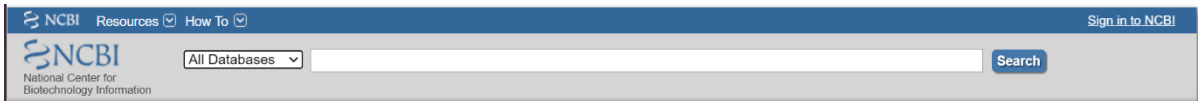
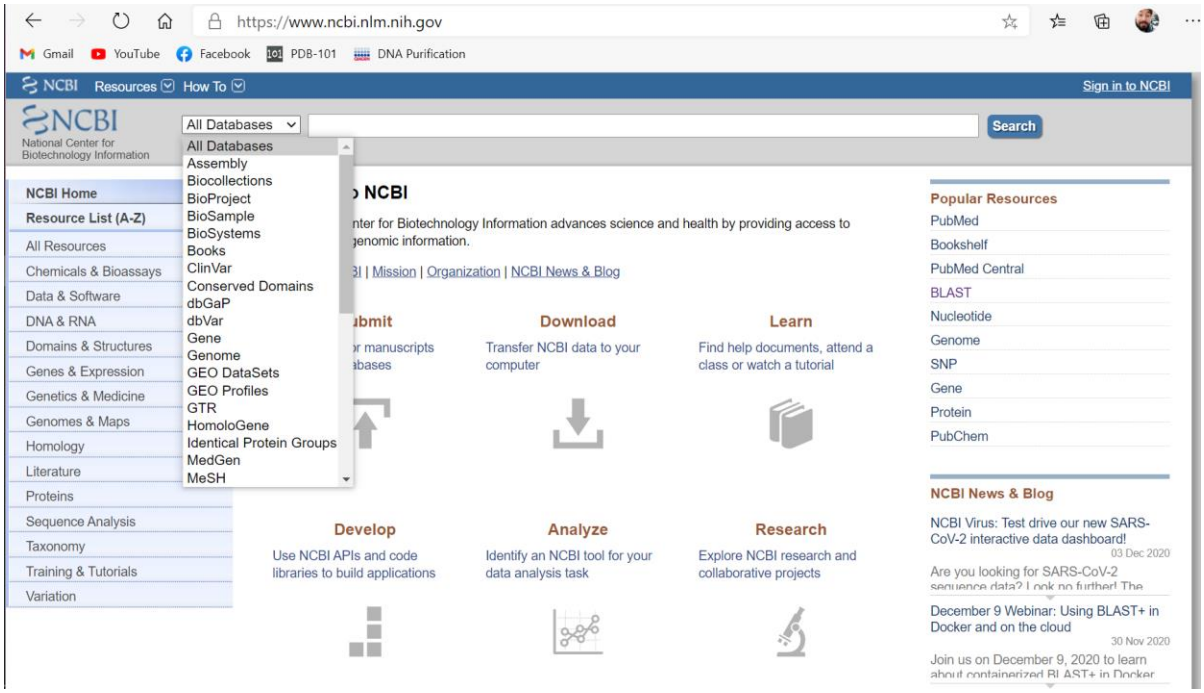
Primer Design

