Using PrimeScript™ Reverse Transcriptase for Quantification of shRNA Knockdown of a Low Abundance Target

Data kindly provided by: H.L., Graduate Student (Baylor College of Medicine)

PrimeScript Reverse Transcriptase is a high efficiency enzyme that is ideal for preparing cDNA for the analysis of low abundance transcripts. In this experiment, Cdc14A mRNA, which is expressed at low levels, was quantified in mouse embryonic fibroblast (MEF) cells that had been transduced with either a control shRNA or a Cdc14A-specific shRNA. The PrimeScript RT Reagent Kit (Perfect Real Time) (Cat. #RR037A) and a reverse transcriptase from another supplier (Company I) were used to generate cDNA templates for measuring Cdc14A expression by real-time PCR using SYBR® Green detection.

Methods

Total RNA was prepared from MEF cells tranduced with either a control shRNA or a Cdc14-targeting shRNA using TRIzol Reagent extraction according to the manufacturer's recommended protocol. One microgram of total RNA was used for reverse transcription.

cDNA was synthesized using either the PrimeScript RT Reagent Kit (Perfect Real Time) or a reverse transcriptase from Company I according to the recommended instructions for each kit. Both oligo-dT and random 6-mers were used to prime the reactions. Reverse transcription was performed with a thermal cycler using the following reaction conditions:

PrimeScript RTase	37°C	15 min.	**Company I RTase**	25°C	10 min.
	85°C	5 sec.		50°C	50 min.
	4°C	hold		85°C	5 min.
	(total ~15 min.)			4°C	hold
				(total ~65 min.)	

The reactions were treated with 1 µl RNaseH at 37°C for 20 min. The expression of 18S rRNA (housekeeping gene) and Cdc14A were analyzed using SYBR® Green for detection. One microliter of the RT products (1:3 dilution for Cdc14A and 1:3,000 for 18S rRNA) were used for qPCR. Two different primer sets were used to analyze Cdc14A (m14A1 and m14A5). Reactions were run in triplicate. Reactions were run on an ABI

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PRISM 7000 Sequence Detection system using the following cycling conditions:

50°C	2 min.	
95°C	10 min.	
95°C	15 sec.	45 cycles (Detection)
60°C	1 min.	
95°C	15 sec.	
60°C	20 sec.	1 cycle (Dissociation)
95°C	15 sec.	

Amplification and dissociation curves were generated, and Ct values were used to assess Cdc14A expression and knockdown in MEF cells.

Results

Good amplification of the 18S rRNA target was obtained from cDNA templates generated by both RTases. However, the Ct values for the PrimeScript RTase reactions were lower than those for Company I RTase (Table 1). For Cdc14, poor amplification was obtained with cDNA generated by Company I RTase for both the control and the knockdown shRNA (Figure 1) resulting in Ct values >40 (Table 1). In contrast, Cdc14 was efficiently amplified from the cDNA templates synthesized with PrimeScript RTase in both the control and Cdc14A shRNA samples (Figure 1 and Table 1).

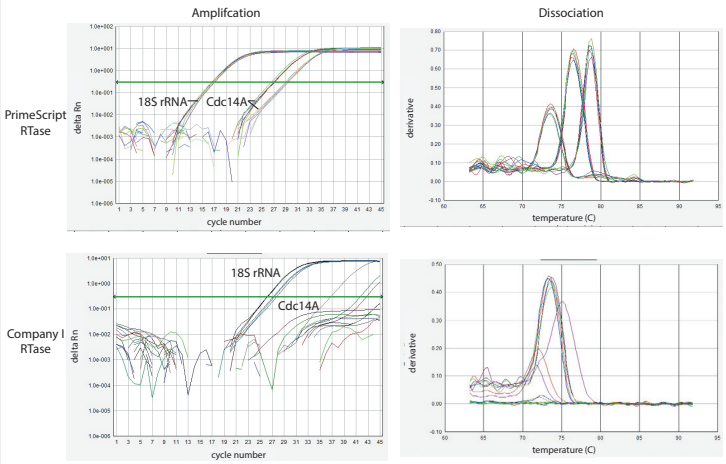

Figure 1. Amplification curves and dissociation curves for Cdc14A and 18S rRNA expression. cDNA templates for qPCR were generated with either PrimeScript RTase (top) or a RTase from Company I (bottom).

Table 1. Ct Values for Cdc14A and 18S rRNA

	Company I		Takara	
shRNA	control	Cdc14A	control	Cdc14A
m14A1* Ct	Undetermined Undetermined Undetermined	36.76 Undetermined Undetermined	26.96 27.09 26.96	28.98 28.96 29.18
m14A5* Ct	42.71 Undetermined Undetermined	41.66 Undetermined Undetermined	26.52 27.10 27.02	29.35 29.18 29.03
18S rRNA Ct	26.21 26.13 26.20	27.71 27.16 27.32	16.51 17.05 16.56	16.72 16.70 16.95

* Two primer sets were used to measure Cdc14A (m14A5 and m14A1).

Conclusions

PrimeScript RTase was able to quickly generate cDNA (total reaction time ~15 minutes) that could be used to quantify the expression of both 18S rRNA, a housekeeping gene that is expressed at relatively high levels, and low level Cdc14A expression by qPCR. Furthermore, PrimeScript RTase outperformed Company I's RT and provided higher sensitivity for the Cdc14A target and allowed quantification of Cdc14A knockdown in MEF cells. When determining the remaining target gene expression level after knockdown, efficiency of cDNA synthesis is critical. PrimeScript RTase allowed sufficient RNA-to-cDNA conversion of rare Cdc14A transcripts to allow meaningful analysis of shRNA knockdown.