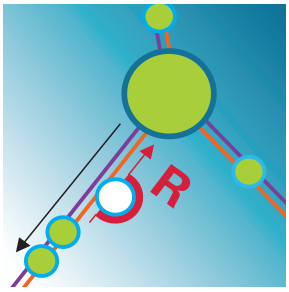


PrimeScript™ Reverse Transcriptase

Extending the Boundaries of Reverse Transcription

that's GOOD science!™

PrimeScript Reverse Transcriptase is a recombinant, RNase H Minus, modified MMLV (Moloney Murine Leukemia Virus) enzyme developed by Takara Bio. PrimeScript RT is robust and versatile, and is well-suited for all applications that require full-length first strand cDNA. PrimeScript RT offers:

- Strong strand displacement and extension capability: Synthesizes long, full-length cDNA molecules (up to 12 kb)
- High specificity: Capable of synthesizing cDNA at 42°C, which allows high specificity, high yield, and reduced risk of RNA degradation
- Outstanding accuracy: Low error rate compared to other commercially available reverse transcriptases
- Fast reactions: Reverse transcription reactions can be completed in as little as 15 minutes

Why does PrimeScript RT provide outstanding performance?

RNA secondary structure can interfere with cDNA synthesis. Conventional RTs can anneal nonspecifically to higher order structures of RNA. Nonspecific products derived from such mispriming can impair overall RT efficiency and reduce the yield of full-length cDNA. To minimize these effects, reactions with conventional RTs are frequently performed at higher temperatures so that RNA secondary structures are partially or completely denatured. However, because high temperatures also increase the risk of RNA degradation, these reaction conditions are not ideal. In contrast, PrimeScript RT can be used at 42°C.

PrimeScript RT reduces mispriming events by avoiding nonspecific annealing and preventing primer-dimer formation. Furthermore, the enzyme has strong strand displacement activity. This tech note introduces the key features of PrimeScript RT and provides experimental examples for PrimeScript RT and kits.

Strong strand displacement activity of PrimeScript RT results in excellent extension of long targets

cDNA was prepared by primer extension using either PrimeScript RT or an RT from Company L at 50°C. Products originating from an oligo-dT primer and an internal, specific primer were analyzed on an alkaline denaturing gel (Figure 1).

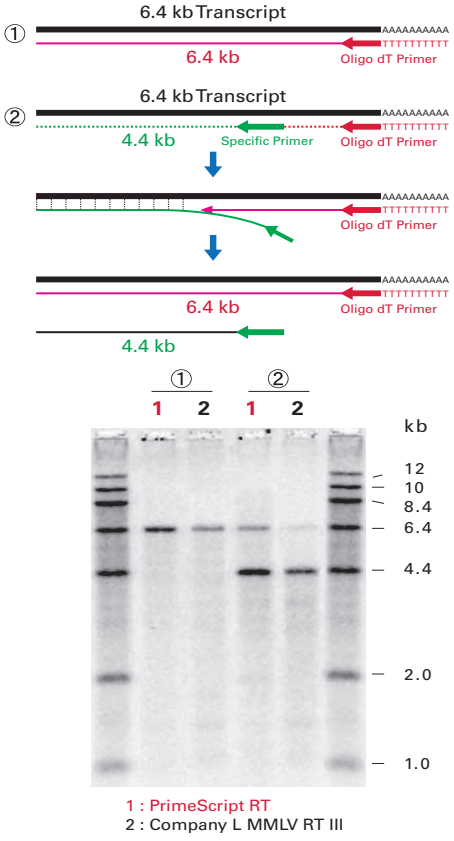


Figure 1: Comparison of strand displacement and elongation activity.

PrimeScript RT synthesizes cDNA at 42°C with high specificity and yield

Extension products obtained with PrimeScript RT were compared with products generated by six other commercially available RTs. An RNA ladder (containing 1, 2, 4.4, 6.4, 8.4, 10, and 12 kb fragments) was used as the template for first strand cDNA synthesis. Each reaction was assembled and performed according to the manufacturer's recommendations. Equivalent amounts of cDNA were loaded onto an alkaline denaturing gel. After electrophoresis, the gel was stained with SYBR® Green II, and the products were detected by fluorescence imaging (Figure 2).

The yield of full-length cDNAs was far higher for PrimeScript RT than any of the other enzymes. In addition, although the reaction temperature for PrimeScript RT was lower (42°C), the resulting cDNA had significantly less background arising from non-specific priming events.

