Genetic Data Analysis using R on BRCA1 gene

i) Algorithm to find all possible numbers of ORF in the gene for all the 6 Possibilities for BRCA1 gene.

Attached is python code CountORF.py.

Explanation: The sequence is read and for all the start codons 'ATG' or equivalently, 'AUG', an algorithm is designed to find sequences AUG......(until stop codon). No of ORFs are calculated for 6 possible orientations and the output is:

```
visheshrangwani@Visheshs-MacBook-Air Question 2 % python3 CountORF.py
No of ORF while reading from sequence in forward direction from 1st index: 330
No of ORF while reading from sequence in forward direction from 2nd index: 318
No of ORF while reading from sequence in forward direction from 3rd index: 303
No of ORF while reading from reverse complement from 1st index: 303
No of ORF while reading from reverse complement from 2nd index: 301
No of ORF while reading from reverse complement from 3rd index: 324
Total no of ORFs = 1879
```

CountORF_different_algo.py considers that if inside an ORF, if we find another start codon we consider that also as an ORF. So the o/p is:

```
visheshrangwani@Visheshs-MacBook-Air Question 2 % python3 CountORF2.py
No of ORF while reading from sequence in forward direction from 1st index: 452
No of ORF while reading from sequence in forward direction from 2nd index: 405
No of ORF while reading from sequence in forward direction from 3rd index: 395
No of ORF while reading from reverse complement from 1st index: 386
No of ORF while reading from reverse complement from 2nd index: 372
No of ORF while reading from reverse complement from 3rd index: 422
Total no of ORFs = 2432
```

ii) Calculation of the possible number of exons

Python Code in the attached file **CountExons.py**. In this, the ORFs containing more than 30 nucleotide sequences(including the 3 nucleotides of the stop codon, if not to be considered, the code can easily be modified to I>=33) are counted. The ORFs are counted as per CountORF.py code. The no of exons are counted for all 6 possibilities of reading the sequence. The output on running the code is:

```
visheshrangwani@Visheshs-MacBook-Air Question 2 % python3 CountExons.py
No of ORF while reading from sequence in forward direction from 1st index: 215
No of ORF while reading from sequence in forward direction from 2nd index: 204
No of ORF while reading from sequence in forward direction from 3rd index: 190
No of ORF while reading from reverse complement from 1st index: 200
No of ORF while reading from reverse complement from 2nd index: 198
No of ORF while reading from reverse complement from 3rd index: 213
Total no of ORFs = 1220
```

iii) Finding number of homologous structural proteins possible

Suppose k exons form a protein and the total number of exons are n. So the no of different possibilities, by Permutation and combination are k! * C(n, k). We take all the 6 possibilities of different ways of reading the sequence and add them.

Python Code in CountProteinStructures.py file. Output:

```
[visheshrangwani@Visheshs-MacBook-Air Question 2 % python3 CountProteinStructures]
.py
a) When 5 exons combine to form protein: 1731935722560 possible structures
b) When 3 exons combine to form protein: 42324750 possible structures
c) When 10 exons combine to form protein: 555188214470132308665600 possible structures
d) When 6 exons combine to form protein: 348736467532320 possible structures
```

iv) Obtaining structure of BRCA1 gene

BRCA1 gene's fasta in 'BRCA1 sequence.fasta'.

