

# Shazam: Tuning clonal assignment thresholds with nearest neighbor distances

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Estimating the optimal distance threshold for partitioning clonally related sequences is accomplished by calculating the distance from each sequence in the data set to its nearest neighbor and finding the break point in the resulting bi-modal distribution that separates clonally related from unrelated sequences. This is done via the following steps:

1. Calculating of the nearest neighbor distances for each sequence.
2. Generating a histogram of the nearest neighbor distances followed by either manual inspect for the threshold separating the two modes or automated threshold detection.

## Example data

A small example Change-O database is included in the **alakazam** package. Calculating the nearest neighbor distances requires the following fields (columns) to be present in the Change-O database:

- SEQUENCE\_ID
- V\_CALL
- J\_CALL
- JUNCTION
- JUNCTION\_LENGTH

```
# Subset example data to one sample
library(shazam)
data(ExampleDb, package="alakazam")
```

## Calculating nearest neighbor distances

The function for calculating distance between every sequence and its nearest neighbor takes a few parameters to adjust how the distance is measured. If a genotype has been inferred using the methods in the **tigger** package, and a **V\_CALL\_GENOTYPED** field has been added to the database, then this column may be used instead of the default **V\_CALL** column by specifying the **vCallColumn** argument. This will allows the more accurate V call from **tigger** to be used for grouping of the

sequences. Furthermore, for more leniency toward ambiguous V(D)J segment calls, the parameter **first** can be set to **FALSE**. Setting **first=FALSE** will use the union of all possible genes to group sequences, rather than the first gene in the field. The **model** parameter determines which underlying SHM model is used to calculate the distance. The default model is single nucleotide Hamming distance with gaps considered as a match to any nucleotide (**ham**). Other options include a human Ig-specific single nucleotide model similar to a transition/transversion model (**hh\_s1f**) and the corresponding 5-mer context model from Yaari et al, 2013 (**hh\_s5f**), an analogous pair of mouse specific models from Cui et al, 2016 (**mk\_rs1nf** and **mk\_rs5nf**), and amino acid Hamming distance (**aa**).

**Note:** Human and mouse distance measures that are backward compatible with SHazaM v0.1.4 and Change-O v0.3.3 are also provide as **hs1f\_compat** and **m1n\_compat**, respectively.

For models that are not symmetric (e.g., distance from A to B is not equal to the distance from B to A), there is a **symmetry** parameter that allows the user to specify whether the average or minimum of the two distances is used to determine the overall distance.

```
# Use nucleotide Hamming distance and normalize by junction length
dist_ham <- distToNearest(ExampleDb, vCallColumn="V_CALL_GENOTYPED",
                          model="ham", normalize="len", nproc=1)

# Use genotyped V assignments, a 5-mer model and no normalization
dist_s5f <- distToNearest(ExampleDb, vCallColumn="V_CALL_GENOTYPED",
                          model="hh_s5f", normalize="none", nproc=1)
```

## Using nearest neighbor distances to determine clonal assignment thresholds

The primary use of the distance to nearest calculation in SHazaM is to determine the optimal threshold for clonal assignment using the **DefineClones** tool in Change-O. Defining a threshold relies on distinguishing clonally related sequences (represented by sequences with close neighbors) from singletons (sequences without close neighbors), which show up as two modes in a nearest neighbor distance histogram.

Thresholds may be manually determined by inspection of the nearest neighbor histograms or by using one of the automated threshold detection algorithms provided by the **findThreshold** function. The available methods are **density** (smoothed density) and **gmm** (gamma/Gaussian mixture model), and are chosen via the **method** parameter of **findThreshold**.

