Instrumentation for analysis and sorting of fluorescence patterns in transgenic Drosophila embryos

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1. Introduction

An automated method for sorting of *Drosophila* embryos on the presence or absence of fluorescence has been used for high throughput exon trapping (M.H. Buszczak et al. and S.S. Gisselbrecht et al., 43rd Annual *Drosophila* Research Conference, 2002). The instrumentation used in these experiments was the COPAS™ SELECT (Union Biometrica, Somerville, MA). The COPAS technology platform is a liquid flow-based system that carries embryos individually through a flow cell for analysis of optical density and fluorescence levels. Tens of thousands of embryos can be interrogated one at a time as they pass through the laser light focused at the center of the flow cell. Fluorescence can be measured from any of several reporter proteins (GFP, EGFP, YFP, EYFP, DsRed) and the fluorescent embryos collected for further analysis.

We describe new technology, called Profiler, for the COPAS instrumentation. This technology allows for the collection of optical density and fluorescence values in slices along the axial length of the embryo. We analyzed embryos with specific fluorescence positional information with the Profiler and sorted these from embryos expressing a different pattern of expression in proof of principle reconstruction experiments. We show that we are able to identify differences in fluorescence peak heights, peak widths, and variants with alterations in these profiles. This technology will enable genetic screening for mutations that alter the expression pattern of genes. It brings an increase in speed and automation to the screening process.

2. Methods

Data collection was on COPAS Select with Profiler.

Fly strains:

caster/twist-GFP TM3 Sb (expressing GFP)
YET 31-1 (Gisselbrecht & Michelson, HHMI/Harvard Med. School)
YET 18-1 (Gisselbrecht & Michelson, HHMI/Harvard Med. School)
WeeP114b (P. Clyne & G. Davis, UCSF)
WeeP20 (P. Clyne & G. Davis, UCSF)
WeeP29-26 (P. Clyne & G. Davis, UCSF)
WeeP59 (P. Clyne & G. Davis, UCSF)
WeeP1-13 (P. Clyne & G. Davis, UCSF)

(For data on Wee-P constructs, see Abstract 149, this meeting, PJ Clyne et al.)

Methods Continued

Embryo collection:

Several different collection schedules were tested. For twist-GFP, YET 31-1 and YET 18-1, adult flies were allowed to lay eggs at 25°C for six hours in the day. The plates were then removed and stored overnight at 20°C. The embryos were collected for testing on the next day. For transgenics with the Wee-P construct, adult flies were allowed to lay eggs overnight at 20°C and embryos were collected for testing.

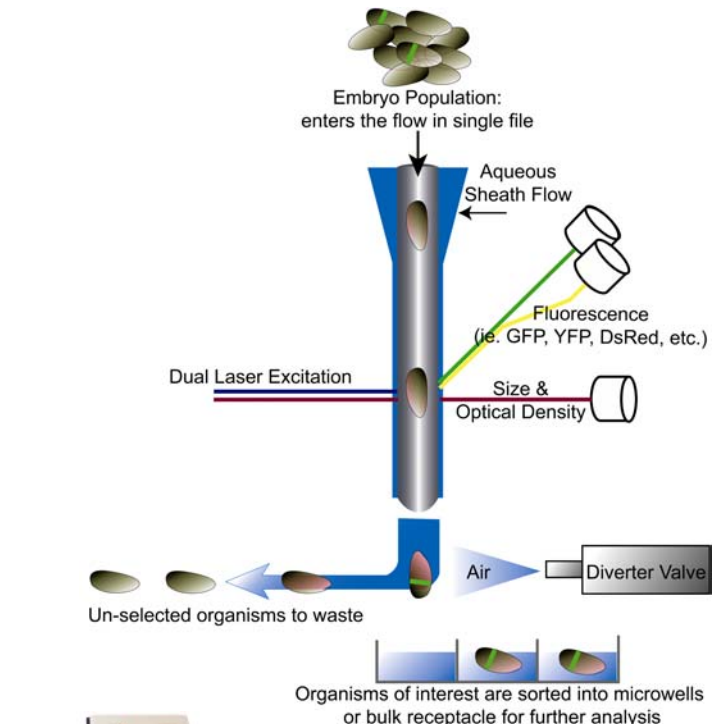
Data collection:

For GFP strain: excitation wavelength, 488nm; Profiler channel, green; PMT level, green 450V, red 500V; signal and integration gains, 150.

For YFP strains: excitation wavelength, 514nm; Profiler channel, yellow; PMT level, yellow 450V, red 500V; signal and integration gains, 150.

Data were stored as txt files and then analyzed using Microsoft® Excel®.

