4. Melting curve:
   (1) If a single peak appears in the melting curve, it indicates that the reaction specificity is good and can be quantified. But if the melting curve has a double peak or multiple peaks, it cannot be quantified.
   (2) If double peaks appear in the melting curve, it is necessary to determine whether the non-target peak is a primer-dimer or a non-specific amplification. In the case of primer-dimers, it is recommended to reduce the primer concentration or redesign the primer with high amplification efficiency. For non-specific amplification, increase the annealing temperature or redesign the primers with higher specificity.

Primer Design Guidelines

1. Primer length of approximately 25 bp is recommended. An optimal amplicon length of 150 bp is preferred, and selection can be made within the range of 100 bp to 300 bp.
2. The difference between the Tm values of the forward primer and the reverse primer should not exceed 2 ℃. Primer Tm value of 56 ℃-65 ℃ is preferred.
3. The primer base distribution should be uniform, avoid 4 consecutive identical bases, and control the GC content at about 50%. The last base at the 3' end is preferably G or C.
4. It is preferred to avoid complementary sequences with more than 3 bases inside the primer or between the two primers.
5. Primer specificity needs to be checked with the NCBI BLAST program. Avoid non-specific complementarity with more than 2 bases at the 3' end of primers.
6. The designed primers need to be tested for amplification efficiency, and only primers with the same amplification efficiency can be used for quantitative comparative analysis.

Related Products

Cat. No.	Product Name
CWY129	Magbead Blood DNA Kit
CW2298	Universal Genomic DNA Kit
CW0531	NuClean Plant Genomic DNA Kit
CWY036	Cell-Free DNA Storage Tube (glass, 10 mL)
CWY041	Fecal DNA Storage Tube
CW3371	HiFiScript All-in-one RT MasterMix for qPCR
CW0612	RNase-Free Water

Lyophilized SuperFast Universal SYBR Master Mix

Cat. No. CW3364

Introduction

Lyophilized SuperFast Universal SYBR Master Mix is supplied lyophilized as a proprietary mix of SYBR GreenI chimeric fluorescence method for qPCR reactions. It can be shipped and stored at room temperature before use. This master mix contains all the components required for PCR amplification except primers and DNA/cDNA samples, and can be used with only nuclease-free water for redissolution, which has many advantages such as strong specificity, high detection sensitivity, and high amplification yield. It is suitable for quantitative and qualitative analysis of DNA and cDNA samples, and can accurately detect in the dynamic range of up to 6 logs. This mix contains ROX Reference Dye, which is compatible with a wide range of qPCR instruments.

Storage Conditions: Store at room temperature

Storage Conditions: Store at room temperature
This product can be stored at room temperature for 12 months. For longer periods, it can be stored at 2-8 ℃ for 24 months. Ensure that the aluminum foil bag is in a vacuum state during storage. After opening the caps of the 8-strip tubes/cryovials or glass bottles, this product should be redissolved with nuclease-free water within 1 h (see the procedure for operations). The PCR master mix made after redissolution can be stored at -20 ℃ for 3 months and at 4 ℃ for 2 weeks.

Components

Component	CW3364T 24rxns	CW3364S 48rxns	CW3364M 100rxns
Lyophilized SuperFast Universal SYBR Master Mix	24rxns	48rxns	100rxns

Procedure

Redissolution

When using, remove the caps of the 8-strip tubes/cryovials or glass bottles, and add 10 μL of nuclease-free water per reaction (CW0612 is recommended). For 8-strip tube, add 10 μL of nuclease-free water for each tube to redissolve. For cryovials, add 270 μL of nuclease-free water for each tube to redissolve. For glass bottles, add 1100 μL of nuclease-free water for each bottle to redissolve. After redissolution, mix well by gently pipette or vortex to prepare 2 × master mix.

PCR Reaction System

1. After redissolution of Lyophilized SuperFast Universal SYBR Master Mix lyophilized reagent as described above, the system can be prepared directly. To use the redissolved master mix, remove it from -20 ℃ and thaw it completely at room temperature, and then configure the system after a short mixing and centrifugation.
2. According to the table below, determine the total volume for the appropriate reaction cycles, and prepare the PCR mixture for all components excluding DNA/cDNA templates.

Component	20 μL System	Final Concentration
Lyophilized SuperFast Universal SYBR Master Mix (2× Master Mix)	10μL	1×
Forward Primer，10 μM	0.4μL	0.2μM
Reverse Primer，10 μM	0.4μL	0.2μM
Template DNA	XμL	<1 μg(total DNA)
Nuclease-free water	Up to 20μL	

Note: If you choose CW3364S lyophilized 8-strip tubes product, you can add primers and templates directly.

3. Once the reaction system is set, gently pipette or vortex to mix well, and the liquid is collected to the bottom of the tube after a brief centrifugation.
4. During the preparation of the reaction system or aliquot into qPCR tubes/96-well plates, to obtain the best experimental results, it is necessary to ensure that the pipetting volume is basically the same during the operation process and minimize the generation of air bubbles.
5. When adding the DNA/cDNA template to the qPCR tube/96-well plates, seal with a transparent lid with good light transmittance and no breakage, and use a fluorescence PCR transparent sealing film. Paying special attention to tight the sealing lid and ensure that the edge corners of the plate are completely sealed to prevent aerosol contamination due to evaporation.
6. Before starting the reaction, the qPCR tubes/96-well plates should be centrifuged instantaneously (1 min at 2500-3000 rpm) to remove air bubbles and collect all the liquid to the bottom.

PCR Reaction Program

Use the SYBR®Green Reagents "Full Channel Scan" mode on the real-time PCR instrument.
For faster results, "Fast" programs can be used depending on the instrument (e.g., Applied Biosystems StepOnePlus, QuantStudio, 7500, etc.).

Steps	Temperature	Time	Cycle Number
Pre-Denaturation[1)]	95℃	5-30s	1
Denaturation[2)]	95℃	5-15s	40-45cycles
Annealing/Extension[3)]	60℃	10-30s *	
	95℃	15s	
Melting Curve[4)]	60℃	1min *	
	95℃	1s	

Note:1) This pre-denaturing condition is suitable for most amplification reactions. The standard procedure can choose 30 s, and the shortest option for fast procedure can be 5 s. If the template structure is complex, the pre-denaturation time can be extended to 3 min to improve the pre-denaturation effect.
2) The standard procedure can choose 10 s, and the shortest option for fast procedure can be 5 s.
3) The standard procedure can choose 30 s. For fast procedure, the shortest option can be 10 sec for amplicons within 200 bp, and 30 sec is recommended for amplicons over 200 bp.
4) Please set the recommended program of the fluorescence PCR instrument used for the analysis of the melting curve. This procedure is set with the ABI-Q5 fluorescence quantitative PCR instrument as the reference.
Set the signal acquisition at the * mark.

Result Analysis

1. For basic information on data analysis in a real-time PCR instrument, please refer to the user manual of the instrument.
2. Once the run is complete, check the amplification curve to ensure that the baseline threshold is set within the interval corresponding to the PCR index and above any background signal.
3. Amplification curve: The standard amplification curve is S-shaped.
   (1) Quantitative analysis is most accurate when the Ct value falls between 20-30.
   (2) If the Ct value is less than 10, the template needs to be diluted and the experiment should be re-performed.
   (3) When the Ct value is between 30-35, it is necessary to increase the template concentration or increase the volume of the reaction system to improve the amplification efficiency and ensure the accuracy of the analysis.
   (4) When the Ct value is greater than 35, the test results cannot quantitatively analyze the expression of genes, but can be used for qualitative analysis.