Comparison of Two Protocols for Single-Cell Gene Expression on Dynamic Arrays

The following is a supplement to the application note *Fluidigm Dynamic Array for Single-Cell Gene Expression Analysis* (MRKT00075b) and the *BioMark Advanced Development Protocol 5*. The supplement describes experiments performed with dynamic arrays to compare two protocols for single cell analysis — one recently developed by Fluidigm, the other a traditional two-step Reverse Transcription (RT) preamplification (PreAmp) protocol. Both experiments were performed with RNA standards, instead of real cells, to control for the reproducibility of sample concentration.

Experiment One

Muscle total RNA was prepared using a 10-fold serial dilution to produce samples of 10 ng, 1 ng, 0.1 ng, 0.01 ng, and 0.00 1 ng concentrations (the lower concentrations resembling the total RNA contents of a single-cell). Each preparation was setup as triplicate reactions for each protocol, against 48 assays. Replicate assays were performed to enhance data reliability. The data was collected on the BioMark system.

Results

Standard curves show that both the Fluidigm single-cell analysis protocol and the standard two-step RT-PreAmp protocol have excellent linearity and reproducibility. The C_t and Sigma C_t values demonstrate good sensitivity. Only the Fluidigm protocol registers a C_t value at the lowest concentration. Dynamic array heat maps show the same gene expression pattern for both protocols.

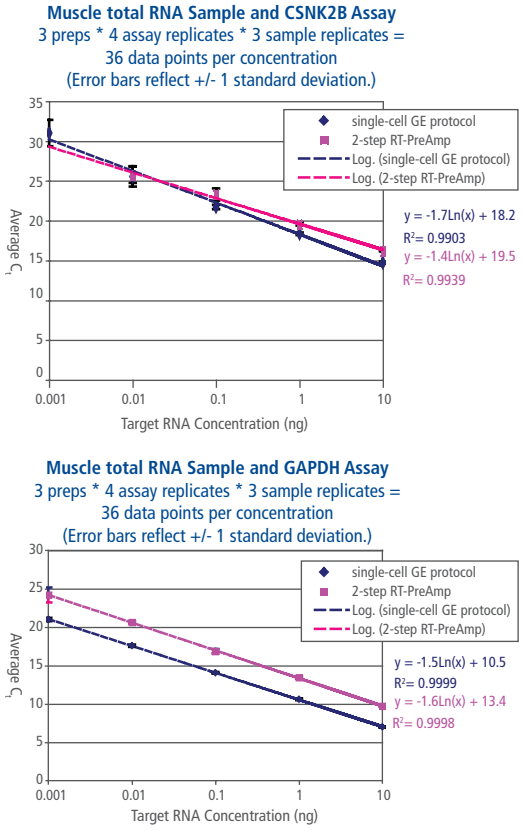


Figure 1. Standard Curves for CSNK2B and GAPDH assays.

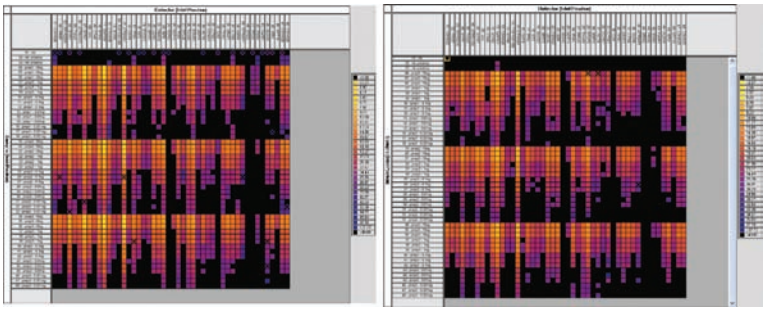


Figure 2. Heat maps for Fluidigm single-cell gene expression and two-step with muscle only samples.

CSNK2B							
	target concentration	10	1	0.1	0.01	0.001	ng
Average C_t	single-cell GE protocol	14.7	18.3	21.7	25.5	31.0	
	2-step RT-PreAmp	16.2	19.5	23.2	25.7	n/a	
Sigma C_t	single-cell GE protocol	0.14	0.18	0.29	1.30	1.66	
	2-step RT-PreAmp	0.09	0.20	0.75	1.02	n/a	
GAPDH							
	target concentration	10	1	0.1	0.01	0.001	ng
Average C_t	single-cell GE protocol	7.0	10.6	14.0	17.6	21.0	
	2-step RT-PreAmp	9.7	13.4	16.9	20.6	24.2	
Sigma C_t	single-cell GE protocol	.10	0.12	0.11	0.15	0.22	
	2-step RT-PreAmp	0.09	0.16	0.29	0.14	0.98	

Table 1. Average C_t and Sigma C_t values.