Converted to mRNA and primary structure of protein using this website.

```
DNA:

GCT GAG ACT_ TCC TGG ACG GGG GAC AGG CTG TGG GGT TTC TCA GAT AAC TGG GCC CCT GCG CTC AGG AGG CCT TCA CCC TCT GCT CTG GGT AAA GGT AG

MRNA:

CGA CUC UGA_ AGG ACC UGC CCC CUG UCC GAC ACC CCA AAG AGU CUA UUG ACC CGG GGA CGC GAG UCC UCC GGA AGU GGG AGA CGA GAC CCA UUU CCA UC

Protein:

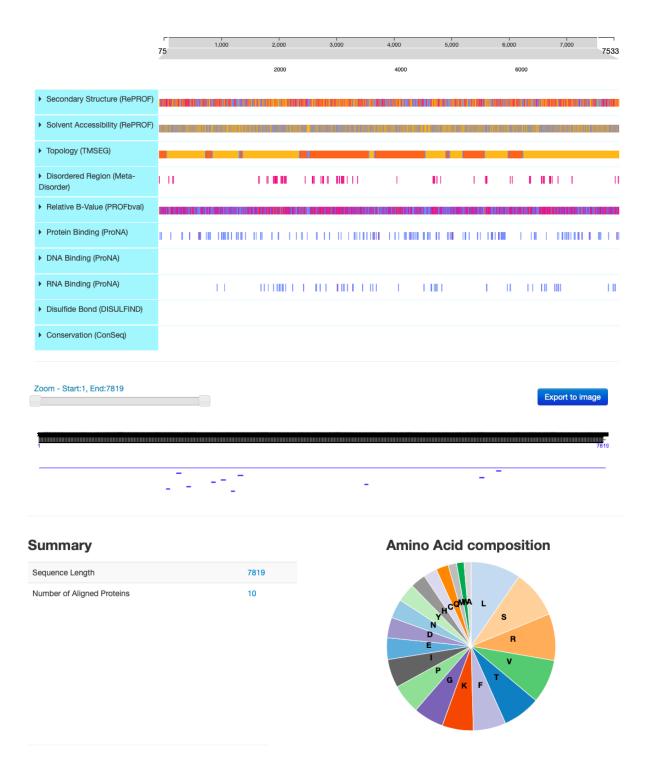
ARG LEU STOP ARG THR CYS PRO LEU SER ASP THR PRO LYS SER LEU LEU THR ARG GLY ARG GLU SER SER GLY SER GLY ARG ARG ASP PRO PHE PRO SEI
```

Results attached in BRCA1mRNA.txt for mRNA and prProtein.txt for primary structure of protein.

In order to translate mRNA for 6 frames $\underline{\text{this}}$ website is used. The results are stored as: 5'->3'(1).txt, 5'->3'(2).txt, 5'->3'(3).txt, 3'->5'(1).txt, 3'->5'(2).txt, 3'->5'(3).txt for each corresponding frame.

These 6 are the different possible translation to amino acid sequence from mRNA. We convert 5'->3'(2).txt to secondary and tertiary protein structures.

This is done using this site. (Since it didnt allow sequences of length > 8000, some last amino acids were trimmed).



<u>Primary Structure of Protein</u>: We know that proteins are formed by the peptide bonds in the sequence of amino acids. This sequence of amino acids is the primary structure of the protein.

<u>Secondary structure of protein</u>: These long amino acids sequences, fold over each other and form polypeptide linkages between atoms. This folding of the amino acid sequence is the scondary structure of protein

<u>Tertiary Structure of Protein</u>: There are further linkages due to H-bonds, electrostatic forces, van der Waals forces of attraction and disulphide linkages. These cause further folding of the secondary structure of protein and is called tertiary structure.

v) Finding the number of genes on each chromosomes

Attached is an R file named BioMart.R.

i) A loop is run over all 22 chromosomes and the no of genes on each chromosome is stored in a vector 'v'. The no of genes is found using the getBM() function in which we find the number of all possible gene IDs on each of the 22 normal chromosomes and then for the 'X' and 'Y' chromosomes. Then the values of these number of genes is printed.

```
[1] "No of genes on chromosome 1 5557"
[1] "No of genes on chromosome 2 4274"
[1] "No of genes on chromosome 3 3252"
[1] "No of genes on chromosome 4 2704"
[1] "No of genes on chromosome 5 3036"
[1] "No of genes on chromosome 6 3125"
[1] "No of genes on chromosome 7 3061"
[1] "No of genes on chromosome 8 2523"
[1] "No of genes on chromosome 9 2361"
[1] "No of genes on chromosome 10 2380"
[1] "No of genes on chromosome 11 3401"
[1] "No of genes on chromosome 12 3091"
[1] "No of genes on chromosome 13 1422"
[1] "No of genes on chromosome 14 2318"
[1] "No of genes on chromosome 15 2249"
[1] "No of genes on chromosome 16 2588"
[1] "No of genes on chromosome 17 3086"
[1] "No of genes on chromosome 18 1254"
[1] "No of genes on chromosome 19 3018"
[1] "No of genes on chromosome 20 1480"
[1] "No of genes on chromosome 21 892"
[1] "No of genes on chromosome 22 1404"
> print(paste("No of genes on chromosome X", v[23]))
[1] "No of genes on chromosome X 2452"
> print(paste("No of genes on chromosome Y", v[24]))
[1] "No of genes on chromosome Y 522"
>
```

