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COPAS™ Application Note S-01

RAPID *DROSOPHILA* EMBRYO SORTING

Automated High Speed Analysis and Sorting of *Drosophila* Embryos

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Objective

This application describes how *Drosophila melanogaster* embryos can be sorted from a large population, based on phenotypic and morphological characteristics, using the COPAS™ *SELECT* system (Union Biometrica, Inc.).

Introduction

Drosophila melanogaster has been widely studied by the research community in the attempt to understand how genetics are involved in basic animal development (Lawrence, 1992). With the recent advancements in gene chip technology, large-scale preparations of embryos are required for the study of gene expression during embryo development.

Recently, the principle of automated sorting of living *Drosophila* embryos was demonstrated on flow cytometry technology developed at Stanford University (Furlong et al., 2001). The Union Biometrica COPAS *SELECT* system also separates *Drosophila* embryos expressing genes of interest, from initially large populations of embryos (Figure 1). In this application, the COPAS *SELECT* sorts embryos exhibiting genetic expression by measuring the fluorescence intensity of the protein reporter expressed during early embryo development. Specifically, a population of embryos containing GFP-tagged balancer chromosomes was used in our experiments (Casso et al., 2000). Those embryos not expressing GFP were selected and sorted from the original population, with a high level of accuracy and purity.

The COPAS *SELECT* also has the ability to isolate embryos displaying specific morphological characteristics. For example, an embryo can be sorted out of a population based on its size. The Time of Flight (TOF) parameter of the COPAS *SELECT* displays the relative size of each embryo analyzed. This information may be used in conjunction with fluorescence intensity, to isolate very unique embryos.

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

Figure 1. Picture of the COPAS *SELECT* System

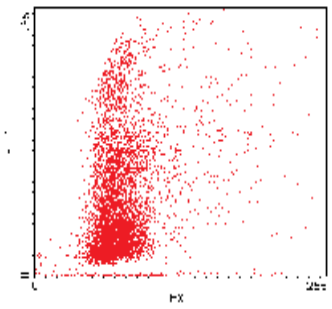

Figure 2. Dot plot showing the entire population of embryos analyzed by the COPAS *SELECT*. FLU1 is green fluorescence intensity and EXT is extinction.

Note:

The sort parameters are set on the user interface screen

Method

Samples were prepared according the *Drosophila* sample preparation protocol (see **SAMPLE PREPARATION PROTOCOL SPS01**). The *Drosophila* embryos are 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 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, Green Fluorescence Intensity (FLU1) and Extinction (EXT), are used to initially analyze the population. EXT is a measure of optical density and FLU is a measure of fluorescence including autofluorescence, fluorescent protein expression, or fluorescent markers. Figure 2 is a dot plot, with FLU1 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 will be isolated from the whole population. The sort region was defined using 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 embryo population of interest, the COPAS *SELECT* will sort only those embryos that are displayed within the user-selected region.

Time of Analysis:
Assuming 250 mL are present in the sample cup and a sample concentration of 500 embryos/mL, 125,000 embryos may be analyzed in a single run. If the flow rate of the instrument has been set to 20 embryos/sec, approximately 31,000 embryos (25% of the population) can be analyzed within 2 hours.

Accuracy:
The accuracy of the instrument is directly related to the Sort Width and Sort Delay parameters of the instrument. Once these parameters have been set to appropriate values, a sort accuracy of 100% can be achieved.

Optimal Yield and Purity Combination:
The population of interest can be identified after an initial run of the embryos on the COPAS *SELECT* by using quantitative parameters such as Extinction (EXT) and Fluorescence intensity (FLU). Figure 4 shows the initial polygonal region around the population of interest on the Gating Dot Plot. As a result of the polygonal region set on the Gating Dot Plot, the Sorting Dot Plot (Figure 5) only displays those embryos of interest. While using these methods, we have observed greater than 95% purity when 15 to 25% of the total population was selected.

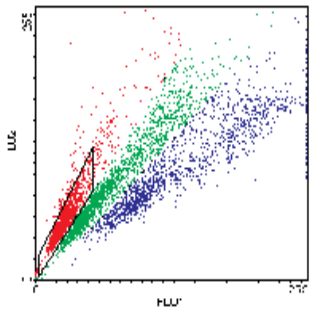

Figure 3. Dot plot showing a polygonal region around the embryos to be extracted from the total population. FLU1 is green fluorescence intensity and FLU2 is red fluorescence intensity.

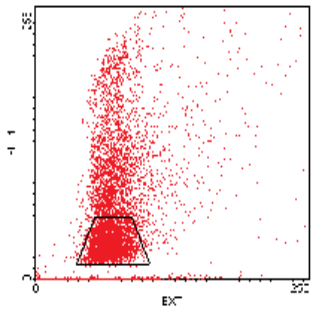

Figure 4. A polygonal region placed around the embryos of interest on the Gating Dot Plot. FLU1 is green fluorescent intensity and EXT is extinction.

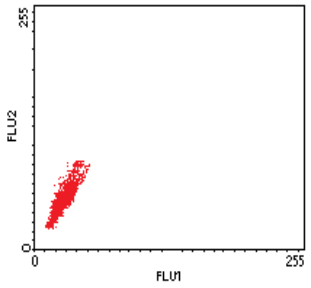

Figure 5. The embryos of interest displayed on the Sorting Dot Plot. Only embryos from within this population will be dispensed into the designated collection vial. FLU1 is green fluorescence intensity and FLU2 is red fluorescence intensity.

Coincidence:
In order to minimize coincidence (extras), it is recommended that the Coincidence Check feature on the user interface be selected and the embryo concentration be carefully measured prior to experimentation.

Discussion

The COPAS system is a technology that can sort embryos of interest, with a high level of accuracy and purity, from an initially large population. Based on our results, we have been able to achieve purity greater than 95% and a sort accuracy greater than 97%. With the recent adoption of gene chip technology by the *Drosophila* research community, the COPAS *SELECT* system is the only commercially available technology that can perform rapid isolation of large quantities of embryos for use on gene chip microarrays. The COPAS *SELECT* can also be integrated with a robotic arm such as a Zymark Twister™ for automated plate handling (96 well plates) onto the system stage.

References

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