732A51 Bioinformatics

Lab 1

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Contents

```r
library(seqinr)
library(stringr)
```

1 Hardy–Weinberg equilibrium

We consider a gene locus with two possible alleles (say A and a) and a diploid population with N individuals. Hence, there are 2N alleles in the population. Let p be the proportion of As in the allele population and q the population of as (of course p + q = 1). A population is said to be in Hardy–Weinberg equilibrium if the proportion of AA homozygotes is p 2, aa homozygotes is q2 and the proportion of heterozygotes (Aa) is 2pq.

1.1 Question 1.1

Show that with random mating (i.e. both alleles of the offspring are just randomly, with proportions p and q, drawn from the parental allele population) Hardy–Weinberg equilibrium is attained in the first generation. What is the proportion of A and a alleles in the offspring population? Hence, with random mating, can a population in Hardy–Weinberg equilibrium ever deviate from it?

$$P(AA) = p^2$$

$$P(Aa) = 2pq$$

$$P(aa) = q^2$$

$$p_{offspring} = p$$

$$q_{offspring} = q$$

When its random mating the proportions will always stay the same for the next generation because the allele frequencies are preserved.

1.2 Question 1.2

We look at the MN blood group (https://en.wikipedia.org/wiki/MNS_antigen_system), it has two possible co–dominating (both contribute to heterozygotes) alleles L M (denoted M) and L N (denoted N). In a population of 1000 Americans of Caucasian descent the following genotype counts were observed, 357 individuals were MM, 485 were MN and 158 were NN. Use a chi– square goodness of fit test to test if the population is in Hardy–Weinberg equilibrium.

chi– square goodness of fit test:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

$$df = 3 - 1$$

$$p - value = P(\chi^2 > Observed\chi^2)$$

```r
# data
MM <- 357
NM <- 485
NN <- 158

# all M's

p <- (2*MM+NM)/(2*1000)
q <- 1-p

# expected values
exp_mm <-p*p*1000
exp_nm <-2*p*q*1000
exp_nn <- q*q*1000

# built in function
# chisq.test(c(MM,NM,NN), p=c(p*p,2*p*q,q*q))
act <- c(MM,NM,NN)
expected <- c(exp_mm,exp_nm,exp_nn)

# Calculate Chi-Square statistic manually
chi_sq_statistic <- sum((act-expected)^2 / expected)

df <- 3-1
p_value <- 1 - pchisq(chi_sq_statistic, df)

p_value
```

```
## [1] 0.951259
```

The p-value is very high, which indicates that there is no significant difference between the groups and we cant reject that the population is in Hardy–Weinberg equilibrium.

2 Question 2: Exploring a genomic sequence

In this exercise, you will use GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the default "Nucleotide" database. We will be interested in the sequence with accession number in GenBank: MK465080. You will find the relevant information in the FEATURES section of the returned record and access the nucleotides of the sequence under CDS (protein coding sequence, from CoDing Sequence). Remember that the coding strand (https://en.wikipedia.org/wiki/Coding_strand) is the strand of the gene that is identical to the transcript (see the lecture slides for the genetic code—translation of DNA triples to amino acids). The complimentary to it strand is called the template strand.

2.1 Question 2.1

From what species does the sequence come from? Name the protein product of the CDS.

The protein product of the CDS is "cytochrome c oxidase subunit I"(COI) and the species is branchipus schaefferi, which seem to be a kind of crustacean.

2.2 Question 2.2

```r
# importing fasta file

branchipus <- read.fasta('branchipus_schaefferi.fasta')

sequence <- branchipus$MK465080.1
print(branchipus)
```

```
## $MK465080.1
##   [1] "t" "c" "t" "c" "c" "t" "a" "g" "g" "a" "g" "a" "t" "g" "a" "c" "c" "a"
##  [19] "a" "c" "t" "t" "t" "a" "t" "a" "a" "c" "g" "t" "c" "a" "t" "t" "g" "t"
##  [37] "t" "a" "c" "t" "g" "c" "t" "c" "a" "c" "g" "c" "t" "t" "t" "t" "g" "t"
##  [55] "a" "a" "t" "g" "a" "t" "t" "t" "t" "c" "t" "t" "c" "a" "t" "a" "g" "t"
##  [73] "t" "a" "t" "a" "c" "c" "a" "a" "t" "c" "c" "t" "t" "a" "t" "t" "g" "g"
##  [91] "a" "g" "g" "a" "t" "t" "t" "g" "g" "a" "a" "a" "t" "t" "g" "a" "t" "t"
## [109] "a" "g" "t" "c" "c" "c" "t" "t" "t" "a" "a" "t" "a" "c" "t" "a" "g" "g"
## [127] "g" "g" "c" "t" "c" "c" "t" "g" "a" "t" "a" "t" "g" "g" "c" "t" "t" "t"
## [145] "c" "c" "c" "c" "c" "g" "a" "c" "t" "a" "a" "a" "t" "a" "a" "c" "t" "t"
## [163] "a" "a" "g" "c" "t" "t" "t" "t" "g" "a" "a" "t" "a" "c" "t" "t" "c" "c"
## [181] "t" "c" "c" "c" "t" "c" "a" "t" "t" "a" "a" "c" "t" "c" "t" "t" "c" "t"
## [199] "a" "g" "t" "g" "g" "c" "c" "a" "g" "c" "t" "c" "a" "a" "t" "g" "g" "t"
## [217] "a" "g" "a" "a" "a" "g" "a" "g" "g" "g" "g" "t" "a" "g" "g" "a" "a" "c"
## [235] "a" "g" "g" "a" "t" "g" "a" "a" "c" "a" "g" "t" "t" "t" "a" "t" "c" "c"
## [253] "a" "c" "c" "c" "c" "t" "a" "t" "c" "t" "g" "c" "t" "g" "c" "t" "a" "t"
## [271] "t" "g" "c" "c" "c" "a" "t" "g" "c" "t" "g" "g" "t" "c" "c" "t" "t" "c"
## [289] "t" "g" "t" "t" "g" "a" "t" "t" "t" "a" "g" "c" "a" "a" "t" "c" "t" "t"
## [307] "t" "t" "c" "a" "c" "t" "t" "c" "a" "c" "c" "t" "c" "g" "c" "a" "g" "g"
## [325] "g" "a" "t" "c" "t" "c" "t" "t" "c" "a" "a" "t" "t" "t" "t" "a" "g" "g"
## [343] "a" "g" "c" "t" "g" "t" "a" "a" "a" "t" "t" "t" "c" "a" "t" "t" "a" "c"
## [361] "a" "a" "c" "t" "g" "t" "a" "a" "t" "t" "a" "a" "t" "a" "t" "a" "c" "g"
## [379] "g" "c" "c" "t" "c" "a" "t" "t" "c" "c" "a" "t" "a" "a" "g" "a" "t" "t"
## [397] "a" "g" "a" "c" "c" "g" "a" "a" "t" "a" "c" "c" "t" "t" "t" "a" "t" "t"
## [415] "t" "g" "c" "a" "t" "g" "a" "g" "c" "g" "g" "t" "a" "g" "t" "t" "a" "t"
## [433] "c" "a" "c" "a" "g" "c" "a" "g" "t" "t" "c" "t" "t" "c" "t" "t" "c" "t"
## [451] "c" "c" "t" "t" "t" "c" "t" "c" "t" "c" "c" "c" "a" "g" "t" "a" "t" "t"
## [469] "a" "g" "c" "a" "g"
## attr(,"name")
## [1] "MK465080.1"
## attr(,"Annot")
## [1] ">MK465080.1 Branchipus schaefferi isolate ST2-2 cytochrome c oxidase subunit I (COI) gene, parti
## attr(,"class")
## [1] "SeqFastadna"
```

Save (and submit) the nucleotide sequence of the coding strand that corresponds to these amino acids as a FASTA format file.. Use transeq (https://www.ebi.ac.uk/Tools/st/emboss_transeq/) to translate the nucleotides to a protein. Do you obtain the same protein sequence? Check what is the ORF and codon table (these are provided by GenBank in the FEATURES section). Use backtranseq (https://www.ebi.ac.uk/Tools/st/emboss_backtranseq/) to obtain the sequence from the protein sequence.

