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")
db <- subset(ExampleDb, SAMPLE == "-1h")
```

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(db, model="ham", first=FALSE, normalize="len",
                          nproc=1)

# Use genotyped V assignments, a 5-mer model and no normalization
dist_s5f <- distToNearest(db, vCallColumn="V_CALL_GENOTYPED", model="hh_s5f",
                          first=FALSE, 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-bygroup` 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.

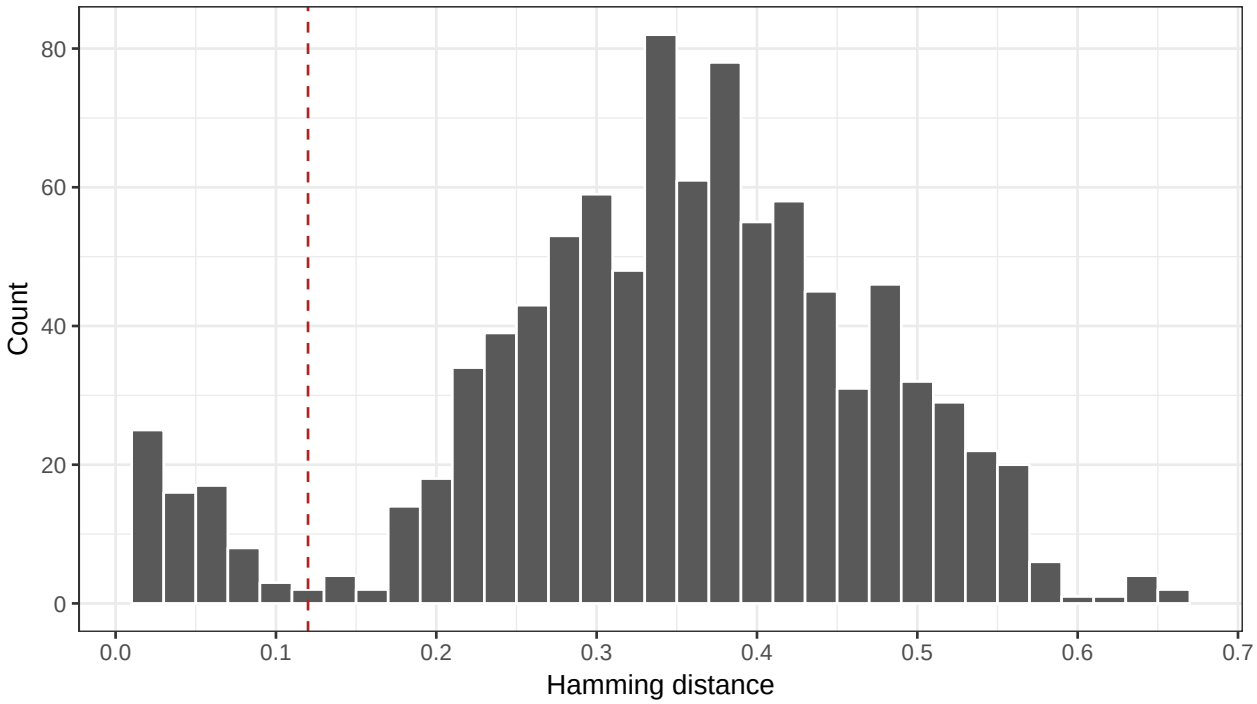
Thresholds may be manually determined by inspection of the nearest neighbor histograms or by using one of the automated