## Threshold determination by manual inspection

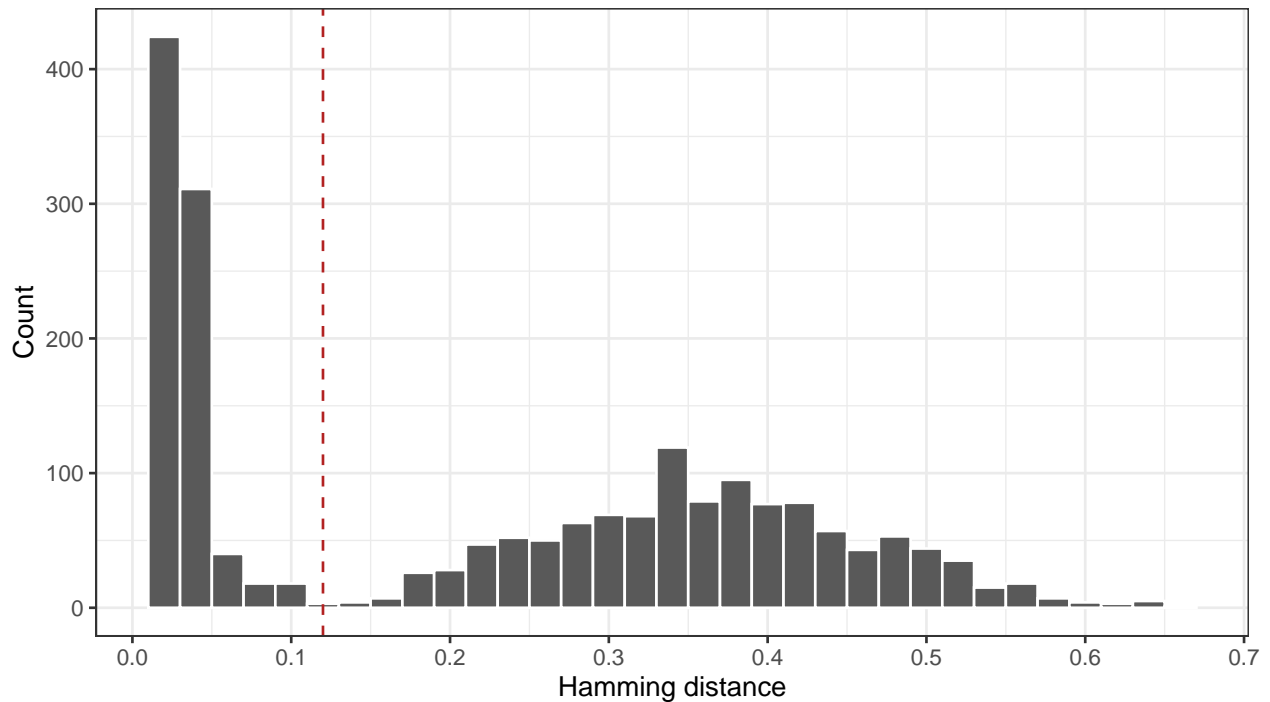
Manual threshold detection simply involves generating a histogram for the values in the **DIST\_NEAREST** column of the **distToNearest** output and selecting a suitable value within the valley between the two modes.

```
# Generate Hamming distance histogram
library(ggplot2)
p1 <- ggplot(subset(dist_ham, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
  theme_bw() +
```

```

xlab("Hamming distance") +
ylab("Count") +
scale_x_continuous(breaks=seq(0, 1, 0.1)) +
geom_histogram(color="white", binwidth=0.02) +
geom_vline(xintercept=0.12, color="firebrick", linetype=2)
plot(p1)

