DraftGenomeMineR

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Introduction

Genome sequencing and genomics are rapidly growing disciplines in biology. As our abilities to sequence and assemble larger, more complicated genomes, tools for analyzing genomes must also be developed. This set of R scripts, dubbed **DraftGenomeMineR**, will help users identify genes of interest, annotate scaffolds, and perform analyses, such as phylogenetic reconstructions and promoter element analyses. The motivation behind this suite of scripts was to create a *reproducible* and *easy-to-use* pipeline to facilitate analyses on draft genomes. Please note that working versions of BLAST+, MAFFT, and RAxML must be installed locally to run parts of these scripts.

DraftGenomeMineR can be used to:
- Identify scaffolds that contain genes of interest
- Extract scaffolds
- Identify and annotate ORFs of genes of interest
- Assemble amino acid sequences
- Align sequences and perform phylogenetic analyses
- Summarize promoter element content and assess for differentiation between genes

Module 1: Set up the environment for genome mining

The "RequireLibraries.R" script contains a list of packages that will be installed, if needed, and load the libraries. Then, set the working directory to the "project" directory, in which all work shall be conducted.

```
## Loading required package: ape
```

```
## Loading required package: Biostrings
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'
```

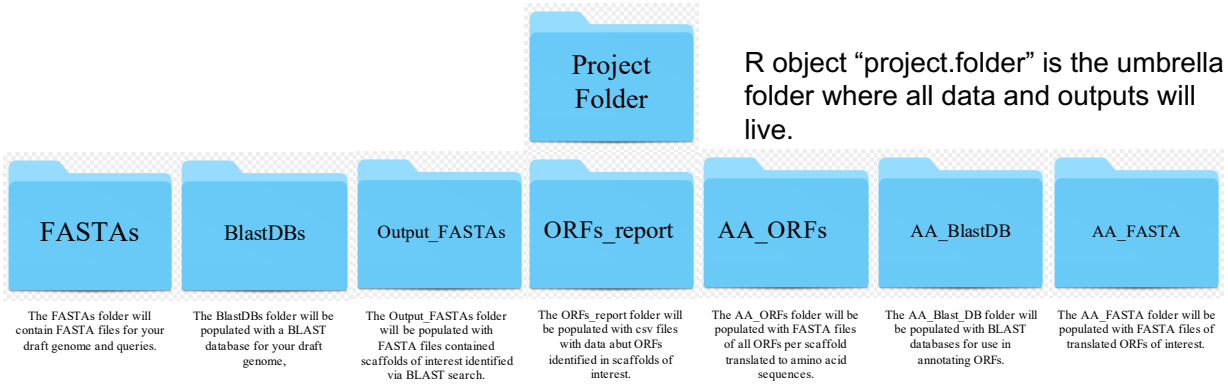
```
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which, which.max, which.min

## Loading required package: S4Vectors

## Loading required package: stats4

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:base':
##
##     expand.grid

## Loading required package: IRanges

## Loading required package: XVector

##
## Attaching package: 'Biostrings'

## The following object is masked from 'package:ape':
##
##     complement

## The following object is masked from 'package:base':
##
##     strsplit

## Loading required package: dplyr

##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:Biostrings':
##
##     collapse, intersect, setdiff, setequal, union

## The following object is masked from 'package:XVector':
##
##     slice

## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union

## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union

## The following objects are masked from 'package:BiocGenerics':
##
##     combine, intersect, setdiff, union

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

## Loading required package: FastaUtils

## Loading required package: ORFik

## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: GenomicAlignments

## Loading required package: SummarizedExperiment

## Loading required package: Biobase

## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

## Loading required package: DelayedArray
```

```
## Loading required package: matrixStats

##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

## The following object is masked from 'package:dplyr':
##
##     count

## Loading required package: BiocParallel

##
## Attaching package: 'DelayedArray'

## The following objects are masked from 'package:matrixStats':
##
##     colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges

## The following objects are masked from 'package:base':
##
##     aperm, apply, rowsum

## Loading required package: Rsamtools

##
## Attaching package: 'GenomicAlignments'

## The following object is masked from 'package:dplyr':
##
##     last

## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg   ggplot2

## Loading required package: readr

## Loading required package: tidyr

##
## Attaching package: 'tidyr'

## The following object is masked from 'package:S4Vectors':
##
##     expand
```

```
## Loading required package: rBLAST

## Loading required package: seqinr

##
## Attaching package: 'seqinr'

## The following object is masked from 'package:matrixStats':
##
##     count

## The following object is masked from 'package:dplyr':
##
##     count

## The following object is masked from 'package:Biostrings':
##
##     translate

## The following objects are masked from 'package:ape':
##
##     as.alignment, consensus

## Loading required package: stringr
```

