NOT FINISHED

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CRISPR:

In this exercise, we are going to extent the work you completed for Module 3 Week 10 Homework.

⦁ This time, we are going to run WU-CRISPR a second time, but with the first option.

⦁ Go to http://crispr.wustl.edu/

⦁ Choose “Option 1: Search for predesigned gRNA to target human or mouse genes:” and type “HBB” in the search window

⦁ Click on “Go”

⦁ Inspect the results obtained from running WU-CRISPR and answer the following questions.

1) How many gRNA’s are reported by WU-CRISPR this time? 4.

2) Why do think there is a (big) difference between the number of reported gRNA when using different options?

Click on “Details” of the second hit (with a Potency Score of 90)

3) Explain in your own words the “Off-Target Analysis”.

Analysis observing whether or not the base pair template used by the protein would result in off target error.

B) Detecting gRNAs in Human HBB with CHOPCHOP:

• Go to http://chopchop.cbu.uib.no/

• Type HBB in the “Target” window and keep the default species and PAM in the other two

windows: “In” and “Using”

• Click on “Find Target Sites!”

• Inspect the results obtained from running CHOPCHOP and answer the following

questions:

4) How many gRNA’s are reported by CHOPCHOP? 92.

Consider the gRNA with “Ranking” value of 7.

5) What is the PAM sequence of that gRNA? AGG.

6) How many off-target hits does this gRNA have? 7.

• Click on the row of gRNA with ranking 7 to go to a new page with more information on the off-target sequences.

• Examine the table that reports the off-targets and answer the following question:

7) Compare these off-target hits with those reported by WU-CRISPR.

WU-CRISPR reported 4 while CHOPCHOP reported 7

C) Detecting gRNAs in Human HBB with GPP:

• Go to https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design

• Select “human” from the “Select Target Taxon”

• Type HBB in the input window

• Check the “I’m not a robot” box

• Click on “Submit”

• Since the two output files, reported under “Download Results” are “tab-delimited text files, click on “sgRNA Picking Results” and save it in an excel spreadsheet that you name “sgRNA\_Picking\_Results\_HBB.xls”

8) How many gRNA’s are reported by GPP? 79.