```

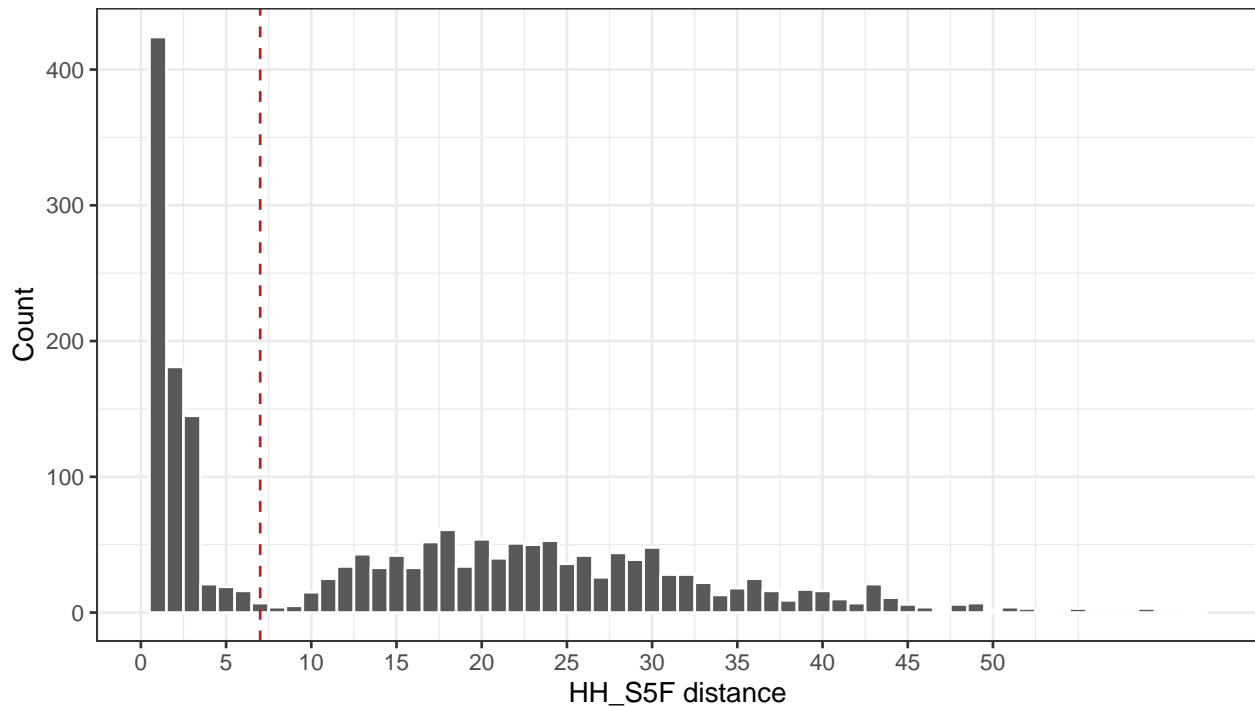


By manual inspection, the length normalized ham model distance threshold would be set to a value near 0.12 in the above example.

```

# Generate HH_S5F distance histogram
p2 <- ggplot(subset(dist_s5f, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
  theme_bw() +
  xlab("HH_S5F distance") +
  ylab("Count") +
  scale_x_continuous(breaks=seq(0, 50, 5)) +
  geom_histogram(color="white", binwidth=1) +
  geom_vline(xintercept=7, color="firebrick", linetype=2)
plot(p2)

```



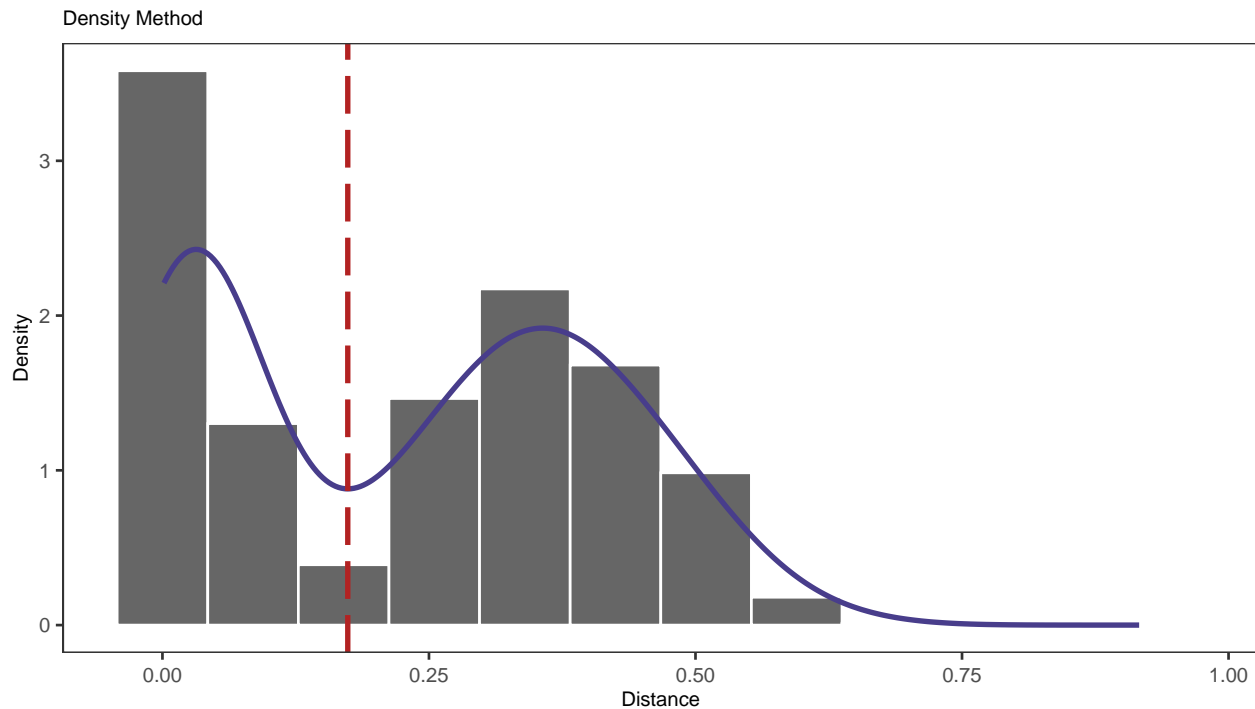
In this example, the unnormalized `hh_s5f` model distance threshold would be set to a value near 7.

