Upsize your clustering with Clusterize

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Contents

1 Introduction to supersized clustering 1
2 Getting started with Clusterize 2
3 Optimize your inputs to Clusterize 2
4 Visualize the output of Clusterize 6
5 Specialize clustering for your goals 9
6 Resize to fit within less memory 12
7 Clustering both nucleotide strands 13
8 Finalize your use of Clusterize 14

1 Introduction to supersized clustering

You may have found yourself in a familiar predicament for many bioinformaticians: you have a lot of sequences and you need to downsize before you can get going. You may also theorize that this must be an easy problem to solve—given sequences, output clusters. But what can you utilize to solve this problem? This vignette will familiarize you with the Clusterize function in the DECIPHER package. Clusterize will revolutionize all your clustering needs!

Why Clusterize?:

• Scalability - Clusterize will linearize the search space so that many sequences can be clustered in a reasonable amount of time.

• Simplicity - Although you can individualize Clusterize, the defaults are straightforward and should meet most of your needs.

• Accuracy - Clusterize will maximize your ability to extract biologically meaningful results from your sequences.

This vignette will summarize the use of Clusterize to cluster DNA, RNA, or protein sequences.
2 Getting started with Clusterize

To get started we need to load the DECIPHER package, which automatically mobilize a few other required packages.

```r
> library(DECIPHER)
```

There’s no need to memorize the inputs to Clusterize, because its help page can be accessed through:

```r
> ? Clusterize
```

Note that, while it’s easy to fantasize about using Clusterize, if you only have a moderate number of homologous sequences (« 100k) then it’s more accurate to use TreeLine with a distance matrix created from a multiple sequence alignment. This function provides hierarchical clustering (i.e., single-linkage, UPGMA, or complete-linkage) that is impossible to criticize as inexact.

3 Optimize your inputs to Clusterize

Clusterize requires that you first digitize your sequences by loading them into memory. For the purpose of this vignette, we will capitalize on the fact that DECIPHER already includes some built-in sets of sequences.

```r
> # specify the path to your file of sequences:
> fas <- "<<path to training FASTA file>>"
> # OR use the example DNA sequences:
> fas <- system.file("extdata", "50S_ribosomal_protein_L2.fas", package="DECIPHER")
> # read the sequences into memory
> dna <- readDNAStringSet(fas)
> dna
DNAStringSet object of length 317:

<table>
<thead>
<tr>
<th>width</th>
<th>seq</th>
<th>names</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>819</td>
<td>ATGGGTTTTAAAAAATTTTAATC...</td>
</tr>
<tr>
<td>[2]</td>
<td>822</td>
<td>ATGGGAAATAGCAACATCAAGC...</td>
</tr>
<tr>
<td>[3]</td>
<td>822</td>
<td>ATGGGAAATAGCAACATCAAGC...</td>
</tr>
<tr>
<td>[4]</td>
<td>822</td>
<td>ATGGGAAATAGCAACATCAAGC...</td>
</tr>
<tr>
<td>[5]</td>
<td>819</td>
<td>ATGGCTATCGTTAAATGTAAGC...</td>
</tr>
</tbody>
</table>

... ... ...

[313] 819 ATGGCAATTGTTAAATGTAAC... TATCGTACGTCGCCGTACTAA... Pectobacterium at...
[314] 822 ATGGCAATTGTTAAATGTAAC... TATCGTACGTCGCCGTACTAA... Pectobacterium sp....
[315] 864 ATGGGCAATTGTTAAATGTAAC... TATCGTACGTCGCCGTACTAA... Thermosynechococc...
[316] 831 ATGGGCAATTGTTAAATGTAAC... TATCGTACGTCGCCGTACTAA... Bradyrhizobium ja...
[317] 840 ATGGGCAATTGTTAAATGTAAC... TATCGTACGTCGCCGTACTAA... Gloeobacter viola...

The Clusterize algorithm will generalize to nucleotide or protein sequences, so we must choose which we are going to use. Here, we hypothesize that weaker similarities can be detected between proteins and, therefore, decide to use the translated coding (amino acid) sequences. If you wish to cluster at high similarity, you could also strategize that nucleotide sequences would be better because there would be more nucleotide than amino acid differences.

