Life Sciences Outreach Fall Faculty Speaker Series
Teacher Professional Development Activity
Designing CRISPR Experiments

THE DEREK BOK CENTER FOR TEACHING AND LEARNING
HARVARD

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Overview

This activity was designed for an audience of classroom high school biology teachers as part of a professional development program to further their knowledge in a research field. It has not been formally formatted or tested for a high school student audience because we believe that teachers are the best interpreters of content for their students. Therefore, we welcome teachers to adapt this activity for their own classroom needs.

Introduction

CRISPR technologies confer the capacity to precisely edit and manipulate genomes, revolutionizing both basic biological and biomedical research. Understanding how this technology works and is implemented at a fundamental level is important to appreciating progress in a field impacting our daily lives.

Objective

The goal of this activity is to introduce a fundamental step in CRISPR experiments: designing guide RNAs (gRNAs) that will target Cas9 to a particular spot in the genome. We will be walking through a model experiment and utilizing CHOPCHOP, a free online resource used by scientists to plan CRISPR projects.

References

Kornel L, Montague T, Krause M, Torres Cleuren YN, Tjeldnes H, Valen E. 2019. CHOPCHOP v3: expanding the CRISPR web toolbox beyond genome editing. Nucleic Acids Research: 47(W1), W171–W174. https://doi.org/10.1093/nar/gkz365

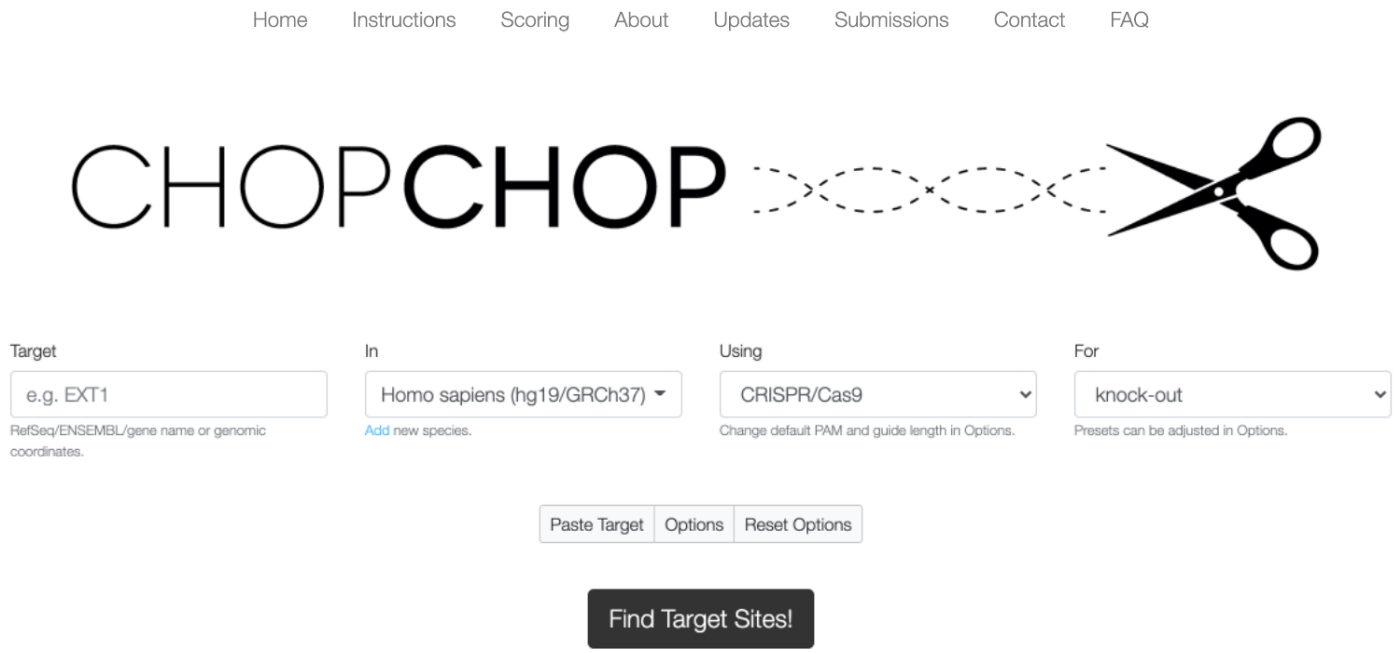
Bialk P, Wang Y, Banas K, Kmiec EB. Functional Gene Knockout of NRF2 Increases Chemosensitivity of Human Lung Cancer A549 Cells In Vitro and in a Xenograft Mouse Model. 2018. Molecular Therapy Oncolytics: 11, 75-89. https://www.cell.com/molecular-therapy-family/oncolytics/pdf/S2372-7705(18)30026-3.pdf

Shen MW, Arbab M, Hsu JY, Worstell D, Culbertson SJ, Krabbe O, Cassa CA, Liu DR, Gifford DK, Sherwood RI. Predictable and precise template-free CRISPR editing of pathogenic variants. 2018. Nature: 563(7733), 646-651. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6517069/

Activity

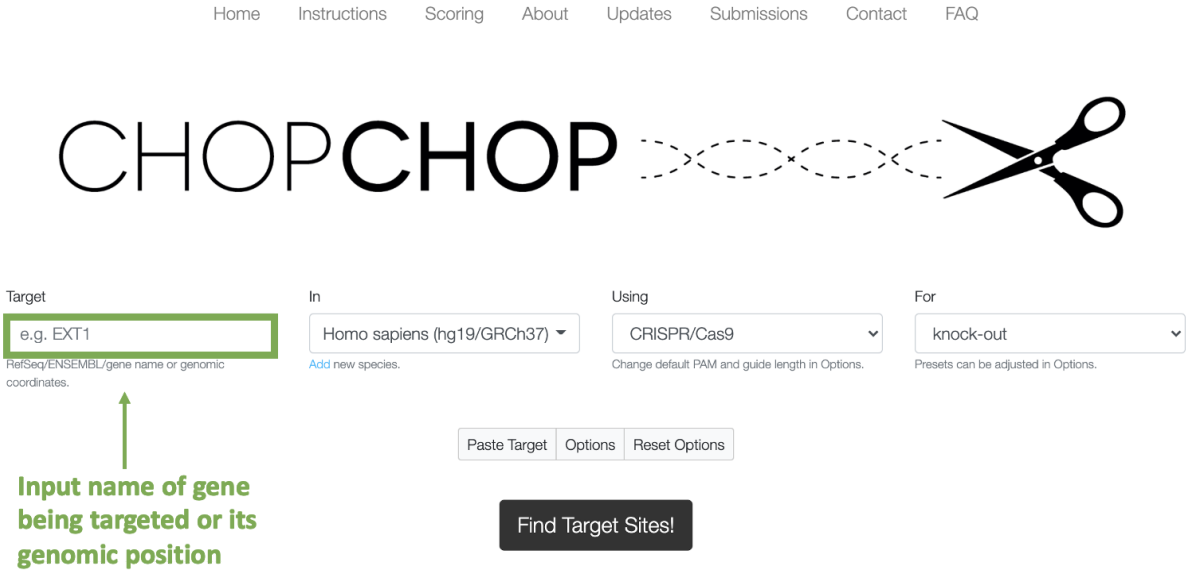
Part A: Introduction to the CHOPCHOP homepage

Visit CHOPCHOP and orient yourself to the homepage (https://chopchop.cbu.uib.no/).

