DROSOPHILA FLUORESCENT-IMAGINAL DISC ANALYSIS AND SORTING BY COPAS FLOW CYTOMETRY TECHNIQUE

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INTRODUCTION

COPAS SELECT and EXPRESS flow cytometry instruments are able to analyze and sort *Drosophila* embryos on the basis of size and fluorescence signals. Another potential application of interest is to use the COPAS systems to reliably analyze and sort fluorescent tissues from *Drosophila* larvae. For example, the ability to isolate imaginal discs would be beneficial. Currently this is most often done manually and is a tedious task. We have set out to test whether we can create a simpler way of harvesting a relatively pure sample of fluorescent discs. We have tested two protocols for disrupting larvae for the extraction of intact imaginal discs. We have used the samples prepared by these methods and analyzed them using COPAS flow analysis instruments to accurately isolate relatively pure populations of imaginal discs.

SAMPLE PREPARATION

Sample Preparation Using Douncer and Pestle: All preparations were carried out on ice and with solutions at 4°C. Third instar larvae were washed from culture bottles (100 ml culture flasks) with 10% NaCl. The larvae were transferred onto a 600-micron mesh and washed thoroughly with water, then with BSS (buffered salt solution). The larvae were resuspended in 30 ml BSS and split into two 50 ml Falcon tubes. The larvae from the first tube were ground in the dounce tube with the pestle for 2-3 minutes. The grindate (disrupted larval tissues) was filtered over a 350-micron mesh into a large beaker on ice. The larvae from the second tube where then processed in the same manner and the two samples pooled. At each step, a forceful stream of cold BSS was used to wash the sample forcing small larvae parts (discs) through the mesh.

Since the samples were still turbid, half of the sample was further cleaned by the following series of washing and settling steps: 120 ml of the larval tissue sample was diluted to 1 liter and allowed to settle on ice for 20 minutes. The liquid was aspirated to 500 ml and made up again to 1 liter with BSS. The sample was stirred and allowed to settle for 15 minutes. The sample was aspirated to 200 ml, transferred to a 600 ml beaker, and made up to 500 ml. The sample was allowed to settle for 10 minutes. Sample was aspirated to 100 ml, transferred to a 250 ml beaker, and made up to 200 ml. The sample was allowed to settle for 10 minutes. The sample was aspirated to 50 ml and brought to 200 ml with BSS, settled again for 10 minutes. Finally the sample was aspirated to 30 ml.

Both samples were then analyzed on the COPAS Express Instrument.

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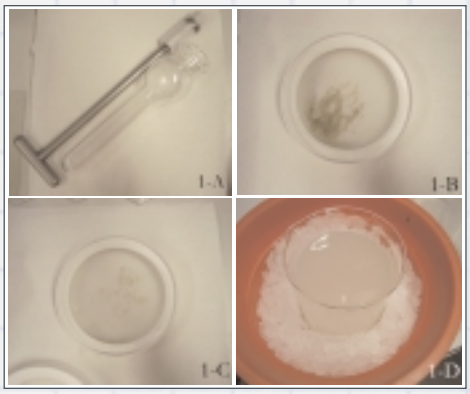

Figure 1: Tools used to carry out preparations using dounce tube and pestle.

Sample Preparation Using "Chopping" and "Pasta" Machine: For this test of sorting imaginal discs prepared by a combination of chopping and a "pasta machine", the strain we used contained GFP-labeled eye discs (SoGFP # 46, 2nd chromosome and SoGFP # 43, 3rd chromosome, provided by Lydia Michaut). Third instar larvae were collected on a 279 µm screen to wash away food and other debris. The larvae were then transferred using cold Ringer's to a Petri dish. The sample was cleaned of further contaminants (pupa or carcasses) and the larvae were sliced, roughly, with either a razor or "chopper machine", to break open the cuticle. The broken larvae were then processed twice through the pasta machine on a very fine setting (using a feeler gauge the separation between rollers was between about 0.152 mm to 0.254 mm) to separate the internal tissue from the cuticle. The Wolfner pasta machine was provided by Steve Russell, University of Cambridge, UK. This sample was collected in a beaker and filtered, in sequence, first through a 279 µm and and then through a 198 µm mesh. At each step, a forceful stream of cold Ringer's was used to wash the sample forcing small larvae parts (discs) through the mesh. The tissue containing flow-through was then collected on 70 µm cell strainers and transferred to a Ringer's soaked 50 ml falcon tube. Using cold Ringer's the volume was brought up to 40 ml and the disc tissues were allowed to settle to the bottom for twenty minutes at 4°C. The top portion was removed and discarded and once or twice more times the volume was brought up and discs were allowed to settle. After the final removal of floating debris the sample was ready for sorting and analysis by the COPAS Select.

Purification of fluorescent imaginal discs on the COPAS Select was achieved using a two step procedure – first, enrichment for the fluorescent discs by setting the sort trigger on green fluorescence and a subsequent second sort using extinction as the sort trigger to separate disc from non-disc material.

Figure 2: Tools used to carry out preparations using chopper (or pasta) machine.

RESULTS:

The results from the samples prepared using the douncer and pestle are as follows: The percentage of positive selected events in the first sample was 29% at a speed of processing 1-3 events per second. Sort results showed that 30% of the events were labeled discs. Number of discs and contaminating objects collected were determined using a stereomicroscope. This low purity is due to the fact that fluorescent parts of gut and salivary gland are present in this strain. In the second sample the concentration of positive events had increased to 32%. A recovery loss of almost 50% of positive events by the settling steps was calculated based on the comparison of the two samples. However, from this sample further purified by a series of settling steps, 80% of the collected events were discs. This indicated that the settling steps eliminated most of the contaminating events sorted in the first sample.