Using frame 2 and codon table 5 'Invertebrate Mitochondrial'.

```r
branchipus_2 <- read.fasta('emboss_transeq-I20241115-093926-0606-64392434-p1m.fasta')
seqq <- branchipus_2$MK465080.1_2
print(seqq)
```

```
##   [1] "l" "l" "g" "d" "d" "q" "l" "y" "n" "v" "i" "v" "t" "a" "h" "a" "f" "v"
##  [19] "m" "i" "f" "f" "m" "v" "m" "p" "i" "l" "i" "g" "g" "f" "g" "n" "w" "l"
##  [37] "v" "p" "l" "m" "l" "g" "a" "p" "d" "m" "a" "f" "p" "r" "l" "n" "n" "l"
##  [55] "s" "f" "w" "m" "l" "p" "p" "s" "l" "t" "l" "l" "v" "a" "s" "s" "m" "v"
##  [73] "e" "s" "g" "v" "g" "t" "g" "w" "t" "v" "y" "p" "p" "l" "s" "a" "a" "i"
##  [91] "a" "h" "a" "g" "p" "s" "v" "d" "l" "a" "i" "f" "s" "l" "h" "l" "a" "g"
## [109] "i" "s" "s" "i" "l" "g" "a" "v" "n" "f" "i" "t" "t" "v" "i" "n" "m" "r"
## [127] "p" "h" "s" "m" "s" "l" "d" "r" "m" "p" "l" "f" "a" "w" "a" "v" "v" "i"
## [145] "t" "a" "v" "l" "l" "l" "l" "s" "l" "p" "v" "l" "a" "x"
## attr(,"name")
## [1] "MK465080.1_2"
## attr(,"Annot")
## [1] ">MK465080.1_2 Branchipus schaefferi isolate ST2-2 cytochrome c oxidase subunit I (COI) gene, par
## attr(,"class")
## [1] "SeqFastadna"
```

```r
true_seq <- str_split(tolower("LLGDDQLYNVIVTAHAFVMIFFMVMPILIGGFGNWLVPLMLGAPDMAFPRLNNLSFWMLPPSLTLLVASSMVE

print(true_seq==seqq)
```

```
##   [1]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [13]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [25]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [37]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [49]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [61]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [73]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [85]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
##  [97]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [109]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [121]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [133]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [145]  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
## [157]  TRUE FALSE
```

We get the same protein sequence as this is the sequence from https://www.ncbi.nlm.nih.gov/nuccore/MK465080 .

“LLGDDQLYNVIVTAHAFVMIFFMVMPILIGGFGNWLVPLMLGAPDMAFPRLNNLSFWMLPPSLTLLVASSMVESGVGT
WTVYPPLSAAIAHAGPSVDLAIFSLHLAGISSILGAVNFITTVINMRPHSMSLDRMPLFAWAVVITAVLLLLSLPVLA”

Then in the end of the sequence we got there is an X which i guess is a stop codon.

To use backtranseq we couldn’t find the correct codon table so we use ‘Homo’ Sapiens’ and got the following results.

```r
branchipus_3 <- read.fasta('emboss_backtranseq-I20241115-101532-0798-77094578-p1m.fasta')

code_seq <- branchipus_3$MK465080.1_2
print(code_seq)
```

```
##   [1] "c" "t" "g" "c" "t" "g" "g" "g" "c" "g" "a" "c" "g" "a" "c" "c" "a" "g"
##  [19] "c" "t" "g" "t" "a" "c" "a" "a" "c" "g" "t" "g" "a" "t" "c" "g" "t" "g"
##  [37] "a" "c" "c" "g" "c" "c" "c" "a" "c" "g" "c" "c" "t" "t" "c" "g" "t" "g"
##  [55] "a" "t" "g" "a" "t" "c" "t" "t" "c" "t" "t" "c" "a" "t" "g" "g" "t" "g"
##  [73] "a" "t" "g" "c" "c" "c" "a" "t" "c" "c" "t" "g" "a" "t" "c" "g" "g" "c"
##  [91] "g" "g" "c" "t" "t" "c" "g" "g" "c" "a" "a" "c" "t" "g" "g" "c" "t" "g"
## [109] "g" "t" "g" "c" "c" "c" "c" "t" "g" "a" "t" "g" "c" "t" "g" "g" "g" "c"
## [127] "g" "c" "c" "c" "c" "c" "g" "a" "c" "a" "t" "g" "g" "c" "c" "t" "t" "c"
## [145] "c" "c" "c" "a" "g" "g" "c" "t" "g" "a" "a" "c" "a" "a" "c" "c" "t" "g"
## [163] "a" "g" "c" "t" "t" "c" "t" "g" "g" "a" "t" "g" "c" "t" "g" "c" "c" "c"
## [181] "c" "c" "c" "a" "g" "c" "c" "t" "g" "a" "c" "c" "c" "t" "g" "c" "t" "g"
## [199] "g" "t" "g" "g" "c" "c" "a" "g" "c" "a" "g" "c" "a" "t" "g" "g" "t" "g"
## [217] "g" "a" "g" "a" "g" "c" "g" "g" "c" "g" "t" "g" "g" "g" "c" "a" "c" "c"
## [235] "g" "g" "c" "t" "g" "g" "a" "c" "c" "g" "t" "g" "t" "a" "c" "c" "c" "c"
## [253] "c" "c" "c" "c" "t" "g" "a" "g" "c" "g" "c" "c" "g" "c" "c" "a" "t" "c"
## [271] "g" "c" "c" "c" "a" "c" "g" "c" "c" "g" "g" "c" "c" "c" "c" "a" "g" "c"
## [289] "g" "t" "g" "g" "a" "c" "c" "t" "g" "g" "c" "c" "a" "t" "c" "t" "t" "c"
## [307] "a" "g" "c" "c" "t" "g" "c" "a" "c" "c" "t" "g" "g" "c" "c" "g" "g" "c"
## [325] "a" "t" "c" "a" "g" "c" "a" "g" "c" "a" "t" "c" "c" "t" "g" "g" "g" "c"
## [343] "g" "c" "c" "g" "t" "g" "a" "a" "c" "t" "t" "c" "a" "t" "c" "a" "c" "c"
## [361] "a" "c" "c" "g" "t" "g" "a" "t" "c" "a" "a" "c" "a" "t" "g" "a" "g" "g"
## [379] "c" "c" "c" "c" "a" "c" "a" "g" "c" "a" "t" "g" "a" "g" "c" "c" "t" "g"
## [397] "g" "a" "c" "a" "g" "g" "a" "t" "g" "c" "c" "c" "c" "t" "g" "t" "t" "c"
## [415] "g" "c" "c" "t" "g" "g" "g" "c" "c" "g" "t" "g" "g" "t" "g" "a" "t" "c"
## [433] "a" "c" "c" "g" "c" "c" "g" "t" "g" "c" "t" "g" "c" "t" "g" "c" "t" "g"
## [451] "c" "t" "g" "a" "g" "c" "c" "t" "g" "c" "c" "c" "g" "t" "g" "c" "t" "g"
## [469] "g" "c" "c" "n" "n" "n"
## attr(,"name")
## [1] "MK465080.1_2"
## attr(,"Annot")
## [1] ">MK465080.1_2 Branchipus schaefferi isolate ST2-2 cytochrome c oxidase subunit I (COI) gene, par
## attr(,"class")
## [1] "SeqFastadna"
```