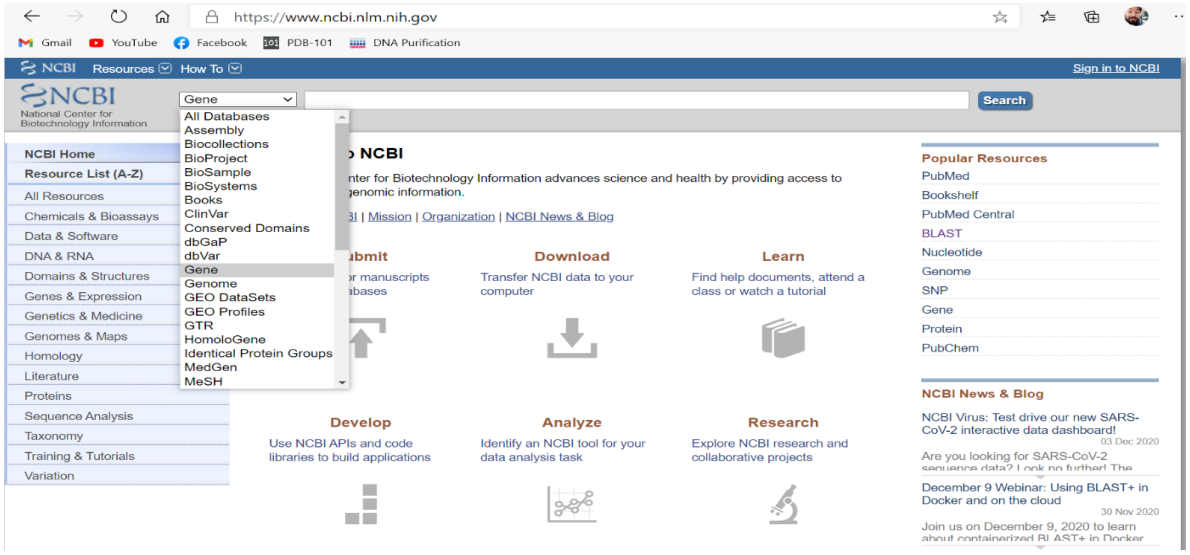
1. **Go to NCBI (National Center for Biotechnology Information) website (National Center for Biotechnology Information (nih.gov)**
2. **On the upper part of the page you will find:**



