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. Calculate the nearest neighbor distances for each sequence.
2. Generate a histogram of the nearest neighbor distances and inspect for the threshold separating the two modes.

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 `hs1f`, a human Ig-specific single nucleotide model similar to a transition/transversion model (Yaari et al, 2013). Other options include nucleotide Hamming distance (`ham`), amino acid Hamming distance (`aa`), single nucleotide (`m1n`) and 3-mer (`m3n`) mouse models (Smith et al, 1996), and a 5-mer model inferred from human data (`hs5f`) (Yaari et al, 2013). 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 taking the average or the minimum of the two distances to determine the overall distance.

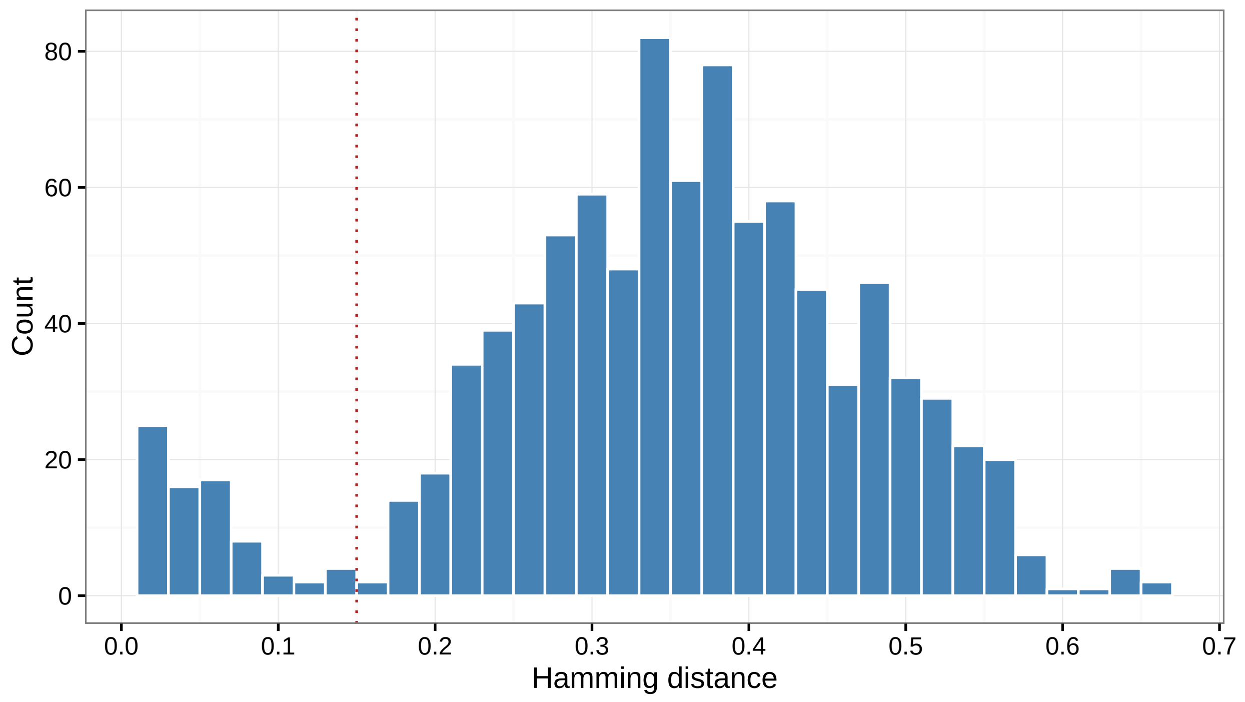
```r
# Use nucleotide Hamming distance and normalize by junction length
dist_ham <- distToNearest(db, model="ham", first=FALSE, normalize="length",
                          nproc=1)

# Use genotyped V assignments and 5-mer model
dist_hs5f <- distToNearest(db, vCallColumn="V_CALL_GENOTYPED", model="hs5f",
                           first=FALSE, normalize="none", nproc=1)
```