We get a different sequence compared to the one we saved from https://www.ncbi.nlm.nih.gov/nuccore/MK465080.1?report=fasta.

2.3 Question 2.3

Compare your obtained coding strand sequence with the nucleotide sequence provided (when following the CDS link). Are they the same or do they differ? Try reversing and taking the complement (e.g., http://arep.med.harvard.edu/labgc/adnan/projects/Utilities/revcomp.html or http://www.bioinformatics.nl/cgi-bin/emboss/revseq or write your own code) of the your coding strand DNA. Consider also backtranseqambiq (https://www.ebi.ac.uk/Tools/st/emboss_backtranseqambig/ and check if the resulting nucleotide sequence is compatible with the true one. Do not forget to check the codon table. Explain what happened and why. Save (and submit) the nucleotide sequence of the template strand that corresponds to these amino acids as a FASTA format file.

Results from backtranseqambiq with codon table 5 'Invertebrate Mitochondrial'.

```
branchipus_4 <- read.fasta('emboss_backtranambig-I20241115-101314-0071-49255505-p1m.fasta')

print(branchipus_4$MK465080.1_2)
```

```
##   [1] "y" "t" "n" "y" "t" "n" "g" "g" "n" "g" "a" "y" "g" "a" "y" "c" "a" "r"
##  [19] "y" "t" "n" "t" "a" "y" "a" "a" "y" "g" "t" "n" "a" "t" "y" "g" "t" "n"
##  [37] "a" "c" "n" "g" "c" "n" "c" "a" "y" "g" "c" "n" "t" "t" "y" "g" "t" "n"
##  [55] "a" "t" "r" "a" "t" "y" "t" "t" "y" "t" "t" "y" "a" "t" "r" "g" "t" "n"
##  [73] "a" "t" "r" "c" "c" "n" "a" "t" "y" "y" "t" "n" "a" "t" "y" "g" "g" "n"
##  [91] "g" "g" "n" "t" "t" "y" "g" "g" "n" "a" "a" "y" "t" "g" "r" "y" "t" "n"
## [109] "g" "t" "n" "c" "c" "n" "y" "t" "n" "a" "t" "r" "y" "t" "n" "g" "g" "n"
## [127] "g" "c" "n" "c" "c" "n" "g" "a" "y" "a" "t" "r" "g" "c" "n" "t" "t" "y"
## [145] "c" "c" "n" "c" "g" "n" "y" "t" "n" "a" "a" "y" "a" "a" "y" "y" "t" "n"
## [163] "w" "s" "n" "t" "t" "y" "t" "g" "r" "a" "t" "r" "y" "t" "n" "c" "c" "n"
## [181] "c" "c" "n" "w" "s" "n" "y" "t" "n" "a" "c" "n" "y" "t" "n" "y" "t" "n"
## [199] "g" "t" "n" "g" "c" "n" "w" "s" "n" "w" "s" "n" "a" "t" "r" "g" "t" "n"
## [217] "g" "a" "r" "w" "s" "n" "g" "g" "n" "g" "t" "n" "g" "g" "n" "a" "c" "n"
## [235] "g" "g" "n" "t" "g" "r" "a" "c" "n" "g" "t" "n" "t" "a" "y" "c" "c" "n"
## [253] "c" "c" "n" "y" "t" "n" "w" "s" "n" "g" "c" "n" "g" "c" "n" "a" "t" "y"
## [271] "g" "c" "n" "c" "a" "y" "g" "c" "n" "g" "g" "n" "c" "c" "n" "w" "s" "n"
## [289] "g" "t" "n" "g" "a" "y" "y" "t" "n" "g" "c" "n" "a" "t" "y" "t" "t" "y"
## [307] "w" "s" "n" "y" "t" "n" "c" "a" "y" "y" "t" "n" "g" "c" "n" "g" "g" "n"
## [325] "a" "t" "y" "w" "s" "n" "w" "s" "n" "a" "t" "y" "y" "t" "n" "g" "g" "n"
## [343] "g" "c" "n" "g" "t" "n" "a" "a" "y" "t" "t" "y" "a" "t" "y" "a" "c" "n"
## [361] "a" "c" "n" "g" "t" "n" "a" "t" "y" "a" "a" "y" "a" "t" "r" "c" "g" "n"
## [379] "c" "c" "n" "c" "a" "y" "w" "s" "n" "a" "t" "r" "w" "s" "n" "y" "t" "n"
## [397] "g" "a" "y" "c" "g" "n" "a" "t" "r" "c" "c" "n" "y" "t" "n" "t" "t" "y"
## [415] "g" "c" "n" "t" "g" "r" "g" "c" "n" "g" "t" "n" "g" "t" "n" "a" "t" "y"
## [433] "a" "c" "n" "g" "c" "n" "g" "t" "n" "y" "t" "n" "y" "t" "n" "y" "t" "n"
## [451] "y" "t" "n" "w" "s" "n" "y" "t" "n" "c" "c" "n" "g" "t" "n" "y" "t" "n"
## [469] "g" "c" "n" "n" "n" "n"
## attr(,"name")
## [1] "MK465080.1_2"
## attr(,"Annot")
## [1] ">MK465080.1_2 Branchipus schaefferi isolate ST2-2 cytochrome c oxidase subunit I (COI) gene, par
## attr(,"class")
## [1] "SeqFastadna"
```

Results from backtranseqambiq with codon table 5 ‘Invertebrate Mitochondrial’

reverse and complemented sequence: “ ““GATCATCACGAAGGCGTGGGCGGTCACGATCACGTTGTACAGCTGGTCGT CATCAGGGGCACCAGCCAGTTGCCGAAGCCGCCGATCAGGATGGGCATCACCATGAAGAA GGGCAGCATCCAGAAGCTCAGGTTGTTCAGCCTGGGGAAGGCCATGTCGGGGGCGCCCAG CCAGCCGGTGCCCACGCCGCTCTCCACCATGCTGCTGGCCACCAGCAGGGTCAGGCTGGG GGCCAGGTCCACGCTGGGGCCGGCGTGGGCGATGGCGGCGCTCAGGGGGGGGTACACGGT GGTGATGAAGTTCACGGCGCCCAGGATGCTGCTGATGCCGGCCAGGTGCAGGCTGAAGAT CCAGGCGAACAGGGGCATCCTGTCCAGGCTCATGCTGTGGGGCCTCATGTTGATCACGGT NNNGGCCAGCACGGGCAGGCTCAGCAGCAGCAGCACGGCGGTGATCACCACGGC”“ ”