```r
> aa <- translate(dna)
> aa
```
AAStrongSet object of length 317:

<table>
<thead>
<tr>
<th>width</th>
<th>seq</th>
<th>names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 [1]</td>
<td>MALKNFPITPSLRELVQVDKT...STGKKTURKNKRTSKFIVKKKR</td>
<td>Rickettsia prowaz...</td>
</tr>
<tr>
<td>2 [2]</td>
<td>MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIERRKK</td>
<td>Porphyromonas gin...</td>
</tr>
<tr>
<td>3 [3]</td>
<td>MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIERRKK</td>
<td>Porphyromonas gin...</td>
</tr>
<tr>
<td>4 [4]</td>
<td>MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIERRKK</td>
<td>Porphyromonas gin...</td>
</tr>
<tr>
<td>5 [5]</td>
<td>MAIVKCKPTSAGRRHVVKIVNP...TKGGKTRHKRTDKFIIVRRRGK</td>
<td>Pasteurella multo...</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>313]</td>
<td>MAIVKCKPTSAGRRHVVKIVNP...TKGGKTRHKRTDKFIIVRRRGK</td>
<td>Pectobacterium at...</td>
</tr>
<tr>
<td>314]</td>
<td>MPIQKCKPTSAGRRHVVKIVNP...TKGGKTRHKRTDKFIIVRRRGK</td>
<td>Acinetobacter sp....</td>
</tr>
<tr>
<td>315]</td>
<td>MGIRVYRPYTPGVRQKRTVSDF...SDALIVRRKKSSKGRGGGQS</td>
<td>Thermosynechococc...</td>
</tr>
<tr>
<td>316]</td>
<td>MALTFNPTPPQQRQLMVDRS...KTRSKSTNFILLSRHKRK</td>
<td>Bradyrhizobium ja...</td>
</tr>
<tr>
<td>317]</td>
<td>MGIRKYPMTPGTRQSGADFA...KRRKPSKFIIRRRTASGGRG</td>
<td>Gloeobacter viola...</td>
</tr>
</tbody>
</table>

> seqs <- aa # could also cluster the nucleotides
> length(seqs)

[1] 317

Now you can choose how to parameterize the function, with the main arguments being myXStringSet and cutoff. In this case, we will initialize cutoff at seq(0.5, 0, -0.1) to cluster sequences from 50% to 100% similarity by 10%’s. It is important to recognize that cutoffs can be provided in ascending or descending order and, when descending, groups at each cutoff will be nested within the previous cutoff’s groups.

We must also choose whether to customize the calculation of distance. The defaults will penalize gaps as single events, such that each consecutive set of gaps (i.e., insertion or deletion) is considered equivalent to one mismatch. If you want to standardize the definition of distance to be the same as most other clustering programs then set: penalizeGapLetterMatches to TRUE (i.e., every gap position is a mismatch), method to "shortest", minCoverage to 0, and includeTerminalGaps to TRUE. It is possible to rationalize many different measures of distance – see the DistanceMatrix function for more information about alternative distance parameterizations.
Figure 1: The most important parameters (in **bold**) to customize your use of Clusterize.
We can further personalize the inputs as desired. The main function argument to emphasize is *processors*, which controls whether the function is parallelized on multiple computer threads (if *DECIPHER* was built with OpenMP enabled). Setting *processors* to a value greater than 1 will speed up clustering considerably, especially for large *size* clustering problems. Once we are ready, it's time to run *Clusterize* and wait for the output to materialize!

