TB Green™ *Premix Ex Taq* ™ GC (Perfect Real Time) (Code RR071A)

Components [200 times (50 μℓ reaction system)]

1. TB Green™ *Premix Ex Taq*™ GC (Perfect Real Time) (2×conc.)[*1] 1 mℓ× 5
2. ROX Reference Dye (50×conc.)[*2] 200 μℓ
3. ROX Reference Dye II (50×conc.)[*2] 200 μℓ

*1 : Contains *TaKaRa Ex Taq* HS, dNTP Mixture, Mg^{2+} and TB Green™.

*2 : ROX Reference Dye and ROX Reference Dye II are intended to be used with instruments that correct for between-well fluorescent signal, such as the real-time PCR devices by Life Technologies.

◆ Use the ROX Reference Dye
- StepOnePlus™ Real-Time PCR System (Life Technologies)

◆ Use the ROX Reference Dye II
- Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies)

◆ Do not use this component
- Thermal Cycler Dice® Real Time System II (Code TP900/TP960)
- Smart Cycler® System/ Smart Cycler® II System (Cepheid)
- LightCycler®/LightCycler® 480 System (Roche Diagnostics)

Protocol

[For the Thermal Cycler Dice® Real Time System]

1. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume		Final conc.
TB Green™ *Premix Ex Taq*™ GC (2×)	12.5	μℓ	1 ×
PCR Forward Primer (10 μM)	0.5	μℓ	0.2 μM *1
PCR Reverse Primer (10 μM)	0.5	μℓ	0.2 μM *1
Template (<100 ng)	2	μℓ	*2
dH_2O (sterile distilled water)	9.5	μℓ	
Total	25	μℓ*3	

*1 : A final primer concentration of 0.2 μM is likely to yield good results. However, if there is insufficient reactivity, use a primer concentration between 0.1 and 1.0 μM.

*2 : The optimal quantity varies depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate template amount and use no more than 100 ng of DNA template. Furthermore, if cDNA (RT reaction mixture) is used as the template, the volume of the RT reaction mixture should not exceed 10% of the PCR mixture.

*3 : The recommended reaction volume is 25 μℓ.

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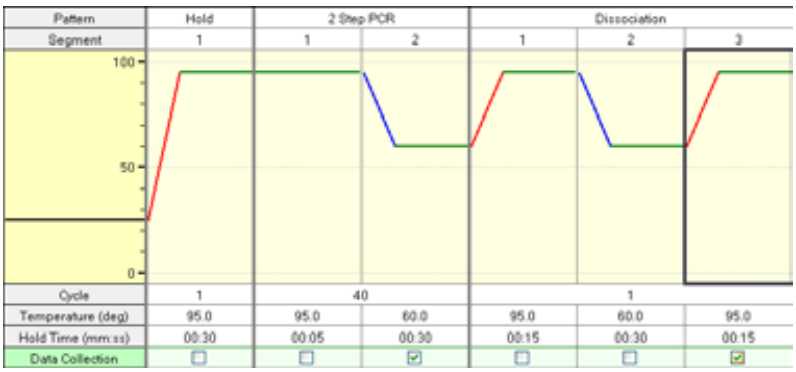
2. Start the reaction

The shuttle PCR standard protocol is recommended for PCR. Try this protocol first and optimize PCR conditions as necessary. To optimize PCR conditions further, please see "About the PCR conditions" on the bottom of this page.

Shuttle PCR standard protocol



Hold (initial denaturation)

Cycle: 1

95 ℃ 30 sec.

2 Step PCR

Cycle: 40

95 ℃ 5 sec.

60 ℃ 30 sec.

Dissociation

※ Note

・ *TaKaRa Ex Taq* HS is a hot start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95℃ for 30 sec. Longer heat treatment may decrease enzyme activity and affect amplification efficiency and quantification.

・ For initial denaturation before PCR, 95℃ for 30 sec. is sufficient.

3. After the reaction is complete, check the amplification and melting curves and plot a standard curve if quantification will be performed.

Refer to the instrument's instruction manual for specific analysis methods.

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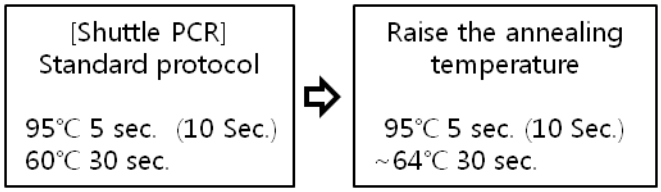
About the PCR conditions

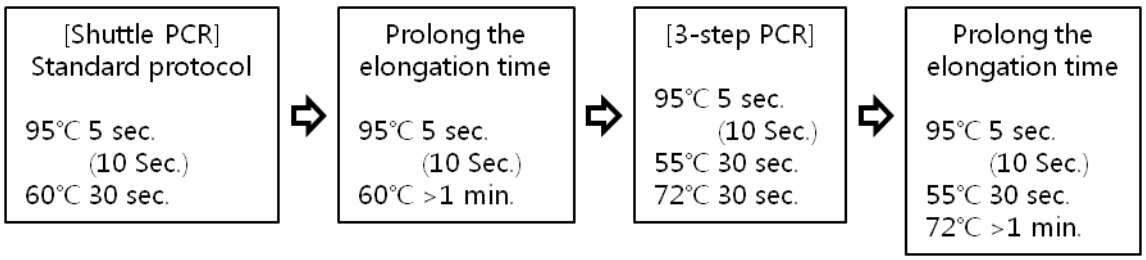
[To increase the reaction specificity]

Raising the annealing temperature may improve reaction specificity. Perform optimization while checking the balance with respect to amplification efficiency.



[To improve amplification efficiency]

Increasing the elongation time or switching to a 3-step PCR may improve amplification efficiency. Perform optimization using the steps below.



[Initial denaturation]

Generally, 95℃ for 30 sec. is sufficient for initial denaturation, even for difficult to denature templates such as circular plasmids and genomic DNA. This procedure may be extended to 1 to 2 min. at 95℃ depending on the template. However, prolonging this step may inactivate the enzyme. Therefore, it is recommended to avoid initial denaturation steps > 2 min.

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