Results from backtranseqambiq:

“GGNGCNACNTGYGCNACNTGYGCNTGYGGNGCNGCNGGNGGNTGYGGNACNGGNGGNGGN TGYGGNGGNACNTGYGCNTGYGGNGCNACNTGYGCNTGYGGNACNACNGGNACNGCNTGY GCNGGNTGYACNGGNGGNACNTGYGGNACNTGYGGNTGYTGYTGYGCNGGNTGYGCNGGN TGYGCNACNTGYGCNGGNGGNGGNGGNTGYGCNTGYTGYGCNGGNTGYTGYGCNGGNACN ACNGGNTGYTGYGGNGCNGCNGGNTGYTGYGGNTGYTGYGGNGCNACNTGYGCNGGNGGN GCNACNGGNGGNGGNTGYGCNACNTGYGCNTGYTGYGCNACNGGNGCNGCNGGNGCNGCN GGNGGNGGNTGYGCNGGNTGYGCNACNTGYTGYGCNGGNGCNGCNGGNTGYACNTGYGCN GGNGGNACNACNGGNACNACNTGYGCNGGNTGYTGYACNGGNGGNGGNGGNGCNGCNGGN GGNTGYTGYGCNACNGGNACNTGYGGNGGNGGNGGNGGNTGYGGNTGYTGYTGYGCNGGN TGYTGYGCNGGNTGYTGYGGNGGNACNGGNTGYTGYTGYGCNTGYGGNTGYTGYGGNTGY ACNTGYACNTGYTGYGCNTGYTGYGCNACNGGNTGYACNGGNTGYACNGGNGGNTGYTGY GCNTGYTGYGCNGGNTGYGCNGGNGGNGGNACNTGYGCNGGNGGNTGYACNGGNGGNGGN GGNGGNTGYTGYGCNGGNGGNACNTGYTGYGCNTGYGGNTGYACNGGNGGNGGNGGNTGY TGYGGNGGNTGYGGNACNGGNGGNGGNTGYGGNGCNACNGGNGGNTGYGGNGGNTGYGGN TGYACNTGYGCNGGNGGNGGNGGNGGNGGNGGNGGNACNGCNTGYGCNTGYGGNGGNACN GGNGGNACNGGNGCNACNGGNGCNGCNGGNACNACNTGYGCNTGYGGNGGNTGYGGNTGY TGYTGYGCNGGNGGNGCNACNGGNTGYACNGGNTGYACNGGNGCNACNGGNTGYTGYGGN GGNTGYTGYGCNGGNGGNACNGGNTGYGCNGGNGGNTGYACNGGNGCNGCNGGNGCNACN

TGYTGYGCNGGNGGNTGYGGNGCNGCNTGYGCNGGNGGNGGNGGNTGYGCNACNTGYTGY
ACNGGNACNTGYTGYGCNGGNGGNTGYACNTGYGCNACNGGNTGYACNGGNACNGGNGGN
GGNGGNTGYTGYACNTGYGCNACNGGNACNACNGGNGCNACNTGYGCNTGYGGNGGNACN
AAYAAYAAYGGNGGNTGYTGYGCNGGNTGYGCNTGYGGNGGNGGNTGYGCNGGNGGNTGY
ACNTGYGCNGGNTGYGCNGGNTGYGCNGGNTGYGCNGGNTGYGCNTGYGGNGGNTGYGGN
GGNACNGGNGCNACNTGYGCNTGYTGYGCNTGYGGNGGNTGY”

Does not look close to the true sequence have in the fasta file were supposed to save. The reason we think is behind this is that the codon table that we have used does not correspond to the protein sequence of the species, making it fail at transcribing the nucleotide sequence to the correct sequence. We also gets lots of N’s, this is because backtranseqambiq tries to cover all possible codon options, as N stands for any nucleotide (A,T,G,C), Y also stand for either Cytosine or Thymine, these ambiguities make the resulting sequence less specific and harder to compare directly with the true nucleotide sequence.

3 Question 3: Exploring a genomic sequence

Eukaryotic genes are commonly divided into exons and introns and these needed to be spliced in order to produce an mRNA that can be translated into a protein. The gene starts with the promoter ("region of DNA that initiates transcription, i.e. DNA→RNA, of a particular gene", see https://en.wikipedia.org/wiki/Promoter_(genetics)), the the first exon, first intron, second exon, e.t.c. Multiple introns an alternative splicing (a single gene codes for different proteins, through different exons used, see https://en.wikipedia.org/wiki/Alternative_splicing) of mRNAs can make it difficult to identify genes. Finding genes and how they are organized is often due to searching for similar nucleotide sequences within already know protein amino acids, or rather their nucleotide sequences and the corresponding full–length cDNAs (complementary DNA—DNA synthesized from a single stranded RNA, see https://en.wikipedia.org/wiki/Complementary_DNA). cDNAs come from back transcription (reverse–transcription) of mRNAs and hence are without introns—and can be considered as equivalent to mRNA sequences. Comparing a part of the genome (that contains introns) with its cDNA will show the introns' start and end points. GenBank (https://www.ncbi.nlm.nih.gov/genbank/) contains both cDNA sequences and corresponding genomic sequences (if available). If order to discover the structure of the gene we need to compare the cDNA with the genomic sequence. In the file 732A51 BioinformaticsHT2024 Lab01Ex03.fasta you can find a genomic sequence from the species C. elegans, that contains a particular gene. You will use the Basic Local Alignment Sequence Tool (BLAST), to find the gene's organization. BLAST is used to compare a query sequence all sequences (i.e., cDNA sequences) in GenBank. Usually the top scoring hit is the one you want. The next ones will be less and less similar. It can happen that all hits have 100% identity—then consider the percent coverage.

3.1 Question 3.1

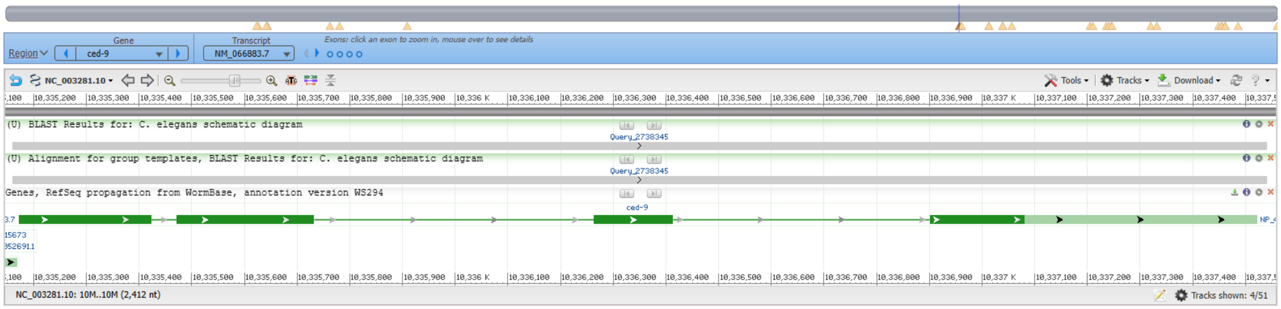
Read up on C. elegans and in a few sentences describe it and why it is such an important organism for the scientific community.