threshold detection algorithms provided by the `findThreshold` function. The available methods are `density` (smoothed density) and `gmm` (Guassian mixture model), and are chosen via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

Threshold determination by manual inspection

Manual threshold detection simply involves generating a histrogram for the values in the `DIST_NEAREST` column of the `distToNearest` output and selecting a suitable value within the valley between the two modes.

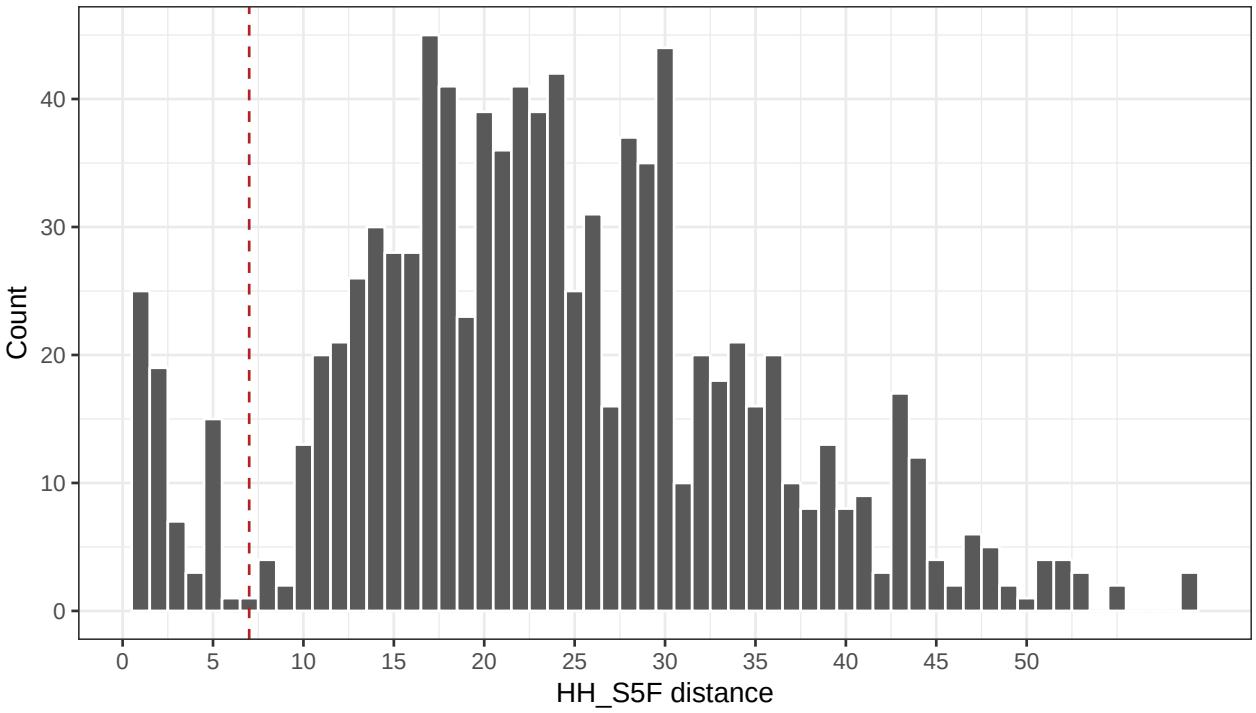
```
# Generate Hamming distance histogram
library(ggplot2)
p1 <- ggplot(subset(dist_ham, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
    theme_bw() +
```

