Novel Enhancer Trap Lines Localizing Gene Expression by Flippase Mosaics

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Abstract

Understanding even the simplest brain is difficult due to the diversity and dense interconnectivity of cells in the nervous system. However, new techniques enable the manipulation of the function of neurons in the brains of living animals. The Flippase Inducible Gal 80 Repression (FINGR) method combines the genetic manageability of *Drosophila* for an insect with a nervous system rich in many cell types, to generate mosaics within neuronal circuits (e.g., some parts of the circuit can be "turned off" while others are "left on") and measure the effect mosaics have on behavior disruption. To "turn off" parts of the circuit, this mixed-method study employs an Enhancer Trap Flippase (ET-FLP) and a FRT (Flippase Recognition Target)-dependent GAL80. The ET-FLPs, when combined with these FRT dependent Gal80, randomly remove parts of the circuit. This mosaic circuit can then be examined for two interesting behaviors: wing inflation and courtship. Preliminary results have yielded approximately 37 new enhancer trap lines. These new ET-FLP lines are expected to break the circuit in up to 37 different ways or mosaic patterns.

Background

What is an enhancer trap?

An enhancer trap is a mobile genetic element (or transposon) that inserts into various chromosomal regions throughout the fruit fly genome.

Gene pattern that is critical for the behavior:

1. the courtship within the fruitless gene-defined circuit
2. the wing inflation in the *bursicon* gene-defined circuit.

Research Questions

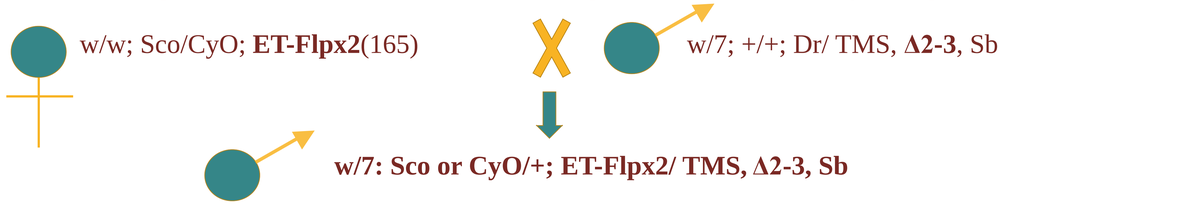
How does a researcher subdivide a circuit?

Can a student successfully subdivide a neuronal circuit successfully by using mosaics created by the Enhancer Trap method?

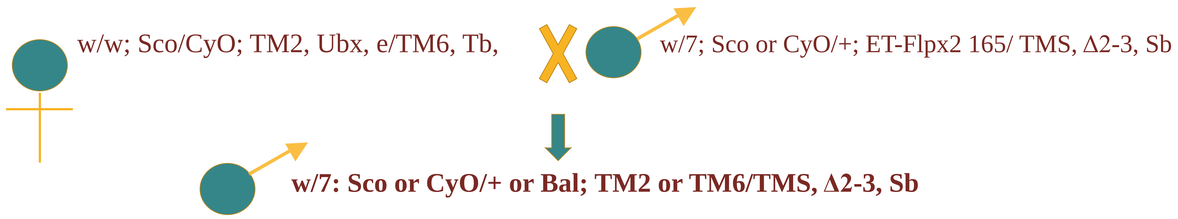
Methods

Strains of *D. melanogaster* and Their Fundamental Components:

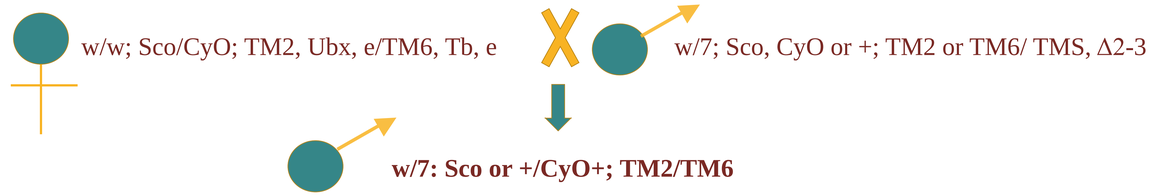
Step 1: Isolate Males with Mobilized Transposons: Female, Existing ET-Flpx2



Step 2: Cross to Balancer to remove Original ET-Flpx2: Female is DBE



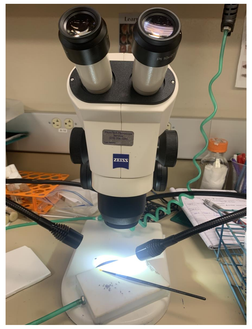
Step 3: Cross to Balancer to remove Transposase (Δ2-3): Female is DBE

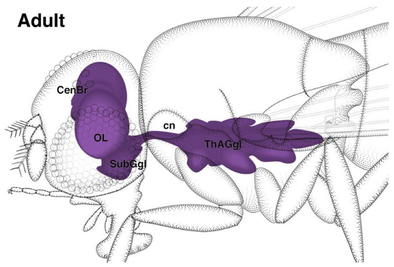


Existing stocks of the following types are to be used: tester(Delta2-3, x ET-Flp,), (F1 Offspring (Mosaic) x W; DBE), (F2 Offspring x W; DBE)

At room temperature (22°C or 72°F), stocks and genetic crosses are maintained via fly food vials, consisting of cornmeal, sucrose, yeast, and agar medium.

Viewing and analyzing stocks is done under a stereo-microscope using carbon dioxide platforms to make the flies immobile.

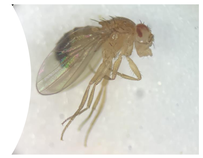




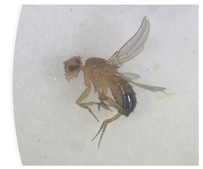
(Hartenstein, 1993)

Preliminary Results and Conclusion

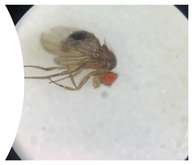
1st Cross Result:



2nd Cross Result:



3rd Cross Result:



Results pertaining to this experiment currently have **37 Enhancer Trap lines.** Work still to be done includes testing the mobilization and start performing shuttering on/off the circuit. This is expected to be completed around Fall 2022.

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Acknowledgements

Thank you to the Ronald E. McNair Scholars Program for their continued financial support. Also, sincere gratitude is extended to the Department of Biological and Health Science and Neurogenetics Lab at Texas A&M University-Kingsville for assisting in this project.

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