Generating histograms of nearest neighbor distances

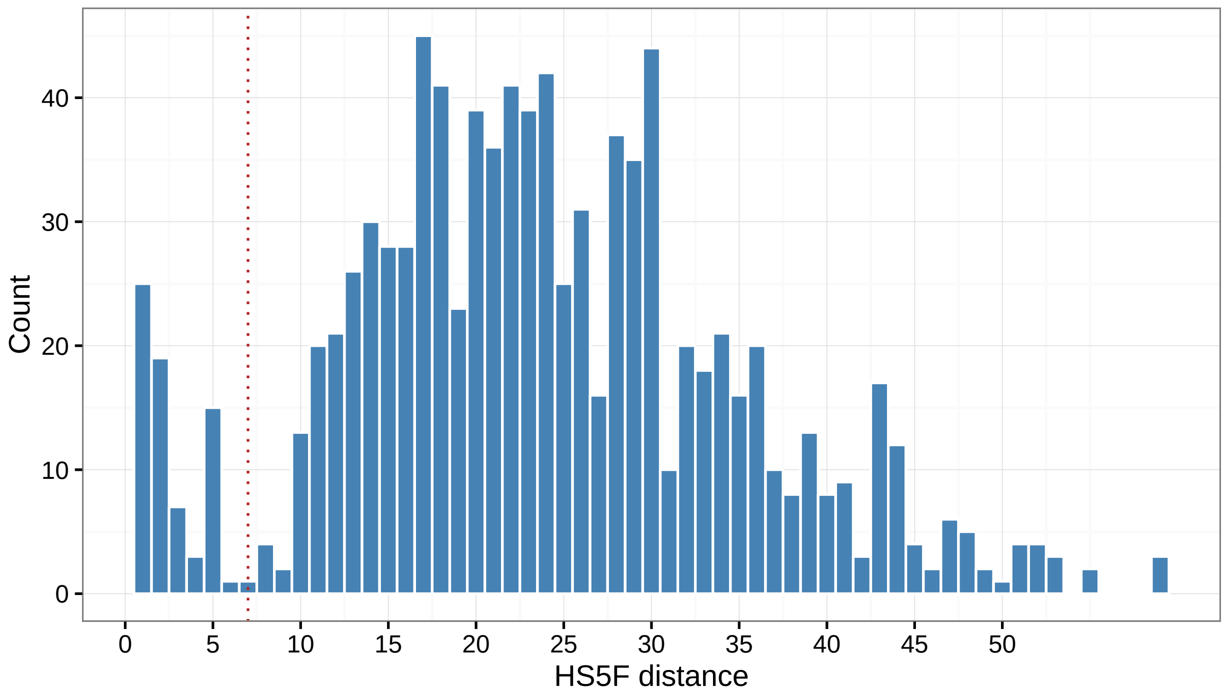
The primary use of the distance to nearest calculation in the Change-O pipeline is to determine the optimal threshold for separating clonally related sequences (represented by sequences with “near”" neighbors) from singletons (sequences without “near”" neighbor), which show up as two modes in a histogram.

```r
# 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(fill="steelblue", color="white", binwidth=0.02) +
    geom_vline(xintercept=0.15, color="firebrick", linetype=3)
plot(p1)
```



In this example, the length normalized `ham` model distance threshold would be set to a value near 0.15.

```
# Generate hs5f distance histogram
p2 <- ggplot(subset(dist_hs5f, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
    theme_bw() + xlab("HS5F distance") + ylab("Count") +
    scale_x_continuous(breaks=seq(0, 50, 5)) +
    geom_histogram(fill="steelblue", color="white", binwidth=1) +
    geom_vline(xintercept=7, color="firebrick", linetype=3)
plot(p2)
```



In this example, the unnormalized `hs5f` model distance threshold would be set to a value near 7.

Calculating nearest neighbor distances independently for subsets of data

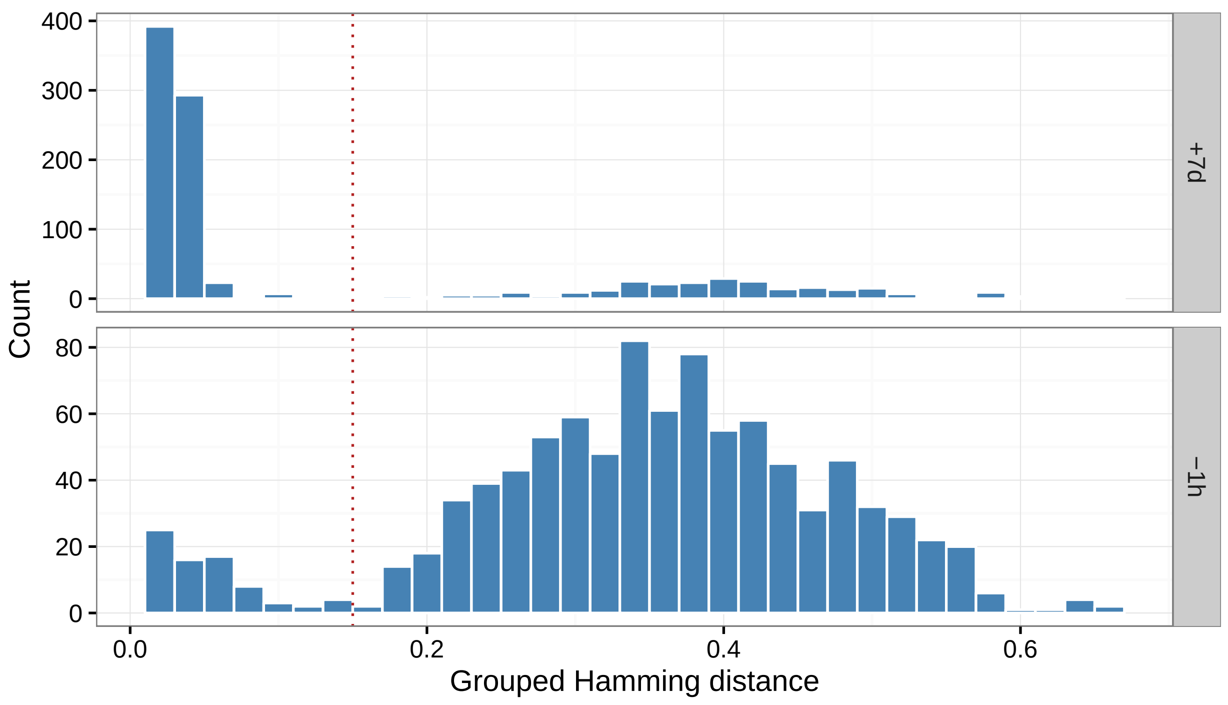
The `fields` argument to `distToNearest` will split the input `data.frame` in groups based on values in the specified fields (columns) and will treat them independently. For example, if the input data has multiple samples, then `fields="SAMPLE"` would allow each sample to be analyzed separately.