### Automated threshold detection via smoothed density

The `density` method will look for the minimum in the valley between two modes of a smoothed distribution based on the input vector (`distances`), which will generally be the `DIST_NEAREST` column from the `distToNearest` output. Below is an example of using the `density` method for threshold detection.

```
# Find threshold using density method
output <- findThreshold(dist_ham$DIST_NEAREST, method="density")
threshold <- output@threshold

# Plot distance histogram, density estimate and optimum threshold
plot(output, title="Density Method")
```



```
# Print threshold
print(output)
## [1] 0.1738391
```

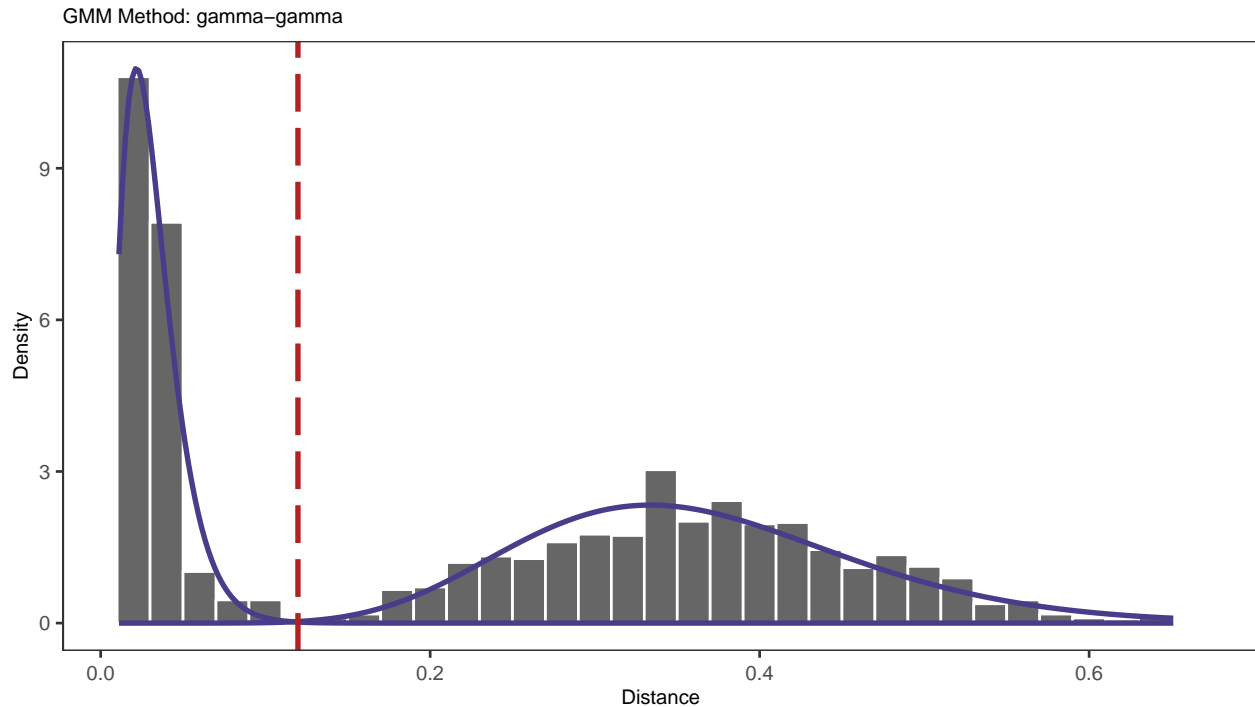
## Automated threshold detection via a mixture model

