

COPAS™ Application Note S-02

RARE *DROSOPHILA* EMBRYO SORTING

Isolation of Rare Fluorescent Expressing Embryos from a Large Population

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Objective

Rare *Drosophila melanogaster* embryos can be isolated from a large population, based on fluorescent intensity, using the COPAS *SELECT* system (Union Biometrica, Inc.).

Introduction

Rare embryos, expressing GFP, have been analyzed and isolated from a large population using the COPAS *SELECT* system (figure 1). Approximately 99.8% of the embryos analyzed in this experiment were not expressing GFP. The remaining 0.2% of the embryos expressed GFP and were removed from the population into a collection container.

Materials

COPAS *SELECT* (Union Biometrica pn 335-5000-000)
COPAS *SELECT* Sheath Fluid (pn 335-5070-000)
Embryo Sample Solution (pn 335-5075-000)
Drosophila embryos
Collection Container

Methods

Samples were prepared according to the *Drosophila* sample preparation protocol (see **Sample Protocol SPS01**). The *Drosophila* embryos are to be diluted to approximately 500 embryos/ml, with the Embryo Sample Solution (ESS). The embryo concentration can be checked by extracting a known volume of liquid out



Figure 1: Picture of the COPAS *SELECT* system.

from the preparation and by counting the amount of embryos present. Multiple aliquots of the solution should be extracted in order to confirm the recommended concentration of embryos. The diluted embryos are then added to the sample cup and analyzed using the COPAS *SELECT* system.

Two parameters of optical characteristics, Time of Flight (TOF) and Extinction (EXT), are used to initially analyze the population. Figure 2 is a dot plot, with TOF and EXT as the two gating parameters, displaying all of the embryos analyzed during the experiment. The Sorting Dot Plot in figure 3 displays a polygonal region positioned around the embryos that were isolated from the whole population. The sorting region was defined on a dual fluorescence parameter plot, with FLU1 (green fluorescence intensity) and FLU2 (red fluorescence intensity) as the two parameters. A collection vial was used to gather the embryos of interest.

Results

Once the user has identified the embryos of interest, the COPAS *SELECT* will sort only those embryos that are displayed within the user-selected region.

Accuracy

In this experiment, rare event embryos expressing GFP were dispensed from a larger population into a collection container (figure 3). Of the 10,044 embryos analyzed, exactly 25 embryos were dispensed. The presence of fluorescence was confirmed with the use of a fluorescence microscope.

Coincidence

Coincidence occurs when two or more organisms are analyzed at the same time and appear as a single organism. Contamination of a particular sort can occur when embryos, not displayed within the sorting region, are dispensed into the collection vial.

Due to the nature of a rare event screen, some contamination may be acceptable when isolating rare embryos expressing GFP. For that reason, the Coincidence Check feature on the software user interface should not be activated when isolating rare biological events.

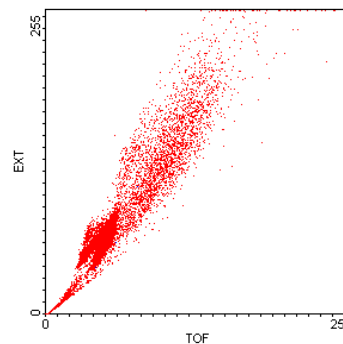


Figure 2: Dot plot showing the entire population of embryos analyzed by the *SELECT*. TOF is Time of Flight and EXT is Extinction.

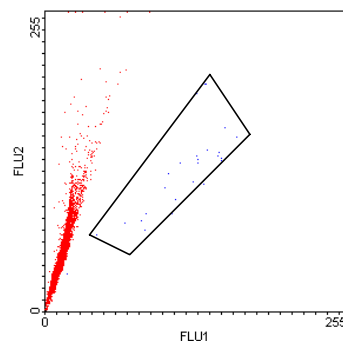


Figure 3: A graph showing data from a rare event screen where three embryos expressing GFP (green pixels, selected) were sorted out from the larger population of non-fluorescent embryos (red pixels). FLU1 represents green fluorescence intensity and FLU2 represents red fluorescence intensity.

Discussion

We have sorted rare *Drosophila* embryos expressing GFP using the COPAS *SELECT* technology platform. Once the sorting region was set around that population of interest, the instrument selected and dispensed only embryos within that population with purity greater than 98%. Based on these data, the results obtained were within the manufacturer's specifications of the instrument.