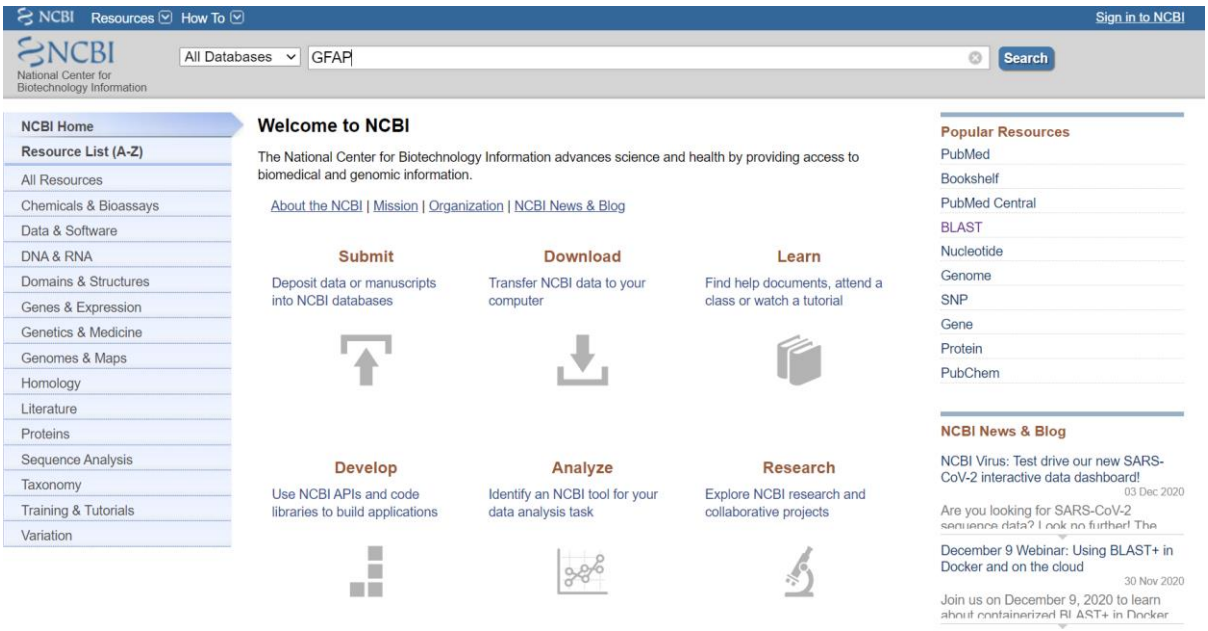
3. **Click on All Databases**



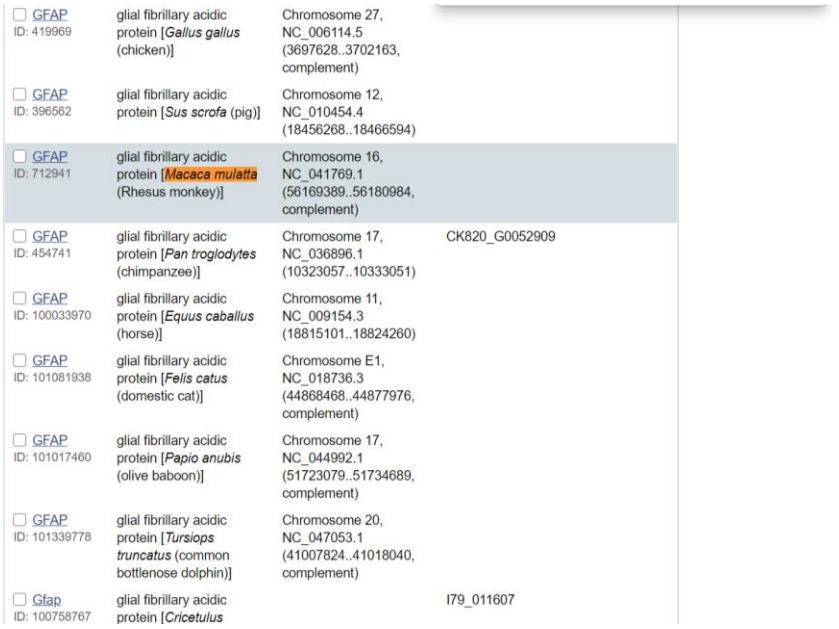
4. **Select 'Gene'**



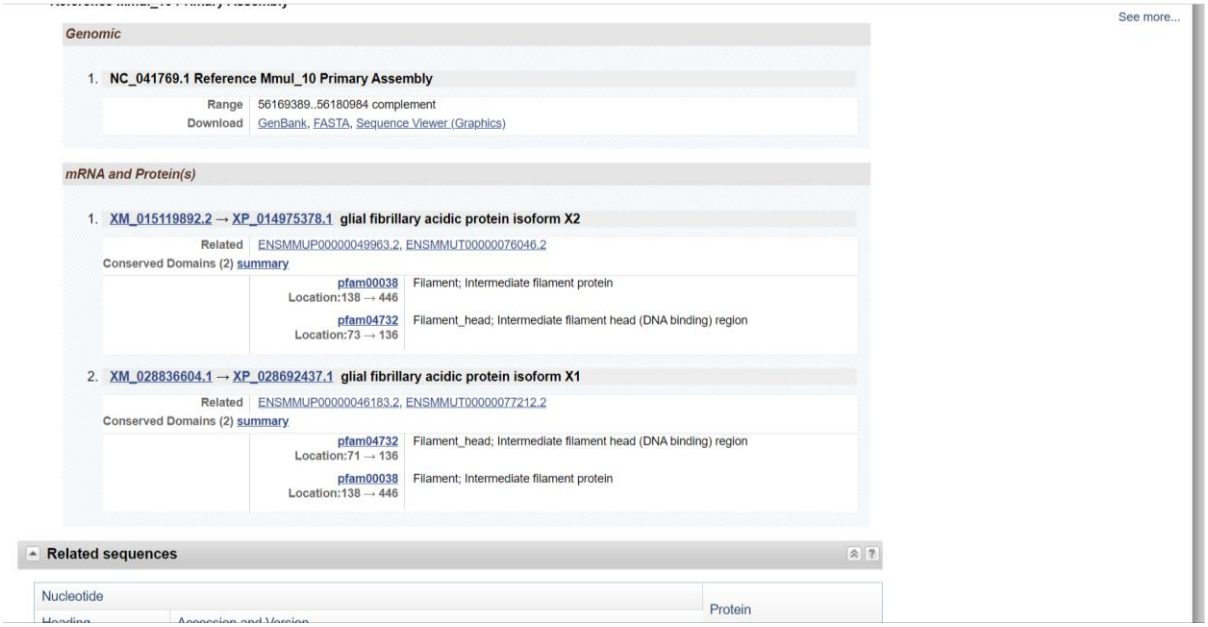
5. **Type the name of your gene of interest in the query box (GFAP for example)**



