### Gold Multiplex PCR Mix

Project number: G666077

#### Storage conditions: -20° C.

#### Products content

Component	G666077
	5 ml
$2 \times Gold$ Multiplex PCR Mix	5  imes 1 ml
ddH20	5 imes 1 ml

#### **Product Introduction**

Gold Multiplex PCR Mix is a premixed system consisting of GoldStar DNA Polymerase, Mg<sup>2+</sup>, dNTPs, PCR stabilizers and enhancers. This product eliminates the need to optimize PCR reaction conditions, and allows for easy multiplex PCR reactions by simply exploring the conditions. GoldStar DNA Polymerase is a chemically modified hot-start enzyme that reduces non-specific amplification due to primer mismatches at the beginning of the PCR reaction. The enzyme is activated by incubation at 95° C for 10 minutes. The enzyme is coupled with a PCR enhancer that improves reaction specificity and a unique buffer system that allows for efficient extension of all primers in the reaction system without additional optimization. The MasterMix also includes a GC Enhancer, which helps to achieve efficient amplification of "difficult" templates, such as those with high GC content. Gold Multiplex PCR Mix is suitable for all types of multiplex PCR reactions, such as microsatellite analysis, genotyping and SNP detection.

#### quality control

No exogenous nuclease activity was detected; no host residual DNA was detected by PCR; no significant change in activity after storage at  $2-8^{\circ}$  C for three months.

#### Usage

The following are examples of conventional PCR reaction systems and conditions, which should be improved and optimized according to the template, primer structure and fragment size.

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1. PCR reaction system

reagents	50 µ 1	final
	reaction	concentr
	system	ation
$2 \times Gold$ Multiplex PCR	25 µl	$1 \times$
Mix		
Primer Mix, 10 µM each	1 µl	0.2 µM
Template DNA	appropriate	
	amount	
ddH20	up to 50 $\mul$	
ddH20	up to 50 µl	

Note: When designing primers, try to minimize the difference between the Tm of each primer, and keep the difference within 5°C. Please use the final concentration of 0.05-0.2  $\mu$ M as a reference for setting the concentration of each primer. If the amplification efficiency is not high, the concentration of primers can be increased; if non-specific amplification occurs, the concentration of primers can be decreased to optimize the reaction system.

1. PCR reaction conditions

move	temp	timing	/
premutability	95° C	10 min	/
denaturation	95° C	30 s	30-40 cycles
annealing (metallurgy)	55-65° C	30 s	30-40 cycles
reach	72° C	1 kb/min	30-40 cycles
ultimate extension	72° C	5 min	/

#### Attention:

(1) In general, the annealing temperature is  $5^{\circ}$  lower than the melting temperature of the amplification primer, Tm, and when the desired amplification efficiency cannot be obtained, the annealing temperature should be lowered appropriately; and when a non-specific reaction occurs, the annealing temperature should be increased, thus optimizing the reaction conditions.

(2) The extension time should be set according to the size of the amplified fragments, and the amplification efficiency of GoldStar DNA Polymerase included 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 low, the amount of amplification will be insufficient; if the number of cycles is too high, the chance of mismatch will increase

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and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.

(4) This product achieves enzyme activation under the condition of pre-denaturation  $95^{\circ}$  C, 10 min.