Live/Dead Staining of *C. elegans* with Sytox Green and Sytox Orange

1. Prepare a 10µM solution of Sytox Green in 1X M9
    a. Stock from Molecular Probes is 5mM - Dilute this 1:500 to get the 10X solution (1µl dye / 500µl M9)
    b. You will need 500µl of 10X solution per sample that you are staining
    c. Note - If animals are expressing GFP, use Sytox Orange
2. Make the 1X working dye solution by mixing 500µl of 10X dye to 4.5ml M9 per sample (i.e. for 10 samples, 5ml of 10X dye + 45ml M9)
3. Wash animals of plates with M9
4. Wash once to eliminate bacteria
5. Resuspend in 5ml of 1X dye solution
    a. Reserve some N2s for a "No dye" control
6. Mix samples on the rotator for 15 minutes at room temperature
7. Add the sample directly to the sample cup of the worm sorter
8. Dilute with M9 in sample cup until worms are at the appropriate concentration (~1 animal per µl)
9. Load the 'Sytox Green Adult Assay' setting program
10. Process until the sample cup is empty (or >1000 gated events)
11. At the end of each sample run, store all data in a folder that includes the date
12. Analyze the data in Microsoft Excel and plot results in Origin.

COPAS™ Application Note B-02

C.elegans SORTING: LIVE vs. DEAD

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.

Figure 1. Picture of the COPAS *BIOSORT* System

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.

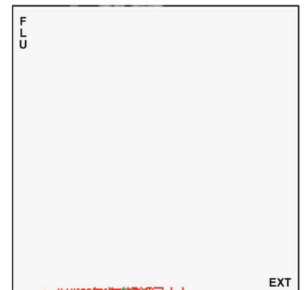

Figure 2. A Dot-Plot image of the COPAS software showing live animals treated with stain displaying auto-fluorescence only.

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

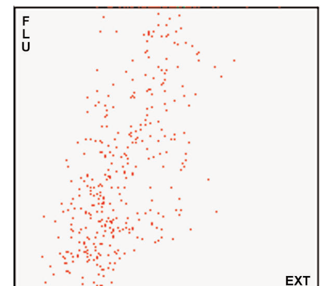

Figure 3. A Dot-Plot image of the COPAS software showing the uptake of the fluorescent dye after the population died.

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.

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 µM. 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).

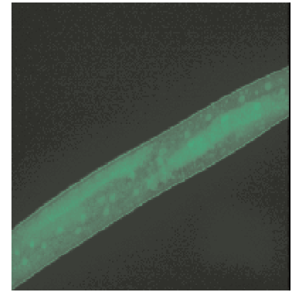

Figure 4. Fluorescent image of heat killed *C.elegans*. The green emission is due to the absorption of the Molecular Probes SYTOX® Green nucleic acid stain in the dead organism.

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.

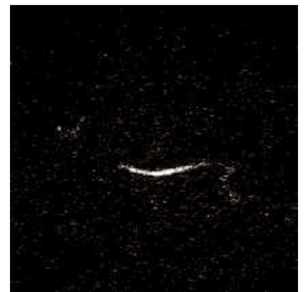

Figure 5. Fluorescent image of a dead *C.elegans*

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).

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

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 7. Overlay of Figures 5 & 6.