BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

NCBI gene ID: WWOX

Organism : *Homo sapiens*

Full name: WW domain containing oxidoreductase

END-TERM PROJECT BIO-INFORMATICS (BIO F242)

Submitted to,

Prof. Shibasish Chowdhury

Ashish Kumar

2017B1A70854P

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Introduction: WWOX gene

WWOX gene encodes a member of the short chain dehydrogenases/reductases (SDR) protein family.

- <u>Location –</u> In Chromosome 16 spanning base pairs 78,099,654 to 79,212,667 i.e. 16q23.1 – q23.2.
  (See Figure-1) →
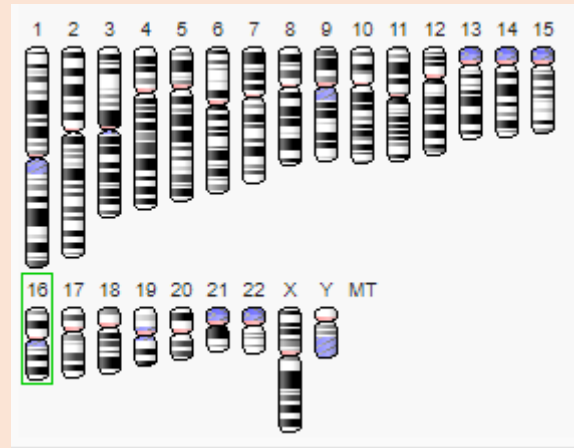
- **Gene type-** Protein coding. This gene produces a translated product which has important function as discussed below.

- **Organism-** Homo *sapiens*

- The gene spans the FRA16D common chromosomal fragile site and appears to function as a tumour suppressor gene. FRA16D is within regions of frequent loss of heterozygosity in certain cancers, and is homozygous deletions of this 270kb FRA16D locus is reported in adenocarcinomas of stomach, lung and ovary.

WWOX protein: 1 WMV function

- 1 WMV is an oxidoreductase. In biological systems, an oxidoreductase acts as a catalyst for oxidation reduction reactions. The enzyme WMV transfers electron from one molecule, known as reductant to another known as oxidant, the electron acceptor.

- The **residue count** of 1 WMV is 54.

- The protein is an intracellular **protein.**

- **Molecular function of WWOX gene-**
  - o Acts as a tumour suppressor gene and plays a role in apoptosis.
  - o Required for a normal bone development

- o Evidence has been obtained that the gene functions synergistically with p53/TP53 to control genotoxic stress-induced cell death.
- o Plays a role in TGFB1 signalling and TGFB1-mediated cell death and also plays a factor in Tumour Necrosis Factor (TNF)-mediated cell death. The protein translocate to the nucleus upon genotoxic stress or TNF stimulation.
- o Inhibits *Wnt* signalling, probably by sequestering DVL2 in the cytoplasm.
- Interacting proteins for WWOX Gene –
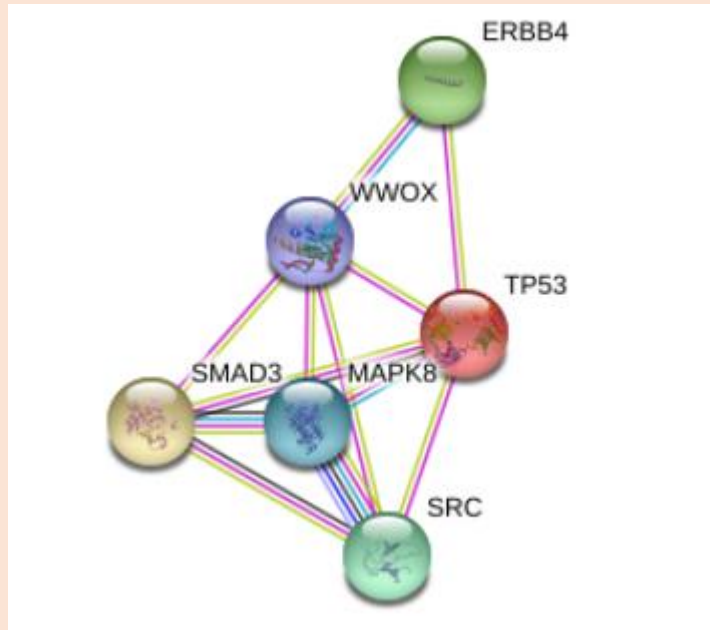
Gene Sequence

(NOTE: To skip the sequence jump to Page 287)

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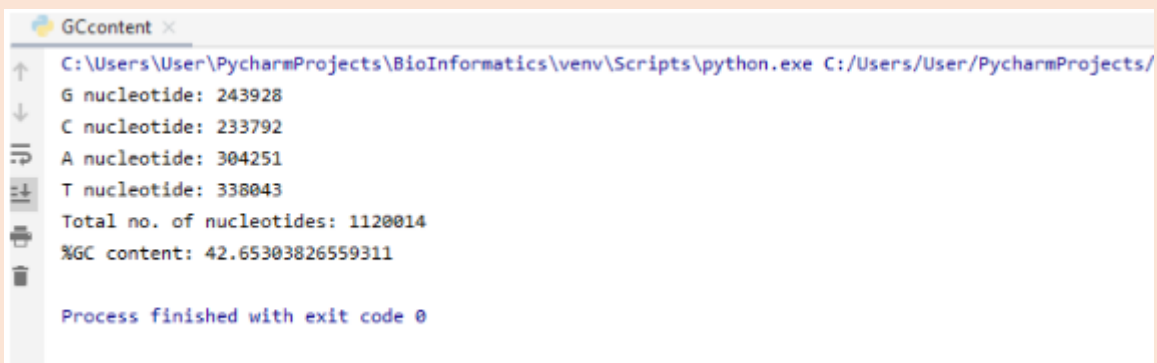
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```

```
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1113001  atgcttgaat  atca
```

GC content in the WWOX whole genome = 42.65%.

```
GCcontent ×

C:\Users\User\PycharmProjects\BioInformatics\venv\Scripts\python.exe C:/Users/User/PycharmProjects/
G nucleotide: 243928
C nucleotide: 233792
A nucleotide: 304251
T nucleotide: 338043
Total no. of nucleotides: 1120014
%GC content: 42.65303826559311

Process finished with exit code 0
```

- o   The GC content of the DNA is <50%. Hence, one can infer that the gene is not thermostable.

- o   GC rich isochores typically include many protein coding regions within them. Existence of GC rich regions can be mapped to gene rich regions. In WWOX raw DNA sequence, about 42% of the sequence contains genes.

- o   The average GC-content in human genomes ranges from 35% to 60% across 100-Kb fragments, with a mean of 41%. The GC-content of Yeast (Saccharomyces cerevisiae) is 38%, and that of another common model organism, thale cress (Arabidopsis thaliana), is 36%.

- o   Because of the nature of the genetic code, it is virtually impossible for an organism to have a genome with a GC-content approaching either 0% or 100%. However, a species with an extremely low GC-content is Plasmodium falciparum (GC% = ~20%), and it is usually common to refer to such examples as being AT-rich instead of GC-poor.

- o   Our sequence is close to the mean of the Human beings which is in line to the data that the sequence is indeed from human beings.

mRNA sequence

(Source: NCBI RefSeq transcripts for WWOX)

https://www.ncbi.nlm.nih.gov/nuccore/NM_016373.4,NM_130791.4,NM_001291997.2,NR_120435.2,NR_120436.2

- The NCBI site lists down 5 transcript variants for the gene WWOX. These transcript variants are before splicing up and removal of intronic sequences.

Transcript variant – 1

➔ **Summary:** This variant (1) represents the longest transcript and encodes the longest isoform (1).

➔ The transcript is a 2242 bp linear mRNA.

- **Sequence –**

```
   1 gcagtgcgca ggcgtgagcg gtcgggcccc gacgcgcgcg ggtctcgttt ggagcgggag
  61 tgagttcctg agcgagtgga cccggcagcg ggcgataggg gggccaggtg cctccacagt
 121 cagccatggc agcgctgcgc tacgcggggc tggacgacac ggacagtgag gacgagctgc
 181 ctccgggctg ggaggagaga accaccaagg acggctgggt ttactacgcc aatcacaccg
 241 aggagaagac tcagtgggaa catccaaaaa ctggaaaaag aaaacgagtg gcaggagatt
 301 tgccatacgg atgggaacaa gaaactgatg agaacggaca agtgtttttt gttgaccata
 361 taaataaaag aaccacctac ttggacccaa gactggcgtt tactgtggat gataatccga
 421 ccaagccaac cacccggcaa agatacgacg gcagcaccac tgccatggaa attctccagg
 481 gccgggattt cactggcaaa gtggttgtgg tcactggagc taattcagga atagggttcg
 541 aaaccgccaa gtcttttgcc ctccatggtg cacatgtgat cttggcctgc aggaacatgg
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 721 ccaagaatgt gcctcttcat gtgcttgtgt gcaacgcagc aacttttgct ctaccctgga
 781 gtctcaccaa agatggcctg gagaccacct ttcaagtgaa tcatctgggg cacttctacc
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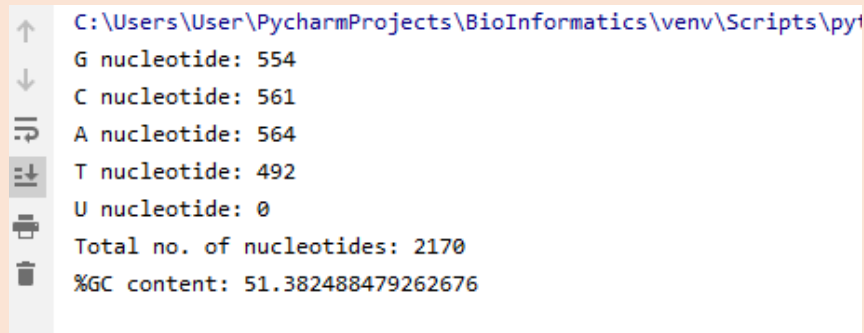
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2101 ttttgggggg cagagaataa aacgttagtt aatccctttg tctgtcaatc acagtctcag
2161 ttctcttgct ttcacattgt acttaaacct cctgctgtgc ctcgcatcct atgcttaata
2221 aaagaacatg cttgaatatc a
```

→ **GC content = 51.38%**

```
    C:\Users\User\PycharmProjects\BioInformatics\venv\Scripts\py
    G nucleotide: 554
    C nucleotide: 561
    A nucleotide: 564
    T nucleotide: 492
    U nucleotide: 0
    Total no. of nucleotides: 2170
    %GC content: 51.382488479262676
```

Transcript variant – 2

➔ **Summary -** Transcript Variant: This variant (2) lacks several exons and uses an alternate 3'-terminal exon, compared to variant 1. The encoded isoform (2) has a shorter and distinct C-terminus, compared to isoform 1.

➔ The transcript is a 758 bp linear mRNA.

➔ **Sequence –**

```
  1 gcagtgcgca ggcgtgagcg gtcgggcccc gacgcgcgcg ggtctcgttt ggagcgggag
 61 tgagttcctg agcgagtgga cccggcagcg ggcgataggg gggccaggtg cctccacagt
121 cagccatggc agcgctgcgc tacgcggggc tggacgacac ggacagtgag gacgagctgc
181 ctccgggctg ggaggagaga accaccaagg acggctgggt ttactacgcc aatcacaccg
241 aggagaagac tcagtgggaa catccaaaaa ctggaaaaag aaaacgagtg gcaggagatt
301 tgccatacgg atgggaacaa gaaactgatg agaacggaca agtgtttttt gttgaccata
361 taaataaaag aaccacctac ttggacccaa gactggcgtt tactgtggat gataatccga
421 ccaagccaac cacccggcaa agatacgacg gcagcaccac tgccatggaa attctccagg
481 gccgggattt cactggcaaa gtggttgtgg tcactggagc taattcagga atagggttcg
541 aaaccgccaa gtcttttgcc ctccatggtg cacatgtgat cttggcctgc aggaacatgg
601 caagggcgag tgaagcagtg tcacgcattt tagaagaatg gaaaacaaaa taccaccctc
661 cgccagaaaa gtgcagaata aaaattttcc actagcaaaa gaaggaaaaa ataaaagatc
721 ttgaatagtc tcatcaatta catcttcttt tgtgggta
```

➔ **GC content = 49.78%**

```
C:\Users\User\PycharmProjects\BioInformatics\venv\Scr
G nucleotide: 190
C nucleotide: 157
A nucleotide: 213
T nucleotide: 128
U nucleotide: 0
Total no. of nucleotides: 697
%GC content in transcript2.txt : 49.78479196556672
```

Transcript variant – 3

➔ **Summary:** This variant is a non-coding RNA (ncRNA). This variant (3) is intron less and extends past a splice site that is used in variant 1. This variant is represented as non-coding because the predicted protein does not meet RefSeq quality criteria.

➔ The transcript is 546 bp linear transcribed RNA.

➔ **Sequence:**

```
1    gcagtgcgca ggcgtgagcg gtcgggcccc gacgcgcgcg ggtctcgttt ggagcgggag
61   tgagttcctg agcgagtgga cccggcagcg ggcgataggg gggccaggtg cctccacagt
121  cagccatggc agcgctgcgc tacgcggggc tggacgacac ggacagtgag gacgagctgc
181  ctccgggctg ggaggagaga accaccaagg acggctgggt ttactacgcc aagtaagggg
241  gccgcagtgg ggccgcggac gcacctggga ccctgcacag cccacggacg ccacctgcgc
301  ggggaggacg cgcactccag cgcagcgcgt gcggtgcaaa gtgaaagtaa ctgttaagga
361  gcttcaggga aaagggtcca gggttcccag taggggccgg cccccttggt gggcctcggg
421  tccagcgggg gtcacctggt ggcttcccgg cgcgccctct gctgttcagg atgcagcact
481  gcgcggcgcg gcgagggcaa agcggcctca tccccgccaa aaaataaaga tgttttaaaa
541  agcgca
```

➔ **GC content – 65.97%**

```
C:\Users\User\PycharmProjects\BioInformatics\venv\Scri
G nucleotide: 177
C nucleotide: 141
A nucleotide: 96
T nucleotide: 62
U nucleotide: 0
Total no. of nucleotides: 482
%GC content in transcript3.txt : 65.97510373443983
```

Transcript variant – 4

➔ **Summary:** Transcript Variant: This variant (4) differs in its 5' UTR and uses a downstream start codon, compared to variant 1. The encoded isoform (4) has a shorter N-terminus, compared to isoform 1.

➔ The transcript is a 2176 bp linear mRNA.

➔ **Sequence -**

```
   1 gcagtgcgca ggcgtgagcg gtcgggcccc gacgcgcgcg ggtctcgttt ggagcgggag
  61 tgagttcctg agcgagtgga cccggcagcg ggcgataggg gggccaggtg cctccacagt
 121 cagccatggc agcgctgcgc tacgcggggc tggacgacac ggacagtgag gacgagctgc
 181 ctccgggctg ggaggagaga accaccaagg acggctgggt ttactacgcc aaatttgcca
 241 tacggatggg aacaagaaac tgatgagaac ggacaagtgt tttttgttga ccatataaat
 301 aaaagaacca cctacttgga cccaagactg gcgtttactg tggatgataa tccgaccaag
 361 ccaaccaccc ggcaaagata cgacggcagc accactgcca tggaaattct ccagggccgg
 421 gatttcactg gcaaagtggt tgtggtcact ggagctaatt caggaatagg gttcgaaacc
 481 gccaagtctt ttgccctcca tggtgcacat gtgatcttgg cctgcaggaa catggcaagg
 541 gcgagtgaag cagtgtcacg cattttagaa gaatggcata aagccaaggt agaagcaatg
 601 accctggacc tcgctctgct ccgtagcgtg cagcattttg ctgaagcatt caaggccaag
 661 aatgtgcctc ttcatgtgct tgtgtgcaac gcagcaactt ttgctctacc ctggagtctc
 721 accaaagatg gcctggagac cacctttcaa gtgaatcatc tggggcactt ctaccttgtc
 781 cagctcctcc aggatgtttt gtgccgctca gctcctgccc gtgtcattgt ggtctcctca
 841 gagtcccatc gatttacaga tattaacgac tccttgggaa aactggactt cagtcgcctc
 901 tctccaacaa aaaacgacta ttgggcgatg ctggcttata acaggtccaa gctctgcaac
 961 atcctcttct ccaacgagct gcaccgtcgc ctctccccac gcggggtcac gtcgaacgca
1021 gtgcatcctg aaatatgat gtactccaac attcatcgca gctggtgggt gtacacactg
1081 ctgtttacct tggcgaggcc tttcaccaag tccatgcaac agggagctgc caccaccgtg
1141 tactgtgctg ctgtcccaga actggagggt ctgggaggga tgtacttcaa caactgctgc
1201 cgctgcatgc cctcaccaga agctcagagc gaagagacgg cccggaccct gtgggcgctc
1261 agcgagaggc tgatccaaga acggcttggc agccagtccg gctaagtgga gctcagagcg
1321 gatgggcaca cacacccgcc ctgtgtgtgt cccctcacgc aagtgccagg gctgggcccc
1381 ttccaaatgt ccctccaaca cagatccgca agagtaaagg aaataagagc agtcacaaca
1441 gagtgaaaaa tcttaagtac caatgggaag cagggaattc ctgggggtaaa gtatcacttt
1501 tctggggctg ggctaggcat aggtctcttt gctttctggt ggtggcctgt ttgaaagtaa
1561 aaacctgctt ggtgtgtagg ttccgtatct ccctggagaa gcaccagcaa ttctcttctc
1621 tttactgtta tagaatagcc tgaggtcccc tcgtcccatc cagctaccac cacggccacc
1681 actgcagccg ggggctggcc ttctcctact tagggaagaa aaagcaagtg ttcactgctc
1741 cttgctgcat tgatccagga gataattgtt tcattcatcc tgaccaagac tgagccagct
1801 tagcaactgc tggggagaca aatctcagaa ccttgtccca gccagtgagg atgacagtga
1861 cacccagagg gagtagaata cgcagaacta ccaggtggca aagtacttgt catagactcc
1921 tttgctaatg ctatgcaaaa aattctttag agattataac aaattttttca aatcattcct
1981 tagatacctt gaaaggcagg aagggaagcg tatatactta agaatacaca ggatattttg
2041 ggggcagag aataaaacgt tagttaatcc ctttgtctgt caatcacagt ctcagttctc
2101 ttgctttcac attgtactta aacctcctgc tgtgcctcgc atcctatgct taataaaaga
2161 acatgcttga atatca
```

➔ **GC content: 50.74%**

```
C:\Users\User\PycharmProjects\BioInformatics\venv\Scri
G nucleotide: 535
C nucleotide: 549
A nucleotide: 536
T nucleotide: 486
U nucleotide: 0
Total no. of nucleotides: 2136
%GC content in transcript4.txt : 50.749063670411985
```

Transcript variant – 5

➔ **Summary:** This variant too is non-coding RNA. This variant (5) uses an alternate splice site in the 5'-terminal exon, lacks several exons, and uses an alternate 3'-terminal exon, compared to variant 1. This variant is represented as non-coding because the predicted protein does not meet RefSeq quality criteria.

➔ The transcript is 872 bp linear transcribed RNA.

➔ **Sequence:**

```
  1 gcagtgcgca ggcgtgagcg gtcgggcccc gacgcgcgcg ggtctcgttt ggagcgggag
 61 tgagttcctg agcgagtgga cccggcagcg ggcgataggg gggccaggtg cctccacagt
121 cagccatggc agcgctgcgc tacgcggggc tggacgacac ggacagtgag gacgagctgc
181 ctccgggctg ggaggagaga accaccaagg acggctgggt ttactacgcc aagtaagggg
241 gccgcagtgg ggccgcggac gcacctggga ccctgcacag cccacggacg ccacctgcgc
301 ggggaggacg cgcactccag cgcagcgcgt gcggtgcaaa gtgaaatcac accgaggaga
361 agactcagtg ggaacatcca aaaactggaa aaagaaaacg agtggcagga gatttgccat
421 acggatggga acaagaaact gatgagaacg gacaagtgtt ttttgttgac catataaata
481 aaagaaccac ctacttggac ccaagactgg cgtttactgt ggatgataat ccgaccaagc
541 caaccacccg gcaaagatac gacggcagca ccactgccat ggaaattctc cagggccggg
601 atttcactgg caaagtggtt gtggtcactg gagctaattc aggaataggg ttcgaaaccg
661 ccaagtcttt tgccctccat ggtgcacatg tgatcttggc ctgcaggaac atggcaaggg
721 cgagtgaagc agtgtcacgc attttagaag aatggaaaac aaaataccac cctccgccag
781 aaaagtgcag aataaaaatt ttccactagc aaaagaagga aaaaataaaa gatcttgaat
841 agtctcatca attacatctt cttttgtggg ta
```

➔ **GC content = 52.89%**

```
C:\Users\User\PycharmProjects\BioInformatics\venv\Script
G nucleotide: 235
C nucleotide: 195
A nucleotide: 235
T nucleotide: 137
U nucleotide: 0
Total no. of nucleotides: 813
%GC content in transcript5.txt : 52.89052890528906
```

➢ **<u>Summary of the GC content in the RefSeq and transcript variants</u>**

Sequence name	Length (bp)	GC content (%)
RefSeq Gene	1120014	42.65%
Transcript - 1	2242	51.38%
Transcript – 2	758	49.78%
Transcript - 3	546	65.97%
Transcript - 4	2176	50.74%
Transcript - 5	872	52.89%

➢ **Transcript variants combined analysis –**

o We see that the ncRNA have higher GC content compared to the coding mRNAs. But according to prevalent literature, lncRNAs prevail in the nucleus and have a lower GC content. (***Source:*** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3312569/).

  ▪ It has been mentioned in this (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4338330/) paper that the exon region of lncRNA are GC rich whereas intronic regions are AT rich. Thus, combining the information from both the sources, we come to the conclusion that transcript variant – 3 has more of the exonic regions. The transcript variant-5 has lesser exonic region and more of the intronic regions.

Protein Sequence

(Source: NCBI WWOX RefSeq protein

https://www.ncbi.nlm.nih.gov/protein/NP_057457.1,NP_570607.1,NP_001278926.1)

➤ The NCBI web site lists down 3 protein sequence for the gene.

Isoform – 1

➜ The length of the protein is 414 amino acids.

➜ This is the longest isoform out of all the 3 isoforms produced from the transcripts.

➜ This isoform is coded by translation of the 2242 nucleotide transcript variant-1.