3. Assay principle



COPAS Instrument

4. Results

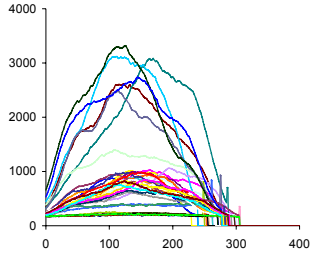


Fig. 1: Green fluorescence profiles collected from *castor/twisterGFP embryos*.

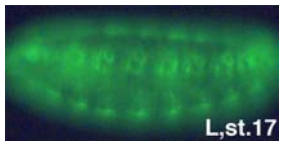


Fig. 2a: Expression pattern of YET 31-1. Photo supplied by Steve Gisselbrecht.

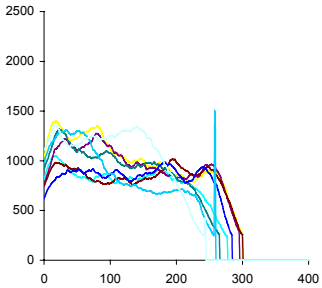


Fig 2b: YFP fluorescence profiles collected from YET31-1 embryos.

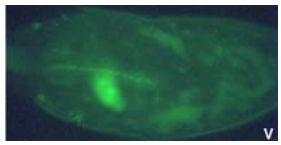


Figure 3a. Expression pattern of YET 18-1. Photo supplied by Steve Gisselbrecht.

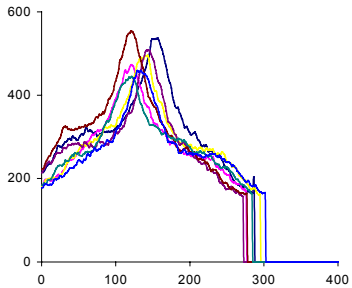
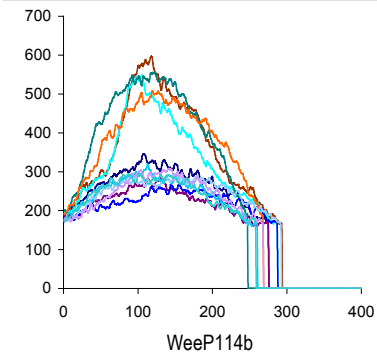
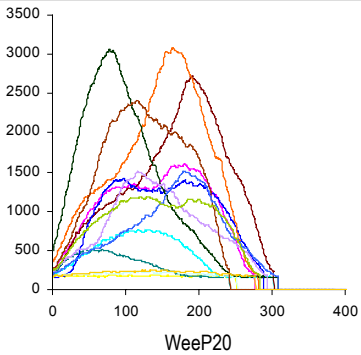
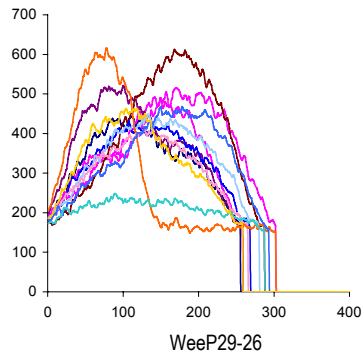
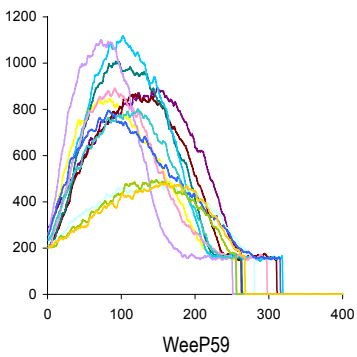


Figure 3b. YFP fluorescence profiles collected from YET 18-1 embryos.









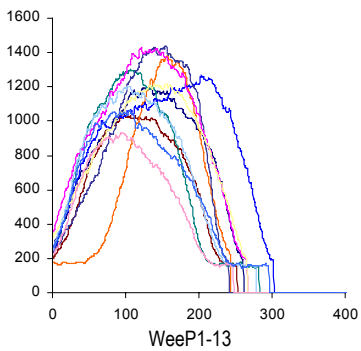


Figure 4. Fluorescence profiles collected from GFP-expressing strains provided by P. Clyne and G. Davis.

5. Conclusions

The Profiler module allows for the collection of positional fluorescence information along the axial length of *Drosophila* embryos. The Profiler collects about 250 to 300 measurements per embryo. These measurements can be used to distinguish patterns in a number of ways. These include peak height, peak width, and the position of fluorescence relative to another positional marker, such as the end of the embryo. The Profiler module can analyze a population of embryos for their pattern of fluorescence. It can also be used to select embryos with altered patterns. This technology will enable genetic screening for mutations that alter the expression pattern of genes. It brings an increase in speed and automation to the screening process.

For more information: Union Biometrica, Inc. (Booth 401)
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