High throughput collection of *Drosophila* embryos for homozygous lethal mutants based on *deformed* driven YFP expression

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Introduction

COPAS Select and Express flow cytometry instruments are able to analyze and dispense *Drosophila* embryos on the basis of size and fluorescent signals. One application of this technology is to sort embryos or larvae that have homozygous lethal mutations maintained over a balancer chromosome. By marking the balancer chromosome with a gene expressing a fluorescent protein, homozygous mutant embryos can be collected based on the absence of a fluorescent signal relative to the other embryos of the sample. The COPAS technology allows automation of this selection process, increasing throughput and reducing human error. Here we show the result of using this technology for collecting homozygous nonfluorescent embryos based on *deformed(dfd)* promoter-driven YFP-labeled balancer chromosome. Additionally, our data shows this instrument is able to recognize and record the expression patterns along the longitudinal axis of an embryo, making it possible for high content screening applications.

Methods

Materials and Methods

Drosophila strains were provided by Dr. Greg Beitel of Northwestern University. An eYFP construct was inserted into the *Drosophila* genome under the control of *dfd* promoter (for details, see poster 1035C). Data collected from one of the strains: w; +/+; TM3P/TM6Sb, with the YFP construct inserted into the TM3 chromosome, are reported in the poster. Embryos were collected from egg-plates and were dechorionated prior to analysis on the COPAS Select. Dispensed embryos were examined under a fluorescent microscope to verify the expression patterns. A COPAS instrument with 500 μm flow cell was used in the experiments. An argon laser with 514 nm wavelength was used for fluorescence excitation. For emission detection, a 545/25 nm band pass filter was used.

Results

Separation based on fluorescence intensity level

Embryos were collected and aged (14-18 hrs) at room temperature before sorting. Fluorescence data was collected for individual embryos and displayed as dot plots in real time. As shown in Figure 1A, two populations emerge on the Yellow-Red dot plot. Microscopic examination of embryos dispensed from these two populations confirmed that they represent embryos with or without fluorescent signals. Better separations of the two populations were achieved by displaying the data in log scales (Figure 1B). Sorting based on either scale was 100% accurate (50/50) for both the nonfluorescent and fluorescent embryo collections.

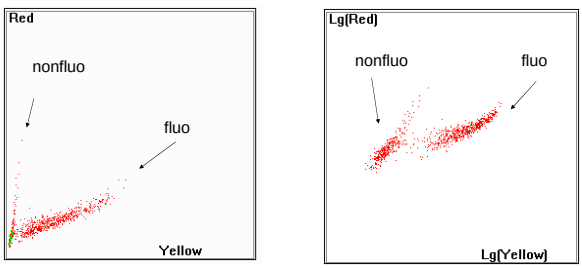

Figure 1. Separation of homozygous non-fluorescent embryos from their fluorescent siblings based on YFP fluorescence level. Yellow: yellow fluorescence at 545 nm. Red: red fluorescence at 610 nm. nonfluo: embryos with no fluorescence. fluo: fluorescent embryos. A. fluorescence intensity in linear scale. B. fluorescence intensity in log scale.

Fluorescence profile

The COPAS Profiler, an add on module to the sorter, is able to record fluorescence positional information along the longitudinal axis of an embryo. Expression driven by the *dfd* promoter starts from the head region (stage 14-15) and then spreads to the rest of the body in a striped pattern (stage 16-17). As shown in Figure 2, fluorescence profiles generated by the Profiler match with the corresponding expression patterns.

Results

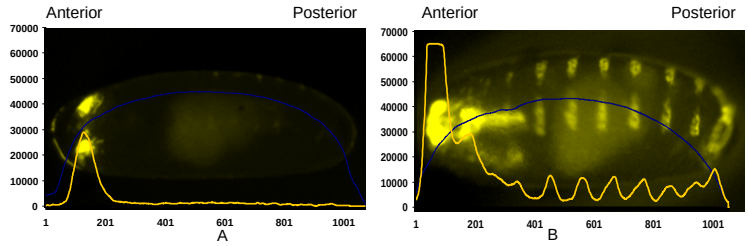

Figure 2.Fluorescence profile based dfdYFP expression. X-axis: Longitudinal axis of an embryo (Time Of Flight). Y-axis: yellow fluorescence intensity level. Blue line: optical density. Yellow line: yellow fluorescence. Embryo head is on the left for both images. A. stage 14-15 embryos. B. stage 16-17 embryos.

Separation based on fluorescence peak height

As shown in Figure 2, *dfdYFP* expression is strongest in the head region, leading to a large spike on the fluorescence profile. With the Profiler, the COPAS instrument is able to sort on either global fluorescence intensity or maximum fluorescence peak height. Given the large signal/noise ratio for the fluorescent peak, we tested sorting based on peak heights instead of the overall fluorescence intensity levels in a mixed stage embryo sample. As shown in Figure 3, the dot plot for peak heights shows a greater separation of populations when compared to global fluorescence intensity based dot plot (Figure 1). Therefore, it is possible to separate embryos with different levels of fluorescence intensities (Figure 4).

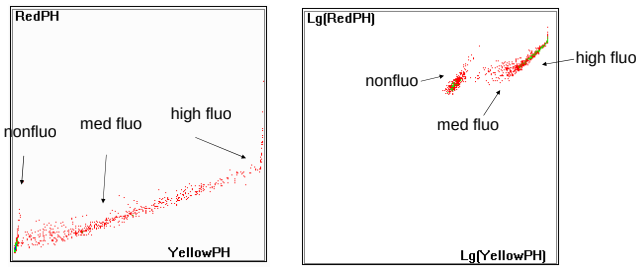

Figure 3. Separation of homozygous nonfluorescent embryos from their fluorescent siblings based on YFP fluorescence peak heights. YellowPH: peak height for highest peak in fluorescence profile. RedPH: peak height for red fluorescence profile. nonfluo: embryos with no fluorescence. med fluo: embryos with intermediate peak heights. high fluo: embryos with high peak heights. A. linear scale. B. log scale.

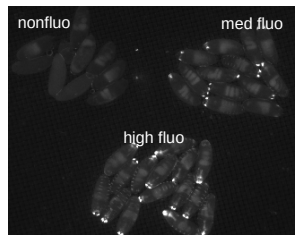

Figure 4. Collection of three populations of embryos based on fluorescent peak heights. Sorting regions were based on regions shown in Figure 3.

Conclusions

We have shown that the COPAS instrument is able to detect *dfd*-driven YFP fluorescence. A *Drosophila* strain with *dfd*YFP marked balancer chromosome was tested and successfully sorted into at least two fluorescent-specific populations – nonfluorescent and fluorescent. Therefore, it is possible to use *dfd*-marked balancer chromosome to maintain lethal mutations and use the COPAS instrument to automate collection of the homozygous mutant embryos. We also believe that the *dfd*YFP strain serves as an excellent alternative to current GFP labeled balancer chromosomes because of the following reasons: 1) This marker is not in a GAL4/UAS system and therefore does not interfere with the use of other UAS constructs. 2) Using the longer excitation wavelength of YFP results in reduced autofluorescence levels generally seen in GFP-based strains. 3) Distinct profile patterns specific to this balancer construct may make it useful for additional applications, such as staging of embryos.

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