The `findThreshold` function includes approaches for automatically determining a clonal assignment threshold. The "gmm" method (gamma/Gaussian mixture method) of `findThreshold` (`method="gmm"`) performs a maximum-likelihood fitting procedure over the distance-to-nearest distribution using one of four combinations of univariate density distribution functions: "norm-norm" (two Gaussian distributions), "norm-gamma" (lower Gaussian and upper gamma distribution), "gamma-norm" (lower gamma and upper Gaussian distribution), and "gamma-gamma" (two gamma distributions). By default, the threshold will be selected by calculating the distance at which the average of sensitivity and specificity reaches its maximum (`cutoff="optimal"`). Alternative threshold selection criteria are also providing, including the curve intersection (`cutoff="intersect"`), user defined sensitivity (`cutoff="user", sen=x`), or user defined specificity (`cutoff="user", spc=x`).

In the example below the mixture model method (`method="gmm"`) is used to find the optimal threshold for separating clonally related sequences by fitting two gamma distributions (`model="gamma-gamma"`). The red dashed-line shown in figure below defines the distance where the average of the sensitivity and specificity reaches its maximum.

```
# Find threshold using gmm method
output <- findThreshold(dist_ham$DIST_NEAREST, method="gmm", model="gamma-gamma")

# Plot distance histogram, Gaussian fits, and optimum threshold
plot(output, binwidth=0.02, title="GMM Method: gamma-gamma")
```



```
# Print threshold
print(output)

## [1] 0.1197552
```

**Note:** The shape of histogram plotted by `plotGmmThreshold` is governed by the `binwidth` parameter. Meaning, any change in bin size will change the form of the distribution, while the `gmm` method is completely bin size independent and only engages the real input data.

## Calculating nearest neighbor distances independently for subsets of data

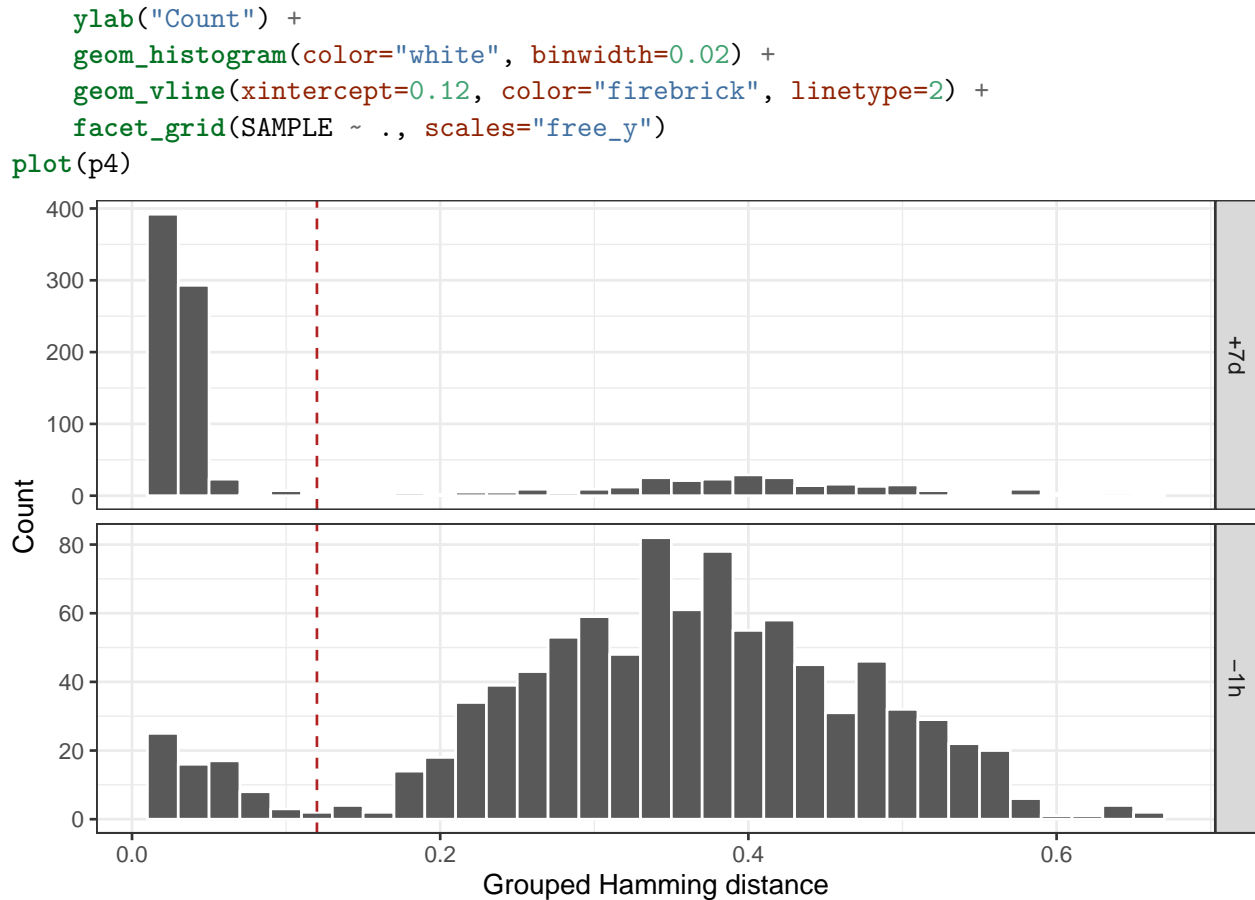
The `fields` argument to `distToNearest` will split the input `data.frame` into groups based on values in the specified fields (columns) and will treat them independently. For example, if the input data has multiple samples, then `fields="SAMPLE"` would allow each sample to be analyzed separately.