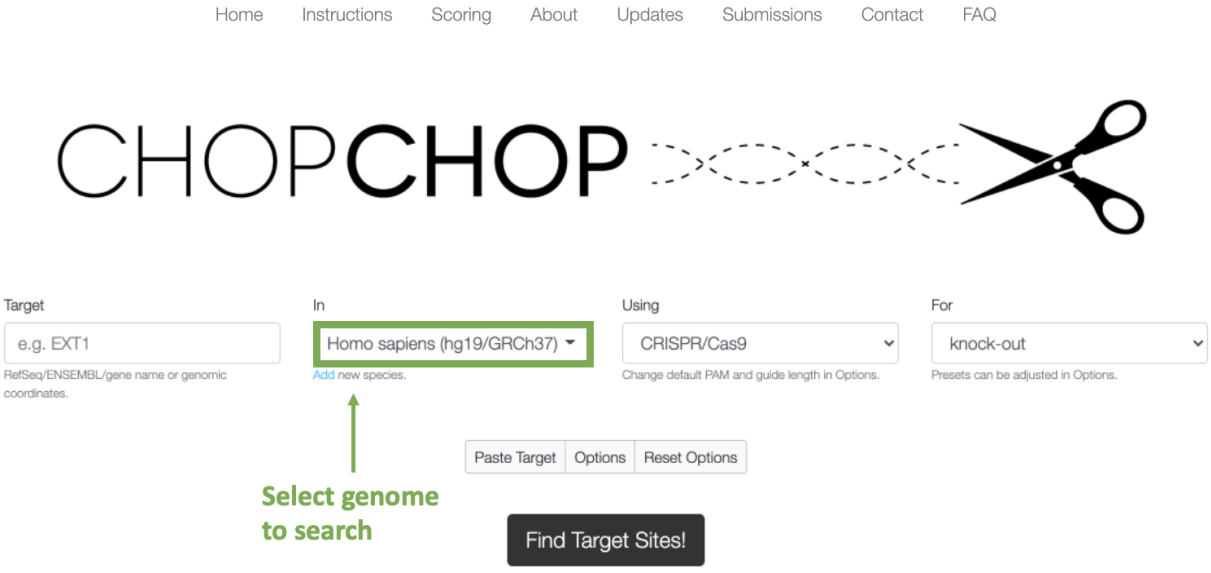


CHOPCHOP is a resource that allows you to design guide RNA (gRNA) sequences. gRNAs are short sequences that direct the enzyme, Cas9, to particular genes or genomic coordinates. With CHOPCHOP, you can design gRNAs for many different kinds of CRISPR experiments. On the CHOPCHOP homepage, there are several boxes to input information about the specific experiment you are planning. We will walk through some of the more important choices on this page.

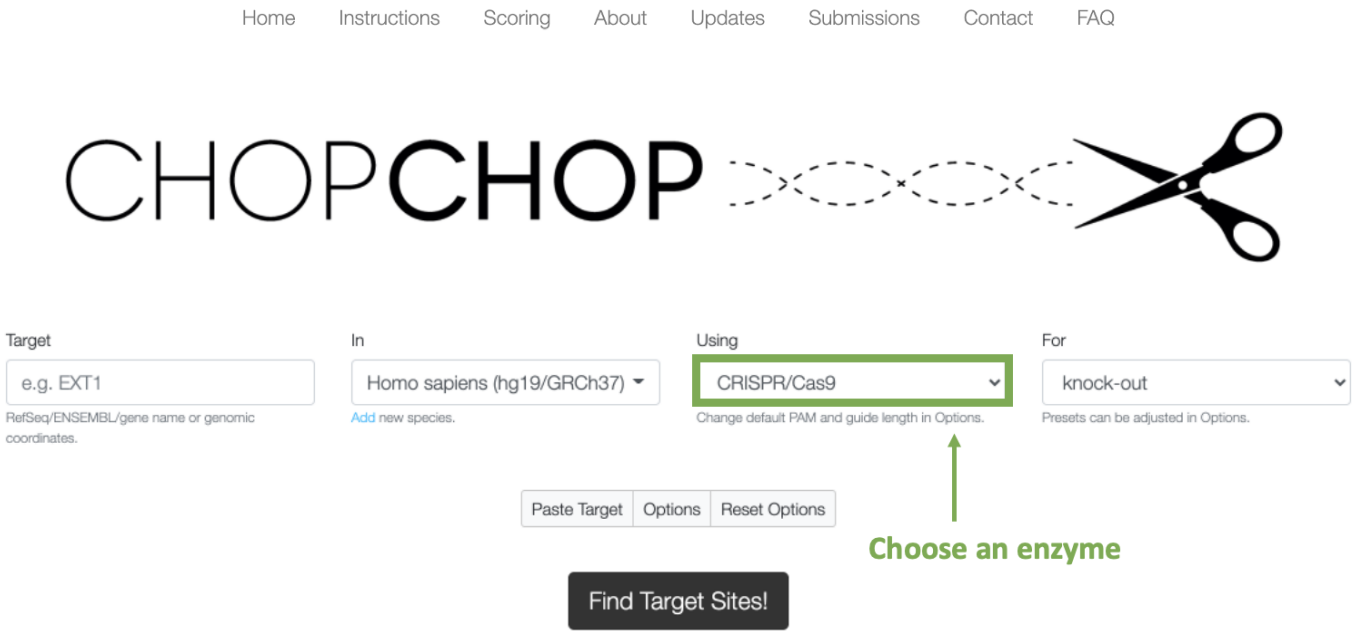
The first box allows you to enter the gene being targeted. For some genes, the name may be entered (i.e. BRCA2). For other, less commonly studied genes, codes assigned on RefSeq or ENSEMBL should be entered instead. It is also possible to input the precise genomic coordinates being targeted if known. Alternatively, if "Paste Target" is selected, the sequence you wish to target can be directly pasted into the box.



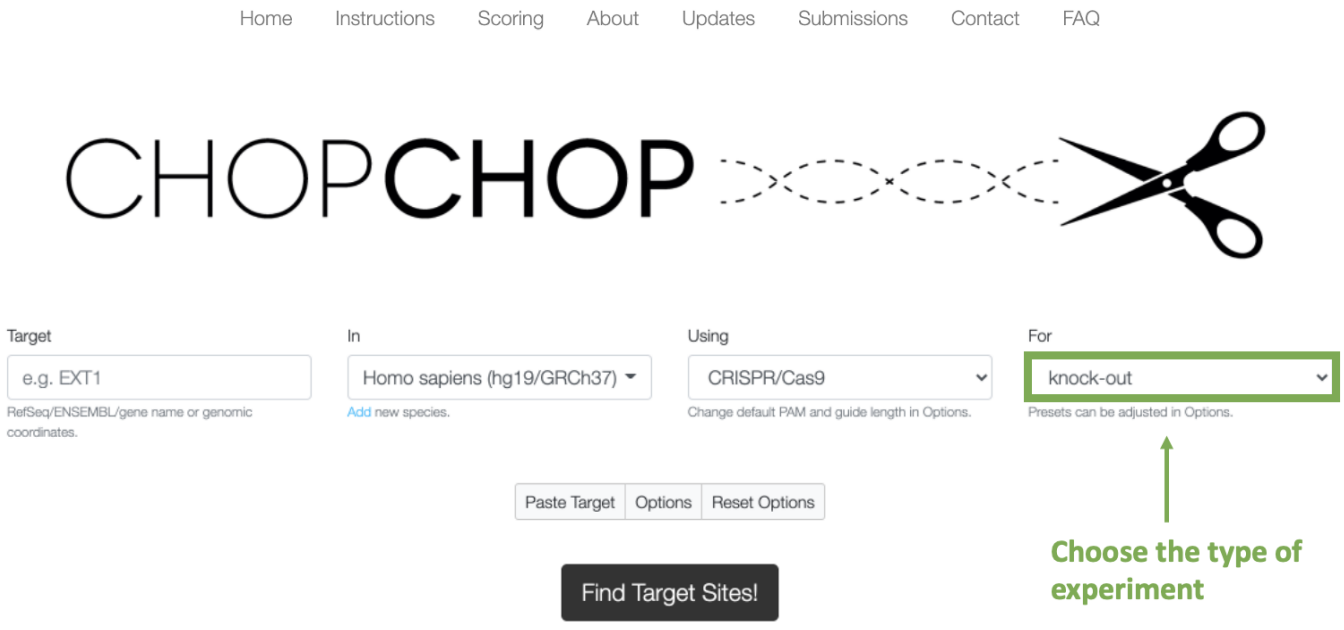
The second box allows you to select the genome from the species you are studying. Genomes from a wide array of organisms are available.



The third box is where the enzyme you are planning to use for editing is selected. If you are interested in learning more about the different enzyme choices, see Table 1 in Useful Resources.