C. elegans is a kind of a small, transparent, nematode or roundworm, feeding on microorganisms and it can mate both with males and through self-fertilization. The research started on C elegans as it has multiple advantageous attributes like its transparency, short life span(easy to follow from egg to death) and relative small genome that reproduces quickly.

3.2 Question 3.2

```r
c_el <- read.fasta('732A51_BioinformaticsHT2024_Lab01Ex03.fasta')
```

Use the nucleotide BLAST tool to construct a schematic diagram that shows the arrangement of introns and exons in the genomic sequence. In the BLAST tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch, choose database RefSeq Genome Database and remember that the species source of the genomic sequence is Caenorhabditis elegans. Use the Genome Data Viewer button. Alternatively you may use https://wormbase.org/tools/blast_blat.



Gene: ced-9

Transcript: NM_066883.7

Exons: click an exon to zoom in, mouse over to see details

(U) BLAST Results for: C. elegans schematic diagram

(U) Alignment for group templates, BLAST Results for: C. elegans schematic diagram

Genes, RefSeq propagation from WormBase, annotation version WS294

NC_003281.10: 10M..10M (2,412 nt)

Tracks shown: 4/51

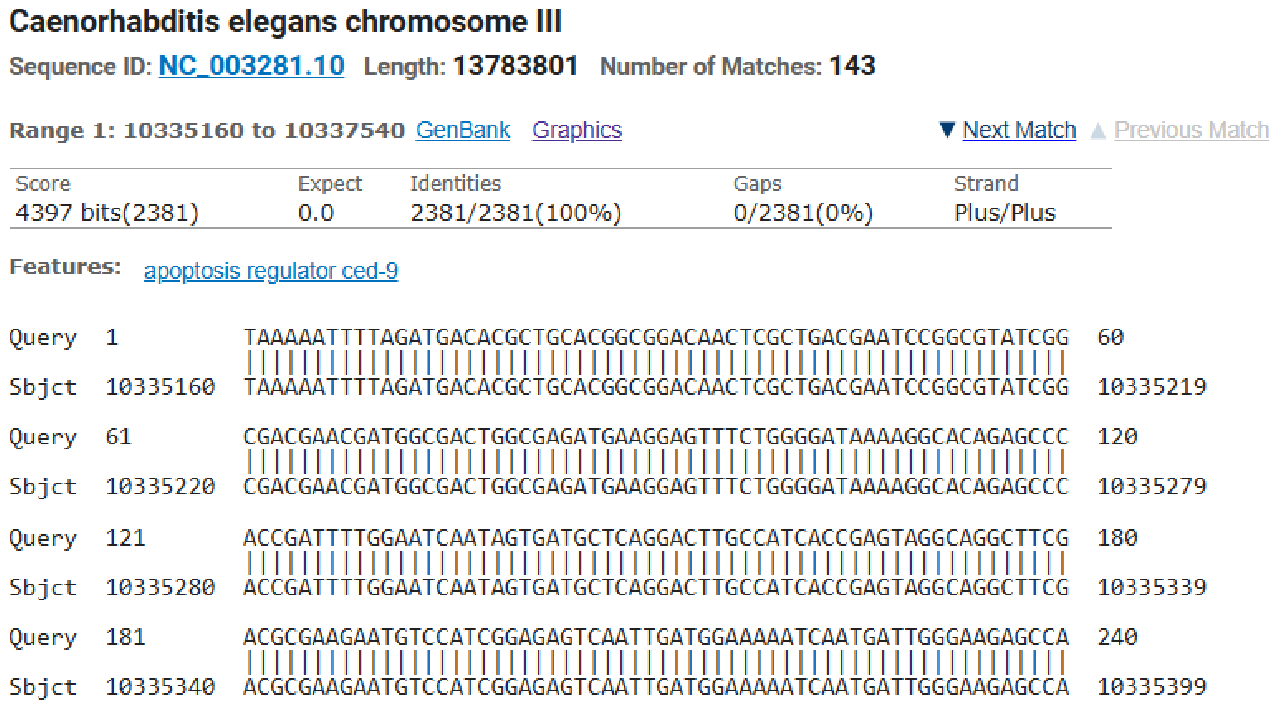
Figure 1: “C.elegans schematic diagram”

Good sequence alignment found with a max score of 4397 with 100% identities and 0% gaps.

3.3 Question 3.3

How are the sequences numbered in the alignment (i.e., pairing of query and database sequences)? Are the directions the same? What would happen if you reverse complement your query sequence (e.g., http://arep.med.harvard.edu/labgc/adnan/projects/Utilities/revcomp.html or http://www.bioinformatics.nl/cgi-bin/emboss/revseq or write your own code) and do the search with such a reverse complemented sequence?

The sequences seems to be numbered after the chromosome's coordinate system, where the start and end positions of the alignment are specific to the reference genome's coordinate structure.



Caenorhabditis elegans chromosome III

Sequence ID: NC_003281.10 Length: 13783801 Number of Matches: 143

Range 1: 10335160 to 10337540 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
4397 bits(2381)	0.0	2381/2381(100%)	0/2381(0%)	Plus/Plus

Features: apoptosis regulator ced-9

```
Query  1         TAAAAATTTTAGATGACACGCTGCACGGCGGACAACTCGCTGACGAATCCGGCGTATCGG  60
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10335160  TAAAAATTTTAGATGACACGCTGCACGGCGGACAACTCGCTGACGAATCCGGCGTATCGG  10335219

Query  61        CGACGAACGATGGCGACTGGCGAGATGAAGGAGTTTCTGGGGATAAAAGGCACAGAGCCC  120
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10335220  CGACGAACGATGGCGACTGGCGAGATGAAGGAGTTTCTGGGGATAAAAGGCACAGAGCCC  10335279

Query  121       ACCGATTTTGGAATCAATAGTGATGCTCAGGACTTGCCATCACCGAGTAGGCAGGCTTCG  180
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10335280  ACCGATTTTGGAATCAATAGTGATGCTCAGGACTTGCCATCACCGAGTAGGCAGGCTTCG  10335339

Query  181       ACGCGAAGAATGTCCATCGGAGAGTCAATTGATGGAAAAATCAATGATTGGGAAGAGCCA  240
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10335340  ACGCGAAGAATGTCCATCGGAGAGTCAATTGATGGAAAAATCAATGATTGGGAAGAGCCA  10335399
```

