2xEsTaqMasterMix

Project number: E666027

Storage conditions: -20° C.

Product Content:

Component	E666027 5ml
2 × EsTaqMasterMix (forPAGE)	5 x 1ml
ddH2O	5 x 1ml

Note: 2 × EsTaqMasterMix contains EsTaqDNAPolymerase, 3mMMgCl2 and 400μMeachdNTP.

Products

It is a premixed system consisting of EsTaqDNAPolymerase, Mg^{2+}, dNTPs, as well as PCR stabilizers and enhancers at a concentration of 2×. EsTaqDNAPolymerase has the excellent performance of high amplification efficiency and low mismatch rate. The original MasterMix formulation makes the whole reaction system very stable, more than 98% of PCR amplification can be successful at one time, while complex templates can also be amplified effectively, and can minimize human error and contamination. The product does not contain dyes, and can be used for electrophoresis after the PCR program by adding appropriate amount of sample buffer as needed. Most of the amplified PCR products have an "A" base at the 3′ end, so they can be used directly for T/A cloning. It is mainly suitable for routine PCR reactions and gene cloning experiments requiring high fidelity, and the PCR amplified products are specialized for polyacrylamide gel electrophoresis detection.

quality control

Tested to be free of exogenous nuclease activity; no host residual DNA detected by PCR method; can efficiently amplify single-copy genes in a variety of genomes.

Usage

The following example shows the PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template, which should be improved and optimized according to the template, primer structure and size of the target fragment in actual operation.

1. PCR reaction system

reagents	50 μ l reaction system	final concentration
2 x EsTaqMasterMix (forDye)	25 μl	1×
ForwardPrimer, 10μM	2μl	0.4μM
ReversePrimer, 10μM	2μl	0.4μM
TemplateDNA	<0.5μg	<0.5μg/50 μl
ddH20	upto50μl	

Note: Please use the final concentration of 0.1-1.0μM as a reference for setting the range of primer concentration. If the amplification efficiency is not high, the concentration of primer can be increased; if a non-specific reaction occurs, the concentration of primer can be decreased, thus optimizing the reaction system.

2. PCR reaction conditions

move	temp	timing	/
premutability	94° C	2 min	/
denaturation	94° C	30 s	25-35 cycles
annealing (metallurgy)	55-65° C	30 s	25-35 cycles
reach	72° C	30 s	25-35 cycles
ultimate extension	72° C	2 min	/

Attention:

(1) In general, the annealing temperature is 5℃ lower than the melting temperature of the amplification primer Tm, and when the ideal amplification efficiency cannot be obtained, the annealing temperature is appropriately lowered; when a non-specific reaction occurs, the annealing temperature is increased, thus optimizing the reaction conditions.

2) The extension time should be set according to the size of the fragment being amplified, and the amplification efficiency of EsTaqDNAPolymerase is 2kb/min.

3) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, the amplification is insufficient; if the number of cycles is too high, the chance of mismatch will increase and the non-specific background will be serious. Therefore, the number of cycles should be minimized under the premise of ensuring the product yield.