

Upsize your clustering with Clusterize

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1 Introduction

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

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

```
> library(DECIPHER)
```

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

```
> ? Clusterize
```

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.

```
> # 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:
      width seq                                     names
[1]    819 ATGGCTTTAAAAATTTTAATC...ATTTATTGTAAAAAAGAAAA Rickettsia prowaz...
[2]    822 ATGGGAATACGTAAACTCAAGC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gin...
[3]    822 ATGGGAATACGTAAACTCAAGC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gin...
[4]    822 ATGGGAATACGTAAACTCAAGC...CATCATTGAGAGAAGGAAAAAG Porphyromonas gin...
[5]    819 ATGGCTATCGTTAAATGTAAGC...CATCGTACGTCGTCGTGGTAAA Pasteurella multo...
...    ...
[313] 819 ATGGCAATTGTTAAATGTAAAC...TATCGTACGTCGCCGTAATAA Pectobacterium at...
[314] 822 ATGCCTATTCAAAAATGCAAAC...TATTCGCGATCGTCGCGTCAAG Acinetobacter sp....
[315] 864 ATGGGCATTTCGCGTTTACCGAC...GGGTCGCGGTGGTCGTCAGTCT Thermosynechococc...
[316] 831 ATGGCACTGAAGACATTCAATC...AAGCCGCCACAAGCGGAAGAAG Bradyrhizobium ja...
[317] 840 ATGGGCATTTCGCAAATATCGAC...CAAGACGGCTTCCGGGCGAGGT 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 substitutions.

```
> aa <- translate(dna)
> aa
AAStringSet object of length 317:
      width seq                                     names
[1]    273 MALKNFPITPSLRELQVDKT...STGKKTRKNKRTSKFIVKKRK Rickettsia prowaz...
[2]    274 MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIIERRKK Porphyromonas gin...
[3]    274 MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIIERRKK Porphyromonas gin...
[4]    274 MGIRKLKPTTPGQRHKVIGAFD...KGLKTRAPKKHSSKYIIERRKK Porphyromonas gin...
[5]    273 MAIVKCKPTSAGRRHVVKIVNP...TKGKKTRHNKRTDKFIVRRRGK Pasteurella multo...
...    ...
[313] 273 MAIVKCKPTSPGRRHVVKVNP...TKGKKTRSNKRTDKFIVRRRTK Pectobacterium at...
[314] 274 MPIQKCKPTSPGRRFVEKVVS...KGYKTRTNKRTTKMIIRDREV Acinetobacter sp....
[315] 288 MGIRVYRPYTPGVRQKTVDFA...SDALIVRRRKSSKRGGRGRQS Thermosynechococc...
[316] 277 MALKTFNPTTPGQRQLVMVDRS...KKTRSNKSTNKFILLSRHKRKK Bradyrhizobium ja...
[317] 280 MGIRKYRPMTPGTRQSGADFA...RKRKPSKFIIRRRKTASGRG Gloeobacter viola...
> seqs <- aa # could also cluster the nucleotides
```

Now you can choose how to parameterize the function, with the only required 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.

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*!

```
> clusters <- Clusterize(seqs, cutoff=seq(0.5, 0, -0.1), processors=1)
Ordering sequences by 4-mer similarity:

iteration 2 of up to 51 (100.0% stability)

Time difference of 0.38 secs

Clustering sequences by similarity:
=====

Time difference of 0.28 secs
> 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  4 4 4 4 4 4 4 3 3 3 ...
 $ cluster_0_4: int  1 6 6 6 4 4 4 10 10 10 ...
 $ cluster_0_3: int  52 37 37 37 44 44 41 27 27 27 ...
 $ cluster_0_2: int  1 24 24 24 12 12 16 37 37 37 ...
 $ cluster_0_1: int  87 54 54 54 69 69 64 40 40 40 ...
 $ cluster_0   : int  2 45 45 45 24 24 32 59 59 59 ...
> apply(clusters, 2, max) #number of clusters per cutoff
cluster_0_5 cluster_0_4 cluster_0_3 cluster_0_2 cluster_0_1 cluster_0
           4          26          52          70          87         102
```

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

```
> o <- order(clusters[[2]], width(seqs), decreasing=TRUE) # 40% cutoff
> o <- o[!duplicated(clusters[[2]])]
> aa[o]
AAStringSet object of length 26:
      width seq                                     names
[1]    274 MAVRKLLKPTTPGQRHKIIGTFEE...KGLKTRAPKKQSSKYIIERRKK Bacteroides sp. 1...
[2]    274 MAVRKLLKPTTPGQRHKIIGTFEE...KGLKTRAPKKQSSKYIIERRKK Bacteroides theta...
```

