

High Throughput Screening and Sorting of Viable *C.elegans* From a Mixed Live and Dead Population

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Objective

The purpose of this application is to determine the viability of organisms by using a fluorescence staining method with the COPAS BIOSORT. The live vs. dead application uses Molecular Probes SYTOX® Green nucleic acid stain, designed for rapid identification of living organisms in a mixed live and dead population.

Introduction

The COPAS BIOSORT is a high throughput system that analyzes and sorts *C.elegans* using parameters of size and fluorescence (Figure 1). In this example, the stain was first used on a population that was heat killed to demonstrate the feasibility of discriminating dead organisms by fluorescent emission. A second experiment was done on a natural starved population, containing a small number of dead adults and a relatively high number of dauer larvae. This method enables high throughput screening and discrimination of live versus dead *C.elegans* at rates as high as 100,000 organisms per hour.

Materials

COPAS BIOSORT (Union Biometrica, pn 350-5000-000)
Liquid *C.elegans* culture medium
M9 buffer with 0.01% Triton X-100
Molecular Probes SYTOX Green nucleic acid stain (S-7020)
15 mL conical tubes

Method

Prepare a 10 µM dye stock solution (10X) of Molecular Probes SYTOX Green nucleic acid stain. Wash animals off agar medium using M9 buffer with 0.01% Triton X-100. In the event of heavy contamination of the culture or if liquid medium is used, the sample should be passed through an 180µm nylon mesh to remove larger clumps of eggs and debris (See Sample Preparation Protocols SPB01 and SPB02 for details). Pellet worms by centrifugation (at low speed) or by settling, decant supernatant, and re-suspend in 15ml of M9 buffer.



Figure 1. Picture of the COPAS BIOSORT System

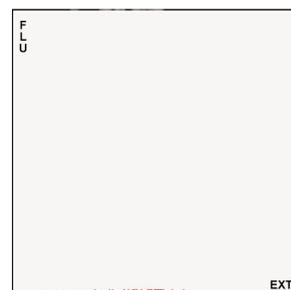


Figure 2. A Dot-Plot image of the COPAS software showing live animals treated with stain displaying auto-fluorescence only.

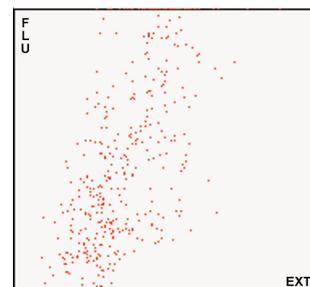


Figure 3. A Dot-Plot image of the COPAS software showing the uptake of the fluorescent dye after the population died.

Place an aliquot of the animal suspension in a second tube to use as an unstained control. Place another aliquot of the animal suspension in a third tube and heat kill these animals. Add the stock dye solution to the heat killed animal suspension and to the live control animal sample so that the final concentration of dye is 1 μ M. Place these suspensions on a laboratory rocker. Let them mix for 15 minutes.

After the 15-minute incubation period, dilute the stained animal suspensions to a final concentration of 500 to 1500 organisms/mL with M9 buffer. Also dilute the unstained control to a final concentration of 500 to 1500 organisms/ml with M9 buffer.

Set up the instrument gains using the unstained control sample. Clean the sample cup thoroughly. Process the two stained test samples (live control animals and heat killed animals).

Results

Figure 2 shows a Dot Plot of Extinction (EXT), which represents the optical density of the organism, versus green fluorescence (FLU) for the unstained control. The organisms display only background auto-fluorescence, illustrated by the low level of fluorescence intensity on the FLU axis. After heat treatment and a staining period of 15 minutes, the killed sample is run. Figure 3 shows a Dot Plot of EXT versus FLU. All organisms absorbed the stain and show a high level of green fluorescence intensity. Microscopic inspection confirmed that they were all dead.

A microscopic image of a stained worm is shown on figure 4. The experiment was repeated using a natural starved population (Figures 5, 6, 7).

Discussion

The unstained and stained worms were run on the COPAS BIOSORT system. The parameters TOF (size) and EXT (optical density) and FLU (fluorescence) were used to analyze the organisms. First, a region was chosen on a TOF histogram that excluded debris. Analysis of the unstained sample within this selected region was used to set the base settings (Figure 2).

The stained population was analyzed after all organisms had died as a result of a heat challenge. The Dot Plot in figure 3 shows clearly that all organisms have absorbed the stain and have levels of fluorescence greater than the live "control" population.

Staining of a normal N2 population that had starved for several days is shown in Figures 5-7. The dauer larvae and living organisms are not stained at all, while the dead worm is stained brightly. This allows easy and fast separation based on size versus fluorescence on the COPAS BIOSORT system.

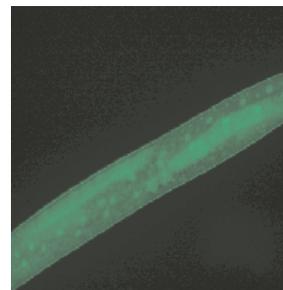


Figure 4. Fluorescent image of heat killed *C.elegans*. The green emission is due to the absorption of the Molecular Probes SYTOX® Green nucleic acid stain in the dead organism.



Figure 5. Fluorescent image of a dead *C.elegans*



Figure 6. Microscopic image of a mixed *C.elegans* population.



Figure 7. Overlay of Figures 5 & 6.