```

```
## # A tibble: 5 x 7
## # Groups:   sample_id, c_call [2]
##   sample_id c_call clone_id seq_count copy_count seq_freq copy_freq
##   <chr>    <chr>    <dbl>    <int>    <dbl>    <dbl>    <dbl>
## 1 +7d     IGHA      3128      88      651    0.331    0.497
## 2 +7d     IGHG      3100      49      279    0.0928   0.173
## 3 +7d     IGHA      3141      44      240    0.165    0.183
## 4 +7d     IGHG      3192      19      141    0.0360   0.0874
## 5 +7d     IGHG      3177      29      130    0.0549   0.0806
```

While `countClones` will report observed abundances, it will not provide confidence intervals. A complete clonal abundance distribution may be inferred using the `estimateAbundance` function with confidence intervals derived via bootstrapping.

This output may be visualized using the `plotAbundanceCurve` function.

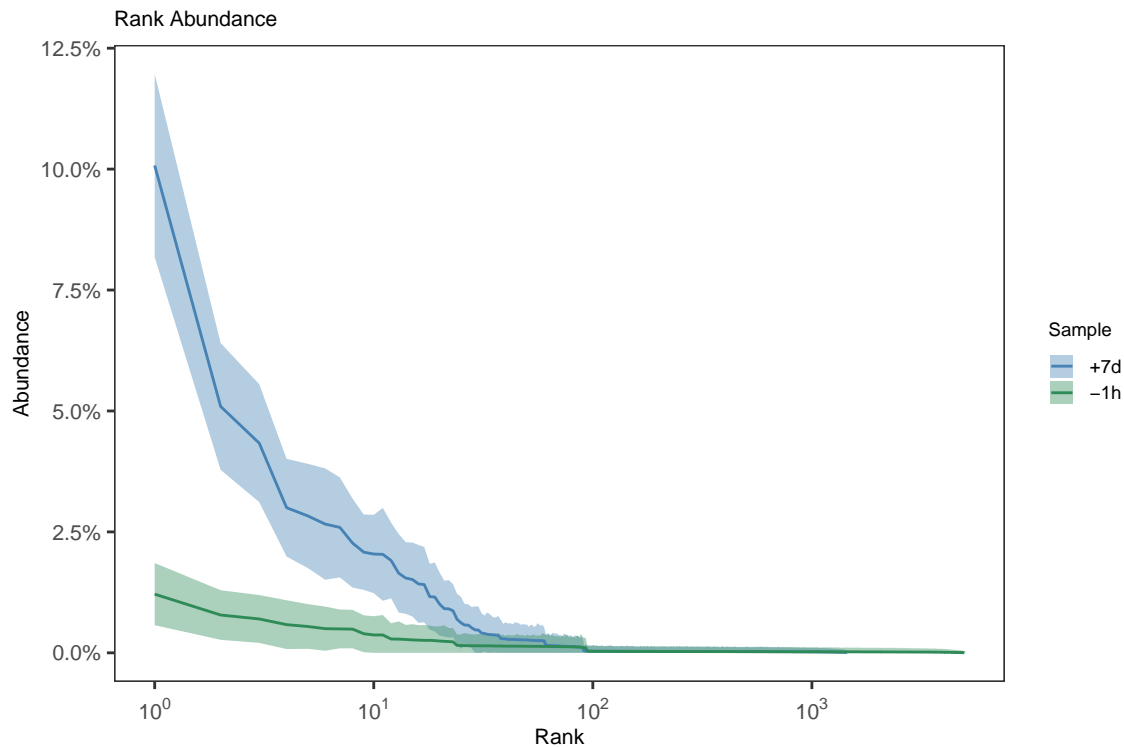
```
# Partitions the data on the sample column
```

```

# Calculates a 95% confidence interval via 100 bootstrap realizations
set.seed(123) # For reproducibility of example bootstrap results
curve <- estimateAbundance(ExampleDb, group="sample_id", ci=0.95, nboot=100, clone="clone_id")

# Plots a rank abundance curve of the relative clonal abundances
sample_colors <- c("-1h"="seagreen", "+7d"="steelblue")
plot(curve, colors = sample_colors, legend_title="Sample")

```



## Generate a diversity curve

The function `alphaDiversity` performs uniform resampling of the input sequences and recalculates the clone size distribution, and diversity, with each resampling realization. Diversity (D) is calculated over a range of diversity orders (q) to generate a smooth curve.

