Using PrimeScript™ Reverse Transcriptase for Single Cell Gene Expression Analysis

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Analysis of gene expression from single cells by quantitative RT-PCR has a number of challenges. For instance, the small number of transcripts in single-cell samples requires high reverse transcription efficiency for detection and quantification by qPCR. In this experiment, Takara® PrimeScript RT Master Mix (Perfect Real Time) (Cat. # RR036A) was compared to another manufacturer's RT Kit for analysis of the expression of 28s rRNA and PTGES in single CP-A (Barrett's esophagus cell line) cells.

Methods

Single cells were collected using a pick-and-place single-cell manipulation robot as described previously[1]. Shear force on the cells during aspiration and dispensing was minimized to avoid damaging the cells. RNA extraction was performed on four individual cells using the ZR RNA Micro-Prep Kit (Zymo Research) following the manufacturer's protocol. RNA was eluted from the column in a volume of 6 µl and was used immediately for reverse transcription.

cDNA was synthesized using either the PrimeScript RT Master Mix (Perfect Real Time) kit or a cDNA synthesis kit from Company I according to the instructions for each kit. Reverse transcription was performed with an Eppendorf PCR thermal cycler using the following reaction conditions:

PrimeScript	37°C 15 min. 85°C 5 sec.	(total reaction time ~15 min.)
Company I	25°C 10 min. 42°C 60 min. 85°C 5 min.	(total reaction time ~75 min.)

The expression of 28s rRNA and PTGES were analyzed by qPCR using SYBR® Green for detection using a StepOnePlus Real-Time PCR system with the following cycling conditions:

95°C	5 min.	
95°C	10 sec.	40 cycles
60°C	1 min.	

Reactions were run in triplicate. Both amplification products were ~300 bp.

Results

cDNA synthesized with the PrimeScript kit had greater specificity and generated lower Ct values as compared to Company I's RT kit (Figure 1).

Conclusions

PrimeScript RT Master Mix (Perfect Real Time) was able to quickly generate cDNA (total reaction time 15 minutes) from single cells that could be used to quantify gene expression by qPCR. Furthermore, the PrimeScript kit outperformed Company I's RT kit and provided higher sensitivity and specificity for the targets tested.

Reference

1. Anis, Y.H., Holl, M.R., Meldrum, D.R. (2010) Automated selection and placement of single cells using vision-based feedback control. *IEEE Trans. Autom. Sci. Eng.* **7**:598–606.

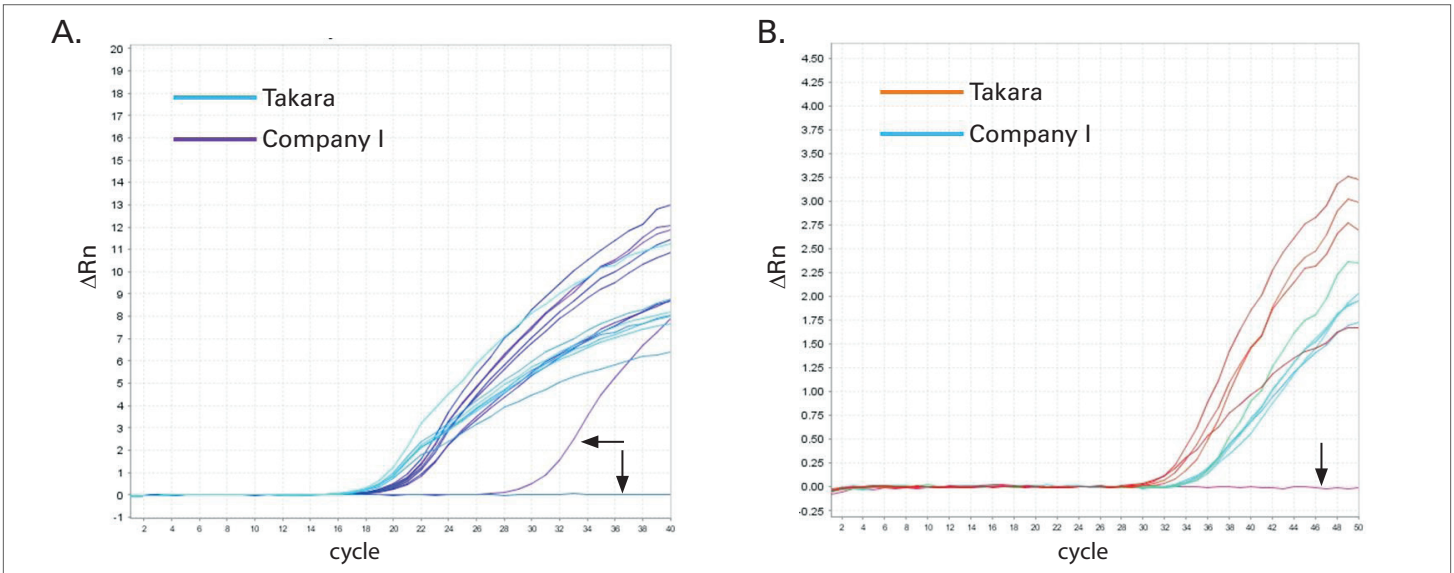

Figure 1. Amplification plots for 28S rRNA (A) and PTGES (B) from individual CP-A cells. The black arrows indicate no template controls.