**CRISPR Gene Editing: Designing the gRNA and Donor Template**

**Piwi Matters Adaptation**

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***Acknowledgements:****This activity was built off of and modified from a CRISPR-based project created by Dr. Jodi Schottenfeld-Roames at Princeton University for MOL350: Laboratory in Molecular Biology.*

**Purpose:**

In this project, you will create CRISPR/Cas9 tools to create the Piwi mutation that you previously described in the *Piwi Matters* case study. In the process, you will learn about the CRISPR/Cas9 bacterial immune system and how it has been modified to facilitate gene editing. Afterwards, you can submit your CRISPR/Cas9 tools to a crowd-sourcing library so that your Piwi mutations can be made in flies.

**By the end of this project, you should be able to:**

* Describe the immune function of the bacterial CRISPR/Cas9 system.
* Identify and describe the function of each component of CRISPR/Cas9.
* Understand which CRISPR/Cas9 components are necessary for gene editing in eukaryotic cells.
* Design both a gRNA target sequence and a donor template to make a desired mutation.
* Decide the most suitable gRNA target from a list of computer-generated targets.
* Persuade others to use your specific combination of a gRNA target and donor template.
* Evaluate the effectiveness of various gRNA target and donor template combinations.

**Homework Assignment:**

To learn about CRISPR/Cas9, review the videos and article listed below. Pay particular attention to the differences of the CRISPR/Cas9 system when used as an immune response in bacteria and a gene editing tool in eukaryotic cells. Afterwards, answer the questions on the next page.

*Videos*

1. Video about CRISPR’s use in gene editing - Mayo Clinic YouTube Video, *CRISPR Explained* <https://www.youtube.com/watch?v=UKbrwPL3wXE>
2. Video about CRISPR’s function in bacteria- Bozeman Science, *What is CRISPR?* <https://www.youtube.com/watch?v=MnYppmstxIs&t=20s>

*Review Article*

1. Thurtle-Schmidt, D.M. and Lo, T. 2018 Biochemistry and Molecular Biology Education. Molecular Biology at the Cutting Edge: A review on CRISPR/CAS9 Gene Editing for Undergraduates. <https://iubmb.onlinelibrary.wiley.com/doi/full/10.1002/bmb.21108>

*Questions*

1. What are three molecules (either RNA or protein) from the *S. pyogenesis* genome that interact with each other to defend the cell against viral infections? What is the function of each component?
2. Briefly describe how bacteria use CRISPR/Cas:
3. during the adaptation phase of immunity
4. during the interference phase of immunity
5. What is a PAM sequence and why is it required for the function of Cas9?
6. Relative to the PAM site, where does Cas9 create a double-strand break?
7. What are two different ways that eukaryotic cells use to repair DNA after a Cas9 mediated double-strand break occurs?
8. What differences between bacteria and eukaryotic cells are important to consider in order to adapt CRISPR/Cas9 for gene editing?
9. What CRISPR/Cas9 components need to be introduced into flies to generate targeted mutations? What is the function of each component?
10. What steps need to be followed to use CRISPR to create a mutation?

The following sections will be addressed in class.

**gRNA target design**

To design an ideal gRNA target sequence, you will review and follow the steps published in the paper: Gratz, S.J. et al 2016. Current Protocols in Molecular Biology. CRISPR‐Cas9 Genome Editing in *Drosophila*<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4506758/>. In this paper, please read the section titled, “Target Site Selection“ and list at least two constraints to consider when choosing the gRNA target.

With these constraints in mind, you will design a gRNA sequence by following steps 6-11 of the protocol. You will start at step 6 because steps 1-5 describe the methods to determine the specific sequence of Piwi in Cas9 transgenic flies. Instead, you will want to refer to the sequence of NCBI accession number AF104355: <https://www.ncbi.nlm.nih.gov/nuccore/AF104355>.

**After finding the Piwi sequence and searching for gRNA sequences, write out the sequence of your desired gRNA:**

**Briefly explain why you chose this gRNA sequence:**

**Single-strand DNA donor template design**

In order to create your mutation, you will also need a donor template. To design a short single-strand DNA template, you will want to follow steps published in the paper: Gratz, S.J. et al 2016. Curr Protoc Mol Biol. CRISPR‐Cas9 Genome Editing in *Drosophila*<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4506758/>. In this paper, please read the section titled, ”Alternate Protocol 1 HDR with single-stranded DNA donors“ and list four constraints to consider when choosing your gRNA target.

**Once you have followed the steps of the protocol and designed a donor template, write out the sequence of your single-strand DNA template. Mark any mutated nucleotides and denote whether the mutations 1) create your desired mutation or 2) alter the PAM site.**

**Briefly explain the strengths and pitfalls of your template:**

**Submitting student mutations, gRNA sequences, and donor templates to our database:**

To have your Piwi mutation made and studied in the lab, you can submit your Piwi mutation, gRNA target sequence, and donor template sequence to our crowd-sourcing library.

To submit your design, fill out the form at: <https://forms.gle/FLcSkE56iA8j9HhP9>.