2.2 Annealing temperature: It is recommended to use two-step PCR, and set the annealing temperature as 60°C. To increase the specificity, increase the annealing temperature, which should be between 60-64°C. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program. The annealing temperature of the three- step PCR program should be between 56°C and 64°C.

2.3 Extension time: It is recommended to use two-step PCR and set the extension time as 1 minute. To increase amplification efficiency, increase the extension time, or try three-step PCR.

Note! The pre-denaturation reaction of this product must be completed at 95°C for 10 minutes!

Three-step QPCR method (this program uses ABI 7500 as an example)

Procedure	Temperature	Time	
Pre-denaturation	95°C	10 min	
Denaturation	95°C	10 s	35-40 cycles
Annealing	56-64°C	30 s	
Extension	72°C	32 s	
Melting curve analysis	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	60°C	15 s	

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95°C for 10 minutes to activate the enzyme.

2) If good amplification efficiency cannot be achieved, lower the annealing temperature appropriately. If there is non-specific reaction, increase the annealing temperature.

3) To increase amplification efficiency, increase extension time appropriately.

4) This program uses ABI 7500 as an example. The melting curve analysis should be set according to the procedure recommended by the Real-time PCR instrument used.

Version: 09/2023

UltraSYBR Mixture

Cat. No. : CW0957M CW2601M CW2602M (5 mL)

Shipping and Storage : -20°C; if used frequently, store at 2-8°C to avoid repeated freezing and thawing.

Components

Component	CW0957M (5 mL)	CW2601M (5 mL)	CW2602M (5 mL)
2×UltraSYBR Mixture	5×1 mL	-	-
2×UltraSYBR Mixture (Low ROX)	-	5×1 mL	-
2×UltraSYBR Mixture (High ROX)	-	-	5×1 mL
ddH_2O	5×1 mL	5×1 mL	5×1 mL

Principle

The UltraSYBR Mixture is a 2 × premixed reagent designed for SYBR Green I based fluorescence quantitative PCR assays. It contains GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, and Mg^{2+}. The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product contains the fluorescent dye SYBR Green I which can bind with all double-strand DNA, so that the product can be used for the detection of different target sequences without the need for the synthesis of specific labeled probes. The GoldStar Taq DNA Polymerase in the mixture is a chemically-modified, new efficient hot-start enzyme that has no polymerase activity at room temperature which prevents non-specific amplification efficiently, and it is activated by incubation at 95°C for 10 minutes. The combination of a unique PCR buffer system and a hot-start enzyme effectively inhibits non-specific PCR amplification and significantly increases the amplification efficiency of PCR.

Instruments that do not require ROX correction（CW0957）

Such as Roche LightCycler 480, Roche LightCyler 96, Bio-Rad iCyler iQ, iQ5, CFX96.

Instruments requiring Low ROX correction (CW2601)

Such as ABI Prism7500/7500 Fast, QuantStudio 3 System, QuantStudio 5 System, QuantStudio 6 Flex System, QuantStudio 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000.

Instruments requiring High ROX correction (CW2602)

Such as ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus.

Features

1. This product uses a new high-performance hot start enzyme (GoldStar Taq DNA Polymerase) and a unique PCR buffer system. This product significantly improves the PCR amplification efficiency and has high sensitivity and specificity.
2. This product is suitable for quantitative PCR detection and can accurately quantify and detect the target gene.

Precautions

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye. Avoid strong light irradiation when storing this product or preparing PCR reaction solution.
3. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may affect product performance.
4. This product cannot be used for probe-based qPCR.
5. When preparing the reaction solution, use new or non-contaminated pipette tips and centrifuge tubes to prevent contamination.

Protocol

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system

Reagent	50 μL system	Final concentration
2×UltraSYBR Mixture/2×UltraSYBR Mixture (Low ROX)/2×UltraSYBR Mixture(High ROX)	25 μL	1 ×
Forward Primer, 10μM	1 μL	0.2 μM
Reverse Primer, 10μM	1 μL	0.2 μM
DNA template	2 μL	
ddH_2O	Up to 50 μL	

Note: 1) Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and 1.0 μM.
2) Usually the amount of DNA template is 10-100 ng for genomic DNA or 1-10 ng for cDNA. As the template of different species contains different copies of the target gene, the template can be diluted by gradient to determine the best amount of template used.
3) The recommended reaction volume is 50 μL, and the reaction volume can also be scaled up or down according to actual experimental requirements.

2. PCR reaction program

Note! The pre-denaturation reaction of this product must be completed at 95°C for 10 minutes!
It is recommended to use two-step PCR reaction program. This program uses ABI 7500 as an example. If a good result cannot be obtained due to the low melting temperature (Tm) of the primers, please try a three-step PCR program.

Procedure	Temperature	Time	
Pre-denaturation	95°C	10 min	
Denaturation	95°C	15 s	35-40 cycles
Annealing/Extension	60°C	1 min	
Melting curve analysis	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	60°C	15 s	

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95°C for 10 minutes to activate the enzyme.
2) The annealing temperature should be between 60-64°C. If there is non-specific reaction, increase the annealing temperature.
3) This program uses ABI 7500 as an example. The melting curve analysis should be set according to the procedure recommended by the Real-time PCR instrument used.

Optimization of reaction conditions

When optimizing the qPCR reaction conditions, different aspects such as the concentration of the primer, the annealing temperature, and the extension time should be considered, to improve the reaction specificity and amplification efficiency.

1. The experimental system with high reaction specificity and high amplification efficiency should be as the following conditions:

1.1 High specificity: no non-specific amplification such as primer dimers for negative control; no other amplification beyond the target fragment.

1.2 High amplification efficiency: low Ct value; amplification efficiency of PCR is high, close to the theoretical value of 100%.

2. Optimization method of reaction conditions:

2.1 Primer concentration: Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 μM. To increase the specificity of the reaction, decrease the concentration of the primer; to increase the amplification efficiency, increase the concentration of the primer.