2. Primer concentration setting:
   The primer concentration can be set between 0.1 μM and 1.0 μM. Too low primer concentration may result in fewer amplification products. High primer concentration inhibits specific amplification and may lead to non-specific amplification.
3. Annealing temperature setting:
   In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, so the annealing temperature can be appropriately reduced when the ideal amplification efficiency cannot be obtained. The annealing temperature can be raised appropriately when non-specific reaction occurs. For complex templates, it is necessary to adjust the annealing temperature to achieve efficient amplification.
4. Extension time setting:
   The extension time should be set according to the size of the amplified fragment. The recommended extension time is as follows:
   Simple template such as plasmid: 5-15 s/ kb; Routine genome and cDNA template: 10-15 s/ kb; Complex template, rough template: 20-30 s/ kb;
   (The extension time should not be too short, at least 5 s/ kb or more than 30 s/ kb).
5. Cycle number setting:
   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 high, the mismatch rate will increase, and the non-specific background will be serious. Therefore, the number of cycles should be reduced as far as possible before ensuring the product yield.

Version: 09/2022

2×Flash PCR MasterMix(Dye)

Cat. No. : CW3009M (5 mL)
CW3009H (40 mL)

Storage Conditions: -20°C

Components

Component	CW3009M 5 mL	CW3009H 40 mL
2×Flash PCR MasterMix (Dye)	5×1 mL	40×1 mL
ddH_2O	5×1 mL	40×1 mL

Introduction

The product is a new, highly efficient, fast DNA Polymerase premix system of Mg^{2+}, dNTPs, PCR stabilizers and enhancers at a concentration of 2×. This product is a new fast DNA polymerase created by Cowin. It has high amplification speed and stability, with an extension speed up to 5 s/kb and a minimum of 15 minutes to complete PCR. Long fragments (more than 3 kb) or complex templates can use 10-30 s/ kb extension speed or more cycles. The original MasterMix formula makes the whole reaction system very stable, while complex templates can be effectively amplified, more than 98% of PCR amplification can be successful at one time. When using, it only needs to add DNA template and primer, and then make up the water to react, which can minimize human error, reduce pollution and save time.

This product has been added dye (blue), after the reaction can be directly electrophoresis detection. The amplified PCR product with an "A" base attached at the 3 'end can be directly used for T/A cloning, and is suitable for Kangwei Seamless cloning kit (CW3034), T4 linkage kit (CW0805) and sensitive product (CW0812).

This product is mainly suitable for ultra-fast PCR, complex template, complex secondary structure, high fidelity gene cloning and other experiments and large-scale gene testing.

Quality control

No exogenous nuclease activity was detected. PCR was used to detect residual DNA without host. It can effectively amplify single copy genes in multiple genomes.

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.

PCR Reaction System

Reagent	50 μL	25 μL	20 μL	Final Conc.
2×Flash PCR MasterMix (Dye)	25 μL	12.5 μL	10 μL	1×
Forward Primer, 10 μM	2 μL	1 μL	0.8 μL	0.4 μM
Reverse Primer, 10 μM	2 μL	1 μL	0.8 μL	0.4 μM
Template DNA	<0.5 μg	<0.25 μg	<0.2 μg	<0.5 μg/50 uL
ddH_2O	up to 50 μL	up to 25 μL	up to 20 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.

PCR Reaction Condition

Step	Temperature	Time	
Initialization	98°C	30 s	
Denaturation	94°C	10 s	30-35 cycles
Annealing	55-65°C	15 s	
Elongation	72°C	5-15 s/kb	
Final elongation	72°C	1 min	

Note:

For simple templates, the pre-denaturation time can be controlled at 30 s-1 min, and for complex templates such as bacterial solution, the pre-denaturation time can be increased to 2 min.

Optimization parameter setting

1. Template DNA amount setting:

   Excessive templates may result in nonspecific amplification or smear. Template DNA in 50 μ L PCR reaction system is recommended

   Dosages is as follows:

   -Human genome DNA 5 ng-500 ng

   -E. coli genomic DNA 50 pg-100 ng

   -Plasmid DNA 10 pg-1 ng