➜ **Sequence:**

```
1    maalryagld dtdsedelpp gweerttkdg wvyyanhtee ktqwehpktg krkrvagdlp
61   ygweqetden gqvffvdhin krttyldprl aftvddnptk pttrqrydgs ttameilqgr
121  dftgkvvvvt gansgigfet aksfalhgah vilacrnmar aseavsrile ewhkakveam
181  tldlallrsv qhfaeafkak nvplhvlvcn aatfalpwsl tkdglettfq vnhlghfylv
241  qllqdvlcrs aparvivvss eshrftdind slgkldfsrl sptkndywam laynrsklcn
301  ilfsnelhrr lsprgvtsna vhpgnmmysn ihrswwvytl lftlarpftk smqqgaattv
361  ycaavpeleg lggmyfnncc rcmpspeaqs eetartlwal serliqerlg sqsg
```

➜ **Amino acid count:** ➜

```
Count        Percentage
Asp : 20     4.77326968973747
Thr : 33     7.875894988066825
Ser : 27     6.443914081145586
Glu : 27     6.443914081145586
Pro : 17     4.05727923627685
Gly : 25     5.966587112171838
Ala : 37     8.83054892601432
Cys : 8       1.9093078758949882
Val : 27     6.443914081145586
Met : 10     2.386634844868735
Ile : 10     2.386634844868735
Leu : 41     9.785202863961814
Tyr : 13     3.1026252983293556
Phe : 17     4.05727923627685
His : 14     3.341288782816229
Lys : 18     4.295942720763723
Arg : 27     6.443914081145586
Trp : 10     2.386634844868735
Gln : 14     3.341288782816229
Asn : 19     4.534606205250596

Process finished with exit code 0
```

➔ **3D structure -**
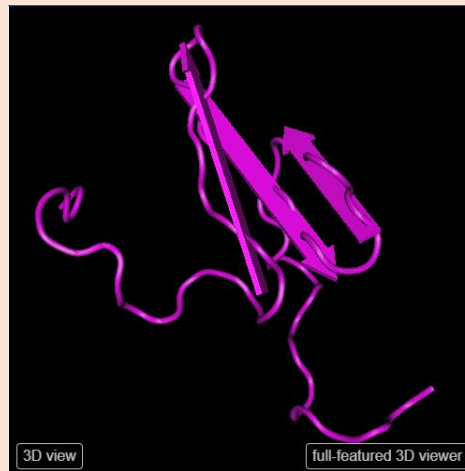
Isoform-2

➔ The length of the protein is 189 aa.

➔ **Summary:** This variant (2) lacks several exons and uses an alternate 3'-terminal exon, compared to variant 1. The encoded isoform (2) has a shorter and distinct C-terminus, compared to isoform 1.

➔ Translated product of transcript – 2.

➔ **Sequence:**

```
  1 maalryagld dtdsedelpp gweerttkdg wvyyanhtee ktqwehpktg krkrvagdlp
 61 ygweqetden gqvffvdhin krttyldprl aftvddnptk pttrqrydgs ttameilqgr
121 dftgkvvvvt gansgigfet aksfalhgah vilacrnmar aseavsrile ewktkyhppp
181 ekcrikifh
```

➔ **Amino acid count:** ➔

```
Count          Percentage
Asp : 13       6.806282722513089
Thr : 19       9.947643979057592
Ser : 6        3.1413612565445024
Glu : 16       8.37696335078534
Pro : 10       5.2356020942408374
Gly : 14       7.329842931937172
Ala : 15       7.853403141361256
Cys : 2        1.0471204188481675
Val : 11       5.75916230366649215
Met : 3        1.5706806282722512
Ile : 7        3.664921465968586
Leu : 10       5.2356020942408374
Tyr : 7        3.664921465968586
Phe : 7        3.664921465968586
His : 7        3.664921465968586
Lys : 13       6.806282722513089
Arg : 13       6.806282722513089
Trp : 5        2.6178010471204187
Gln : 5        2.6178010471204187
Asn : 6        3.1413612565445024

Process finished with exit code 0
```

➔ 3D structure:

Isoform – 4

➔ The length of the peptide is 301 aa.

➔ Coded by the transcript variant 4.

➔ Sequence:

```
1   meilqgrdft gkvvvvtgan sgigfetaks falhgahvil acrnmarase avsrileewh
61  kakveamtld lallrsvqhf aeafkaknvp lhvlvcnaat falpwsltkd glettfqvnh
121 lghfylvqll qdvlcrsapa rvivvssesh rftdindslg kldfsrlspt kndywamlay
181 nrsklcnilf snelhrrlsp rgvtsnavhp gnmmysnihr swwvytllft larpftksmq
241 qgaattvyca avpeleglgg myfnnccrcm pspeaqseet artlwalser liqerlgsqs
301 g
```

➢ Amino acid count: ➔

Count	Percentage
Asp : 8	2.622950819672131
Thr : 18	5.901639344262295
Ser : 25	8.19672131147541
Glu : 17	5.573770491803279
Pro : 10	3.278688524590164
Gly : 17	5.573770491803279
Ala : 30	9.836065573770492
Cys : 8	2.622950819672131
Val : 22	7.213114754098362
Met : 9	2.9508196721311477
Ile : 9	2.9508196721311477
Leu : 35	11.475409836065573
Tyr : 7	2.2950819672131146
Phe : 14	4.590163934426229
His : 11	3.606557377049181
Lys : 11	3.606557377049181
Arg : 19	6.229508196721312
Trp : 6	1.9672131147540985
Gln : 10	3.278688524590164
Asn : 15	4.918032786885246

→Structure →
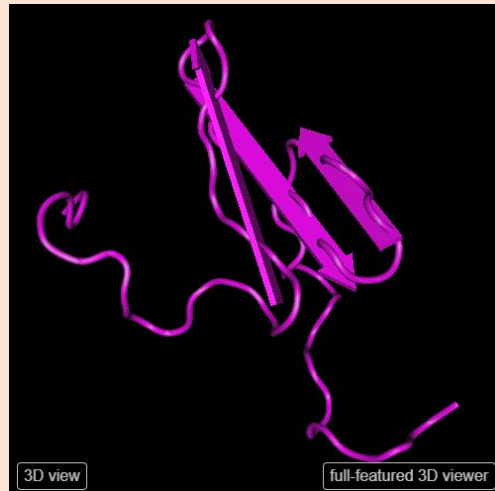
> **Combined Analysis of isoform 1, isoform 2 and isoform 4-**
>   o The three isoforms have similar structures; hence I propose that the functions performed by the 3 isoforms must be similar by the **structure function hypothesis.**
>   o The residue count of the 1 WMV functional protein is 54 aa. This infers one to say that all the 3 isoforms, specifically, isoform 1, isoform 2, isoform 4 are subject to **post-translational modifications** as the length of final protein is less than the aa count of the isoforms.

Exons/Introns in the gene

➜ Exon count -10 (source NCBI).

➜ List of Exons, Protein coding regions in the exons from ncbi sequence text view –

➜

Exon Numb	Exon start	Exon end	Co din	CDS start	CDS end
Exon -1	78,099,654	78,099,885	CDS	78,099,779	78,099,885
Exon-2	78,108,424	78,108,488	CDS	78,108,424	78,108,488
Exon-3	78,109,779	78,109,836	CDS	78,109,779	78,109,836
Exon-4	78,114,977	78,115,155	CDS	78,114,977	78,115,155
Exon-5	78,164,184	78,164,291	CDS	78,164,184	78,164,291
Exon-6	78,386,861	78,386,949	CDS	78,386,861	78,386,949
Exon-7	78,424,871	78,425,056	CDS	78,424,871	78,425,056
Exon-8	78,432,489	78,432,753	CDS	78,432,489	78,432,753
Exon-9	79,211,608	79,212,667	CDS	79,211,608	79,211,796

- **Analysis of intronic regions -** We can clearly see from the table that the intronic sequence length vastly outnumbers the exonic sequence length. The gap between exon-8 and exon-9 exceeds 750,000 base pairs (Quarter of a million bps).

  This might be paradoxical to the enery conserving nature of our cells as maintaining a large number of these non-coding sequences require vast energy investments from our body.

  But having these intronic sequences have various evolutionary benefits which justifies the energy cost in maintaining them, such as –

  o Introns regulate alternative splicing

  o Introns harbor several kinds of noncoding functional RNA genes such as miRNAs, siRNAs, piRNAs.

o Introns act as a buffer to dampen the mutation ill effects on the exons.
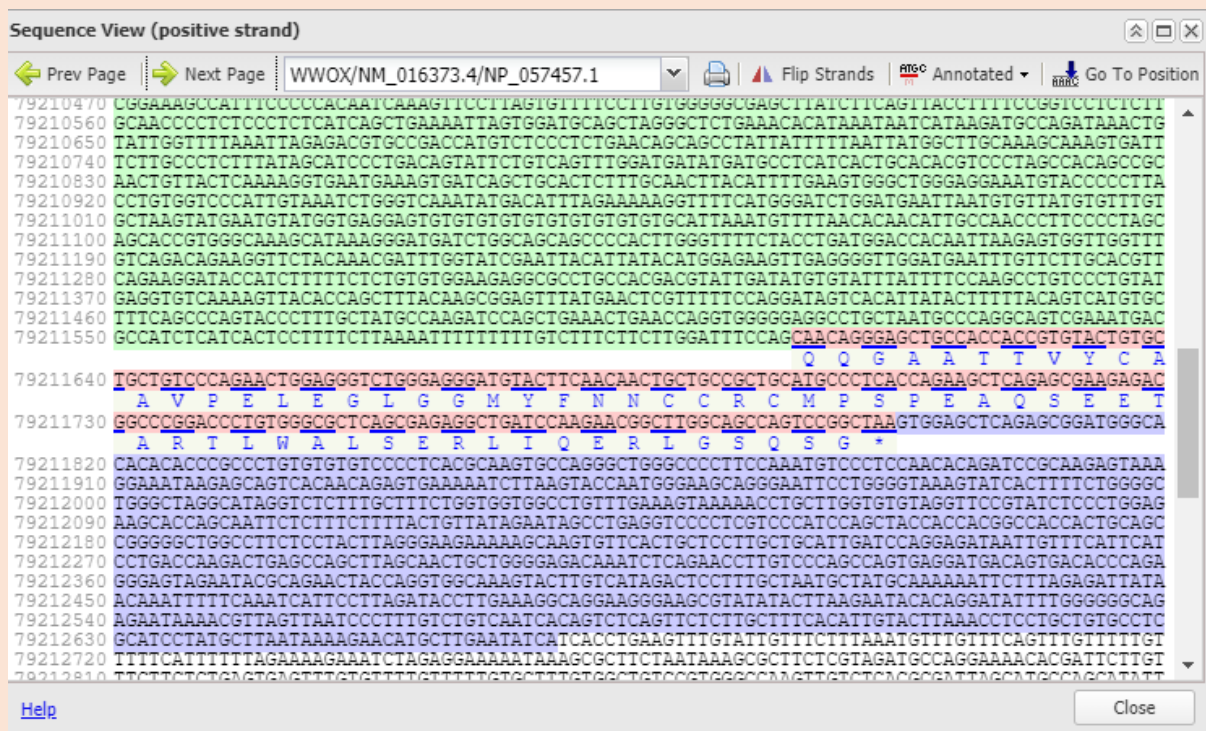
Figure 1 (above)

Screenshot of the sequence view of WWOX gene (part from 79,210,470 – 79,212,810)

Green – Intronic sequence; Red – Coding Domain sequence;

Blue – Non coding mRNA which is after/before transcription stop site/transcription start site respectively

Splice sites of the exons

Exon number	Start splice site	End splice site
Exon – 1	GGC,GCA	CAA,**GT**A
Exon – 2	C**AG**,TCA	GAG,**GT**T
Exon – 3	C**AG**,ATT	TGA,**GT**A
Exon – 4	C**AG**,CCA	TAG,**GT**A
Exon – 5	T**AG**,GGT	TGG,**GT**A
Exon – 6	C**AG**,CAT	TGT,**GT**G
Exon – 7	C**AG**,GCC	TCG,**GT**G
Exon – 8	A**AG**,ATT	ATG,**GT**A
Exon – 9	C**AG**,CAA	TAA,**GT**G

➤ Presence of **AG** at the acceptor splice of the intronic splice site. Acceptor site is the splicing site at the end of an intron, intron 3' right end.

➤ Presence of **GT** at the donor site of the exonic splice site. Donor site is the splicing site at the beginning of an intron, intron 5' left end.

→ Thus, the **GT/AG RNA rule is applicable for WWOX gene**.

Promoter regions of the gene

- **Procedure -** As told in the lab session, a promoter was looked from the ncbi site. The promoter sequence is a repeating sequence of at least three nucleotides present between the indices of -35 to -10 upstream of the gene WWOX.

    In the Blue box enclosed is the twice repeating sequence **CGGCGG,** which is the sequence to which the promoter binds.

  - The promoter sequence is unique in the sense, it does not contain a TATA box, a common feature to many promoters.
  - The promoter protein must spend a lot of energy to open the GCCGCC sequence to transcribe the gene.

- This gene might be transcribed at a lower rate due to presence of strong G-C bond at the promoter sequence.

Boundary sites of gene WWOX
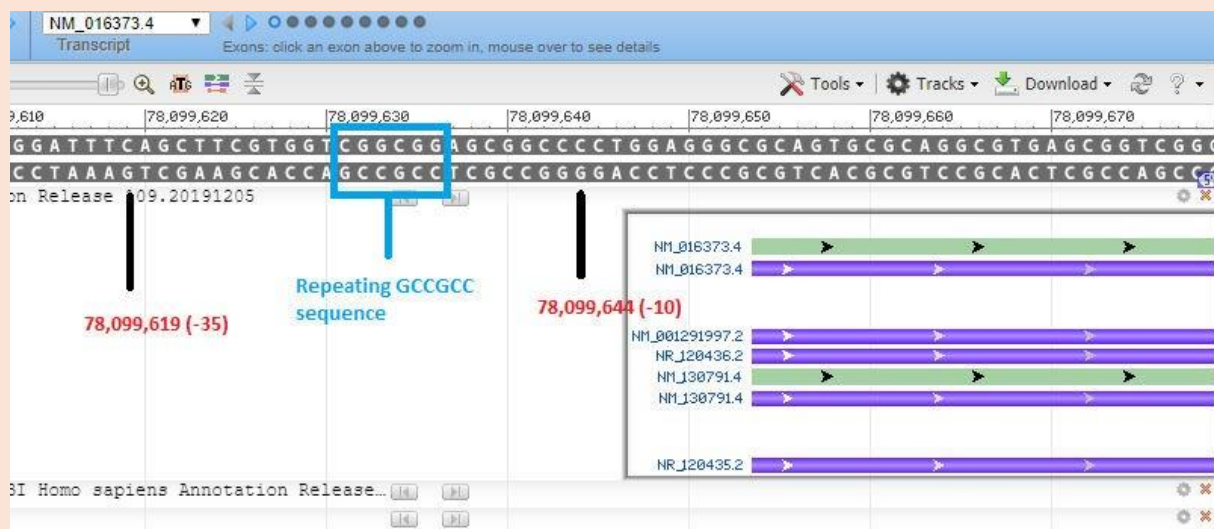
- **5 prime flanking sequence –**
    - CCGCCTTCCTCTCCAGGCCCTGCCCCTTTGACGCCGGCCG TCGCGATATTGCGGAGACTGGATTTCAGCTTCGTGGT**CG GCGG**AGCGGCCCCTGGAGGGC
- **3 prime flanking sequence –**
    - ATGCTTGAATATCATCACCTGAAGTTTGTATTGTTTCTTT AAATGTTTGTTTCAGTTTGTTTTTGTTTTTCATTTTTTAGA AAAGAAATCTAGAGGAAAAATAAA
- We can see the presence of many T's and A's at the end of the gene. This may be due to the fact that the flanking region immediately precedes the promoter region of gene after WWOX.

Dot-Plots

(Source: https://www.bioinformatics.nl/cgi-bin/emboss/dotmatcher)

1) WWOX gene self-plot

➢ The dot plot was performed by emboss dottup tool-

Dottup: fasta::emboss-dottup–I20200503–221505–0810–38402...
Sun 3 May 2020 22:15:23

➢ The gene doesn't show any special features such as palindromic repeats, direct or indirect repeats in its dot plot.

➢ There exists none low complexity sites in my gene which is established by the absence of any box like regions in the dot plot.

2) Transcript Dot Plots

➢ **Transcript- 1 dotplot:**

Transcript 1
(windowsize = 20, threshold = 40.00   03/05/20)
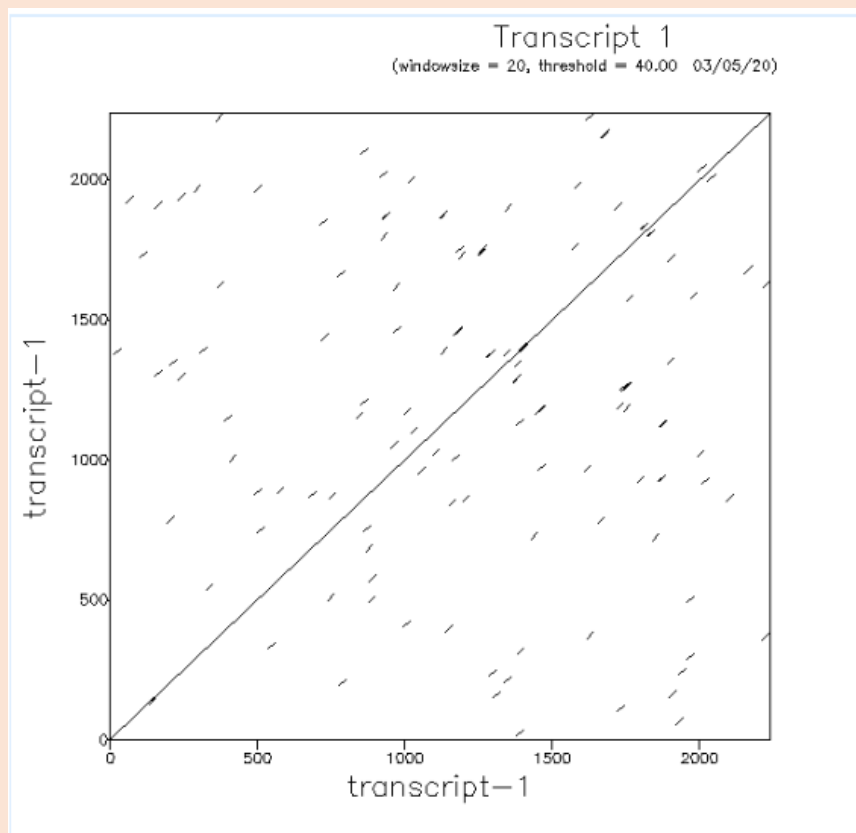
- **Analysis –**
  - o The single diagonal exists because the sequences on the X and Y axis are exactly similar.

➢ **Transcript-2 self-dot plot:**

Transcript 2
(windowsize = 15, threshold = 30.00 03/05/20)
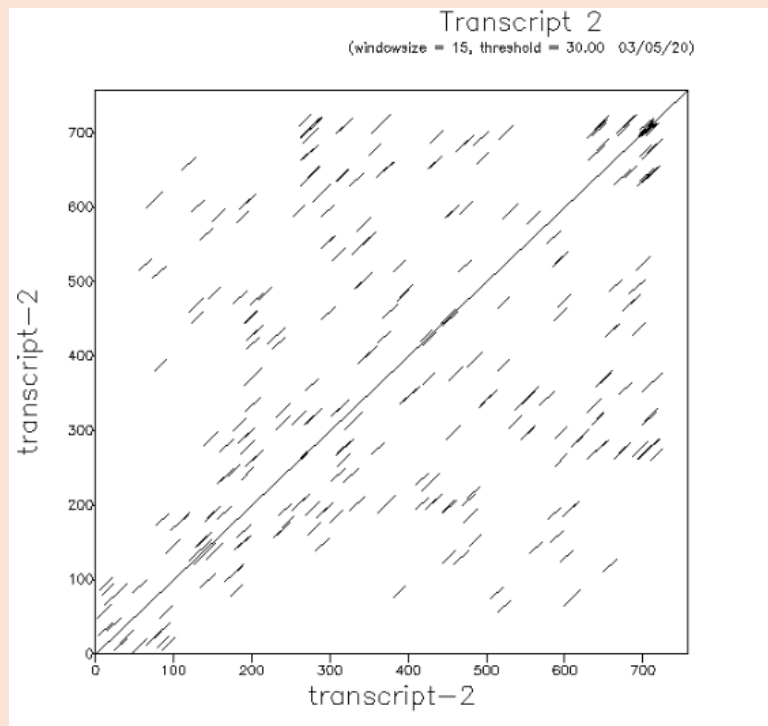
➢ **Transcript – 3 self-dot plot:**

Transcript 3
(windowsize = 15, threshold = 30.00 03/05/20)
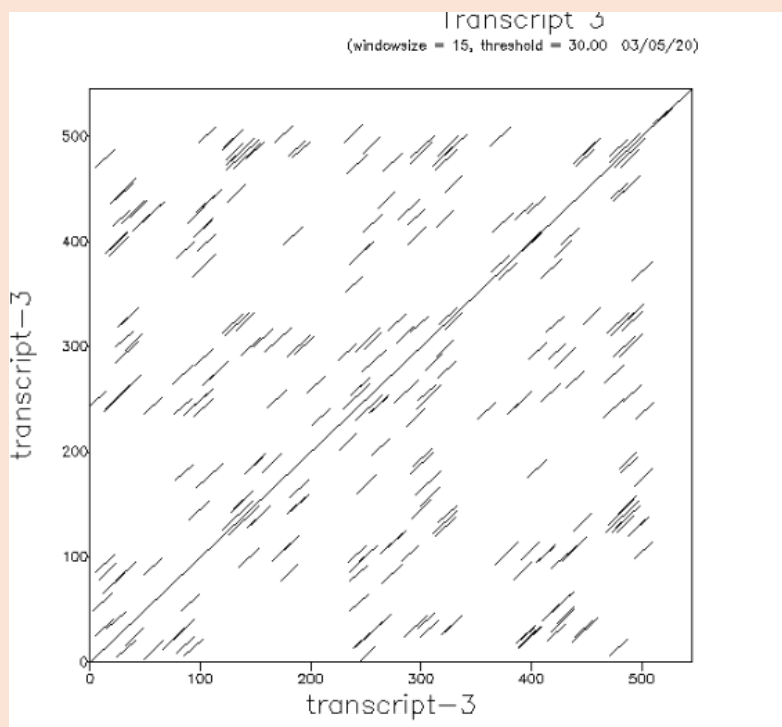
- Transcript 3 indeed contains many repeats under stringent conditions.
- The two sides divided by the diagonal are symmetric due to the fact that the dot plots have been performed for two exactly similar sequence.