PrimeScript RT is highly accurate

Several different RTs were used to synthesize first-strand cDNA from human placenta total RNA (Clontech® Cat. #636527) using oligo-dT primers. Reactions were performed following each manufacturer's recommended protocol. After cDNA synthesis, PCR amplification of the TF gene was performed with high-fidelity PrimeSTAR® HS DNA Polymerase (Clontech Cat. # R010A). The 500 bp amplified fragments were then cloned into vectors, and multiple clones were chosen for DNA sequence analysis. The error rate was defined as the number of errors per total number of bases sequenced (~200,000 bases). A PCR fragment directly amplified from human genomic DNA was used as a control. PrimeScript RT resulted in only 7 errors out of 200,000 bases (an error rate of just 0.0035%), the highest accuracy of all RTs analyzed (Figure 3).

PrimeScript RT facilitates easy workflow

During synthesis of long RNA products by reverse transcription, conventional enzymes require that the RNA template be denatured first, followed by addition of RT. This is intended to avoid the suppression of cDNA synthesis that can occur when RT anneals to double-stranded RNA secondary structures. In contrast, PrimeScript RT does not require template denaturation, allowing greater flexibility in workflow.

The yield of an 8 kb dystrophin cDNA product produced by PrimeScript RT and Company L's RT was examined by RT-PCR. Three different protocols were compared as shown in Figure 4: 1) denaturation of RNA template followed by addition of RT on ice; 2) pre-incubation at the RT reaction temperature (42°C for PrimeScript RT or 50°C for Company L's RT) followed by addition of RT at the reaction temperature, but without prior template denaturation; or 3) addition of RT to template on ice, then shifting to the reaction temperature. While PrimeScript RT produced cDNA with all protocols, Company L's RT generally showed poor yield and absence of product when RT was added without prior template denaturation (protocol 3).

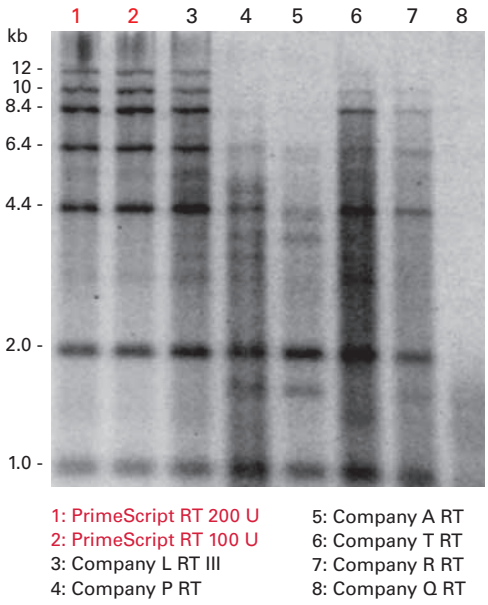


Figure 2: First strand cDNA synthesis with various RTs.

RT	Bases sequenced	Error (bases)	Error rate (%)
PrimeScript RT	**201,297**	**7**	**0.0035**
Company L RT II	166,227	9	0.0054
Company L RT III	161,409	11	0.0068
Company A RT	132,962	13	0.0098
M-MLV (Takara)	144,504	15	0.0104
Control (PCR only)	156,188	1	0.0006

Figure 3: Accuracy of various RTs.

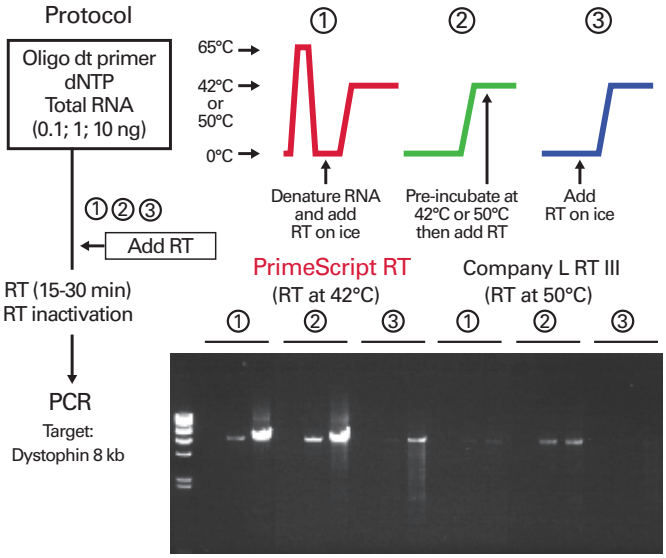


Figure 4: cDNA synthesis suppression by addition of RT to non-denatured RNA.