6. **Find your species of interest. Click ctrl+F and type in the name of the species (in our case this is *Macaca mulatta (Rhesus monkey)***



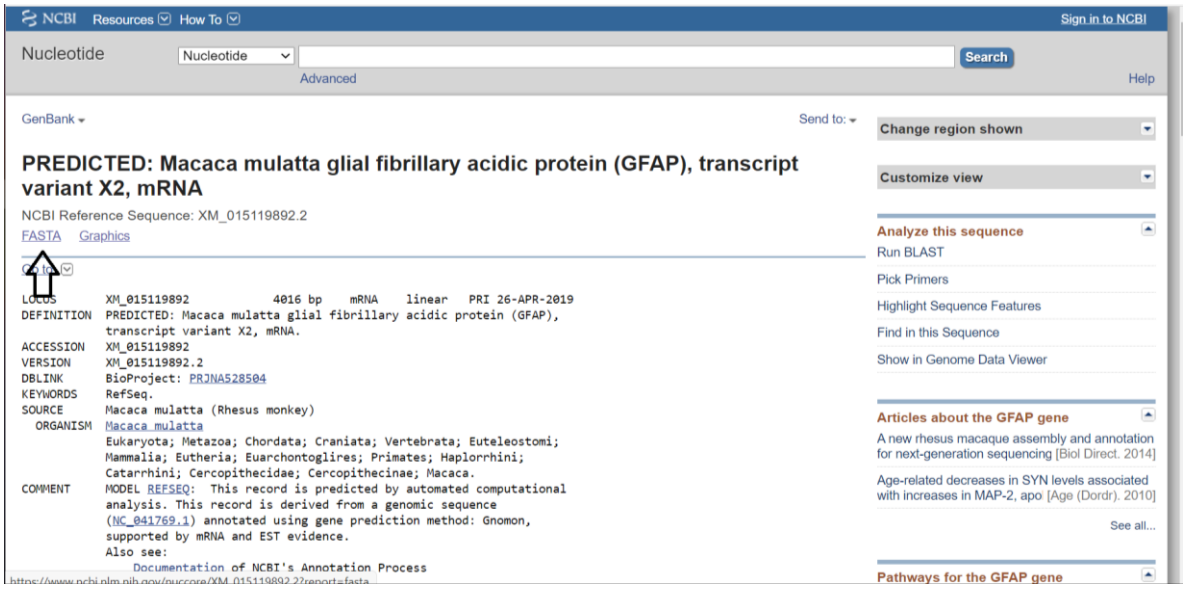
7. **Click on the gene name. In the following page you should find *mRNA and Protein(s) section***