```

# Compare diversity curve across values in the "sample" column
# q ranges from 0 (min_q=0) to 4 (max_q=4) in 0.05 increments (step_q=0.05)
# A 95% confidence interval will be calculated (ci=0.95)
# 100 resampling realizations are performed (nboot=100)
set.seed(123) # For reproducibility of example alphaDiversity results
sample_curve <- alphaDiversity(ExampleDb, group="sample_id", clone="clone_id",
                               min_q=0, max_q=4, step_q=0.1,
                               ci=0.95, nboot=100)

# Compare diversity curve across values in the c_call column
# Analyse is restricted to c_call values with at least 30 sequences by min_n=30
# Excluded groups are indicated by a warning message
isotype_curve <- alphaDiversity(ExampleDb, group="c_call", clone="clone_id",
                                min_q=0, max_q=4, step_q=0.1,

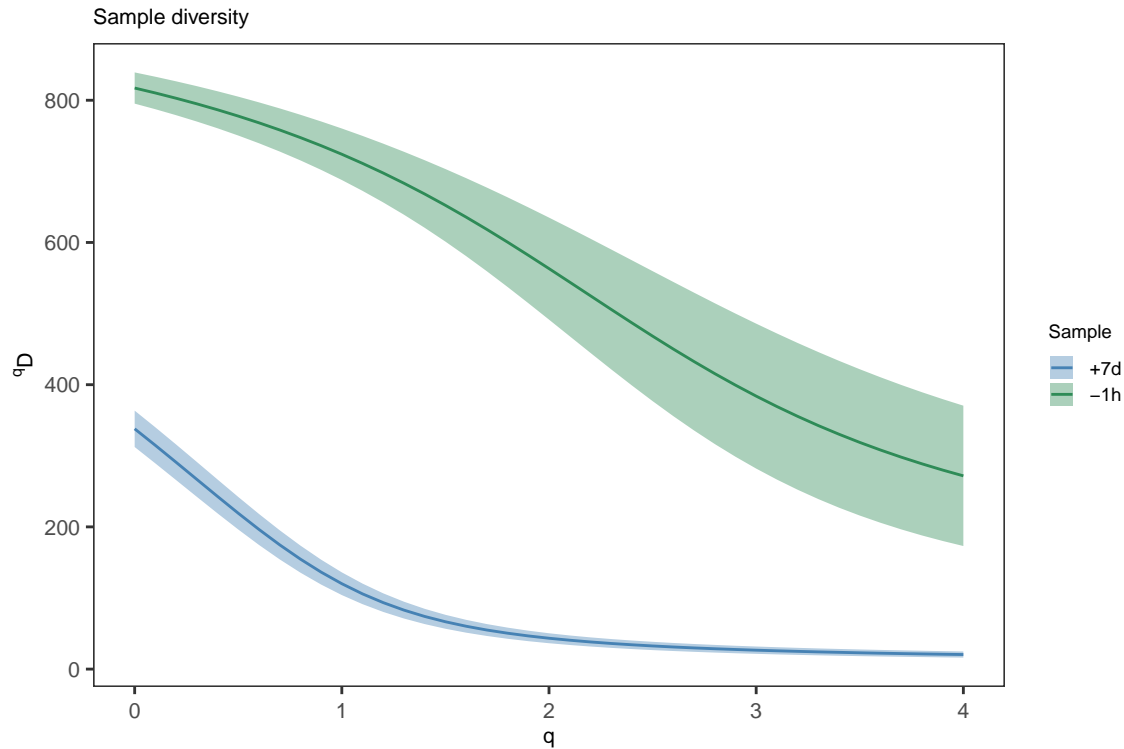
```

```

ci=0.95, nboot=100)