```

[3] 276 MAIRKMKPITNGTRHMSRLVNDE...LGIKTRGRKTSDFIVRRRNEK Fusobacterium nuc...
[4] 280 MAIRKYKPTTPGRRASSVSMFTE...KPKRYSDDMIVRRRRANKNKKR Corynebacterium g...
[5] 277 MGIKTYKPKTSSLRYKTTLSFDD...KGYKTRKKKRYSDKFIIKRRNK Borrelia burgdorf...
...
[22] 277 MAIKKYKPSSNGRRGMTTSDFAE...EFKTRKQKNKSDKFIVRRRKNK Bacillus subtilis...
[23] 276 MGIKKYNPTTNGRRNMTTNDFAE...LGFKTRKKNKASDKFIVRRRKK Bacillus thuringi...
[24] 278 MALKSFNPTTPSQRLVIVSRAG...KRTRSNDKSTDKFIMRSRHQRKK Sinorhizobium mel...
[25] 276 MSVIKCNPTSPGRRHVVKLVNGG...KGKRTSNKRTDKFILCRRKKK Candidatus Blochm...
[26] 274 MAIVKCKPTSAGRRHVVKVNVAD...TKGYKTRSNKRTDKYIVRRRNK Vibrio cholerae 1...
> dna[o]
DNASet object of length 26:
      width seq
[1] 822 ATGGCAGTACGTAAATTAAAGCC...CATTATTGAGAGAAGAAAAAAG Bacteroides sp. 1...
[2] 822 ATGGCAGTACGTAAATTAAAGCC...CATTATTGAGAGAAGAAAAAAG Bacteroides theta...
[3] 828 ATGGCTATTAGAAAAATGAAACC...CGTAAGAAGAAGAAACGAAAAA Fusobacterium nuc...
[4] 840 ATGGCTATTCGTAAGTACAAGCC...TGCTAACAAGAACAAGAAGCGC Corynebacterium g...
[5] 831 ATGGGTATTAAGACTTATAAGCC...TATTATTAAGAAGAATAATAA Borrelia burgdorf...
...
[22] 831 ATGGCGATTAAAAAGTATAAACC...CGTACGTCGTCGTAAAAATAAA Bacillus subtilis...
[23] 828 ATGGGAATCAAAAAGTATAATCC...CATCGTTCGTCGTCGTAAAAA Bacillus thuringi...
[24] 834 ATGGCATTGAAAAGTTTCAATCC...CTCGCGTCACCAGCGCAAGAAG Sinorhizobium mel...
[25] 828 ATGTCTGTTATAAAATGTAATCC...TTTATGTCGTCGTAAAGAAAAA Candidatus Blochm...
[26] 822 ATGGCTATTGTTAAATGTAAGCC...CATCGTACGTCGTCGTAATAAG Vibrio cholerae 1...

```

4 Visualize the output of Clusterize

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

```

> t <- table(clusters[[1]]) # select the clusters at a cutoff
> t <- sort(t, decreasing=TRUE)
> head(t)
 4  3  1  2
153 105 45 14
> w <- which(clusters[[1]] == names(t[1]))
> AlignSeqs(seqs[w], verbose=FALSE)
AAStringSet object of length 153:
      width seq
[1] 287 -MALKNFPNITPSLRELVQVDK...TR-KNKRTSKFIVKKRK----- Rickettsia prowaz...
[2] 287 -MGIRKLKPTTPGQRHKVIGAF...TRAPKKHSSKYIIERRKK---- Porphyromonas gin...
[3] 287 -MGIRKLKPTTPGQRHKVIGAF...TRAPKKHSSKYIIERRKK---- Porphyromonas gin...
[4] 287 -MGIRKLKPTTPGQRHKVIGAF...TRAPKKHSSKYIIERRKK---- Porphyromonas gin...
[5] 287 -MAIVKCKPTSAGRRHVVKIVN...TR-HNKRTDKFIVRRRGK---- Pasteurella multo...
...
[149] 287 -MAFKHFNPTTPGQRQLVIVDR...TR-SNKATDKFIMHTRHQRKK- Bartonella quinta...
[150] 287 -MAFKHFNPTTPGQRQLVIVDR...TR-SNKATDKFIMHTRHQRKK- Bartonella quinta...
[151] 287 -MAIVKCKPTSPGRRHVVKVNV...TR-SNKRTDKFIVRRRTK---- Pectobacterium at...
[152] 287 -MPIQKCKPTSPGRRFVEKVVH...TR-TNKRTTKMIIRDRVK--- Acinetobacter sp....
[153] 287 -MALKTFNPTTPGQRQLVMVDR...TR-SNKSTNKFILLSRHKRKK- Bradyrhizobium ja...

```

