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Using CRISPR to Induce a Knock-out of dPRL-1 in Drosophila melanogaster

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Using CRISPR to Induce a Knock-out of dPRL-1 in *Drosophila melanogaster*

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Abstract

Phosphatase of regenerating liver (PRL) is a protein that controls cell processes such as growth and division which has unknown targets. PRL has been found to have both oncogenic and tumor suppressive properties. This study aimed to create a knock out of PRL in *Drosohpila melanogaster* in order to assess its role in development and in order to illuminate its activity when it is expressed in cancers. We hypothesize that dPRL-1 plays an important role in embryogenesis and that the progeny which lack this gene will be unviable. The CRISPR/Cas9 system was selected as the method in which to create a knock-out of this gene due to the specificity that it provides. Guide RNAs were designed in order to knock-out dPRL-1 and a gene called Yellow. The purpose of knocking out Yellow is that its absence leads to flies being yellow in color, which would serve as a positive marker. The gRNA for dPRL-1 was successfully integrated into a vector to cause expression in *Drosophila,* but the gRNA for Yellow was not able to be inserted. We await the arrival of the transgenic gRNA expressing lines to cross with a line of Cas9 expressing flies. The resulting progeny which lack dPRL-1 which will aid in our understanding of the role it plays in *Drosophila* development and its possible function in humans.

Background

- Phosphatase of regenerating liver (PRL) proteins regulate cell processes by likely removing phosphate groups to targets.
- PRL has been implicated as an oncogene and a tumor suppressor.
- Humans and mice have three copies of PRL. Knock-out studies that have removed 2 copies of PRL in mice and have shown mild phenotypes related to embryogenesis and spermatogenesis.
- *Drosophila* have only one copy of PRL – dPRL1 which makes them a simpler system for a knock-out study.

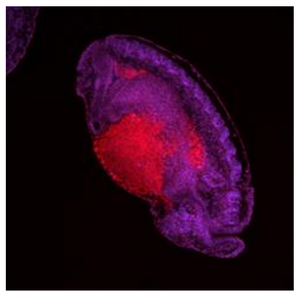

Figure 1. Expression of dPRL-1 in *Drosophila* embryo. During embryogenesis, dPRL-1 (red) is expressed at high levels before the morphogenic event of dorsal closure. Image from the Saucedo Lab.

Project Goals

- Create a knock-out of dPRl-1 in *Drosophila melanogaster* using CRISPR CAS9.
- Determine if and when dPRL-1 becomes necessary for *Drosophila* development.
- If knock-out embryos are viable, determine if phenotypes can be seen in tissues known to express the gene such as egg chambers.

Methods

- We will use PCR to insert specific gRNA sequences into vectors.
- Transgenic flies will be made by the company Best Gene.
- Antibody staining will be used to assess the amount of protein expressed in embryos.

CRISPR Cas9 Gene Editing

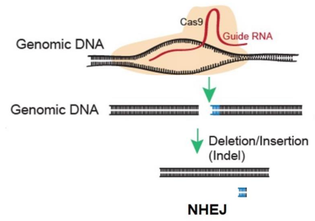

Figure 2. Deletion of PRL using CRISPR Cas9 gene editing. CRISPR allows site specific editing of DNA. Guide RNA (gRNA) and the Cas9 protein form a complex. The gRNA guides the complex to location of PRL in the genome. The Cas9 protein cuts both strands of DNA near PRL, causing a double stranded break. The cell repairs the broken DNA through a process called non-homologous end joining (NHEJ) which leads to DNA loss. Image adapted from cellsystems.de

Experimental Design

- gRNAs were designed for dPRL-1 and for Yellow. Yellow is a marker gene that results in yellow coloring to more easily identify flies where Cas9 had made cuts to the genome.
- Flies expressing gRNA will be crossed with flies that express the Cas9 protein. Resulting offspring from the cross will no longer produce d-PRL1 and be yellow in color.

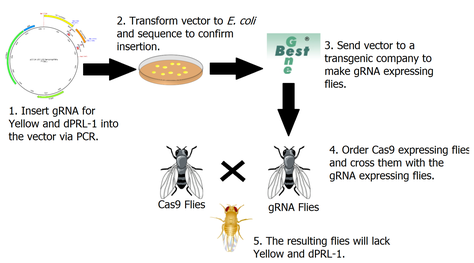

Figure 3. Insertion of vector into *Drosophila.* gRNA will be inserted through PCR and then transformed to *E. coli.* Sequencing will confirm the insertion. BestGene will create the *Drosophila* transgenics that express the gRNA.

Results

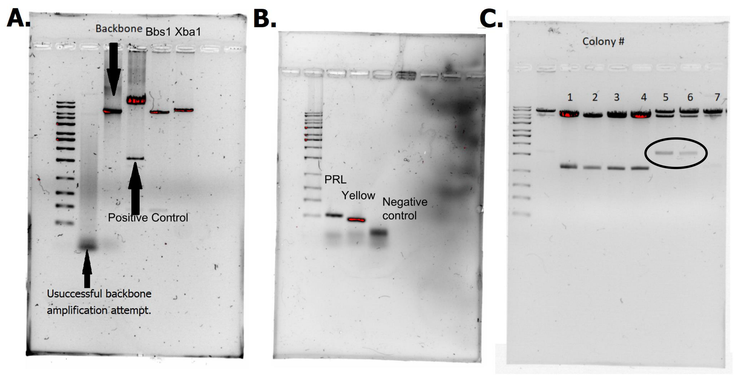

Figure 4. gRNA Vector Insertion and *E. coli* Transformation. A. Amplification of the backbone of the vector. A restriction enzyme digest of the plasmid was also performed in order to confirm the size of the fragment. **B.** Amplification of the gRNA for Yellow and dPRL-1. These linear pieces were later connected through Gibson Cloning. **C.** After transformation to *E. coli* a restriction enzyme digest using XbaI and BglII was preformed to determine if the gRNAs were successfully integrated. Colonies with fragments of 1,600 base pairs were sequenced because their size corresponded to both gRNAs being added.

- Based on sequencing colonies we were able to insert to the vector the gRNA for dPRL-1, but not for Yellow.

BestGene Trasngenics Timeline			
July 31st	**August 7th**	**August 12th**	**August 27th**
• 200 Embryos injected with vector.	• 100 Larvae Survived.	• G0 Adults crossed with stock.	• G1 Adult count is 2.

Future Directions

- We await the transgenic flies which will express the gRNA for dPRL-1 to cross with Cas9 expressing flies.
- The resulting offspring will be stained for dPRL-1 to assess the success of the gene editing.
- If the deletion of dPRL-1 leads to unviable offspring, we will assess with Cas9 expressed in tissue specific lines.

References

Gratz, S.J. et al. Mol Biol. 2015.
Pagarigan et al. PLoS One. 2013.

Acknowledgements

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