In the previous examples we used a subset of the original example data. In the following example, we will use the two available samples, `-1h` and `+7d`, and will set `fields="SAMPLE"`. This will reproduce previous results for sample `-1h` and add results for sample `+7d`.

```
dist_fields <- distToNearest(ExampleDb, model="ham", normalize="len",
                             fields="SAMPLE", nproc=1)
```

We can plot the nearest neighbor distances for the two samples:

```
# Generate grouped histograms
p4 <- ggplot(subset(dist_fields, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
  theme_bw() +
  xlab("Grouped Hamming distance") +
```



In this case, the threshold selected for `-1h` seems to work well for `+7d` as well.

## Calculating nearest neighbor distances across groups rather than within a groups

Specifying the `cross` argument to `distToNearest` forces distance calculations to be performed across groups, such that the nearest neighbor of each sequence will always be a sequence in a different group. In the following example we set `cross="SAMPLE"`, which will group the data into `-1h` and `+7d` sample subsets. Thus, nearest neighbor distances for sequences in sample `-1h` will be restricted to the closest sequence in sample `+7d` and vice versa.

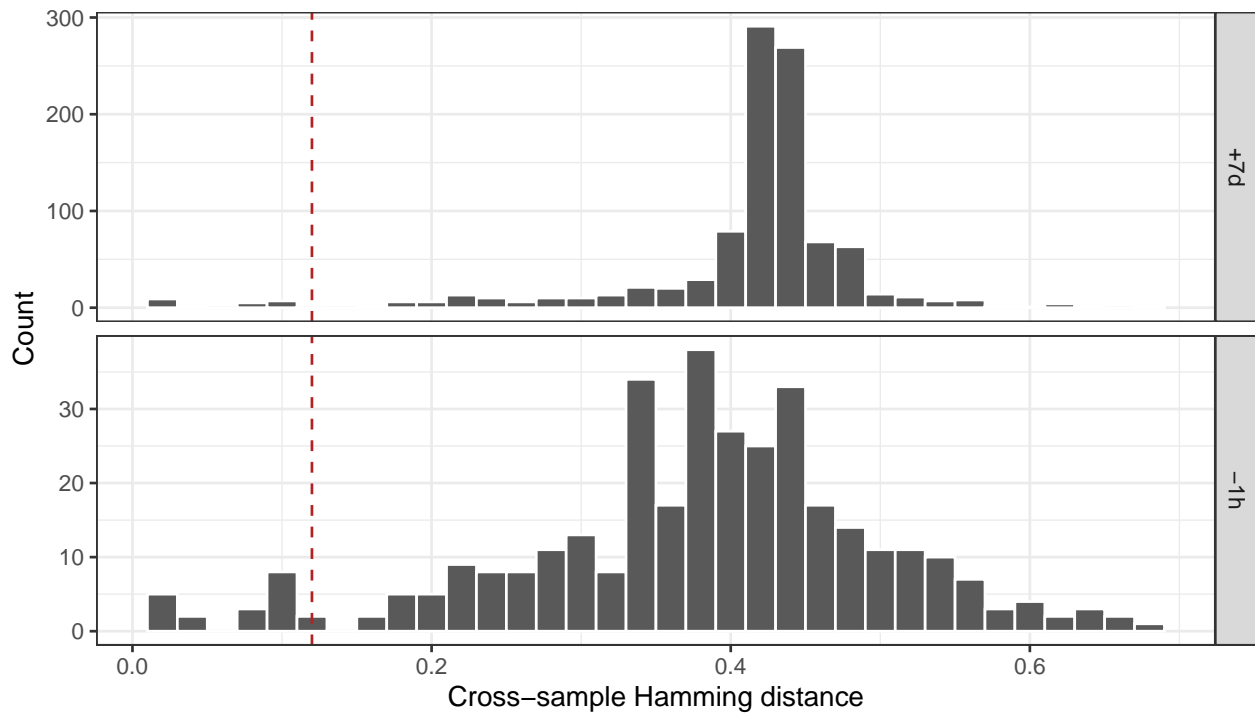
```

dist_cross <- distToNearest(ExampleDb, model="ham", first=FALSE,
                           normalize="len", cross="SAMPLE", nproc=1)

# Generate cross sample histograms
p5 <- ggplot(subset(dist_cross, !is.na(CROSS_DIST_NEAREST)),
            aes(x=CROSS_DIST_NEAREST)) +
  theme_bw() +
  xlab("Cross-sample Hamming distance") +
  ylab("Count") +
  geom_histogram(color="white", binwidth=0.02) +
  geom_vline(xintercept=0.12, color="firebrick", linetype=2) +
  facet_grid(SAMPLE ~ ., scales="free_y")

```

```
plot(p5)
```



This can provide a sense of overlap between samples or a way to compare within-sample variation to cross-sample variation.

