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COPAS™ Application Note B-04
C.elegans Dual Color Fluorescence Sorting

Automated Analysis and Sorting of *C.elegans* from a Mixed ZsGreen and ZsYellow Expressing Population

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Objective

Two populations of *C.elegans,* one expressing ZsGreen and the other ZsYellow fluorescent reporters (Clontech, Palo Alto, CA) were mixed together, then analyzed and sorted using the COPAS *BIOSORT*. The COPAS *BIOSORT* is capable of detecting and differentiating between the two populations using a 488 nm multiline argon laser and two Photo Multiplier Tubes for fluorescence detection.

Introduction

The COPAS *BIOSORT* is a high throughput system that analyzes and sorts *C.elegans* based on physical and optical parameters (Figure 1). A population of transgenic animals expressing ZsGreen in the pharynx and a seperate population expressing ZsYellow in the pharynx (see Figure 3) were used in this application.

The COPAS *BIOSORT* is equipped with two lasers. A red diode laser is used to analyze two physical parameters of the organism, Time of Flight (TOF) and Extinction (EXT). TOF is a measure of the relative length of each organism, and EXT provides a measurement of its optical density. The second laser is a multiline argon laser. The 488 nm line of this laser is used to excite both the ZsGreen and ZsYellow fluorescent reporters.

The optical design of the COPAS *BIOSORT* allows the user to simultaneously measure emission parameters. In this experiment, FLU1 (498-522 nm emission) corresponds to fluorescence from ZsGreen and FLU2 (530-560 nm emission) corresponds to fluorescence from ZsYellow. The optical emission filters have been designed to prevent spectrum overlap and allow for clear resolution between ZsGreen and ZsYellow.

Materials

COPAS *BIOSORT* (Union Biometrica, pn 350-5000-000)
M9 buffer with 0.01% Triton X-100
UB168 *C.elegans* organisms expressing ZsGreen protein
UB185 *C.elegans* organisms expressing ZsYellow protein
50 mL conical tubes
Fluorescence dissecting microscope (Leica)

Figure 1. The COPAS *BIOSORT* was used to analyze *C.elegans*.

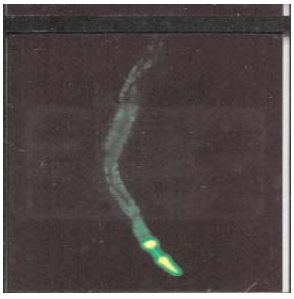

Figure 2. Image of the ZsGreen positive sorted *C.elegans* population.

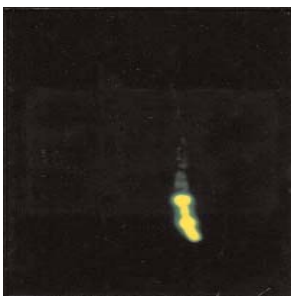

Figure 3. Image of a ZsYellow *C.elegans*.

Method

We started with two populations of worms, one expressing ZsGreen and the other expressing ZsYellow. Samples were prepared individually by washing the worms off an agar plate using M9 buffer. The worms were then collected in 50 mL tubes. **(SEE SAMPLE PREPARATION PROTOCOLS SP01 and SP02.)** The ZsGreen expressing samples were placed into the COPAS *BIOSORT* sample cup. Two parameters, Time of Flight (TOF) and Extinction (EXT), were used to analyze the population.

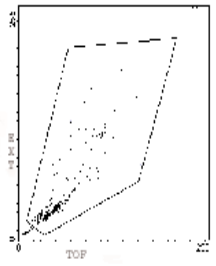

Figure 4. Dot plot displaying TOF (length) and EXT (density) for a mixed *C. elegans* population.

Results

A gating region (R1) was drawn on an EXT versus TOF dot-plot (Figure 4) to eliminate eggs or debris. The sorting dot plot was set so that the FLU1 (ZsGreen signal) and FLU2 (ZsYellow signal) parameters were displayed. Figure 5 displays the FLU2 versus FLU1 dot plot for the ZsGreen population only. This data shows the distribution of fluorescence values for the animals with ZsGreen fluorescence. Compare with Figure 6.

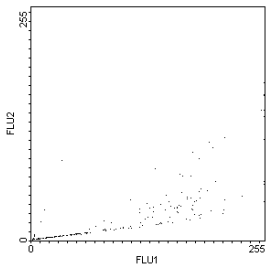

Figure 5. Dot plot of the ZsGreen fluorescent *C.elegans* population.

After analysis was performed on the ZsGreen sample, the animals were removed from the sample cup and replaced with worms expressing ZsYellow. Figure 6 shows a dot plot with the parameters FLU2 (Yellow) versus FLU1 (Green) displayed. The data points represent worms expressing ZsYellow only. Compare with Figure 5.

After the individual populations were analyzed, a ZsGreen sample and ZsYellow sample mix was added to the sample cup and analyzed.

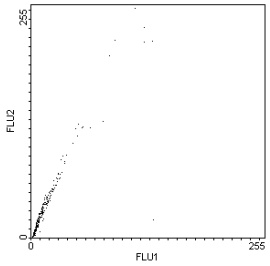

Figure 6. Dot plot of the ZsYellow expressing population.

Figure 7 is a dot plot showing both ZsGreen and ZsYellow worms. The two populations are clearly resolved from one another and can easily be discriminated for sorting. No worms containing ZsYellow were detected in the sorted ZsGreen population and visa versa as confirmed by microscopy.

Discussion

This application demonstrates the capability of the COPAS technology to sort worm populations expressing different fluorescent reporters. Sorting can be done with one or two color fluorescence analysis. In this application, the sorting of a mixed worm population was demonstrated with green and yellow fluorescent markers.

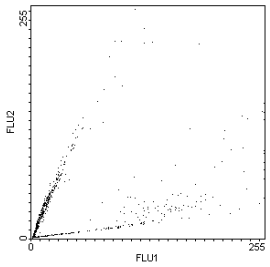

Figure 7. Dot plot of the mixed ZsGreen and ZsYellow expressing populations.

References

Clontech, Palo Alto, CA
WinMDI, Joe Trotter (The Scripps Institute, Flow Cytometry Core Facility)