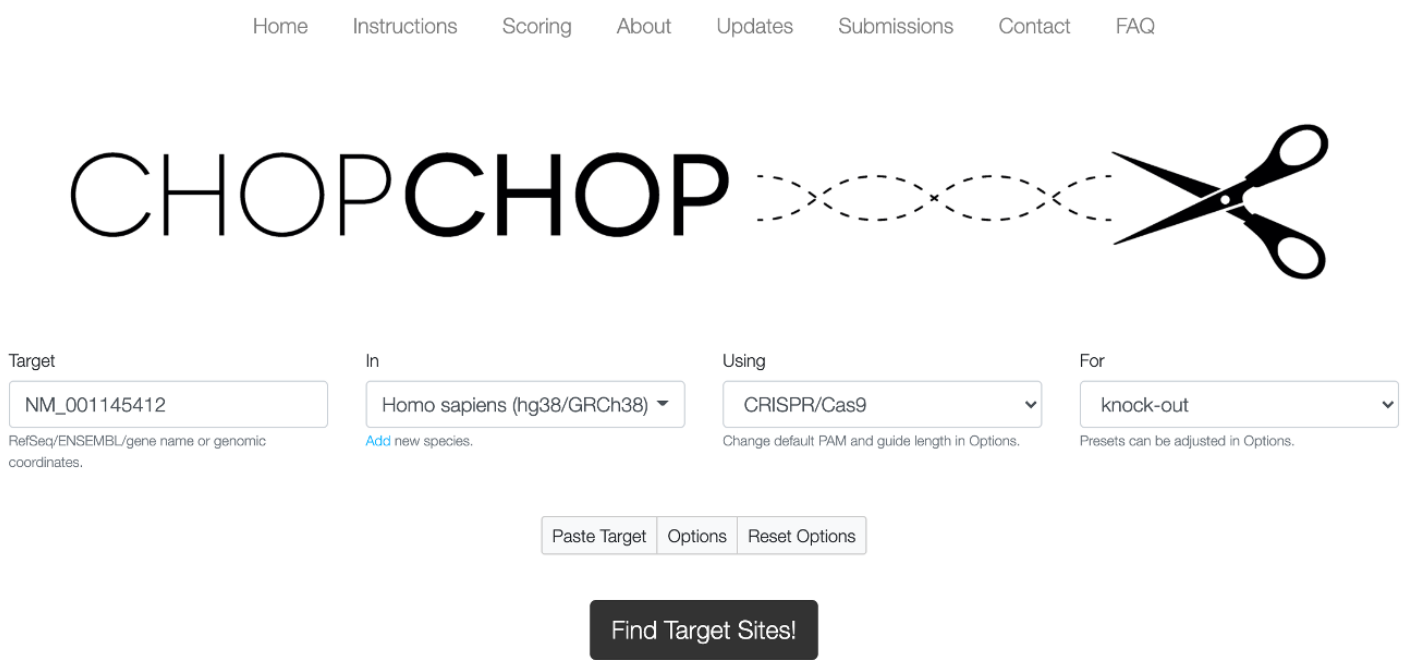


The fourth box is where you select the type of experiment you are trying to conduct. The experiments that might be selected here will vary depending on the type of enzyme you have chosen and its capabilities. If you are interested in learning more about the different types of experiments that appear in the dropdown menu, see Table 2 in Useful Resources. In addition, you can find information about the different DNA repair pathways that might be activated when conducting CRISPR experiments in Table 3 in Useful Resources.



Part B: Introduction to the CHOPCHOP results page

1. Input the following information on the CHOPCHOP home page.
    a. Under gene target, write in the gene name **CFTR** (this is the cystic fibrosis transmembrane conductance regulator, which when mutated can lead to cystic fibrosis).
    b. Select **Homo sapiens (hg38/GRCh38)** for the genome.
    c. Select **CRISPR/Cas** as the enzyme.
    d. Select **knock-out** for the type of experiment.
    e. Press **Find Target Sites!**

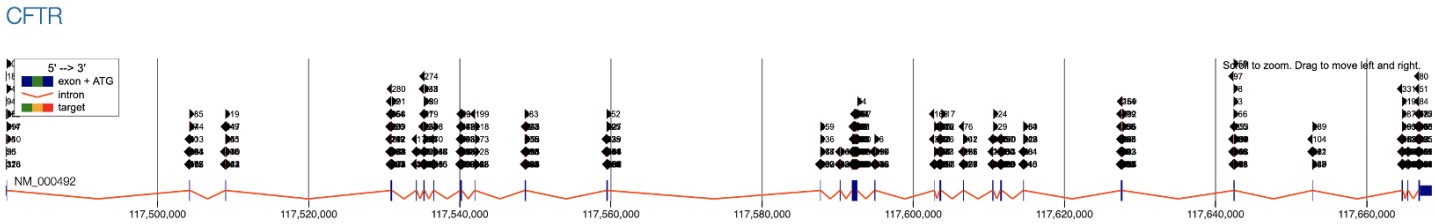


    f. CHOPCHOP is now processing your request. The time this process takes varies from a few seconds to several minutes depending on how many other people are using CHOPCHOP. **Screenshots from the output page that will eventually appear have been copied into this document and may be used to complete the rest of this worksheet.** When your CHOPCHOP job completes, you can look through the exercise using the actual output if you are interested.

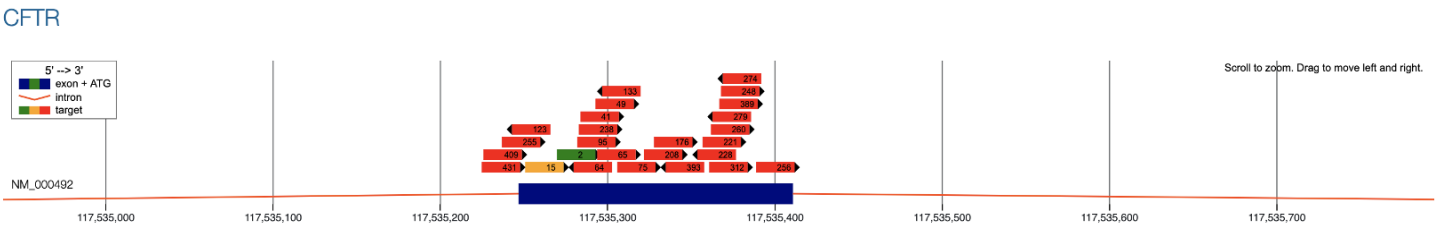
2. The output page will look like the image below.

Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CACCTGTGGTATCACTCCAAAGG	chr7:117642574	-	50	0	0	0	0	0	61.05
2	GTGGATCGCTCCTTTGCAAGTGG	chr7:117535271	+	55	0	0	0	0	0	57.97
3	CAAGCTATCCACATCTATGCTGG	chr7:117614691	-	45	0	0	0	0	0	50.73
4	CGCATCAGCGTGATCAGCACTGG	chr7:117592418	+	60	0	0	0	0	0	49.31
5	CTCCTTCGTGCCTGAAGCGTGGG	chr7:117592442	-	60	1	0	0	0	0	38.34
6	CGCTCTATCGCGATTTATCTAGG	chr7:117530974	+	45	0	0	0	0	0	37.22
7	CGGTCACTCGGCAATTTCCCTGG	chr7:117540276	+	60	0	0	0	0	0	33.34
8	GGCCAGTGACACTTTTCGTGTGG	chr7:117592536	-	55	2	0	0	0	1	59.83
9	AAAGTAAGGCTGCCGTCCGAAGG	chr7:117611641	-	55	5	0	0	0	1	60.11
10	ATCGCGATTTATCTAGGCATAGG	chr7:117530980	+	40	1	0	0	0	1	53.91
11	CACATAATACGAACTGGTGCTGG	chr7:117603597	-	45	0	0	0	0	1	48.70
12	GAGCGTTCCTCCTTGTTATCCGG	chr7:117530956	-	50	1	0	0	0	1	35.06
13	TGTGGACAGTAATATATCGAAGG	chr7:117594986	-	35	0	0	0	0	2	65.45
14	TCCAGAAAAAACATCGCCGAAGG	chr7:117509085	-	45	0	0	0	0	2	58.68

The top of the page has a schematic of the gene. Exons are in blue and introns in red. Above the gene schematic, the locations of possible targets are shown. You can drag this part of the screen with your mouse to see more of the gene or scroll to zoom in and out.



Zooming in on one of the exons shows the targets that CHOPCHOP has identified as candidates for your CRISPR experiment. Targets are colored by how highly ranked they are as candidates for CRISPR (green for the best-ranked, yellow for mid-ranked, and red for the lowest-ranked).



The table at the bottom of the page has a list of candidate CRISPR targets with several pieces of information about the nature of the sequences.

Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CACCTGTGGTATCACTCCAAAGG	chr7:117642574	-	50	0	0	0	0	0	61.05
2	GTGGATCGCTCCTTTGCAAGTGG	chr7:117535271	+	55	0	0	0	0	0	57.97
3	CAAGCTATCCACATCTATGCTGG	chr7:117614691	-	45	0	0	0	0	0	50.73
4	CGCATCAGCGTGATCAGCACTGG	chr7:117592418	+	60	0	0	0	0	0	49.31
5	CTCCTTCGTGCCTGAAGCGTGGG	chr7:117592442	-	60	1	0	0	0	0	38.34
6	CGCTCTATCGCGATTTATCTAGG	chr7:117530974	+	45	0	0	0	0	0	37.22
7	CGGTCACTCGGCAATTTCCCTGG	chr7:117540276	+	60	0	0	0	0	0	33.34
8	GGCCAGTGACACTTTTCGTGTGG	chr7:117592536	-	55	2	0	0	0	1	59.83
9	AAAGTAAGGCTGCCGTCCGAAGG	chr7:117611641	-	55	5	0	0	0	1	60.11
10	ATCGCGATTTATCTAGGCATAGG	chr7:117530980	+	40	1	0	0	0	1	53.91
11	CACATAATACGAACTGGTGCTGG	chr7:117603597	-	45	0	0	0	0	1	48.70
12	GAGCGTTCCTCCTTGTTATCCGG	chr7:117530956	-	50	1	0	0	0	1	35.06
13	TGTGGACAGTAATATATCGAAGG	chr7:117594986	-	35	0	0	0	0	2	65.45
14	TCCAGAAAAAACATCGCCGAAGG	chr7:117509085	-	45	0	0	0	0	2	58.68
15	TTGCATTGGCACATTTCGTGTGG	chr7:117535252	+	45	0	0	0	0	2	55.64
16	GCACTTGCTTGAGTTCCGGTGGG	chr7:117667017	-	55	1	0	0	0	2	51.18
17	CTATGGACACTTCGTGCCTTCGG	chr7:117611625	+	50	2	0	0	0	2	51.25
18	AAGGTAGGTATACATCGCTTGGG	chr7:117592655	+	40	0	0	0	0	2	47.61

“Target Sequence” has the sequences identified as candidate CRISPR targets. These targets will all end in NGG (A, T, C, or G followed by GG). The NGG is a PAM sequence, which is a specific DNA sequence that needs to be adjacent to the target sequence for Cas9 to be able to cut at this location.

Rank	Target sequence
1	CACCTGTGGTATCACTCCAAAGG
2	GTGGATCGCTCCTTTGCAAGTGG
3	CAAGCTATCCACATCTATGCTGG
4	CGCATCAGCGTGATCAGCACTGG
5	CTCCTTCGTGCCTGAAGCGTGGG

“Genomic location” has the precise coordinates of each candidate target sequence.

Genomic location
chr7:117642574
chr7:117535271
chr7:117614691
chr7:117592418
chr7:117592442

“Strand” lists which strand of the double-stranded DNA the target sequence can be found on. Targets for this kind of experiment can be on either strand since Cas9 is going to cleave through both strands once it is directed to the spot.

Strand
-
+
-
+
-

“GC content (%)” tells us the percentage of bases in this target sequence that are either guanine or cytosine relative to the number of adenine and thymine. The GC content can influence CRISPR efficiency, so it is ideal to design gRNAs around 40-70% if possible.

GC content (%)
50
55
45
60
60

“Self-complementarity” describes whether a sequence is likely to bind to itself. Self-complementarity within the gRNA can inhibit CRISPR efficiency. The number indicates the number of regions of self-complementarity longer than 3 nucleotides that have been predicted.

Self-complementarity
0
0
0
0
1

“MM” columns specify the number of off-target genes that the gRNA could bind to outside of the target gene. Off-target editing describes the unintended cleavage of genes that can sometimes occur if those sequences are too similar to the one being targeted. MM0 lists the number of off-target genes that completely share the sequence being targeted (0 mismatches). MM1 lists the number of off-targets that have the sequence being targeted except for 1 base pair difference (1 mismatch). MM2 and MM3 columns list the number of off-targets with 2 and 3 mismatches respectively.

MM0	MM1	MM2	MM3
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0

“Efficiency” is the efficiency score generated by an algorithm. This algorithm was originally developed based on experimental evidence collected in large-scale screens of gRNA effectiveness. This score describes how effective a gRNA is predicted to be at maximizing on-target activity and minimizing off-target activity (a high score means a gRNA is more effective).

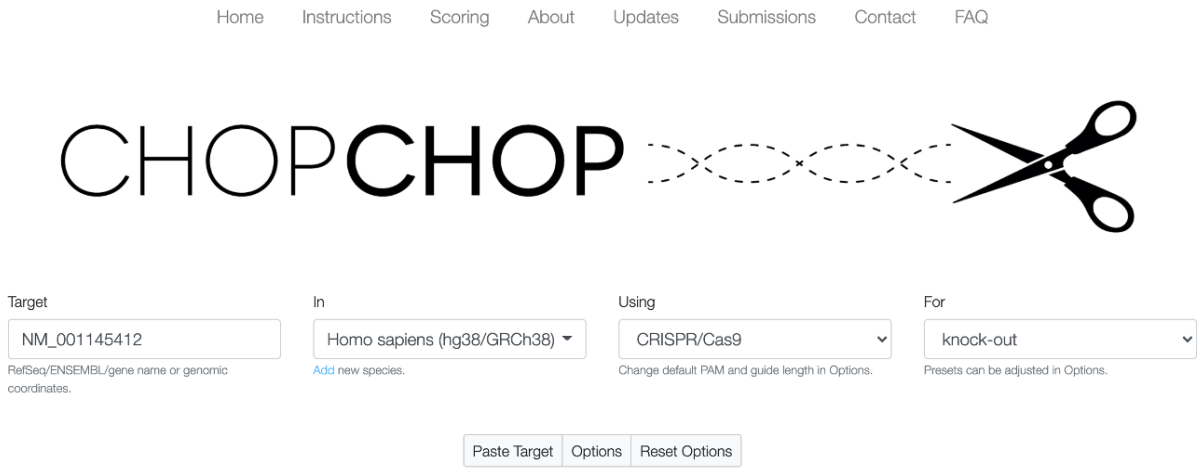
Efficiency
61.05
57.97
50.73
49.31
38.34

Rankings of the guide RNAs are determined by (i) efficiency score, (ii) number of off-targets and whether they have mismatches, (iii) existence of self-complementarity regions, (iv) GC-content, (v) location of sgRNA within a gene.

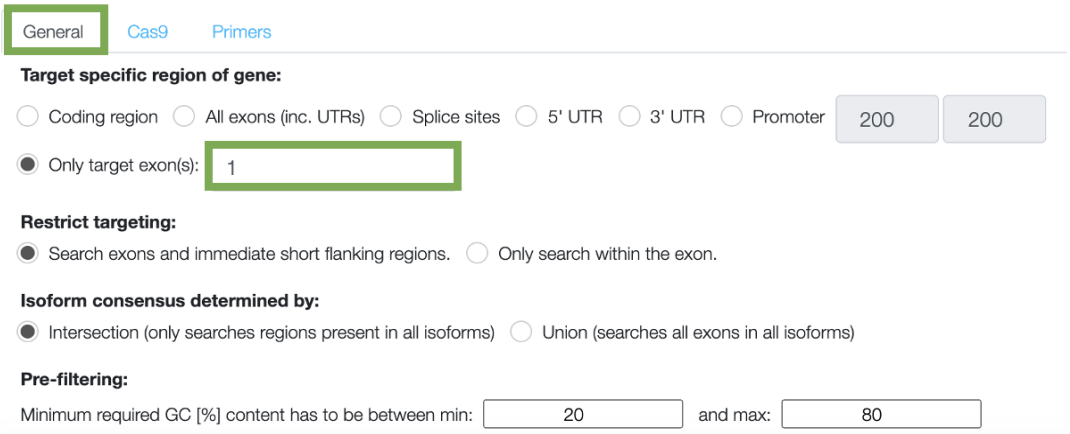
Part C: Designing guide RNAs

You are studying a human gene called Nuclear Factor Erythroid 2-Related Factor (NRF2). This gene regulates the expression of proteins involved in cellular responses to stress. Increased expression of NRF2 leads to increased resistance of lung cancer cells to chemotherapy. You are conducting a CRISPR experiment to knock out the function of NRF2 in human cells and test whether this makes cells more susceptible to cancer treatment. To knock out this gene, you plan to generate a single cut to disrupt its function.

1. Input the following information on the CHOPCHOP home page (https://chopchop.cbu.uib.no/).
    a. Under gene target, copy in the Refseq ID for NRF2: **NM_001145412 .**
    b. Select **Homo sapiens (hg38/GRCh38)** for the genome.
    c. Select **CRISPR/Cas9** as the enzyme.
    d. Select **knock-out** for the type of experiment.

