A Report of Flow Cytometric Analyses in 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 family of research instrumentation comprises a collection of large particle cytometers utilizing at least 1 excitation laser and up to 8 channels of fluorescence collection. Unique to COPAS instrumentation is the Profiling feature which graphically plots the fluorescence intensity changes along the length of the object as it passes through the laser(s). Large objects up to 1.5mm in diameter can be analyzed for physical and fluorescence characteristics and gently dispensed into a multi-well plate or other collection container for further investigation or reuse. The COPAS Vision also has equipped a camera to take an image of the object inside the flow channel. This image accompanies the cytometry data and can be analyzed using Union Biometrica's FlowPilot software or other image analysis tools.

In this experiment, the COPAS BIOSORT instrument features a 250µm 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 was 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

Distinguish Embryos with Strong GFP vs. Weak GFP

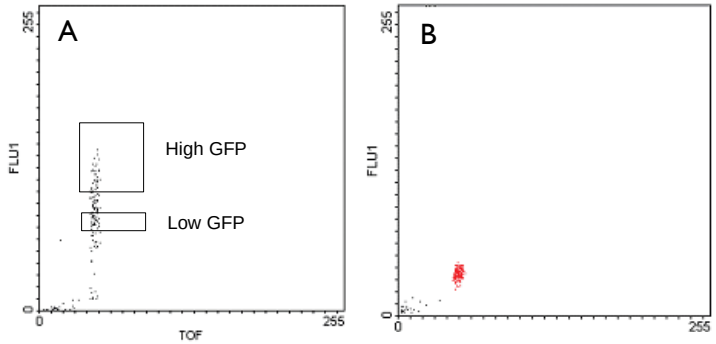

Figure 1A: *HE-GFP injected embryos. Boxes represent the gating regions used for sorting*

Figure 1B: *Uninjected embryos (controls)*

Microinjection into sea urchin embryos produces mosaic expressions. It was therefore expected that the COPAS instrument would be able to distinguish and quantitatively 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).

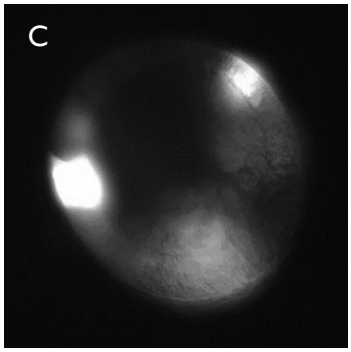

Figure 1C: *a microscopy image of a sorted high GFP embryo.*

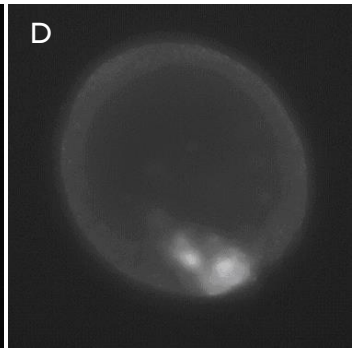

Figure 1 D: *a microscopy image of a sorted low GFP embryo*

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.

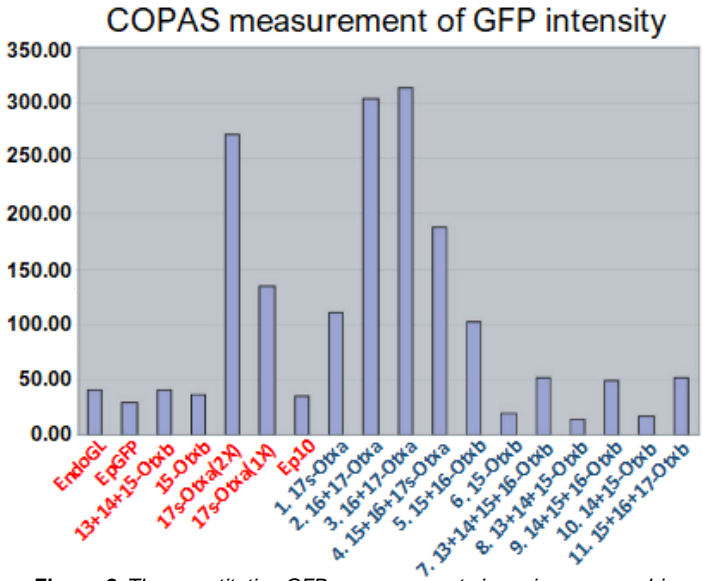

Figure 2. *The quantitative GFP measurements in various sea urchin embryo samples. Note that the values of GFP presented are the values after subtracting the baseline fluorescence, such as from*

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 (R2=0.91, N>100).

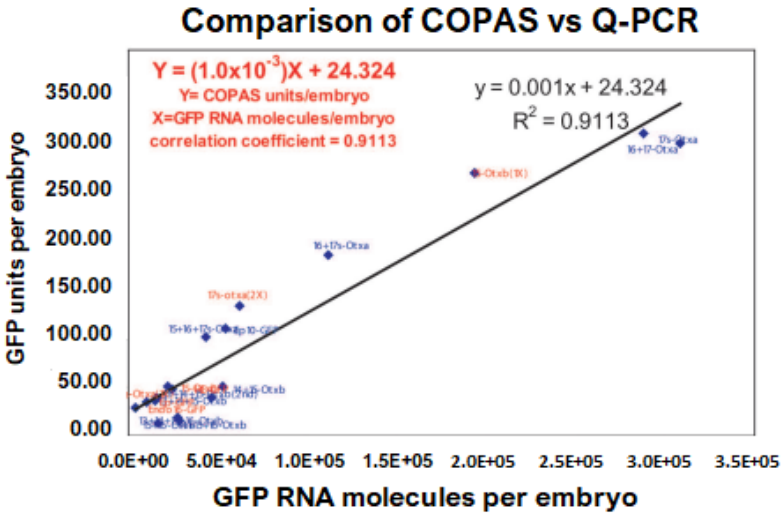

Figure 3*. The correlation between COPAS measurement and Q-PCR measurement of GFP levels. Using 10 different samples, we compare the results using the two methodologies. More than 100 sea urchin embryos from each sample were subjected to either quantitative PCR or COPAS analysis, respectively.*

Conclusions

The COPAS instrument brings several advantages to sea urchin research. One of the greatest advantages of using COPAS to collect quantitative measurements is the multiplexing capability. Three fluorescence colors can be analyzed simultaneously. In addition, size and optical density information can also be collected. The older sea urchin embryos appear to be optically denser than younger ones (data not shown). One may be able to survey a mixed population of sea urchin embryos on fluorescence and

extract the data for each age group.

Another advantage of using COPAS is the high throughput capability. Using ReFlx sampler module, we were able to collect data at a rate of 1 minute per sample (>100 embryos per sample), compared to ~1-4 hrs/sample using Q-PCR or quantitative imaging. Sea urchin has become a model organism for system biology. The needs for high-content and high throughput studies on gene regulation and biochemical pathway have become increasingly large. The results presented in this report indicate the potential and feasibility of using COPAS sorter to conduct multiplexing and high throughput analysis on sea urchin embryos.

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