This is a diagram that shows the required folder nesting for running DraftGenomeMineR. The project folder is the highest folder, with the other fodlers nested within. This is the most basic folder requirement for the scripts - others will be added as more functionality is added into the package. Your local folders should be organized and named the same as in the diagram, as the scripts currently have this arrangement coded into them.



Module 2: Load data and perform a blast search

The following are variables that are listed in a function to perform a blast search. Some are specific files, paths to folders, and specific paramters such as blast type and thresholds. The following is an expanded version of the *DoBlastSearch* function in DraftGenomeMineR so everyone can see what's going on under the hood and better understand the process of identifying the scaffolds of interest.

```
query.file.path <- "FASTAs/Scaffold151535.fa"
genome.file.name <- "Artemesia_tridentata.hipmer.final_assembly.fa"
genome.path <- "FASTAs/Artemesia_tridentata.hipmer.final_assembly.fa"
blast.db.path <- "BlastDBs/Artemesia_tridentata.hipmer.final_assembly.fa"
AA.BlastDB.folder <- "~/Dropbox/Genome_PlayGround/AA_BlastDB/"
AA.ORF.folder <- "~/Dropbox/Genome_PlayGround/AA_ORFs/"
min.e <- 5E-50
perc.ident <- 90.000
query.type <- "AA"
blast.type <- "tblastn"
make.BlastDB <- T
BlastDB.type <- "nucl"
```

Next, read in the draft genome to be mined. *readLines* will read in the fasta file line by line. Be aware of the return characters in your text files, as different operating systems may read these differently.

```
genome <- readLines(con = genome.path)
head(genome) # Print the top 6 lines of the fasta. There should be no spaces in what is printed. Each h
```

```
## [1] ">Scaffold0"
## [2] "CTTGAGTAGGCATTAATACTGCTAAAACCACAACGACATCACGCGTGCACCAAGTATTTGGCGCCGCTACCGTGGATATAAAAGAATTAGAGCAA
## [3] ">Scaffold2560"
## [4] "TGGGTCACTTATCCAGCAACACAATGGGATTGTCAATCCAATAGATCTATTCATAATATTCGCGCTCACCCGGAAGTGATTAATGGTTCATGATA
## [5] ">Scaffold5120"
## [6] "GATTTTACCGCAATTAAGGGTATGATTGTCTAATCATTGGGTGTCAGAGTGTCAGAGAAGCACAAAAGCACACGAATGGGCTACGTGGAAGCATG
```

Do you need to make a new blast database? Set *make.BlastDB* to T for yes, and F for no.

```
if(make.BlastDB == TRUE){
setwd("BlastDBs/")
makeblastdb(file = genome.file.name, dbtype = BlastDB.type)
}
```

Read in the query. Specify whether the query is a DNA (or RNA; these will be read the same) sequence. If not, it will assume that the query is an amino acid sequence.

```
if (query.type == "DNA") {
  query <- readDNAStringSet(filepath = query.file.path,
    format = "fasta")
} else {
  query <- readAAStringSet(filepath = query.file.path,
  format = "fasta")
}
```

Set up the blast search.

```
bl <- blast(db = blast.db.path, type = blast.type)
```

Perform the blast search.

```
cl <- predict(bl, query)
head(cl)
```

```
##                 QueryID       SubjectID Perc.Ident Alignment.Length Mismatches
## 1 Scaffold151535_PIP1-3  Scaffold151535    100.000              122          0
## 2 Scaffold151535_PIP1-3  Scaffold406567     98.214              112          2
## 3 Scaffold151535_PIP1-3  Scaffold317907     97.321              112          3
## 4 Scaffold151535_PIP1-3  Scaffold246845     93.220              118          7
## 5 Scaffold151535_PIP1-3 Scaffold2537666    100.000               96          0
## 6 Scaffold151535_PIP1-3  Scaffold107293     86.087              115         15
##   Gap.Openings Q.start Q.end S.start S.end        E Bits
## 1            0       1   122   12243 12608 1.73e-75  248
## 2            0       1   112    9513  9848 6.73e-68  226
## 3            0       1   112    5158  4823 2.35e-67  225
## 4            1       1   117    1315   962 1.02e-65  220
## 5            0      20   115       3   290 5.00e-64  194
## 6            1       1   114    1123  1467 8.34e-61  204
```

