Fluidigm®

Fluidigm Dynamic Arrays provide a platform for single-cell gene expression analysis

Historically, single-cell gene expression experiments have been difficult and expensive to perform. Now, however, single-cell gene expression results from single-cell samples can be inexpensive and easily reproducible using Fluidigm's Dynamic Array™ integrated fluidic circuits and BioMark™ system for genetic analysis. This method is ideally suited for high-throughput cell-line studies to determine individual cell behavior in a homozygous population. To demonstrate this capability, we chose single human cells from eight-cell-stage embryos, collected and analyzed for expression of 46 developmental genes.

One of the hottest trends today in life sciences is the analyses of individual cells. What researchers are finding is that each cell needs to be treated as an individual because cells are not necessarily in the same state at every point in time.

Fluidigm Dynamic Arrays enable you to test up to 96 individual cells against 96 genes in a single experiment. Using existing reagents and assays, you can simply sort cells into a standard 384-well plate and then transfer them to the dynamic array. The dynamic array assembles the cDNA material from individual cells and reagents to create individual quantitative PCR (qPCR) reactions.

The ability of Fluidigm's microfluidic devices—called integrated fluidic circuits (IFCs)—to isolate and control individual cells can provide great insight into the development of biological events. Analyzing the stochastic differences between individual cells often requires studying many individual cells and genes. Fluidigm's IFC volume requirements are so low that researchers have been able to study as many as 1,000 genes in an individual cell. The technology facilitates thousands of single-cell experiments, allowing the biological differences to come through.

In this experiment, we manually sorted 15 human stem cells, from the same stage of development, and reverse-transcribed into RT/Specific Target Amplification (STA) master mix solution. The samples were then reverse-transcribed and specific targets amplified. We loaded the resulting cDNA product into the Fluidigm 48.48 Dynamic Array—a microfluidic chip that integrates a diverse set of critical liquid-handling functions on a nanoliter scale to meter, combine, diffuse, fold, mix, separate or pump fluids with precise control and reproducibility (the company also makes a 96.96 Dynamic Array)—and assayed it against 46 genes using TaqMan® Assays (Applied Biosystems). The RNA from the 15 samples was loaded, in triplicate, into 45 sample inlets, and 48 primer/probes (each a combination of forward and reverse PCR primers specific

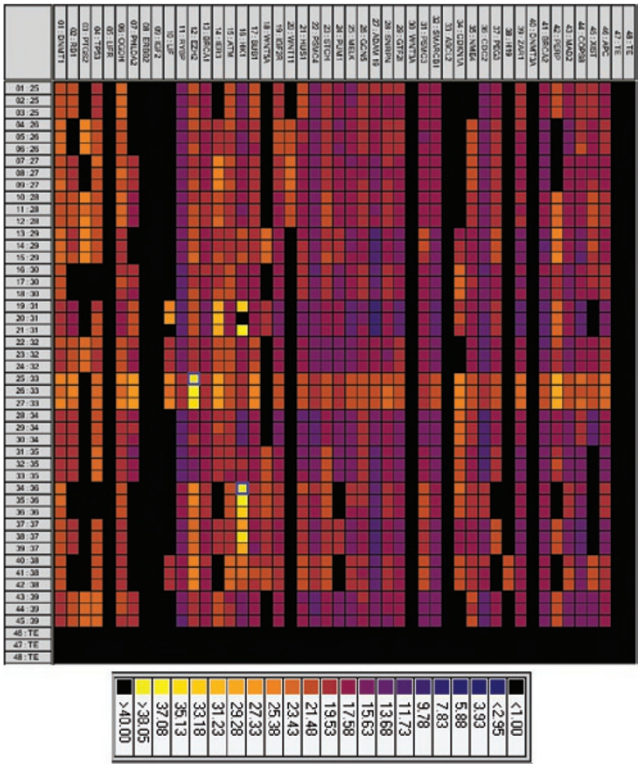

Figure 1 | Results from Fluidigm's Dynamic Array can be presented as a heat map with individual assays on the *x* axis and individual cell samples on the *y* axis. The intersection of each assay and sample is an individual real-time quantitative PCR result. The colors on the heat map correspond to the Ct values, as indicated in the legend. The heat map legend shows color-coded Ct values for every reaction position on the dynamic array.

Martin Pieprzyk & Howard High

Fluidigm Corporation, South San Francisco, California, USA. Correspondence should be addressed to M.P. (martin.pieprzyk@fluidigm.com).

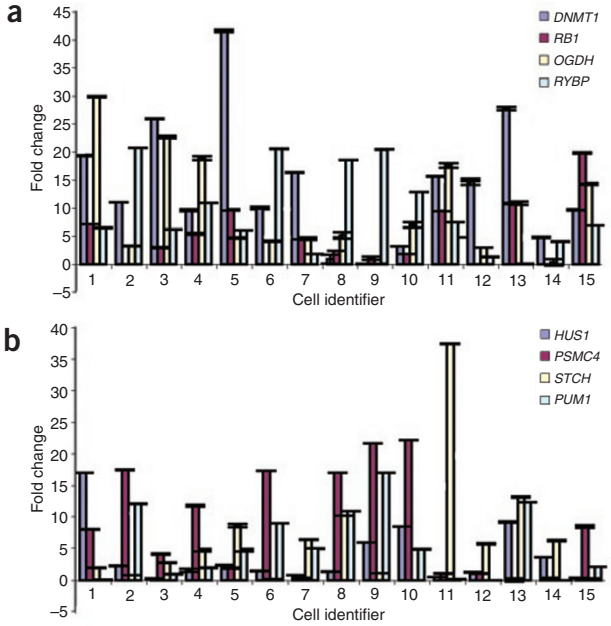

Figure 2 | Fold changes in expression of individual genes. (**a**,**b**) ΔCt values were calculated for different sets of genes using the *APC* gene as a reference, and those values were then converted to fold change values for each of the 15 cell replicates. Error bars, standard deviations based on the Ct standard deviation of the three technical replicates converted to fold change.

to the gene of interest and a fluorescent resonance energy transfer (FRET) hydrolysis probe located between the two primers) were loaded into a separate set of 48 inlets.

The workflow is a simple, five-step procedure: prime, transfer, load, run and analyze. You begin by priming the dynamic array to close the interface valves, which prevents premature mixing of samples and assays. This is as simple as choosing a menu item on the touch-screen of the loader. After the chip has been 'primed', then you pipette your samples—premixed with master mix—into separate sample inlets of the dynamic array. The primer/probe sets are pipetted into separate inlets on the chip. You place the dynamic array on the IFC Controller and the software directs the pressure-loading of the assay components into the reaction chambers. The assay components are automatically combined on-chip. You then place the dynamic array on the Fluidigm Real-Time PCR System for thermal cycling and fluorescence detection. Once the thermal cycling is complete, the real-time qPCR Analysis software provides amplification curves, color-coded heat maps (**Fig. 1**) and cycle threshold (Ct) data for each run.

Our results (**Fig. 2**) showed that there was meaningful variation (defined as more than tenfold) in gene expression among the 15 individual cells from the same stage of early embryonic development.

Conclusion

Using Fluidigm's Dynamic Array for single-cell gene expression analyses is inexpensive and easy to use, giving researchers the ability to test a large number of cells and genes at the same time with data quality rivaling benchmark real-time qPCR results.

This article was submitted to *Nature Methods* by a commercial organization and has not been peer reviewed. *Nature Methods* takes no responsibility for the accuracy or otherwise of the information provided.