```
> heatmap(as.matrix(clusters), scale="column", Colv=NA)
```

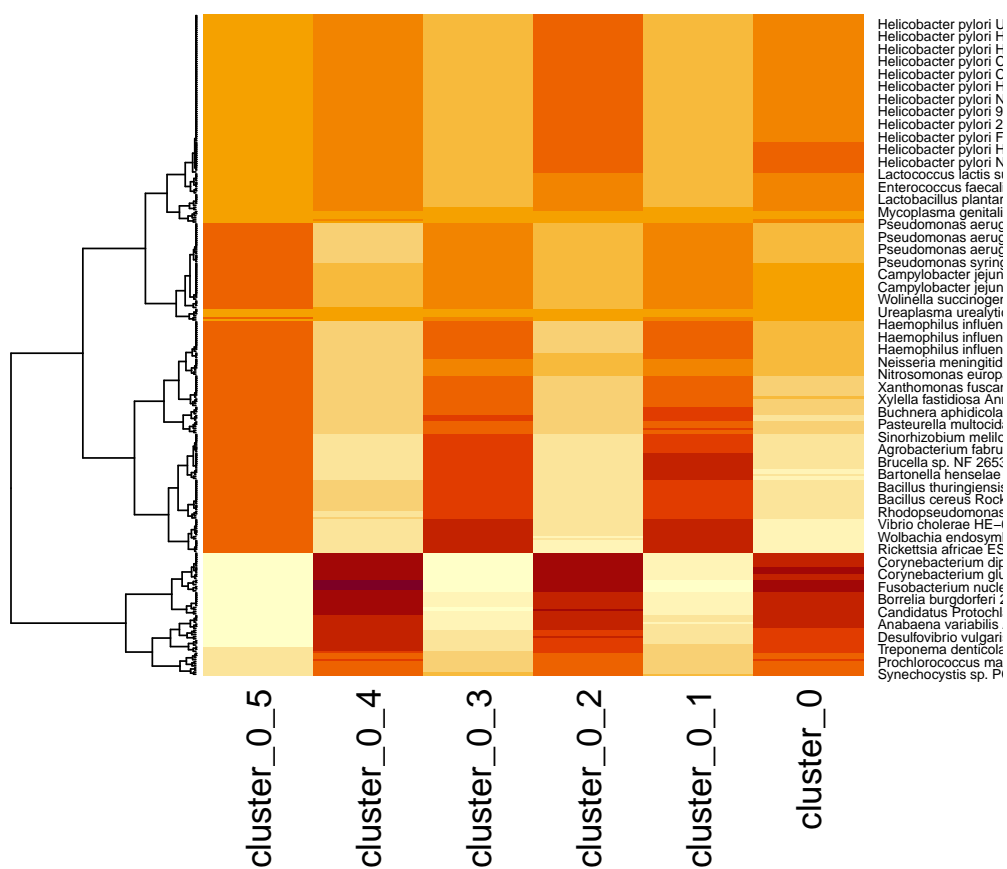


Figure 1: Visualization of the clustering.

It's possible to utilize the `heatmap` function to view the clustering results.

As can be seen in Figure 1, `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 another way to categorize sequences without clustering.

5 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 to guarantee reproducibility of the clusters:

```
> set.seed(123) # initialize the random number generator
> clusters <- Clusterize(seqs, cutoff=seq(0.5, 0, -0.1))
Ordering sequences by 4-mer similarity:
```

```
iteration 2 of up to 51 (100.0% stability)
```

```
Time difference of 0.39 secs
```

```
Clustering sequences by similarity:
```

```
=====
```

```
Time difference of 0.3 secs
```

```
> set.seed(NULL) # reset the seed
```

Now you know how to utilize `Clusterize` to cluster sequences.

6 Session Information

All of the output in this vignette was produced under the following conditions:

- R version 4.2.1 Patched (2022-07-09 r82577), x86_64-apple-darwin17.0
- Running under: macOS Big Sur ... 10.16
- Matrix products: default
- BLAS:
/Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
- LAPACK:
/Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: BiocGenerics 0.44.0, Biostrings 2.66.0, DECIPHER 2.26.0, GenomeInfoDb 1.34.0, IRanges 2.32.0, RSQLite 2.2.18, S4Vectors 0.36.0, XVector 0.38.0
- Loaded via a namespace (and not attached): DBI 1.1.3, GenomeInfoDbData 1.2.9, RCurl 1.98-1.9, Rcpp 1.0.9, bit 4.0.4, bit64 4.0.5, bitops 1.0-7, blob 1.2.3, cachem 1.0.6, cli 3.4.1, compiler 4.2.1, crayon 1.5.2, fastmap 1.1.0, memoise 2.0.1, pkgconfig 2.0.3, rlang 1.0.6, tools 4.2.1, vctrs 0.5.0, zlibbioc 1.44.0