Protocol

TD-P Revision 1.0

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Polymerase Chain Reaction (PCR)

Utilizing Hot Start *Taq* 2x Master Mix, 20 µl and 50 µl reactions

Introduction

Polymerase Chain Reaction (PCR) is a powerful technique used to amplify DNA through the use of the enzyme *Taq* DNA Polymerase. GoldBio Hot Start *Taq* 2x Master Mix is a ready to use premix that contains Hot Start *Taq* DNA Polymerase, dNTPs, $MgCl_2$, and stabilizers with an optimized reaction buffer. GoldBio Hot Start *Taq* is a thermostable DNA polymerase that possesses a 5'→3' polymerase activity and a 5' flap endonuclease activity. Hot Start *Taq* DNA Polymerase has been chemically modified to completely inactivate enzymatic activity until the initial heat activation step at the beginning of the PCR cycle. Hot Start PCR reduces non-specific amplification during the setup of the reaction and helps increase PCR specificity and sensitivity. GoldBio Hot Start *Taq* 2x Master Mix can be used in many applications including routine PCR and RT-PCR, primer extension, colony PCR, genotyping, and amplification of high GC contenct DNA with the 5x GC enhancer. Here, we describe a general protocol for the use of GoldBio Hot Start *Taq* 2x Master Mix in 20 µl or 50 µl reactions.

Materials

- Hot Start *Taq* 2x Master Mix (GoldBio Catalog # T-512 or T-513)
- 5x GC enhancer

Not supplied:

- Primers
- Water 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 Hot Start *Taq* Polymerase.

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

Storage/Handling

- Store GoldBio Hot Start *Taq* 2x Master Mix, and the 5x GC enhancer at -20°C.
- 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.

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- Thaw on ice and mix by gentle vortexing. After thawing, these products should be kept on ice before use. These products can ben refrozen for storage.

Method

1. Thaw the primers, Hot Start *Taq* 2x Master Mix, 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 for 20 µl and 50 µl reactions.

Component	20 µl reaction	50 µl reaction
Template DNA	~1-50 ng	~1-50 ng
Forward primer (5µM)	1 µl	2.5 µl
Reverse primer (5µM)	1 µl	2.5 µl
5x GC enhancer (optional)	4 µl	10.0 µl
Hot Start Taq 2x Master Mix	10 µl	25.0 µl
H_2O	up to 20.0 µl	up to 50.0 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.

Table 2. Sample PCR cycling conditions.

Steps	Temp.	Time	Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	25-35
Annealing	55-60°C	30 sec	

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Extension	72°C	1 min/kb	
Final Extension	72°C	5-10 min	1

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

7. Analyze 5 µl of PCR product 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 – 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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