Homework Assignment

Computational Gene Finding

02-22-2023

Task A

Task B

```
# Reverse complement of the sequence
reverse_comp_seq <- reverseComplement(seq_str)</pre>
print(reverse_comp_seq)
## 29648-letter DNAString object
\verb|## seq: ACTACTAAAATTAATTTTACACATTAGGGCTCTTCC...AAGTTCGTTTAGAGAACAGATCTACAAGAGATCGAG
# Print all the potential ORFs in the reverse complement of
# the sequence
orfs_rev_comp <- findORFs(as.character(reverse_comp_seq))</pre>
print(orfs_rev_comp[, c("start", "end", "length")], max = 10000)
##
          start
                  end
                           length
     [1,] "98"
                  "145"
                           "48"
##
     [2,] "132"
                  "149"
                           "18"
##
     [3,] "158"
                  "163"
                           "6"
##
                  "308"
                           "78"
##
     [4,] "231"
                  "462"
##
     [5,] "301"
                           "162"
##
     [6,] "333"
                  "344"
                           "12"
```

```
[7,] "366"
                    "428"
                             "63"
##
                             "39"
##
      [8,] "478"
                    "516"
      [9,] "495"
                    "566"
                             "72"
##
    [10,] "563"
                    "613"
                             "51"
##
                    "594"
                             "18"
##
    [11,] "577"
##
    [12,] "600"
                    "674"
                             "75"
    [13.] "625"
                    "690"
                             "66"
##
    [14,] "665"
                    "694"
                             "30"
##
##
    [15,] "745"
                    "756"
                             "12"
##
    [16,] "802"
                    "825"
                             "24"
##
    [17,] "1003"
                    "1113"
                             "111"
    [18,] "1128"
                    "1199"
                             "72"
##
    [19,] "1388"
                    "1432"
                             "45"
##
                    "1521"
                             "6"
    [20,] "1516"
##
    [21,] "1521"
##
                    "1589"
                             "69"
    [22,] "1610"
                    "1651"
##
                             "42"
##
    [23,] "1632"
                    "1742"
                             "111"
    [24,] "1706"
                    "1720"
                             "15"
    [25,] "1822"
                    "1875"
                             "54"
##
    [26,] "1836"
                    "1898"
                             "63"
##
##
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                    "1918"
                             "57"
##
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                             "60"
    [29,] "2152"
                    "2190"
                             "39"
##
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                             "54"
##
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##
                    "2344"
                             "66"
##
    [32,] "2375"
                    "2380"
                             "18"
##
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                    "2407"
    [34,] "2413"
                    "2436"
                             "24"
