Isolation and gene expression analysis of single cells using two complementary technologies- Sony SH800 personal cell sorter and Roche real-time PCR system

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Introduction

Gene expression analysis by qPCR with pools of thousands of cells has been used for investigating cell differentiation, disease mechanisms, and a number of other biological processes, however, differences in the gene expression profile of individual cells has so far been neglected. Here we demonstrate a streamlined workflow to measure gene expression levels in singe cells using an easy to use cell sorter and a single-cell qRT-PCR system with reagents for cell lysis and reverse transcription.

Fully Automated Personal Cell Sorter

Cell Sorter SH800 is the first 'flow cytometer' cell analysis instrument developed by Sony for the optical analysis of cells. This product successfully automates optical alignment and sorting set-up by utilizing Sony's technologies cultivated in laser optics, such as Blu-ray Disc, and optical discs. In addition, by incorporating a newly developed plastic cell sorting chip, it offers dramatically greater efficiency with measurement tasks.

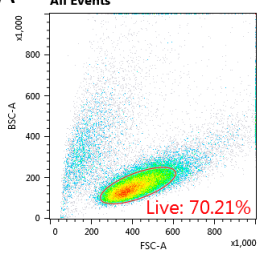
High Performance Compact Real-Time PCR

The Roche Diagnostics LightCycler® 96 system achieves the ideal combination of accuracy, temperature homogeneity, and reproducibility with its innovative optics and thermal block. In addition to standard analysis methods like endpoint genotyping, absolute and relative quantification, dedicated software modules for qualitative detection and advanced high resolution melting analysis are also included.

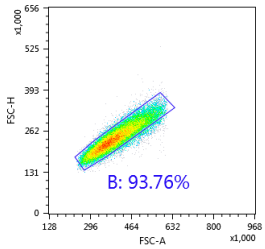
Sorting Experiment

To demonstrate the workflow of cell sorting, target cells were selected by gate [Live] on FSC-A vs BSC-A plot (A). Sort gate B was created on the FSC-A vs. FSC-H plot (B) to mark the single cells. The Sort mode was set as Single Cell Sort mode and either 1, 10, 50 cells were sorted into each well (C).

A



B



C

Well Number	Sort Gate	Sort Mode	Elapsed Time	Sorted Count
A1	B	Single Cell	00:00:01	50
B1	B	Single Cell	00:00:01	50
C1	B	Single Cell	00:00:01	50
D1	B	Single Cell	00:00:01	1
E1	B	Single Cell	00:00:01	1
F1	B	Single Cell	00:00:01	10
G1	B	Single Cell	00:00:01	10
H1	B	Single Cell	00:00:01	10

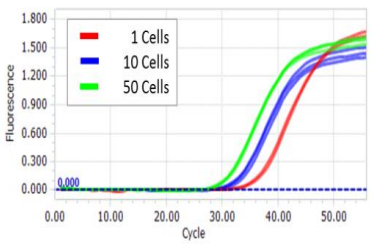
qPCR Experiment

RNA was extracted from Jurkat cells using RealTime ready Cell Lysis Kit (Roche). RNA was reversely transcribed with Transcriptor Universal cDNA Master (Roche). Measurement of GAPDH expression level were performed on a LightCycler® 96 using RealTime ready Catalog Assays and FastStart Essential DNA Probes Master (Roche) according to the instructions.

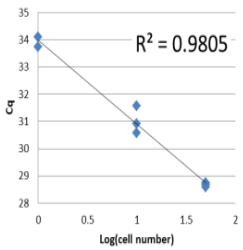
Results

(D) Amplification plots of the qPCR assays. (E) The standard curve was obtained from the amplification plot. Strong correlations can be seen between sorted cell numbers and Cq values for each of the amplification plots (R^2 = 0.9805) . Reproducibility of triplicate samples was excellent, as shown by the overlapping amplification plots.

D



E



Streamlined workflow of gene expression analysis in a single cell

Conclusion

In this work, we showed that the combination of the Sony Cell Sorter SH800 with the Roche Diagnostics LightCycler® 96 system enables researchers to measure gene expression at the single-cell level within just two hours. This is expected to be a powerful tool, not only for measuring gene expression levels in single cells, but also for monitoring individual cell responses to stimuli, drug screening, and identifying novel biomarkers.

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