Protocol

TD-P Revision 1.0

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Polymerase Chain Reaction (PCR)

Utilizing *Taq* DNA Polymerase

Introduction

Polymerase Chain Reaction (PCR) is a powerful technique used to amplify DNA through the use of the enzyme *Taq* DNA Polymerase. GoldBio *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses 5'→3' polymerase activity and 5' flap endonuclease activity. This product is supplied with a 10x PCR reaction buffer containing $MgCl_2$, which produces a final Mg^{2+} concentration of 1.5mM. GoldBio *Taq* DNA Polymerase is ideal for primary extension reaction DNA fragments having dA overhang on 3' ends and can be used in many applications including routing PCR cloning, primer extension, colony PCR, and amplification of high GC content DNA with the GC enhancer. In addition, GoldBio *Taq* DNA Polymerase has an elongation efficiency of 1.0-1.2 kb/minute and can be used to amplify long target DNA. Here, we describe a general protocol for the use of GoldBio *Taq* DNA Polymerase.

Materials

- *Taq* DNA Polymerase (GoldBio Catalog # T-514, T-515 or T-516)
- 10x PCR Buffer with Mg^{2+}
- 5x GC enhancer
- 10mM dNTP (not supplied with T-514 or T-516)

Not supplied:

- Primers
- Water, PCR Grade

Note: The storage buffer contains 50mM Tris-HCl, 50mM DTT, 0.1mM EDTA, and 50% glycerol, pH 7.5 at 25°C.

Note: 10x PCR Buffer with Mg^{2+} contains 100mM Tris-HCl, pH 8.0, 15mM $MgCl_2$, 100mM KCl, 80mM $(NH_4)_2SO_4$, and 0.5% Igepal CA 630.

Note: One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP into acid-insoluble form in 30 minutes at 72°C.

Storage/Handling

- Store GoldBio *Taq* DNA Polymerase, 10x PCR Buffer with Mg^{2+}, 5x GC enhancer, and dNTPs (if supplied) at -20°C.

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

Method

1. Thaw the PCR buffer, dNTPs, primers, 5x GC enhancer (optional) on ice, and mix thoroughly.

2. Prepare a reaction mix according to the following Table 1.

Note: The reaction mix contains all the components needed for the PCR reaction, except the template DNA.

Table 1. PCR reaction set up.

Component	20 µl reaction
Template DNA	~1-50 ng
10x PCR Buffer	2.0 µl
dNTP (10mM)	0.4 µl
Forward primer (3.2µM)	1.0 µl
Reverse primer (3.2µM)	1.0 µl
5x GC enhancer (optional)	4.0 µl
Taq DNA Polymerase (1 U)	1.0 µl
H_2O	up to 20.0 µl

3. Mix the reaction thoroughly.

4. Add template DNA to the individual PCR tubes containing the reaction mixture.

Note: Prepare the reaction mix on ice and immediately place reactions on thermocycler for PCR completion.

Note: Mix gently and spin briefly if necessary to collect the whole volume at the bottom of the tube.

5. Program the thermal cycler according to the manufacturer's instructions using a PCR cycling program similar to the program described in Table 2.

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Table 2. Sample PCR cycling conditions.

Steps	Temp.	Time	Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	25-35
Annealing	55-60°C	40 sec	
Extension	72°C	1-2 min	
Final Extension	72°C	7 min	1
Hold	4-12°C	∞	

6. Place the PCR tubes in the thermal cycler and complete the cycling program.

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)

References

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