Filter out hits to just have unique scaffolds to extract from draft genome (no need to extract the same scaffold multiple times if it has multiple hits). There are several ways to filter: percent identity, E-value, scaffold ID....

```
cl.filt <- subset(x = cl, SubjectID == "Scaffold151535")
cl.filt.unique <- cl.filt[!duplicated(cl.filt[,c('SubjectID')]),] # SubjectID is the column that contai
cl.filt.unique
```

```
##                 QueryID      SubjectID Perc.Ident Alignment.Length Mismatches
## 1 Scaffold151535_PIP1-3 Scaffold151535        100              122          0
##   Gap.Openings Q.start Q.end S.start S.end        E Bits
## 1            0       1   122   12243 12608 1.73e-75  248
```

```
nrow(cl)
```

```
## [1] 57
```

```
nrow(cl.filt)
```

```
## [1] 1
```

```
nrow(cl.filt.unique)
```

```
## [1] 1
```

```
write.csv(x = cl.filt.unique, file = "Unique_Filtered_Blast_Hit_Info.csv", row.names = F)
```

Module 3: Extract scaffolds of interest identified in blast search

This chunk of code uses the output of the previous chunk, the blast search, to find and extract scaffolds of interest from draft genome and make a fasta file. This is the code for the *GetScaffolds* function.

```
cl.filt.unique <- read.csv(file = "Unique_Filtered_Blast_Hit_Info.csv") # Output of Module 1
header <- NULL # Generate empty objects to store the headers and sequences for the scaffolds of interes
seq <- NULL

for(i in 1:nrow(cl.filt.unique)){

  header[i] <- as.character(cl.filt.unique$SubjectID[i])
  seq[i] <- genome[c(match(paste(">", cl.filt.unique$SubjectID[i], sep=''), genome)+1)]

}
x <- dplyr::tibble(name = header, seq = seq) # This will assemble the headers and sequences into an obj
x
```

```
## # A tibble: 1 x 2
##   name          seq
##   <chr>         <chr>
## 1 Scaffold1515~ ATTGGTATGCAAGTCATCGATTATATTATGCATTGGTATGCAAGTCATCTAATACATTGTGCA~
```

```
writeFasta(data = x, filename = "~/Dropbox/Genome_PlayGround/Output_FASTAs/test.fasta")
```

Module 4: Identify ORFs in the extracted scaffolds

Find ORFs in scaffolds; This is pretty memory intense. It will write out a lot of fasta files - one for each ORF. Larger scafolds may not be able to annotated with this on computers without a lot of free hard drive space. This is the code for the *FindORFs* function.

```
#scaffolds <- readLines("Output_FASTAs/test.fasta")
#scaffoldIDs <- grep(pattern = "^>", x = scaffolds, value = T)
#scaffoldIDs <- gsub(pattern = ">", replacement = "", x = scaffoldIDs)
#tryCatch(
#  {
#    for(j in 1:length(scaffoldIDs)){
#      header <- scaffoldIDs[j]
#      seq <- scaffolds[c(match(paste(">", header, sep=''), scaffolds)+1)]
#
#      findORFsTranslateDNA2AA(scaffold = seq, scaffoldID = header)
#     }
#  })

#for (j in 1:length(scaffoldIDs)) {


#  header <- scaffoldIDs[j]
#  seq <- scaffolds[c(match(paste(">", header, sep=''), scaffolds)+1)]

#    findORFsTranslateDNA2AA(scaffold = seq, scaffoldID = header, MinLen = 40)

#}

FindORFs(OutputFasta = "Output_FASTAs/test.fasta", Minimum.Length = 40)
```

Module 5: Annotate ORFs and write out a fasta containing the amino acid sequence for each scaffold

