2. PCR reaction program

Procedure	Temperature	Time	
Pre-denaturation	95°C	20 s	
Denaturation	95°C	3 s	35-40 cycles
Annealing/Extension	60°C	30 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 20 seconds to activate the enzyme. Under this condition, most templates can be unchained well. For the templates with the high GC content and the complex secondary structure, the pre-denaturation time can be extended to 1 minute to fully unchain the starting template. If the high temperature treatment time is too long, it will affect the enzyme activity. The best pre-denaturation time can be determined according to the condition of the template.

2) It is suggested that the two-step PCR reaction procedure should be adopted. The annealing temperature should be 60-64°C as the reference of the set range. When the non-specific reaction occurs, the annealing temperature can be increased. If you can not get good experimental results due to the use of primers with low Tm value, you can try three-step PCR amplification. The annealing temperature should be set in the range of 56°C-64°C.

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.

Version: 09/2023

FastSYBR Mixture

Cat. No. : CW0955M CW2621M CW2622M (5 mL)

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

Components

Component	CW0955M (5 mL)	CW2621M (5 mL)	CW2622M (5 mL)
2×FastSYBR Mixture	5×1 mL	5×1 mL	5×1 mL
50× Low ROX	-	200 μL	-
50× High ROX	-	-	200 μL
ddH_2O	5×1 mL	5×1 mL	5×1 mL

Principle

The FastSYBR Mixture is a 2 × premixed reagent designed for SYBR Green I based fluorescence quantitative PCR assays. It contains Fast 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 Fast Taq DNA Polymerase contained in this product can effectively reduce the non-specific amplification produced by the non-specific binding of primers and templates or the primer dimer at room temperature, and it is activated by incubation at 95°C for 20 seconds. The whole PCR reaction process can save about 40 minutes compared with the ordinary reaction, which greatly shortens the reaction time of PCR. 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.

ROX dye is used to correct the fluorescence signal error between holes of quantitative PCR, and is generally used in Real-time PCR instruments of ABI, Stratagene and other companies. The excitation optical system of different instruments is different, so select the appropriate ROX reference dye according to the Real-time PCR instrument used.

Instruments that do not require ROX correction（CW0955）

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

Instruments requiring Low ROX correction (CW2621)

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 (CW2622)

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

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.

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× FastSYBR Mixture	25 μL	1 ×
Forward Primer, 10μM	1 μL	0.2 μM
Reverse Primer, 10μM	1 μL	0.2 μM
DNA template	2 μL	
50×Low ROX or High ROX（optional）	1 μL	1 ×
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. In the case of low amplification efficiency, the concentration of primers can be increased, and when non-specific reaction occurs, the concentration of primers can be reduced, thus the reaction system can be optimized.

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 excitation optical system of different instruments is different, and 50 × Low ROX or 50 × High ROX is added according to the Real-time PCR instrument used.