

Finding Chimeric Sequences

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July 23, 2013

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1 Introduction

This document illustrates how to detect chimeric sequences using the DECIPHER package through the use of the `FindChimeras` function. The function finds chimeric sequences present in a database (*dbFile*) of sequences by making use of a reference database (*dbFileReference*) of good sequences. The examples in this document are directed towards finding chimeric 16S rRNA sequences, although a similar strategy could be used with any type of sequence. For 16S sequences the functionality of `FindChimeras` is implemented as a web tool available at <http://DECIPHER.cee.wisc.edu>.

2 Chimera Finding Problems

Chimeras are a common type of sequence anomaly that result from the use of PCR amplification. Chimeras masquerade as normal sequences, but actually are made up of several different sequence parts that do not exist together in reality. Undetected chimeras can have insidious effects, such as causing incorrect assessments of sequence diversity or primer/probe specificity. Chimeras are very difficult to detect, and several detection methods have been proposed to varying degrees of success. The basic problem can be described as the search for one or more regions of a sequence that do not belong with the rest of the sequence.

The `FindChimeras` algorithm works by finding suspect sequence fragments that are uncommon in the group where the sequence belongs, but very common in another group where the sequence does not belong. Each sequence in the *dbFile* is tiled into short sequence segments called fragments. If the fragments are infrequent in their respective group in the *dbFileReference* then they are considered suspect. If enough suspect fragments from a sequence meet the specified constraints then the sequence is flagged as a chimera.

3 Steps to Find Chimeras

3.1 Obtaining A Reference Database

The first step necessary to use `FindChimeras` is to obtain a reference database (*dbFileReference*) of good sequences. In order for the algorithm to work accurately, it is crucial that the reference database is free of any chimeras. The examples given in this document use 16S rRNA sequences, and a suitable reference database can be found at <http://DECIPHER.cee.wisc.edu>. The 16S reference database must be unzipped after download.

If a reference database is not present then it is feasible to create a one if a significantly large repository of sequences is available. By using the input database as the reference database it is possible to remove chimeras from the database. Iteratively repeating the process can eventually result in a clean reference database that is chimera free.

The reference database is required to have several fields of information that are not found in a newly created sequence database:

1. *bases* and *nonbases* as provided by the function `IdLengths`.
2. *origin* as provided by the function `FormGroups`. The origin column contains the taxonomic rank above the level shared by the whole group. For example, the origin of the group *Nanoarchaeum* would be *Root*; *Archaea*; *Nanoarchaeota*. Without the taxonomic rank it would be possible to substitute numbers. For example, the rank 3.1.4.2 with a group named 4 that included 4.1 and 4.2 would have origin 3.1.

3.2 Assigning Sequences To A Reference Group

To get started we need to load the DECIPHER package, which automatically loads several other required packages.

```
> library(DECIPHER)
```

Help for the `FindChimeras` function can be accessed through

```
> ? FindChimeras
```

If this vignette was installed on your system then the code in each example can be obtained by

```
> browseVignettes("DECIPHER")
```

3.2.1 Classifying Sequences

In order to assign new sequences to a group in the reference database, it is necessary to first classify the sequences. For this purpose we can make use of the RDP Multiclassifier available from <http://rdp.cme.msu.edu/classifier/>. After downloading the multiclassifier.jar we need to use it to classify our sequences. Be sure to change the path names to those on your system by replacing all of the text inside quotes labeled “<<path to ...>>” with the actual path on your system.

```

> output.file <- tempfile()
> command <- paste("java -Xmx1g -jar '",
  "<<path to multiclassifier.jar>>",
  "' --hier_outfile=/dev/null --conf=0 --assign_outfile='",
  output.file,
  "' '",
  "<<path to FASTA file>>",
  "'",
  sep="")
> cat("\n\n", command, "\n\n", sep="")

java -jar '/multiclassifier.jar' -hier_outfile=/dev/null -conf=0 -assign_outfile='/tmp/file1'
'/seqs.fas'

> system(command)

```

3.2.2 Determining Reference Groups

Next we need to determine which groups are present in the reference database and the number of sequences present in each group.

```

> dbRef <- dbConnect(SQLite(), '<<path to reference database>>')
> t <- dbGetQuery(dbRef, "select distinct rank, id from DNA group by rank")
> y <- dbGetQuery(dbRef, paste("select distinct id,
  count(*) as count from DNA", "where chimera is null group by id"))
> t$count <- 0
> for (i in 1:dim(t)[1])
  t$count[i] <- y$count[which(y$id==t$id[i])]

```

3.2.3 Assign to Groups

We can now assign each of the classified sequences to a group in the reference database.

```

> r <- readLines(output.file)
> z <- data.frame(id=I(character(length(r))),
  row.names=1:length(r))
> confidence <- .51
> for (i in 1:length(r)) {
  u <- unlist(strsplit(r[i], "\t"))

  confidenceLevel <- 0
  for (k in seq(5, length(u), 3)) {
    if (as.numeric(u[k]) < confidence) {
      confidenceLevel <- k
      break
    }
  }

  if (confidenceLevel == 0) { # full confidence
    r[i] <- paste(u[seq(3, length(u), 3)],
      collapse="; ")
  }
}

