**“Introduction”:**

1. Why are Culex mosquitos such a global concern? Cite several specific examples. Culex mosquitoes are a global concern because they can carry a multitude of diseases that are capable of killing humans and animals. Some diseases they carry are West Nile (my grandpa was hospitalized for this), malaria, and lymphatic filariasis.
2. What makes *C. quinquefasciatus* so harmful? Culex mosquitoes can be found everywhere, especially in urban and suburban areas that have high concentrations of people. It also has a high tolerance to polluted water, and this is what they use as a breeding ground. Additionally, it can mate with other similar species and gives it different characteristics. All of these factors make it super hard to get under control.
3. Describe what this group actually created and the mechanism they used. The group used CRISPR to create Culex mutants. CRISPR could be used to prevent the tremendous disease transmission these mosquitoes are responsible for. Additionally, it can be used to reduce the overall Culex mosquito population.
4. In your own words, try to explain why CRISPR-based gene drivers are successful. CRISPR-based genes are successful because they can reproduce by themselves, and it can spread through the Culex population one at a time until it either prevents disease transmission and/or reduces the Culex population.
5. Using the last paragraph, determine how many steps were done in sequence? There were seven steps done in the sequence. I. Generation and validation of transgenes for the expression of CRISPR components, II. Validation of CRISPR reagents in Culex quinquefasciatus ovarian cell line, III. In vivo validation of CRISPR reagents in Culex quinque-fasciatus, IV. Generation of a Cas9-expressing line by site-directed trans-genesis in Culex quinquefasciatus, V. Validation of the Culex quinquefasciatus vasa-Cas9 transgenic line, VI. gRNA scaffold variants improve transgenesis in Drosophila melanogaster, VII. gRNA scaffold variants improve gene drive efficiency in Drosophila melanogaster.

**Results**

**i. Generation and validation of transgenes for the expression of CRISPR components**

1. What was made here? They identified many U6 genes from the Culex specimens, and then cloned these to validate the functionality of the Culex mutants.

**ii. Validation of CRISPR reagents in *Culex quinquefasciatus* ovarian cell line**

1. Define “in vitro.” Using **Fig. 1,** explain how this group used the technique. In vitro means in a cultured environment outside of a specimen's normal environment. In figure one, Hsu cells were cultured for 12 days, the dna undergoes PCR amplification, the eggs are injected with a plasmid mixture. Once the eggs hatch, the larvae are observed. This group used this technique in the first step, where the cells were cultured for 12 days.

**iii. In vivo validation of CRISPR reagents in *Culex quinquefasciatus***

1. Define “in vivo.” Using **Fig. 1,** explain how this group used the technique. In vivo refers to a process that is conducted inside of an organism. In figure one, Hsu cells were cultured for 12 days, the dna undergoes PCR amplification, the eggs are injected with a plasmid mixture. Once the eggs hatch, the larvae are observed. One example of this is when the plasmid mixture was injected into the larvae and they hatched and were observed.

**v. Validation of the *Culex quinquefasciatus* vasa-Cas9 transgenic line**

1. Referencing **Fig. 2,** describe what was done in this step as it relates to genetics. This figure shows where the mutation was in the Culex mosquitoes, and compares the scaffold variants from the original culex mosquitoes to the mutants.

**vi. gRNA scaffold variants improve transgenesis in Drosophila melanogaster**

1. Why is this a big test? I.e.: What future implications could it provide? This is experiment is being done with mosquitoes, but this is a big test because eventually this could also be used on other species, like flies and locusts. Once proven to be effective, this could also be used on many other species.

**Discussion**

1. What do you notice about the discussion and the results section? The Conclusion is that gene drive is an important part of the process used to conduct transgenesis and CRISPR to remove the ability to transfer diseases and reduce population. Some important methods to this are cell culture and transfection, fluorescence activated cell sorting, embryo microinjection, and more.
* How would you evaluate the success of this study? This study must have been very successful, because the transfer of disease through mosquitoes is something that is very relevant to us today, so the use of CRISPR to prevent disease transfer and reduce the population is very successful.