##
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                    "2444"
                             "21"
##
    [36,] "2561"
                    "2608"
                             "48"
##
    [37,] "2605"
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                             "177"
##
##
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                             "42"
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                             "153"
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##
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##
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                             "12"
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##
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##
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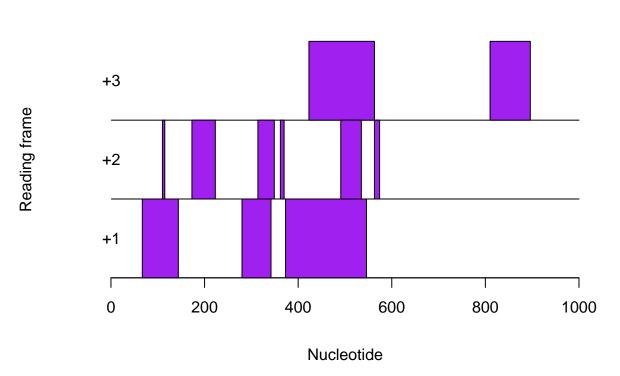
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## [378,] "27996" "28022" "27"
  [379.] "28019" "28126" "108"
## [380,] "28035" "28160" "126"
## [381,] "28245" "28262" "18"
## [382,] "28277" "28345" "69"
## [383.] "28315" "28338" "24"
## [384.] "28354" "28359" "6"
```

```
## [385,] "28394" "28435" "42"
## [386,] "28432" "28470" "39"
## [387,] "28448" "28588" "141"
## [388,] "28501" "28536" "36"
## [389,] "28533" "28571" "39"
## [390,] "28572" "28679" "108"
## [391,] "28715" "28792" "78"
## [392,] "28758" "28763" "6"
## [393,] "28821" "28871" "51"
## [394,] "28928" "28990" "63"
## [395,] "28962" "28997" "36"
## [396,] "29010" "29018" "9"
## [397,] "29021" "29194" "174"
## [398,] "29071" "29211" "141"
## [399,] "29139" "29183" "45"
## [400,] "29211" "29222" "12"
## [401,] "29458" "29544" "87"
```

Task C

```
# Plot potential ORFs in the last 1000 bases
plotORFsinSeq(tail(reverse_comp_seq, 1000))
```

Predicted ORFs



Task D

```
# Extract, translate and print the longest potential gene
max_length <- max(as.numeric(orfs_rev_comp[, "length"]))</pre>
longest_gene <- orfs_rev_comp[as.numeric(orfs_rev_comp[, "length"]) ==</pre>
   max_length, ]
cat("Length of the maximum potential gene:", max_length, "\n")
## Length of the maximum potential gene: 282
cat("There are", dim(longest_gene)[1], "sequences with the maximum length of",
   max_length)
## There are 2 sequences with the maximum length of 282
for (i in 1:dim(longest_gene)[1]) {
    cat("Sequence", i, ": Start =", longest_gene[i, "start"],
        ", End =", longest_gene[i, "end"])
   1_seq <- DNAString(longest_gene[i, "orf.sequence"])</pre>
   print(l_seq)
   protein <- Biostrings::translate(l_seq)</pre>
   cat("\nThe resulting protein sequence:\n")
   print(protein)
   cat("\n")
    cat("--
}
## Sequence 1 : Start = 9852 , End = 10133282-letter DNAString object
## seq: ATGTGCTGGGGCTTCTCTTTTATAGTCCCAGATTAC...TCCTTTATTAACAACATTATAAGCCACATTTTCTAA
##
## The resulting protein sequence:
## 94-letter AAString object
## seq: MCWGFSFIVPDYSISSDINTQIIEYLNLWHWFNVTL...TIYLCVNSVINDGNRCFAGVSIKVSFINNIISHIF*
##
##
##
## Sequence 2 : Start = 10599 , End = 10880282-letter DNAString object
\verb|## seq: ATGTGTAGCATAAGAATAGAAGAGCTCCTCTATTTT...ATCAACGCGCTTAACAAAGCACTCATGGACTGCTAA
## The resulting protein sequence:
## 94-letter AAString object
## seq: MCSIRIEELLYFVSFVTTWLSIVELPFYFSLRHTLD...HVLYFSASRINPQFISYNRVFNRPINALNKALMDC*
## ----
Task E and F
# Identify the significant ORFs using the 95th percentile
```

```
threshold <- quantile(length_vector, 0.95)</pre>