# Plot a log-log (log_q=TRUE, log_d=TRUE) plot of sample diversity
# Indicate number of sequences resampled from each group in the title
sample_main <- paste0("Sample diversity")
sample_colors <- c("-1h"="seagreen", "+7d"="steelblue")
plot(sample_curve, colors=sample_colors, main_title=sample_main,
      legend_title="Sample")

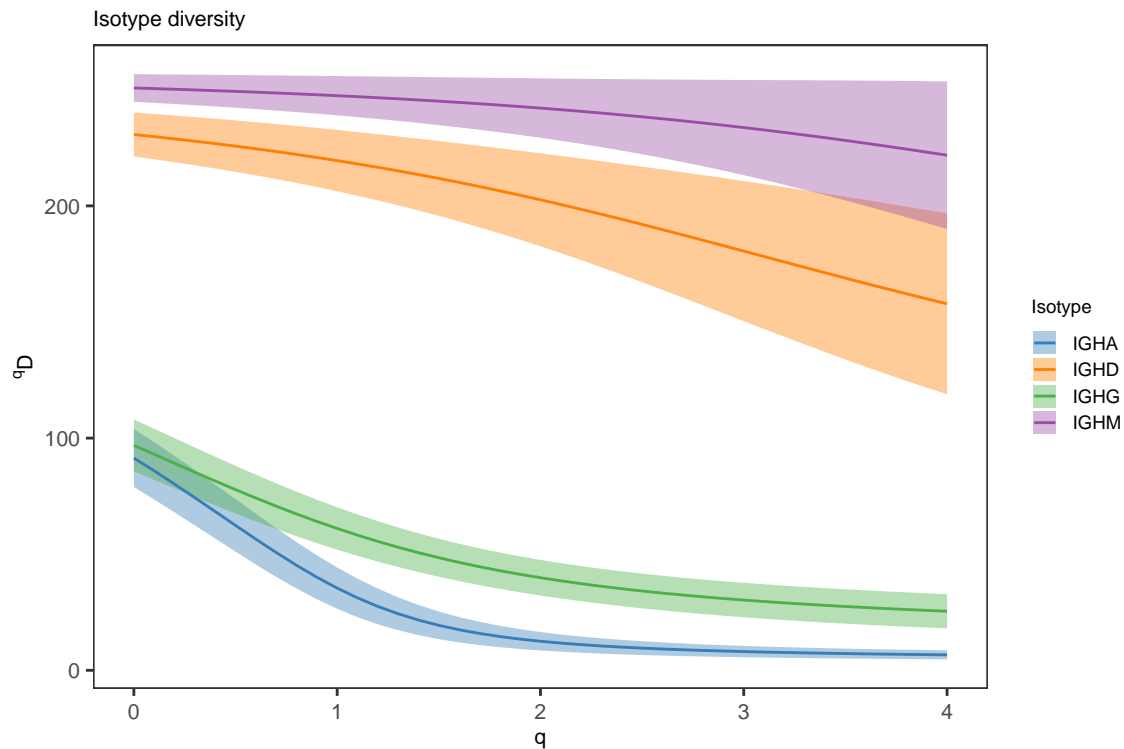
```



```

# Plot isotype diversity using default set of Ig isotype colors
isotype_main <- paste0("Isotype diversity")
plot(isotype_curve, colors=IG_COLORS, main_title=isotype_main,
      legend_title="Isotype")

```



## View diversity tests at a fixed diversity order

Significance testing across groups is performed using the delta of the bootstrap distributions between groups when running `alphaDiversity` for all values of `q` specified.

