Developing RNAi protocol to target genes in the alfalfa leafcutter bee, *Megachile rotundata,* for gene functionality

USDA United States Department of Agriculture

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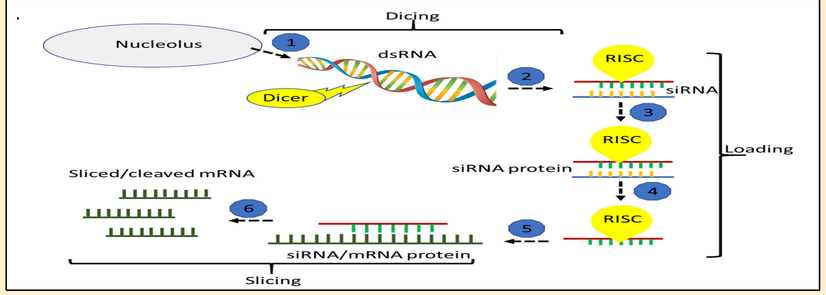
Introduction

- *Megachile rotundata,* alfalfa leafcutter bee is the primary pollinator for alfalfa seed production[1].
- *M. rotundata* goes into diapause as a prepupa or skips diapause to emerge as the parent generation.
- Analysis of gene expression and function could be used to identify the physiological mechanisms behind diapause[1].
- RNA interference (RNAi) is a tool for knocking down the expression of individual genes and has been widely used to study the cellular function of genes[2].
- We have targeted genes of known function using RNAi which'll eventually help to discover the function of genes that help *M. rotundata* diapause.

Objective

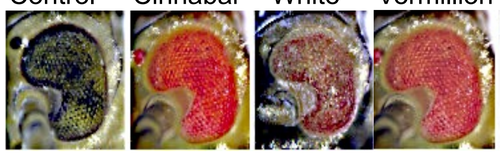
Develop a RNAi protocol by targeting Cinnabar Vermillion, and White in *Megachile rotundata.*

RNAi mechanism



Predictions (Eye pigmentation Defects)

Control Cinnabar White Vermillion



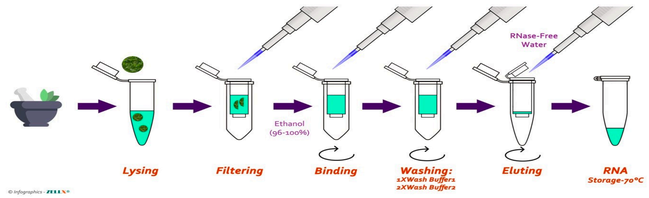
- Expect to see changes in eye pigmentation

Methods

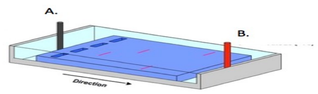
1. Making double stranded RNA against Cinnabar (dsC), Vermillion (dsV) and White (dsW).
2. Injecting dsRNA: Thorax of *M.rotundata* pupae

Control (water) Treatments (dsC, dsV, and dsW)

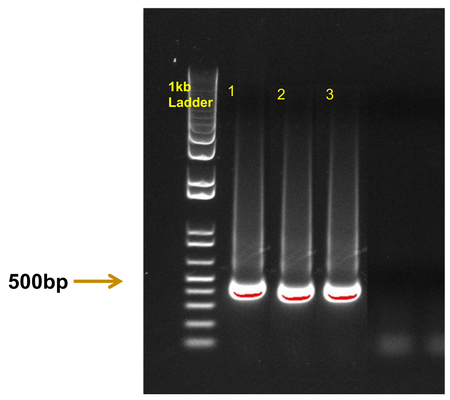
3. RNA extraction and cDNA synthesis



4. Running PCR for targeted genes dsC, dsV, dsW and control (water)
5. Gene Expression Analysis using Gel Agrose Electrophoresis



Results



dsRNA expected size: 460 bp

Agarose gel electrophoresis of dsRNA synthesis against

1. dsCinnabar
2. dsVermillion
3. dsWhite

Conclusions

- There will be lower expression in samples where dsRNA genes was injected against dsC, dsV, and dsW as compared to the control (water)
- Samples injected with dsRNA against genes will show light/faint bands on agarose gel as compared to control after running semi-quantitative PCR.
- Phenotypically, there will be changes in eye pigmentation after knocking down the genes Cinnabar, Vermillion and White.

Future Directions

- Run qPCR again with increases in concentration of the three dsRNA genes
- Select other genes with known function to use for RNAi protocol to be fully developed
- Expression analysis will be done using qPCR after gene knockdown
- This RNAi protocol will be used for gene knockdown of selected genes that might contribute to *M. rotundata* going into diapause.
- This study will help in understanding the function of different gene in solitary bees

Acknowledgements

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References

1. Xu J., James R.R. Temperature stress affects the expression of immune response genes in the alfalfa leafcutting bee, *Megachile rotundata*. *Insect Mol. Biol.* 2012;21:269–280.
2. U.S. National Library of Medicine. (n.d.). RNA interference (rnai*). National Center for Biotechnology Information.*
3. Heu, C. C., Gross, R. J., Le, K. P., LeRoy, D. M., Fan, B., Hull, J. J., Brent, C. S., & Fabrick, J. A. CRISPR-mediated knockout of cardinal and cinnabar eye pigmentation genes in the western tarnished plant bug. *Sci Rep*. 2022, 12: 4817.