Generating a novel fly line to quantify cellular oxidative stress on developing Drosophila melanogaster

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Abstract & Background
The redox condition of the cell, the ratio of reactive oxygen species to reducing agents present, can indicate the likelihood of oncogenesis. There are several genetic pathways present, such as the Nrf2 pathway in humans, which moderate the level of reactive oxygen species (ROS) within the cell. The Nrf2 pathway is an orthologue to the cnC pathway in *Drosophila melanogaster*, making *Drosophila* an ideal model organism to study redox pathways in the cell.

Previous work has shown that cnC alterations can alter oncogene levels in *Drosophila*, however it hasn’t been shown that this manipulation truly alters ROS levels within the cell. To solve this, a fly line was created which could indicate and quantify ROS levels via fluorescence microscopy. This was done through use of the UAS-GAL4 system; a system which allows for the introduction and expression of desired genes within the *Drosophila melanogaster* genome. For this desired purpose, both the UAS-GAL4 indicator and ROS GFP reporter need to be on the same chromosome, so recombination was necessary for the success of this introduction. Through several rounds of genetic crosses, a fly line was established with a UAS-GAL4 indicator and a ROS GFP reporter on the same chromosome within the selected flies. Recombination occurred in 26% of 250 offspring.

This recombination will allow quantification of ROS in developing wings, guts, and testes via GFP fluorescence.

Methods
Flies with both genes of interest were selected and crossed with their-eyed, wild-type flies to easily identify recombination. To perform future experiments on whether cnC alters cellular ROS levels, both the UAS-GAL4 sequence and ROS GFP need to be on the same chromosome. Thus, we selected for offspring in which recombination has occurred, as seen through eye color and fluorescence.

Results
26% of the 250 offspring from heterozygous parents showed recombination of both indicator and reporter on the same chromosome. 24% of the offspring collected had white eyes, indicative of neither indicator nor reporter. The flies with either indicator or reporter were also found in near 1:1 ratio to each other, at approx. 25% of the offspring each.

Conclusions & Future Directions
Several conclusions can be drawn from the results of this experiment.

1. The UAS-Gal4/ROS GFP system is effective at measuring ROS levels in developing wing discs.

2. The UAS-GAL4 indicator and ROS GFP reporter are both on chromosome II, but they are not near each other. This allowed for recombination to occur readily.

Based on these conclusions, further research can be conducted to quantify whether cnC actually alters cellular ROS levels. This will also enable us to investigate the effect of overexpressing cnC in these flies to determine the role cnC plays in managing oxidative stress. This will provide insight into the ability of oncogenes to promote cancer.

This model system can be used to better understand how antioxidants relate to cancer development in the human system. By understanding the role of ROS in oncogene expression, treatments and targets for cancer prevention can be better understood.

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Generating a novel fly line to quantify cellular oxidative stress on developing *Drosophila melanogaster*
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