➢ **Transcript – 4 self dot plot:**
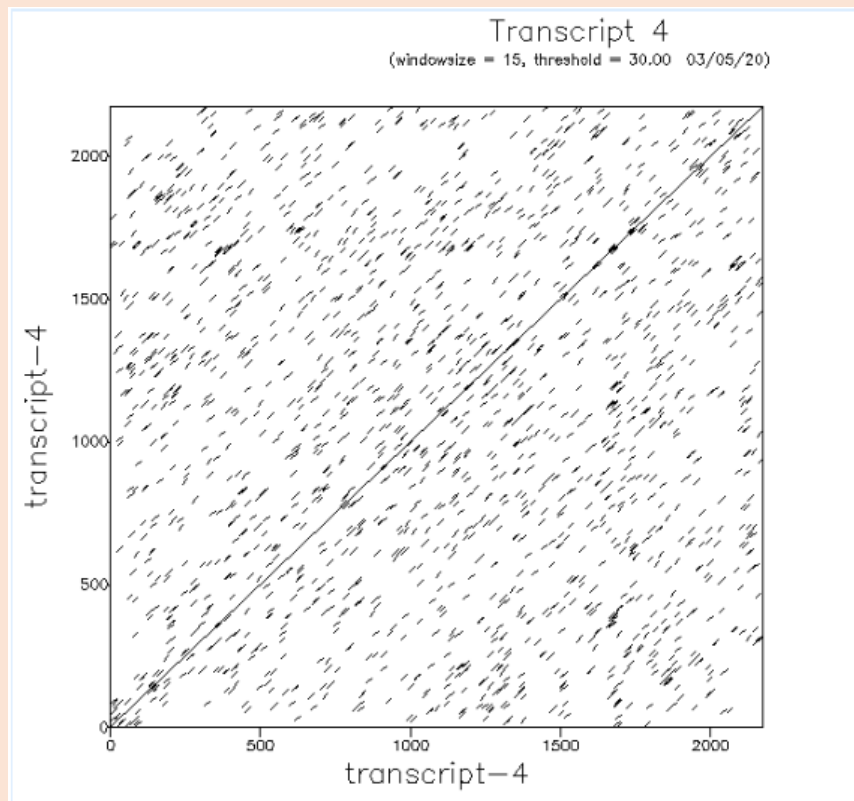
➢ **Transcript – 5 self dot plot:**
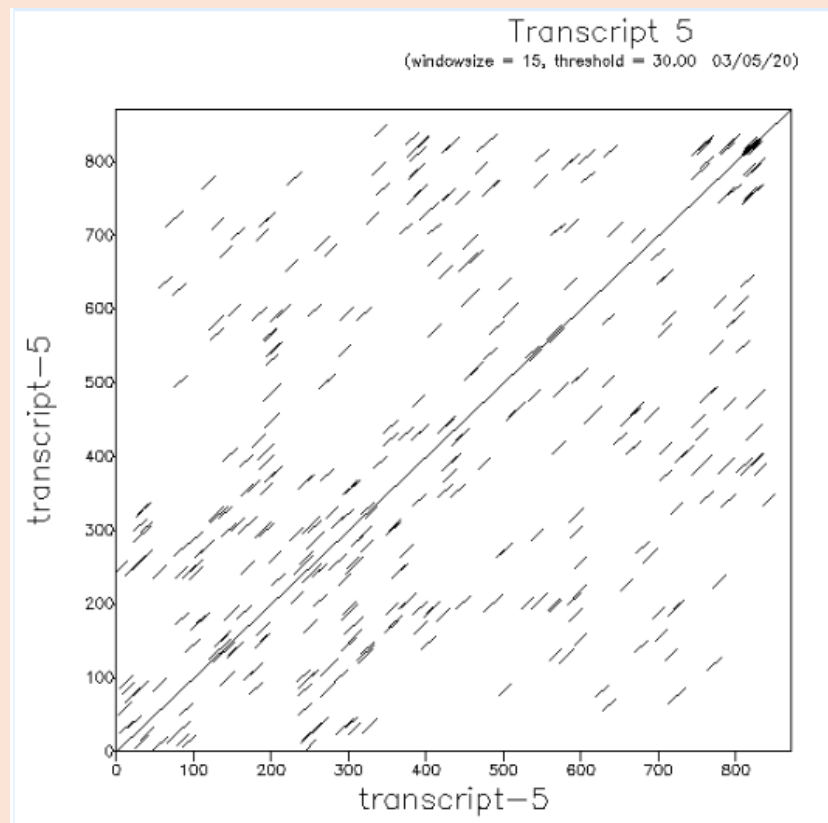
Protein sequence dot plots

- **Isoform 1 self-dot plot:**
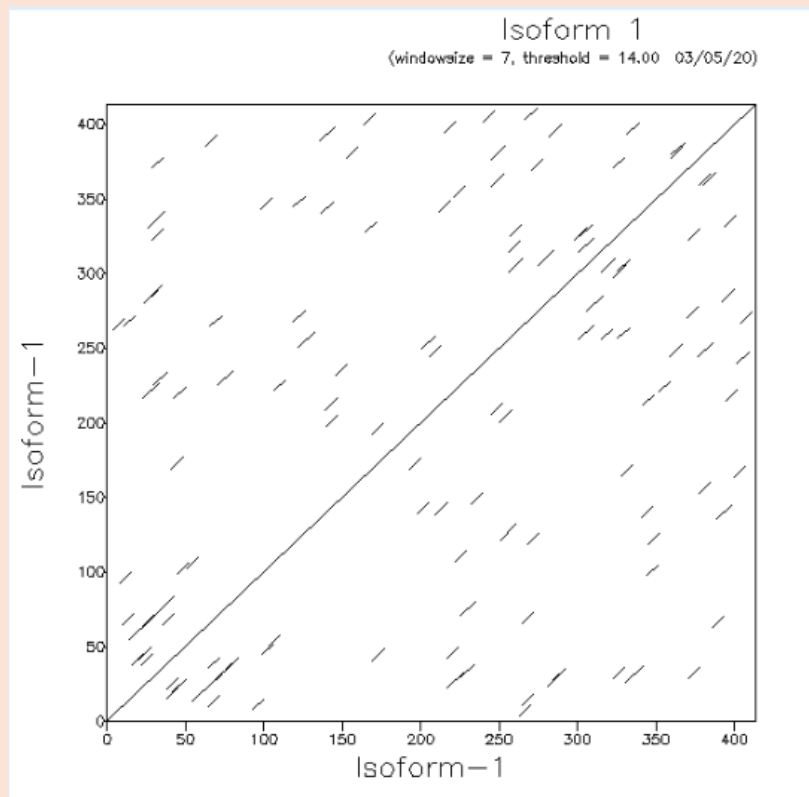
- **Isoform -2 self-dot plot:**
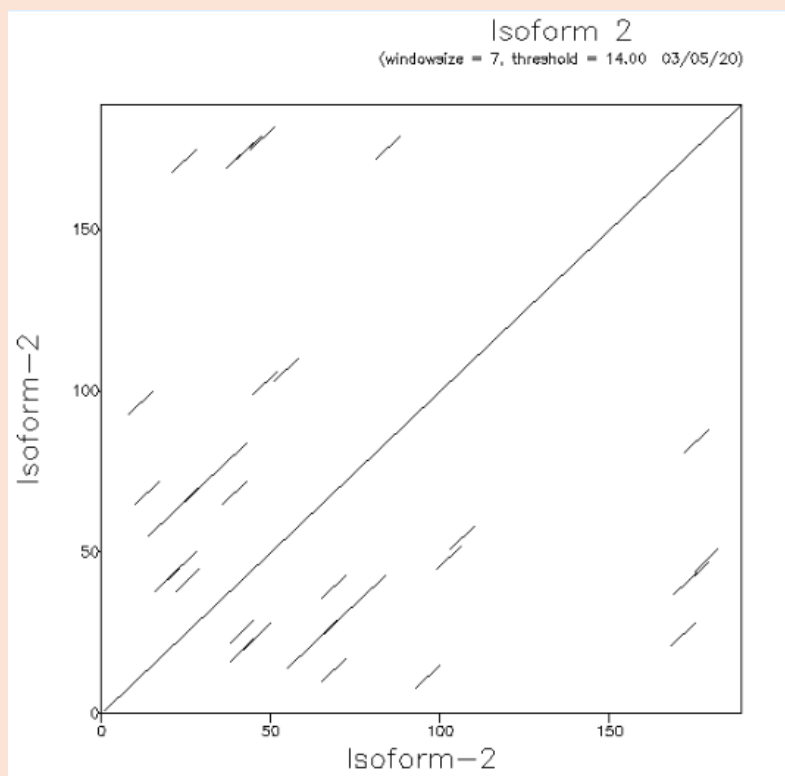
o We observe, that **isoform-2 has a repeated region between index 20-50 and 53-83**. The 30 aa length sequence are very close to each other.

o Upon inspection of transcript-2, which is the mRNA for Isoform-2, we should have found about 150 nt repeated sequence (in the plot, a straight line parallel to the diagonal extending for 150 nt).

   But we find no such line parallel to the diagonal. Thus, the **repetition in the peptide is due to the codon degeneracy and not due to the transcript nucleotide degeneracy**.

- **<u>Isoform 4 self dot plot:</u>**
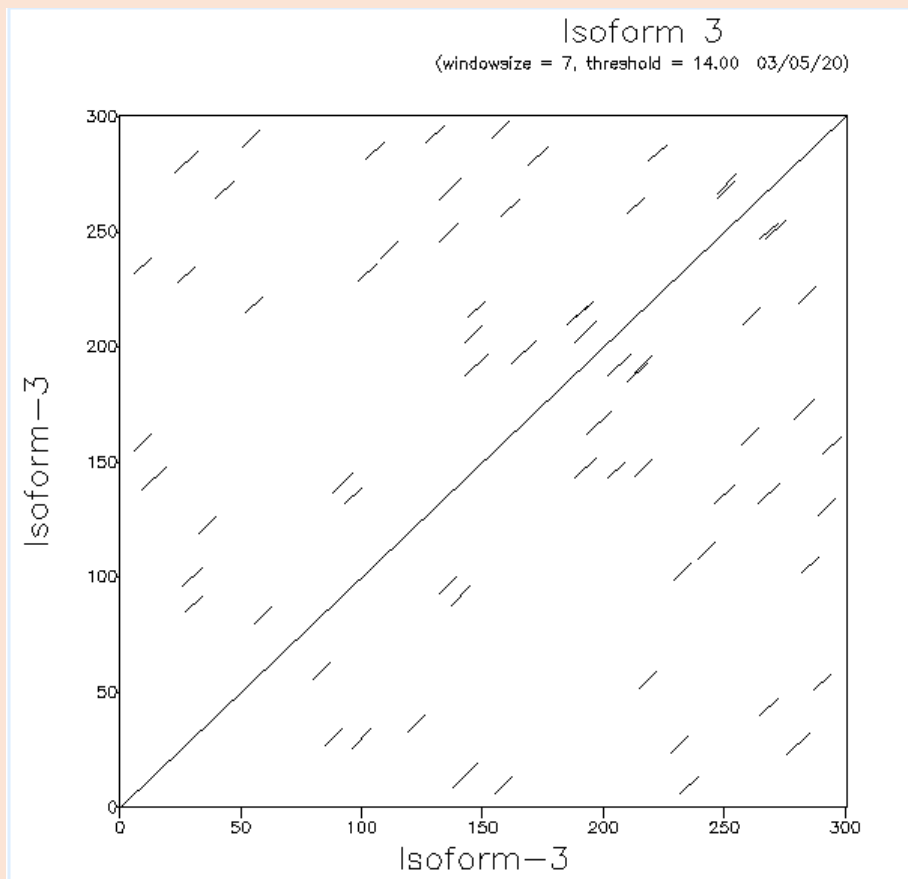
Cross Dot Plots (Between 2 different nt sequence pr different peptide sequence)

- **(Isoform -1) (Isoform – 4) :Cross Dot Plot:**
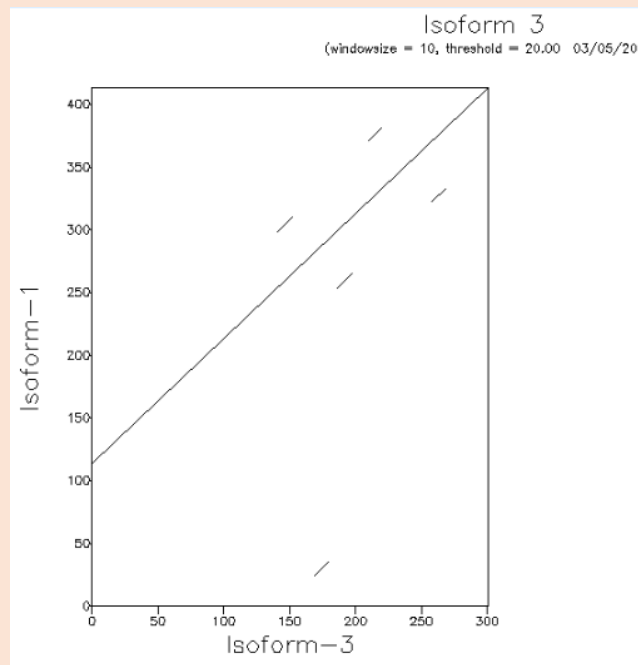
- Isoform 4 is exactly similar to peptide sequence indexed from 110-410 of isoform-4. Thus Isoform 4 is a truncated version of Isoform -1. This is because at index 110 of Isoform-1, the codon must be for methionine. If the tRNA carrying start codon, attaches at this position, then Isoform – 4 is formed, else Isoform -1 is formed. Transcript 1 and Trancript-4 too have a straight line almost passing through the diagonal except   some insertions in t4 due to **frameshifts around 250th nucleotide.**
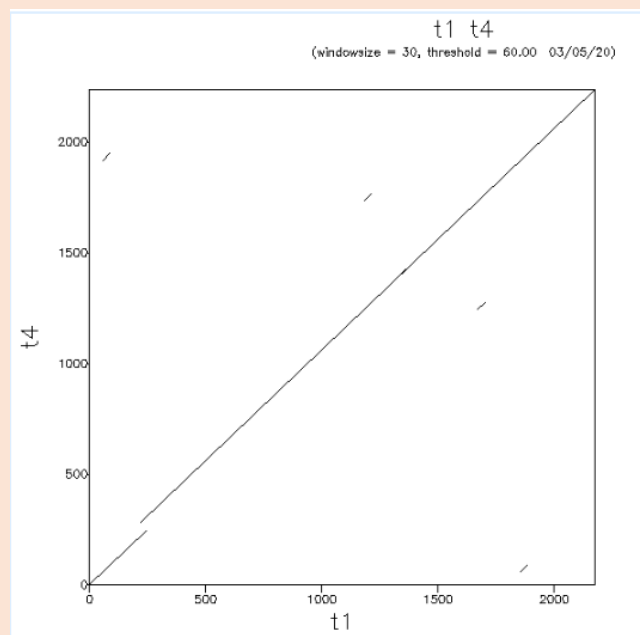
- The same is true for other cross plot also, Isoform -1 X Isoform-2.
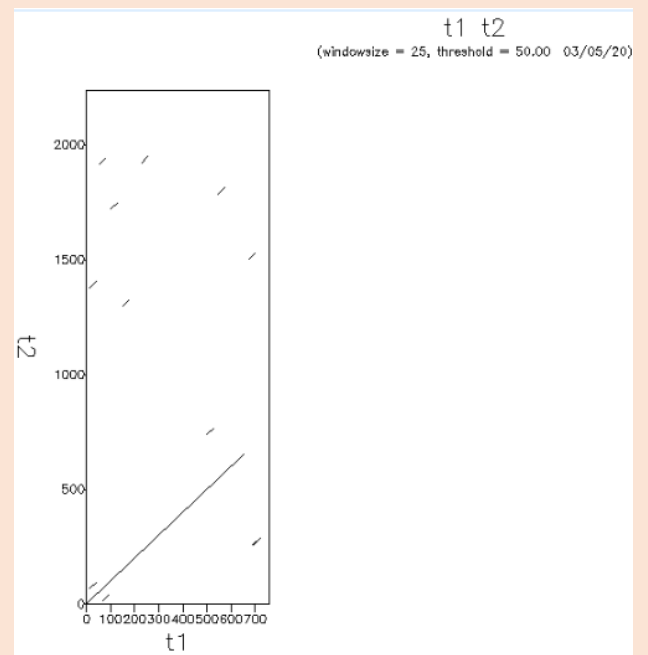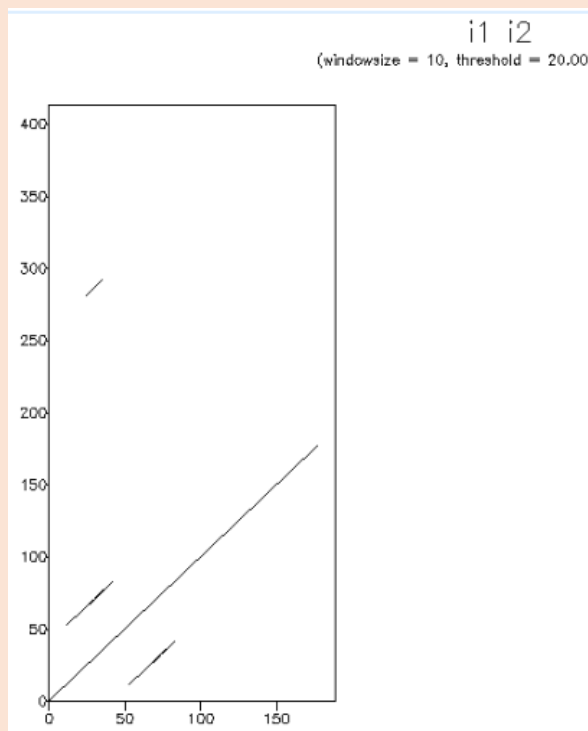
- ## **Cross Plot for Isoform 2 X Isoform 4-**
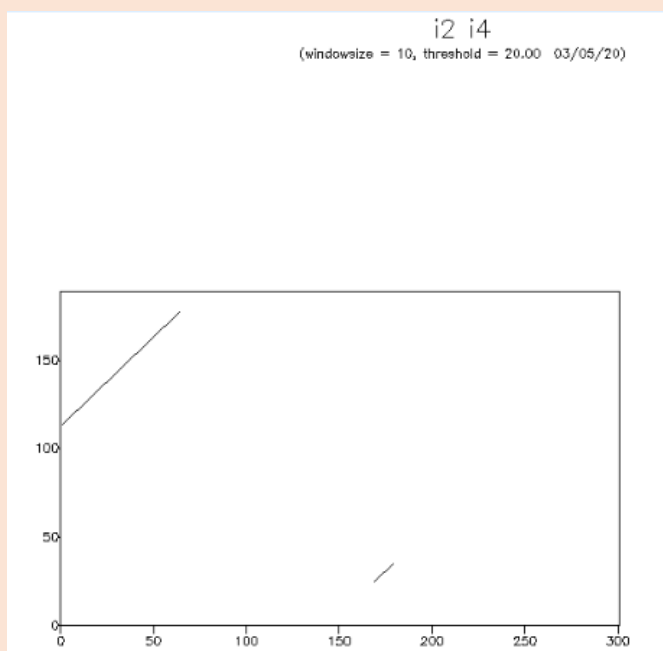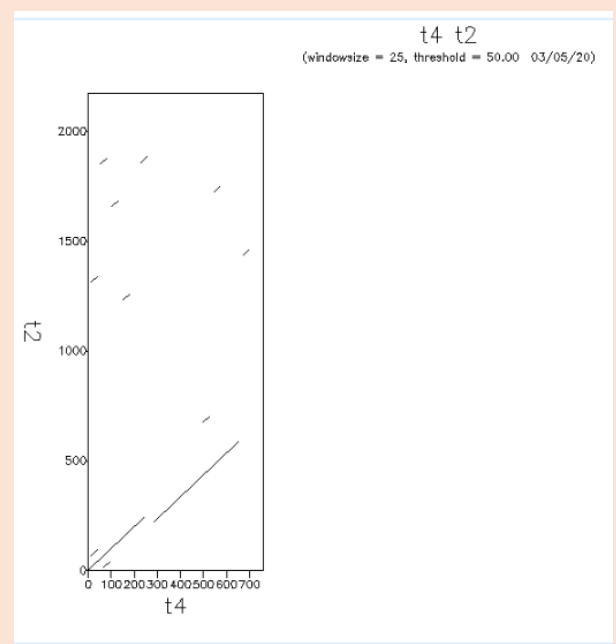
Isoform cross   **Transcript cross**

- But the cross between (Iso-2 and Iso-4) has different results than both the crosses (Iso-1 X Iso-2) and (Iso-1 X Iso-4). We see that a large part of Isoform 4 is different from Isoform-2 in spite of their transcripts sharing approximately 700 nucleotides similarity.

- Thus, I propose that the **start codon for Isoform-2 somewhere around 500th nucleotide** in transcript-2 and for **Isoform -4 at around 700th index in transcript-4 in backward direction** for such dotplots.

- ## Combined analysis of dotplots-

  - **We see a complete absence of –**

    - Low complexity regions i.e. the regions having a redundant nucleotide or amino acid within a small region, in both the transcript and isoform dot plots. (As no box like structure present in dot plots).

    - Inverted repeats (No perpendicular line) and Palindromic regions in the dotplots.

Top 10 prokaryotic and eukaryotic organisms similar by BLAST method

BLASTn for gene

Top 10 eukaryotic organisms-

Organism	Max. score	Total score	Query cover	E – value	% identity
Chlorocebus sabaeus	2361	2361	0 %	0.0	92.04
Pongo abelii [WW domain oxidoreductase]	1622	1622	0 %	0.0	94.49
Nomascus leucogenys [WW domain oxidoreductase]	1528	1528	0 %	0.0	92.69
Colobus angolensis [WW domain oxidoreductase]	1465	1465	0 %	0.0	91.83
Papio anubis [WW domain oxidoreductase]	1452	1452	0 %	0.0	91.60
Theropithecus gelada [WW domain oxidoreductase]	1452	1452	0 %	0.0	91.66
Macaca nemestrina [WW domain oxidoreductase]	1443	1443	0 %	0.0	91.29
Trachypithecus francoisi [WW domain oxidoreductase]	1435	1435	0 %	0.0	91.25
Panthera pardus	1382	1382	0 %	0.0	82.42
Mandrillus leucophaeus	1149	1149	0 %	0.0	94.30

➢ The BLASTn for the whole WWOX gene did not complete. So, instead of the whole gene, I took a **200k fragment of gene spanning from 780212667 to 78212667.** I

chose this fragment because it contains exon-9 which is used next for BLASTn to find prokaryotic matches. Moreover, for MSA and tree formation I needed to link the **200k fragment with exon-9.**

➢ **Conclusion:**

    ○ Top 10 eukaryotic sequence match was obtained by BLASTn.

Top 5 prokaryotic organisms-

○ The method adopted to get prokaryotic organisms is that we need to blast the exonic or coding sequences only, as the prokaryotic organisms are intron less and hence a full gene BLASTn would list them with a high E-value, and hence disregarding them from the database.

○ First, from the *Gene* database of NCBI, I searched for *WWOX* gene. In the table of contents in the upper right corner, go to *NCBI Reference Sequences.* (https://www.ncbi.nlm.nih.gov/gene/51741#reference-sequences).

○ From the reference sequences, we could choose the transcript sequence for BLASTn.

○ But, a more accurate approach would be to choose the exonic sequence as exons are continuous whereas the transcripts may be joined from many sequences.

○ I chose Exon-9 as the sequence to do a BLASTn due to its longer length compared to other exons. **A longer length exon would have a more reliable data as the chances of random matches decrease exponentially with increase in the length of query sequence.**

    • **Exon 9 sequence-**

```
1    caacagggag ctgccaccac cgtgtactgt gctgctgtcc cagaactgga gggtctggga
61   gggatgtact tcaacaactg ctgccgctgc atgccctcac cagaagctca gagcgaagag
121  acggcccgga ccctgtgggc gctcagcgag aggctgatcc aagaacggct tggcagccag
181  tccggctaag tggagctcag agcggatggg cacacacacc cgccctgtgt gtgtcccctc
241  acgcaagtgc cagggctggg cccttccaa atgtccctcc aacacagatc cgcaagagta
301  aaggaaataa gagcagtcac aacagagtga aaatcttaa gtaccaatgg gaagcaggga
361  attcctgggg taaagtatca cttttctggg gctgggctag gcataggtct ctttgctttc
421  tggtggtggc ctgtttgaaa gtaaaaacct gcttggtgtg taggttccgt atctccctgg
481  agaagcacca gcaattctct ttcttttact gttatagaat agcctgaggt cccctcgtcc
541  catccagcta ccaccacggc caccactgca gccgggggct ggccttctcc tacttaggga
601  agaaaaagca agtgttcact gctccttgct gcattgatcc aggagataat tgtttcattc
661  atcctgacca agactgagcc agcttagcaa ctgctgggga gacaaatctc agaaccttgt
```

```
721  cccagccagt gaggatgaca gtgacaccca gagggagtag aatacgcaga actaccaggt
781  ggcaaagtac ttgtcataga ctcctttgct aatgctatgc aaaaaattct ttagagatta
841  taacaaattt ttcaaatcat tccttagata ccttgaaagg caggaaggga agcgtatata
901  cttaagaata cacaggatat tttgggggggc agagaataaa acgttagtta atcccctttgt
961  ctgtcaatca cagtctcagt tctcttgctt tcacattgta cttaaacctc ctgctgtgcc
1021 tcgcatccta tgcttaataa aagaacatgc ttgaatatca
```

➢ When ***Expected threshold = 10*** and ***low complexity regions are masked,*** 0 hits were observed. Thus, we go to ***"Algorithmic parameters"*** in the BLASTn window in the bottom.

