UNION BIOMETRICA
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COPAS™ Application Note B-07
C.elegans Green Color Fluorescence Sorting

Automated Analysis and Sorting of *C.elegans* from a Mixed Wild-type and ZsGreen Expressing Population

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Objective

Individual populations of *C.elegans* expressing ZsGreen fluorescent reporter (Clontech, Palo Alto, CA) and wild-type were mixed together, analyzed, and sorted using the *COPAS BIOSORT* (Union Biometrica, Somerville, MA). The COPAS *BIOSORT* is capable of detecting and differentiating between the two populations using a multiline argon laser.

Introduction

The COPAS *BIOSORT* is a high throughput system that analyzes and sorts *C.elegans* based on physical and optical parameters (Figure 1). A wild-type (non-fluorescent) population of worms and a separate population expressing ZsGreen in the pharynx of the organism (Figure 2) were used in this application. The COPAS *BIOSORT* is equipped with two lasers. A red diode excitation laser is used to analyze the physical parameters of the organism, refered to as Time of Flight (TOF) and Extinction (EXT). Time of Flight is a measure of the relative length of each organism, and Extinction provides a measurement of its optical density. A multiline argon laser is used to excite various fluorphores. In this example the 499 nm line of the laser is used to excite the ZsGreen fluorescent reporter. The design of the COPAS allows simultaneous excitation and collection of optical measurements of two separate populations.

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

Figure 2. Top: Light image of transgenic worm (UB168). Bottom: Fluorescent image of the same worm showing green fluorescence in the pharynx (left). Green autofluorescence can be seen in the gut of the worm myo2::ZsGreen.

Materials

COPAS *BIOSORT* (Union Biometrica, pn 350-5000-000)
M9 buffer with 0.01% Triton X-100
C.elegans transgenic strain expressing ZsGreen protein (UB168)
C.elegans organisms, wild-type (N2)
15 mL conical tubes

Method

Two populations of worms were used. UB168 expresses ZsGreen in the pharynx under the control of the myo-2 promoter and N2, the non-fluorescent wild type control. Samples were prepared individually by washing the worms off an agar plate using M9 buffer. The worms were then collected in 15 mL tubes, (SEE SAMPLE PREPARATION PROTOCOLS SP01and SP02).

Results

After running the ZsGreen expressing samples through the *BIOSORT,* two parameters, Time Of Flight (TOF) and Extinction (EXT), were used to analyze the population. A gating region (R1) was drawn on an EXT versus TOF dot-plot (Figure 3) to eliminate eggs or debris. The sorting dot plot was set so that the FLU1 (ZsGreen signal) and EXT parameters were displayed. Figure 4 displays the FLU1 versus EXT dot plot for the non-fluorescent, wild-type population only.

After analysis of the non-fluorescent, wild-type sample, the animals were removed from the sample cup and replaced with worms expressing ZsGreen. Figure 5 shows a dot plot with the parameters FLU1 (Green) versus EXT displayed. The data points represent worms expressing ZsGreen only.

Subsequent to the analysis of the individual populations, a ZsGreen sample and wild-type sample mix was added to the sample cup and analyzed. Figure 6 is a dot plot showing both ZsGreen and wild-type worms. The two populations are clearly resolved from one another and can easily be discriminated for sorting. Worms were sorted into a 96-well plate from each population. Five worms each were dispensed into 20 wells for a total of 100 sorted events. Figure 2 shows the microscopic analysis of one of the organisms sorted from the ZsGreen population. Non-fluorescent worms were not detected in the sorted ZsGreen population as confirmed by microscopy.

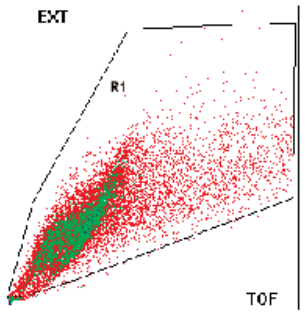

Figure 3. . Dot plot of TOF (length) and EXT (density) of a mixed *C.elegans* population.

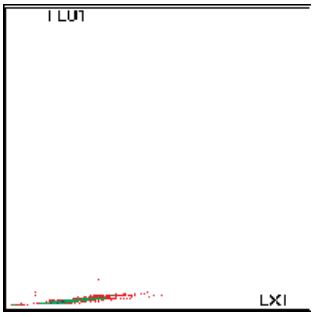

Figure 4. Dot plot of a wildtype, non-fluorescent,population of *C.elegans*.

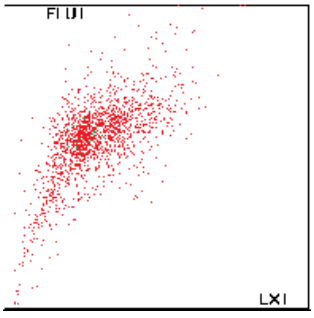

Figure 5. Dot plot of myo2::ZsGreen fluorescent worms, strain UB168.

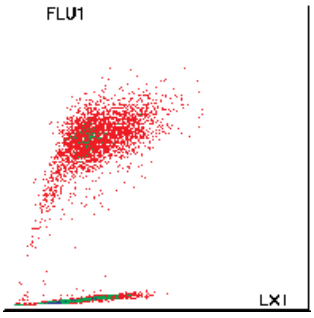

Figure 6. Dot plot of a mixed population containing wildtype and myo2::ZsGreen *C.elegans*. The green fluorescent and non-fluorescent worms are separated in two distinct populations.

Conclusion

This application demonstrates the capability of the COPAS technology to sort worm populations expressing a green fluorescent reporter. In this application, the sorting of a mixed worm population was demonstrated with green fluorescence analysis.

Reference

Clontech, Palo Alto, CA