

A REPORT OF FLOW CYTOMETRIC ANALYSES ON SEA URCHIN EMBRYOS

SUMMARY

Sea urchins have been a model system for developmental biology and marine biology for over a century. Sea urchins are sea creatures found in oceans all over the world. Sea urchins are echinoderms, belonging to the phylum *Echinodermata*, which also includes starfish, sea cucumbers, and brittle-stars. Early embryonic development has been extensively studied in sea urchins providing insights into the fundamental events at these very early stages of the life cycle. A significant technological improvement that increases the scope of biological studies using sea urchins is the ability for quantitative analysis of gene expression of sea urchin embryos. A number of such technological innovations have emerged, allowing for studies of gene regulation during development. Two methods of measuring gene expression include quantitative PCR¹ and quantitative imaging². Both methods are time consuming and low throughput. A third method now commonly implemented involves using a fluorescent reporter protein as a surrogate reporter of gene expression. Here we report the first study that uses the COPAS™ instrument to perform flow cytometric analyses of fluorescence in whole embryos. The COPAS can accurately distinguish and sort embryos with high fluorescence from those with low fluorescence. Furthermore, the quantitative results correlate well with that from quantitative PCR. We conclude that COPAS instrument is a ready-to-use instrument for research on sea urchin embryos.

INTRODUCTION

The COPAS BIOSORT instrument features a 250µl flow cell and is able to analyze and sort large objects (40-200 microns) on the basis of size, density and fluorescence signals. In this experiment we analyzed and sorted mixed populations of HE-GFP injected sea urchin embryos² and then dispensed into 96-well plates. A panel of the sea urchin embryos was then re-analyzed using the ReFlx automatic sampler option. Non-fluorescent sea urchin embryos were used as a negative control. The COPAS's gentle pneumatic sorting mechanism provides a method for analyzing and handling these sensitive organisms with a high level of recovery and a high level of viability.

MATERIALS AND METHODS

The COPAS BIOSORT equipped with ReFlx sampler optional hardware and software was used for these experiments. Filtered (0.2 micron) seawater was used as sheath reagent instead of the standard supplied sheath reagent. Sheath solution is used to produce a stabilized laminar flow and focus the organisms into the center of the flow stream for optimal analysis. Data was acquired using the COPAS Biosort and analyzed using the WinMDI shareware and Microsoft® Excel®.

Quantitative PCRs were performed as previously reported³ as an independent method of determining the levels of gene expression and for comparison to the data from the COPAS Biosort.

RESULTS AND DISCUSSIONS

Distinguish Embryos with Strong GFP vs. Weak GFP

Microinjection into sea urchin embryos produces mosaic expressions. It was therefore expected that the COPAS instrument would be able to distinguish and qualitatively separate embryos with different levels of GFP using the fluorescence measurement (FLU1). Embryos were injected with HE (hatching enzyme)- GFP construct and were grown to 24-hr old. We analyzed the sample on the COPAS instrument using the fluorescence measurement FLU1 and Time of Flight (TOF), which is a size measurement in order to differentiate the embryos with high GFP intensity from those with relatively weak GFP. As shown in Figure 1A, the GFP levels varied among the injected embryos. We sorted the injected embryos with strong GFPs and weak GFPs, respectively. A non-injected sea urchin embryo population was run on the COPAS BIOSORT as a control (shown in Figure 1B).

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Figure 1. Sorted sea urchin embryos on the basis of GFP level. The measurements of GFP signal (shown as FLU1 on the dot plots) and size (shown as TOF) were used.

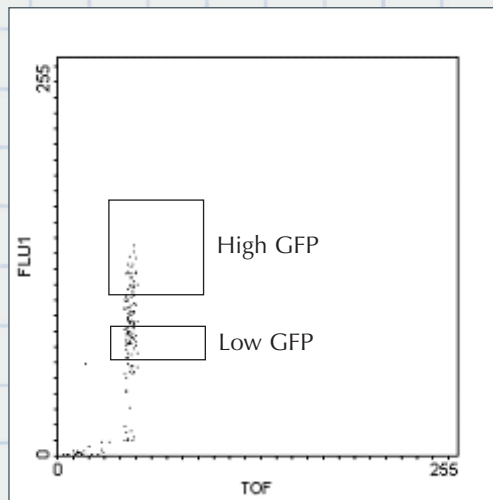


Figure 1 A: HE-GFP injected embryos. The two boxes represent the gating windows used for sorting.

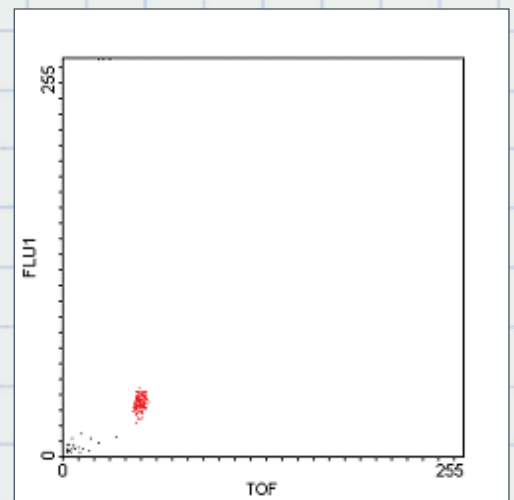


Figure 1 B: Uninjected embryos (controls).

The GFP expression levels were then confirmed via microscopy (Figures 1 C and D).