cat("Threshold:", threshold, "\n\n")
## Threshold: 144
significant_orfs <- orfs_rev_comp[as.numeric(orfs_rev_comp[,</pre>
    "length"]) > threshold, ]
cat("Significant ORFs:\n\n")
## Significant ORFs:
for (i in 1:dim(significant orfs)[1]) {
    cat("Sequence", i, ": Start =", significant_orfs[i, "start"],
        ", End =", significant_orfs[i, "end"], ", Length =",
       significant_orfs[i, "length"])
   print(DNAString(significant_orfs[i, "orf.sequence"]))
    cat("\n")
}
## Sequence 1 : Start = 301 , End = 462 , Length = 162162-letter DNAString object
## Sequence 2 : Start = 2605 , End = 2781 , Length = 177177-letter DNAString object
## seq: ATGAAACATCTGTTGTCACTTACTGTACTAGCAAAG...CTCCTAATTTGTAATAAGAAAGCGTTCGTGATGTAG
## Sequence 3 : Start = 2859 , End = 3011 , Length = 153153-letter DNAString object
## seq: ATGATCACAGCACCAATGACAAGTTCACTTTCCATG...GTACGAGCAAACAGCCTGAAGGAAGCAACGAAGTAG
## Sequence 4 : Start = 3473 , End = 3634 , Length = 162162-letter DNAString object
## seq: ATGGCTAGTGTGACTAGCAAGAATACCACGAAAGCA...AAAGGCACGCTAGTAGTCGTCGTCGGCTCATCATAA
##
## Sequence 5 : Start = 4852 , End = 5004 , Length = 153153-letter DNAString object
## seq: ATGATGCCAATAACGACATCACAATTTCCTGAGACA...ACACCTTCACGAGGGAAGTATGCTTTGCCTTCATGA
## Sequence 6 : Start = 5701 , End = 5901 , Length = 201201-letter DNAString object
## seq: ATGAGATCTCTAGCATTAATATCACCTAGGCATTCG...TTCAAAGTTTGGGGGTTTTGTACATTTGTTTGACTTGA
## Sequence 7 : Start = 6213 , End = 6407 , Length = 195195-letter DNAString object
## seq: ATGGTAACTAGCACAAATGCCAGCTCCAATAGGAAT...TGTAGAAACATCAGTGCAGTTAACATCTTGATATAG
##
## Sequence 8 : Start = 7705 , End = 7935 , Length = 231231-letter DNAString object
## seq: ATGTACTCGAAAGTGCAATTAAATGCATTATCGAAT...CGGACAACATTTGATTTCTCTGTGGCAGCAAAATAA
## Sequence 9 : Start = 9852 , End = 10133 , Length = 282282-letter DNAString object
\verb|## seq: ATGTGCTGGGGCTTCTCTTTTATAGTCCCAGATTAC...TCCTTTATTAACAACATTATAAGCCACATTTTCTAA
## Sequence 10 : Start = 10412 , End = 10588 , Length = 177177-letter DNAString object
## seq: ATGCACTTTTATCGAAAGCTGGAGTGTGGAATGCAT...CGTTACAATTCCAAAACAAACAAACACACCATCAGTGA
## Sequence 11 : Start = 10599 , End = 10880 , Length = 282282-letter DNAString object
## seq: ATGTGTAGCATAAGAATAGAAGAGCTCCTCTATTTT...ATCAACGCGCTTTAACAAAGCACTCATGGACTGCTAA
##
## Sequence 12 : Start = 10774 , End = 10959 , Length = 186186-letter DNAString object
## seq: ATGTGTTGTACTTTTCTGCAAGCAGAATTAACCCTC...CCATGTACCTGGCAATGTTGGTCATGGTTACTCTGA
##
```

```
## Sequence 13 : Start = 11433 , End = 11606 , Length = 174174-letter DNAString object
## seq: ATGACAGCCGGCTACGGCAAAGCCAATCCACGCACG...CATGTCCTTTGGTATGCCTGGTATGTCAACACATAA
## Sequence 14 : Start = 12412 , End = 12591 , Length = 180180-letter DNAString object
## seq: ATGTCAGCAGTTGTTTCTGGCAATGCATTTACAGTG...AATGCCTTTTCACATAGGGCATCAACAGCTGCATGA
## Sequence 15 : Start = 12588 , End = 12746 , Length = 159159-letter DNAString object
## seq: ATGAGAGCATGCCGTATACACTATGCGAGCAGATGG...TTGATAATTTGCAACATTGCTAGAAAACTCATCTGA
##
## Sequence 16 : Start = 14601 , End = 14765 , Length = 165165-letter DNAString object
## seq: ATGAAACTGTCTATTTGTCATAGTACTACAGATAGA...CGCGAAAAGTGCATCTTGATCCTCATAACTCATTGA
## Sequence 17 : Start = 15390 , End = 15602 , Length = 213213-letter DNAString object
## seq: ATGAAGGATACACCTATCCAAACAGTTAATACA...TGCCCTAGTCAAAGTGAGGATGGGCATCAGCAATGA
## Sequence 18 : Start = 16491 , End = 16646 , Length = 156156-letter DNAString object
## seq: ATGTGGCATCTACAATACAGACAACATGAAGCACCA...CAGTTGGTTGATTGGTTGTCCTCCACTTGCTAGGTAA
## Sequence 19 : Start = 17280 , End = 17474 , Length = 195195-letter DNAString object
## seq: ATGAGTTTGGCTGCTGTAGTCAATGGTATGATGTTG...GATCTTGCCTGTTTGTACATTTTGGGTCATAGCCTGA
## Sequence 20 : Start = 17712 , End = 17864 , Length = 153153-letter DNAString object
## Sequence 21 : Start = 19084 , End = 19254 , Length = 171171-letter DNAString object
## seq: ATGCCAAAACATTTAATGTTATGGTTGTGTTGTAC...TATAGCAGAAAGACACGCAATCATAATCAATGTTAA
## Sequence 22 : Start = 19463 , End = 19675 , Length = 213213-letter DNAString object
## seq: ATGCCCAATAACACGAAGTTGAACATTGCCAGCCTG...GGTTACTTGTACCATGCACCCTTCAACTTTGCCTGA
## Sequence 23 : Start = 19557 , End = 19745 , Length = 189189-letter DNAString object
\verb|## seq: ATGTCTTCTGCTGTGCAAATGACATGTCTTGGACAG...ACAGCAGAAGTGATTGATGTCTGTGGTGGTAG
## Sequence 24 : Start = 20808 , End = 21056 , Length = 249249-letter DNAString object
## seq: ATGTTGCCAACAGCACTAAAAACACGAGGTAGAAAA...TCAGTAGAAATGATGTCACGAGTGACACCATCCTGA
## Sequence 25 : Start = 22295 , End = 22462 , Length = 168168-letter DNAString object
## seq: ATGAACATAGCTCTTCCATATGTAGTAGAAAGAAGC...AGCAAAATAGCCAAAGAACACCTGCATTATAGCTGA
## Sequence 26 : Start = 22501 , End = 22659 , Length = 159159-letter DNAString object
## seq: ATGCCAAAACCCACTCAGCGGCCAGACCTAAAATTG...TGCTGCAAGGAAAAGAACCTTCACAGAAATCCATAG
## Sequence 27 : Start = 24290 , End = 24451 , Length = 162162-letter DNAString object
\verb|## seq: ATGGGTCATAGTTTCTCTGACATCACCAAGCTCGCC...TGCATTGAATTTGACTTCAAGCTGTTGAAGTGCTAA|
## Sequence 28 : Start = 24653 , End = 24814 , Length = 162162-letter DNAString object
## seq: ATGATTTACATGAGGTTTAATTTTTGTAACATCAGC...GAACACTTTTATAGTCTTAACCTCCCGCAGGGATAA
##
## Sequence 29 : Start = 26031 , End = 26222 , Length = 192192-letter DNAString object
## seq: ATGCCTGCTGACAACAATGGTGCAAGTAAGATGTCC...CCTCCTACTGTAAGAGGGCCATTTAGCTTAATGTAA
## Sequence 30 : Start = 26241 , End = 26399 , Length = 159159-letter DNAString object
## seq: ATGGCACCATTGGTTGCCTTGTTGAGTGCACCTGCT...CATTTAATGGCAACATTGTCAGTAAGTTTTAAATAA
##
```

```
## Sequence 31 : Start = 27057 , End = 27269 , Length = 213213-letter DNAString object
## seq: ATGAGGCCATTTACACAGACTGGTGTGCCAACGATA...AGTAGTTGCAGCTGCTCCTTGCCACGTATACACTGA
##
## Sequence 32 : Start = 27447 , End = 27593 , Length = 147147-letter DNAString object
## seq: ATGTCAAAAACACCTGTAATGAGAAATTTGAGAATC...TCAACAGTAGTGCCCAAAAGATTAGACAACCACTGA
##
## Sequence 33 : Start = 27672 , End = 27899 , Length = 228228-letter DNAString object
## seq: ATGGCGTCGACAAGACGTAATGACTGTTCAGAAATA...GCACCTTTTACGGGCTTTCCCTTGGTAACTTTATAG
##
## Sequence 34 : Start = 29021 , End = 29194 , Length = 174174-letter DNAString object
## seq: ATGACCACCGGCTCCCTTATTACCGTTCTTACGAAG...CAGCTCAACGACCTTGTGGCCGTGATTGGTGCTTAA
```

Not all of the ORFs found in a DNA sequence correspond to real genes. Some of them occur by chance. To extract the actual genes, lab experimentation is necessary along with bioinformatics. As computer scientists, we can make predictions to extract genes from a sequence. The length of the ORF can be used as a measure, as long ORFs cannot happen by chance. Small ORFs have a high probability of occurring by chance, so we can eliminate those using a threshold. We could have used the 100th percentile (or the largest value) of the ORF length as the threshold, but by reducing the threshold slightly (5% in this case), we improve our chances of finding the actual genes in the sequence.

