Two-Step RT-PCR

Components	RT02-01	RT02-02
PowerScript™ Plus RTase (200 U/ul)	25 µl	100 µl
2X Reaction Mix	300 µl	3 x 1 ml
Nuclease-free H2O	1 ml	4 x 1 ml
2X PCR Taq Plus MasterMix	1 x 1ml	4 x 1 ml
Size	**25 rxns**	**100 rxns**

Product Description

PowerScript™ Plus RTase is a novel recombinant reverse transcriptase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. The PowerScript™ Plus Reverse Transcriptase is engineered to perform under high temperatures (50°C - 55°C), facilitating the elimination of secondary structures associated with GC-rich RNA templates. Due to this unique feature, PowerScript® Plus can synthesize full-length cDNA libraries from RNA templates up to 15 kb in length. In addition, PowerScript™ Plus Reverse Transcriptase has outstanding proofreading ability due to the presence of a fidelity-enhancing subunit, thus making this RTase an excellent choice for whole genome sequencing.

2X PCR Taq Plus MasterMix is a ready-to-use mixture of high-quality Taq Plus DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. PCR products, amplified up to 6 kb in length with Taq Plus DNA Polymerase, contain a mixture of blunt ends and single base (A) 3' overhang. The error rate of this PCR amplification is 7.5 x 10-6 per nucleotide per cycle. The products can be used for direct T/A cloning, but its efficiency is not as high as PCR products amplified with Taq DNA Polymerase alone.

Unit Definition One unit is defined as the amount of enzyme required to incorporate 1 nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C using poly(A) and Oligo(dT) as template and primer, respectively.

Storage Buffer 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01 % (v/v) NP-40, 50 % (v/v) glycerol.

Storage Conditions Store all components at -25°C to -15°C in a non-frost-free freezer. 2X PCR Taq Plus MasterMix is stable at 4°C for three months or for fifteen freeze-thaw cycles. All components are stable for 1 years from the date of shipping when stored and handled properly.

Protocol

Reverse transcription reactions should be assembled in a RNase-free environment. The use of "clean", automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thaw RNA templates and all reagents on ice. Mix each solution by vortexing gently.
2. Prepare the following reaction mixture on ice:

Components	Volume	Final Concentration
Total RNA or poly(A)$^+$ mRNA	Variable	1 ng - 2 µg/rxn 1 pg - 2 ng/rxn
2X Reaction Mix	10 µl	1X
Nuclease-free H2O	Up to 19 µl	-

3. Optional: Heat mixture to 65°C for 5 mins, then incubate on ice for at least 1 min. Collect all components by a brief centrifugation.
4. Add the following:

Components	Volume	Final Concentration
PowerScript™ Plus RTase (200 U/µl)	1 µl	200 U/rxn

5. Mix components well and collect all components (20 µl) by a brief centrifugation. Incubate the tube at 25°C for 10 mins. Perform cDNA synthesis by incubating the tube for either 15 mins (for qPCR) or 50 mins (for PCR) at 50°C.
6. Stop the reaction by heating it at 85°C for 5 mins. Chill on ice. The newly synthesized firststrand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.
7. Assay cDNA yield by spectrophotometry reading.
8. Prepare the following reaction mixture on ice:

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µ
Forward primer (10 µM)	1 - 2.5 µl	200 - 500 nM
Reverse primer (10 µM)	1 - 2.5 µl	200 - 500 nM
2X PCR Taq Plus MasterMix	25 µl	1X
Nuclease-free H2O	25 µl	-

**We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.*

9. Mix contents of tube and centrifuge briefly.

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10. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.

11. Perform 30 - 35 cycles of PCR amplification as follows:
    a. Denature: 94°C for 30 secs
    b. Anneal: 45 - 72°C for 30 secs
    c. Extend: 72°C for 1 min/1 kb template
12. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
13. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or Safe™ gel staining. Use appropriate molecular weight standards.

General Notes

1. RNA samples must be free of genomic DNA contamination, which can be achieved by incubating with DNase I.
2. Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A)$^+$ mRNA may give higher yields and improved purity of final products.
3. Unlike Oligo(dT) priming, which requires little optimization, the ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products.
4. To remove RNA complementary to the cDNA, add 1 µl (2 U) of E. coli RNase H and incubate at 37°C for 20 mins.