```r
> clusters <- Clusterize(seqs, cutoff=seq(0.5, 0, -0.1), processors=1)
Partitioning sequences by 3-mer similarity:
=====================================================================
Time difference of 0.05 secs
Sorting by relatedness within 35 groups:
iteration 34 of up to 34 (100.0% stability)
Time difference of 0.51 secs
Clustering sequences by 5-mer similarity:
=====================================================================
Time difference of 0.15 secs
Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 3-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%
> class(clusters)
[1] "data.frame"
> colnames(clusters)
[1] "cluster_0_5" "cluster_0_4" "cluster_0_3" "cluster_0_2" "cluster_0_1"
[6] "cluster_0"
> str(clusters)
'data.frame': 317 obs. of 6 variables:
$ cluster_0_5: int 3 1 1 1 3 3 2 2 2 2 ...
$ cluster_0_4: int 1 21 21 21 3 3 3 10 10 10 ...
$ cluster_0_3: int 42 1 1 1 35 35 36 23 23 23 ...
$ cluster_0_2: int 1 67 67 67 12 12 9 34 34 34 ...
$ cluster_0_1: int 86 1 1 1 69 69 73 41 41 41 ...
$ cluster_0 : int 2 102 102 102 25 25 20 59 59 59 ...
> apply(clusters, 2, max) # number of clusters per cutoff
class_0_5  class_0_4  class_0_3  class_0_2  class_0_1  class_0
3  21  42  67  86 102
> apply(clusters, 2, function(x) which.max(table(x))) # max sizes
class_0_5  class_0_4  class_0_3  class_0_2  class_0_1  class_0
3  5  30  22  54  45
```

Notice that *Clusterize* will characterize the clustering based on how many clustered pairs came from relatedness sorting versus rare k-mers, and *Clusterize* will predict the effectiveness of clustering. Depending on the input sequences, the percentage of clusters originating from relatedness sorting will equalize with the number originating from rare k-mers, but more commonly clusters will originate from one source or the other. The clustering effectiveness formalizes the concept of “inexact” clustering by approximating the fraction of possible sequence pairs
that were correctly clustered together. You can incentivize a higher clustering effectiveness by increasing `maxPhase3` at the expense of (proportionally) longer run times.

We can now realize our objective of decreasing the number of sequences. Here, we will prioritize keeping only the longest diverse sequences.

```r
> o <- order(clusters[[2]], width(seqs), decreasing=TRUE) # 40% cutoff
> o <- o[!duplicated(clusters[[2]])]
> aa[o]
AAStringSet object of length 21:
   width  seq      names
[1] 274 MGIRKLKPTTPGQRHKVIAGAFDK...KGLKTRAPKHSSKYYIEERRKK Porphyromonas gini...
[2] 274 MGIRKLKPTTPGQRHKVIAGAFDK...KGLKTRAPKHSSKYYIEERRKK Porphyromonas gini...
[3] 274 MAVRKLKPTTPGQRHIIIGTFEE...KGLKTRAPKQSSKYYIEERRKK Bacteroides theta...
[4] 277 MGKYKPKTSSLRYKTLSSFD...KGYKTRKKKRYSDKFIIKRRNK Borrelia burgdof...
[5] 280 MAIRKYKPTTPGRQSSVSMFEE...PNPYSNNMIVQRRTNKSXKR Corynebacterium d...
       ...
[17] 273 MAIVKCKPTSAGHRHVKVINPE...TKGKTRHNKRTDKYIVRRRGK Haemophilus influ...
[18] 273 MAIVKCKPTSAGHRHVKVINPE...TKGKTRHNKRTDKYIVRRRGK Haemophilus influ...
[19] 273 MAIVKCKPTSAGHRFVVKVQNE...QTGKTRSNKRTDMIVRRRK Pseudomonas aerug...
[20] 277 MALPHTNPITPQQLVIVDRE...KKRTSNNKTDFIFIRNRQQK Brucella suis VBI22
[21] 274 MAIVKCKPTSAGHRHVKVINNA...TKGKTRSNKRTDYIVRRRNK Vibrio cholerae PS15
> dna[o]
DNAStringSet object of length 21:
   width  seq      names
[1] 822 ATGGGAATACGTAAACTCAAGCC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gini...
[2] 822 ATGGGAATACGTAAACTCAAGCC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gini...
[3] 822 ATGGGATACGTAAACTCAAGCC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gini...
[4] 831 ATGGGTATTAAGACTTATAAGCC...TATTATTAAAAGAAGAAATAAA Borrelia burgdof...
[5] 831 ATGGGTATTAAGACTTATAAGCC...TATTATTAAAAGAAGAAATAAA Borrelia burgdof...
       ...
[17] 819 ATGGGATAGTGGTAGTATAAGCC...TATCGTACGTCGGCGCAAG Haemophilus influ...
[18] 819 ATGGGATAGTGGTAGTATAAGCC...TATCGTACGTCGGCGCAAG Haemophilus influ...
[19] 819 ATGGGATAGTGGTAGTATAAGCC...TATCGTACGTCGGCGCAAG Haemophilus influ...
[20] 819 ATGGGATAGTGGTAGTATAAGCC...TATCGTACGTCGGCGCAAG Haemophilus influ...
[21] 822 ATGGGATAGTGGTAGTATAAGCC...TATCGTACGTCGGCGCAAG Haemophilus influ...