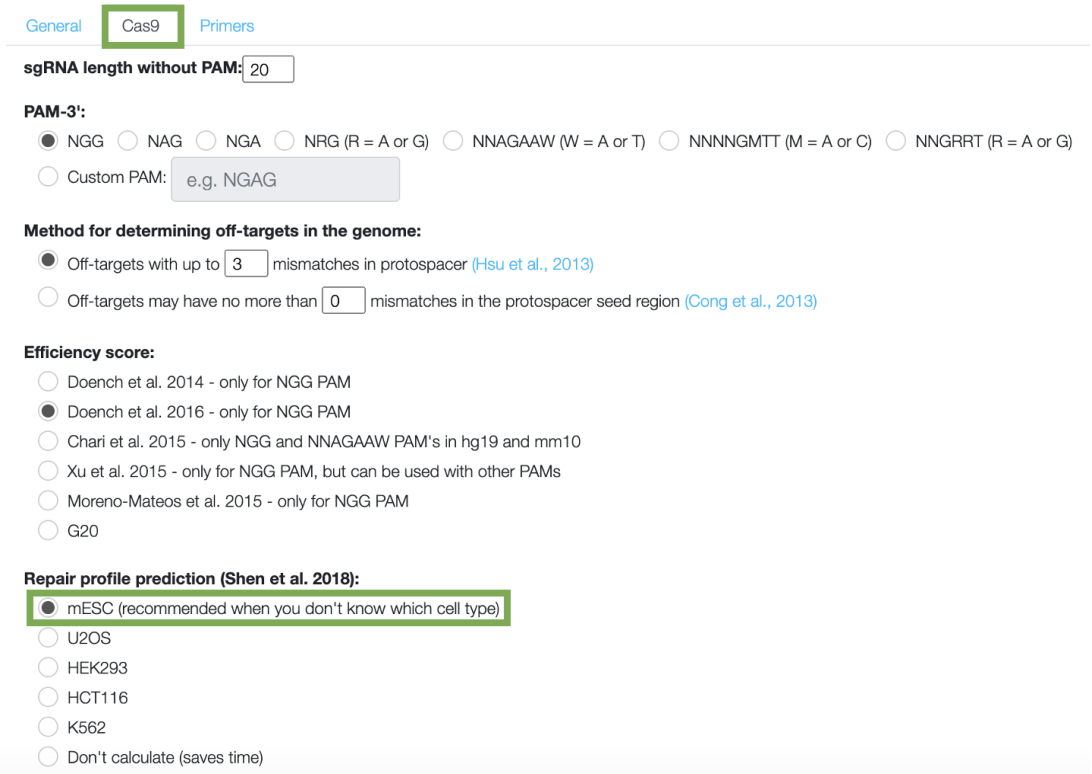


2. Click on “Options”
    a. Select “General”
        i. Under “Target specific region of gene,” select “Only target exon(s)” and write in **1.** This will restrict our search for CRISPR targets just to exon 1.



        ii. Keep other settings as is.

b. Select “Cas9”
   i. Under “Repair profile prediction (Shen et al. 2018)” select **mESC.** This will have CHOPCHOP calculate the most likely edits to occur when choosing a particular gRNA (we will discuss this further later).

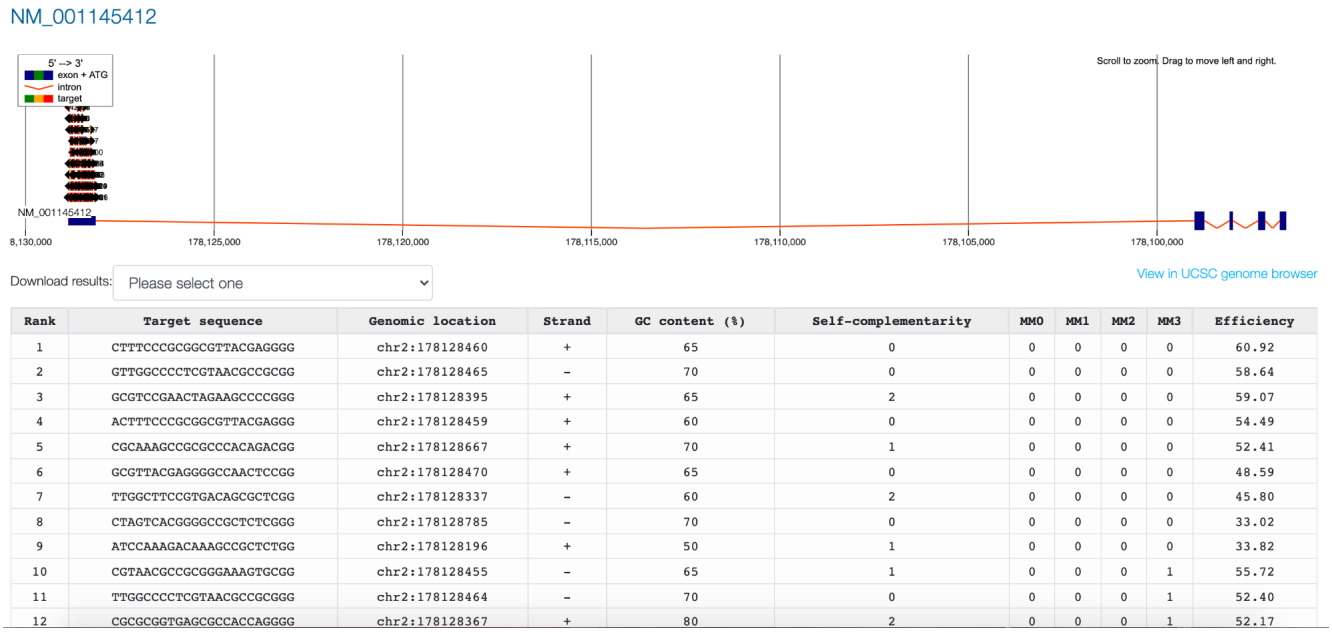


   ii. Keep other settings as is.

3. Scroll to the bottom of the page and click on “**Find Target Sites!**”
   a. CHOPCHOP is now processing your request. The time this process takes varies from a few seconds to several minutes depending on how many other people are using CHOPCHOP. **Screenshots from the output page that will eventually appear have been copied into this document and may be used to complete the rest of this worksheet.** When your CHOPCHOP job completes, you can look through the exercise using the actual output if you are interested.

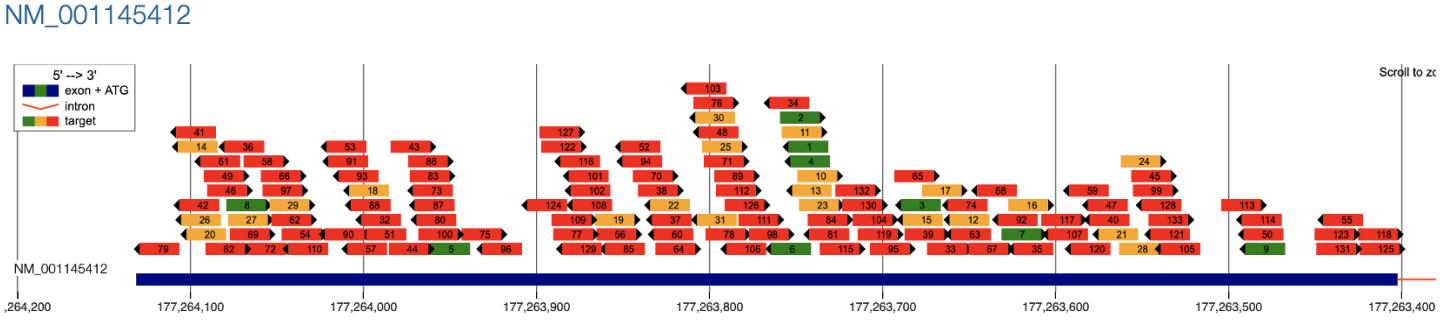
4. **Discussion Question 1**: Why might we want to restrict our search for CRISPR targets just to exon 1 for this experiment? What might be the advantage of targeting the cut at the beginning of the gene if we are interested in knocking out a gene’s function? Can you think of any other strategies for selecting regions of a gene to target for cleavage if we want to render the gene non-functional?

5. The CHOPCHOP output page will look like the image below.



Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CTTTCCCGCGGCGTTACGAGGGG	chr2:178128460	+	65	0	0	0	0	0	60.92
2	GTTGGCCCCTCGTAACGCCGCGG	chr2:178128465	-	70	0	0	0	0	0	58.64
3	GCGTCCGAACTAGAAGCCCCGGG	chr2:178128395	+	65	2	0	0	0	0	59.07
4	ACTTTCCCGCGGCGTTACGAGGG	chr2:178128459	+	60	0	0	0	0	0	54.49
5	CGCAAAGCCGCGCCCACAGACGG	chr2:178128667	+	70	1	0	0	0	0	52.41
6	GCGTTACGAGGGGCCAACTCCGG	chr2:178128470	+	65	0	0	0	0	0	48.59
7	TTGGCTTCCGTGACAGCGCTCGG	chr2:178128337	-	60	2	0	0	0	0	45.80
8	CTAGTCACGGGGCCGCTCTCGGG	chr2:178128785	-	70	0	0	0	0	0	33.02
9	ATCCAAAGACAAAGCCGCTCTGG	chr2:178128196	+	50	1	0	0	0	0	33.82
10	CGTAACGCCGCGGGAAAGTGCGG	chr2:178128455	-	65	1	0	0	0	1	55.72
11	TTGGCCCCTCGTAACGCCGCGGG	chr2:178128464	-	70	0	0	0	0	1	52.40
12	CGCGCGGTGAGCGCCACCAGGGG	chr2:178128367	+	80	2	0	0	0	1	52.17

