2) The extension time should be set according to the size of the amplified fragment. The amplification efficiency of GoldStar Best DNA Polymerase contained in this product is 1-2 kb/min.

3) The number of cycles can be set according to the downstream application of the amplified product. If the number of cycles is too small, the amount of amplification will be insufficient; if the number of cycles is too many, the probability of mismatching will increase and the non-specific background will be severe. Therefore, the number of cycles should be minimized on the premise of ensuring the product yield.

4) This product must be pre-denatured at 95°C for 10 min to activate the enzyme.

Version: 09/2022

GoldStar Best DNA Polymerase

Cat. No. : CW0654S (500 U)
CW0654M (2500 U)
CW0654L (10000 U)

Storage Conditions: -20°C

Components

Component	CW0654S 500 U	CW0654M 2500 U	CW0654L 10000 U
GoldStar Best DNA Polymerase, 5 U/μL	100 μL	5×100 μL	2×1 mL
5×GoldStar PCR Buffer	1.9 mL	5×1.9 mL	8×5 mL

Note: The 5× GoldStar PCR Buffer of this product contains 8.5 mM magnesium ions.

Introduction

This product is a chemically modified hot-start high-fidelity polymerase. The polymerase has 5'-3' DNA polymerase activity, 5'-3' exonuclease activity and 3'-5' exonuclease activity. Under normal PCR conditions, compared with GoldStar DNA Polymerase, it has higher amplification efficiency excellent performance with high and low mismatch rate. The chemically modified enzyme has no polymerase activity at room temperature, which can effectively avoid non-specific amplification caused by the non-specific binding of primer and template or primer dimer under normal temperature conditions. The activation of the enzyme must be incubated at 95°C for 10 minutes his which can be integrated into existing PCR thermal cycling programs. The optimized buffer system maximizes the effect of the enzyme to achieve high fidelity, high specificity, high amplification efficiency, and high sensitivity amplification of the target fragment. Most of the PCR products amplified with this product have an "A" base attached to the 3' end, so they can be directly used for T/A cloning. This product is used in conventional PCR, RT-PCR and multiplex PCR,especially for PCR with high requirements on specificity, fidelity and amplification efficiency.

Active Definition

Using activated salmon sperm DNA as template/primer, the amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid insoluble substances within 30 minutes at 74°C was defined as 1 activity unit (U).

Quality Control

After several column purifications, the purity of the SDS-PAGE test is more than 99%; no exogenous nuclease activity is detected; no host residual DNA is detected by PCR method; it can effectively amplify single-copy genes in the human genome; it can be stored at room temperature for one month , no significant activity changes.

Procedure

The following example is a PCR reaction system and reaction conditions for amplifying a 1 kb fragment using human genomic DNA as a template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR reaction system

Reagent	50 μL reaction system	Final Conc.
5×GoldStar PCR Buffer	10 μL	1×
dNTP Mix，10 mM each	1 μL	200 μM each
Forward Primer，10 μM	2 μL	0.4 μM
Reverse Primer，10 μM	2 μL	0.4 μM
Template DNA	< 0.5 μg	< 0.5 μg/50 μL
GoldStar Best DNA Polymerase ， 5 U/μL	0.5 μL	2.5 U/50 μL
ddH_2O	up to 50 μL	

Note:

Please use the final concentration of 0.1-1.0 μM as the reference for the setting range of primer concentration. When the amplification efficiency is not high, the concentration of primers can be increased; when non-specific reactions occur, the concentration of primers can be decreased to optimize the reaction system.

2. PCR reaction conditions

Step	Temperature	Time	
predenaturation	95°C	3 min	
denaturation	94°C	30 s	30-35 cycles
annealing	55-65°C	30 s	
extend	72°C	60 s	
final extension	72°C	5 min	

Note:

1) In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer. When the ideal amplification efficiency cannot be obtained, the annealing temperature should be appropriately reduced; when non-specific reactions occur, the annealing temperature should be increased to optimize the reaction conditions.