```
    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 a mixture model

A mixture of two univariate density distribution functions is considered:

$$f(x) = \lambda_1 f_1(x|\phi_1) + \lambda_2 f_2(x|\phi_2). \tag{1}$$

Here, the λ_1 and λ_2 represent the mixing weights (sum to one), x represents the nearest neighbor distance (calculated by function `distToNearest`), and ϕ represents the vector of each component parameters; mean and standard deviation (μ, σ) of a Normal distribution, shape and scale (k, θ) of a Gamma distribution.

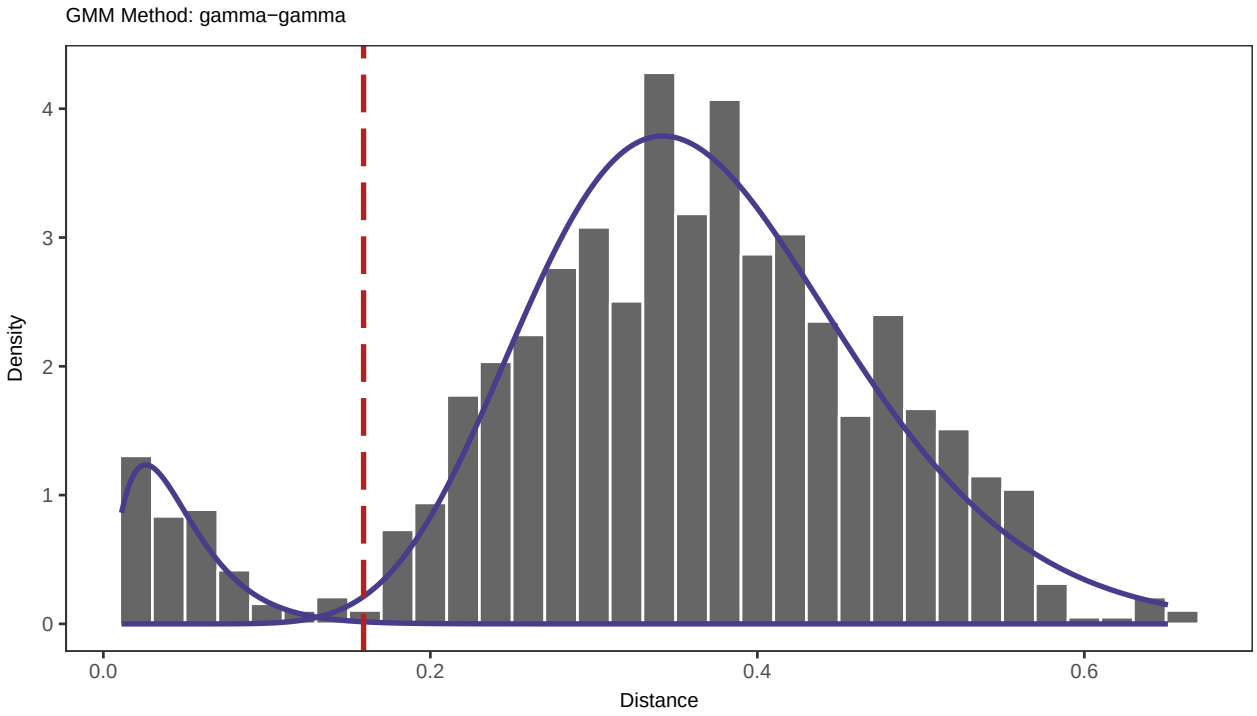
The `"gmm"` (Gamma/Gaussian Mixture Method) is enabled to perform a maximum-likelihood fitting procedure over the dist-to-nearest distribution through one of the four combinations of f_1 and f_2: (1) `"norm-norm"`, (2) `"norm-gamma"`, (3) `"gamma-norm"`, and (4) `"gamma-gamma"`. A Gaussian mixture model, including expectation maximization algorithm, is also implemented into the `findThreshold` function to initialize the fit component parameters automatically. The Gaussian mixture model estimates two sets of values; (1) mixing weight λ_i, (2) mean μ_i, and (3) standard deviation σ_i where $i \in \{1, 2\}$ refers to the first and second curve. These parameters are then used as initial values to begin the fitting procedure (if Gamma distribution is chosen, the initial values are translated accordingly). After maximum-likelihood fitting procedure converges, through an optimization approach, it then calculates the optimum threshold where the average of Sensitivity and Specificity reaches its maximum.

Below is an example showing how the `"gmm"` method is used to find optimal threshold for separating clonally related sequences. The red dashed-line shown in figure below defines the distance where

the average of the Sensitivity plus Specificity reaches its maximum.

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

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




```
# Print threshold
print(output)
```

```
## [1] 0.1591457
```

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.