4 Visualize the output of Clusterize

We can scrutinize the clusters by selecting them and looking at their multiple sequence alignment:

```r
> t <- table(clusters[[1]]) # select the clusters at a cutoff
> t <- sort(t, decreasing=TRUE)
> head(t)
   3  1  2
218 58 41
> w <- which(clusters[[1]] == names(t[1]))
> AlignSeqs(seqs[w], verbose=FALSE)
AAStringSet object of length 218:
   width  seq      names
       ...
```
It’s possible to utilize the heatmap function to view the clustering results. As can be seen in Figure 2, Clusterize will organize its clusters such that each new cluster is within the previous cluster when cutoff is provided in descending order. We can also see that sequences from the same species tend to cluster together, which is an alternative way to systematize sequences without clustering.
> aligned_seqs <- AlignSeqs(seqs, verbose=FALSE)
> d <- DistanceMatrix(aligned_seqs, verbose=FALSE)
> tree <- TreeLine(myDistMatrix=d, method="UPGMA", verbose=FALSE)
> heatmap(as.matrix(clusters), scale="column", Colv=NA, Rowv=tree)

Figure 2: Visualization of the clustering.
5 Specialize clustering for your goals

The most common use of clustering is to categorize sequences into groups sharing similarity above a threshold and pick one representative sequence per group. These settings epitomize this typical user scenario:

```r
> c1 <- Clusterize(dna, cutoff=0.2, invertCenters=TRUE, processors=1)
Partitioning sequences by 5-mer similarity:
================================================================================
Time difference of 0.11 secs
Sorting by relatedness within 34 groups:
iteration 25 of up to 56 (100.0% stability)
Time difference of 2.34 secs
Clustering sequences by 10-mer similarity:
================================================================================
Time difference of 0.4 secs
Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 5-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%
> w <- which(c1 < 0 & !duplicated(c1))
> dna[w] # select cluster representatives (negative cluster numbers)
DNAStringSet object of length 78:

width seq names
[1] 819 ATGGCTTTAAAAATTTTATATCC...ATTTATTGTAAAAGAAAAAAG Rickettsia prowaz...
[2] 822 ATGGGAATAGTAAACTCAAGCC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gin...
[3] 837 GTGGGTATGAAGGTAGTAAACCC...TGTCGCCGTCCAGCAACAC Lactobacillus pla...
[4] 825 ATGCCATTGATGAAGTTCAAACC...CATCGTCCGCGATCGTAGGGGC Xanthomonas axono...
[5] 828 ATGGGTATTCGTAATTATCGGCC...GATTGTCCGCCGTCACTAAA Synechocystis sp....
... ... ...
[74] 831 ATGGCATTTAAAGCAGCCTTTAATTC...TACGCCGTCATCGCCGCCAGAATAA Bartonella quinta...
[75] 843 ATGTGGAAAAATATCGACCTGTG...CGTGAACGTCGAACGAGAAGAAG Candidatus Protoc...
[76] 822 ATGCCATATCCCATAAAAGCTCAAACC...TATTCGCCGATCTGCCTCAAG Acinetobacter sp....
[77] 864 ATGGGGCATTCGCCGTTACCACCC...GCGGCATCGGCCGTCACTCT Thromosynechococc...
[78] 840 ATGGCATTCCGCAATTATCGACCA...CAAGACGGCTTTCCGGCGAGGT Gloeobacter viola...
```