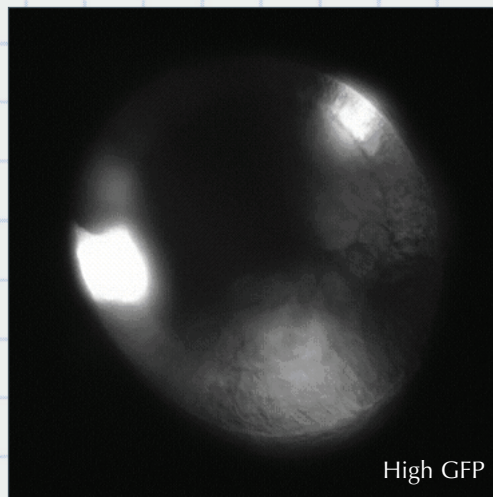


Figure 1 C: a microscopy image of a sorted high GFP embryo.

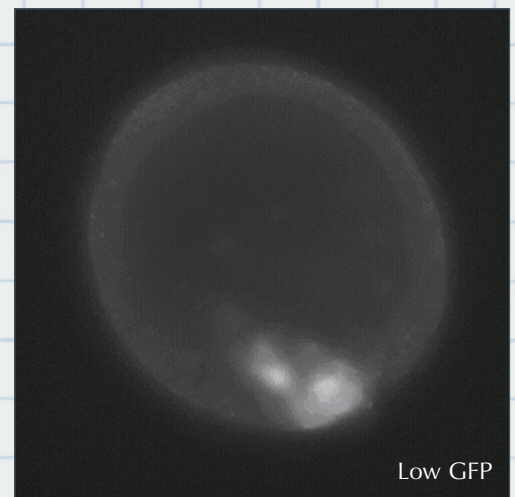


Figure 1 D: a microscopy image of a sorted low GFP embryo.

Measure the expression of endogenous Promoter-driven GFPs

The COPAS BIOSORT was equipped with a ReFlx sampler module to allow quick and efficient automatic re-analysis and sorting for samples on a 96-well plate. We used the module to analyze a panel of sea urchin embryos injected with endogenous promoter-driven GFPs (Table 1).

We left two wells blank between samples to minimize carryover between samples. The sum value of the GFP signals in the three wells (samples + two blanks) corresponding to the sample is calculated as a measurement of the total GFP fluorescence from the sea urchin embryos.

We set the sorting criteria to harvest embryos with weak GFP signals. The population sum of GFP intensity of sorted embryos is collected and displayed in Figure 2. During analysis, these same embryos were collected into another 96-well plate and were analyzed by Q-PCR.

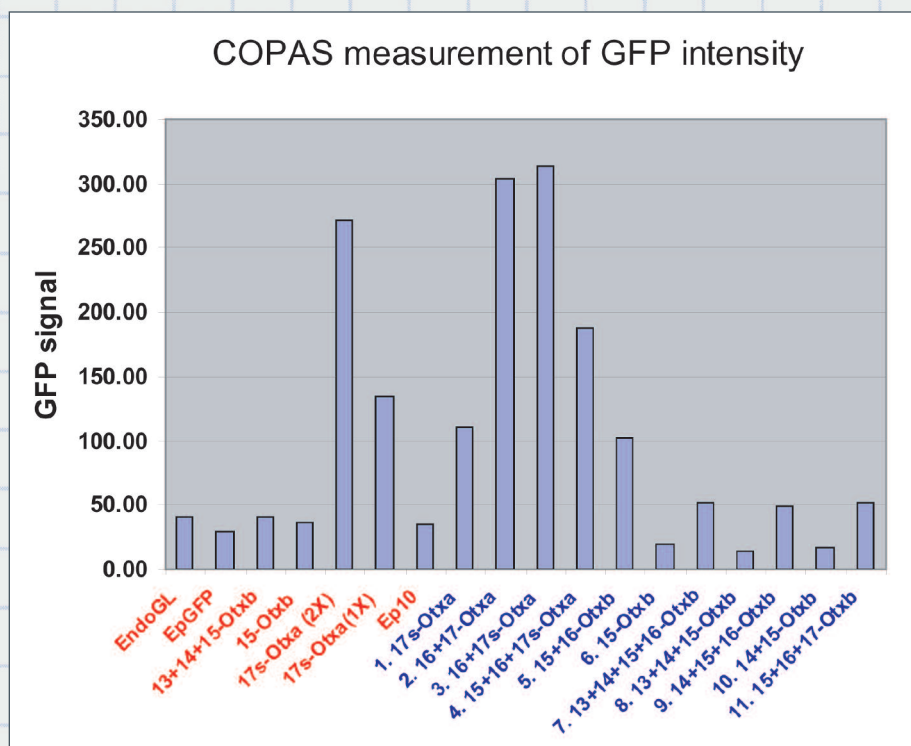


Figure 2. The quantitative GFP measurements in various sea urchin embryo samples. Note that the values of GFP presented are the values after subtracting the baseline fluorescence, such as from autofluorescence.

Correlation between quantitative PCR and COPAS results.

The same batch of injected embryos that was used for COPAS analyses were subject to quantitative PCR that measures the mRNA level of the GFP mRNA in the embryos. Figure 3 shows the correlation between the GFP RNA per embryo measured by quantitative PCR and GFP intensity per embryo measured by COPAS. The measurements obtained using COPAS highly correlate with those using quantitative PCR ($R^2=0.91$, $N>100$).

Figure 3. The correlation between COPAS measurement and Q-PCR measurement of GFP levels. Using 10 different samples, we compare the results using the two methodologies. More than 100 sea urchin embryos from each sample were subjected to either quantitative PCR or COPAS analysis, respectively.