The next chunk of code will make data base of genes of interest to annotate ORFs. This chunk has not been condensed into function form, yet.

```
setwd(AA.ORF.folder)
orf.files <- list.files()
BlastDB.type <- "prot"
blast.type <- "blastp"
#setwd("AA_BlastDB/")
file.copy(orf.files, AA.BlastDB.folder)
```

```
## [1] FALSE
```

```
### All of this will need to be in a loop to loop over each scaffold, generate a db for each, and annot
#genes.seq <- readLines(con = "Scaffold18599_ORFs.fa")
setwd(AA.BlastDB.folder)
for(i in 1:length(orf.files)){
  makeblastdb(file = orf.files[i], dbtype = BlastDB.type)
}
#

# Annotate ORFs of interest and write them to their own fasta
annotated.genes.file <- "~/Dropbox/Genome_PlayGround/FASTAs/Scaffold151535.fa"
AA.FASTA.out.folder <- "~/Dropbox/Genome_PlayGround/AA_FASTA/"
BlastDB.type <- "prot"
blast.type <- "blastp"

setwd(AA.ORF.folder)
orf.files <- list.files()

for(i in 1:length(orf.files)){
  setwd(AA.BlastDB.folder)
  blast.db.path <- orf.files[i]
  annotated.fasta <- readAAStringSet(filepath = annotated.genes.file)
  bl <- blast(db = blast.db.path, type = blast.type)
  cl <- predict(bl, annotated.fasta) #annotated.fasta[1,]
  blast.csv.filename <- paste0(orf.files[i], "_BlastOut.csv")
  write.csv(x = cl, file = blast.csv.filename, row.names = F)

  cl.filt <- subset(x = cl, Perc.Ident >= perc.ident)
  blast.csv.filename <- paste0(orf.files[i], "_FILTERED_BlastOut.csv")
  write.csv(x = cl.filt, file = blast.csv.filename, row.names = F)
}

setwd(AA.ORF.folder)
genes.seq <- readLines(con = orf.files)
header <- NULL
seq <- NULL

setwd(AA.FASTA.out.folder)
```

```
for(i in 1:nrow(cl)){
  header <- as.character(cl.filt$QueryID[i])
  seq <- genes.seq[c(match(paste(">", cl.filt$QueryID[i], sep=''), genes.seq)+1)]
  filename <- paste0(as.character(cl.filt$SubjectID[i]), ".fasta")
  x <- dplyr::tibble(name = header, seq = seq)
  writeFasta(data = x, filename = filename)
}
```

Module 6: Assemble proteins from AA_ORF files

```
protein.fasta.folder <- "~/Dropbox/Genome_PlayGround/Protein_FASTAs/"
setwd(AA.FASTA.out.folder)

AA.orf.files <- list.files()

AA.orfs <- sapply(AA.orf.files, readLines)

orfs <- AA.orfs[c(grep(">", AA.orfs)+1)]

protein <- paste(orfs, collapse="")
protein <- str_replace_all(protein, "[[:punct:]]", "")

header <- ">PIP1-3"
x <- dplyr::tibble(name = header, seq = protein)
setwd("../Protein_FASTAs/")
writeFasta(data = x, filename = "PIP1-3_Scaffold151535.fa")
```

Module 7: Use the blast results to extract promoter sequences for analyses

This function will need some love. Currently, there is one filtering step and one string modifcation step that are example specific. This is the code for the *GetPromoterSequences* function.

```
orfs.report = "ORFs_report/Scaffold151535_ORFs.csv"
blast.out = "AA_BlastDB/Scaffold151535_ORFs.fa_FILTERED_BlastOut.csv"
scaffold.fasta = "Output_FASTAs/test.fasta"
promoter.csv.file.out = "PROMOTER_OUT_TEST.csv"
promoter.sequence.fasta = "PROMOTER_OUT_TEST.fa"

orf.blast.out <- read.csv(blast.out)
orf.blast.out.filt <- filter(orf.blast.out, orf.blast.out$Perc.Ident == 100.000)
orf.blast.out.filt.keep <- str_remove_all(string = orf.blast.out.filt$SubjectID, pattern = "Scaffold151
#

#
csv.full <- read.csv(file = orfs.report, sep = " ")
csv <- csv.full[csv.full$ORFID == orf.blast.out.filt.keep,]
csvsub <- csv.full %>%
```

```
  filter(csv.full$ORFID %in% orf.blast.out.filt.keep)
csv <- csvsub[which(as.numeric(csvsub$start) >= 1500),]

#

#Create data frame and populate it
Promoters <- data.frame(matrix(ncol=6, nrow=nrow(csv)))
colnames(Promoters) <- c("ORFid", "ScaffoldID","Strand", "Start","End", "Sequence")

Promoters$ORFid <- csv$ORFID
Promoters$End <- csv$start
Promoters$ScaffoldID <- as.vector(csv$scaffoldID)
Promoters$Strand <- as.vector(csv$strand)
Promoters$Start <- as.numeric(Promoters$End) - 1500
Promoters$Start[Promoters$start < 0] <- 1

#Read FASTA file (line by line)
scaffold <- readLines(scaffold.fasta)

#Extract sequences to be mined for promoter sequences using PALACE
setwd("Promoters/")
for(i in 1:nrow(Promoters)){
  #Add DNA sequence adapted to strand
  #Extract seq from FASTA file
  seqRaw <- scaffold[c(grep(paste0(">", Promoters$ScaffoldID[i]), scaffold)+1)]

  #Package sequence and extract start and end
  if(Promoters$Strand[i] == "+"){
    Promoters$Sequence[i] <- paste(strsplit(seqRaw, split='')[[1]][as.numeric(Promoters$Start[i]):as.nu
  }
  if(Promoters$Strand[i] == "-"){
    revComp <- as.vector(reverseComplement(DNAStringSet(seqRaw)))
    Promoters$Sequence[i] <- paste(strsplit(revComp, split='')[[1]][as.numeric(Promoters$Start[i]):as.n
  }
}
#
#

#
#Promoters$Start
#

#
write.csv(Promoters, promoter.csv.file.out, row.names = F, quote = F)
#

#
csv <- Promoters
#csv
seqType <- "DNA"
FASTA <- NULL
for(i in 1:nrow(csv)){
  if(seqType == "DNA"){
```

```
    DNA <- paste(paste(">", csv$ScaffoldID[i], "_", csv$Gene_hypothesis[i], sep=''), csv$Sequence[i], se
    FASTA <- rbind(FASTA, DNA)
  }
}
#

#
#FASTA
write.table(FASTA, file = promoter.sequence.fasta, col.names = F, row.names = F, quote = F)
#

head(read.csv("~/Dropbox/Genome_PlayGround/PROMOTER_OUT_TEST.csv"))
```

```
##    ORFid     ScaffoldID Strand Start   End
## 1 ORF_26 Scaffold151535      + 10743 12243
##
## 1 GATCCGGATGAGCCGGATGTACCATAACTTCTTCTGTTTTTACAACCACCGCCTCCGTCGGTCCGGCCTCACAGGGTTCTCTTCTGACCTCTTTTTTC
```

Module X: Align assembled proteins using MAFFT

This function will perform a MAFFT alignment of your mined sequences. Basic set up for now, will add in more functionality later.

```
DoMafftAlignment(align.method = "--auto",
in.file = "~/Dropbox/BSU_Research/Aquaporin/OutFiles_AEM/FASTA/AQP_AEM_trim.fa",
out.file = "~/Desktop/Test.fa")
```

Module X: Use RAxML to reconstruct a phylogeny

Currently, this is just a basic set up. I will add in more functionality of RAxML later.

```
setwd("~/Desktop/")
DoRAxMLReconstruction(algorithm = "a",
                      in.file = "AQP_AEM_trim.fa",
                      out.file = "raxTest",
                      model = "PROTGAMMAGTR",
                      parsimony.random.seed = 12345,
                      bootstrap.random.seed = 12345,
                      BS.rep.count = 100)
```

One thing that I don't have running in R right now is the New Place promoter element detection. Not sure if there is an API we could use to automate this.... Will need to investigate.

Module X: Classifying RDE's identified by New Place

This section will require that you have the New Place database downloaded and stored locally (LINK TO NEW PLACE DATABSE FILE).

