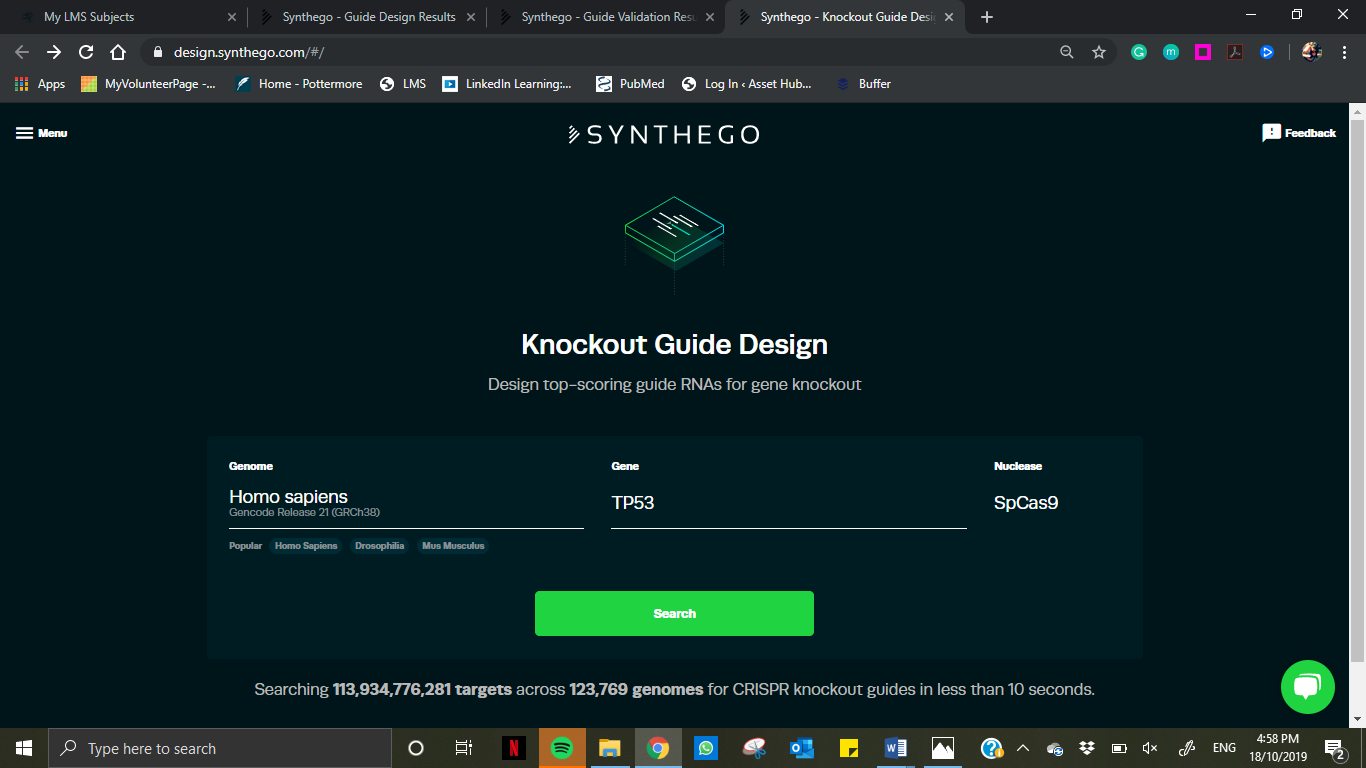
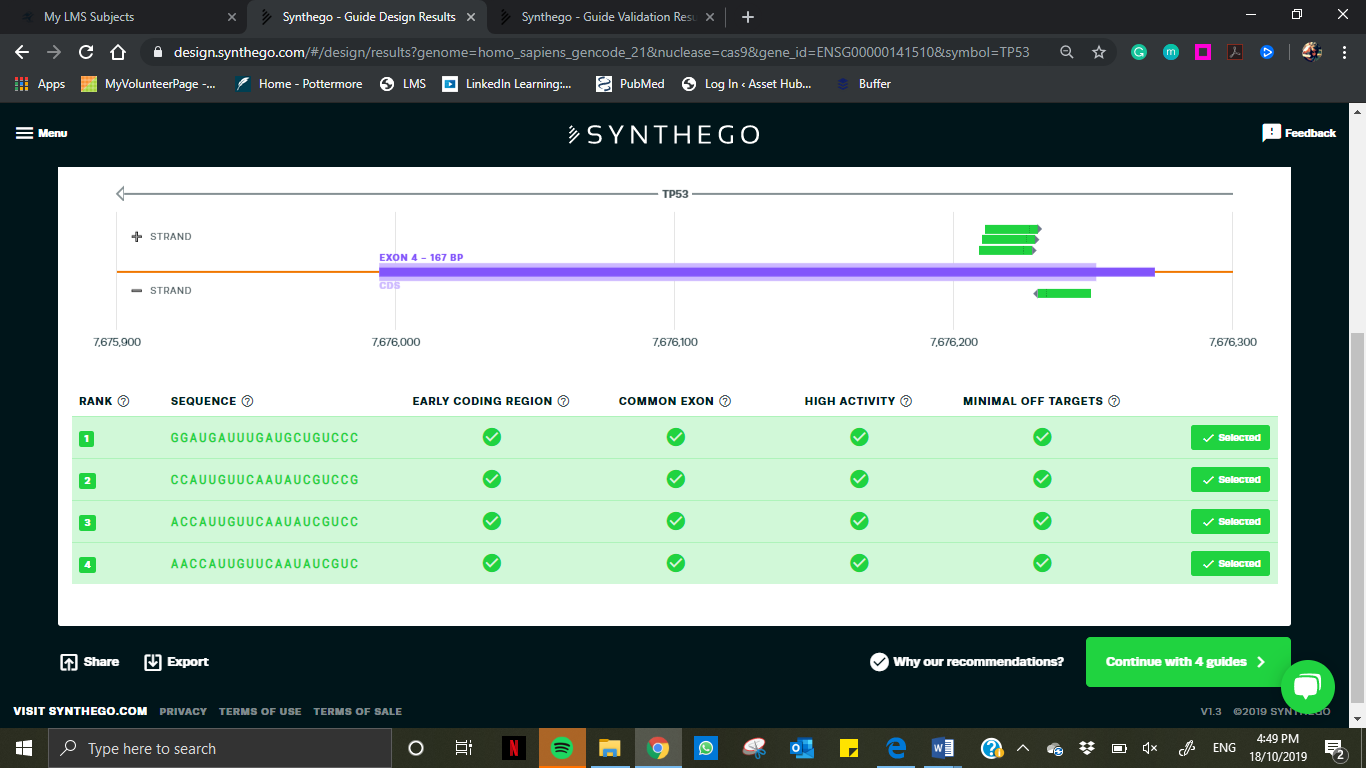
MED3ATB Mouse models worksheet

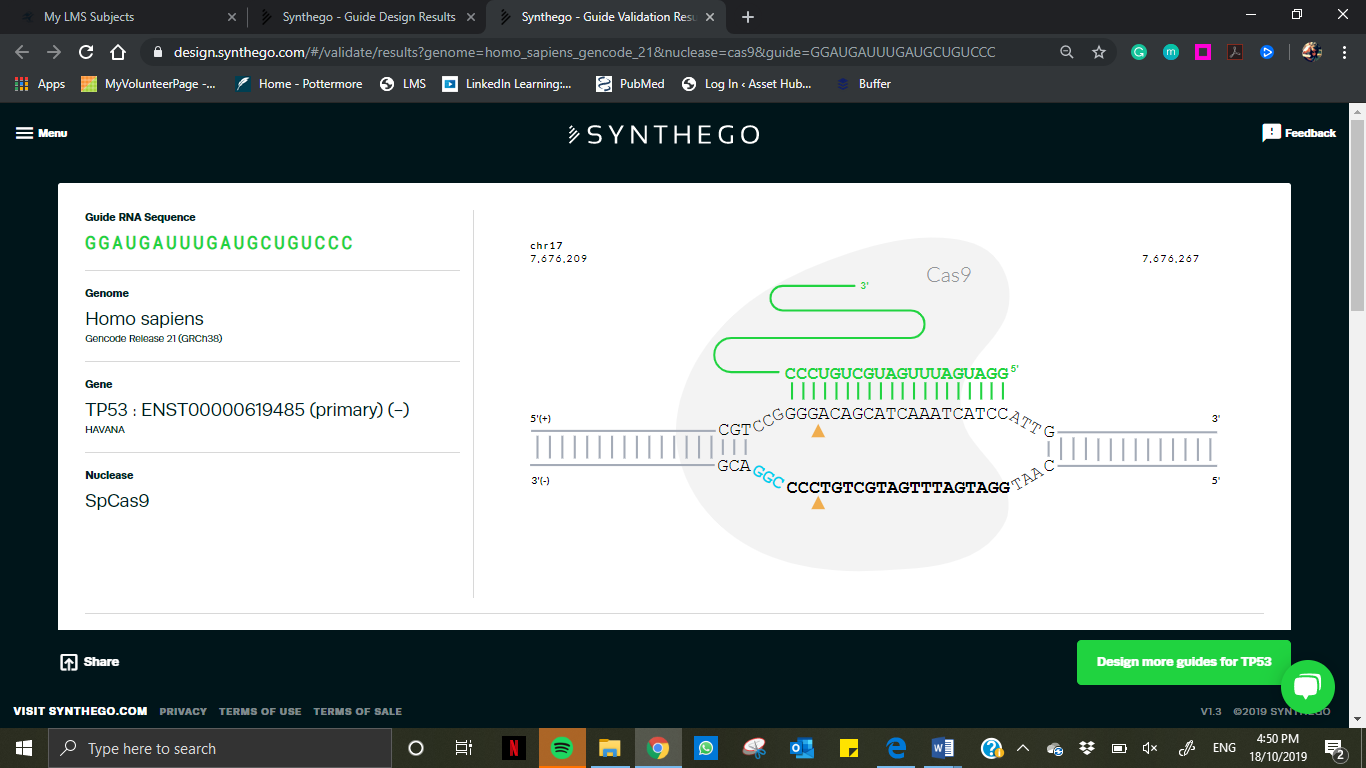
**Step 1.** Go to website: <https://design.synthego.com/#/>



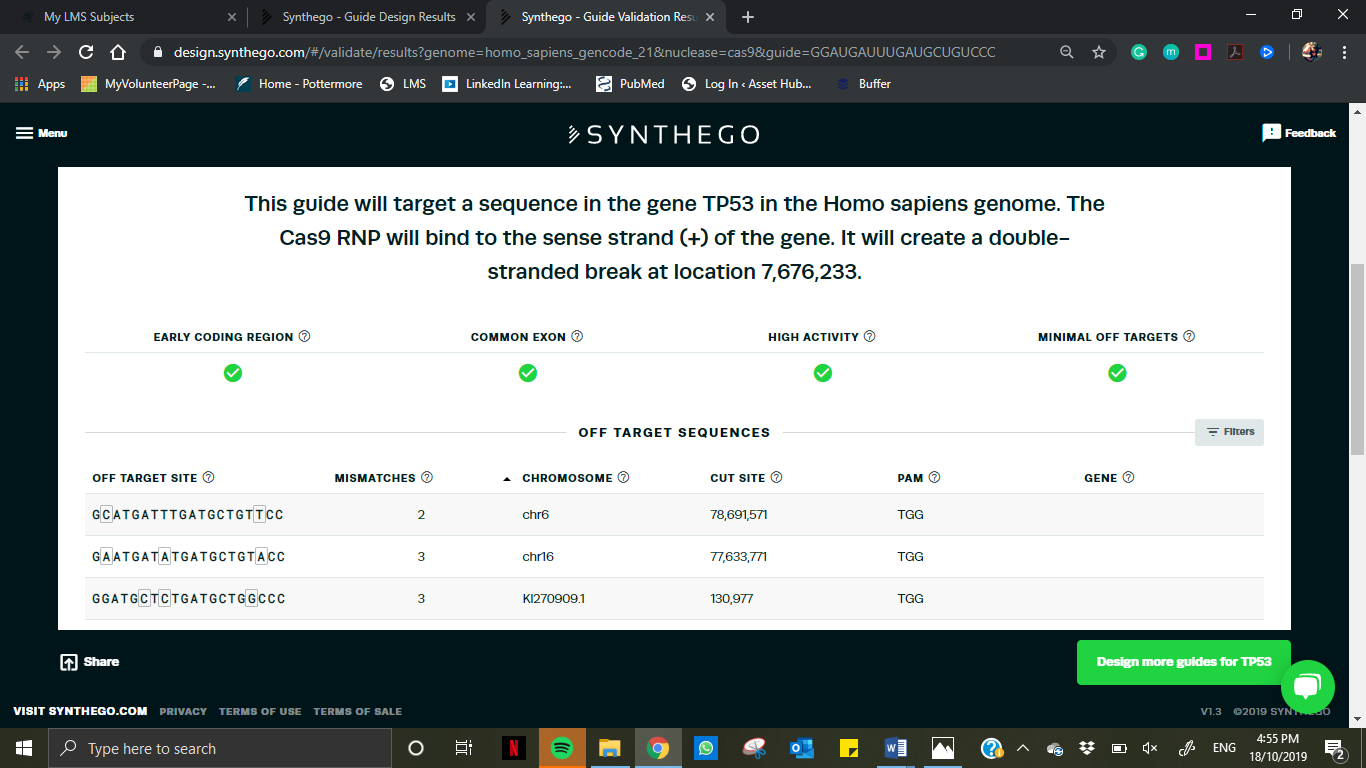
**Step 2:** Take a screenshot of the output.

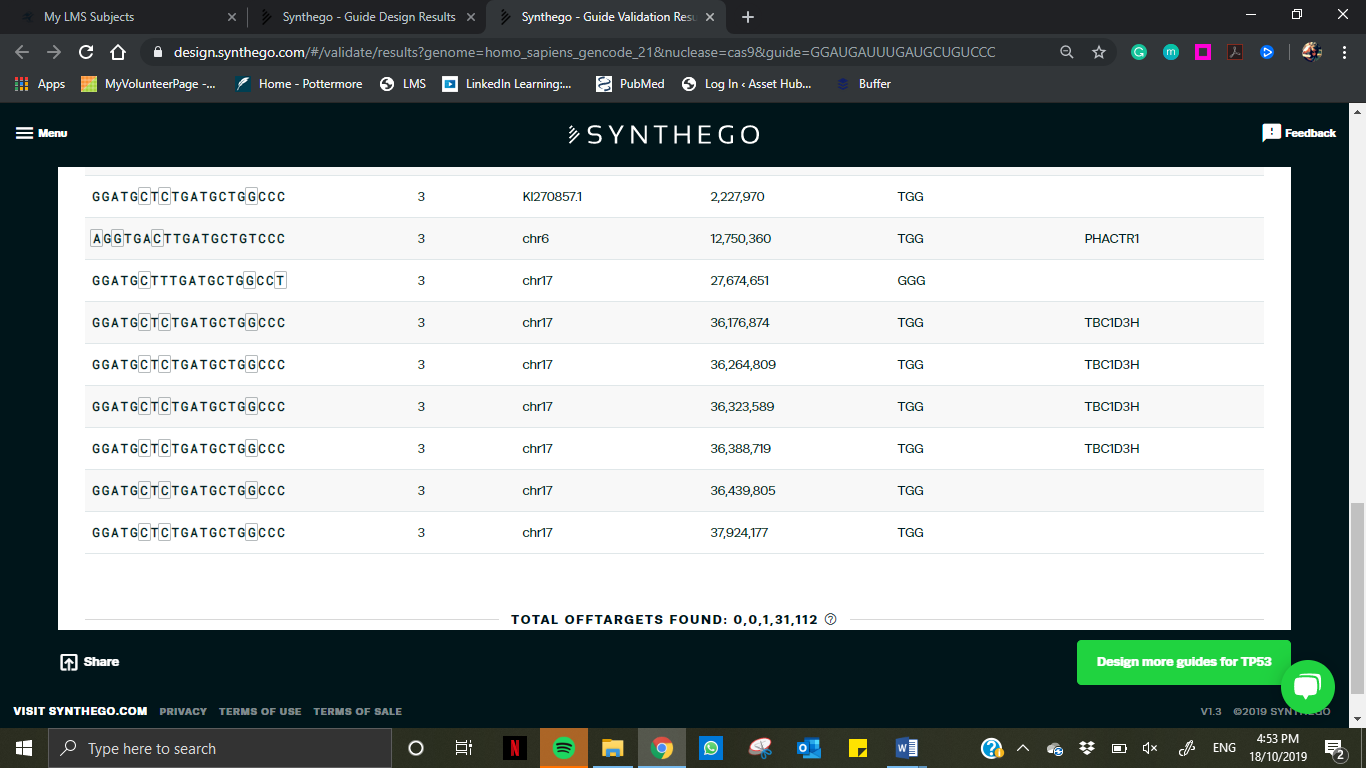


**Step 3:** Mark the PAM sequence.



**Step 4:** Identify the off-target sites.



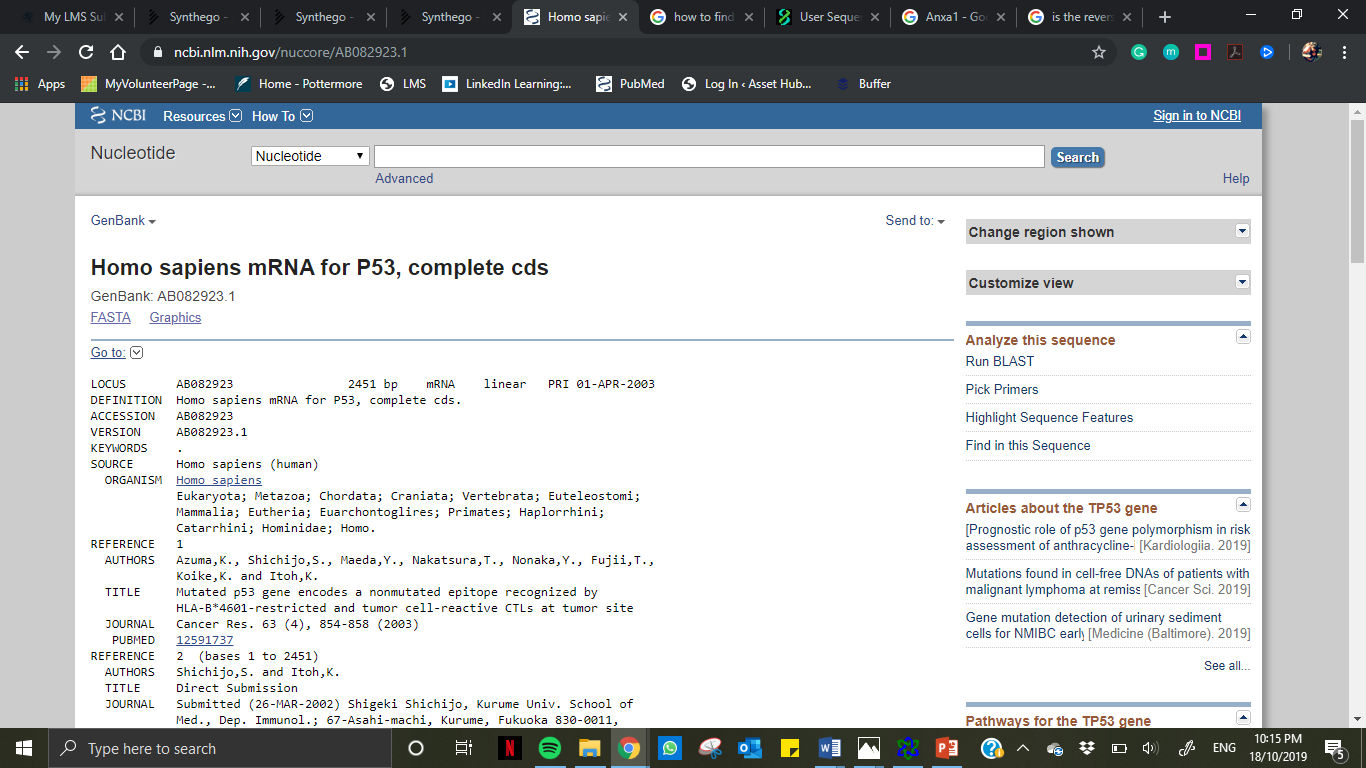


**Step 5:** Explain an off-target effect.

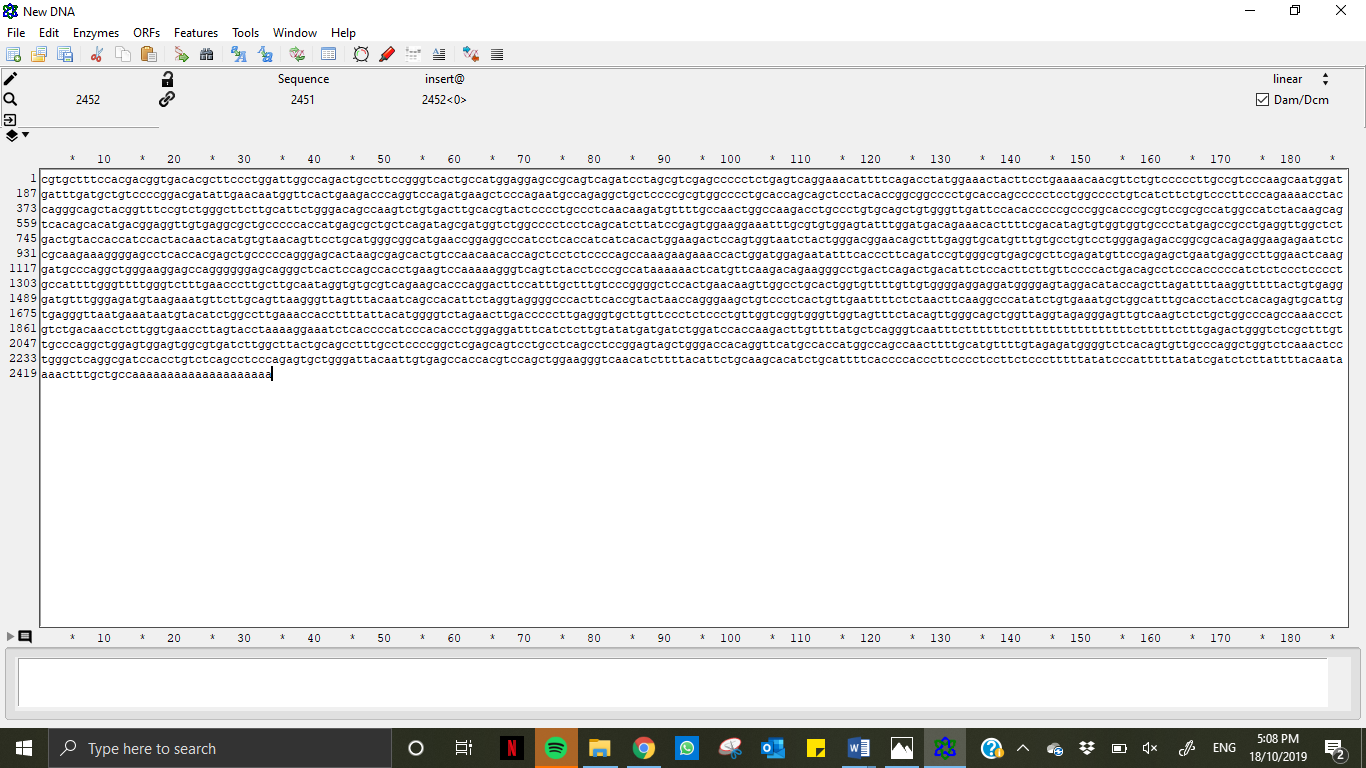
I chose PHACTR1. As PHACTR1 gene has a similar sequence to that of p53, there is a possibility that PHACTR1 too could be knocked out during CRISPR that target p53 (off target effect). PHACTR1 gene encodes for the protein, phosphatase and actin regulator 1. This protein is a member of the actin regulator family of proteins and regulates the reorganization of the actin cytoskeleton, actin stress fibre formation and endothelial cell survival through upon being bound by actin. Polymorphisms of this gene can result in increased susceptibility to myocardial infarction, coronary artery disease and cervical artery dissection. Moreover, multiple transcript variants will be observed through alternative splicing of PHACTR1 gene.

**Step 6:** Search the cDNA sequence using Pubmed (<http://www.ncbi.nlm.nih.gov/pubmed>). Search term is your accession number.

(Searched for “Human p53” in PubMed (nucleotide) instead)



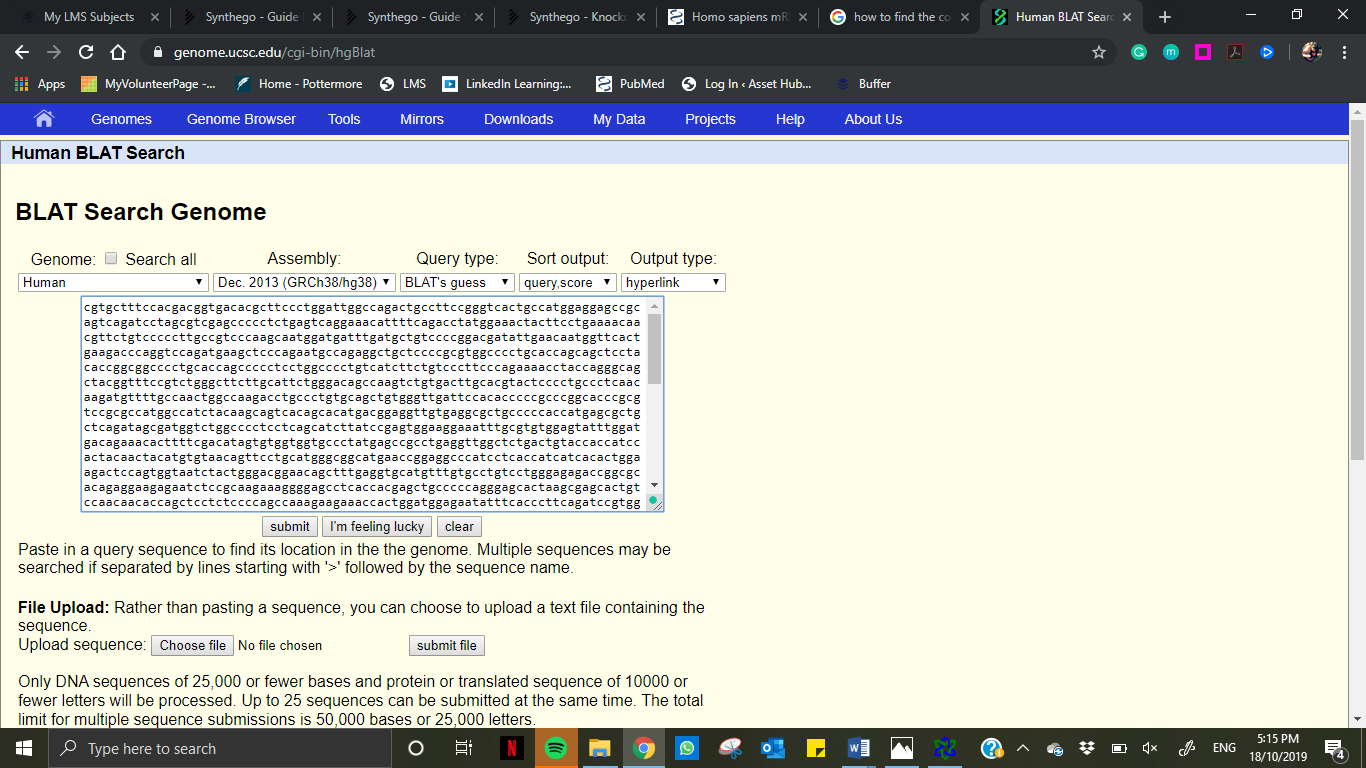
**Step 7**. Copy the sequence and create an Ape.

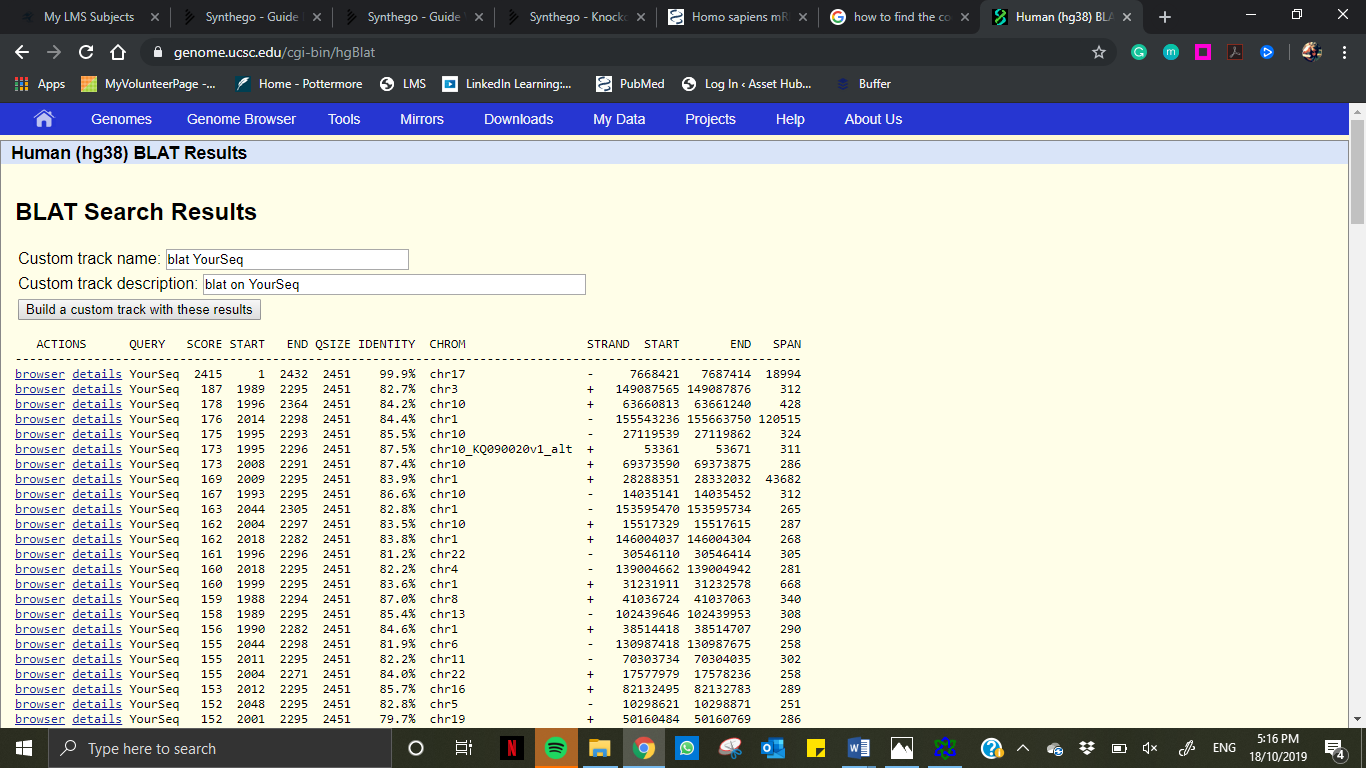


**Step 8**. Find the coding region.

cgtgctttccacgacggtgacacgcttccctggattggccagactgccttccgggtcactgccatggaggagccgcagtcagatcctagcgtcgagccccctctgagtcaggaaacattttcagacctatggaaactacttcctgaaaacaacgttctgtcccccttgccgtcccaagcaatggatgatttgatgctgtccccggacgatattgaacaatggttcactgaagacccaggtccagatgaagctcccagaatgccagaggctgctccccgcgtggcccctgcaccagcagctcctacaccggcggcccctgcaccagccccctcctggcccctgtcatcttctgtcccttcccagaaaacctaccagggcagctacggtttccgtctgggcttcttgcattctgggacagccaagtctgtgacttgcacgtactcccctgccctcaacaagatgttttgccaactggccaagacctgccctgtgcagctgtgggttgattccacacccccgcccggcacccgcgtccgcgccatggccatctacaagcagtcacagcacatgacggaggttgtgaggcgctgcccccaccatgagcgctgctcagatagcgatggtctggcccctcctcagcatcttatccgagtggaaggaaatttgcgtgtggagtatttggatgacagaaacacttttcgacatagtgtggtggtgccctatgagccgcctgaggttggctctgactgtaccaccatccactacaactacatgtgtaacagttcctgcatgggcggcatgaaccggaggcccatcctcaccatcatcacactggaagactccagtggtaatctactgggacggaacagctttgaggtgcatgtttgtgcctgtcctgggagagaccggcgcacagaggaagagaatctccgcaagaaaggggagcctcaccacgagctgcccccagggagcactaagcgagcactgtccaacaacaccagctcctctccccagccaaagaagaaaccactggatggagaatatttcacccttcagatccgtgggcgtgagcgcttcgagatgttccgagagctgaatgaggccttggaactcaaggatgcccaggctgggaaggagccaggggggagcagggctcactccagccacctgaagtccaaaaagggtcagtctacctcccgccataaaaaactcatgttcaagacagaagggcctgactcagactgacattctccacttcttgttccccactgacagcctcccacccccatctctccctcccctgccattttgggttttgggtctttgaacccttgcttgcaataggtgtgcgtcagaagcacccaggacttccatttgctttgtcccggggctccactgaacaagttggcctgcactggtgttttgttgtggggaggaggatggggagtaggacataccagcttagattttaaggtttttactgtgagggatgtttgggagatgtaagaaatgttcttgcagttaagggttagtttacaatcagccacattctaggtaggggcccacttcaccgtactaaccagggaagctgtccctcactgttgaattttctctaacttcaaggcccatatctgtgaaatgctggcatttgcacctacctcacagagtgcattgtgagggttaatgaaataatgtacatctggccttgaaaccaccttttattacatggggtctagaacttgacccccttgagggtgcttgttccctctccctgttggtcggtgggttggtagtttctacagttgggcagctggttaggtagagggagttgtcaagtctctgctggcccagccaaaccctgtctgacaacctcttggtgaaccttagtacctaaaaggaaatctcaccccatcccacaccctggaggatttcatctcttgtatatgatgatctggatccaccaagacttgttttatgctcagggtcaatttcttttttctttttttttttttttttctttttctttgagactgggtctcgctttgttgcccaggctggagtggagtggcgtgatcttggcttactgcagcctttgcctccccggctcgagcagtcctgcctcagcctccggagtagctgggaccacaggttcatgccaccatggccagccaacttttgcatgttttgtagagatggggtctcacagtgttgcccaggctggtctcaaactcctgggctcaggcgatccacctgtctcagcctcccagagtgctgggattacaattgtgagccaccacgtccagctggaagggtcaacatcttttacattctgcaagcacatctgcattttcaccccacccttcccctccttctccctttttatatcccatttttatatcgatctcttattttacaataaaactttgctgccaaaaaaaaaaaaaaaaaaaa

**Step 9.** Find the intronic-exonic boundaries using BLAT algorithm.







**Step 10.** Identify where your CRISPR guide binds and take a screenshot.

