Targeting Drive Associated Genes using CRISPR-Cas9
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Introduction

Teleopsis Dalmani (also known as Stalk-eyed Flies) are creatures that have these long stalks growing out of their heads. Attached to these stalks are their eyes which is not noticeable at first glance. My focus is on selfish gene these flies carry known as a meiotic drive gene. According to Mendel’s Law of segregation in species where chromosomes determine sex, the chances of receiving a male or a female is half and half. However, in this case that half and half is now 10 and 90. Meiotic drive is a cause by a selfish gene on the X-chromosome that also causes specific traits in the same, males have reduced fertility whereas in females have higher fertility. In addition, the way these flies are attracted to each other are due to their stalks. Female stalk-eyed flies are attracted to long stalks on males. Males with the drive associated X-chromosome will have shorter stalks and therefore reduce their chances of reproducing with other females. However, almost nothing is known about the genetic causes of any of these consequences of carrying a meiotic drive chromosome. In my project, we plan on modifying a standard stalk-eye fly and hope to replicate meiotic drive. In addition, we look at impacts on the other traits described above. What we want to do with these stalk-eyed flies is modify candidate genes using CRISPR-Cas9. We'll use CRISPR to induce a frameshift mutation, and that is an approach we are investigating.

Methods

Generate Hypothesis… What Gene Do I Need to Target? What Phenotype?

Find Genes that are associated with meiotic drive

Design and order gRNAs as well as PCR primers

Plasmid DNA Prep

Digest Plasmid

Ligate gRNA insert Transform into bacteria

Colonies

“Mini-Prep” Plasmid DNA, then sequence the DNA

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Inject plasmid and cas9 into fly embryos

References


Conclusion

The Yuri transformation was unsuccessful however, the Chiffon transformation was successful. Moving forward, we want to re-do our Yuri ligation and transformation, sequence colonies from the chifon transformation. We need to troubleshoot our PCR approach and hopefully be ready to inject modified plasmids into fly embryos!