➢ Under "***Algorithmic Parameters***" –

  ▪ **Short query sequences -** Increase the ***Expected Threshold***, because short sequences may have expect value above threshold above default value 10, and hence may not be displayed.

  ▪ **Filtering -** Some of the BLAST programs mask regions of low complexity by default. These regions are not allowed to initiate alignments, so if your query is largely low complexity, the filter may prevent all hits to the database. On the Basic BLAST pages, adjust the filter settings in the section ***'Filters and Masking'***, under ***'Algorithm parameters'***.

➢ **Results- Match=2; Mismatch=-3; Gap-existence=5; extension=2**

Organism	Max. Score	Total Score	Query cover	E-value	% identity
Mycobacterium shinjukuense	48.2	48.2	4 %	0.9	83.33
Peptostreptococcaceae bacterium	48.2	48.2	3 %	0.9	89.47
Clostridium botulinum F str. 230613	46.4	46.4	3 %	3.1	91.18
Fusobacterium varium str NCTC10560	45.5	45.5	3 %	11	91.18
Bacillus velezensis str SGAir0473	45.5	45.5	3 %	11	86.49

➢ Only 5 top sequences were chosen, because of the low % identity of the sequences below. I had already decreased the stringency in the **"*Algorithmic parameters*"** before beginning the BLASTn. Thus, to prevent noise sequences from corrupting the data, I had to limit to top 5 prokaryotes.

➢ **Conclusion:**

○ My hypothesis behind lesser prokaryotic matches for WWOX is that, the WWOX protein 1 WMV has a complex tumour suppressor function in eukaryotes whereas in prokaryotes, it has oxidoreductase function only. Other enzymes for this such as *Molybdoterin oxidoreductase* exist which perform the same function.

○ Thus, this makes WWOX gene protein redundant and is found only in a handful of prokaryotes.

BLASTp for isoform-1

Eukaryotic matches

➢ I chose isoform-1 for BLASTp again because of its long size.

➢ Algorithmic parameters-

    o Expected Threshold = 100

    o Word size = 6

    o No masking and filtering of low complexity regions

➢ **Results:**

Organism	Max. Score	Total score	Query cover	E-value	% identity
Gorilla gorilla	867	867	99 %	0.0	99.76
Pongo abelii	867	867	100 %	0.0	99.28
Hylobates moloch	865	865	100 %	0.0	98.79
Pan troglodytes	865	865	100 %	0.0	99.28
Macaca fascicularis	859	859	100 %	0.0	98.07
Nomascus leucogenys	859	859	100 %	0.0	98.31
Rhinopithecus roxellana	858	858	100 %	0.0	97.83
Circocebus atys	857	857	100 %	0.0	97.83
Papio anubis	857	857	100 %	0.0	97.83
Mandrillus leucophaeus	856	856	100 %	0.0	97.83

➤ The top 10 eukaryotes in BLASTp are primates as it is in the case of BLASTn. This makes the conclusion evident that *Homo sapiens* share ancestral/sibling relationship with most of the primates.

Prokaryotic matches Results:

Organism name [Protein name]	Max. score	Total score	Query cover	E – value	% identity
Planctomycetes bacterium [SDR family oxidoreductase]	236	236	72 %	3e-71	43.05
Gemmatimonadetes bacterium [reductase SDR]	227	227	71 %	9e-68	41.22
Moraxellaceae bacterium [short chain dehydrogenase]	226	226	72 %	2e-67	39.40
SAR86 cluster bacterium [hypothetical protein COC19_02535]	219	219	72 %	5e-65	40.33
Chloroflexi bacterium [short chain dehydrogenase]	216	216	67 %	1e-63	43.10
BD1-7 clade bacterium [SDR family oxidoreductase]	215	215	71 %	4e-63	40.60
Acidobacteria bacterium [short chain dehydrogenase]	208	208	68 %	7e-61	43.11
Actinobacteria bacterium [SDR family NAD(P) dependent oxidoreductase]	209	209	73 %	7e-61	39.44
Acinetobacter johnsonii [SDR family oxidoreductase]	204	204	66 %	2e-59	42.81
Deltaproteobacteria bacterium [SDR family NAD(P) – dependent oxidoreductase]	205	205	73 %	5e-59	37.85

➢ **Conclusion for BLASTp: -** We immediately see that the query cover for prokaryotes dips in comparison to the eukaryotes. This is expected as prokaryotes and eukaryotes **diverged at least 1.5 billion years ago.**

o But, even after diverging, the primary function of WWOX gene protein remains oxidoreductase activity. In the prokaryotes, these enzymes perform the much important task of providing energy by ATP. In the eukaryotes, WWOX provides a tumour suppressor activity.

o The conclusion is that proteins retain their specific activity by 2 mechanisms-

1) Dampening the mutation end effect because of codon degeneracy.

2) Elimination of organisms having mutation severely altering the protein function.

P.T.O

Global alignment (Needleman-Wunsch) for DNA

Organism performing Global alignment with Homo Sapiens	Score	%Identity = (Match/Length)
Chlorocebus sabaeus (E)	11946.5	1.3 %
Pongo abelii (E)	8612.0	1.1 %
Nomascus leucogenys (E)	8547.0	1.1 %
Colobus angolensis (E)	1972.5	0.6 %
Papio anubis (E)	7536	1.0 %
Therapithecus gelada (E)	4697.0	0.5 %
Macaca nemestrina (E)	3495.0	0.4 %
Trachypithecus francoisi (E)	3322.0	0.4 %
Panthera pardus (E)	1287.5	0.4 %
Mandrillus leucophaeus (E)	4034.0	0.5 %
Mycobacterium shinjukuense (P)	162	3.8 %
Peptostreptococcaceae bacterium (P)	148	3.2 %
Clostridium botulinum F str. 230613 (P)	140.5	2.9 %
Fusobacterium varium strain NCTC10560 (P)	73.5	2.3 %
Bacillus velezensis strain SGAir0473 (P)	97.5	2.3 %

- The gene used for alignment with eukaryotic organisms was a part of the WWOX genome. I did this so that the time and the efficiency of the algorithm gets better. The

part used for alignment spanned from **79012667 – 79212667(200k base pairs).** This span of gene contains the **exon-9** which is BLAST alignment to find the eukaryotes.

- For prokaryotic global alignment, **exon – 9** was used.

- E = eukaryotic organisms, P = prokaryotic organisms

- **Analysis and conclusion:**

  ○ The order of closeness of *Homo sapiens* with other organisms is different in the case of BLASTn and DP method. Example – *Papio anubis* has higher DP score than *Colobus angolensis* even though the latter is mentioned above in BLAST list. This might be because of the heuristic nature of BLAST.

P.T.O

Global alignment (Needleman – Wunsch) for protein

Organism	Global alignment score	% identity
Gorilla gorilla (E)	2193.0	99.5
Pongo abelii (E)	2194.0	99.3
Hylobates moloch (E)	2188	98.8
Pan troglodytes (E)	2188	99.3
Macaca fascicularis (E)	2172	98.1
Nomascus leucogenys (E)	2175	98.3
Rhinopithecus roxellana (E)	2169	97.8
Circocebus atys (E)	2167	97.8
Papio anubis (E)	2165	97.8
Mandrillus leucophaeus (E)	2164	97.8
Planctomycetes bacterium (P)	589	31.4
Gemmatimonadetes bacterium (P)	564	29.5
Moraxellceae bacterium (P)	561	28.5
SAR86 cluster bacterium (P)	575.5	29.4
Chloroflexi bacterium (P)	532.5	29.3
BD1-7 clade bacterium (P)	532	29.2
Acidobacteria bacterium (P)	518.5	29.5
Actinobacteria bacterium (P)	533.5	29.4

Acinetobacter bacterium (P)	522.5	29.1
Deltaproteobacteria bacterium (P)	525	28.3

➤ EMBOSS Needle reads two input sequences and writes their optimal global sequence alignment to file. It uses the Needleman-Wunsch alignment algorithm to find the optimum alignment (including gaps) of two sequences along their entire length.

➤ The scoring matrix used is EBLOSUM62

       o Default Gap opening score = -10.0

       o Gap extension score = -0.5

P.T.O

ClustalW MSA

MSA for gene

- To get better analysis of the MSA for WWOX, performed the following approach-

- As, the BLASTn query sequence was different for prokaryotes and the eukaryotes. For prokaryotes, query sequence was **exon-9**, whereas for eukaryotes, query sequence was a **200k fragment of WWOX spanning from 78500000 to 78700000.**

- Thus, we perform 2 different Clustal omega MSA, one with **exon-9** and other prokaryotic sequences and the second MSA of the **200k fragment of WWOX spanning from 78500000 to 78700000** with the eukaryotic sequences.

> ## ClustalW MSA for eukaryotic sequences, with the 200k fragment of WWOX + exon -9

- o As we have BLAST for eukaryotes on **200k fragment** and prokaryotes on **exon-9** on different segments, we perform 2 different global alignments –
  1) MSA of all the sequences i.e. **200k fragment, exon-9,** top 5 prokaryotic BLAST, top 10 eukaryotic blasts.
  2) MSA of prokaryotic sequences and **exon-9.**

The 1st MSA of top 5 prokaryotic BLAST, top 10 eukaryotic BLAST, top 5 prokaryotic sequences, **exon-9, 200k fragment.**

The result was too long and empty. **Hence, I have written here the MSA with at least 2 sequences, rest all omitted parts was empty 200k fragment without any alignment.**

```
Panthera               AACTGACAGCTTCCATCTTGAACAAATAGACTAGTGTTCATTTGGATGTTCTCGGGCTCA    60
Fusobacterium          ------------------------------------------------------------    0
Bacillus               ------------------------------------------------------------    0
Chlorocebus            ------------------------------------------------------------    0
Mycobacterium          ------------------------------------------------------------    0
Clostridium            ------------------------------------------------------------    0
Peptostreptococcaceae  ------------------------------------------------------------    0
Macaca                 ------------------------------------------------------------    0
```

```
Trachypithecus          ------------------------------------------------------------  0
Colobus                 ------------------------------------------------------------  0
Mandrillus              ------------------------------------------------------------  0
Papio                   ------------------------------------------------------------  0
Theropithecus           ------------------------------------------------------------  0
Nomascus                ------------------------------------------------------------  0
Pongo                   ------------------------------------------------------------  0
Exon-9                  ------------------------------------------------------------  0
790212667               ------------------------------------------------------------  0


Panthera                CGTGGCACCCACTCCAACAGCTGCTTGCCGAGTGAATTTAGACAATGTTCTGGATGCCAG  120
Fusobacterium           ------------------------------------------------------------  0
Bacillus                ------------------------------------------------------------  0
Chlorocebus             ------------------------------------------------------------  0
Mycobacterium           ------------------------------------------------------------  0
Clostridium             ------------------------------------------------------------  0
Peptostreptococcaceae   ------------------------------------------------------------  0
Macaca                  ------------------------------------------------------------  0
Trachypithecus          ------------------------------------------------------------  0
Colobus                 ------------------------------------------------------------  0
Mandrillus              ------------------------------------------------------------  0
Papio                   ------------------------------------------------------------  0
Theropithecus           ------------------------------------------------------------  0
Nomascus                ------------------------------------------------------------  0
Pongo                   ------------------------------------------------------------  0
Exon-9                  ------------------------------------------------------------  0
790212667               ---------------------------------------------TATCCAGGCAGAATG  15


Panthera                TTCTCTCATTGCAAATGGGAAGAGATTTACAAAGCAGCAGCCGCTTGTCATGCGGAAATA  180
Fusobacterium           ------------------------------------------------------------  0
Bacillus                ------------------------------------------------------------  0
Chlorocebus             ------------------------------------------------------------  0
Mycobacterium           ------------------------------------------------------------  0
Clostridium             ------------------------------------------------------------  0
Peptostreptococcaceae   ------------------------------------------------------------  0
Macaca                  ------------------------------------------------------------  0
Trachypithecus          ------------------------------------------------------------  0
Colobus                 ------------------------------------------------------------  0
Mandrillus              ------------------------------------------------------------  0
Papio                   ------------------------------------------------------------  0
Theropithecus           ------------------------------------------------------------  0
Nomascus                ------------------------------------------------------------  0
Pongo                   ------------------------------------------------------------  0
Exon-9                  ------------------------------------------------------------  0
790212667               AATAGTCTATG--GAAAGGGCTGGAAGTTCCACAAAGCAGGACGTATACGTGCCCTTTTT  73


Panthera                ATTACGGGTCAAAGTCTCCAAATCCCTGAGTTGCCCCGGGTATGTGTCTCAGTCC----T  236
Fusobacterium           ------------------------------------------------------------  0
Bacillus                ------------------------------------------------------------  0
Chlorocebus             -----------------------------------------TCTGTTCAGCTGA---A  14
Mycobacterium           ------------------------------------------------------------  0
Clostridium             ------------------------------------------------------------  0
Peptostreptococcaceae   ------------------------------------------------------------  0
Macaca                  ------------------------------------------------------------  0
Trachypithecus          ------------------------------------------------------------  0
Colobus                 ------------------------------------------------------------  0
Mandrillus              ------------------------------------------------------------  0
Papio                   ------------------------------------------------------------  0
Theropithecus           ------------------------------------------------------------  0
Nomascus                ------------------------------------------------------------  0
Pongo                   ------------------------------------------------------------  0
Exon-9                  ------------------------------------------------------------  0
790212667               TCCTCCCCTGGTGGCCAGCCAGCCCCTGAAACACAGCAGGTATTCGGTGCAGCCTTCTGT  133


Panthera                GGGAGCCAGAGCACCCCACCCC---AGGACGACCCTCTTCAGAGAGTGAGGGTGGCATA-  292
Fusobacterium           ------------------------------------------------------------  0
Bacillus                ------------------------------------------------------------  0
Chlorocebus             GTTCGTCTGACTCCATTGTCTCGCTTTTTC--CCCTTAGCTAGGCCC-AGGAAATCAGG-  70
```

```
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              TTTCTTCCAGATCCTTTGACTC---CTGCC--CCACCAACATGGCCCGTGGCAGACTGGG  188


Panthera               -CACCCCAAGA-TGACAGGCAAAGGCTAGGAGGCTGGAAGGGAGAAATAAATCCCGAGTC  350
Fusobacterium          ------------------------------------------------------------  0
Bacillus               ------------------------------------------------------------  0
Chlorocebus            -CACCTTCTGTGTTTCATACACTATCT----TTCTCCAGGGCCAAGACCATACCGAGAAC  125
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              CTGCTGTTTGTAAGCCTGGCTGAAGAAAGGCATCACAAAGAAAATGCCACCAGCAGGCTG  248


Panthera               AACGAGAAGG---ATTAAAAAGTCACCTACCATGAGG---------AGAATTCGCACTCC  398
Fusobacterium          ------------------------------------------------------------  0
Bacillus               ------------------------------------------------------------  0
Chlorocebus            CCTGCAGCAACCTAGGAAAAGCACTCCAACCCAATGGCAAGTCACAGGCAAAGGCACAGT  185
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              GGTGTGGTGGCTCATGCCTGTATTCCCAGCCCTTTGGGAGGCCGAGGCGGGTGGATCGCC  308


Panthera               -----ACAAATTTTTCTCGATTGAGTAAATTTAA--------AGAAATAAAACTAGATTG  445
Fusobacterium          ------------------------------------------------------------  0
Bacillus               ------------------------------------------------------------  0
Chlorocebus            AGTG-TCTTGTGGTCCCCGACACATCCAAATTTG--------AAAGACAGAGGTTCAGGA  236
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              TGAGGTCAGGAAGAACCTGGGAGATTGGAGGTTGCAGTGAGCTGAGATCGTGCCACTGCA  368
```

```
Panthera                   AGCATGCGTTTAATACTGGCTGCTATCTTTTGCTCTGCTCTGCTCAGTGGCTTGTTAAAT    505
Fusobacterium              ------------------------------------------------------------    0
Bacillus                   ------------------------------------------------------------    0
Chlorocebus                TGACAGCAGGAG-AAA-GGCCCCTTGCAT---ATCATACCTGGTCCTTGGATTTCTGCCT    291
Mycobacterium              ------------------------------------------------------------    0
Clostridium                ------------------------------------------------------------    0
Peptostreptococcaceae      ------------------------------------------------------------    0
Macaca                     ------------------------------------------------------------    0
Trachypithecus             ------------------------------------------------------------    0
Colobus                    ------------------------------------------------------------    0
Mandrillus                 ------------------------------------------------------------    0
Papio                      ------------------------------------------------------------    0
Theropithecus              ------------------------------------------------------------    0
Nomascus                   ------------------------------------------------------------    0
Pongo                      ------------------------------------------------------------    0
Exon-9                     ------------------------------------------------------------    0
790212667                  TGTCAGCCTGAGCAAC-AGAGCAAGACTC---CGTTTCACAAAAAAAGGAAATGCTACCA    424


Panthera                   ATAA-----AAAGATTTGCCCTGCAAGG------AGACGTGGGGAGAGGCAATCTCAGA-    553
Fusobacterium              ------------------------------------------------------------    0
Bacillus                   ------------------------------------------------------------    0
Chlorocebus                TTCC-----ATCTTGGATCACTCCCAGGTACCTTTGCCTGGTGGATACATTGTTCTCCTC    346
Mycobacterium              ------------------------------------------------------------    0
Clostridium                ------------------------------------------------------------    0
Peptostreptococcaceae      ------------------------------------------------------------    0
Macaca                     ------------------------------------------------------------    0
Trachypithecus             ------------------------------------------------------------    0
Colobus                    ------------------------------------------------------------    0
Mandrillus                 ------------------------------------------------------------    0
Papio                      ------------------------------------------------------------    0
Theropithecus              ------------------------------------------------------------    0
Nomascus                   ------------------------------------------------------------    0
Pongo                      ------------------------------------------------------------    0
Exon-9                     ------------------------------------------------------------    0
790212667                  GTGAGCAGGAAGTTGGTGGTCCTCCAGA----ATTGCCCTGTTGAGGGTATCTCCCTGCC    480


Panthera                   -AGGACCCATCAGCCACCCCGCCCCTCCTCCTTGTAACCCAGGGA---AGACGGACAGCC    609
Fusobacterium              ------------------------------------------------------------    0
Bacillus                   ------------------------------------------------------------    0
Chlorocebus                CATATCACAGAGCATTCCTAGTCCTTCCCGGGTGCAACCACAGGTATCAGTAAATAAACG    406
Mycobacterium              ------------------------------------------------------------    0
Clostridium                ------------------------------------------------------------    0
Peptostreptococcaceae      ------------------------------------------------------------    0
Macaca                     ------------------------------------------------------------    0
Trachypithecus             ------------------------------------------------------------    0
Colobus                    ------------------------------------------------------------    0
Mandrillus                 ------------------------------------------------------------    0
Papio                      ------------------------------------------------------------    0
Theropithecus              ------------------------------------------------------------    0
Nomascus                   ------------------------------------------------------------    0
Pongo                      ------------------------------------------------------------    0
Exon-9                     ------------------------------------------------------------    0
790212667                  CTGGTCTGAAAGCTGCCACTTGCCGCCCACCGCACAGCCCCAGGGCTTTAGCTCACTGTG    540


Panthera                   CTGTTTCATT-CT-----AGCGCC-CCAGCGCCCCGGTCTTGTGTACAGGCCCCAC----    658
Fusobacterium              ------------------------------------------------------------    0
Bacillus                   ------------------------------------------------------------    0
Chlorocebus                CTCATTGAAA-CAGCAGAAGATCA-CTAAGCACTTGTAAAAGAGAATTTGATAATCAGAT    464
Mycobacterium              ------------------------------------------------------------    0
Clostridium                ------------------------------------------------------------    0
Peptostreptococcaceae      ------------------------------------------------------------    0
Macaca                     ------------------------------------------------------------    0
Trachypithecus             ------------------------------------------------------------    0
Colobus                    ------------------------------------------------------------    0
Mandrillus                 ------------------------------------------------------------    0
Papio                      ------------------------------------------------------------    0
Theropithecus              ------------------------------------------------------------    0
Nomascus                   ------------------------------------------------------------    0
Pongo                      ------------------------------------------------------------    0
```

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Exon-9              ------------------------------------------------------------   0
790212667           CAGGAAGAGGCCTTCCTCTGTGCATCCTCCCACAGGGTCGAGAGTGAATGCCTTGCCTTT   600


Panthera            ---------ATG--CGATCACCGGTATCCCCACTGTCT--CTTAATAGAAGG-TGAGATA   704
Fusobacterium       ------------------------------------------------------------   0
Bacillus            ------------------------------------------------------------   0
Chlorocebus         CAATATAAGTTGAACAAAAGTAGGAAACCAAAAAAAAAGCCTAATAAGTAGCCTGGAATG   524
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae  ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           AAAAATAAAATG--CATACGCCTGTCCTCATGCGCTCTGATCTGCCAGCAGGCTGTGCTG   658


Panthera            TAAAAAGCTCATTCAA-----GATGCCAGGAGCTTCCCTTTCTGACCCTGGCTGCCTGAG   759
Fusobacterium       ------------------------------------------------------------   0
Bacillus            ------------------------------------------------------------   0
Chlorocebus         GAGCAAAATTGGACTT-----GAATCCACTTACTAGTTGTGTACACTTGAGAAAGTGACT   579
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae  ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           GGGAGTGGTGAGTAGTACAGGGATGTGTCTGACCCCAGGAGAACTGCCCCGATCCCTATC   718


Panthera            AGGCAAATTG---CATTAGAAAAATGACATGCCATTCATCTCGCGAGTACTTGCAGGAAC   816
Fusobacterium       ------------------------------------------------------------   0
Bacillus            ------------------------------------------------------------   0
Chlorocebus         CAG-TTTCTGTGCCTTGGTTCTTTCATCTCTGAAATCA------GGG--CCAAAATGTCC   630
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae  ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           TTG-TCTCTG---CTCAGTTCTGGTAACTCTTTTATCTTCCCTGGAG--TTATTTTTTC   772


Panthera            ATTTAGCTAATTAAATGATTAATAAATTTAACACTGCTTTAT-AATTTCATTTAGCTGTG   875
Fusobacterium       ------------------------------------------------------------   0
Bacillus            ------------------------------------------------------------   0
Chlorocebus         CCTTTGCTGTGGTGACACATGATTCATAA-ACTATGCAAAGTCTAAGGCATT--------   681
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae  ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
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Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              CCTT---CTTTGTTTCTCTTTTTCTGTGT-GCTCTTCTAAGAGTAAAACGCATGTCCCCC  828


