Protocol

TD-P Revision 1.0

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Polymerase Chain Reaction (PCR)
Utilizing Hot Start *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 Hot Start *Taq* DNA Polymerase 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. 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 can be used in many applications including routine PCR, primer extension, colony PCR, 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* DNA Polymerase.

Materials

- Hot Start *Taq* DNA Polymerase (GoldBio Catalog # T-510 or T-511)
- 10x PCR Buffer with Mg^{2+}
- 5x GC enhancer
- 10mM dNTP (not supplied with T-510)

Not supplied:

- Primers
- H_2O PCR Grade

Note: Hot Start *Taq* DNA Polymerase should be stored at -20°C. 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 10nmoles of dNTP into acid-insoluble form in 30 minutes at 72°C.

Method

1. Thaw the 10x PCR buffer, dNTPs, primers, and 5x GC enhancer (optional) on ice, and mix thoroughly.

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2. Prepare a reaction mix for a 100 µl reaction according to 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 (0.1-0.5 µM)
Reverse primer	x µl (0.1-0.5 µM)
5x GC enhancer (optional)	20.0 µl
Hot Start *Taq* 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 PCR program must start with an initial heat-activation step at 95°C for 15 minutes.

Table 2. Sample PCR cycling conditions.

Steps	Temp.	Time	Cycles
Initial Denaturation	95°C	15 min	1
Denaturation	94°C	30-60 sec	
Annealing	52-66°C	30-60 sec	25-35
Extension	72°C	1-2 min	
Final Extension	72°C	10 min	1
Hold	4-12°C	∞	

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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* 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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