```
# Test diversity at q=0, q=1 and q=2 (equivalent to species richness, Shannon entropy,
# Simpson's index) across values in the sample_id column
# 100 bootstrap realizations are performed (nboot=100)
set.seed(123) # For reproducibility of example alphaDiversity results
isotype_test <- alphaDiversity(ExampleDb, group="c_call", min_q=0, max_q=2, step_q=1, nboot=100)

# Print P-value table
print(isotype_test@tests)
```

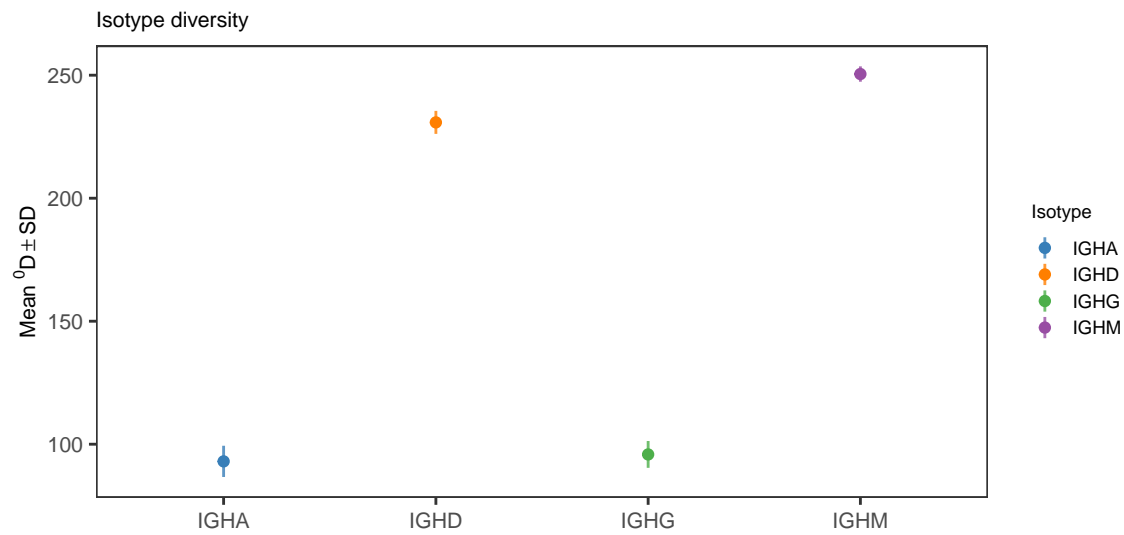
```
## # A tibble: 18 x 5
##   test      q    delta_mean delta_sd pvalue
##   <chr>    <chr>      <dbl>    <dbl>   <dbl>
## 1 IGHA != IGHD 0        138.      7.85    0
## 2 IGHA != IGHD 1        183.      8.34    0
## 3 IGHA != IGHD 2        191.     10.3    0
## 4 IGHA != IGHG 0         2.82      8.65   0.64
## 5 IGHA != IGHG 1         23.6      7.05    0
## 6 IGHA != IGHG 2         26.8      4.85    0
## 7 IGHA != IGHM 0        157.      7.83    0
## 8 IGHA != IGHM 1        210.      7.61    0
## 9 IGHA != IGHM 2        229.      7.31    0
## 10 IGHD != IGHG 0        135.      6.74    0
```

```
## 11 IGHD != IGHG 1      160.      7.81  0
## 12 IGHD != IGHG 2      164.     10.7  0
## 13 IGHD != IGHM 0       19.6      5.86  0
## 14 IGHD != IGHM 1       27.3      8.36  0
## 15 IGHD != IGHM 2       38.4     12.5  0
## 16 IGHG != IGHM 0      155.      6.43  0
## 17 IGHG != IGHM 1      187.      6.60  0
## 18 IGHG != IGHM 2      202.      7.83  0
```

*# Plot results at q=0 and q=2*

*# Plot the mean and standard deviations at q=0 and q=2*

```
plot(isotype_test, 0, colors=IG_COLORS, main_title=isotype_main,
     legend_title="Isotype")
```



```
plot(isotype_test, 2, colors=IG_COLORS, main_title=isotype_main,
     legend_title="Isotype")
```

