Protocol

TD-P Revision 1.0

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Polymerase Chain Reaction (PCR)

Utilizing *Pfu* 2X DNA Polymerase Master Mix

Introduction

Polymerase Chain Reaction (PCR) is a powerful technique used to amplify DNA through the use of the enzyme DNA Polymerase. GoldBio *Pfu* 2x DNA Polymerase Master Mix contains a thermostable *Pfu* DNA polymerase that has 5'→3' DNA polymerase and 3'→5' exonuclease (proofreading) activities. This 2x master mix also contains dNTPs, $MgCl_2$, and stabilizers with an optimized reaction buffer. The *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.

This 2x master mix is supplied with a 5x GC enhancer that enables efficient amplification of GC-rich templates up to 84% and 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* 2x DNA Polymerase Master Mix.

Materials

- GoldBio *Pfu* 2x DNA Polymerase Master Mix (GoldBio Catalog # P-660)
- 5x GC enhancer

Not supplied

- Primers
- H_2O PCR Grade

Note: The 1x Master Mix contains 10mM Tris-HCl, pH 9.0, 50mM KCl, 1.5mM $MgCl_2$, 0.2mM dNTPs, 5% glycerol, 0.08% Igepal CA 630, 0.05% Tween-20 and 100 Units/ml *Pfu* Polymerase.

Storage/Handling

- Store Pfu 2x DNA Polymerase Master Mix and the 5x GC enhancer at -20°C.
- These products 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, 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.

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Method

1. Thaw the primers, 5x GC enhancer (optional) and the *Pfu* DNA Polymerase 2x Master Mix 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
Forward primer	1.0 µl
Reverse primer	1.0 µl
5x GC enhancer (optional)	4.0 µl
Pfu 2x Master Mix	10.0 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.

   Note: The extension rate of *Pfu* DNA Polymerase is 2 minutes/kb. 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 min	1

Denaturation	95°C	30 sec	25-40
Annealing	50-66°C	30 sec	
Extension	72°C	1 min/kb	
Final Extension	72°C	5 min	1
Hold	4-12°C	∞	

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

7. 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* DNA Polymerase (GoldBio Catalog # P-665)
- *Pfu* DNA Polymerase plus dNTP (GoldBio Catalog # P-690)

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.