Panthera               TTGGAAATCACACGGTGAGCTGCGTATGCACCGAATGAGATTAGGT-GGGAGTGAGGGTT  934
Fusobacterium          ------------------------------------------------------------  0
Bacillus               ------------------------------------------------------------  0
Chlorocebus            -GAGTAGCTGCTCAAAAAGCAG--------TGGCCAGGAATTGGGT-TGTTTTTGCCAAG  731
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              CATGAAGTCACTCAAAGCTCAG--------GCGTGTGCAACAGGGACAGCAAAAGCACAC  880


Panthera               TCGGAGGCTGCCC-----TGGTAATTGA-----GATTAGTCTATTACTACAGTGACCTTT  984
Fusobacterium          ------------------------------------------------------------  0
Bacillus               ------------------------------------------------------------  0
Chlorocebus            TCTGGGGAATCCTCCAGGTGAAAACATCTCTG-GAATTGAAAATCTTAGAAGGACCTGCA  790
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              ACAATGATGGCCA-----GAGACACTGCTCAGTGTACTGTCTTCCACCGGGACAGCCCAC  935


Panthera               TTAACTTTTATATTCTCTTGTTAAAGCGAAATAAAATTTTATTC---------ACTTTTA  1035
Fusobacterium          ---------------------TGAAA-AAT-TTATTATA---------TCTTTAA  23
Bacillus               ----------------------TTTGAAA-AAATTTTTATAT---------TCTCAAA  26
Chlorocebus            GCAACGGCTGGATTTCCCTGAACCGAATGAG-GCA-ACATTTACATTTTTCATACCATGG  848
Mycobacterium          ------------------------------------------------------------  0
Clostridium            ------------------------------------------------------------  0
Peptostreptococcaceae  ------------------------------------------------------------  0
Macaca                 ------------------------------------------------------------  0
Trachypithecus         ------------------------------------------------------------  0
Colobus                ------------------------------------------------------------  0
Mandrillus             ------------------------------------------------------------  0
Papio                  ------------------------------------------------------------  0
Theropithecus          ------------------------------------------------------------  0
Nomascus               ------------------------------------------------------------  0
Pongo                  ------------------------------------------------------------  0
Exon-9                 ------------------------------------------------------------  0
790212667              TCACCAAGGGTTAGGTCCTGTGCTGCCTGGG-TC---CCGGGTC---------TCCTCGG  982


Panthera               AGGAATATTTACTTAGAGAAATTTACA----------TGAAATTAAAGAAACACCAAGC  1084
Fusobacterium          AGAATTTTTT------------------------------------------------  33
Bacillus               AGTATTTTTTG-----------------------------------------------  37
Chlorocebus            AAAATTGAGGGATGATCCAGGAAAACAGACAGCTTGAGAAAATTTAAAGGTTCCACAGT-  907
Mycobacterium          ----------------------------------------------------------  0
Clostridium            ----------------------------------------------------------  0
Peptostreptococcaceae  ----------------------------------------------------------  0
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Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           AGGTCGGTTTGCATCACGTGG-ATACACTAATCTACCCAAGTTGACTTGGAGAACCAAT-   1040


Panthera            CCAGAAGCCTACTGGGGCCAGAATCCATGCACTGTTCCTTTTCTAATT-TTCAGAGGCTC   1143
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         -GTTTTTCCATTCAGGGAC---AACAGAAAACAGGAAGGCATTAATAACATGTCACTGTC   963
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           -CTGAAATCAGATGGGAGC---TCCCGGAAGCAAAGCAATTGCAGGTTCATTTGTTACTT   1096


Panthera            TTTAGGAAA-TTGTTCTTGAGCTAGCTGGAGGCAGAGTG--TAGAGCACTGTGGTTTTAG   1200
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         TGACTGCCAGCAGAACCACCCCAGGAAAGCATCTTTTGGGATAAATCACCGGGCGGGCAT   1023
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           TTATTGTCT-CATTTCTTCTTATTTTTTTAGCCCAATTGCCTCCTACATGGGGAAGGTTG   1155


Panthera            AGAGCTCAATCATTCATTCATTCA--CTCAGTAAACACCCAATCCCTGCTGTG-------   1251
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         GGCATTCTCCCATCCGAACCAACG--CTCCCACAACCACCATCTCGGGTAGATGTTGAAA   1081
Mycobacterium       ------------------------------------------------------------   0
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca              ------------------------------------------------------------   0
Trachypithecus      ------------------------------------------------------------   0
Colobus             ------------------------------------------------------------   0
Mandrillus          ------------------------------------------------------------   0
Papio               ------------------------------------------------------------   0
Theropithecus       ------------------------------------------------------------   0
Nomascus            ------------------------------------------------------------   0
Pongo               ------------------------------------------------------------   0
Exon-9              ------------------------------------------------------------   0
790212667           GGGTCCTCTTTATTCTACTTTTCATTCTCGCTCTGCCGCCCTCCCAGGTGTTTGTAATCA   1215


Panthera            TCCTGTAAATGCCTCTAGGTCCTGGTTCTACAGCAATAAACACAACCTGGGTCCCTGCTT   1311
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
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Chlorocebus              ACCTTTCAAACTTGACAAAACCCAGGGC--CTTCCTTAAA------CAGGACCATCCCTT   1133
Mycobacterium            ------------------------------------------------------------   0
Clostridium              ------------------------------------------------------------   0
Peptostreptococcaceae    ------------------------------------------------------------   0
Macaca                   ------------------------------------------------------------   0
Trachypithecus           ------------------------------------------------------------   0
Colobus                  ------------------------------------------------------------   0
Mandrillus               ------------------------------------------------------------   0
Papio                    ------------------------------------------------------------   0
Theropithecus            ------------------------------------------------------------   0
Nomascus                 ------------------------------------------------------------   0
Pongo                    ------------------------------------------------------------   0
Exon-9                   ------------------------------------------------------------   0
790212667                --CATATTTTTCATTTGGTTTCCGGGTC--CAGCGACAAGAACA-CCAGAAACACCTCCT   1270


Panthera                 CCCTGGAGTTTAGGTTATAGCGGGAAAATAGAAATGAGA---AGCAAATAAATAAACAAC   1368
Fusobacterium            ------------------------------------------------------------   33
Bacillus                 ------------------------------------------------------------   37
Chlorocebus              CCC---AGAAAAGATCTTGCTCTCTGCCAGCTCAAAGACCTAGCTCGGGCTAAAGCGAG   1190
Mycobacterium            ------------------------------------------------------------   0
Clostridium              ------------------------------------------------------------   0
Peptostreptococcaceae    ------------------------------------------------------------   0
Macaca                   ------------------------------------------------------------   0
Trachypithecus           ------------------------------------------------------------   0
Colobus                  ------------------------------------------------------------   0
Mandrillus               ------------------------------------------------------------   0
Papio                    ------------------------------------------------------------   0
Theropithecus            ------------------------------------------------------------   0
Nomascus                 ------------------------------------------------------------   0
Pongo                    ------------------------------------------------------------   0
Exon-9                   ------------------------------------------------------------   0
790212667                CCCCAGAGAGGAAGAGAGGATGCTCCGCCGCCTGTAAGG-ATTTTTTGCTCTCCTCCTTT   1329


Panthera                 AAAATAACAGACAGTAATAAGCAC------AGCACAGAGAATTAGAAATAATGATTTCTT   1422
Fusobacterium            ------------------------------------------------------------   33
Bacillus                 ------------------------------------------------------------   37
Chlorocebus              GAAATGCAGGCGATTGCTGAGTTCCCCAGCGGCTCTCCGGAATTGACAGTCTCTAAATTA   1250
Mycobacterium            ------------------------------------------------------------   0
Clostridium              ------------------------------------------------------------   0
Peptostreptococcaceae    ------------------------------------------------------------   0
Macaca                   ------------------------------------------------------------   0
Trachypithecus           ------------------------------------------------------------   0
Colobus                  ------------------------------------------------------------   0
Mandrillus               ------------------------------------------------------------   0
Papio                    ------------------------------------------------------------   0
Theropithecus            ------------------------------------------------------------   0
Nomascus                 ------------------------------------------------------------   0
Pongo                    ------------------------------------------------------------   0
Exon-9                   ------------------------------------------------------------   0
790212667                TGTCAGTTGGCATGCGATATGCTTCTCA--GGCAGTAATTTAGAGACTGTCAATTCCTGA   1387


Panthera                 TAGA--CTGGGGAACCCAAGAAAATCTCTGTTCAGAGATGACCATTCAACTGAGATCTGA   1480
Fusobacterium            ------------------------------------------------------------   33
Bacillus                 ------------------------------------------------------------   37
Chlorocebus              CTGC--CTGAGAAGC------ATATCGCATGCC--AACTGACAAA--AGCAGGAG--AGG   1296
Mycobacterium            ------------------------------------------------------------   0
Clostridium              ------------------------------------------------------------   0
Peptostreptococcaceae    ------------------------------------------------------------   0
Macaca                   ------------------------------------------------------------   0
Trachypithecus           ------------------------------------------------------------   0
Colobus                  ------------------------------------------------------------   0
Mandrillus               ------------------------------------------------------------   0
Papio                    ------------------------------------------------------------   0
Theropithecus            ------------------------------------------------------------   0
Nomascus                 ------------------------------------------------------------   0
Pongo                    ------------------------------------------------------------   0
Exon-9                   ------------------------------------------------------------   0
790212667                GAGCCGCTGGGGAAC------TGAGCAATCGTC--TGCATTTCGT--CGCTTTAGCCCGA   1437
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Panthera              ATTATAAAAAGCAGCCAGCCATGCA-AAGCTCAGAAGGAATGGCACACCAGAATAGAGGA    1539
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           AAAAATCCTTACAGGCGGCAGAGCATCCTCTCTTC--------CTCTCTGGGGAGGAGGT    1348
Mycobacterium         ------------------------------------------------------------    0
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                ------------------------------------------------------------    0
Trachypithecus        ------------------------------------------------------------    0
Colobus               ------------------------------------------------------------    0
Mandrillus            ------------------------------------------------------------    0
Papio                 ------------------------------------------------------------    0
Theropithecus         ------------------------------------------------------------    0
Nomascus              ------------------------------------------------------------    0
Pongo                 ------------------------------------------------------------    0
Exon-9                ------------------------------------------------------------    0
790212667             GCTGGGTCTTTGAGCTGGCAGAGACCAAGATCTTT--------TTCTGGGAAGGGATGGT    1489


Panthera              GACTGGGGGTGAAGGTCCCGAGGAAGGAAGAAGTGGATGTGGTGTGTTCAA------GAA    1593
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ----GTTTCTGGTGTTCTTGTCGCTGGACCCGGAAACCAAATGAAAAATAT------GTG    1398
Mycobacterium         ------------------------------------------------------------    0
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                ------------------------------------------------------------    0
Trachypithecus        ------------------------------------------------------------    0
Colobus               ------------------------------------------------------------    0
Mandrillus            ------------------------------------------------------------    0
Papio                 ------------------------------------------------------------    0
Theropithecus         ------------------------------------------------------------    0
Nomascus              ------------------------------------------------------------    0
Pongo                 ------------------------------------------------------------    0
Exon-9                ------------------------------------------------------------    0
790212667             CCTATTTAAGGAAGGCCCTGGATTTTGTCAAGTTTGAAAGGTTTTCAACATCTTCCCGAG    1549


Panthera              ACAGAAGAAACAGGG---------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ATTACAAACACCTGGGAGGGCGGCAGAGTGAGAATGAAAAGTAGAATAAAGAGGACCCCA    1458
Mycobacterium         ------------------------------------------------------------    0
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                ------------------------------------------------------------    0
Trachypithecus        ------------------------------------------------------------    0
Colobus               ------------------------------------------------------------    0
Mandrillus            ------------------------------------------------------------    0
Papio                 ------------------------------------------------------------    0
Theropithecus         ------------------------------------------------------------    0
Nomascus              ------------------------------------------------------------    0
Pongo                 ------------------------------------------------------------    0
Exon-9                ------------------------------------------------------------    0
790212667             ATGGTGGTCATGGGAGTAGTGGTTAGGGTGGTTATGACGCATGGGACGGGAGAATACCAT    1609


Panthera              ------------------------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ACCTTCCCCGTGTAGGAGGCAATTTG--GCTTAAAAAAATAAGAAGAAATGAGATAATAA    1516
Mycobacterium         ------------------------------------------------------------    0
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                ------------------------------------------------------------    0
Trachypithecus        ------------------------------------------------------------    0
Colobus               ------------------------------------------------------------    0
Mandrillus            ------------------------------------------------------------    0
Papio                 ------------------------------------------------------------    0
Theropithecus         ------------------------------------------------------------    0
Nomascus              ------------------------------------------------------------    0
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Pongo              ------------------------------------------------------------  0
Exon-9             ------------------------------------------------------------  0
790212667          GCCCACCCGGTGATTTATACCAAAAGATGCTTTCCTGGGGTGGTTCTGCTGGCAGTCGGG  1669


Panthera           ------------------------------------------------------------  1608
Fusobacterium      ------------------------------------------------------------  33
Bacillus           ------------------------------------------------------------  37
Chlorocebus        AAGTAACAAATGAACCTGCAATTGCTTTGCTTCCGGGAGCTCCCATCTGATTTCAGATTG  1576
Mycobacterium      ------------------------------------------------------------  0
Clostridium        ------------------------------------------------------------  0
Peptostreptococcaceae ---------------------------------------------------------  0
Macaca             ------------------------------------------------------------  0
Trachypithecus     ------------------------------------------------------------  0
Colobus            ------------------------------------------------------------  0
Mandrillus         ------------------------------------------------------------  0
Papio              ------------------------------------------------------------  0
Theropithecus      ------------------------------------------------------------  0
Nomascus           ------------------------------------------------------------  0
Pongo              ------------------------------------------------------------  0
Exon-9             ------------------------------------------------------------  0
790212667          CAGTGAC--ATGTTATTAATGTTTTCCTGTTTTCTATTGTCCCCGAATGGAAAAACACTG  1727


Panthera           ------------------------------------------------------------  1608
Fusobacterium      ------------------------------------------------------------  33
Bacillus           ------------------------------------------------------------  37
Chlorocebus        GTTCTCC---AAGTCAACTTGGGTAGATTAATGTATCCGCGTGATGCAAACCTAGCTCCG  1633
Mycobacterium      ------------------------------------------------------------  0
Clostridium        ------------------------------------------------------------  0
Peptostreptococcaceae ---------------------------------------------------------  0
Macaca             ------------------------------------------------------------  0
Trachypithecus     ------------------------------------------------------------  0
Colobus            ------------------------------------------------------------  0
Mandrillus         ------------------------------------------------------------  0
Papio              ------------------------------------------------------------  0
Theropithecus      ------------------------------------------------------------  0
Nomascus           ------------------------------------------------------------  0
Pongo              ------------------------------------------------------------  0
Exon-9             ------------------------------------------------------------  0
790212667          TGGAACCTTTAAATTTTCTCAAGCTGTCTATGTTTTCCTGGATCGTCCCTCAATTTTCCG  1787


Panthera           ------------------------------------------------------------  1608
Fusobacterium      ------------------------------------------------------------  33
Bacillus           ------------------------------------------------------------  37
Chlorocebus        AGGAGACCCGG-----------GACCCAGTCAGCACAGGACTTAACCCTTG---------  1673
Mycobacterium      ------------------------------------------------------------  0
Clostridium        ------------------------------------------------------------  0
Peptostreptococcaceae ---------------------------------------------------------  0
Macaca             ------------------------------------------------------------  0
Trachypithecus     ------------------------------------------------------------  0
Colobus            ------------------------------------------------------------  0
Mandrillus         ------------------------------------------------------------  0
Papio              ------------------------------------------------------------  0
Theropithecus      ------------------------------------------------------------  0
Nomascus           ------------------------------------------------------------  0
Pongo              ------------------------------------------------------------  0
Exon-9             ------------------------------------------------------------  0
790212667          TGGTATGAAAAATGTAAATGTTGCTACATTCAGTTCAGGGAAATCCAGCCGTTGCTCCAG  1847