vi) Finding the sum of total number of genes on each chromosomes

```
Z4 ## Loge for part 11) of the question
 25 print(paste("Sum total of all the genes", sum(v)))
 26
 27 ##From the above code, we observe that chromosome 1 has the maximum amount of genes i.e. 5557
 28
 29 #Store all gene ids in 'genes' variable for chromosome 1
 30 genes <- getBM(attributes = "external_gene_name", filters = "chromosome_name", values = "1", mart = mart)
 31
 32 #Finding number of transcripts by iterating over all genes and finding their 'transcript_count'
 33 #also finding gene with max no of transcripts and transcript having maximum length
 34
 35 max_transcripts <- getBM(attributes="transcript_count", values = genes[1,], mart = mart,filters = "external_gene_name")[[1]]
 36 no_of_transcripts <- max_transcripts
 37 max_transcripts_index <- 1
 38 longest_tr <- max(getBM(attributes="transcript_length", values = genes[1,], mart = mart,filters = "external_gene_name"))
 39 longest_tr_idx <- 1
 40 - for (i in 2:dim(genes)) {
25:51 (Top Level) $
                                                                                                                                 R Script :
Console Terminal × Jobs ×
R 4.1.2 · ~/
    "No of genes on chromosome 13 1422"
[1] "No of genes on chromosome 14 2318"
[1] "No of genes on chromosome 15 2249"
[1] "No of genes on chromosome 16 2588"
[1] "No of genes on chromosome 17 3086"
[1] "No of genes on chromosome 18 1254"
[1] "No of genes on chromosome 19 3018"
[1] "No of genes on chromosome 20 1480"
[1] "No of genes on chromosome 21 892"
[1] "No of genes on chromosome 22 1404"
> print(paste("No of genes on chromosome X", v[23]))
[1] "No of genes on chromosome X 2452"
 print(paste("No of genes on chromosome Y", v[24]))
[1] "No of genes on chromosome Y 522"
> print(paste("Sum total of all the genes", sum(v)))
[1] "Sum total of all the genes 61450'
```

vii) Finding the transcript having maximum length

We see that the chromosome with the max no of genes is chromosome 1. We perform further calculations for the above chromosome.

a) In order to find this, we first store all the genes available in biomaRt in a data structure and then we iterate over all those genes and find the 'transcript_count' attribute of all genes by providing the filter as 'external_gene_name' and value as the ith index of the data structure(data frame used). This done in getBM() function. We sum over all these iterations and store and print them in the 'no_of_transcripts' variable.

NOTE: The program runs very slowly and due to this may give a warning. However, after 5-6 tries, it didn't give a warning.

Theoretical rough calculations:

According to the <u>article</u>, there are on average 3.42 transcripts per gene. Since by analysis in part i), there were 5557 genes on chromosome 1. So the total no of transcripts should be roughly 19005 transcripts. However, the program would show a much smaller no as no of genes stored in the 'genes' variable would be much lesser than 5557 as there may not be sufficient data on biomart.

- b) Using the above approach, we find genes having the maximum number of transcripts also. By storing it in a variable 'max_transcript' and its variable in 'max transcript index'.
 - Using this we get the maximum number of transcripts for genes[max_transcript_index,]
- c) For this, we again do the same. We store the gene index with the currently maximum transcript in the 'longest_tr_idx' variable and maximum length in 'longest_tr' variable. We find the max length of transcript for each gene, compare with 'longest_tr' and update 'longest_tr' and 'longest_tr_idx'. Finally, we print its Ensembl transcript ID.

Below is the Screenshot of Code and its o/p for parts a), b) and c):



vii) Translation into mRNA



The code for conversion to protein is written in protein.py file. The output is:



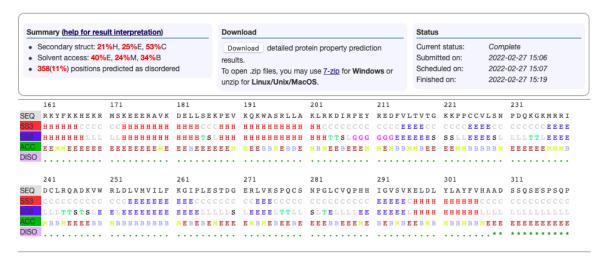
vii) Primary structure output

Primary structure is the output of the program protein.py.