Methods used in this assignment

```
findPotentialStartsAndStops <- function(sequence)</pre>
  # Define a vector with the sequences of potential start and stop codons
  codons <- c("atg", "taa", "tag", "tga")</pre>
  # Find the number of occurrences of each type of potential start or stop codon
  for (i in 1:4)
    codon <- codons[i]</pre>
    # Find all occurrences of codon "codon" in sequence "sequence"
    occurrences <- matchPattern(codon, sequence)
    # Find the start positions of all occurrences of "codon" in sequence "sequence"
    codonpositions <- start(occurrences)</pre>
    # Find the total number of potential start and stop codons in sequence "sequence"
    numoccurrences <- length(codonpositions)</pre>
    if (i == 1)
      # Make a copy of vector "codonpositions" called "positions"
      positions <- codonpositions
      # Make a vector "types" containing "numoccurrences" copies of "codon"
      types <- rep(codon, numoccurrences)</pre>
    }
    else
      # Add the vector "codonpositions" to the end of vector "positions":
      positions <- append(positions, codonpositions, after=length(positions))</pre>
      # Add the vector "rep(codon, numoccurrences)" to the end of vector "types":
      types <- append(types, rep(codon, numoccurrences), after=length(types))</pre>
    }
  }
  # print(positions)
  # Sort the vectors "positions" and "types" in order of position along the input sequence:
  indices <- order((positions))</pre>
```

```
positions <- positions[indices]</pre>
  types <- types[indices]</pre>
  # Return a list variable including vectors "positions" and "types":
  mylist <- list(positions, types)</pre>
  return(mylist)
findORFsinSeq <- function(sequence)</pre>
  require(Biostrings)
  # Make vectors "positions" and "types" containing information on the positions of ATGs in the sequenc
  mylist <- findPotentialStartsAndStops(sequence)</pre>
  positions <- mylist[[1]]</pre>
  types <- mylist[[2]]</pre>
  # Make vectors "orfstarts" and "orfstops" to store the predicted start and stop codons of ORFs
  orfstarts <- numeric()</pre>
  orfstops <- numeric()</pre>
  # Make a vector "orflengths" to store the lengths of the ORFs
  orflengths <- numeric()</pre>
  # Print out the positions of ORFs in the sequence:
  # Find the length of vector "positions"
  numpositions <- length(positions)</pre>
  # There must be at least one start codon and one stop codon to have an ORF.
  if (numpositions >= 2)
    for (i in 1:(numpositions-1))
      posi <- positions[i]</pre>
      typei <- types[i]</pre>
      found <- 0
      while (found == 0)
        for (j in (i+1):numpositions)
          posj <- positions[j]</pre>
          typej <- types[j]</pre>
          posdiff <- posj - posi
          posdiffmod3 <- posdiff %% 3</pre>
          # Add in the length of the stop codon
          orflength <- posj - posi + 3
          if (typei == "atg" && (typej == "taa" || typej == "tag" || typej == "tga") && posdiffmod3 ==
             # Check if we have already used the stop codon at posj+2 in an ORF
            numorfs <- length(orfstops)</pre>
             usedstop <- -1
             if (numorfs > 0)
               for (k in 1:numorfs)
                 orfstopk <- orfstops[k]</pre>
                 if (orfstopk == (posj + 2)) { usedstop <- 1 }</pre>
```

```
if (usedstop == -1)
               orfstarts <- append(orfstarts, posi, after=length(orfstarts))</pre>
               orfstops <- append(orfstops, posj+2, after=length(orfstops)) # Including the stop codon.
               orflengths <- append(orflengths, orflength, after=length(orflengths))</pre>
            found <- 1
            break
          if (j == numpositions) { found <- 1 }</pre>
      }
    }
  }
  # Sort the final ORFs by start position:
  indices <- order(orfstarts)</pre>
  orfstarts <- orfstarts[indices]</pre>
  orfstops <- orfstops[indices]</pre>
  # Find the lengths of the ORFs that we have
  orflengths <- numeric()
  numorfs <- length(orfstarts)</pre>
  for (i in 1:numorfs)
    orfstart <- orfstarts[i]</pre>
    orfstop <- orfstops[i]</pre>
    orflength <- orfstop - orfstart + 1
    orflengths <- append(orflengths,orflength,after=length(orflengths))
  mylist <- list(orfstarts, orfstops, orflengths)</pre>
  return(mylist)
plotORFsinSeq <- function(sequence)</pre>
  # Make vectors "positions" and "types" containing information on the positions of ATGs in the sequence
  mylist <- findPotentialStartsAndStops(sequence)</pre>
  positions <- mylist[[1]]</pre>
  types <- mylist[[2]]</pre>
  # Make vectors "orfstarts" and "orfstops" to store the predicted start and stop codons of ORFs
  orfstarts <- numeric()</pre>
  orfstops <- numeric()</pre>
  # Make a vector "orflengths" to store the lengths of the ORFs
  orflengths <- numeric()
  # Print out the positions of ORFs in the sequence:
  numpositions <- length(positions) # Find the length of vector "positions"