Experiment Two

The second experiment compares the performance of the Fluidigm single-cell gene expression protocol and the two-step RT-PreAmp protocol on multiple RNA samples from various sample types.

Results

Standard curves show the excellent linearity and technical reproducibility of both the Fluidigm single-cell analysis protocol and the standard two-step RT-PreAmp protocol, across a variety of samples and assays. The C_t and Sigma C_t values demonstrate excellent sensitivity and low Sigma C_t values for the single-cell gene expression protocol. The heat maps show the same gene expression patterns for both protocols.

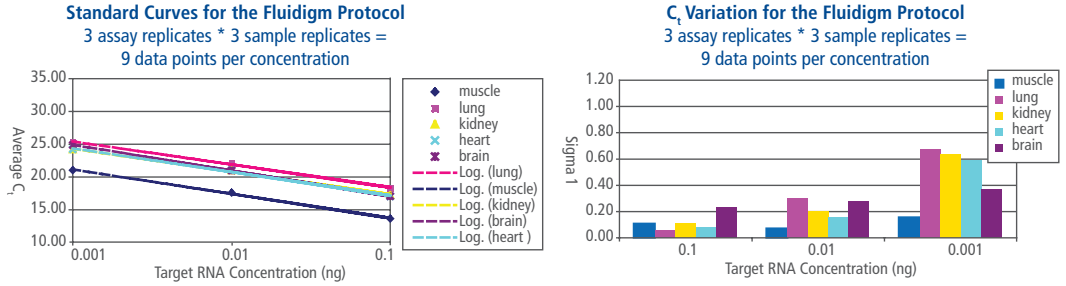


Figure 3. Standard Curves for single-cell gene expression and two-step RT-PreAmp, with muscle, lung, kidney, heart and brain samples.

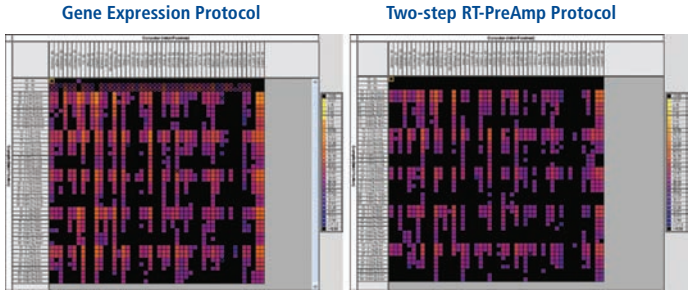


Figure 4. Heat maps for single-cell gene expression and two-step with muscle, lung, kidney, heart and brain samples.

Average C_t	0.100	0.010	0.001	ng
muscle	13.6	17.5	21.0	
lung	18.2	22.1	25.2	
kidney	17.2	20.9	24.1	
heart	17.0	21.0	24.2	
brain	17.0	20.9	24.8	

Sigma C_t	0.100	0.010	0.001	ng
muscle	0.10	0.06	0.15	
lung	0.05	0.30	0.67	
kidney	0.11	0.20	0.64	
heart	0.08	0.15	0.59	
brain	0.23	0.28	0.37	

Table 2. Average C_t and Sigma C_t values for the GAPDH gene.

Conclusion

As the experiments show, Fluidigm single-cell gene expression protocol provides superior sensitivity compared to the two-step RT-PreAmp protocol. The experiments suggest that dynamic arrays, combined with the Fluidigm protocol, provide researchers inherent advantages for optimizing work flow, without sacrificing data quality.

Materials. Total RNA samples were obtained from Origene and the TaqMan® Gene Expression MGB Assays from Applied Biosystems. The single-cell gene expression protocol was preformed with the CellsDirect™ One-Step qRTPCR kit from Invitrogen. The two-step RT-PreAmp protocol used the SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR kit from Invitrogen and the TaqMan® PreAmp MasterMix Kit from Applied Biosystems. Reagent preparation followed Fluidigm protocols and/or the supplier protocol when Fluidigm protocols were absent. Eighteen cycles were used for preamplification.

WORK FLOW

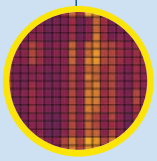
1 Prime.
Prime the dynamic array to close the interface valves, preventing premature mixing of samples and assays.

2 Transfer.
Pipette samples, premixed with master mix, into separate sample inlets and the primer-probe sets into separate primer-probe inlets on the frame of the chip.

3 Load.
Place the dynamic array on the IFC controller, and use the software interface to pressure load the assay components into reaction chambers. Assay components are automatically combined on-chip.

4 Run.
Place the dynamic array on the BioMark Real-Time PCR System for thermal cycling and fluorescence detection.

5 Analyze.
Use Real-Time PCR Analysis software to view and to interact with amplification curves, color-coded heat maps, and C_t data for the run.



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