To further assess the robustness of these enzymes, the ability of each RT to synthesize cDNA after prolonged incubation on ice was analyzed. RT was incubated with denatured RNA template on ice for 0, 5, or 15 minutes before proceeding with reverse transcription (Figure 5). PrimeScript RT produced high yield of dystrophin cDNA even after a 15 minutes incubation on ice, whereas synthesis of cDNA by Company L's RT was completely suppressed under the same condtions.

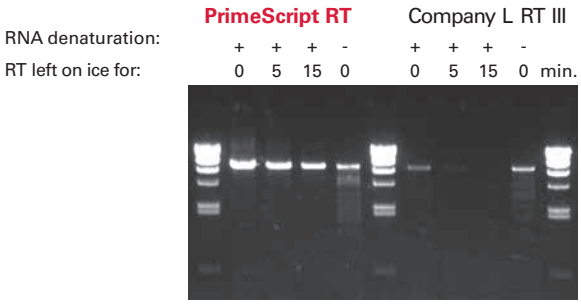


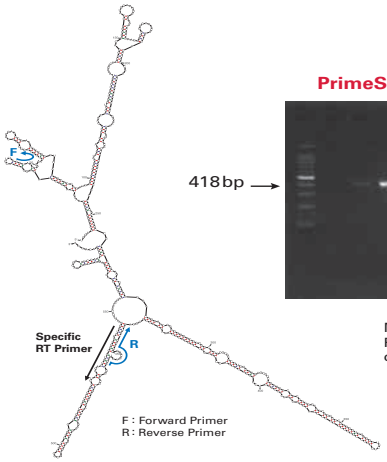
Figure 5: cDNA synthesis after extended pre-incubation on ice.

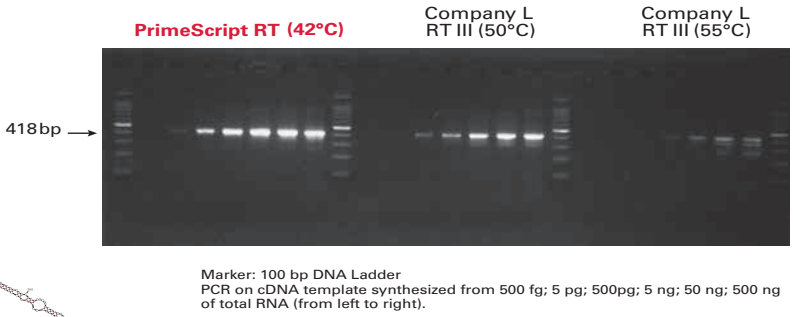
These experiments demonstrate the ability of PrimeScript RT to synthesize cDNA under a variety of protocol conditions. Taken together, the data indicate that PrimeScript RT provides greater flexibility in workflow, which can be particularly advantageous for medium- and high-throughput studies.

Experimental Examples

Example 1: Reverse transcription of template RNA that has strong secondary structure

[Procedure] A 418 bp cDNA product from 28S ribosomal RNA, which contains strong higher order structure (Figure 6, left panel), was synthesized using either PrimeScript RT or Company L's RT. Total RNA from HL60 cells was used as template, and a gene-specific primer was used for cDNA synthesis. PrimeScript RT reactions were performed at 42°C and Company L reactions were performed at 50°C or 55°C using each manufacturer's recommended protocols. The efficiency of cDNA synthesis was evaluated using PCR.





Marker: 100 bp DNA Ladder
PCR on cDNA template synthesized from 500 fg; 5 pg; 500pg; 5 ng; 50 ng; 500 ng of total RNA (from left to right).

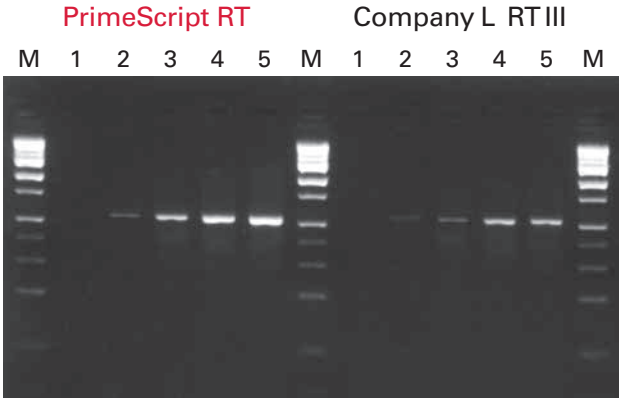
Figure 6: Reverse transcription of RNA with high secondary structure.

[Result] As shown in Figure 6, PrimeScript RT had the highest sensitivity and transcription efficiency. These results are consistent with the high RT efficiency of PrimeScript RT for RNA that contains strong secondary structure.

