

Intro to Bioinformatics - The flu

BIOS P-13532

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Bioinformatics: An Example in Influenza

Let's get started! We'll use influenza sequence data from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). I have created a GitHub repository with the files needed for this assignment. In Rstudio click the terminal tab and type `git clone https://github.com/DiGiacomoAA/Omics_Class.git` then click enter. If successful, you'll see a folder appear in the "Files" panel to the right called "Omics_Class".

Now in the top left corner click the dropdown to make a new file (it looks like a sheet of paper with a green +) and select "R Script". Once you've done that we will set the working directory (the path to the directory that we want to work in) to be the folder we just downloaded by typing

```
setwd("/cloud/project/Omics_Class")
```

Complete the below code block to set up our environment in R and load the data files you've just downloaded into R.

```
# First we will install the BiocManager so that we can
# easily install packages from Bioconductor in our R
# environment. If prompted with 'update all/some/none?',
# enter a If prompted with 'Do you want to install from
# sources the packages which need compilation?' enter n
if (!requireNamespace("BiocManager", quietly = TRUE)) install.packages("BiocManager")
# We will then use BiocManager to install the remaining
# packages needed for this tutorial
BiocManager::install(c("msa", "tidyverse", "Biostrings", "ape",
  "seqinr", "ggtree", "RColorBrewer", "ggmsa"))
# Once they are installed the libraries can be loaded into
# R. In the future, if you have already installed a
# program on your computer as we did above, you can simply
# proceed for the library(package) commands
library(msa)
library(tidyverse)
library(Biostrings)
library(ape)
library(seqinr)
library(ggtree)
library(RColorBrewer)
library(ggmsa)

# Excellent! The Rstudio environment is set up and we're
# ready to go!

# We will now create variables for the names of the
# sequence and metadata in your computer so R can find this
# later.
```

```

fasta_file <- "BVBRG_genome_sequence.fasta"
metadata_file <- "BVBRG_genome.csv"

# Now that we have those variables we can load the data as
# follows:
sequences <- readDNASTringSet(fasta_file)

## Error in .Call2("new_input_filexp", filepath, PACKAGE = "XVector"): cannot open file 'BVBRG_genome_s
# For the metadata information we will assign the GenBank
# Accession number column as the row names (the identifiers
# for the row data) to coordinate with the ids in our
# sequence data
metadata_df <- read_csv(metadata_file) %>%
  column_to_rownames("GenBank_Accessions") %>%
  as.data.frame()

## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
# Let's take a look and see what our data looks like so
# far.

# If we type the name of the variable for our sequences,
# Rstudio will print them to the console below and we can
# see a shortened version of our sequences
sequences

## Error in eval(expr, envir, enclos): object 'sequences' not found
# If we want to see the raw sequence data we can type
view(sequences)

## Error in eval(expr, envir, enclos): object 'sequences' not found
# If we want to investigate the set of our DNA FASTA
# sequences we can tell R to open a new tab with the
# information using
View(sequences)

## Error in eval(expr, envir, enclos): object 'sequences' not found
# Run the same commands for metadata and see what
# information you find

```

Using the separate table that the View(metadata_df) command yields, look at some of the details the metadata contains. What is the host? Where did the strains originate? How many sequences are contained in our sample? Are these all the same type of flu or do they differ?

Once we get a handle on what we're working with, we can proceed to align the flu sequences and start asking some questions.

```

# Assign the metadata to the sequence data so they're
# associated
metadata[sequences] <- metadata_df[match(names(sequences), rownames(metadata_df)),
]

## Error in eval(expr, envir, enclos): object 'metadata_df' not found
# Now run View(sequences) again. What has changed?
View(sequences)

