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Locating the mRFPruby Transgene in *Drosophila melanogaster*

Background

In the fruit fly *Drosophila melanogaster*, primordial germ cells (PGCs) develop on the posterior end of the embryo and then migrate to their final position in the gonads. During this migration, some PGCs undergo cell death[1]. The study of programmed cell death has potential applications for future cancer treatments. In order to identify and image PGCs in live embryos, we have created transgenic fly lines that express the fluorophore mRFPruby in PGCs. A challenge with these fly lines is that mRFPruby cannot be observed early enough, so the PGCs have almost completed their migration by the time mRFPruby is visible using multiphoton imaging (Figure 1). I am attempting to increase expression of mRFPruby so the PGCs are visible earlier in migration.

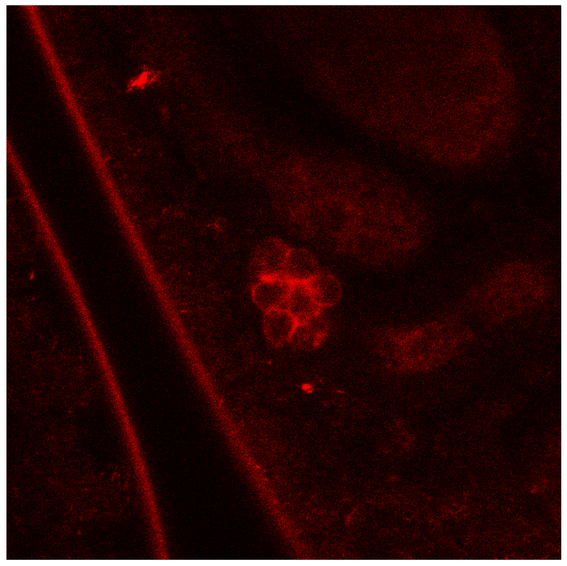

Figure 1: Primordial germ cells expressing the mRFPruby fluorophore in a live embryo.

Objectives

- To locate the site of insertion of the mRFPruby transgene in two lines of fruit flies
- To create a protocol for screening future lines for the presence of the mRFPruby transgene

Methods

- I used a technique called inverse PCR to isolate the section of the genome that contained the insert
- I used gel electrophoresis to visualize the results of the PCR experiments.
- I used DNA sequencing and a genome analysis tool BLAST to identify the area of genomic sequence flanking the construct
- I designed primers that would allow me to confirm the presence of this insert in future tests

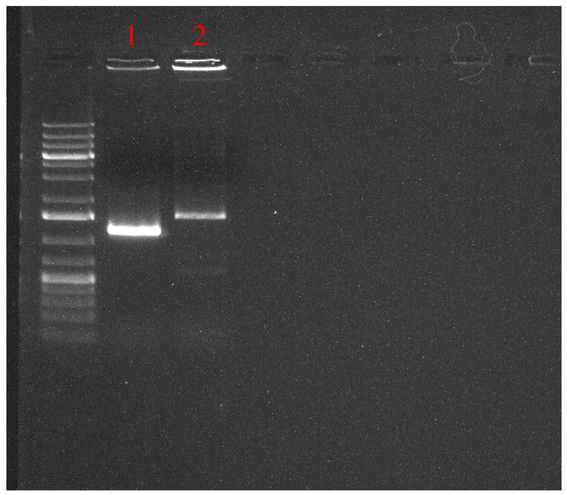

Figure 2: Agarose gel confirming presence of the transgene from an upstream direction (lane 1) and downstream direction (lane 2).

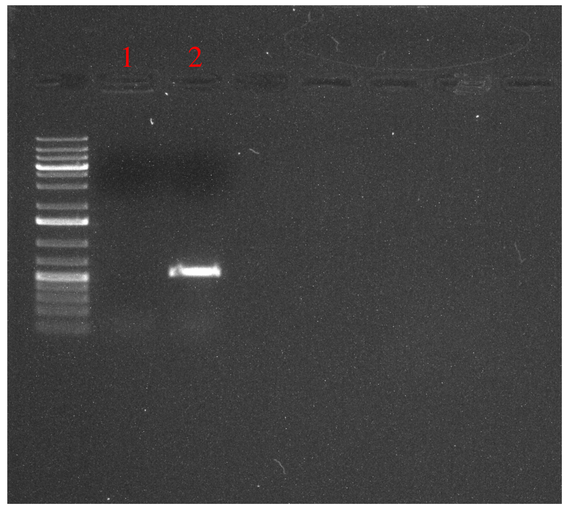

Figure 3: Agarose gel showing initial screen of 1-3 flies in lane 1 and control flies in lane 2.

Results

I focused my project on two lines of flies: 1-3 and 1-7. Using PCR, I was able to confirm the location of the transgene in 1-7 flies on the right arm of the third chromosome (Figure 2). I have preliminary data pointing towards a location of the transgene within 1-3 flies (Figure 3), but more testing is needed to confirm this.

Conclusion/Future Directions

We can be confident that the mRFPruby transgene is present in 1-7 flies on the right arm of the third chromosome. Once a protocol is determined to screen for inserts in both fly lines, we can perform fly crosses in an attempt to recombine multiple copies of the transgene onto the same chromosome. Increasing the copy number of this gene will hopefully increase expression of the mRFPruby fluorophore allowing for earlier visualization of PGCs.

References: 1. Yukiko Yamada, Keri D. Davis, and Clark R. Coffman (2008). Programmed cell death of primordial germ cells in Drosophila is regulated by p53 and the Outsiders monocarboxylate transporter. Development. 135 207-216.