Example 2: Reverse Transcription of GC-rich RNA

[Procedure] cDNA synthesis from human heart total RNA primed with oligo-dT was performed using either PrimeScript RT or Company L's RT. The reactions were assembled and performed according to each manufacturer's recommended conditions. A 520 bp fragment of the APOE gene (~75% GC content) was amplified by PCR.

[Result] A higher yield of APOE cDNA was obtained using PrimeScript RT (Figure 7). In contrast, Company L's RT gave lower yield of cDNA from the GC-rich APOE template.



Template cDNA amount used (corresponding to total RNA)

1 : 0 ng
2 : 0.125 ng
3 : 1.25 ng
4 : 12.5 ng
5 : 125 ng
M: 100 bp ladder

Figure 7: RT-PCR of GC rich region (APOE gene).

Example 3: Long RT-PCR

[Procedure] cDNA was synthesized from total RNA from HL60 cells with oligo-dT primers using either PrimeScript RT or one of four other commercially-available RTs. The reactions were assembled and performed according to each manufacturer's recommended conditions. Dystrophin cDNA products (2 kb, 6 kb, or 12kb) were amplified by PCR.

[Result] As shown in Figure 8, a higher yield was generally observed for the PrimeScript RT-generated cDNA samples, in particular the 6 kb and 12 kb cDNA products which could not be amplified from cDNA synthesized with most of the other commercially available enzymes. These results indicate that PrimeScript RT provides superior yield of long cDNA products.

Example 4: RT-PCR using random primers

[Procedure] cDNA was synthesized from 0 ng, 50 ng, 500 ng, or 1 µg of input RNA (human heart total RNA, Clontech Cat. # 636532) using PrimeScript RT with random primers. After cDNA synthesis, 6 kb or 12 kb fragments of the Dystrophin gene were amplified by PCR.

[Result] As shown in Figure 9, PrimeScript RT was capable of synthesizing long cDNA (6 kb and 12 kb) with random primers.

Description of Select PrimeScript Kits

PrimeScript 1st strand cDNA Synthesis Kit

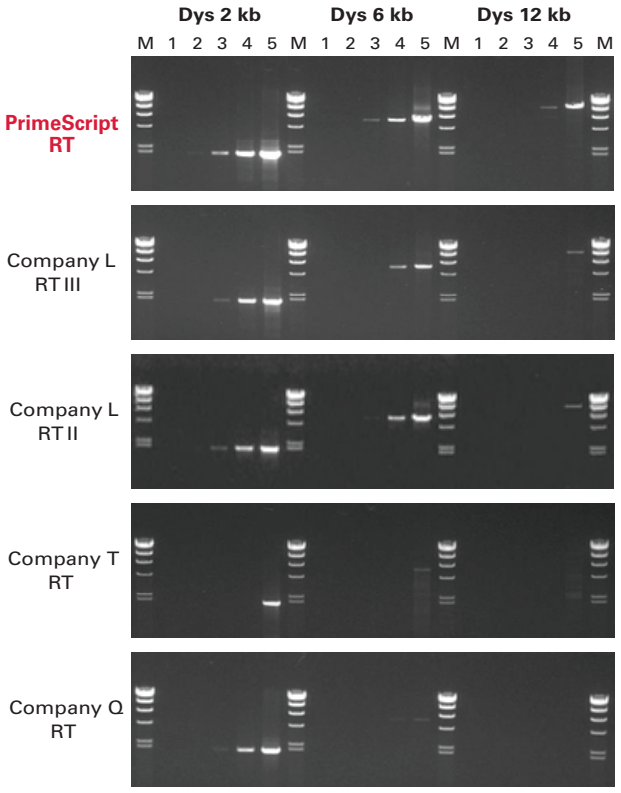
This kit (Clontech Cat. # 6110A) contains all components required for first-strand cDNA synthesis. It is designed for long and sensitive first strand cDNA synthesis from total RNA or polyA$^+$ RNA using PrimeScript RT. cDNA prepared using this kit can be used for second-strand cDNA synthesis, PCR amplification, or other applications such as cDNA library preparation.

PrimeScript RT-PCR Kit

Perform two-step RT-PCR efficiently on any RNA template with this kit (Clontech Cat. # RR014A), which includes an optimized combination of *TaKaRa Ex Taq*® HS and PrimeScript RT. The combination of enzymes allows excellent extension and highly efficient amplification. This kit provides all of the reagents required for reverse transcription and PCR (Figure 10), and is best suited for general RT-PCR applications.