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="length", fields="SAMPLE",
                             nproc=1)
```

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

```
# Generate grouped histograms
p3 <- ggplot(subset(dist_fields, !is.na(DIST_NEAREST)),
             aes(x=DIST_NEAREST)) +
    theme_bw() + xlab("Grouped Hamming distance") + ylab("Count") +
    geom_histogram(fill="steelblue", color="white", binwidth=0.02) +
    geom_vline(xintercept=0.15, color="firebrick", linetype=3) +
    facet_grid(SAMPLE ~ ., scales="free_y")
plot(p3)
```



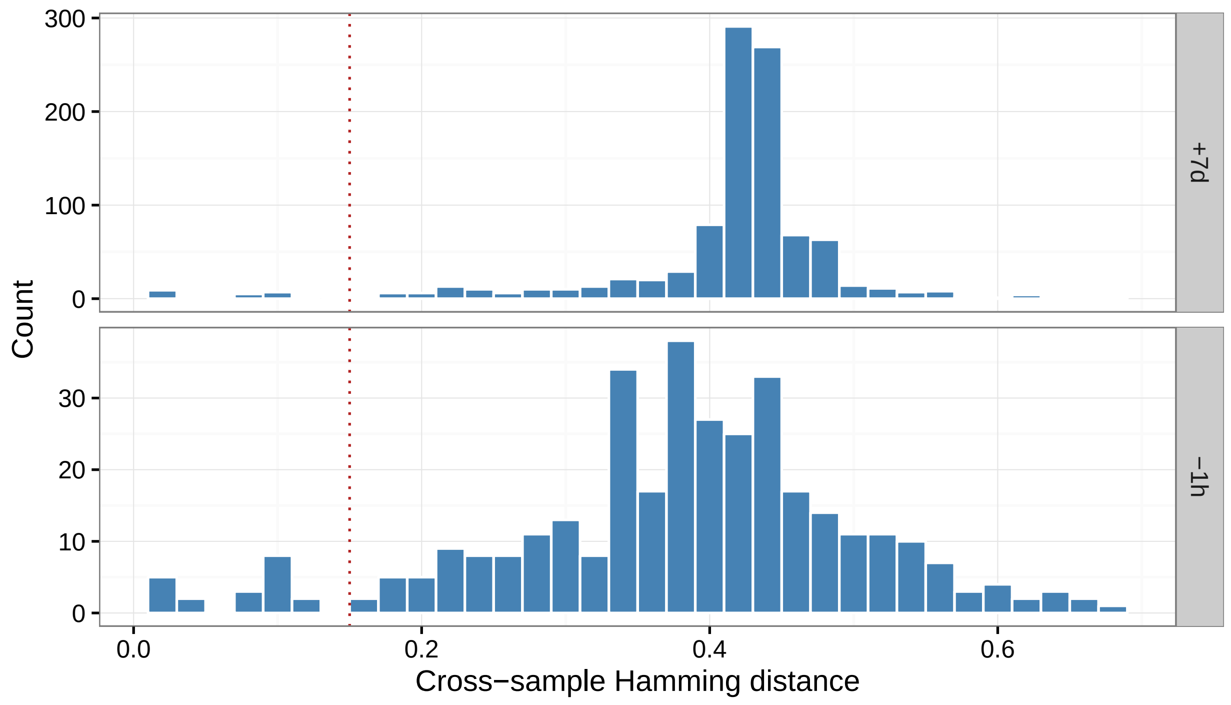
In this case, the threshold selected for `+7d` is not noticeably different from that selected for `-1h`.

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 grouped 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.

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

# Generate cross sample histograms
p4 <- 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(fill="steelblue", color="white", binwidth=0.02) +
    geom_vline(xintercept=0.15, color="firebrick", linetype=3) +
    facet_grid(SAMPLE ~ ., scales="free_y")
plot(p4)
```



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