  # There must be at least one start codon and one stop codon to have an ORF.
  if (numpositions >= 2)
    for (i in 1:(numpositions-1))
      posi <- positions[i]</pre>
```

```
typei <- types[i]</pre>
          found <- 0
          while (found == 0)
              for (j in (i+1):numpositions)
                   posj <- positions[j]</pre>
                   typei <- types[i]</pre>
                   posdiff <- posj - posi
                   posdiffmod3 <- posdiff %% 3</pre>
                    orflength <- posj - posi + 3 # Add in the length of the stop codon
                   if (typei == "atg" && (typej == "taa" || typej == "tag" || typej == "tga") && posdiffmod3 ==
                         # Check if we have already used the stop codon at posj+2 in an ORF
                        numorfs <- length(orfstops)</pre>
                        usedstop <- -1
                         if (numorfs > 0)
                             for (k in 1:numorfs)
                                  orfstopk <- orfstops[k]</pre>
                                   if (orfstopk == (posj + 2)) { usedstop <- 1 }</pre>
                         if (usedstop == -1)
                             orfstarts <- append(orfstarts, posi, after=length(orfstarts))</pre>
                             orfstops <- append(orfstops, posj+2, after=length(orfstops)) # Including the stop codon.
                             orflengths <- append(orflengths, orflength, after=length(orflengths))</pre>
                        found <- 1
                        break
                    if (j == numpositions) { found <- 1 }</pre>
    }
}
# Sort the final ORFs by start position:
indices <- order(orfstarts)</pre>
orfstarts <- orfstarts[indices]</pre>
orfstops <- orfstops[indices]</pre>
# Make a plot showing the positions of ORFs in the input sequence:
# Draw a line at y=0 from 1 to the length of the sequence:
x <- c(1,nchar(sequence))</pre>
y < -c(0,0)
plot(x, y, ylim=c(0,3), type="l", axes=FALSE, xlab="Nucleotide", ylab="Reading frame", main="Predicted to the state of the
segments(1,1,nchar(sequence),1)
segments(1,2,nchar(sequence),2)
# Add the x-axis at y=0:
axis(1, pos=0)
# Add the y-axis labels:
text(0.9,0.5,"+1")
```

```
text(0.9, 1.5, "+2")
  text(0.9, 2.5, "+3")
  # Make a plot of the ORFs in the sequence:
  numorfs <- length(orfstarts)</pre>
  for (i in 1:numorfs)
    orfstart <- orfstarts[i]</pre>
    orfstop <- orfstops[i]</pre>
    remainder <- (orfstart-1) %% 3</pre>
    if (remainder == 0) # +1 reading frame
      rect(orfstart,0,orfstop,1,col="purple",border="black")
    }
    else if (remainder == 1)
      rect(orfstart,1,orfstop,2,col="purple",border="black")
    else if (remainder == 2)
      rect(orfstart,2,orfstop,3,col="purple",border="black")
  }
}
generateSeqsWithMultinomialModel <- function(inputsequence, X)</pre>
  # Change the input sequence into a vector of letters
  require("seqinr") # This function requires the SeqinR package.
  inputsequencevector <- s2c(inputsequence)</pre>
  # Find the frequencies of the letters in the input sequence "inputsequencevector":
  mylength <- length(inputsequencevector)</pre>
  mytable <- table(inputsequencevector)</pre>
  # Find the names of the letters in the sequence
  letters <- rownames(mytable)</pre>
  numletters <- length(letters)</pre>
  probabilities <- numeric() # Make a vector to store the probabilities of letters
  for (i in 1:numletters)
    letter <- letters[i]</pre>
    count <- mytable[[i]]</pre>
    probabilities[i] <- count/mylength</pre>
  # Make X random sequences using the multinomial model with probabilities "probabilities"
  seqs <- numeric(X)</pre>
  for (j in 1:X)
    seq <- sample(letters, mylength, rep=TRUE, prob=probabilities) # Sample with replacement</pre>
    seq \leftarrow c2s(seq)
    seqs[j] <- seq</pre>
  # Return the vector of random sequences
  return (as.list(seqs))
```

```
get_max_seq_vector <- function (x) {
  return (as.numeric(x[,"length"]))
}</pre>
```