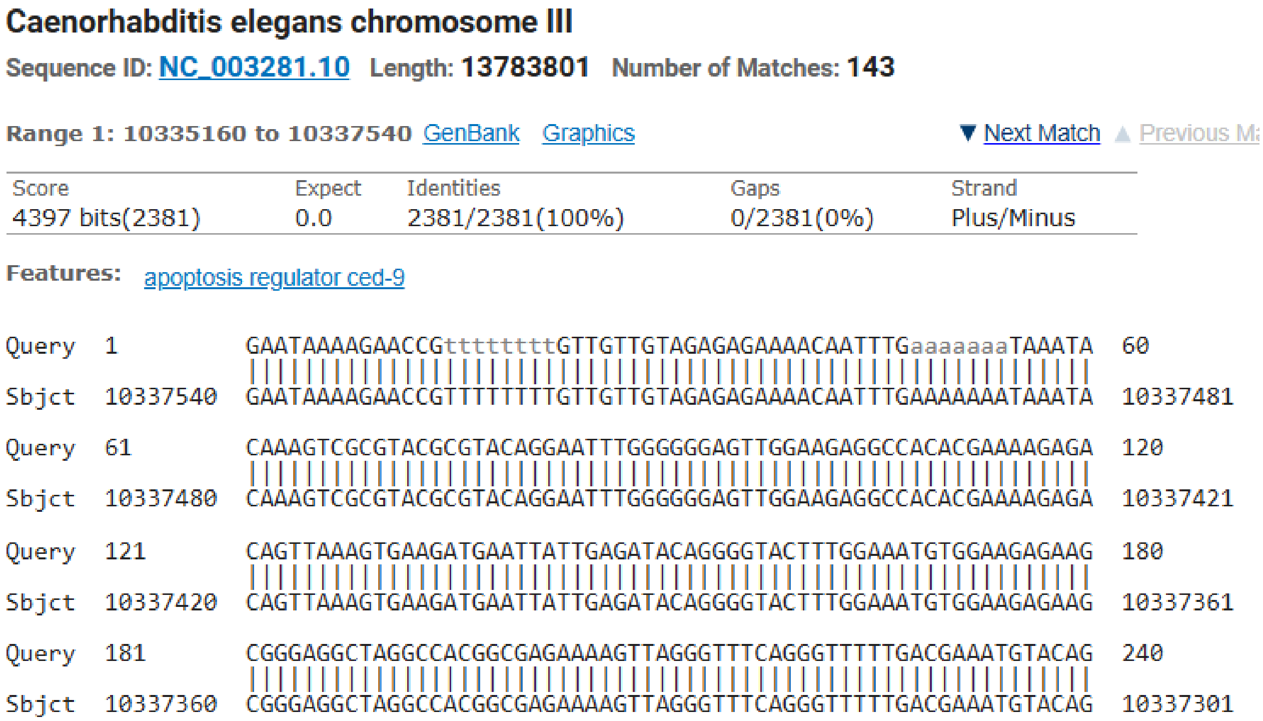
Figure 2: "Sequence aligment"

The Strand being Plus/Plus means both sequences move in the same directions, and identities being 100% and gaps 0% means they are aligned well.

Reverse complemented sequence is as below:

```
"GAATAAAAGAACCGTTTTTTTTGTTGTTGTAGAGAGAAAACAATTTGAAAAAAATAAATA
CAAAGTCGCGTACGCGTACAGGAATTTGGGGGGAGTTGGAAGAGGCCACACGAAAAGAGA
CAGTTAAAGTGAAGATGAATTATTGAGATACAGGGGTACTTTGGAAATGTGGAAGAGAAG
CGGGAGGCTAGGCCACGGCGAGAAAAGTTAGGGTTTCAGGGTTTTTGACGAAATGTACAG
AACACGGAAAAGTTGATATTACGCGGAAGTAGGGTTTGGCCAAAAAAAAAGGCAATCGGA
AAAAATTGCAGTAAAATCGTAAATCGACACAGAGAGCCGTAATTTGTGGCCTAGCTTTCC
CACTTCTTCCAGTTCAAAGGATGAGAGAATCAGTGAGCAAAAATGAGATTCAAATGTAAG
GAGTTGTACATAAATTAATTATTTACACAAATTGAATACGTTACTTCAAGCTGAACATCA
TCCGCCCACACACGACGACTCCAACGATTCCAATGGCTCCAGCTGTTACTCCAGCGCCAA
TCATCGACCACCGTCTGTTCTGCTTCCGGCGTCCCACTTTTTCAGCTTCTGCTCGTTCGT
AGTCCTCTTTCATTTGTTTTCCGAGTGTCATGAAGTCGTCCTGCAATTTTTTTCTTGATT
TTTTATTTTCACAAATAATGCTTTAGAGCATATTCCGGGGAACACAAAAATCCGTGAATG
CGTATTTCACAACATATTTGAGGCGGAAATATCTCGTAGCGAAAACTACAGTAATTCTTT
AAATGCCTACTGTAGTCGATTTACGGGCTCGATTTTTGAAATTAATTTCTTTTGATTAGT
GGTTATTTTCTTTAATTGTAAAAATTTTAAAAATTTAAAGAATTACTGTAGTTTTCTCTA
CTGGATATTTTGCGCGTCAAATATGTTGTGAAATACGCATTCTGCGAATTTTGTATTCCC
GTAACAATAACATTATCATAAACAGTAATATACTTGAATATGTTTCCAGCTGTCCCCCCA
AGAGGCTACCGAACCCTGTAATTATCGGCTTACCAACACCCCGGGAAGTCAAGGGTACTA
GGGAAAGGGGGATATTGACTTCTAATGTCTATGCAAATACTCCTTACCCAGCTCCGATTG
TGTTCCTTCCAGTTGTTGCGGATCCGCGTTTTGATGAACAGCGATGTGTAAACGAAGAGG
TTTCGCACTTGTCCCTGCAGTTCCACGGATTCCATCATTTTTGCAGCTACGAAACCGCCG
AACGAGATTAGACCTATCTGAAAATTAGAAATTTGCAATTTAATTCGTTCATTTCTGGCG
GAAAATTAAGCTTAAAAAAACGATTTTTCTGGTTTTTAATGTTCGAAAATTGAATTTTTG
GGGTTTTTCGTTCAGCAAATCAGCAGAAAAACCTGTTTTTCGAAGAAAAAAGTTCAAAAA
TCGAATTTTAATGTATTTTCCACAAATTTTACATTTAAAAACCGATTTTTGATAAATCAG
CAGATCTTTTTCGCAAATACAAATCAAGGAAAATATGCTTTAAAAATATTCGAATAGAAA
ATAACGTTTTTAATCGAATTCGTAAAGTTTTCTGCTAATTTATCAACAAAAAAAATTACA
ATCCGGGATCAAAATTCGTGGTTTTTTAATGGAAAACTTTCAAAAAAGACACATAATTGG
GCTATTTTTGACCAAGTTTTGCTGATTTCCTGAAGGAAAACAAACAATTTTTGCAAATGT
TTTTTCTGCCACCTTTCTGGCGAATTTTCGAATTTTTGCAAACTTTTTTTCAGTATTTTC
TCCCTTACCAAACGTCCATAAGACATTGGACATTGATCTGTCTGTGCATTTCCAACCGTC
CGAACCACATCCTGATACAGTGAAAATGAGATTCTGGGCACTGCGAGCAGCTGCTCACAG
AAGGTCTCAAAATTTTCCGCGTGCTTCTTCTCGAATATCGTTCCCATAACTCGCATCATT
TCGTGCTCCGGTTGCACTCCACACGGCAATCCCGGTGCTCCAAACCATTCCATTCCGTTT
TGCCGGATTCGGTGCGTGAAATAGTCGACCTGGAAGCAGCAGGAAATTTTATTTACAAAA
AAAAATTAAAAAATTACCACAAATCCCTCGATATCAAGCCTTGGCTCTTCCCAATCATTG
ATTTTTCCATCAATTGACTCTCCGATGGACATTCTTCGCGTCGAAGCCTGCCTACTCGGT
GATGGCAAGTCCTGAGCATCACTATTGATTCCAAAATCGGTGGGCTCTGTGCCTTTTATC
CCCAGAAACTCCTTCATCTCGCCAGTCGCCATCGTTCGTCGCCGATACGCCGGATTCGTC
AGCGAGTTGTCCGCCGTGCAGCGTGTCATCTAAAATTTTTA"
```