PrimeScript One-Step RT-PCR Kit

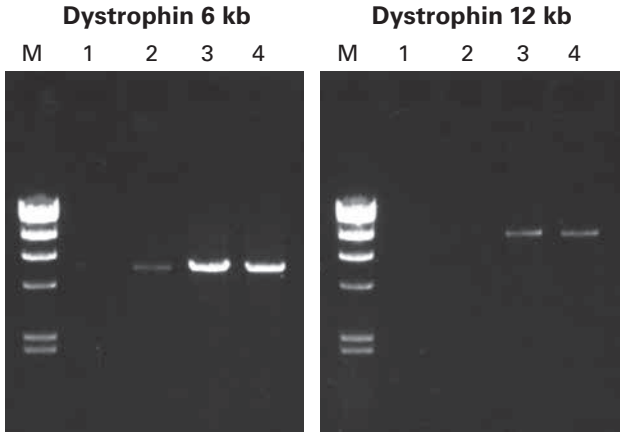
The PrimeScript One Step RT-PCR Kit (Clontech Cat. # RR055A) combines PrimeScript RT and *TaKaRa Ex Taq* HS for excellent performance and ease of use. This kit allows all reagents for reverse transcription and PCR to be assembled in one tube, making handling simple and greatly minimizing the risk of contamination (Figure 11).



*PCR was performed under the same conditions (30 cycles)

Template cDNA amount used in PCR (corresponding to total RNA)
1: 0; 2: 125 pg; 3: 1.25 ng; 4: 12.5 ng; 5: 125 ng; M:λ-*Hin*d III

Figure 8: Comparison of RT-PCR using various RTs.



RT reaction: 50 min.
1: 0
2: 50 ng
3: 500 ng
4: 1 µg
M: λ-*Hin*d III

Figure 9: Reverse transcription using random 6-mer primers.

PrimeScript High Fidelity RT-PCR Kit

This two-step RT-PCR kit (Clontech Cat. # R022A) provides outstanding accuracy by combining PrimeSTAR Max DNA polymerase and PrimeScript RT. PrimeSTAR Max is a hot-start PCR enzyme with the highest accuracy of any commercially available enzyme. The PrimeScript High Fidelity RT-PCR Kit is compatible with a broad range of template RNA levels. This kit is recommended for any RT-PCR application that requires high accuracy.

PrimeScript RT Reagent Kit (Perfect Real Time) for qRT-PCR

This kit (Clontech Cat. # RR037A) is designed for two step real time RT-PCR. It allows easy, rapid preparation of template cDNA using PrimeScript RT, followed by efficient cDNA amplification using SYBR *Premix Ex Taq*™ (Cat. #RR420A) or *Premix Ex Taq* (Clontech Cat. #RR390A) (Figure 12).

PROTOCOL

20 µl RT reactions were performed using various amounts of total RNA. Then, 2 µl of each RT reaction was used in a 50-µl PCR reaction.

RESULT

The PrimeScript RT-PCR Kit performed better than the previous generation Takara RT-PCR Kit with AMV RT. This result is likely due to the higher RT efficiency imparted by PrimeScript RT versus conventional AMV RT.

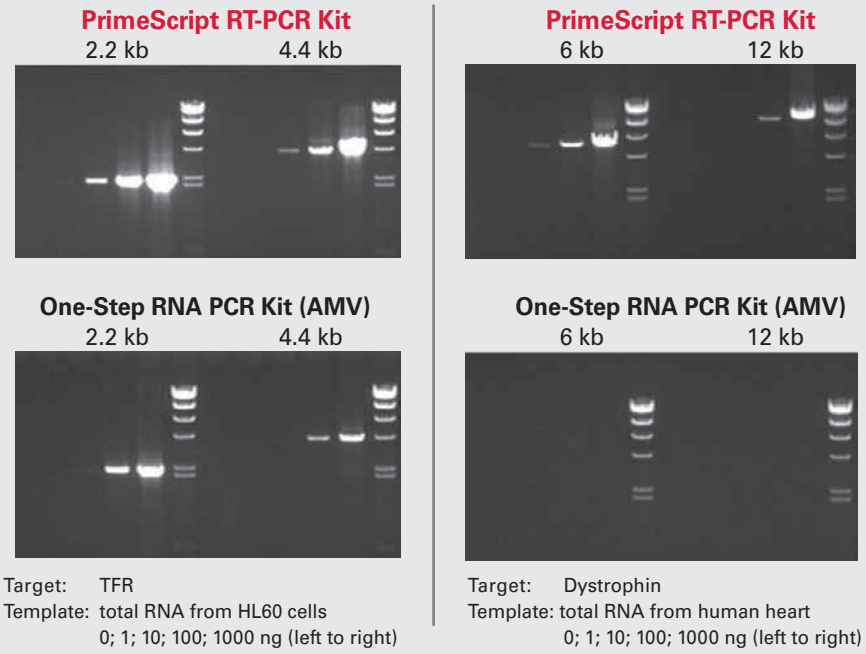


Figure 10. Comparison between PrimeScript RT-PCR Kit and a kit containing a conventional AMV RT.