Panthera           ------------------------------------------------------------  1608
Fusobacterium      ------------------------------------------------------------  33
Bacillus           ------------------------------------------------------------  37
Chlorocebus        ------------------------------------------------------------  1673
Mycobacterium      ------------------------------------------------------------  0
Clostridium        ------------------------------------------------------------  0
Peptostreptococcaceae ---------------------------------------------------------  0
Macaca             ------------------------------------------------------------  0
Trachypithecus     ------------------------------------------------------------  0
Colobus            ------------------------------------------------------------  0
```

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 Mandrillus        ------------------------------------------------------------   0
 Papio             ------------------------------------------------------------   0
 Theropithecus     ------------------------------------------------------------   0
 Nomascus          ------------------------------------------------------------   0
 Pongo             ------------------------------------------------------------   0
 Exon-9            ------------------------------------------------------------   0
 790212667         ATCCTTCTAAGATTTTTCAATTCCAGAGATGTTTTCACCTGGAAGATTCCCCAGATTTGG   1907
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Panthera              ------------------------------------------------------------   1608
Fusobacterium         ------------------------------------------------------------   33
Bacillus              ------------------------------------------------------------   37
Chlorocebus           ------------------------------------------------------------   1673
Mycobacterium         ------------------------------------------------------------   0
Clostridium           ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca                -------------------------------------------------TCCATGCAACAG   12
Trachypithecus        -------------------------------------------------TCCATGCAACAG   12
Colobus               -------------------------------------------------TCCATGCAACAG   12
Mandrillus            -------------------------------------------------TCCATGCAACAG   12
Papio                 -------------------------------------------------TCCATGCAACAG   12
Theropithecus         -------------------------------------------------TCCATGCAACAG   12
Nomascus              -------------------------------------------------TCCATGCAACAG   12
Pongo                 ----------------------------------------------------GCAACAG   7
Exon-9                -----------------------------------------------------CAACAG   6
790212667             TCTCATCACTCCTTTTCTTAAAATTTTTTTTTGTCTTTCTTCTTGGATTTCCAGCAACAG   198947


Panthera              ------------------------------------------------------------   1608
Fusobacterium         ------------------------------------------------------------   33
Bacillus              ------------------------------------------------------------   37
Chlorocebus           ------------------------------------------------------------   1673
Mycobacterium         -----------------------------------ACTGCAGGGTCTGGGCGGGGTG   22
Clostridium           ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca                GGAGCTGCCACCACCGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Trachypithecus        GGAGCTGCCACCACCGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Colobus               GGAGCTGCCACCACCGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Mandrillus            GGAGCTGCCACCACTGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Papio                 GGAGCTGCCACCACTGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Theropithecus         GGAGCTGCCACCACTGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTCGGAGGGATG   72
Nomascus              GGAGCTGCCACCACCGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   72
Pongo                 GGAGCTGCCACCACCGTGTACTGTGCTGCTGCCCCAGAACTGGAGGGTCTGGGAGGGATG   67
Exon-9                GGAGCTGCCACCACCGTGTACTGTGCTGCTGTCCCAGAACTGGAGGGTCTGGGAGGGATG   66
790212667             GGAGCTGCCACCACCGTGTACTGTGCTGCTGTCCCAGAACTGGAGGGTCTGGGAGGGATG   199007


Panthera              ------------------------------------------------------------   1608
Fusobacterium         ------------------------------------------------------------   33
Bacillus              ------------------------------------------------------------   37
Chlorocebus           ------------------------------------------------------------   1673
Mycobacterium         TACTCCGACATCTACCTGCCGCTGCA----------------------------------   48
Clostridium           ------------------------------------------------------------   0
Peptostreptococcaceae ------------------------------------------------------------   0
Macaca                TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCACAGAGCGAAGAGACGGC   131
Trachypithecus        TATTTCAACAACTG-CTGCCGCTGTATGCCCTCGCCAGAAGCACAGAGCGAAGAGACGGC   131
Colobus               TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCACAGAGCGAAGAGACGGC   131
Mandrillus            TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCACAGAGCGAAGAGACGGC   131
Papio                 TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCACAGAGTGAAGAGACGGC   131
Theropithecus         TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCACAGAGTGAAGAGACGGC   131
Nomascus              TACTTCAACAACTG-CTGCCGGTGCATGCCCTCGCCAGAAGCTCAGAGCGAAGAGACGGC   131
Pongo                 TATTTCAACAACTG-CTGCCGCTGCATGCCCTCGCCAGAAGCTCAGAGCGAAGAGACGGC   126
Exon-9                TACTTCAACAACTG-CTGCCGCTGCATGCCCTCACCAGAAGCTCAGAGCGAAGAGACGGC   125
790212667             TACTTCAACAACTG-CTGCCGCTGCATGCCCTCACCAGAAGCTCAGAGCGAAGAGACGGC   199066
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Panthera              ------------------------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ------------------------------------------------------------    1673
Mycobacterium         ------------------------------------------------------------    48
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                CCGGGCCCTGTGGGCACTCAGTGAGAGGCTCATCCAAGAACGGCTTGGCAGCCAGTCCGG    191
Trachypithecus        CCGGGCCCTGTGGGCACTCAGTGAGAGGCTCATCCAAGAACGGCTTGGCAGCCAGTCCGG    191
Colobus               CCGGGCCCTGTGGGCACTCAGTGAGAGGCTCATCCAAGAACGGCTTGGCAGCCAGTCTGG    191
Mandrillus            CCGGGCCCTGTGGGCGCTCAGTGAGAGGCTCATCCAAGAACGGCTTGGCAGCCAGTCCGG    191
Papio                 CCGGGCCCTGTGGGCACTCAGTGAGAGGCTCATCCAAGAACGGCTTGGCAGCCAGTCCGG    191
Theropithecus         CCGGGCCCTGTGGGCACTCAGTGAGAGGCTCATCCAAGAACGGCTCGGCAGCCAGTCCGG    191
Nomascus              CCGTACCCTGTGGGCGCTCAGTGAGAGGCTGATCCAAGAACGGCTTGGCAGCCAGTCCGG    191
Pongo                 CCGGACCCTGTGGGCGCTCAGTGAGAGGCTGATCCAAGAACGGCTTGGCAGCCAGTCCGG    186
Exon-9                CCGGACCCTGTGGGCGCTCAGCGAGAGGCTGATCCAAGAACGGCTTGGCAGCCAGTCCGG    185
790212667             CCGGACCCTGTGGGCGCTCAGCGAGAGGCTGATCCAAGAACGGCTTGGCAGCCAGTCCGG    199126


Panthera              ------------------------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ------------------------------------------------------------    1673
Mycobacterium         ------------------------------------------------------------    48
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                CTAAAAGGAGCTTGGAGTGGATGGGCGCAGACACCCACCCTGTGTGTGTGTCCGCTCACG    251
Trachypithecus        CTAAGAGGAGCTCGGAGCGGATGGGCGCAGACACCCGCCTTGTG----TGTCCGCTCACG    247
Colobus               CTAAGAGGAGCTCGGAGCGGATGGGCGCAGACACCCGCCTTGTG--TGTCCCCGCTCACG    249
Mandrillus            CTGAGAGGATCTCAGAGTGGATGGGCGCAGACACCCGCCCTGTG--TGTGTCCGCTCACG    249
Papio                 CTAAGAGGAGCTCAGAGTGGATGGGCGCAGACACCCGCCCTGTGTGTGTGTCCGCTCACG    251
Theropithecus         CTAAGAGGAGCTCAGAGTGGATGGGCGCAGACACCCGCCCTGTGTGTGTGTCCGCTCACG    251
Nomascus              CTAAGTGGAGCTCGGAGCGGATGGGCACACACACCCGCCC--TGTGTGTGTCTGCTCACG    249
Pongo                 CTAAGTGGAGCTCAGAGCGGATGGGCACACACACCCGCCC--TGTGTGTGTCCCCTCACG    244
Exon-9                CTAAGTGGAGCTCAGAGCGGATGGGCACACACACCCGCCC--TGTGTGTGTCCCCTCACG    243
790212667             CTAAGTGGAGCTCAGAGCGGATGGGCACACACACCCGCCC--TGTGTGTGTCCCCTCACG    199184


Panthera              ------------------------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ------------------------------------------------------------    1673
Mycobacterium         ------------------------------------------------------------    48
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                CAAGTGCCAGAGCTGGGCCCCTTCCAAATGTCCCTCCAATGCAGATCCGCAAGAGTGAAG    311
Trachypithecus        CAAGTGCCAGAGCTGGGCTCCTTCCAAATGTCCCTCCAACACAGATCCGCAACAGTGAAG    307
Colobus               CAAGTGCCAGAGCTGGGCTCCTTCCAAATGTCCCTCCAACACAGATCCGCAACAGTGAAG    309
Mandrillus            CAAGTGCCAGAGCTGGGCCCCTTCCA-ATGTCCCTCCAACACAGATCCGCAAGAGTGAAG    308
Papio                 CAAGTGCCAGAGCTGGGCCCCTTCCA-ATGTCCCTCCAACACAGATCCGCAAGAGTGAAG    310
Theropithecus         CAAGTGCCAGAGCTGGGCCCCTTCCA-ATGTCCCTCCAACACAGATCCGCAAGAGTGAAG    310
Nomascus              CAAGTGCCAGGGCTGGGCCCCTTCCAAATGTCCCTCCAACACAGATCCACAAGAGTAAAG    309
Pongo                 CAAGTGCCAGGGCTGGGACCCTTCCAAATGTCCCTCCAACACAGATCCGCAAGAGTAAAG    304
Exon-9                CAAGTGCCAGGGCTGGGCCCCTTCCAAATGTCCCTCCAACACAGATCCGCAAGAGTAAAG    303
790212667             CAAGTGCCAGGGCTGGGCCCCTTCCAAATGTCCCTCCAACACAGATCCGCAAGAGTAAAG    199244


Panthera              ------------------------------------------------------------    1608
Fusobacterium         ------------------------------------------------------------    33
Bacillus              ------------------------------------------------------------    37
Chlorocebus           ------------------------------------------------------------    1673
Mycobacterium         ------------------------------------------------------------    48
Clostridium           ------------------------------------------------------------    0
Peptostreptococcaceae ------------------------------------------------------------    0
Macaca                GAAATAAGAGCAGTCACAGTAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    371
Trachypithecus        GAAATAAGAGCAGTCACCACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    367
Colobus               GAAATAAGAGCAGTCACAACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    369
Mandrillus            GAAATAAGAGCAGTCACAACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    368
Papio                 GAAATAAGAGCAGTCACAACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    370
Theropithecus         GAAATAAGAGCAGTCACAACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    370
Nomascus              GAAGTAAGAGCAGTCACGACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT    369
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Pongo              GAAATAAGAGCGGTCACAACAGAGTGAAAAATGTTAAGTACCAATGGGAAGCAGGGAATT   364
Exon-9             GAAATAAGAGCAGTCACAACAGAGTGAAAAATCTTAAGTACCAATGGGAAGCAGGGAATT   363
790212667          GAAATAAGAGCAGTCACAACAGAGTGAAAAATCTTAAGTACCAATGGGAAGCAGGGAATT   199304


Panthera           ------------------------------------------------------------   1608
Fusobacterium      ------------------------------------------------------------   33
Bacillus           ------------------------------------------------------------   37
Chlorocebus        ------------------------------------------------------------   1673
Mycobacterium      ------------------------------------------------------------   48
Clostridium        ------------------------------------------------------------   0
Peptostreptococcaceae ---------------------------------------------------------   0
Macaca             CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTAAGGTCTCTTTGCTTTCTG   431
Trachypithecus     CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTGAGGTCTCTCTGCTTTCTG   427
Colobus            CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTAAGGTCTCTTTGCTTTCTG   429
Mandrillus         CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTAAGGTCTCTTTGCTTTCTG   428
Papio              CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTAAGGTCTCTTTGCTTTCTG   430
Theropithecus      CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCCTAAGGTCTCTTTGCTTTCTG   430
Nomascus           CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGT-CATAGGTCTCTTTGCTTTCGG   428
Pongo              CCTGGGGTAAATGATCACTTTTCTGGGGCTGAGCTAGG-CATAGGTCTCTTTGCTTTCTG   423
Exon-9             CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGG-CATAGGTCTCTTTGCTTTCTG   422
790212667          CCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGG-CATAGGTCTCTTTGCTTTCTG   199363


Panthera           ------------------------------------------------------------   1608
Fusobacterium      ------------------------------------------------------------   33
Bacillus           ------------------------------------------------------------   37
Chlorocebus        ------------------------------------------------------------   1673
Mycobacterium      ------------------------------------------------------------   48
Clostridium        ------------------------------------------------------------   0
Peptostreptococcaceae ---------------------------------------------------------   0
Macaca             ATGGTGGCCTGTTTGAAAGTA---TAAAACCTGGTTGGTGTGTAGGTTCCATA----TCT   484
Trachypithecus     ATGGTGGCCTGTTTGAAAGTA---TAAGGCCTGGTTGGTGTGTAGGTTCCCTATCTATCT   484
Colobus            ATGGTGGCCTGTTTGAAAGTA---TAAGACCTGGCTGGTATGTAGGTTCCCTA----TCT   482
Mandrillus         ATGGTGGCCTGTTTGAAAGTA---TAAAACCTGGTTGGTGTGTAGGTTCCATA----TCT   481
Papio              ATGGTGGCCTGTTTGAAAGTA---TAAAACCTGGTTGGTGTGTAGGTTCCATA----TCT   483
Theropithecus      ATGGTGGCCTGTTTGAAAGTA---TAAAACCTGGTTGGTGTGTAGGTTCCATA----TCT   483
Nomascus           GTGGTGGCC----GGTTTGAAAGTAAAAACCTGGTTGGTGTGTAGGTTCCGTA----TCT   480
Pongo              GTGGTGGCCTGTTTGTTTGAAAGTAAAAACCTGGTTGGTGTGTAGGTTCCGTA----TCT   479
Exon-9             GTGGTGGCC----TGTTTGAAAGTAAAAACCTGCTTGGTGTGTAGGTTCCGTA----TCT   474
790212667          GTGGTGGCC----TGTTTGAAAGTAAAAACCTGCTTGGTGTGTAGGTTCCGTA----TCT   199415


Panthera           ------------------------------------------------------------   1608
Fusobacterium      ------------------------------------------------------------   33
Bacillus           ------------------------------------------------------------   37
Chlorocebus        ------------------------------------------------------------   1673
Mycobacterium      ------------------------------------------------------------   48
Clostridium        ------------------------------------------------------------   0
Peptostreptococcaceae ---------------------------------------------------------   0
Macaca             CCCTGGAGAAGCACCAGCAATTGTTTTT----CTTTTACTGTTGAATAGCCTGAGGTCCC   540
Trachypithecus     CCCTGGAGCAGCACCAGCAATTGTCTTTCTTTCTTTTACTGTTGAATAGCCTGAGGTCCC   544
Colobus            CCCTGGAGAAGCACCAGCAATTGTCTTT----CTTTTACTGTGGAATAGCCTGAGGTCCC   538
Mandrillus         CCCTGGAGAAGCACCAGCAATTGTCTTT----CTTTTACTGTTGAATAGCCTGAGGTCCC   537
Papio              CCCTGGAGAAGCACCAGCAATTGTCTTT----CTTTTACTGTTGAATAGCCTGAGGTCCC   539
Theropithecus      CCCTGGAGAAGCACCAGCAATTGTCTTT----CTTTTACTGTTGAATAGCCTGAGGTCCC   539
Nomascus           CCCTGGAGAAGCACCAGCAATTGTCTTTTACT-GTTGTTGTAGAATAGCCTGAGGTCCC   539
Pongo              CCCTGGAGAAGCACCAGCAATTGTCTTTCTTT-TACTGTTATAGAATAGCCTGAGGTCCC   538
Exon-9             CCCTGGAGAAGCACCAGCAATTCTCTTTCTTT-TACTGTTATAGAATAGCCTGAGGTCCC   533
790212667          CCCTGGAGAAGCACCAGCAATTCTCTTTCTTT-TACTGTTATAGAATAGCCTGAGGTCCC   199474


Panthera           ------------------------------------------------------------   1608
Fusobacterium      ------------------------------------------------------------   33
Bacillus           ------------------------------------------------------------   37
Chlorocebus        ------------------------------------------------------------   1673
Mycobacterium      ------------------------------------------------------------   48
Clostridium        ------------------------------------------------------------   0
Peptostreptococcaceae ---------------------------------------------------------   0
Macaca             CTCGTCCCATCCAGCTATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   600
Trachypithecus     GTCGTCCCATCCAGCTATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   604
Colobus            GTCGTCCCATCCAGCTATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   598
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Mandrillus          CTCGTCCCATCCAGCTATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   597
Papio               CTCGTCCCATCCAGATATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   599
Theropithecus       CTCGTCCCATCCCGATATCACCACGGCCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   599
Nomascus            CTCGTCCCATCCAGCTA---------CCACCACGGCCACCAGGGGCTGGCTTTCTCCTAC   590
Pongo               CTCGTCCCATCCAGCCA---------CCACCACTGCAGCCAGGGGCTGGCTTTCTCCTAC   589
Exon-9              CTCGTCCCATCCAGCTACCACCACGGCCACCACTGCAGCCGGGGGCTGGCCTTCTCCTAC   593
790212667           CTCGTCCCATCCAGCTACCACCACGGCCACCACTGCAGCCGGGGGCTGGCCTTCTCCTAC   199534


Panthera            ------------------------------------------------------------   1608
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         ------------------------------------------------------------   1673
Mycobacterium       ------------------------------------------------------------   48
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ----------------------------------------------------------   0
Macaca              TTAGGGAAGAAAAAGCAACTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   660
Trachypithecus      TTAGCGAAGAAAAAGCAACTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   664
Colobus             TTAGGGAAGAAAAAGCAACTGTTCACTGCTCCTTGCTGCAATGATCCAGGAGATAATTGT   658
Mandrillus          TTAGGGAAGAAAAATCAACTGTTCACTGCTCCTTACTGCATTGATCCAGGAGATAATTGT   657
Papio               TTAGGGAAGAAAAATCAACTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   659
Theropithecus       TTAGGGAAGAAAAATCAACTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   659
Nomascus            TTAGGGAAGAAAAAGCAAGTGTTCACCTCTCCTTGCTGCATTGATCCAGGAGATAATTGT   650
Pongo               TTAGGGAAGAAAAAGCAAGTGTTCACCGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   649
Exon-9              TTAGGGAAGAAAAAGCAAGTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   653
790212667           TTAGGGAAGAAAAAGCAAGTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGT   199594


Panthera            ------------------------------------------------------------   1608
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         ------------------------------------------------------------   1673
Mycobacterium       ------------------------------------------------------------   48
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ----------------------------------------------------------   0
Macaca              TGCATTCATCCTGACTGAGCCTGAGTCAGTTTAGCAACGGCTGGGGAGACAAATCCCAGA   720
Trachypithecus      TTCATTCATCCTGACCAAGCCTGAGCCAGTTTAGCAACTGCTGGGGAGACAAATCCCAGA   724
Colobus             TTCATTCATCCTGACCAAGCCCGAGCCAGTTTAGCAACTGCTGGGGAGACAAATCCCAGA   718
Mandrillus          TTCATTCATCCTGACCAAGCCTGAGCCAGTTTAGCAACTGCTGGGGAGACAAATCCCAGA   717
Papio               TTCATTCATCCTGACCAAGCCTGAGCCAGTTTAGCAACTGCTGGGGAGACAAATCCCAGA   719
Theropithecus       TTCATTCATCCTGACCAAGCCTGAGCCAGTTTAGCAACTGCTGGGGAGACAAATCCCAGA   719
Nomascus            TTCATTCATCCTGACCAAGCCTGCGCCAGCTTGGTCACTGCTGGGGAGACAAATCTCAGA   710
Pongo               TTCATTCATCCTGACCAAGCCTGAGCCAGCTTGGCAACTGCTGGGGAGACAAATCTCAGA   709
Exon-9              TTCATTCATCCTGACCAAGACTGAGCCAGCTTAGCAACTGCTGGGGAGACAAATCTCAGA   713
790212667           TTCATTCATCCTGACCAAGACTGAGCCAGCTTAGCAACTGCTGGGGAGACAAATCTCAGA   199654


Panthera            ------------------------------------------------------------   1608
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         ------------------------------------------------------------   1673
Mycobacterium       ------------------------------------------------------------   48
Clostridium         ------------------------------------------------------------   0
Peptostreptococcaceae ----------------------------------------------------------   0
Macaca              ACCCTGTCCCAGCGAGCTAGGATGGCGGTGACACCCAGACAGAGTAGAATACCCAGAGCT   780
Trachypithecus      ACCTTGTCCCAGCCAGGCAGGATGGCGATGACACCCAGACGGAGTAGAATACGCAGAGCT   784
Colobus             ACCTTGTCCCAGCCAGCCAGGATGGCGGTGACACCCAGACGGAGTAGAATACGCAGAGCT   778
Mandrillus          ACCTTGTCCCAGCCAGCCAGGATGGCGGTGACAC------------------------   751
Papio               ACCTTGTCCCAGCCAGCCAGGATGGCGGTGACACCCAGACAGAGTAGAATACACAGAGCT   779
Theropithecus       ACCTTGTCCCAGCCAGCCAGGATGGCGGTGACACCCAGACAGAGTAGAATACACAGAGCT   779
Nomascus            ACCTTGTCCCAGCCAGTGAGGATGGCAGTGACACCCAGACGGAGTAGAATACGGGGAACT   770
Pongo               ACCTTGTCCCAGCCAGTGAGGATG-----------TGACGGAGTAGAATACGCAGAACT   757
Exon-9              ACCTTGTCCCAGCCAGTGAGGATGACAGTGACACCCAGAGGGAGTAGAATACGCAGAACT   773
790212667           ACCTTGTCCCAGCCAGTGAGGATGACAGTGACACCCAGAGGGAGTAGAATACGCAGAACT   199714


Panthera            ------------------------------------------------------------   1608
Fusobacterium       ------------------------------------------------------------   33
Bacillus            ------------------------------------------------------------   37
Chlorocebus         ------------------------------------------------------------   1673
Mycobacterium       ------------------------------------------------------------   48
Clostridium         ---------------------------------------------------AAAAAATTCTT   11
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Peptostreptococcaceae    ----------------------------------------CTA-TC-AAAGAATTCTT        16
Macaca                   TCCAGGTGGCAAAGTACTTGTCACAGCCTCCCT-CGCCACTGCTATACAAAAAATT-CTT     838
Trachypithecus           ACCAGGTGGCAAAGTACTTGTCACAGACTCCCT-CACCAATGCTATACAAAAAATTCT--     841
Colobus                  ACCAGGTGGCAAAGTACTTGTCACAGACTCCCT-CGCCAATGCTATACAAAAAATTC-T-     835
Mandrillus               -----------------------------------------------------------     751
Papio                    ACCTGGTGGCAAAGTACTTGTCACAGACTCCCT-CGCCACTGCTATACAAAAAAATTCTT     838
Theropithecus            ACCTGGTGGCAAAGTACTTGTCACAGACTCCTT-CGCCACTGCTATACAAAAAAATTCTT     838
Nomascus                 ACCACCTGGCAAAGTACTTGTCATAGACTCCCT-CGCTCATGCTATACAAAAATT-CTT     828
Pongo                    ACCAGGTGGCAGAGTACTTGTCATAGACTCCTTCTTCTAATGCTATACAAAAATT-CTT     816
Exon-9                   ACCAGGTGGCAAAGTACTTGTCATAGACTCCTT-TGCTAATGCTATGCAAAAATT-CTT     831
790212667                ACCAGGTGGCAAAGTACTTGTCATAGACTCCTT-TGCTAATGCTATGCAAAAATT-CTT  199772


Panthera                 -----------------------------------------------------------    1608
Fusobacterium            -----------------------------------------------------------      33
Bacillus                 -----------------------------------------------------------      37
Chlorocebus              -----------------------------------------------------------    1673
Mycobacterium            -----------------------------------------------------------      48
Clostridium              TAGAGATAGTATAACAAAATTTT------------------------------------      34
Peptostreptococcaceae    TAAAGATTT--TAACAAATTTTT------------------------------------      37
Macaca                   TAAAGATTA--TAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG     882
Trachypithecus           ------TTG--TAACACATTTTTC-------------AAATCATTCCTTAGATACCTTG     879
Colobus                  ------TTG--TAACACATTTTTC-------------AGATCATTCCTTACATACCTTG     873
Mandrillus               -----------------------------------------------------------     751
Papio                    TAAAGATTA--TAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG     882
Theropithecus            TAAAGATTA--TAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG     882
Nomascus                 TAGAGATTA--TAACAAGTTTTTCAAATCATTCCTTAGAAATCATTCCTTAGATACCTTG     886
Pongo                    TAGAGATTA--CAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG     860
Exon-9                   TAGAGATTA--TAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG     875
790212667                TAGAGATTA--TAACAAATTTTTC-------------AAATCATTCCTTAGATACCTTG  199816


Panthera                 -----------------------------------------------------------    1608
Fusobacterium            -----------------------------------------------------------      33
Bacillus                 -----------------------------------------------------------      37
Chlorocebus              -----------------------------------------------------------    1673
Mycobacterium            -----------------------------------------------------------      48
Clostridium              -----------------------------------------------------------      34
Peptostreptococcaceae    -----------------------------------------------------------      37
Macaca                   AGACTCAGGAAGGGAAGCGTGTGTACTTAAGAATACACAGGATCTTCTGGG--GGGCGGA     940
Trachypithecus           AGAGGCAGGAAGGGAAGCGTGTGTACTTAAGAATACACAGAATCTTTTGGGG--GGTGGA     937
Colobus                  AGAGGCAGGAAGGGAAGCGTGTGTACTTAAGAATACACAGGATCTTTTGGG--GGGCGGA     931
Mandrillus               -----------------------------------------------------------     751
Papio                    AGACTCAGGAAGGGAAGCGTGTGTACTTAAGAATACACAGGATCTTTTGGGGGGGGCGGA     942
Theropithecus            AGACTCAGGAAGGGAAGCGTGTGTACTTAAGAATACACAGGATCTTTTGGG--GGGCGGA     940
Nomascus                 AGAGGCAGGAAGGGTAGCGTGTGTATACTTAAGAATACACAGGATTTTTGGG--GGGCAGA     944
Pongo                    AAAGGCAGGAAGGGAAGCGTGTATACTTAAGAATACACAGGATATTTTGGG--GGGCAGA     918
Exon-9                   AAAGGCAGGAAGGGAAGCGTATATACTTAAGAATACACAGGATATTTTGGG--GGGCAGA     933
790212667                AAAGGCAGGAAGGGAAGCGTATATACTTAAGAATACACAGGATATTTTGGG--GGGCAGA  199874


Panthera                 -----------------------------------------------------------    1608
Fusobacterium            -----------------------------------------------------------      33
Bacillus                 -----------------------------------------------------------      37
Chlorocebus              -----------------------------------------------------------    1673
Mycobacterium            -----------------------------------------------------------      48
Clostridium              -----------------------------------------------------------      34
Peptostreptococcaceae    -----------------------------------------------------------      37
Macaca                   GAAT--------AAAAAGTTAATCCCTTTGT----CAGTCACAGTCTCCGTTCTCGTGCT     988
Trachypithecus           GAAT--------AAAAAGTTAATCCCTTTGTCCGTCAGTCACAGTCTCAGTTCTGTTGCT     989
Colobus                  GAAT--------AAAAAGTTAATCCCTCTGTCCGTCAGTCACAGTCTCAGTTCTGTTGCT     983
Mandrillus               -----------------------------------------------------------     751
Papio                    GAAT--------AAAAAGTTAATCCCTTTGTCCGTCAGTCACAGTCTCCATTCTCTTGCT     994
Theropithecus            GAAT--------AAAAAGTTAATCCCTTTGTCCGTCAGTCACAGTCTCCGTTCTCTTGCT     992
Nomascus                 GAATAAAA----AGTTAGTTAATCCCTTTGTCAG----TCACAGTCTCAGTTCTCTTGCT     996
Pongo                    GAATAAAAAGTTAGTTAGTTAATCCCTTTGTCTGTCAGTCACAGTCTCAGTTCTCTTGCT     978
Exon-9                   GAATAAAA----CGTTAGTTAATCCCTTTGTCTGTCAATCACAGTCTCAGTTCTCTTGCT     989
790212667                GAATAAAA----CGTTAGTTAATCCCTTTGTCTGTCAATCACAGTCTCAGTTCTCTTGCT  199930


Panthera                 -----------------------------------------------------------    1608
Fusobacterium            -----------------------------------------------------------      33
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Bacillus                   ------------------------------------------------------------  37
Chlorocebus                ------------------------------------------------------------  1673
Mycobacterium              ------------------------------------------------------------  48
Clostridium                ------------------------------------------------------------  34
Peptostreptococcaceae      ------------------------------------------------------------  37
Macaca                     TTCACATTCTGCTTAAAATTCCTGCTGTGCCTCCCATCCTATGCTTAATAAAAGAACATG  1048
Trachypithecus             TTCACATTCTGCTTAAACTTCCTGCCGTGCCTCCCATCCTATGCTTAATAAA--AACATG  1047
Colobus                    TTCACATACTGCTTAAACTTCCTGCCGTGCCTCTCATCCTATGCTTAATAAAAGAACATG  1043
Mandrillus                 ------------------------------------------------------------  751
Papio                      TTTA------------CAGTCTGCTGTGCCTCCCATCCTATGCTTAATAAAAGAACATG   1041
Theropithecus              TTTA------------CAGTCTGCTGTGCCTCCCATCCTATGCTTAATAAAAGAACATG   1039
Nomascus                   TTCACATTCTGCTTCAACCTCCTGCTCTGCCTCCCATCCTATGCTTAATAAAAGAACATG  1056
Pongo                      TTCACGGTCTGCTTAAACCTCCTGCTGTGCCTCCCATCCTATGCTTAATAAAAGAACATG  1038
Exon-9                     TTCACATTGTACTTAAACCTCCTGCTGTGCCTCGCATCCTATGCTTAATAAAAGAACATG  1049
790212667                  TTCACATTGTACTTAAACCTCCTGCTGTGCCTCGCATCCTATGCTTAATAAAAGAACATG  199990


Panthera                   ----------   1608
Fusobacterium              ----------   33
Bacillus                   ----------   37
Chlorocebus                ----------   1673
Mycobacterium              ----------   48
Clostridium                ----------   34
Peptostreptococcaceae      ----------   37
Macaca                     CTTGAATAT--  1057
Trachypithecus             CTTGAATAT--  1056
Colobus                    CTTGAA-----  1049
Mandrillus                 ----------   751
Papio                      CTTGAATAT--  1050
Theropithecus              CTTGAAT----  1046
Nomascus                   CTTGAATAT--  1065
Pongo                      CTTGAATATCA  1049
Exon-9                     CTTGAATATCA  1060
790212667                  CTTGAATATCA  200001
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> **Analysis and conclusion for Gene CLUSTAL omega:**

- o *Fusobacterium* and *Bacillus* align with intronic region instead of exonic-9 region due to a better alignment score. This can be ignored due to short size of the query sequence.

- o We observe that the primates align better with the exonic sequence of human gene WWOX. They must be having similarity with the intronic regions too, but considering the alignment here, we can say the former. This concludes that **mutations in the exon region is quickly eliminated from race** whereas **mutation in intron region is not.** Lesser mutations mean better alignment.

> ## ClustalW MSA for prokaryotic sequences, with the exon-9 of Homo sapiens-