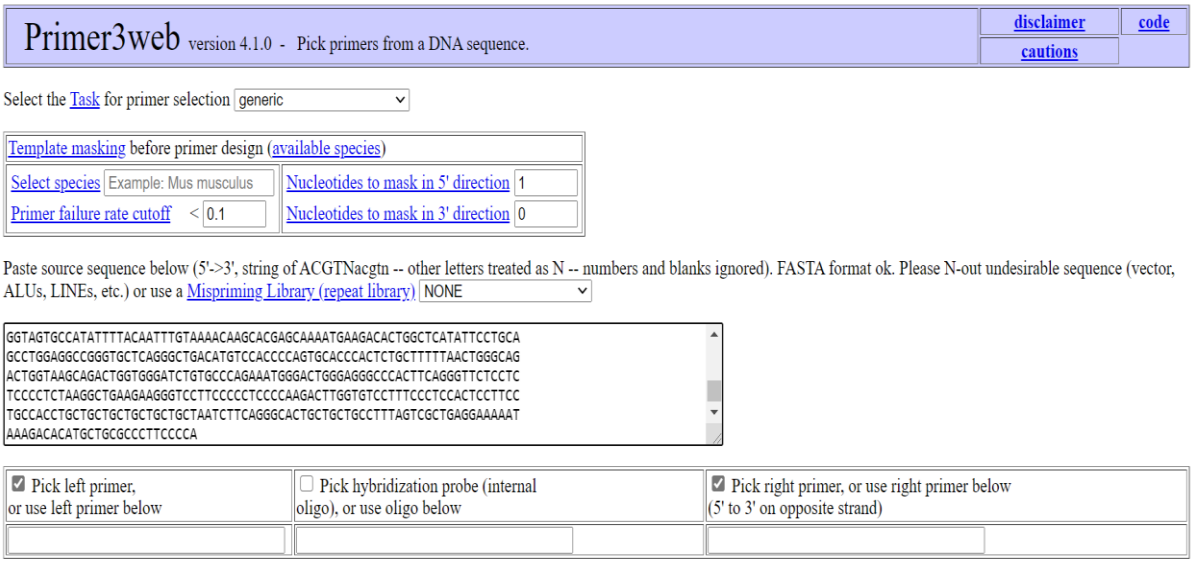
8. **Click XM_015119892.2 which is accession number of the mRNA sequence**
   N.B. XP_014975378.1 is the protein sequence ;
   prefix XM_ (mRNA), XR_ (non-coding RNA), and XP_ (protein)

9. **The following page will show you the all the information about the mRNA of the gene that you are searching.**

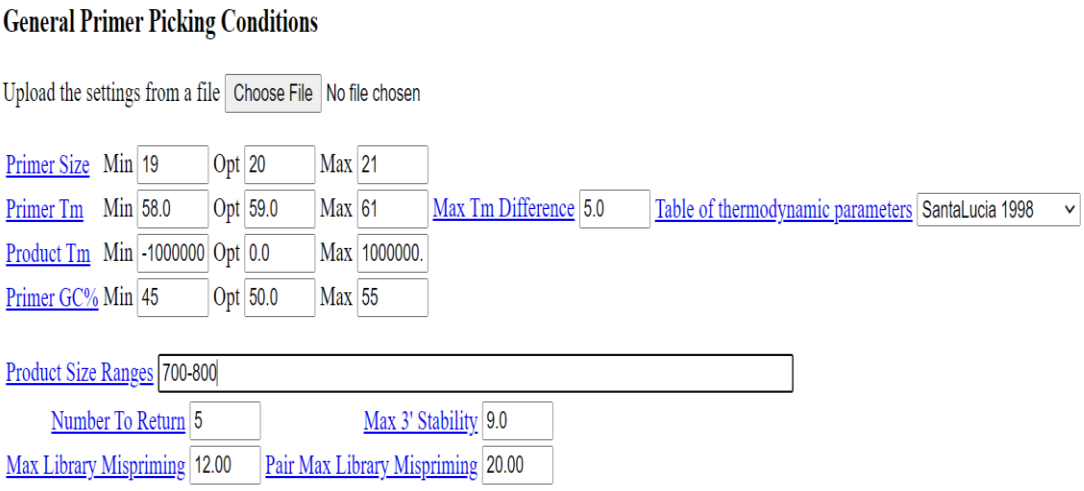
10. **Next click FASTA to obtain the full sequence of the mRNA of your gene of interest.**



11. **Copy the sequence and transfer it into a word file**
12. **Go to Primer3 Input website**
13. **Paste the sequence in the box**



