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2×GoldStar Best MasterMix (Dye)

Cat. No. : CW0655M (5 ml)

Storage Condition: -20℃.Store at 2-8℃ for frequent use.

Components

Component	CW0655M 5 ml
2×GoldStar Best MasterMix (Dye)	5×1 ml
ddH_2O	5×1 ml

Note: 2×GoldStar Best MasterMix contains GoldStar Best DNA Polymerase, 3.4 mM MgCl2 and 400 μM each dNTP.

Introducntion

The product is a premix system composed of GoldStar Best DNA Polymerase, Mg^{2+}, dNTPs, PCR stabilizer and reinforcer, with a concentration of 2×. It has the advantages of simple and rapid operation, high sensitivity, strong specificity, good stability, and can minimize human error and contamination. The GoldStar Best DNA Polymerase included in the product is a chemically modified heat-activated hi-fi Polymerase. The Polymerase has 5 '-3' DNA Polymerase activity, 5 '-3' exonuclease activity and 3 '-5' exonuclease activity. Compared with the GoldStar Taq DNA Polymerase, the Polymerase has higher amplification efficiency and lower mismatch rate under normal PCR conditions. The chemically modified enzyme had no polymerase activity at room temperature and is effective in avoiding the nonspecific amplification caused by the nonspecific binding of primer and template or primer dimer at room temperature. The activation of the enzyme required incubation at 95℃ for 10 min, which could be integrated into the existing PCR thermal cycle program. The optimized buffer system maximizes the effect of the enzyme and achieves high-fidelity, high-specificity, high-amplification and high-sensitivity amplification of the target fragment. This product has been added dye (blue), and can be directly electrophoresis detected after the reaction. Most PCR products obtained by amplification have an "A" base attached to the 3 'end, so they can be directly used for T/A cloning. It is suitable for conventional PCR reaction and high fidelity gene cloning and other experiments.

Quality control

No exogenous nuclease activity was detected. The PCR method detected no residual host DNA. It can efficiently amplify single-copy genes from various genomes. There was no obvious change in activity when stored at 2-8℃ for 3 months.

Procedure

The following examples are the PCR reaction system and reaction conditions for the amplification of 1 KB fragment using human genomic DNA as template. In actual operation, corresponding improvements and optimization should be made according to the template, primer structure and the size of the target fragment.

1. PCR reaction system

reagent	50 μl reaction system	final concentration
2×GoldStar Best MasterMix（Dye）	25 μl	1×
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
ddH_2O	up to 50 μl	

Note: Primer concentration should take final concentration 0.1-1.0 μM as reference for setting range. When the amplification efficiency is not high, the primer concentration can be increased. When nonspecific reactions occur, the concentration of primers can be reduced to optimize the reaction system.

2. PCR reaction condition

Step	Temperature	Time	
Pre-denaturation	95℃	10 mins	
Denaturation	94℃	30 s	} 30-40 cycles
Annealing	55-65℃	30 s	
Extension	72℃	60 s	
Ternimal Extension	72℃	5 mins	

Note:

1) In general experiments, the annealing temperature is 5℃ lower than the melting temperature Tm of the amplification primer, and the annealing temperature should be lowered appropriately when the ideal amplification efficiency cannot be obtained. When the nonspecific reaction occurs, the annealing temperature is increased to optimize the reaction conditions.

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

3) Cycle number can be set according to downstream application of amplified products. If the number of cycles is too small, the increment of expansion is insufficient; If the number of cycles is too many, the mismatch rate will increase, and non-specific background will be serious; Therefore, the number of cycles should be reduced as far as possible under the premise of ensuring the product yield.

4) The product must be pre-denaturated at 95℃ for 10 min to activate the enzyme.