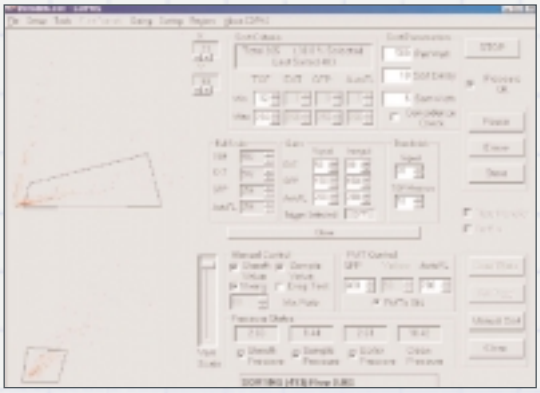

Figure 3: Screen capture of COPAS software screen showing results of first sample run.

Figure 4: The imaginal discs remain intact as shown in the image of a wing-disc after collection. The color is inverted since the GFP on the black background is difficult to see. The darker right side is GFP positive.

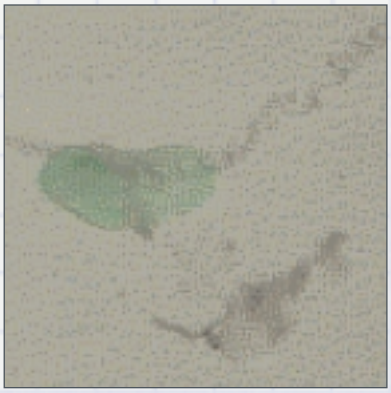

Figure 5: Image of imaginal eye disc from SoGFP #46 expressing GFP.

The results from the samples prepared using the "Chopping" and "Pasta" Machine are as follows: We performed seven tests involving the collection of 100 objects per run. The number of discs and number of contaminating objects per 100 objects collected were determined using a stereo-microscope. Total number of discs dispensed was 554 or about 79% purity (554/700). There were a total of 92 discs counted in the waste container after these sorts. Taken together, 646 imaginal discs passed through the flow cell from the prepared sample containing imaginal discs. This indicates that about 85% of the all imaginal discs were sorted (554/646). From the original two preparations we estimate that about 12% of all discs were recovered. In two experiments that started with 6000 embryos (two eye discs per embryo or a total of 12,000 discs), we collected 1869 fluorescent objects. We estimate that 79% of these sorted objects are eye discs.

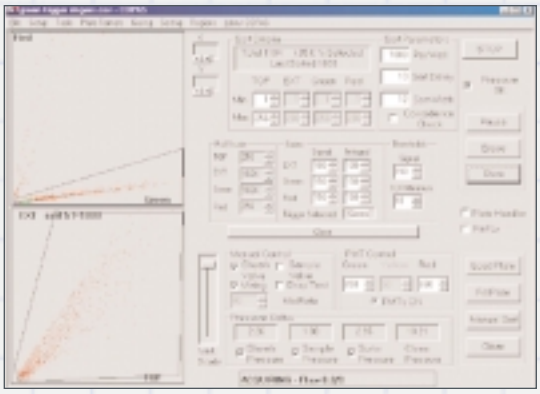

Figure 6: Screen capture of COPAS software screen showing first step enrichment – trigger on green fluorescence.

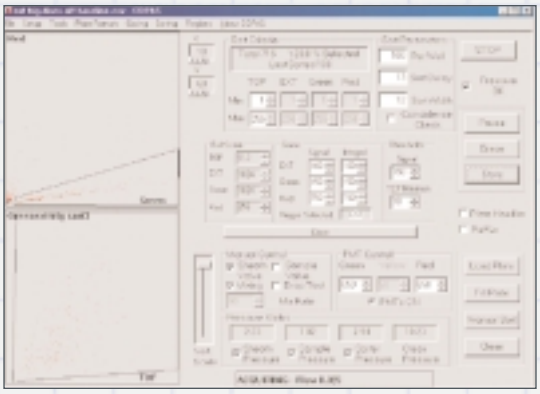

Figure 7: Screen capture showing second step purification – trigger on extincting.

CONCLUSION

Using the COPAS Select or Express for analysis and sorting on the basis of fluorescence allows for greatly enriched fluorescent imaginal discs preparation from a crude tissue sample from *Drosophila* larvae. In one experiment, using a dounce tube and pestle, the yield of the sorting was 11% of the total events. The strain (engal4;UAS::GFPgpi, provided by Daniela Panakova) expressed GFP

in the posterior half of 3 pairs of imaginal discs, part of the gut and salivary gland. The purity of the collected events depended on the sample treatment and reached a level of about 80%. The purity and yield were affected by the presents of fluorescent body parts (gut and salivary gland) other than the GFP-fluorescent imaginal discs. Using a separate protocol, we also tested a strain containing labeled eye discs (SoGFP # 46, 2nd chromosome and SoGFP # 43, 3rd chromosome, provided by Lydia Michaut). The mass isolation of the discs in this experiment was done with a combination of a chopping machine and the Wolfner "pasta-machine" (provided by Steve Russell, University of Cambridge, UK). We used a two-step purification of the fluorescent discs with the COPAS Select. First an enrichment, then a purification. The recovery of imaginal discs from the larval preparation was estimated to be about 12% (essentially the same as the experiment using the dounce tube and pestle). Following a two-step purification method the purified samples were determined to be 79% imaginal disc. These data show that the COPAS instrument can be used for sample preparation of fluorescent imaginal discs from *Drosophila*.

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