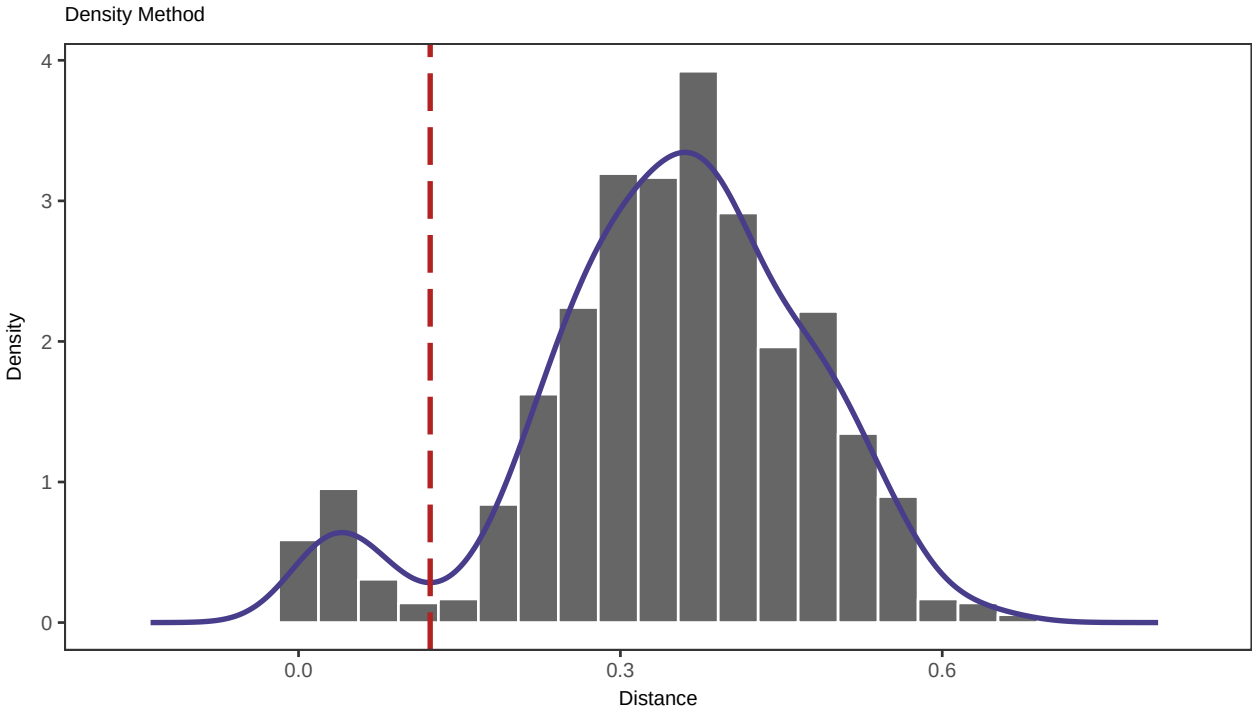
Automated threshold detection via smoothed density

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. Determining the optimal bandwidth parameter for smoothing the distribution can be computationally intensive. The bandwidth tuning is typically robust when subsampling down to 15,000 distances, though the ideal subsampling count will depend upon the data set. The input vector can be subsampled to the size specified using the `subsample` parameter. 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] 0.1226913
```

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.

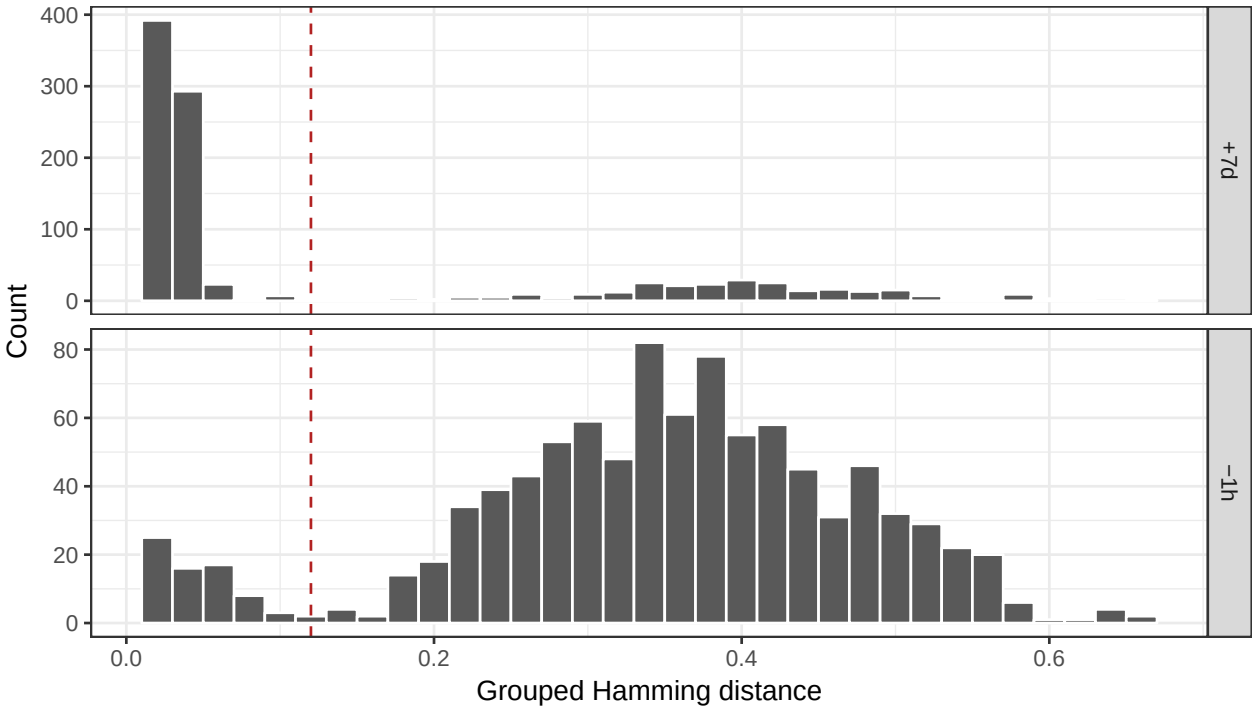
In the previous examples we used a subset of the original example data. In the following example, we will use the two available samples, `-1h` and `+7d`, and will set `fields="SAMPLE"`. This will reproduce previous results for sample `-1h` and add results for sample `+7d`.