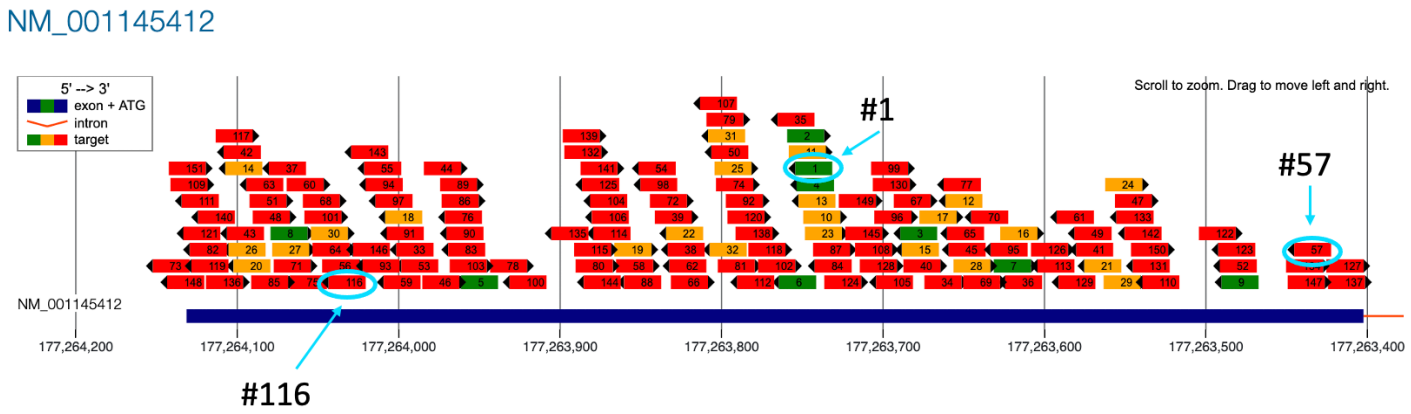
Zooming in on the schematic of the gene at the top of the page shows the targets that CHOPCHOP has identified as candidates for your CRISPR experiment.



The table at the bottom of the page has the sequences of the candidate CRISPR targets.

Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CTTTCCCGCGGCGTTACGAGGGG	chr2:177263732	+	65	0	0	0	0	0	60.92
2	GTTGGCCCCTCGTAACGCCGCGG	chr2:177263737	-	70	0	0	0	0	0	58.64
3	GCGTCCGAACTAGAAGCCCCGGG	chr2:177263667	+	65	2	0	0	0	0	59.07
4	ACTTTCCCGCGGCGTTACGAGGG	chr2:177263731	+	60	0	0	0	0	0	54.49
5	CGCAAAGCCGCGCCCACAGACGG	chr2:177263939	+	70	1	0	0	0	0	52.41
6	GCGTTACGAGGGGCCAACTCCGG	chr2:177263742	+	65	0	0	0	0	0	48.59
7	TTGGCTTCCGTGACAGCGCTCGG	chr2:177263609	-	60	2	0	0	0	0	45.80
8	CTAGTCACGGGGCCGCTCTCGGG	chr2:177264057	-	70	0	0	0	0	0	33.02
9	ATCCAAAGACAAAGCCGCTCTGG	chr2:177263468	+	50	1	0	0	0	0	33.82
10	CGTAACGCCGCGGGAAAGTGCGG	chr2:177263727	-	65	1	0	0	0	1	55.72

6. **Discussion Question 2**: Look more closely at the candidate CRISPR targets ranked #1, #57, and #116 (relevant information about the properties and locations of these targets have been copied below). Speculate as to why #57 and #116 might be less effective choices than #1 for your experiment.



Rank	Target sequence	GC content	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	CTTTCCCGCGGCGTTACGAGGGG	65%	0	0	0	0	0	60.92
57	ATATCCCGTCTTACACATTTTGG	35%	0	0	0	0	5	19.58
116	GGCTCCGGGTCCCAGCCCGAAGG	80%	3	0	0	1	17	60.01

Part D: Predicting CRISPR-mediated genomic changes

You selected the highest-ranked gRNA from the CHOPCHOP output, generated the gRNA, and transfected it into cells expressing Cas9 to achieve CRISPR knockout. However, not all CRISPR-mediated edits that arise from this experiment will necessarily achieve your goal of knocking out NRF2 function.

1. **Discussion Question 3**: What kinds of mutations at the cut site will most likely achieve the goal of knocking out NRF2? Discuss your ideas with your group.

2. If your CHOPCHOP job has finished running, **click on the candidate CRISPR target ranked #1**. A new output page will appear. If the job is still in progress, you can follow along with the screenshots in this document.

We have algorithms that can predict the types of genomic changes most likely to occur when using a particular gRNA. We told CHOPCHOP to implement one of these algorithms at the start of the activity when we asked the program to provide a “Repair Profile Prediction.” Scrolling down to the bottom of the current page will show the output of this algorithm for the gRNA that we have selected, which is called a repair profile. This repair

profile contains a lot of information, some of which we didn't have time to cover today. We will focus on just a few elements of this table on the next page.

Shen et al. 2018 predictions of repair profile - statistics	
Reference sequence	GTGCCGCCGACTCCGGCCTCAGAGTCCACTGCCCTCGCCCGCACTTTCCCGCGGCGTTAC<>GAGGGGCCAACTCCGGGTGCCCGAGCCCGAACCCCTCCCCGGCCGAGAAAGTGCGCGGGG
Frameshift frequency	67.70
Precision score	0.43
Frame +0 frequency	32.30
Frame +1 frequency	36.36
Frame +2 frequency	31.33
1-bp ins frequency	9.97
Highest del frequency	14.47
Highest ins frequency	8.45
Highest outcome frequency	14.47
Microhomology deletion frequency	64.10
Microhomology-less deletion frequency	25.94

3. **Discussion Question 4**: Compare a subset of the values we get for the repair profile for the gRNA we selected (#1) to that of another candidate gRNA (#126). A table of values for both gRNAs can be found below. Does one gRNA seem like a better choice for the type of experiment we are planning today? Would one of these gRNAs be better if we were planning an experiment where instead of trying to completely knock out gene function we only wanted to remove a small portion of the sequence?

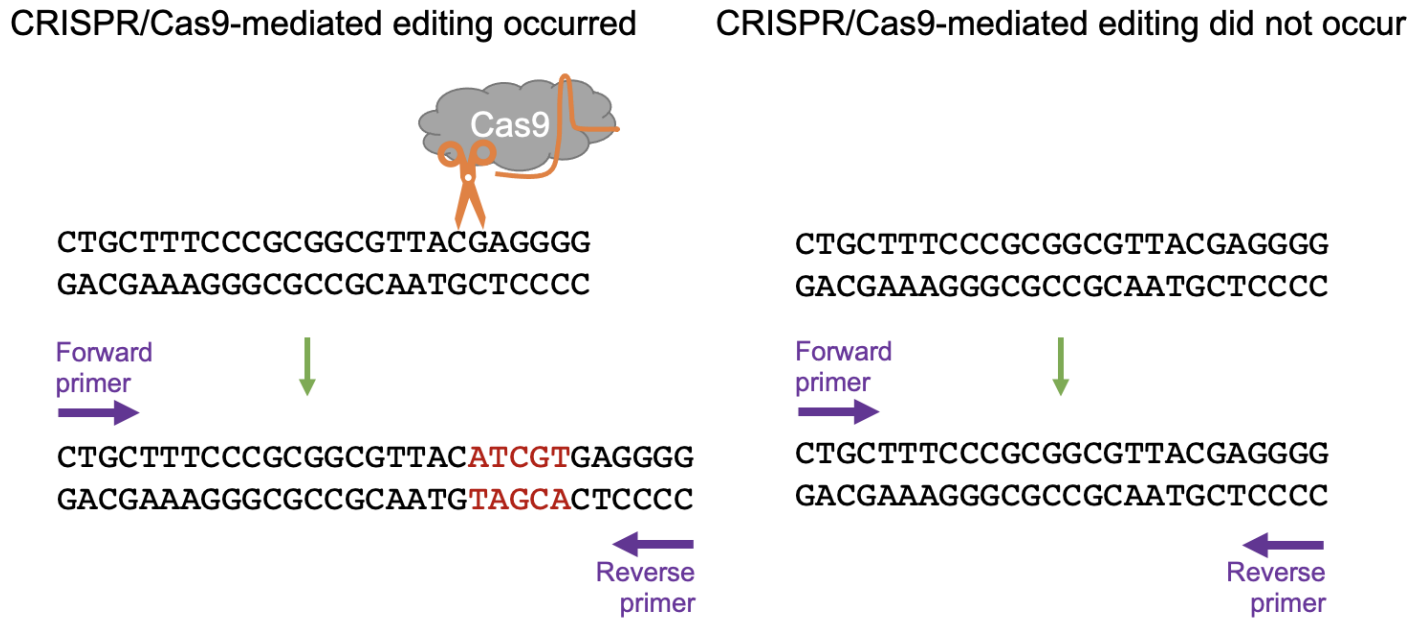
	gRNA #1	**gRNA #138**
Frameshift frequency (the predicted frequency of frameshift mutations that will occur)	67.70	43.60
Frame +0 frequency (predicted frequency of mutations that will shift the reading frame by 0 bases)	32.30	56.40
Frame +1 frequency (predicted frequency of mutations that will shift the reading frame by 1 base)	36.36	21.40
Frame +2 frequency (predicted frequency of mutations that will shift the reading frame by 2 bases)	31.33	22.20

Part E: Screening for CRISPR activity

After selecting your gRNA and conducting your CRISPR experiment, you will need to identify cells in which the desired CRISPR-mediated edit occurred.