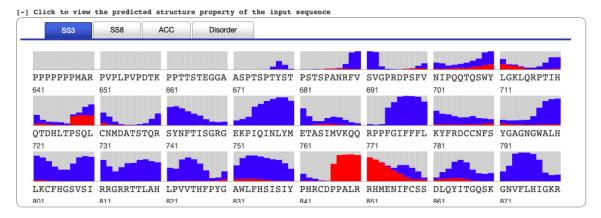
To find secondary, tertiary and quaternary structure, <u>this</u> website was used and the results obtained are downloaded in the folder 'Structure of Protein'.

This is the link to the result of the job.

Following is a screenshot of the result's summary:



Section II. Detailed Prediction Results (see the result by clicking on it)



A more holistic and clear result was obtained from this website.

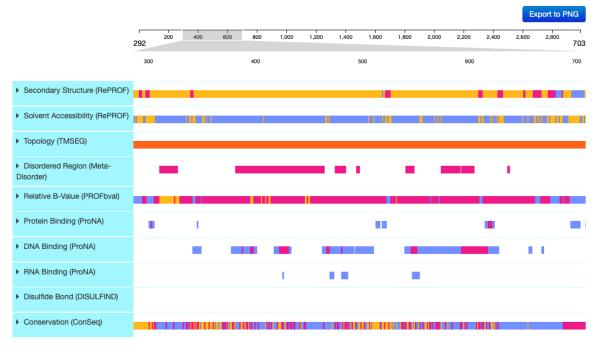
Results occur in various formats.

Stored in ProteinStruct.png.

Screenshot:

Predicted features

What am I seeing Here? This viewer lays out predicted features that correspond to regions within the queried sequence. Mouse over the different colored boxes to learn more about the annotations

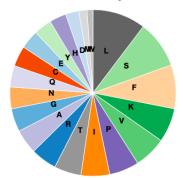




Summary

Sequence Length	2989
Number of Aligned Proteins	31

Amino Acid composition

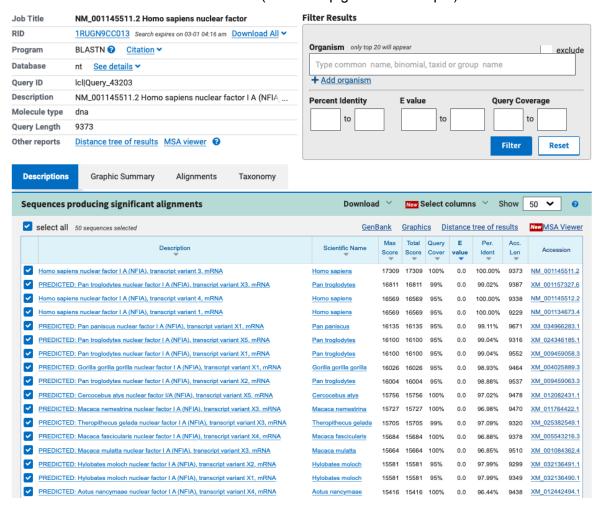


viii) BLAST queries on NCBI

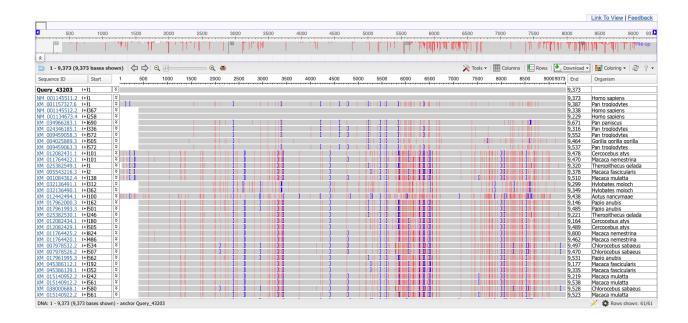
To find this, we blast the transcript sequence on NCBI website and analyze the result.

5 organisms sharing similarity:

- Homo Sapiens (Humans)
- Pan troglodytes (Chimpanzee)
- Pan paniscus (Bonobo Great Ape)
- Gorilla gorilla (Gorilla)
- Cercocebus atys (sooty mangabey a type of monkey)
- Macaca nemestrina (Southern pig-tailed macaque)



The top 50 aligned sequences are downloaded in file named '50AlignedSequences.txt'. MSA done on NCBI website. Downloaded in file named MSA.pdf. Screenshot:



viii) Phylogenetic Trees (NCBI)

The phylogenetic tree is made on NCBI website itself. Downloaded in file named 'PhylTree.pdf'. Screenshot:

