**Workshop – Mouse models**

The objective of this workshop is to design CRISPR oligos for knock out of the tumor suppressor gene p53 (Homo sapiens, accession number AB082923). The example given in this is for the gene BOK.

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

Enter the following details:

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



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



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



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

**For example,** I chose ACAA1 gene as a potential target that may be affected.

ACAA1 gene encodes acetyl-CoA acyltransferase 1 involved in beta oxidation. Beta oxidation in eukaryotes is responsible for fatty acid metabolism. Fatty acids get broken down into acetyl-CoA which is fed into TCA cycle to generate NADH and FADH2. Mutations in beta oxidation pathway could lead to seizures, coma and sometimes death. Also, deficiency of ACAA1 could lead to various developmental abnormalities.

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

**Step 7**. Copy the sequence and create an Ape. file.(<http://jorgensen.biology.utah.edu/wayned/ape/>)

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

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

 (<https://genome.ucsc.edu/FAQ/FAQblat.html>).

A:



B: *The sequence you enter here is only the coding sequence!!*



C: BLAT results



D: Sequence alignment



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

Example: Anxa1 guide is on mouse chromosome 19, inside the second exon of the coding region.

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The final report (a word file) should include:

Your name:

Your student number:

Your worksheet should have screenshots of steps 1-9.

GGAUGAUUUGAUGCUGUCCC

Convert all the Us into Ts

GGATGATTTGATGCTGTCCC

Off target gene – PHACTR1 -search in Pubmed for the gene and write what would happen if this gene gets knocked out

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). Phosphatase and actin regulator 1 (PHACTR1) are a gene that codes for the protein,

Find the cDNA for p53 using the accession number – switch to “nucleotide” and search for “Human p53”

CGTGCTTTCC ACGACGGTGA CACGCTTCCC TGGATTGG

CCAGACTGCCTTCCGGGTCACTGCCATGGAGGAGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTTTCAGACCTATGGAAACT

ACTTCCTGA AAACAACGTT CTG

TCCCCCTTGCCGTCCCAAGCAATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCcCGTGGCCCCTGCACCAGCAGCTCCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGTCATCTTCTGTCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCCAAGTCTGTGACTTGCACG

TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATG

GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG

GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG

TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCgTGTTTGTGCCTGTCCTGGGAGAGACCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCGAG

CACTGcCCAACAACACCAGCTCCTCTCCCCAGCCAAAGAAGAAACCACTGGATGGAGAATATTTCACCCTTCAG

ATCCGTGGGCGTGAGCGCTTCGAGATGTTCCGAGAGCTGAATGAGGCCTTGGAACTCAAGGATGCCCAGGCTGGGAAGGAGCCAGGGGGGAGCAGGGCTCACTCCAG

CCACCTGAAGTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGACAGAAGGGCCTGACTCAGACTGACATTCTCCACTTCTTGTTCCCCACTGACAGCCTCCCACCCCCATCTCTCCCTCCCCTGCCATTTTGGGTTTTGGGTCTTTGAACCCTTGCTTGCAATAGGTGTGCGTCAGAAGCACCCAGGACTTCCATTTGCTTTGTCCCGGGGCTCCACTGAACAAGTTGGCCTGCACTGGTGTTTTGTTGTGGGGAGGAGGATGGGGAGTAGGACATACCAGCTTAGATTTTAAGGTTTTTACTGTGAGGGATGTTTGGGAGATGTAAGAAATGTTCTTGCAGTTAAGGGTTAGTTTACAATCAGCCACATTCTAGGTAGGGGCCCACTTCACCGTACTAACCAGGGAAGCTGTCCCTCACTGTTGAATTTTCTCTAACTTCAAGGCCCATATCTGTGAAATGCTGGCATTTGCACCTACCTCACAGAGTGCATTGTGAGGGTTAATGAAATAATGTACATCTGGCCTTGAAACCACCTTTTATTACATGGGGTCTAGAACTTGACCCCCTTGAGGGTGCTTGTTCCCTCTCCCTGTTGGTCGGTGGGTTGGTAGTTTCTACAGTTGGGCAGCTGGTTAGGTAGAGGGAGTTGTCAAGTCTCTGCTGGCCCAGCCAAACCCTGTCTGACAACCTCTTGGTGAACCTTAGTACCTAAAAGGAAATCTCACCCCATCCCACACCCTGGAGGATTTCATCTCTTGTATATGATGATCTGGATCCACCAAGACTTGTTTTATGCTCAGGGTCAATTTCTTTTTTCtTTTTTTTTTTTTTTTTTCTTTTTCTTTGAGACTGGGTCTCGCTTTGTTGCCCAGGCTGGAGTGGAGTGGCGTGATCTTGGCTTACTGCAGCCTTTGCCTCCCCGGCTCGAGCAGTCCTGCCTCAGCCTCCGGAGTAGCTGGGACCACAGGTTCATGCCACCATGGCCAGCCAACTTTTGCATGTTTTGTAGAGATGGGGTCTCACAGTGTTGCCCAGGCTGGTCTCAAACTCCTGGGCTCAGGCGATCCACCTGTCTCAGCCTCCCAGAGTGCTGGGATTACAATTGTGAGCCACCACGTCCAGCTGGAAGGGTCAACATCTTTTACATTCTGCAAGCACATCTGCATTTTCACCCCACCCTTCCCCTCCTTCTCCCTTTTTATATCCCATTTTTATATCGATCTCTTATTTTACAATAAAACTTTGCTGCCA