1. **Discussion Question 5**: How might we be able to detect CRISPR-mediated gene editing? Consider how we expect the DNA to be repaired in this experiment. Consider the tools we have at our disposal to identify changes in the genome at a particular position.

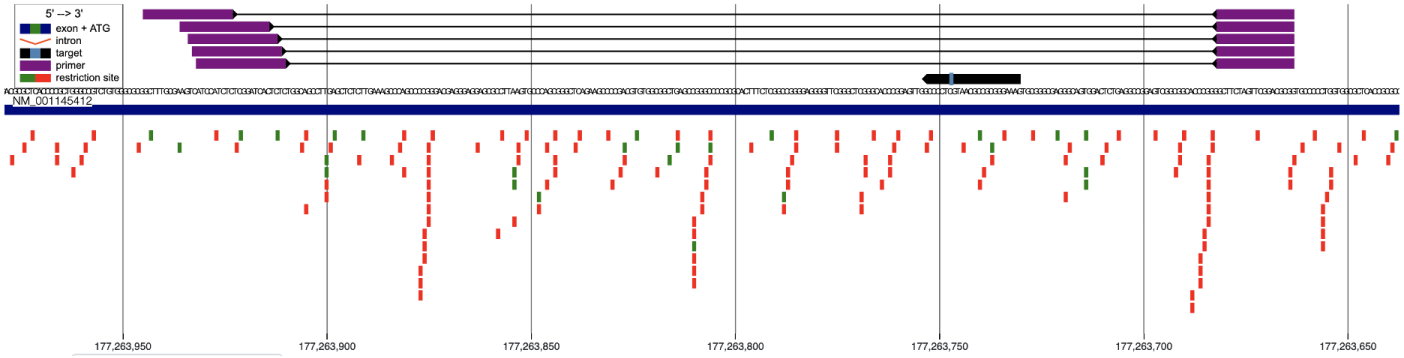
To screen for successful CRISPR editing, you decide to extract DNA from the cells and then conduct PCR with primers on either side of the target region. This will allow you to amplify the region and sequence it.



2. Click on the candidate CRISPR target ranked #1. A new output page will appear.

   The schematic at the top of the page now contains new information.
   - **Black** box: target sequence we have selected
   - **Blue** box: gene schematic
   - **Purple** boxes: candidate primer locations for PCR reactions that could amplify this region for sequencing.
   - **Red** and **green** boxes: sites at which various restriction enzymes cut (another possible resource that we could have used to screen for successful CRISPR events).

Target: **NM_001145412**
Rank: **1**
Target sequence: **CTTTCCCGCGGCGTTACGAGGGG**



The table below shows information about the primers, including their sequences, coordinates, off-targets, and the products predicted to be generated by PCR.

Pair	Left primer coordinates	Left primer	Left primer Tm	Left primer off-targets	Right primer coordinates	Right primer	Right primer Tm	Right primer off-targets	Pair off-targets	Product size
1	chr2:177263911-177263933	TCCATCTCTCGGATCACTCTCT	60.4	0	chr2:177263664-177263683	ACCGCGTCCGAACTAGAAG	60.4	0	0	269
2	chr2:177263912-177263934	ATCCATCTCTCGGATCACTCTC	59.7	0	chr2:177263664-177263683	ACCGCGTCCGAACTAGAAG	60.4	0	0	270
3	chr2:177263913-177263935	CATCCATCTCTCGGATCACTCT	60.6	0	chr2:177263664-177263683	ACCGCGTCCGAACTAGAAG	60.4	0	0	271
4	chr2:177263915-177263937	GTCATCCATCTCTCGGATCACT	60.5	0	chr2:177263664-177263683	ACCGCGTCCGAACTAGAAG	60.4	0	0	273
5	chr2:177263924-177263946	CTTTGCGAAGTCATCCATCTCT	60.8	0	chr2:177263664-177263683	ACCGCGTCCGAACTAGAAG	60.4	0	0	282

Part F: Continuing to predict CRISPR-mediated genomic changes

The scientists who designed the algorithm we implemented to generate the repair profile prediction have a website called **inDelphi**. This site provides additional information about the predicted genetic changes resulting from CRISPR at particular target sites. If you are interested in learning more, try inputting specific target sites or genes of interest into inDelphi to see the types of predictions we can make using this tool.

Answers to Discussion Questions

Discussion Question 1: **Why might we want to restrict our search for CRISPR targets just to exon 1 for this experiment? What might be the advantage of targeting the cut at the beginning of the gene if we are interested in knocking out a gene's function? Can you think of any other strategies for selecting regions of a gene most likely to knock out its function if targeted for cleavage?**
For this experiment, we are aiming to generate a single cut in this gene that will lead to loss of function. Because we are not providing a template for repair, this cut in the DNA will activate the non-homologous end joining (NHEJ) repair pathway. NHEJ is an error-prone process, so bases will be randomly added and deleted in the region where the cut happens. If an addition or deletion of bases is a non-multiple of three, this will shift the reading frame during translation, which can generate an early stop codon and consequently truncate the protein prematurely. The random changes that happen at the cut site might even encode a stop codon right at the region where the cut was made. In either case, we might be more likely to generate a non-functional protein if the premature stop happens earlier in the protein (the less of the protein that is translated, the more likely it is that it is not going to be a functional protein product). Aiming to generate a cut at the start of the gene, in the first exon, could therefore help us select a target more likely to abrogate this protein's function.

An alternative strategy would be to target domains of the gene known to be associated with its function. For genes where certain portions of its sequence are known to be required for its function, choosing CRISPR targets within such domains would be a good strategy for this experiment.

Discussion Question 2: Look more closely at the candidate CRISPR targets ranked #1, #57, and #116. Speculate as to why #57 and #116 might be less effective choices than #1 for your experiment.
Several factors influence the likelihood that a guide RNA will work as expected for your experiment. Certain qualities of targets #57 and #116 suggest they are less likely to achieve your goals.
Target #57: The %GC content is slightly below the ideal range, which can negatively affect the activity of the gRNA. There are five places in the genome with nearly the same sequence as this target site (just three bases different between those sequences and the target), which may lead to off-target editing. Finally, the efficiency score, which predicts how effective different gRNAs will be at maximizing on-target activity and minimizing off-target activity, is lower. The target site for this gRNA will be farther downstream in the gene than #1, so it may not truncate the protein as early as #1.
Target #116: The %GC content is slightly above the ideal range. The target has three regions of self-complementarity, which may inhibit gRNA efficiency. There are also multiple potential off-targets. 17 places in the genome have the same sequence except for 3 bases, and 1 place has the same sequence except for 2 bases. Finally, the efficiency score for this gRNA is slightly lower than that of #1.

Discussion Question 3: What kinds of genomic changes at the cut site will likely achieve the goal of knocking out NRF2?
Generating a frameshift mutation is one way in which we might knock out NRF2. To achieve a frameshift, we would want to add or subtract bases by a number that is a non-multiple of three. Remember that ribosomes read "codons" or groups of three bases at a time, so changing the sequence by a multiple of three will only add or subtract a couple of amino acids while leaving the rest of the sequence intact. This kind of genetic change might lead to a change in protein structure. However, we are more likely to achieve our goal of eliminating this

protein's function if we shift the reading frame entirely since this often leads to an early stop codon being encoded. Alternatively, a nonsense mutation could effectively knock out this gene.

