3. **Some primers have no amplification or low amplification efficiency**
   (1) Increase the concentration of corresponding primers.
   (2) Excluding whether there is degradation of the DNA template. The DNA template can be diluted again after absolute quantification.
   (3) There may be a large difference in Tm value between primers, or a large difference in GC content between the target fragments, so the primers can be redesigned for amplification detection.
4. **The uniformity of library assays is poor**
   (1) The yield of shorter amplicon (≤100 bp) is low: The magnetic beads input can be increased in the PCR product purification process.
   (2) The yield of longer amplicons (≥500 bp) is low: Excluding whether there is degradation of the DNA template. The DNA template can be diluted again after absolute quantification. Or the extension time during the reaction procedure can be extended.
   (3) The yield of amplicon with high GC content (65%~75%) of the target fragment is low: The pre-denaturation time can be extended to 5-10 min, the denaturation time can be extended to 30 s, and the annealing time can be extended. Or 3%-6% DMSO can be added to the reaction system to promote the amplification of the high-GC target fragment.
   (4) The yield of amplicon with high AT content (65%~75%) of the target fragment is low: The annealing temperature can be reduced or the annealing time can be extended.

Related Products

Cat. No.	Product Name
CWY129	Magbead Blood DNA Kit
CW2298	Universal Genomic DNA Kit
CWY036	Cell-Free DNA Storage Tube
CWY041	Fecal DNA Storage Tube
CW3171	DNA Purification Magbeads (for NGS Size Selection)
CW3141	RNase and DNA Remover

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

TFP Multiplex PCR Mix

Cat. No. CW3351

Introduction

TFP Multiplex PCR Mix is a 2× master mix designed for multiplex PCR experiments and contains components such as DNA polymerase, PCR buffer, dNTPs, Mg^{2+}, stabilizers and enhancers. It is simple and rapid to operate.

The DNA polymerase contained in the TFP Multiplex PCR Mix is a genetically engineered recombinant enzyme with 5'→3' DNA polymerase activity and no 5'→3' exonuclease activity. It is a hot-start enzyme modified by novel antibodies, and can effectively reduce non-specific amplification (produced by the non-specific binding of primers and templates or primer dimers at room temperature), and has the excellent characteristics of short activation time, strong amplification ability, high sensitivity and good stability. The unique combination of PCR buffer system and hot-start enzyme significantly improves the amplification efficiency of PCR, with higher sensitivity and stronger inhibitor tolerance.

TFP Multiplex PCR Mix can be used in a wide range of applications for multiplex PCR experiments such as amplicon library preparation, microsatellite analysis, genotyping, and SNP detection.

Storage Conditions: -20±5 ℃
Storage Conditions: 2~8 ℃

Components

Component	CW3351S 1mL	CW3351M 5mL
2×TFP Multiplex PCR Mix	1mL	5mL
RNase-Free Water	1mL	5mL

Procedure

The following examples show the conventional reaction system and reaction conditions, which should be improved and optimized according to the different templates, primer structures, target fragment sizes and amplification effect.

1. PCR reaction system

Reagent	20 μL System	50 μL System
2×TFP Multiplex PCR Mix	10μL	25μL
Primer Mix[1)]	XμL	XμL
Template DNA[2)]	XμL	XμL
RNase-Free Water	Up to 20μL	Up to 50μL

Note:
1) The recommended final concentration of each primer in the reaction system is 0.2 μM, which can be adjusted between 0.1-0.3 μM. When designing primers, the difference between the Tm of each primer should be reduced as much as possible, and the difference should be controlled within 5 ℃ as much as possible. The primer concentration can be increased when the amplification efficiency is not high, and the primer concentration can be reduced in the case of non-specific reactions, so that the reaction system can be optimized. For optimal amplification, it is recommended that the primer mixture be used after brief centrifugation after vortexing for 10 seconds.
2) The amount of DNA input is recommended to be between 1 ng and 500 ng. Too low input may result in no detection of target fragments, and too high input may inhibit primers amplification. DNA contamination should be avoided during the operation, and it is recommended to set up a group of negative controls (no DNA) during the experiment.

2. PCR reaction program

Steps	Temperature	Time	Number of Cycles
Hot Lid	105℃	On	
Pre-denaturation	95℃	3-10min	1
Denaturation	95℃	10s	28-35cycles[2)]
Annealing	60℃[1)]	30s-4min[3)]	
Extension	72℃	1kb/min	
Terminal Extension	72℃	5min	1
Heat Preservation	4℃	Hold	

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 appropriately reduced when the ideal amplification efficiency cannot be obtained. When nonspecific reactions occur, increase the annealing temperature to optimize reaction conditions.
2) The number of cycles can be set according to the downstream application of the amplification product. If the number of cycles is too low, there is insufficient amplification. If the number of cycles is too high, the mismatch rate increases, leading to significant nonspecific background. Therefore, the number of cycles should be minimized on the premise of ensuring the yield of the product.
3) Adjust the annealing time based on the number of primers in the amplification system. When using a higher number of primers, consider extending the annealing time accordingly.
4) PCR products are prone to aerosol contamination, which leads to problems such as inaccurate experimental results and low reliability. It is recommended to physically isolate the PCR reaction system preparation area from the PCR reaction area, use special pipettes and other equipment, and clean each experimental area regularly (CW3141 RNase and DNA Remover are recommended) to ensure the reliability of the experimental results.

Frequently Asked Questions and Solutions

1. **The amplification yield is low**
   (1) Increase the number of cycles.
   (2) Reduce annealing temperature.
   (3) After absolute quantification of the DNA template, diluted the template again.
   (4) When the number of primers is large, the annealing time can be appropriately extended, and the PCR annealing time can be referred to the following table:

Number of Primers in Single Tube	Annealing Time
3-10	30s~1min
10-30	2min
30-800	4min
800 or more	8min

   (5) When purifying PCR products, the beads should not be dried for too long, as too much drying of the beads may make the product difficult to elute.

2. **Non-specific amplification is present**
   (1) Increase the annealing temperature.
   (2) Reduce the number of cycles.
   (3) Reduce primer input.
   (4) Reduce annealing and extension time.
   (5) Redesign primers.