```

```

    } else { # unclassified at some level
      r[i] <- paste(u[seq(3, (confidenceLevel - 3), 3)],
                   collapse="; ")
      r[i] <- paste(r[i],
                   "unclassified",
                   sep="; ")
    }

    index <- grep(r[i],
                 t$rank,
                 fixed=TRUE)

    if (length(index) == 0)
      z$id[i] <- "unknown_lineage"
    else
      z$id[i] <- as.character(t$id[index[1]])
  }

```

At this point we should have the group assignment for each sequence.

```

> head(z)
      id
1  Lysinibacillus
2  Staphylococcus
3  Staphylococcus
4  Carnobacteriaceae
5  Peptococcaceae
6  Erysipelotrichaceae

```

3.3 Forming the Sequence Database

3.3.1 Importing Sequences

Next, we need to load the sequences into a sequence database (*dbFile*). This database will be used directly by the `FindChimeras` function. Here we can either set `dbConn` to a file location to write the database, or if there is enough memory simply create the database in memory.

```

> # specify a path for where to write the sequence database
> dbConn <- "<<path to write sequence database>>"
> # OR create the sequence database in memory
> dbConn <- dbConnect(SQLite(), ":memory:")
> Seqs2DB("<<path to FASTA file>>", "FASTA", dbConn, "unknown")

```

Reading FASTA file from line 0 to 99999

```

175 total sequences in database.
Time difference of 0.2 secs

```

```

[1] 175

```

3.3.2 Adding Group Names

At this point we can add the group names that we determined previously to the sequence database we just created.

```
> Add2DB(z, dbConn)
```

3.4 Finding Chimeras

Finally, we can use the `FindChimeras` function to search for chimeras in our sequences. Two sets of parameters are available for 16S sequences. The defaults apply to sequences longer than 1,000 nucleotides, while the second command applies to sequences that are shorter in length.

```
> # full-length sequences
> chimeras <- FindChimeras(dbConn, dbFileReference=dbRef, add2tbl=TRUE,
  maxGroupSize=1e4)
> # OR for short-length sequences
> chimeras <- FindChimeras(dbConn, dbFileReference=dbRef, minLength=40,
  minSuspectFragments=2, add2tbl=TRUE, maxGroupSize=1e4)
```

```
Group of Sequence (Number of Sequences):
|=====| 100%
```

```
Searching Other Groups:
|=====| 100%
```

```
Found 19 possible chimeras.
Added to table DNA: "chimera".
Time difference of 1665.8 secs
```

If any chimeras were found then they are stored in the `chimeras data.frame`. There are several ways to determine which sequences were found to be chimeric.

```
> # outputs the number of chimeras that were found
> cat("Number of chimeras =", dim(chimeras)[1])
Number of chimeras = 19
> # get the ordering of chimeric sequences in the set
> row.names(chimeras)
[1] "147" "89" "87" "90" "88" "132" "139" "97" "5" "120" "123" "13"
[13] "118" "114" "173" "174" "175" "18" "124"
> # allows viewing the chimera results in a web browser
> BrowseDB(dbConn)
[1] TRUE
> # returns the FASTA description of the chimeric sequences
> dbGetQuery(dbConn, "select description from DNA where chimera is not null")
1      uncultured bacterium; Fin_CL-100633_OTU-22.
2      uncultured bacterium; Fin_CL-030731_OTU-12.
3      uncultured bacterium; Fin_CL-050647_OTU-9.
4      uncultured bacterium; Pro_CL-100643_OTU-10.
5      uncultured bacterium; L01_CL-100643_OTU-3.
6      uncultured bacterium; L03_CL-100641_OTU-3.
```

```

7      uncultured bacterium; UWH_CL-010711_OTU-13.
8  uncultured bacterium; Chlplus_CL-120529_OTU-35.
9      uncultured bacterium; UWH_CL-110523_OTU-35.
10     uncultured bacterium; UWH_CL-100636_OTU-11.
11 uncultured bacterium; Chlminus_CL-090519_OTU-14.
12     uncultured bacterium; UWL_CL-080514_OTU-34.
13     uncultured bacterium; UWL_CL-080545_OTU-12.
14     uncultured bacterium; Pro_CL-100633_OTU-7.
15     uncultured bacterium; UWL_CL-110645_OTU-15.
16     uncultured bacterium; UWL_CL-09061_OTU-5.
17     uncultured bacterium; Fin_CL-03079_OTU-21.
18     uncultured bacterium; UWL_CL-110518_OTU-11.
19     uncultured bacterium; UWL_CL-110548_OTU-32.

```

```

> dbDisconnect(dbRef)
[1] TRUE
> dbDisconnect(dbConn)
[1] TRUE
> unlink(output.file)

```

4 Session Information

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

- R version 3.0.1 (2013-05-16), x86_64-apple-darwin10.8.0
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: BiocGenerics 0.7.3, Biostrings 2.29.13, DBI 0.2-7, DECIPHER 1.7.7, IRanges 1.19.18, RSQLite 0.11.4, XVector 0.1.0
- Loaded via a namespace (and not attached): KernSmooth 2.23-10, stats4 3.0.1, tools 3.0.1