```
CLUSTAL O(1.2.4) multiple sequence alignment


Mycobacterium          --------------------------------------------ACTGCAGGGTCTGGGC   16
Clostridium            ------------------------------------------------------------   0
Homo                   CAACAGGGAGCTGCCACCACCGTGTACTGTGCTGCTGTCCCAGAACTGGAGGGTCTGGGA   60
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          GGGGTGTACTCCGACATCTACCTGCCGCTGCA----------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   GGGATGTACTTCAACAACTG-CTGCCGCTGCATGCCCTCACCAGAAGCTCAGAGCGAAGA   119
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   GACGGCCCGGACCCTGTGGGCGCTCAGCGAGAGGCTGATCCAAGAACGGCTTGGCAGCCA   179
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   GTCCGGCTAAGTGGAGCTCAGAGCGGATGGGCACACACACCCGCCCTGTGTGTGTCCCCT   239
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   CACGCAAGTGCCAGGGCTGGGCCCCTTCCAAATGTCCCTCCAACACAGATCCGCAAGAGT   299
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   AAAGGAAATAAGAGCAGTCACAACAGAGTGAAAAATCTTAAGTACCAATGGGAAGCAGGG   359
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   AATTCCTGGGGTAAAGTATCACTTTTCTGGGGCTGGGCTAGGCATAGGTCTCTTTGCTTT   419
Peptostreptococcaceae  ------------------------------------------------------------   0
Fusobacterium          ------------------------------------------------------------   0
Bacillus               ------------------------------------------------------------   0


Mycobacterium          ------------------------------------------------------------   48
Clostridium            ------------------------------------------------------------   0
Homo                   CTGGTGGTGGCCTGTTTGAAAGTAAAAACCTGCTTGGTGTGTAGGTTCCGTATCTCCCTG   479
Peptostreptococcaceae  ------------------------------------------------------------   0
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```
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------    0
Homo                 GAGAAGCACCAGCAATTCTCTTTCTTTTACTGTTATAGAATAGCCTGAGGTCCCCTCGTC  539
Peptostreptococcaceae --------------------------------------------------------    0
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------    0
Homo                 CCATCCAGCTACCACCACGGCCACCACTGCAGCCGGGGGCTGGCCTTCTCCTACTTAGGG  599
Peptostreptococcaceae --------------------------------------------------------    0
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------    0
Homo                 AAGAAAAAGCAAGTGTTCACTGCTCCTTGCTGCATTGATCCAGGAGATAATTGTTTCATT  659
Peptostreptococcaceae --------------------------------------------------------    0
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------    0
Homo                 CATCCTGACCAAGACTGAGCCAGCTTAGCAACTGCTGGGGAGACAAATCTCAGAACCTTG  719
Peptostreptococcaceae --------------------------------------------------------    0
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------    0
Homo                 TCCCAGCCAGTGAGGATGACAGTGACACCCAGAGGGAGTAGAATACGCAGAACTACCAGG  779
Peptostreptococcaceae --------------------------------------------------------    0
Fusobacterium        --------------------------------------------------------    0
Bacillus             --------------------------------------------------------    0


Mycobacterium        --------------------------------------------------------   48
Clostridium          -------------------------------------AAAAAATTCTTTAGAGATA   19
Homo                 TGGCAAAGTACTTGTCATAGACTCCTTTGCTAATGCTATGCAAAAAATTCTTTAGAGATT  839
Peptostreptococcaceae -----------------------------CTATCAAAGAATTCTTTAAAGATT   24
Fusobacterium        -------------------------TGAAAAA----T-TTATTATATCTTTAAAGAAT   28
Bacillus             -----------------------TTTGAAAAA----ATTTTTATATTCTCAAAAGTAT   31


Mycobacterium        --------------------------------------------------------   48
Clostridium          GTATAACAAAATTTT-----------------------------------------   34
Homo                 A--TAACAAATTTTTCAAATCATTCCTTAGATACCTTGAAAGGCAGGAAGGGAAGCGTAT  897
Peptostreptococcaceae T--TAACAAATTTTT-----------------------------------------   37
Fusobacterium        TTTT----------------------------------------------------   32
Bacillus             TTTT----------------------------------------------------   35


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------   34
Homo                 ATACTTAAGAATACACAGGATATTTTGGGGGGCAGAGAATAAAACGTTAGTTAATCCCTT  957
Peptostreptococcaceae --------------------------------------------------------   37
Fusobacterium        -----------------------T--------------------------------   33
Bacillus             -----------------------TG-------------------------------   37


Mycobacterium        --------------------------------------------------------   48
Clostridium          --------------------------------------------------------   34
Homo                 TGTCTGTCAATCACAGTCTCAGTTCTCTTGCTTTCACATTGTACTTAAACCTCCTGCTGT 1017
Peptostreptococcaceae --------------------------------------------------------   37
```

```
Fusobacterium              ---------------------------------------------------------  33
Bacillus                   ---------------------------------------------------------  37


Mycobacterium             ------------------------------------------  48
Clostridium               ------------------------------------------  34
Homo                      GCCTCGCATCCTATGCTTAATAAAAGAACATGCTTGAATATCA  1060
Peptostreptococcaceae     ------------------------------------------  37
Fusobacterium             ------------------------------------------  33
Bacillus                  ------------------------------------------  37
```

P.T.O

ClustalW MSA for protein

➢ For proteins, we need not perform different MSAs. This is because the proteins exist in all the organisms unlike DNA which has introns exclusively to eukaryotes.

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CLUSTAL O(1.2.4) multiple sequence alignment


Actinobacteria        ---------------------------------------------------------     0
Deltaproteobacteria   ---------------------------------------------------------     0
Acinetobacter         ---------------------------------------------------------     0
Chloroflexi           ---------------------------------------------------------     0
Acidobacteria         ---------------------------------------------------------     0
Mandrillus            MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Papio                 MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Cercocebus            MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Macaca                MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Rhinopithecus         MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Nomascus              MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Hylobates             MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Pan                   MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Pongo                 MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Gorilla               MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Homo                  MAALRYAGLDDTDSEDELPPGWEERTTKDGWVYYANHTEEKTQWEHPKTGKRKRVAGDLP    60
Planctomycetes        ---------------------------------------------------------     0
BD1-7                 ---------------------------------------------------------     0
Gemmatimonadetes      ---------------------------------------------------------     0
Moraxellaceae         ---------------------------------------------------------     0
SAR86                 ---------------------------------------------------------     0


Actinobacteria        -----------------------------------------TTNSGFSATSTTDEVLAGI    19
Deltaproteobacteria   -----------------------------------------KFGAETTASEVAEGI    15
Acinetobacter         ---------------------------------------------------------     0
Chloroflexi           ---------------------------------------------------------     0
Acidobacteria         ---------------------------------------------------------     0
Mandrillus            YGWEQETDENGQVFFVDHINKRTTYVDPRLAFTVDDNPTKPTTQQRYDGSTTAMEILQGR   120
Papio                 YGWEQETDENGQVFFVDHINKRTTYVDPRLAFTVDDNPTKPTTQQRYDGSTTAMEILQGR   120
Cercocebus            YGWEQETDENGQVFFVDHINKRTTYVDPRLAFTVDDNPTKPTTQQRYDGSTTAMEILQGR   120
Macaca                YGWEQETDENGQVFFVDHINKRTTYVDPRLAFTVDDNPTKPTTQQRYDGSTTAMEILQGR   120
Rhinopithecus         YGWEQETDENGQVFFVDHINKRTTYVDPRLAFTVDDNPTKPTTQQRYDGSTTAMEILQGR   120
Nomascus              YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPATRQRYDGSTTAMEILQGR   120
Hylobates             YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPTTRQRYDGSTTAMEILQGR   120
Pan                   YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPTTRQRYDGSTTAMEILQGR   120
Pongo                 YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPTTRQRYDGSTTAMEILQGR   120
Gorilla               YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPTTRQRYDGSTTAMEILQGR   120
Homo                  YGWEQETDENGQVFFVDHINKRTTYLDPRLAFTVDDNPTKPTTRQRYDGSTTAMEILQGR   120
Planctomycetes        ---------------------------------------RRRFGKRTRCDAVVAGV    17
BD1-7                 ---------------------------------------KKFKRSTTSDDVLSGI    16
Gemmatimonadetes      ----------------------------------------FGARTTADEAVADR    14
Moraxellaceae         ---------------------------------------KFGARTAADNVLQDQ    15
SAR86                 ---------------------------------------KFGSRTTADQVLKDK    15


Actinobacteria        DLQGRRILVTGASGGLGLETSRSLAAKGAIVVMAARDHAKNETAAAGIRA-AHPDAKLES    78
Deltaproteobacteria   DLSGKVALITGGSSGLGQETARVLAERGAHVILTARDMPKGQAVAAAIRA-STGNQHVEV    74
Acinetobacter         -LNGKVALVTGANSGIGAVTVRELALQGYHVFLACRDADKAQQVINAIDQQSRGQAKAEF    59
Chloroflexi           ----KTVVVTGGNNGIGLETAVGLAGLGAHVVITARNQAKGEAALADIKK-RSGSDDIEL    55
Acidobacteria         -MTGKVCVVTGANAGIGLETARGLAEMGATVVMVCRDRERGEAAQREIKQ-STGNDAVEL    58
Mandrillus            DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Papio                 DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Cercocebus            DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARAREAVSRILE-EWHKAKVEA   179
Macaca                DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Rhinopithecus         DFTGKVVMVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Nomascus              DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Hylobates             DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
Pan                   DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILG-EWHKAKVEA   179
Pongo                 DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA   179
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Gorilla             DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA    179
Homo                DFTGKVVVVTGANSGIGFETAKSFALHGAHVILACRNMARASEAVSRILE-EWHKAKVEA    179
Planctomycetes      DLRGKAYAITGANAGIGYETARALASRGARVYLLCRHPLRGSLAAERIRR-AHPGAWVEV     76
BD1-7               DLSGKRIIVTGANTGIGFETARALAGAGATVALACRSLETGNAAAQRIRE-AYSGADVCC     75
Gemmatimonadetes    DLSGQLMIVTGANSGIGFHTARALASAGARVVFACRDSAAGAAAVERTLA-AHPGVLAEV     73
Moraxellaceae       DLSGKIIVVTGANAGIGFETARSLAAVGAKVIFACRDKQKGLAAVDKAKK-AHPQCDVEF     74
SAR86               DLSSKNIVVTGANTGIGYEAARALAAAGANVIFACRDKAKGLAAVARAKQ-AHPGCEAQF     74
                    :    :**.. *:*  :   :*   *   * : .*          .

Actinobacteria      LILDLGSLKSVRAAAAEFMSRNVPLHGLILNAGIMATPLGHTVDGFEQQFGVDHLGHFLL    138
Deltaproteobacteria EELELGSLKQIRAFARRFLARHPTLHILVNNAGVMACPRAKTADGFELQFGSNHVGHFVT    134
Acinetobacter       IALDLASLDSIRACAAQFLAKKLPLHLLICNAGLA-GHKGMTKSGFELTFGVCHVGHFLL    118
Chloroflexi         MLADFASLESIRGFAEKFKANHDRLDVLVNNAGGINTSRSETLDGFETTFGVNHLGYFLV    115
Acidobacteria       MVCDLASQASMRQFAADFKQRHDRLDVLVNNAGVLLRERSLNEDGIESTFAVNHLGYFLV    118
Mandrillus          MALDLALLRSVQRFAEAFKAKNVPLHVLVCNAAAFALPWSLTKDGLETTFQVNHLGHFYL    239
Papio               MALDLALLRSVQHFAEAFKAKNVPLHVLVCNAAAFALPWSLTKDGLETTFQVNHLGHFYL    239
Cercocebus          MALDLALLRSVQHFAEAFKAKNVPLHVLVCNAAAFALPWSLTKDGLETTFQVNHLGHFYL    239
Macaca              MALDLALLRSVQHFAEAFKAKNVPLHVLVCNAAAFALPWSLTKDGLETTFQVNHLGHFYL    239
Rhinopithecus       MALDLALLRSVQHFAEAFKAKNVPLHVLVCNAAAFALPWSLTKDGLETTFQVNHLGHFYL    239
Nomascus            MTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFALPWILTKDGLETTFQVNHLGHFYL    239
Hylobates           MTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFAVPWSLTKDGLETTFQVNHLGHFYL    239
Pan                 MTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFALPWSLTKDGLETTFQVNHLGHFYL    239
Pongo               VTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFALPWSLTKDGLETTFQVNHLGHFYL    239
Gorilla             MTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFALPWSLTKDGLETTFQVNHLGHFYL    239
Homo                MTLDLALLRSVQHFAEAFKAKNVPLHVLVCNAATFALPWSLTKDGLETTFQVNHLGHFYL    239
Planctomycetes      LSLDLASLRSVRACAKRFPERL--LEGLIANAGVFGGSYRETEDGFERTVGVCHFGHFAL    134
BD1-7               KQLDLGSLPAVVAFVEALKDSA--IDILVCNAGSMSFEYAETEQGFERTLGVCHIGHFLL    133
Gemmatimonadetes    SLLDLGSRQSISDFAQRIDADS--VDTLICNAGLFGGPYQEVDGALERTVGVSHVGHFLL    131
Moraxellaceae       QQLDLASFDSIRSFCEKFSYEK--VDILIANAGLFSATYKETENNIERTVGVCHFGHFLL    132
SAR86               IQLDLSSFENIRNFCENYPFEK--LDILIANAGLFSTGYGQTQDNIEATVGVCHLGHFLL    132
                    ::.   :            :. *: **.            :*  .   *.*:*

Actinobacteria      ARDLLPRLVESAPARVVVLSSAGHRMG-------DIDFDDVNFERRDY---EPFLAYGAA    188
Deltaproteobacteria TCLLAPALCQGAPSRVVSVSSRGHHMS-------PVVFDDIQFEHRPY---DKWLAYGQA    184
Acinetobacter       TQLLTEKLIESKPARVVVVSSKAHRHA------KGIDFDAVIRPTESM---GALKEYAVA    169
Chloroflexi         TNLLLDLIKASAPARVVSVSSGAHLRA------KGMNFDDLNA-EQGY---SGMGVYGKS    165
Acidobacteria       TNLLLDVLKKSSPSRIVNVSSTAHKYG-------KLDIGGWVA-GRDY---SAFGAYSNS    167
Mandrillus          VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSKNDY---WAMLAYNRS    296
Papio               VQLLQDGLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSKNDY---WAMLAYNRS    296
Cercocebus          VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSKNDY---WAMLAYNRS    296
Macaca              VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSKNDY---WAMLAYNRS    296
Rhinopithecus       VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSKNDY---WAMLAYNRS    296
Nomascus            VQLLQDVLCHSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSRNDY---WAMLAYNRS    296
Hylobates           VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPSRNDY---WAMLAYNRS    296
Pan                 VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPTKNDY---WAMLAYNRS    296
Pongo               VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPTKNDY---WAMLAYNRS    296
Gorilla             VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPTKNDY---WAMLAYNRS    296
Homo                VQLLQDVLCRSAPARVIVVSSESHRFTDINDSLGKLDFSRLSPTKNDY---WAMLAYNRS    296
Planctomycetes      FLGLWERLRAAERPRLVVVSSGSHRSP------PRLDFEHFLRPRERY---RPLVAYGQA    185
BD1-7               VKRLMPKLLASGTPRVVMVSSIAHQNP------KTLDFSNLPYTESAY---SVMGAYGQA    184
Gemmatimonadetes    VRALLPELLAXGGGRVVMVASESHRTP------SKLDFDRLPLTAAQY---SGFVAYGQA    182
Moraxellaceae       TNLLMPKLLAADKPRVVMVSSESHRMP------AKINFNRFPLNSKDIIAVATMVAYGQA    186
SAR86               TQGLMDKLLKANKPRIVMVSSESHRSP------KELDFDNLPLKKTNY---SAFTAYGQA    183

                    *   :      *:: ::* .*            : :               *  :

Actinobacteria      KTCNVLHAVEIDRRYRDQGVRAFAVHPGVIHTELSRYMTEETLTSLISRLSERPTAMAWK    248
Deltaproteobacteria KTANVLFAVGLERRLGARGVHANALHPGGIMTELSRHLQPEDMQF----LQARTRGMKFK    240
Acinetobacter       KLANVLFVKELGRRLYGTGVTAYAVHPGVVSTNVWRSL---------PKPMVKVLSRWMI    220
Chloroflexi         KLANVLFTYELARRLEGSGVTANCLHPGVVRSGFGQNNGGL--IAIVFKAAYTLMTPFTK    223
Acidobacteria       KLANVLFTYELARRLQGTGVTANCLHPGGVGTNLFRGLPGF--LQT-------LIKLVTI    218
Mandrillus          KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Papio               KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Cercocebus          KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Macaca              KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Rhinopithecus       KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Nomascus            KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Hylobates           KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Pan                 KLCNILFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Pongo               KLCNVLFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Gorilla             KLCNILFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
Homo                KLCNILFSNELHRRLSPRGVTSNAVHPGNMMYSNIHRSWWV------YTLLFTLARPFTK    350
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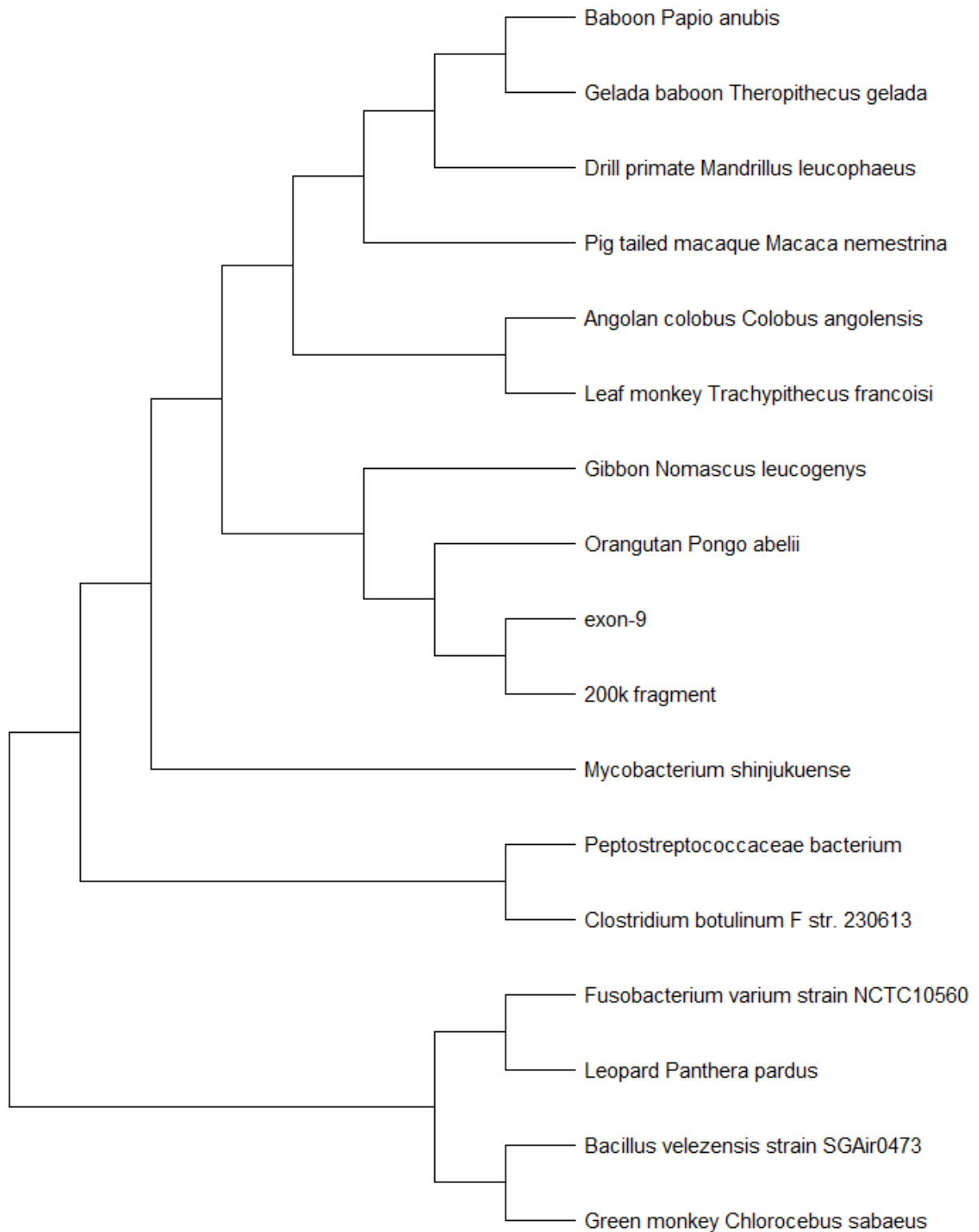
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Planctomycetes           KLCNVLFAAEAQRRFGAEGLTAVSLHPGPLIATDIGRDAWY------ARLAIALARPFSR    239
BD1-7                    KLCNILMALELQNRYGAQGLTACSVHPGSGVSTDFGRESGF------MKVALKLISPFTK    238
Gemmatimonadetes         KLCNVLFASELQRRYGGEGLTACSLHPGSMIPTNIARSSSL------AGFVMKLISPFTK    236
Moraxellaceae            KLCNILFANELQRRFGEKGLNACSLHPGALITTDLFRNSAF------GSVIMKVISPFTK    240
SAR86                    KLCNVLFAKELQRRFEDKGLSACSLHPGTLITTDIGRNSFW------VSLGMKIISPFTK    237
*  .*:*      .*     *:  :  .:***

Actinobacteria           SPEQGAATSVWAATSPLLDGRGGEYCEDCNVSAVVPDDELDDGGVAARAVDPARATALWA    308
Deltaproteobacteria      SVEAGAATSVFAATAPELEGRGGLYLEDCHVAAVNDAPD-ALDGVKSYALDPQSAERLWE    299
Acinetobacter            SPEEGAQTTLYCATQPQLSGETGMYYDNCKVTASSG-----------TAQDIQLARLLW-    268
Chloroflexi              SNAQGAATSIHLAASPDVEGVTGKYFADSKETPSSA-----------ASHDEEAARRLWK    272
Acidobacteria            GPERGARTSIYLASSPEVEGITGKYFVRRRQQKSSE-----------TSRNEEAARRLWE    267
Mandrillus               SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARALWA    399
Papio                    SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARALWA    399
Cercocebus               SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARALWA    399
Macaca                   SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARALWA    399
Rhinopithecus            SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARALWA    399
Nomascus                 SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWA    399
Hylobates                SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWA    399
Pan                      SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWV    399
Pongo                    SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWA    399
Gorilla                  SMQQGAATTVYCAAAPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWA    399
Homo                     SMQQGAATTVYCAAVPELEGLGGMYFNNCCRCMPSP-----------EAQSEETARTLWA    399
Planctomycetes           SVVQGAATSVYCATAPEVEEHGGGYFRDCAPCLPSS-----------EARDPEVARRLWE    288
BD1-7                    SANQGAATSVLCAVHEPAEDLAAGYFSHCQLAQCTP-----------EARDVNVAKALWE    287
Gemmatimonadetes         DLNTGAATSIVCATHPHLEEISGRYFSDCRPKLPSA-----------EASDVAVAERLWK    285
Moraxellaceae            NTNQGASTTVYCAAYASEEEIAGNYFSHCKIAKATD-----------EANNPEVAKKLWE    289
SAR86                    TANQGASTSVYCAAYASETEIAGHYFSHCQKASSSR-----------AAKSTETATRLWQ    286
** *::   *         . *                     : .    *   **

Actinobacteria           LSEKLV---------  314
Deltaproteobacteria      VSEKLVGERF-----  309
Acinetobacter            ---------------  268
Chloroflexi              LSEKM----------  277
Acidobacteria            VSEEL----------  272
Mandrillus               LSERLIQERLGSQSG  414
Papio                    LSERLIQERLGSQSG  414
Cercocebus               LSERLIQERLGSQSG  414
Macaca                   LSERLIQERLGSQSG  414
Rhinopithecus            LSERLIQERLGSQSG  414
Nomascus                 LSERLIQERLGSQSG  414
Hylobates                LSERLIQERLGSQSG  414
Pan                      LSERLIQERLGSQSG  414
Pongo                    LSERLIQERLGSQSG  414
Gorilla                  LSERLIQERLGSQS-  413
Homo                     LSERLIQERLGSQSG  414
Planctomycetes           LSEDLV---------  294
BD1-7                    RSE------------  290
Gemmatimonadetes         RTE------------  288
Moraxellaceae            FSEKI----------  294
SAR86                    LSEQL----------  291
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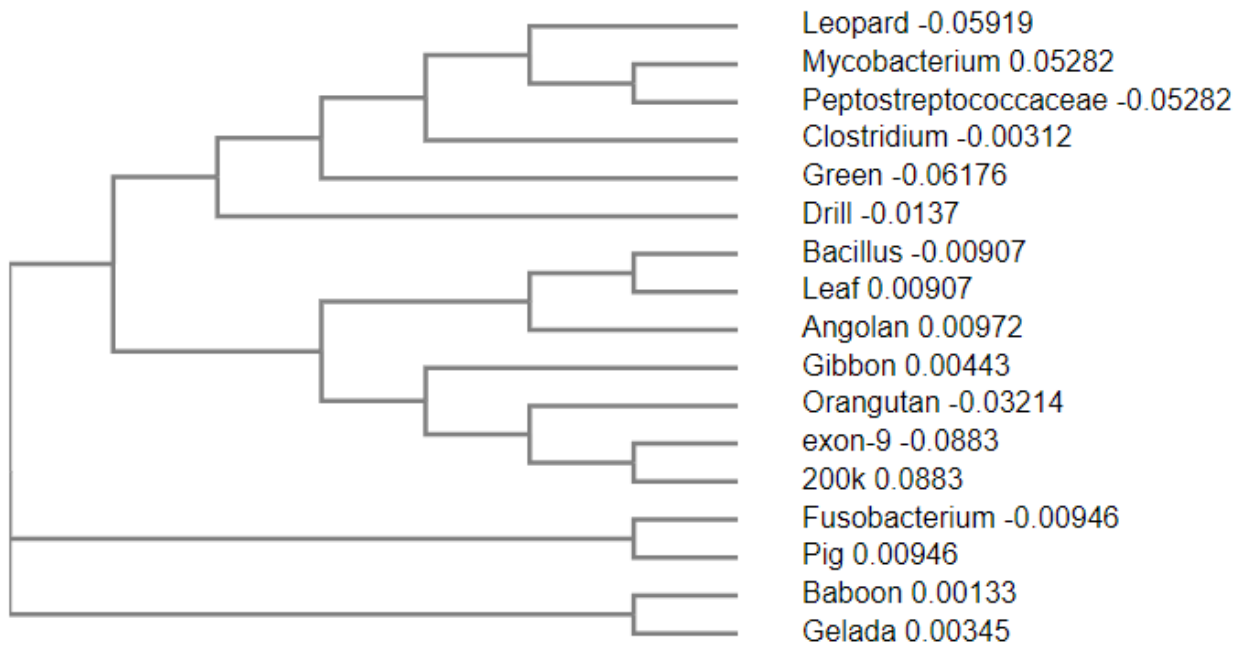
> ➤ **Analysis and conclusion:**

- o There is **not a single conserved site for the 15 species in the gene MSA**. But there exist **many conserved sites** in the protein MSA marked by *. There are exactly 36 conserved sites in the protein MSA.

- o Thus, **proteins are conserved more compared to genes.** The mutations which don't alter the function of the protein are incorporated whereas others eliminated.

- o Moreover, the regions of alignment for proteins and gene is different for both the MSAs. This can be attributed to codon degeneracy.
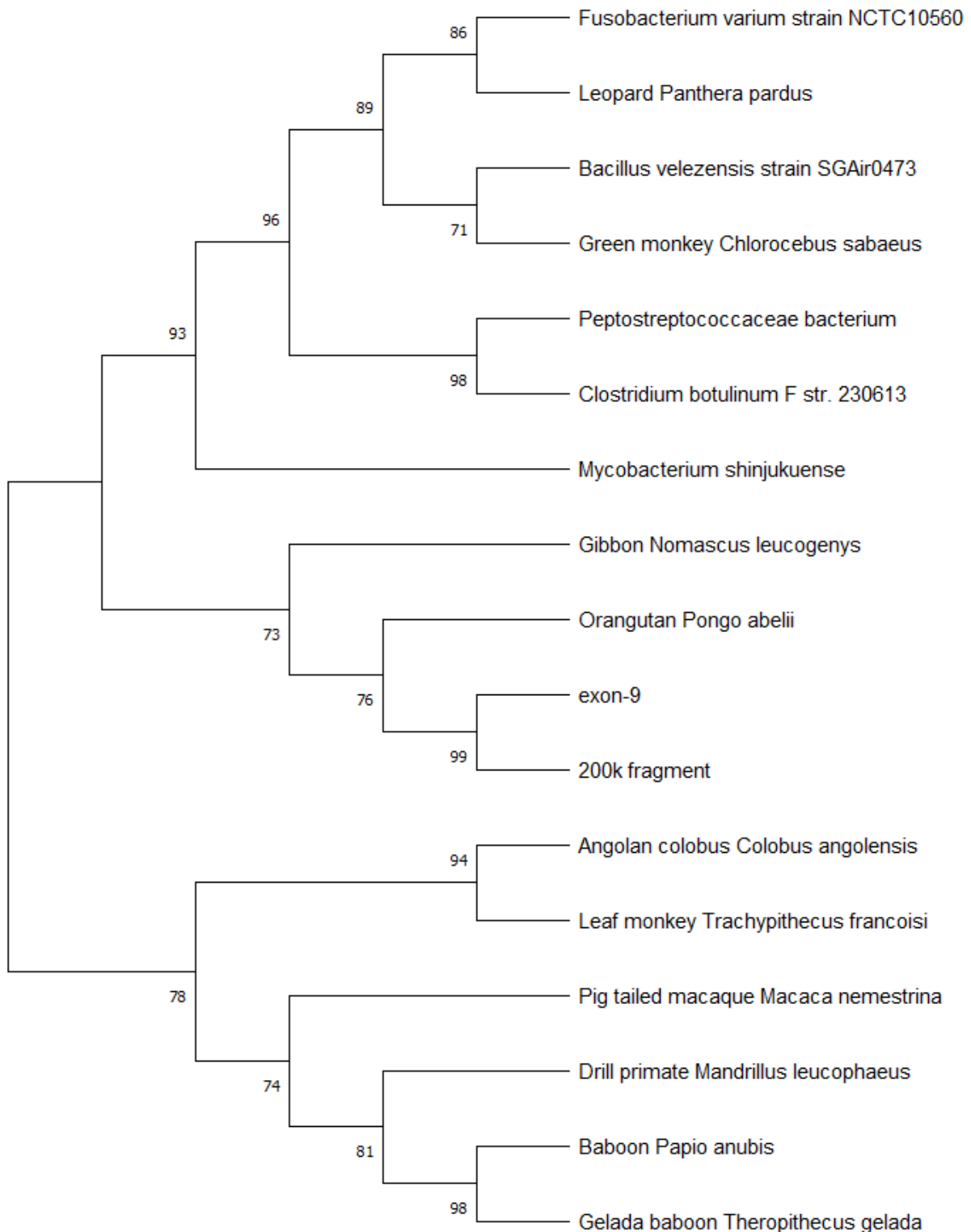
Evolutionary trees (Gene MSA)

➢ Maximum likelihood tree for genes (MEGA-X)

➢ **Neighbour Joining Tree for gene (CLUSTAL omega)**