```
dist_fields <- distToNearest(ExampleDb, model="ham", first=FALSE,
                             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") +
    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(p4)
```



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

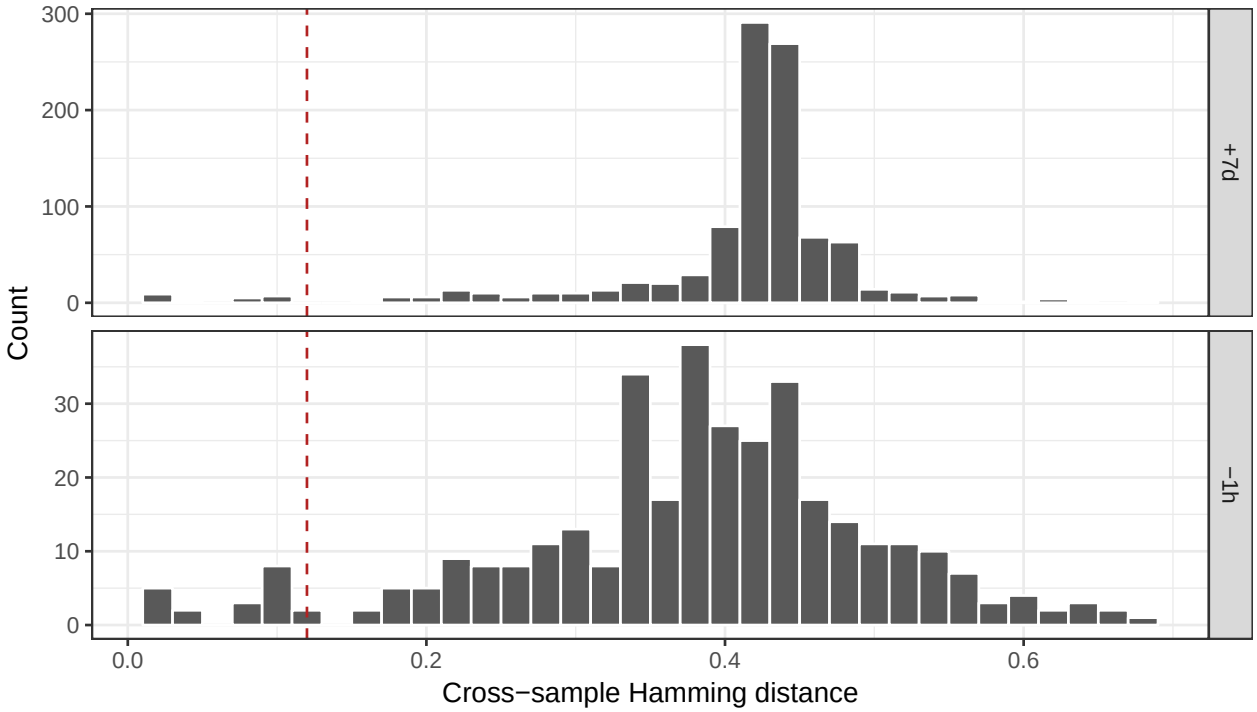
Calculating nearest neighbor distances across groups rather than within a groups

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.