PROTOCOL

Various sizes of targets were amplified by RT-PCR. 1 µg of total human RNA was used as a template for cDNA synthesis. Each RT-PCR reaction was performed according to the supplier's recommended conditions.

RESULT

The PrimeScript One Step RT-PCR Kit resulted in better amplification of longer products (up to 8 kb) than the other kits tested.

Template : human total RNA

Lane		
	1 : TFR	0.5 kb
	2 : CCND2	2.1 kb
	3 : CCND2	2.8 kb
	4 : TFR	4.4 kb
	5 : Dystrophin	6 kb
	6 : Dystrophin	8 kb
	M : λ-*Hin*d III digest	

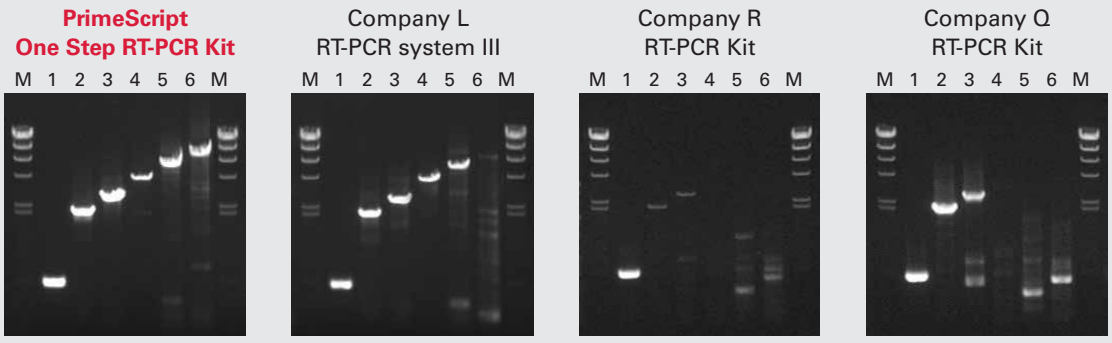


Figure 11. Performance of PrimeScript One Step RT-PCR Kit and One Step RT-PCR kits from other suppliers for products up to 8kb long.

PROTOCOL

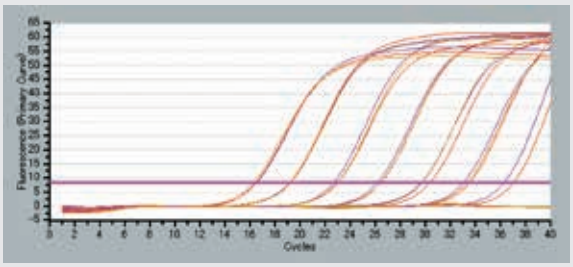
[RT]
Template: mouse liver total RNA 2 pg—2 µg
or distilled water (negative control)
Volume: 10 µl
Primer: Random 6mers
Reaction conditions: 15, 30, or 60 minutes at 37°C;
5 seconds at 85°C; 4°C.

[Real-Time PCR]
Reagent used: SYBR *Premix Ex Taq* (Perfect Real Time)
Template: 2 µl of above the RT reaction
Final volume: 25 µl
Target gene: mouse *Actb*
Instrument: Thermal Cycler Dice™ Real Time System
(not available in all geographic regions)

RESULT

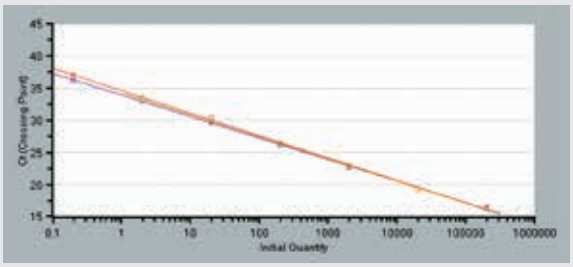
The results obtained using various RT reaction times indicate excellent efficiency over a wide range of template concentrations.

Amplification curve



RT 15 min. RT 30 min. RT 60 min.

Standard curve



RT 15 min. Rsq: 0.999 Eff: 98.7% Y = 3.552×LOG(X) + 33.94
RT 30 min. Rsq: 0.999 Eff: 93.3% Y = 3.495×LOG(X) + 34.61
RT 60 min. Rsq: 0.999 Eff: 95.2% Y = 3.441×LOG(X) + 34.28

Figure 12. Performance of PrimeScript RT reagent Kit (Perfect Real Time) with various RT reaction lengths.