```
Leopard -0.05919
Mycobacterium 0.05282
Peptostreptococcaceae -0.05282
Clostridium -0.00312
Green -0.06176
Drill -0.0137
Bacillus -0.00907
Leaf 0.00907
Angolan 0.00972
Gibbon 0.00443
Orangutan -0.03214
exon-9 -0.0883
200k 0.0883
Fusobacterium -0.00946
Pig 0.00946
Baboon 0.00133
Gelada 0.00345
```

➢ **Maximum Parsimony tree for genes (MEGA-X)-**
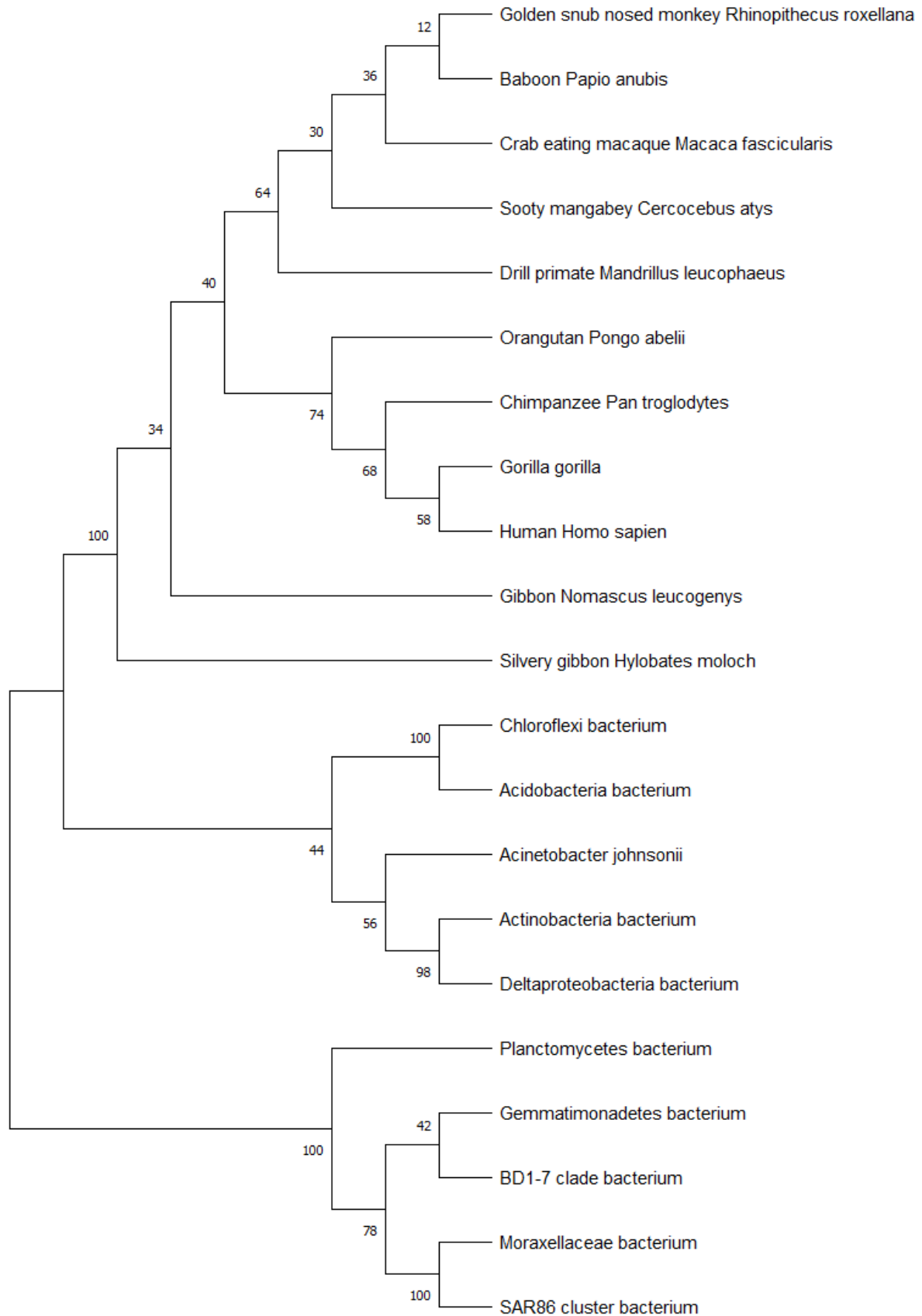

➢ **Analysis and Conclusion:**

o The exon-9 and the 200k fragment are rightly aligned closest in all the 3 methods.

- o In the NJ tree, *Fusobacterium* has been shown to be close to the primates. But, in the ML tree, *Fusobacterium* has been grouped with prokaryotes. In case of such contrasting results, we go with the results of the ML tree as the math behind the ML tree better models the natural evolution. **NJ method is fast but ML method is more reliable.**
- o Guidelines for the methods to use for phylogenetic trees-
    - NJ method is a heuristic method and it very quickly gives one an idea of the shape of the tree.
    - Use MP, again with bootstrapping to get an idea of character variation across the tree, in particular, which characters influence which branches.
    - Finally, use ML method to generate the final tree.

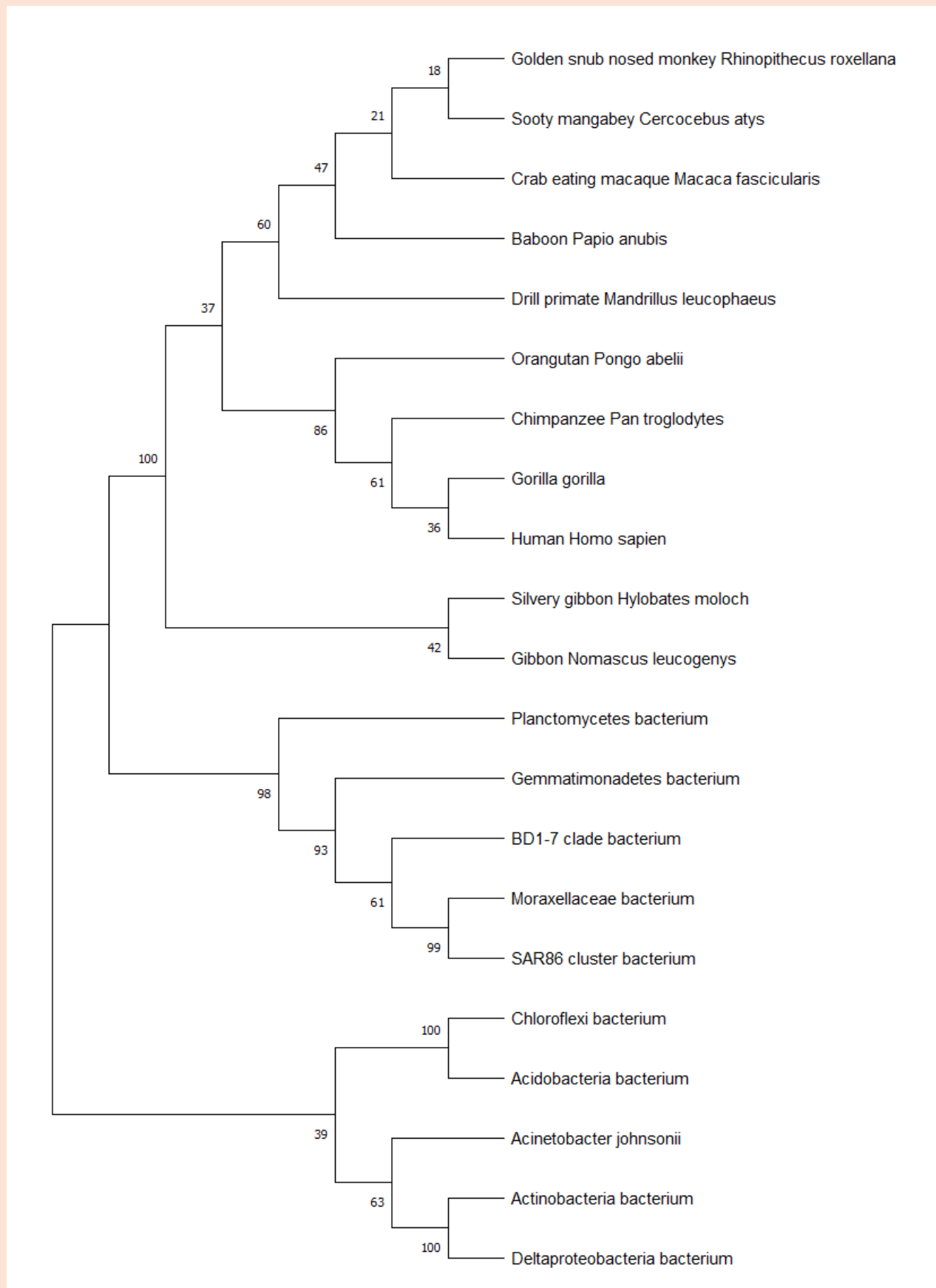➢ Human WWOX gene has been most closely evolved from orangutan according to ML tree.

P.T.O
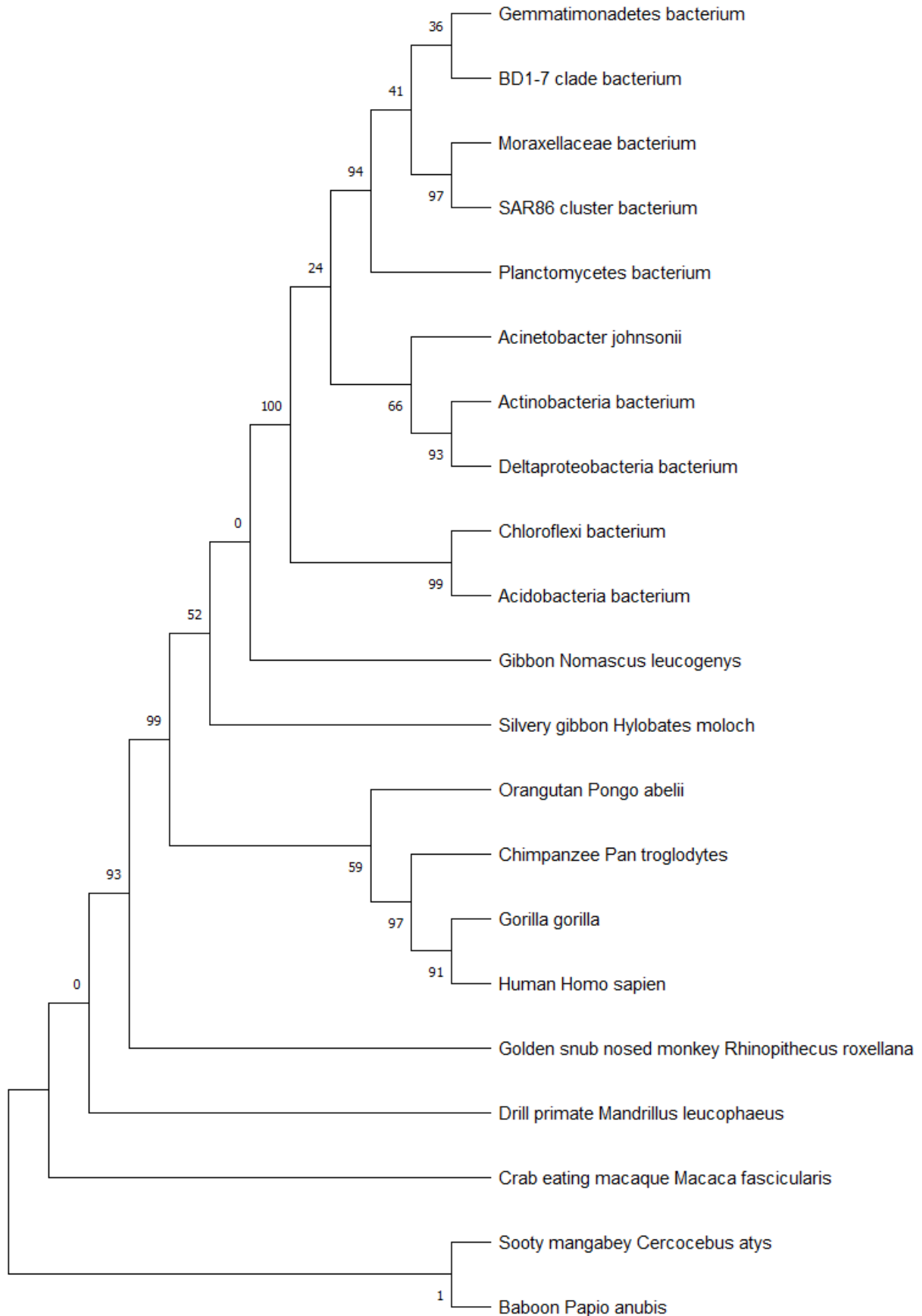
Phylogenetic trees for protein BLAST

➢ **Maximum likelihood tree for proteins (MEGA-X)**

➢ Neighbour joining tree for proteins (MEGA-X)

	Bootstrap	Taxon

18 — Golden snub nosed monkey Rhinopithecus roxellana
21 — Sooty mangabey Cercocebus atys
47 — Crab eating macaque Macaca fascicularis
60 — Baboon Papio anubis
37 — Drill primate Mandrillus leucophaeus
Orangutan Pongo abelii
86 — Chimpanzee Pan troglodytes
61 — Gorilla gorilla
36 — Human Homo sapien
Silvery gibbon Hylobates moloch
42 — Gibbon Nomascus leucogenys
100
Planctomycetes bacterium
Gemmatimonadetes bacterium
98 — BD1-7 clade bacterium
93 — Moraxellaceae bacterium
61 — SAR86 cluster bacterium
99
Chloroflexi bacterium
100 — Acidobacteria bacterium
Acinetobacter johnsonii
39 — Actinobacteria bacterium
63 — Deltaproteobacteria bacterium
100

> **Maximum parsimony tree for proteins (MEGA-X)**

➤ Analysis and conclusion for protein phylogenetic tree-

→ **1** WMV is predicted to be phylogenetically evolved from Gorilla by the ML tree.

→ The phylogenetic tree for the protein is found to be different from the phylogenetic tree of the gene.

→ Primates are clustered together, and the prokaryotes are clustered together.

P.T.O

ACKNOWLEDGMENT

I would like to thank Prof. Shibasish Chowdhury for giving me an opportunity to work on WW domain containing oxidreductase as end term project

REFERENCES

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   https://www.uniprot.org/citations/10861292

2) *"WWOX gene: Genecards"*

   https://www.genecards.org/cgi-bin/carddisp.pl?gene=WWOX#summaries

3) *"Computational analysis of functional long noncoding RNAs reveals lack of peptide-coding capacity and parallels with 3' UTRs"*

   https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3312569/