```

```
## Error in eval(expr, envir, enclos): object 'sequences' not found
```

```
# If you'd like to view the metadata for a specific  
# sequence you can type  
# sequences@metadata['ACCESSION_NUMBER',] for example
```

```
sequences@metadata["OK047469", ]
```

```
## Error in eval(expr, envir, enclos): object 'sequences' not found
```

```
# Now that we've combined our data let's make an alignment.  
# Let's see what our options for aligning these sequences  
# are by pulling up the help panel for our aligner.  
`?`(msa)
```

```
# What different options do we have for this alignment  
# algorithm? Do you think we need to specify them all?
```

```
# Given the time constraints, we'll run an alignment using  
# the Muscle algorithm and assign it to a variable called  
# 'alignment'. Below this code block is information about  
# the different alignment algorithms; why msa runs, take  
# some time to read about the differences.
```

```
alignment <- msa(sequences, method = "Muscle")
```

```
## Error in eval(expr, envir, enclos): object 'sequences' not found
```

The MUSCLE and Clustal Omega aligner information below is from Geneious, a commonly used sequence aligner. Information about this and other aligners used by Geneious can be found [here](#). The ClustalW aligner information is from Mega.

MUSCLE

MUSCLE is a progressive aligner that features rapid sequence distance estimation using k-mer counting, progressive alignment using a profile function termed the log-expectation score, and refinement using tree-dependent restricted partitioning of the sequences. The algorithm is described at <http://nar.oxfordjournals.org/content/32/5/1792.full.pdf+html> and a full manual is available at <http://www.drive5.com/muscle/manual/index.html>.

Suitable for medium-large alignments up to 1000 sequences. Not suitable for sequences with low homology N-terminal and C-terminal extensions.

Clustal Omega

Clustal Omega is a fast, accurate aligner suitable for alignments of any size. It uses mBed guide trees and pair HMM-based algorithm which improves sensitivity and alignment quality. A full description of the algorithms used by Clustal Omega is available in the Molecular Systems Biology paper [Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega](#). Latest additions to Clustal Omega are described in [Clustal Omega for making accurate alignments of many protein sciences](#). See also the Clustal Omega website for further details.

Suitable for very large datasets of over 2000 sequences Multi-threaded for faster alignment. Suitable for sequences with long, low homology N-terminal or C-terminal extensions Not suitable for alignment of sequences with large internal indels

ClustalW

ClustalW is a widely used system for aligning any number of homologous nucleotide or protein sequences. For multi-sequence alignments, ClustalW uses progressive alignment methods. In these, the most similar sequences, that is, those with the best alignment score are aligned first. Then progressively more distant groups of sequences are aligned until a global alignment is obtained. This heuristic approach is necessary because finding the global optimal solution is prohibitive in both memory and time requirements. ClustalW performs very well in practice. The algorithm starts by computing a rough distance matrix between each pair of sequences based on pairwise sequence alignment scores. These scores are computed using the pairwise alignment parameters for DNA and protein sequences. Next, the algorithm uses the neighbor-joining method with midpoint rooting to create a guide tree, which is used to generate a global alignment. The guide tree serves as a rough template for clades that tend to share insertion and deletion features. This generally provides a close-to-optimal result, especially when the data set contains sequences with varied degrees of divergence, so the guide tree is less sensitive to noise.

```
# Once the alignment has finished running we can take a
# peak at our data. We can check what type of data that
# variable contains
class(alignment)

## Error in eval(expr, envir, enclos): object 'alignment' not found

is(alignment)

## Error in eval(expr, envir, enclos): object 'alignment' not found

# We can view the direct output from the msa alignment but
# it ay not be very interesting given the amount of data
# and our screen size.
alignment

## Error in eval(expr, envir, enclos): object 'alignment' not found

# If we'd like to view more we can use the print the full
# alignment using print(alignment, show='complete')

# What do you notice when we do this? How do you even being
# to sort this into meaningful information? Let's extract
# the alignment data from our alignment object and save it
# to a fasta file as a backup.

alignment_clean <- unmasked(alignment)

## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
writeXStringSet(alignment_clean, "alignment.fasta", append = FALSE,
               compress = FALSE, compression_level = NA, format = "fasta")

## Error in eval(expr, envir, enclos): object 'alignment_clean' not found

# How does this look now?
alignment_clean

## Error in eval(expr, envir, enclos): object 'alignment_clean' not found

# Let's visualize this a bit more nicely. This is a lot of
# data so this may take a few minutes!

png("alignment.png", width = 2000, height = 6000)
```

```
ggmsa(alignment_clean, 300, 350, color = "Clustal", font = "DroidSansMono",
      char_width = 0.5, seq_name = TRUE)
```

```
## Error in eval(expr, envir, enclos): object 'alignment_clean' not found
```

```
dev.off()
```

```
## pdf
```

```
## 2
```