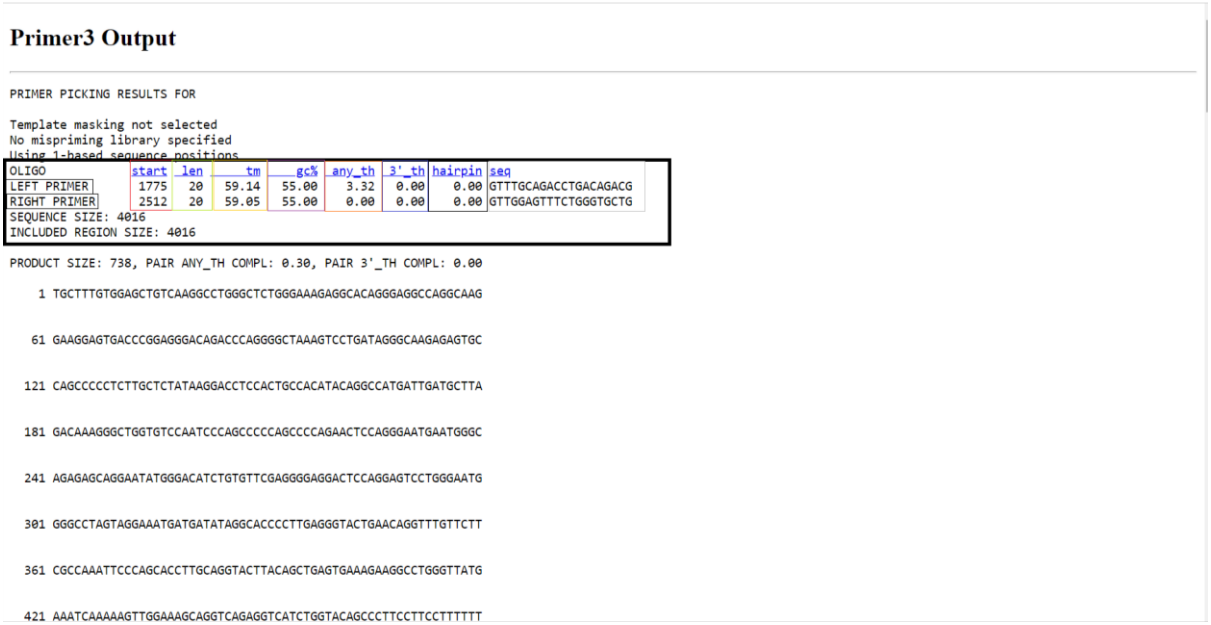
14. **Next go down and find General Primer Picking Conditions. Using the information that we have provided in Primer Design Rules file, fill the spaces as indicated.**



N.B. Choose your product size range accordingly to the length of your mRNA sequence. Optimal range amplicon length product is between 600-1000bp. For genes with shorter than 600bp of length we usually design amplicon which ranges between 60-70% of the total length. Note that for those smaller gene there is no strict rule about the length of the amplicon.

15. **Scroll down in the page, find, and click the button PICK PRIMERS.**

16. The page that will open will show you the primes that the program picked

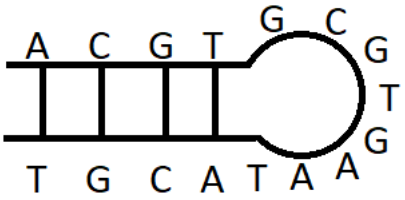


You can see some information about the primers that the program picked.

1. In the red box (start) is the information about the position of the 5' base of the primer.
2. In the green box (len) is the information regarding the length of the primer.
3. In the yellow box (Tm) is the information regarding the melting temperature of the primer.
4. In the violet box (gc%) is the information about the GC% content of the primers or the percent of G or C bases in the primer.
5. In the orange box -any th (Self-Complementarity) and **brown box** -(hairpin) in the information about the tendency to primers to anneal to itself or to form secondary structure (hairpins). The lower the value the better.

Self-Complementarity

5' ACGTGCGTGAATACGT 3`



6. In the violet box (3`th)
   The self-complementarity of the primer at the 3` ends (primer dimers formation with itself). The lower the value the better.

7. The final Gray box (seq) represent the primers sequence.

8. Copy and paste all the information from the BLACK BOX into your word file.

9. Add T7 promotor sequence to the forward primer-

   T7 GCGTAATACGACTCACTATAGGG + Forward PRIMER

10. Add Sp6 promotor sequence to the reverse primer-

    Sp6 GCGATTTAGGTGACACTATAG + Reverse PRIMER