Discussion Question 4: Compare a subset of the values we get for the repair profile for the gRNA we selected (#1) to that of another candidate gRNA (#138). A table of values for both gRNAs can be found below. Does one gRNA seem like a better choice for the type of experiment we are planning today? Would one of these gRNAs be better if we were planning an experiment where instead of trying to completely knock out gene function we only wanted to remove a small portion of the sequence?
gRNA #1 looks like a more promising candidate for this experiment based on the values in the table since higher frequencies are predicted for frameshifts that are non-multiples of three compared to the frequencies predicted for gRNA #126. Based on these values, gRNA #126 looks like it would be a better candidate when planning an experiment in which we wanted to only remove a small portion of a gene's sequence since it has lower predicted frequencies of frameshift mutations.

Discussion Question 5: How might we be able to detect CRISPR-mediated gene editing? Consider how we expect the DNA to be repaired in this experiment. Consider the tools we have at our disposal to identify changes in the genome at a particular position.
We could use PCR to amplify the region where we targeted a cut and then sequence the PCR product to find out the exact sequence of the genome after editing. If we cut at a position where a restriction enzyme recognition site was located, we could also do a restriction enzyme digest on the PCR product and run the digest on a gel. If we disrupted the recognition site, we would expect the digest to yield products of a different size than a product with an unmodified site. There are also newer methods for detecting variations in nucleic acid sequences, such as high resolution melting analysis and surveyor nuclease assays.

Useful Resources

Table 1: Different enzymes available to select in CHOPCHOP

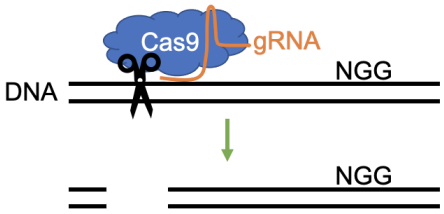
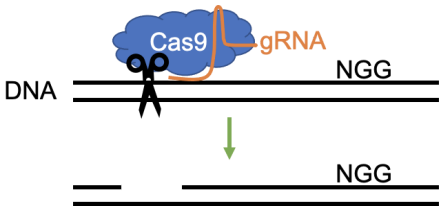
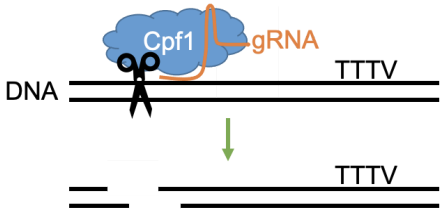
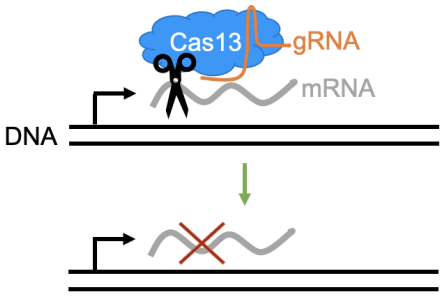
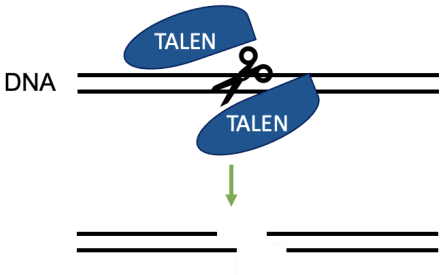
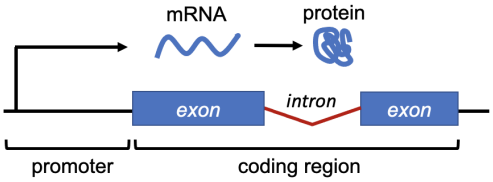
CRISPR/Cas9	**Cas9** enzyme is directed by gRNA(s) to a particular genomic location to generate a **double-stranded** cut in the genome; the most common type of CRISPR experiment	
CRISPR/Cas9 nickase	**Cas9** enzyme is directed by gRNA(s) to a particular genomic location to generate a **single-stranded** cut in the genome; used with two gRNAs to reduce the likelihood of off-target editing compared to traditional CRISPR/Cas9	
CRISPR/Cpf1 or CasX	**Cpf1** or **CasX** enzymes are directed by gRNA(s) to a particular genomic location to generate a **double-stranded** cut in the genome; different from Cas9 in several ways, such as **how they cut DNA** and the sequences of the **recognition sites** adjacent to where a cut is being targeted	
CRISPR/Cas13	**Cas13** enzyme is directed by gRNA(s) to a **RNA** sequence to generate a cut that **"knocks down" expression** of a gene without actually editing the genome	
TALEN	TALEN enzymes can be **engineered** to generate a **double-stranded cut** in a specific sequence of DNA; technology used before CRISPR	

Table 2: Experiments that can be designed for CRISPR/Cas9 in CHOPCHOP



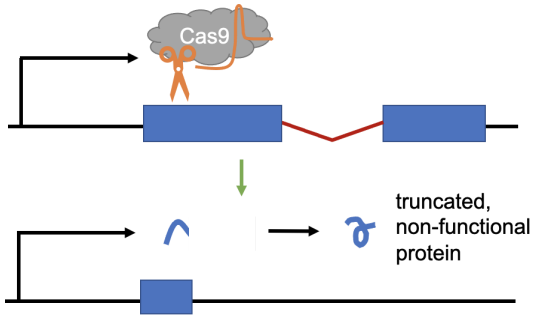
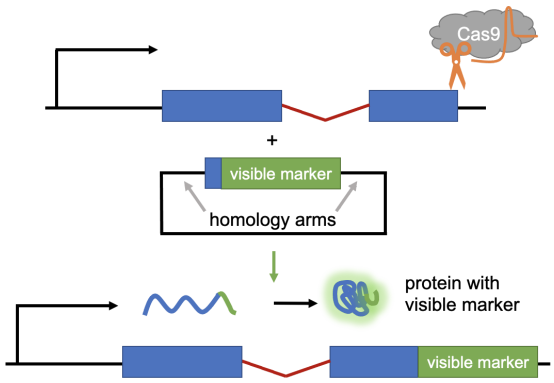
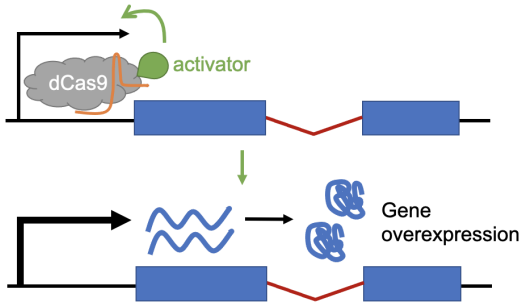
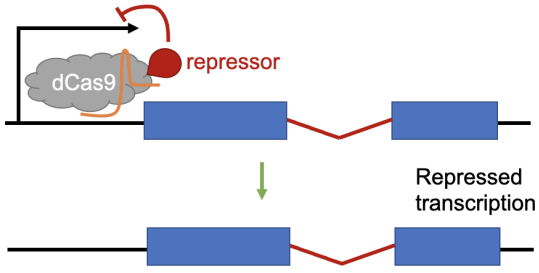
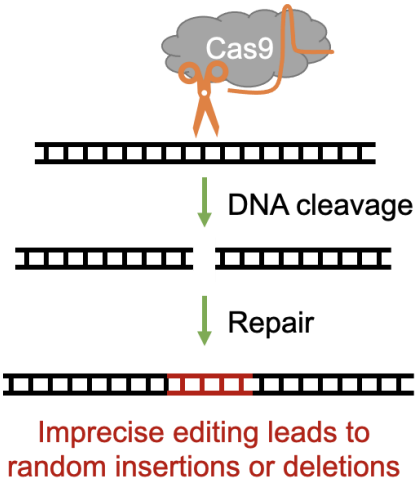
Knock-out	Generate a cut in the coding region of the gene with Cas9; likely to cause a frameshift mutation, which would prevent the protein from being translated properly and may render it non-functional	
Knock-in	Generate a cut with Cas9 and provide a template with a novel sequence flanked by homology arms (the sequences on either side of the targeted cut) to incorporate a new DNA sequence into a locus of interest; an example of something to knock-in could be a visible marker to visualize the protein with microscopy	
Activation	Target a deactivated Cas9 (no DNA cutting ability) fused to a transcriptional activator to the promoter of a gene of interest to overexpress that gene	
Repression	Target a deactivated Cas9 (no DNA cutting ability) fused to a transcriptional repressor to the promoter of a gene of interest to repress transcription of that gene	

Table 3: DNA repair pathways

Non-homologous end joining (NHEJ)	Pathway that directly repairs the two broken ends of DNA after cleavage An error-prone process that introduces small insertions or deletions at the cleavage site Common pathway leveraged in knock-out experiments	
Homology directed repair (HDR)	Pathway that uses a DNA sequence as a template for precise repair Repair template needs homology arms (sequences that match the sequences on either side of the cleavage site in the genome) May be used to edit just one base or to incorporate large pieces of new DNA sequence into the genome Common pathway leveraged in knock-in experiments