Protocol

TD-P Revision 1.0

Creation Date: 2/18/2019
Revision Date: 4/24/2019

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Utilizing RT-PCR Kit

Introduction

Our RT-PCR Kit combines two powerful mixtures: i) 5x Master Mix and ii) Hot start Taq 2x master mix with a standard buffer for 2-step RT-PCR. The two mixtures require minimal handling during the reaction setup and offer consistent and robust RT-PCR reactions.

First strand cDNA synthesis is achieved by using the 5x Master Mix, which contains Reverse Transcriptase, recombinant RNase inhibitor, dNTPs, an optimized buffer, $MgCl_2$ and protein stabilizers. Reverse Transcriptase is a recombinant MMLV reverse transcriptase with reduced RNase H activity and increased thermostability. This kit also provides two optimized primers and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}VN$] forces the primer to anneal to the beginning of the polyA tail and the random hexamer primer mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. In addition, this kit is highly efficient at producing full-length cDNA from long RNA templates at temperatures between 42-55°C.

The amplification step features a high quality Hot Start Taq DNA Polymerase, which offers higher fidelity and better amplification in a master mix format. RT-PCR product can be generated up to 6 kb. Our RT-PCR Kit can be used for cDNA synthesis followed by gene expression data validation. The advantages of our RT-PCR Kit include robust and active cDNA synthesis at temperatures up to 55°C and high efficiency at producing full-length cDNA from as little as 50 pg of total RNA. Here, we describe a general protocol for the use of our RT-PCR Kit.

Materials

RT-PCR Kit (GoldBio Catalog # R-920)

- 5x Master Mix
- Oligo $d(T)_{23}$ VN primer (50µM)
- Random hexamer primer mix (60µM)
- Hot start Taq 2x master mix
- Nuclease free H_2O

Storage and Handling

- Store RT-PCR Kit at -20°C.

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- This product may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, this product should be stable for at least 1 year from the date of receipt.
- Thaw on ice and mix by gentle vortexing. After thawing, this product should be kept on ice before use. This product can be refrozen for storage.

Method

First strand cDNA synthesis

1. In a sterile micro-centrifuge tube, add the following components on ice:

Component	Volume
Total RNA	Up to 1.0 µg
5x Master Mix	4.0 µl
Primer: $d(T)_{23}VN$ (50µM) and/or random primer mix (60µM) or Gene specific primer (10µM)	2.0 µl
Nuclease free H_2O	Up to 20.0 µl

2. If using random hexamers, incubate the reaction mixture at 25°C for 10 minutes, then proceed to step 3.

3. Incubate the reaction mixture at temperatures between 42°C to 55°C for 30-60 minutes.

4. Inactivate the reaction by incubating at 65°C for 20 minutes.

5. Proceed to PCR amplification step.

PCR amplification

1. Prepare a reaction mix according to the following table:

PCR reaction set up:	
Component	**Volume**
Diluted cDNA	1-5 µl
Forward primer (5µM)	1.0 µl
Reverse primer (5µM)	1.0 µl
Hot start Taq 2x master mix	10.0 µl
H_2O up to	20.0 µl

2. Mix the reaction mixture thoroughly.

3. Program the thermal cycler according to the manufacturer's instructions.

4. A typical PCR cycling program is outlined in the following table.

5. Place the PCR tubes in the thermal cycler and start the cycling program.

PCR cycling conditions:			
Steps	**Temperature**	**Time**	**Cycles**
Initial denaturation	95°C	15 min	1
Denaturation	94°C	30 sec	25-40
Annealing	50-66°C	30 sec	
Extension	72°C	1 min/kb	
Final extension	72°C	5 min	1

6. Analyze 5 µl of PCR products by agarose gel electrophoresis.

Associated Products

- dNTP mix (GoldBio Catalog # D-900)
- Hot Start Taq DNA Polymerase (GoldBio Catalog # T-510)
- Hot Start Taq DNA Polymerase plus dNTP (GoldBio Catalog # T-511)
- Hot Start Taq 2x Master Mix – 50 µl reaction (GoldBio Catalog # T-512)
- Hot Start Taq 2x Master Mix – 20 µl reaction (GoldBio Catalog # T-513)
- Taq DNA Polymerase (GoldBio Catalog # T-514)
- Taq DNA Polymerase plus dNTP (GoldBio Catalog # T-515)
- Taq DNA Polymerase with Dye (GoldBio Catalog # T-516)
- Taq DNA Polymerase with Dye plus dNTP (GoldBio Catalog # T-517)
- Taq DNA Polymerase 2x Premix with Dye (GoldBio Catalog # T-518)
- Hot Start Pfu DNA Polymerase (GoldBio Catalog # P-650)
- Hot Start Pfu DNA Polymerase plus dNTP (GoldBio Catalog # P-655)
- Pfu 2x DNA Polymerase Master Mix (GoldBio Catalog # P-660)
- Pfu DNA Polymerase (GoldBio Catalog # P-665)
- Pfu DNA Polymerase plus dNTP (GoldBio Catalog # P-690)
- One Step RT-PCR Kit (GoldBio Catalog # R-910)
- RT-qPCR Kit (GoldBio Catalog # R-925)
- One Step RT-qPCR Kit (GoldBio Catalog # R-915)
- qPCR 2x Master Mix with SYBR® Green (GoldBio Catalog # M-915)
- Reverse Transcriptase (GoldBio Catalog # R-900)
- 1 kb DNA Ladder (GoldBio Catalog # D010)
- 1 kb PLUS™ DNA Ladder (GoldBio Catalog # D011)

- 100 bp DNA Ladder (GoldBio Catalog # D001)
- 100 bp PLUS™ DNA Ladder (GoldBio Catalog # D003)
- 50 bp DNA Ladder (GoldBio Catalog # D100)
- VersaLadder™, 100-10,000 bp (GoldBio Catalog # D012)
- Agarose LE (Molecular Biology Grade) (GoldBio Catalog # A-201)