PROTOCOL

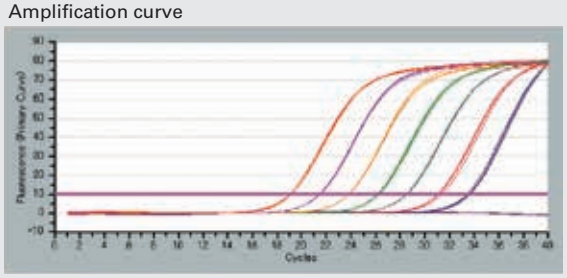
Reagent: One Step SYBR PrimeScript RT-PCR Kit
(Perfect Real Time)
Template: Mouse liver total RNA 6.4 pg—100 ng
Target gene: rat *Rplp2* (ribosomal protein, large P2)
Instrument: Thermal Cycler Dice Real Time System
(not available in all geographic locations)
Reaction conditions:

1) RT step	42°C 5 min.	
	95°C 10 sec.	
2) PCR step	95°C 5 sec.	40 cycles
	60°C 30 sec.	
3) Melting step	60°C to 94°C	

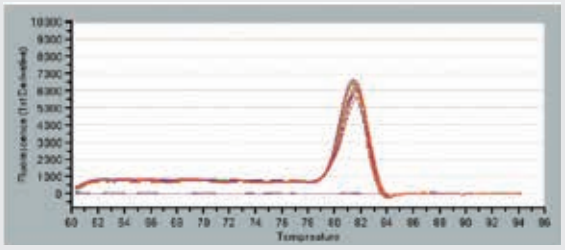
RESULT

Rplp2 was detected with total RNA inputs ranging from 6.4 pg—100 ng. Good linearity of the standard curve was obtained. These results indicate that the One Step SYBR PrimeScript RT-PCR Kit (Perfect Real Time) provided accurate quantification across the range of input RNA amounts tested.