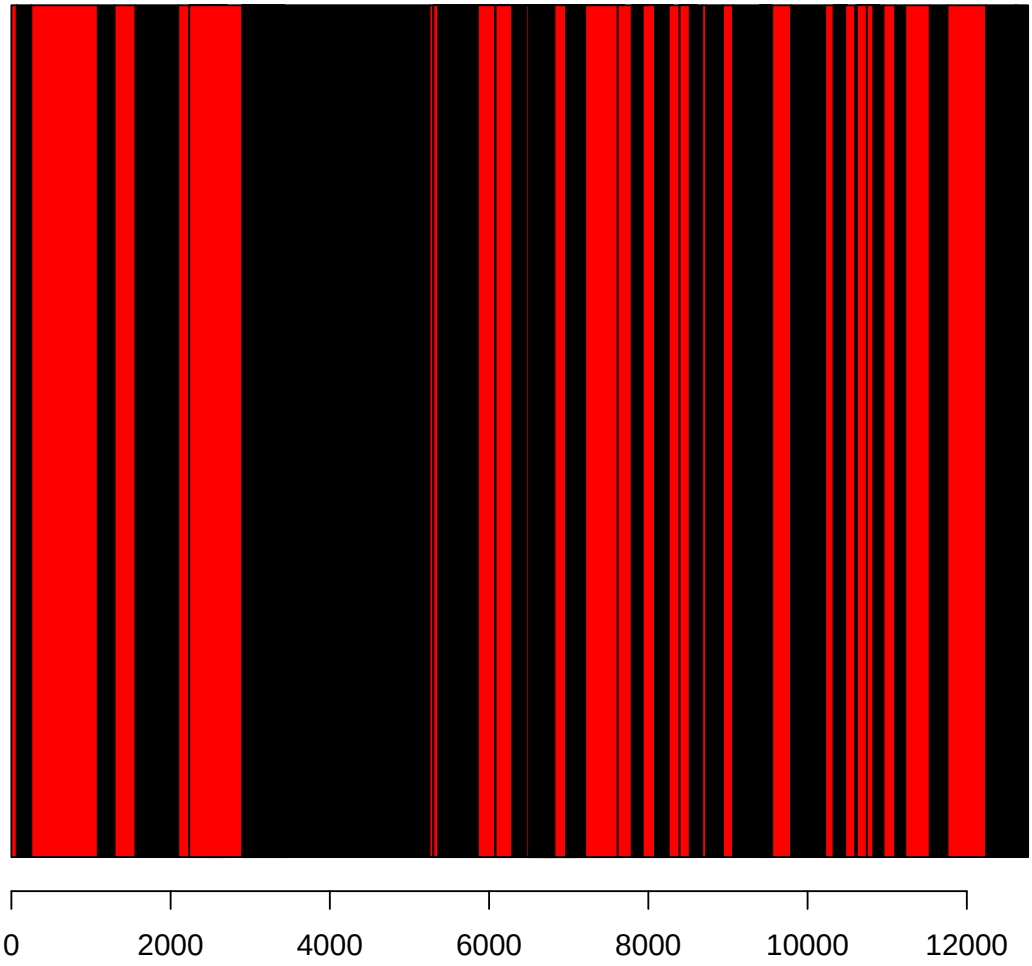
```
ClassifyPromoterElements(New.Place.DB = "~/Dropbox/BSU_Research/Aquaporin/OutFiles_AEM/Promoters/New_PL
                         out.file = "~/Desktop/testPromoEleClass.csv")
```

```
GetPromoterElementsPerScaffold(Promoters.Folder <- "~/Dropbox/BSU_Research/Aquaporin/OutFiles_AEM/Promo
                               Promoters <- "Promoters_sequences_AEM.csv",
                               New.Place.Folder <- "New_PLACE_txt/",
                               Output.Folder <- "~/Desktop/Promoter_CSV_TEST/",
                               Output.Filename <- "~/Desktop/RDE_per_Scaffold_Counts.csv")
```

```
GetORFMap(scaffold.file = "~/Dropbox/Genome_PlayGround/Output_FASTAs/test.fa",
          ORF.data.file = "~/Dropbox/Genome_PlayGround/ORFs_report/Scaffold151535_ORFs.csv",
          user.sep = " ",
          out.file = "~/Desktop/TEST.pdf")
```

```
## pdf
##   2
```

```
img_path <- "~/Desktop/TEST.pdf"
include_graphics(path = img_path, dpi = 300)
```


0
2000
4000
6000
8000
10000
12000