## Speeding up pairwise-distance-matrix calculations with subsampling

The `subsample` option in `distToNearest` allows to speed up calculations and reduce memory usage.

If there are very large groups of sequences that share V call, J call and junction length, `distToNearest` will need a lot of memory and it will take a long time to calculate all the distances. Without subsampling, in a large group of  $n=70,000$  sequences `distToNearest` calculates a  $n*n$  distance matrix. With subsampling, e.g. to  $s=15,000$ , the distance matrix for the same group has size  $s*n$ , and for each sequence in `db`, the distance value is calculated by comparing the sequence to the subsampled sequences from the same V-J-junction length group.

```
# Explore V-J-junction length groups sizes to use subsample
# Show the size of the largest groups
library(dplyr)

##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##   filter, lag

## The following objects are masked from 'package:base':
##
##   intersect, setdiff, setequal, union
```



```

library(alakazam)
top_10_sizes <- ExampleDb %>%
  group_by(JUNCTION_LENGTH) %>% # group by junction length
  do(alakazam::groupGenes(., first=TRUE)) %>% # group by V and J call
  mutate(GROUP_ID=paste(JUNCTION_LENGTH,VJ_GROUP, sep="_")) %>% # Create group ids based on
  ungroup() %>%
  group_by(GROUP_ID) %>% # group by GROUP_ID
  distinct(JUNCTION) %>% # for each group, we want to count unique junctions, so keep distinct
  summarize(SIZE=n()) %>% # get the size of the group, the number of sequences
  arrange(desc(SIZE)) %>% # sort by decreasing size
  select(SIZE) %>%
  top_n(10) # show the top 10

## Selecting by SIZE

top_10_sizes

## # A tibble: 10 x 1
##   SIZE
##   <int>
## 1     90
## 2     37
## 3     36
## 4     34
## 5     33
## 6     33
## 7     32
## 8     26
## 9     25
## 10    25

# Use 30 to subsample
# NOTE. This is a toy example. To use 30 with real data
# is probably not a good choice.
dist <- distToNearest(ExampleDb,
  vCallColumn="V_CALL_GENOTYPED",
  model="ham",
  first=FALSE, normalize="len",
  subsample = 30)

```