Reversing and complementing the sequence results in the strands being Plus/Minus which is expected as they now move in different directions. However, identities and gaps are still maintained and thus they are still aligned.



Caenorhabditis elegans chromosome III

Sequence ID: NC_003281.10 Length: 13783801 Number of Matches: 143

Range 1: 10335160 to 10337540 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
4397 bits(2381)	0.0	2381/2381(100%)	0/2381(0%)	Plus/Minus

Features: apoptosis regulator ced-9

```
Query  1         GAATAAAAGAACCGttttttttGTTGTTGTAGAGAGAAAACAATTTGaaaaaaaTAAATA  60
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10337540  GAATAAAAGAACCGTTTTTTTTGTTGTTGTAGAGAGAAAACAATTTGAAAAAAATAAATA  10337481

Query  61        CAAAGTCGCGTACGCGTACAGGAATTTGGGGGGAGTTGGAAGAGGCCACACGAAAAGAGA  120
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10337480  CAAAGTCGCGTACGCGTACAGGAATTTGGGGGGAGTTGGAAGAGGCCACACGAAAAGAGA  10337421

Query  121       CAGTTAAAGTGAAGATGAATTATTGAGATACAGGGGTACTTTGGAAATGTGGAAGAGAAG  180
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10337420  CAGTTAAAGTGAAGATGAATTATTGAGATACAGGGGTACTTTGGAAATGTGGAAGAGAAG  10337361

Query  181       CGGGAGGCTAGGCCACGGCGAGAAAAGTTAGGGTTTCAGGGTTTTTGACGAAATGTACAG  240
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  10337360  CGGGAGGCTAGGCCACGGCGAGAAAAGTTAGGGTTTCAGGGTTTTTGACGAAATGTACAG  10337301
```

Figure 3: “Sequence aligment reversed”

3.4 Question 3.4

On what chromosome and what position is the query sequence found? At which position does the gene begin and end in your query sequence?

The query sequence is found on chromosome III at positions 10335160 to 10337540.

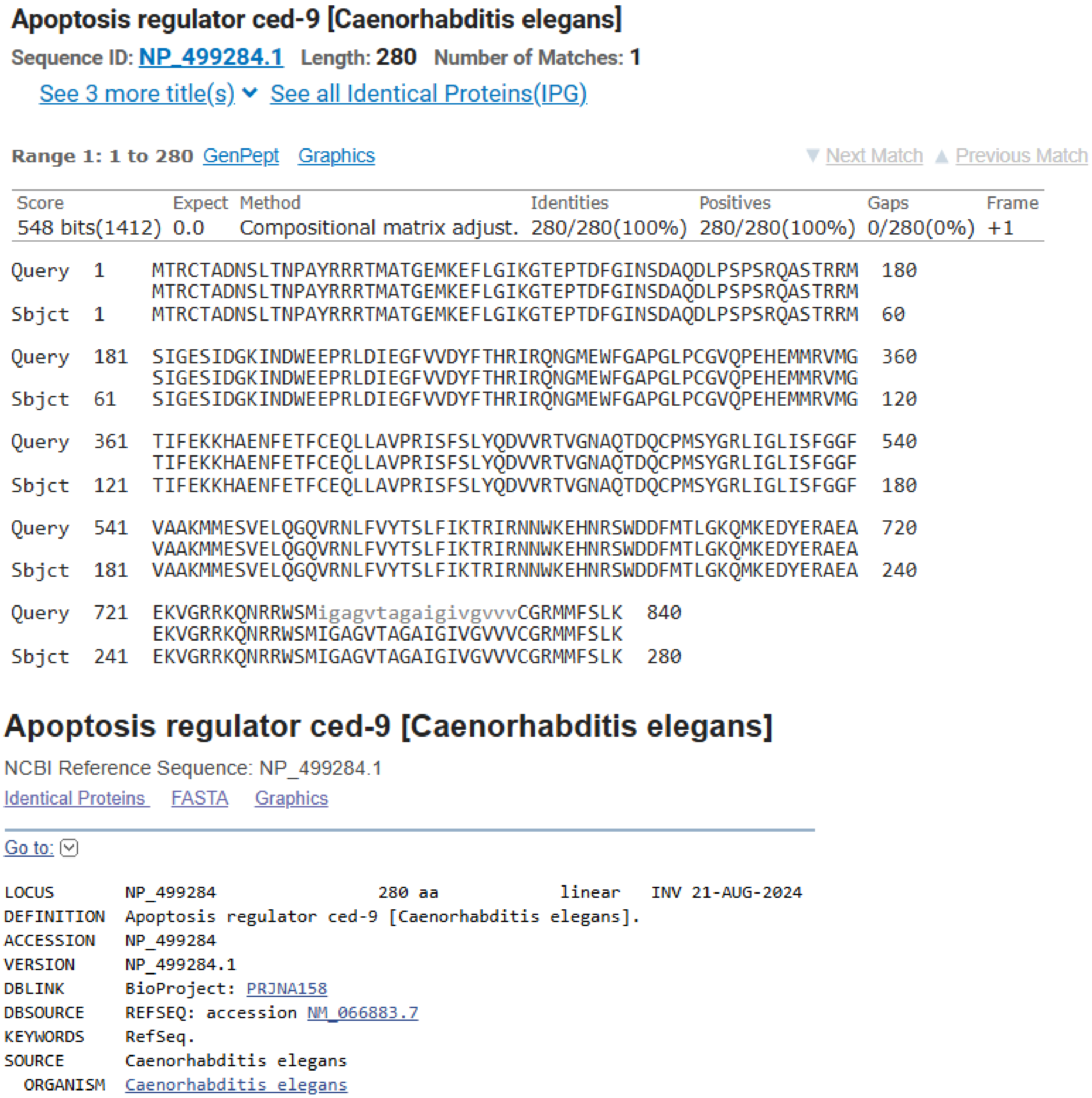
3.5 Question 3.5

Extract the DNA code of each exon and using transeq (https://www.ebi.ac.uk/Tools/st/ emboss_transeq/) find the protein code of the gene. You can also use blastx (https://blast. ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAG or https://wormbase.org/tools/blast_blat) to obtain protein sequences. How do they compare to your translation?

