Protocol

TD-P Revision 1.0

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Polymerase Chain Reaction (PCR)

Utilizing *Pfu* DNA Polymerase

Introduction

Polymerase Chain Reaction (PCR) is a powerful technique used to amplify DNA through the use of the enzyme DNA Polymerase. GoldBio *Pfu* DNA Polymerase is a thermostable enzyme that has 5'→3' DNA polymerase and 3'→5' exonuclease (proofreading) activities. GoldBio *Pfu* DNA Polymerase retains the high fidelity, sensitivity and processivity with an error rate six-fold lower than *Taq* DNA polymerase. In addition, GoldBio *Pfu* DNA Polymerase exhibits significantly lower error rates than most other proofreading enzymes or DNA polymerase mixtures.
GoldBio *Pfu* DNA Polymerase is supplied with a 10x PCR reaction buffer, containing $MgCl_2$, which produces a final Mg^{2+} concentration of 1.5mM and 5x GC enhancer that enables efficient amplification of GC-rich templates up to 84%. GoldBio *Pfu* DNA Polymerase can be used in many applications including routine PCR cloning, primer extension, colony PCR, genotyping and amplification of high GC content DNA with the GC enhancer. Here, we describe a general protocol for the use of GoldBio *Pfu* DNA Polymerase.

Materials

- GoldBio *Pfu* DNA Polymerase (GoldBio Catalog # P-665 or P-690)
- 10x PCR Buffer with Mg^{2+}
- 5x GC enhancer
- 10mM dNTP (not supplied with P-665)

Not supplied

- Primers
- H_2O PCR Grade

Note: The storage buffer contains 50mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol, pH 7.5 at 25°C.

Note: 10x PCR Buffer with Mg^{2+} contains 100mM Tris-HCl, pH 9.0, 15mM $MgCl_2$, 100mM KCl, 80mM $(NH_4)_2SO_4$, 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

Gold Biotechnology
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- Store GoldBio *Pfu* DNA Polymerase, 10x PCR Buffer with Mg^{2+}, 5x GC enhancer and dNTPs (if supplied) at -20°C.
- These products may be shipped in blue ice and should be stored at -20°C immediately upon arrival. 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 10x PCR buffer, dNTPs, primers and 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	100 µl reaction
Template DNA	x µl (0.01-0.5 µg)
10x PCR Buffer	10.0 µl
dNTP (10mM)	2.0 µl
Forward primer	x µl (0.1-0.5µM)
Reverse primer	x µl (0.1-0.5µM)
5x GC enhancer (optional)	20.0 µl
Pfu DNA Polymerase (5 U/µl)	0.5 µl
H_2O	up to 100.0 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.

Note: The extension rate of *Pfu* DNA Polymerase is 1 kb/2 minutes. The minimum extension time is 1 minute. Thus, the extension time may be adjusted according to the length of template DNA.

Table 2. Sample PCR cycling conditions.

Steps	Temp.	Time	Cycles
Initial Denaturation	95°C	3-5 min	1
Denaturation	94°C	30-60 sec	25-35
Annealing	52-66°C	30-60 sec	
Extension	72-74°C	1-2 min	
Final Extension	72-74°C	10 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)

References

Frey, B. and Suppmann, B. (1995). Demonstration of the expand PCR system's greater fidelity and higher yields with a lacI-based PCR fidelity assay. *Biochemica* 2: 34–35.