Relationships of Influenza Strains

Now that we have an alignment of our sequences, we'll build a phylogenetic tree to look at how they're related and which strains likely arose from common ancestors.

```
# To build a tree we need to quantify how different our
# sequences are from each other. To do this. we calculate a
# **distance matrix**. This calculates a distance value for
# each pair of sequences and saves them into a Q matrix.
dist_matrix <- as.DNABin(alignment_clean) %>%
  dist.dna()
```

```
## Error in eval(expr, envir, enclos): object 'alignment_clean' not found
```

```
# Once we've calculated these values, we can build a tree.
# There are many models for how best to build phylogenetic
# trees, but this topic can (and does!) take whole classes
# to cover in depth. For the purpose of this tutorial,
# we'll build a neighbor joining tree. Neighbor Joining is
# one method of tree building which works by iteratively
# joining the most closely related pairs of species or
# sequences, creating a tree where the distances between
# the branches represent the genetic or evolutionary
# differences. This approach allows us to visualize and
# understand the evolutionary connections among different
# organisms or genetic sequences.
```

```
tree <- nj(dist_matrix)
```

```
## Error in eval(expr, envir, enclos): object 'dist_matrix' not found
```

```
# Now that we've built a tree, let's create a png of the
# tree and take a look! We'll plot the tree and also load
# our metadata in so we can color by different information.
```

```
# To use our metadata we need a column called 'taxa' with
# the accession numbers. To do this we'll run
metadata_df <- rownames_to_column(metadata_df, var = "taxa")
```

```
## Error in eval(expr, envir, enclos): object 'metadata_df' not found
```

```
# You can change the 'Isolation_Country' column name in '
# aes(color=Isolation_Country) ' to a different column name
# color by different data traits. To view columnnames enter
colnames(metadata_df)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
```



```

# Check if it is a SNP (more than one unique
# nucleotide)
if (length(nucleotides) > 1) {
  # Store the SNPs in the dataframe
  snp_df$SNPs[i] <- paste(nucleotides, collapse = "/")
}
}

```

Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f

```

# Remove positions without SNPs
snp_df <- snp_df %>%
  filter(!is.na(SNPs))

```

Error in eval(expr, envir, enclos): object 'snp_df' not found

```

# To view all of your SNPs you can print the dataframe by
# typing print(snp_df)

```

```

# It may be easier to visualize this over the consensus
# sequence so we can see where in the overall alignment
# these SNPs are occurring.

```

```

# First we need to extract the consensus sequence from our
# original multisequence alignment

```

```

consensus <- msaConsensusSequence(alignment) %>%
  as.character()

```

Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f

```

# Then we create a dataframe for printing
print_df <- data.frame(Position = snp_df$Position, Consensus = unlist(strsplit(consensus,
"")), SNP_Alternate = snp_df$SNPs, stringsAsFactors = FALSE)

```

Error in eval(expr, envir, enclos): object 'snp_df' not found

```

# And finally we create a loop to print the position in the
# first column, the consensus sequence base in the second
# column and the SNPs in parentheses the 3rd column Print
# the SNP analysis Define the output file path and name
output_file <- "snp_analysis.tsv"

```

```

# Open the output file for writing
file_conn <- file(output_file, "w")

```

```

# Loop through each row of the dataframe and write to the
# output file
for (i in 1:nrow(print_df)) {
  position <- print_df$Position[i]
  consensus_base <- print_df$Consensus[i]
  snp_alternate <- print_df$SNP_Alternate[i]

  # Write the SNP analysis to the file
  cat(position, "\t", consensus_base, "\t", "(", snp_alternate,
      ")", "\n", file = file_conn)
}

```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for f
# Close the output file connection
close(file_conn)

# Go to your working directory. You can now open this file
# in a plain text editor or excel to view.
```