Below is the extracted combined DNA code for all 4 exons.

```
"ATGACACGCTGCACGGCGGACAACTCGCTGACGAATCCGGCGTATCGGCGACGAACGATGGCGACTGGCG
AGATGAAGGAGTTTCTGGGGATAAAAGGCACAGAGCCCACCGATTTTGGAATCAATAGTGATGCTCAGGA
CTTGCCATCACCGAGTAGGCAGGCTTCGACGCGAAGAATGTCCATCGGAGAGTCAATTGATGGAAAAATC
AATGATTGGGAAGAGCCAAGGCTTGATATCGAGGGATTTGTGGTCGACTATTTCACGCACCGAATCCGGC
AAAACGGAATGGAATGGTTTGGAGCACCGGGATTGCCGTGTGGAGTGCAACCGGAGCACGAAATGATGCG
AGTTATGGGAACGATATTCGAGAAGAAGCACGCGGAAAATTTTGAGACCTTCTGTGAGCAGCTGCTCGCA
GTGCCCAGAATCTCATTTTCACTGTATCAGGATGTGGTTCGGACGGTTGGAAATGCACAGACAGATCAAT
GTCCAATGTCTTATGGACGTTTGATAGGTCTAATCTCGTTCGGCGGTTTCGTAGCTGCAAAAATGATGGA
ATCCGTGGAACTGCAGGGACAAGTGCGAAACCTCTTCGTTTACACATCGCTGTTCATCAAAACGCGGATC
CGCAACAACTGGAAGGAACACAATCGGAGCTGGGACGACTTCATGACACTCGGAAAACAAATGAAAGAGG
ACTACGAACGAGCAGAAGCTGAAAAAGTGGGACGCCGGAAGCAGAACAGACGGTGGTCGATGATTGGCGC
TGGAGTAACAGCTGGAGCCATTGGAATCGTTGGAGTCGTCGTGTGTGGGCGGATGATGTTCAGCTTGAAG
TAACGTATTCAATTTGTGTAAATAATTAATTTATGTACAACTCCTTACATTTGAATCTCATTTTTGCTCA
CTGATTCTCTCATCCTTTGAACTGGAAGAAGTGGGAAAGCTAGGCCACAAATTACGGCTCTCTGTGTCGA
TTTACGATTTTACTGCAATTTTTTCCGATTGCCTTTTTTTTTGGCCAAACCCTACTTCCGCGTAATATCA
ACTTTTCCGTGTTCTGTACATTTCGTCAAAAACCCTGAAACCCTAACTTTTCTCGCCGTGGCCTAGCCTC
CCGCTTCTCTTCCACATTTCCAAAGTACCCCTGTATCTCAATAATTCATCTTCACTTTAACTGTCTCTTT
TCGTGTGGCCTCTTCCAACTCCCCCCAAATTCCTGTACGCGTACGCGACTTTGTATTTATTTTTTTCAAA
TTGTTTTCTCTCTACAACAACAAAA"
```

The protein sequence for the extraced DNA code is on the page below. It has a max score of 548 with 100% identities and 0% gaps, but only 65% query cover. It is identified as the NP_499284.1 protein sequence. Seems to compare well to the translation.



Apoptosis regulator ced-9 [Caenorhabditis elegans]

Sequence ID: NP_499284.1 Length: 280 Number of Matches: 1

See 3 more title(s) See all Identical Proteins(IPG)

Range 1: 1 to 280 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
548 bits(1412)	0.0	Compositional matrix adjust.	280/280(100%)	280/280(100%)	0/280(0%)	+1

```
Query  1    MTRCTADNSLTNPAYRRRTMATGEMKEFLGIKGTEPTDFGINSDAQDLPSPSRQASTRRM  180
            MTRCTADNSLTNPAYRRRTMATGEMKEFLGIKGTEPTDFGINSDAQDLPSPSRQASTRRM
Sbjct  1    MTRCTADNSLTNPAYRRRTMATGEMKEFLGIKGTEPTDFGINSDAQDLPSPSRQASTRRM  60

Query  181  SIGESIDGKINDWEEPRLDIEGFVVDYFTHRIRQNGMEWFGAPGLPCGVQPEHEMMRVMG  360
            SIGESIDGKINDWEEPRLDIEGFVVDYFTHRIRQNGMEWFGAPGLPCGVQPEHEMMRVMG
Sbjct  61   SIGESIDGKINDWEEPRLDIEGFVVDYFTHRIRQNGMEWFGAPGLPCGVQPEHEMMRVMG  120

Query  361  TIFEKKHAENFETFCEQLLAVPRISFSLYQDVVRTVGNAQTDQCPMSYGRLIGLISFGGF  540
            TIFEKKHAENFETFCEQLLAVPRISFSLYQDVVRTVGNAQTDQCPMSYGRLIGLISFGGF
Sbjct  121  TIFEKKHAENFETFCEQLLAVPRISFSLYQDVVRTVGNAQTDQCPMSYGRLIGLISFGGF  180

Query  541  VAAKMMESVELQGQVRNLFVYTSLFIKTRIRNNWKEHNRSWDDFMTLGKQMKEDYERAEA  720
            VAAKMMESVELQGQVRNLFVYTSLFIKTRIRNNWKEHNRSWDDFMTLGKQMKEDYERAEA
Sbjct  181  VAAKMMESVELQGQVRNLFVYTSLFIKTRIRNNWKEHNRSWDDFMTLGKQMKEDYERAEA  240

Query  721  EKVGRRKQNRRWSMigagvtagaigivgvvvCGRMMFSLK  840
            EKVGRRKQNRRWSMIGAGVTAGAIGIVGVVVCGRMMFSLK
Sbjct  241  EKVGRRKQNRRWSMIGAGVTAGAIGIVGVVVCGRMMFSLK  280
```

Apoptosis regulator ced-9 [Caenorhabditis elegans]

NCBI Reference Sequence: NP_499284.1

Identical Proteins FASTA Graphics

Go to:

```
LOCUS       NP_499284               280 aa            linear   INV 21-AUG-2024
DEFINITION  Apoptosis regulator ced-9 [Caenorhabditis elegans].
ACCESSION   NP_499284
VERSION     NP_499284.1
DBLINK      BioProject: PRJNA158
DBSOURCE    REFSEQ: accession NM_066883.7
KEYWORDS    RefSeq.
SOURCE      Caenorhabditis elegans
  ORGANISM  Caenorhabditis elegans
```

Figure 4: “Protein sequence and GenBank reference”

3.6 Question 3.6

What gene is in the query sequence? Hovering over an exon you should see links to View GeneID and View WormBase. These point to pages with more information on the gene. Follow them and write a few sentences about the gene.

The gene in the query sequence is the ced-9. The official full name is Apoptosis regulator ced-9 which was originated by Jonathan Hodgkin. It is a gene type for protein coding. Enables GTPase activator activity and protein sequestering activity. Involved in several processes, including positive regulation of brood size; positive regulation of cellular component organization; and positive regulation of egg-laying behavior. Located in several cellular components, including mitochondrial outer membrane; neuronal cell body; and perinuclear region of cytoplasm. Is expressed in several structures, including gonad.