By default, Clusterize will cluster sequences with linkage to the representative sequence in each group, but it is also possible to tell Clusterize to minimize the number of clusters by establishing linkage to any sequence in the cluster (i.e., single-linkage). This is often how we conceptualize natural groupings and, therefore, may better match alternative classification systems such as taxonomy:

```r
> c2 <- Clusterize(dna, cutoff=0.2, singleLinkage=TRUE, processors=1)
Partitioning sequences by 5-mer similarity:
================================================================================
Time difference of 0.11 secs
Sorting by relatedness within 34 groups:

iteration 22 of up to 56 (100.0% stability)

Time difference of 1.99 secs

Clustering sequences by 10-mer similarity:

Time difference of 0.85 secs

Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 5-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%

> max(abs(c1)) # center-linkage
[1] 78
> max(c2) # single-linkage (fewer clusters, but broader clusters)
[1] 76

It is possible to synthesize a plot showing a cross tabulation of taxonomy and cluster number. We may idealize the clustering as matching taxonomic labels [3], but this is not exactly the case.
> genus <- sapply(strsplit(names(dna), " "), `[`, 1)
> t <- table(genus, c2[[1]])
> heatmap(sqrt(t), scale="none", Rowv=NA, col=hcl.colors(100))

Figure 3: Another visualization of the clustering.
6 Resize to fit within less memory

What should you do if you have more sequences than you can cluster on your midsize computer? If there are far fewer clusters than sequences (e.g., cutoff is high) then it is likely possible to resize the clustering problem. This is accomplished by processing the sequences in batches that miniaturize the memory footprint and are at least as large as the final number of clusters. The number of sequences processed per batch is critical to atomize the problem appropriately while limiting redundant computations. Although not ideal from a speed perspective, the results will not jeopardize accuracy relative to as if there was sufficient memory available to process all sequences in one batch.

```
> batchSize <- 2e2 # normally a large number (e.g., 1e6 or 1e7)
> o <- order(width(seqs), decreasing=TRUE) # process largest to smallest
> c3 <- integer(length(seqs)) # cluster numbers
> repeat {
    m <- which(c3 < 0) # existing cluster representatives
    m <- m[!duplicated(c3[m])] # remove redundant sequences
    if (length(m) >= batchSize)
        stop("batchSize is too small")
    w <- head(c(m, o[c3[o] == 0L]), batchSize)
    if (!any(c3[w] == 0L)) {  # done
        if (any(c3[-w] == 0L))
            stop("batchSize is too small")
        break
    }
    m <- m[match(abs(c3[-w]), abs(c3[m]))]
    c3[w] <- Clusterize(seqs[w], cutoff=0.05, invertCenters=TRUE)[[1]]
    c3[-w] <- ifelse(is.na(c3[m]), 0L, abs(c3[m]))
}
```

Partitioning sequences by 3-mer similarity:
===========================================

Time difference of 0.02 secs

Sorting by relatedness within 4 groups:
iteration 1 of up to 29 (100.0% stability)
Time difference of 0.02 secs

Clustering sequences by 5-mer similarity:
===========================================

Time difference of 0.12 secs

Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 3-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%

Partitioning sequences by 3-mer similarity:
===========================================

Time difference of 0.03 secs
Sorting by relatedness within 97 groups:
Clustering sequences by 5-mer similarity:

Time difference of 0.25 secs

Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 3-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%

> table(abs(c3)) # cluster sizes

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|   | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 3  | 1  | 1  | 1  | 1  | 1  | 7  | 1  | 1  | 1  | 1  | 1  | 1  | 3  | 1  | 1  | 1  | 1  |
|   | 27| 28| 29| 30| 31| 32| 33| 34| 35| 36| 37| 38| 39| 40| 41| 42| 43| 44| 45| 46| 47| 48| 49| 50| 51| 52 |
|   | 3 | 5 | 1 | 3 | 2 | 6 | 3 | 3 | 1 | 2 | 1 | 6 | 1 | 7 | 1 | 1 | 1 | 2 | 8 | 3 | 17| 3 | 2 | 2 | 2 | 1 |
|   | 53| 54| 55| 56| 57| 58| 59| 60| 61| 62| 63| 64| 65| 66| 67| 68| 69| 70| 71| 72| 73| 74| 75| 76| 77| 78 |
|   | 1 | 1 | 1 | 3 | 12| 1 | 75| 4 | 1 | 1 | 11| 3 | 1 | 1 | 1 | 1 | 1 | 5 | 6 | 3 | 3 | 2 | 1 | 1 |
|   | 79| 80| 81| 82| 83| 84| 85| 86| 87| 88| 89| 90| 91|
|   | 1 | 1 | 1 | 17| 13| 1 | 6 | 3 | 1 | 1 | 1 | 1 | 1 |

7 Clustering both nucleotide strands

Sometimes the input sequences are present in different orientations and it is necessary to harmonize the clusterings from both strands. Without trying to hyperbolize how easy this is to do, here’s an example of clustering both strands:

```r
> # simulate half of strands having opposite orientation
> s <- sample(c(TRUE, FALSE), length(dna), replace=TRUE)
> dna[s] <- reverseComplement(dna[s])
> # cluster both strands at the same time
> clus <- Clusterize(c(dna, reverseComplement(dna)), cutoff=0.2, processors=1)
```

Partitioning sequences by 5-mer similarity:

Time difference of 0.24 secs

Sorting by relatedness within 142 groups:

iteration 28 of up to 50 (100.0% stability)

Time difference of 4.57 secs

Clustering sequences by 10-mer similarity:

Time difference of 1.23 secs

Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 5-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%
> clus <- match(clus[[1]], clus[[1]]) # renumber clusters ascending
> # if needed, reorient all clustered sequences to have the same orientation
> strand <- clus[seq_len(length(clus)/2)] >= clus[-seq_len(length(clus)/2)]
> dna[strand] <- reverseComplement(dna[strand])
> # renumber clusters across both strands and compare to original clustering
> clus <- pmin(clus[seq_len(length(clus)/2)], clus[-seq_len(length(clus)/2)])
> org <- match(abs(c1[[1]]), abs(c1[[1]])) # renumber original clustering
> mean(clus == org) # some differences expected due to algorithm stochasticity
[1] 0.9842271
> # verify the largest cluster is now back in the same orientation
> dna[clus == which.max(tabulate(clus))]

DNAStringSet object of length 75:

<table>
<thead>
<tr>
<th>width</th>
<th>seq names</th>
</tr>
</thead>
<tbody>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>...</td>
<td></td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>828</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
<tr>
<td>78</td>
<td>ATGGCGATAAAACTTATAAGCC...CATTTCCAGAAAGAAACACAAA Helicobacter pyl...</td>
</tr>
</tbody>
</table>

8 Finalize your use of Clusterize

Notably, Clusterize is a stochastic algorithm, meaning it will randomize which sequences are selected during pre-sorting. Even though the clusters will typically stabilize with enough iterations, you can set the random number seed (before every run) to guarantee reproducibility of the clusters:

> set.seed(123) # initialize the random number generator
> clusters <- Clusterize(seqs, cutoff=0.1, processors=1)

Partitioning sequences by 3-mer similarity:

Time difference of 0.03 secs

Sorting by relatedness within 35 groups:

iteration 1 of up to 34 (100.0% stability)

Time difference of 0.02 secs

Clustering sequences by 5-mer similarity:

Time difference of 0.22 secs
Clusters via relatedness sorting: 100% (0% exclusively)
Clusters via rare 3-mers: 100% (0% exclusively)
Estimated clustering effectiveness: 100%

> set.seed(NULL) # reset the seed

Now you know how to utilize Clusterize to cluster sequences. To publicize your results for others to reproduce, make sure to provide your random number seed and version number:

- **R version 4.4.0 beta (2024-04-15 r86425), x86_64-pc-linux-gnu**
- **Running under: Ubuntu 22.04.4 LTS**
- **Matrix products: default**
- **BLAS: /home/biocbuild/bbs-3.19-bioc/R/lib/libRblas.so**
- **LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0**
- **Base packages: base, datasets, graphics, grDevices, methods, stats, stats4, utils**
- **Other packages: BiocGenerics 0.50.0, Biostrings 2.72.0, DECIPHER 3.0.0, GenomeInfoDb 1.40.0, IRanges 2.38.0, S4Vectors 0.42.0, XVector 0.44.0**
- **Loaded via a namespace (and not attached): compiler 4.4.0, crayon 1.5.2, DBI 1.2.2, GenomeInfoDbData 1.2.12, htttr 1.4.7, jsonlite 1.8.8, R6 2.5.1, tools 4.4.0, UCSC.utils 1.0.0, zlibbioc 1.50.0**