Amplification curve



Denaturation curve



Standard curve

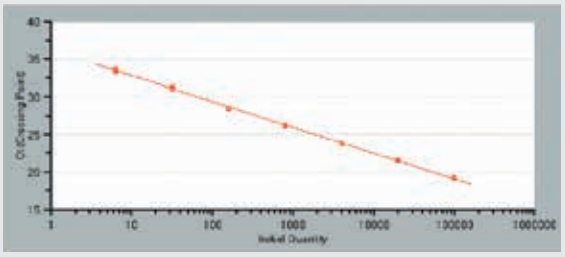


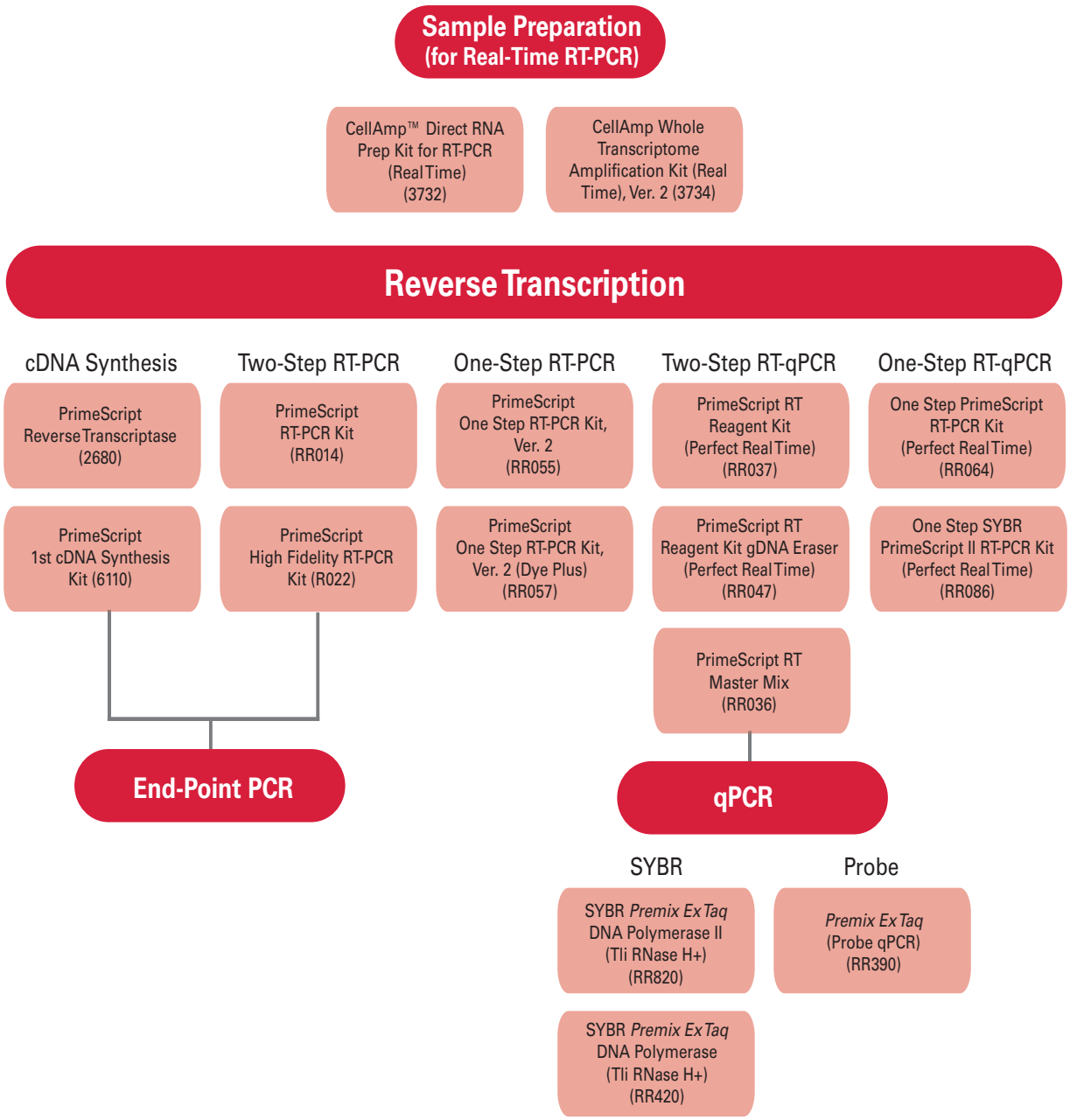
Figure 13. Performance of Real-Time One Step RT-PCR with SYBR Green I detection.

One Step SYBR PrimeScript RT-PCR Kit II (Perfect Real Time) & One Step PrimeScript RT-PCR Kit (Perfect Real Time)

These systems are designed for one-step RT-PCR using PrimeScript RT for cDNA synthesis and *TaKaRa Ex Taq* HS for PCR amplification.

Two kits are available: one for detection with SYBR Green I (Clontech Cat. #RR086A), and the other for detection with a TaqMan® probe (Clontech Cat. #RR064A). Because these systems provide excellent amplification rate and reaction specificity, they are highly recommended when working with small amounts of RNA or RNA viruses (Figure 13).

Choosing the Best PrimeScript Kit for Your Experiment



PRODUCTS

Cat. #	Product	Package Size
2680A 2680B	PrimeScript Reverse Transcriptase	10,000 Units 40,000 Units
6110A 6110B	PrimeScript 1st strand cDNA Synthesis Kit	50 rxns 200 rxns
R022A R022B	PrimeScript High Fidelity RT-PCR Kit	50 rxns 200 rxns
RR014A RR014B	PrimeScript RT-PCR Kit	50 rxns 200 rxns
RR036A RR036B	PrimeScript RT Master Mix (Perfect Real Time)	200 rxns 800 rxns
RR037A RR037B	PrimeScript RT Reagent Kit (Perfect Real Time)	200 rxns 800 rxns
RR047A RR047B	PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time)	200 rxns 800 rxns
RR055A RR055B	PrimeScript One Step RT-PCR Kit, Ver. 2	50 rxns 200 rxns
RR057A RR057B	PrimeScript One Step RT-PCR Kit, Ver. 2 (Dye Plus)	50 rxns 200 rxns
RR064A RR064B	One Step PrimeScript RT-PCR Kit (Perfect Real Time)	100 rxns 500 rxns
RR086A RR086B	One Step PrimeScript RT-PCR Kit II (Perfect Real Time)	100 rxns 500 rxns
3734	CellAmp Whole Transcriptome Amplification Kit (Real Time), Ver. 2	100 rxns

RELATED PRODUCTS

Cat. #	Product	Package Size
R050A	PrimeSTAR GXL DNA Polymerase	250 Units
R045A	PrimeSTAR Max DNA Polymerase	100 rxns
RR390A	*Premix Ex Taq* (Probe qPCR)	200 rxns
RR420A	SYBR *Premix Ex Taq* (Tli RNase H Plus)	200 rxns
RR820A	SYBR *Premix Ex Taq* II (Tli RNase H Plus)	200 rxns

Please check www.clontech.com for current pricing.