Experimental Procedures

Resuspension Protocol

1

Resuspension of primer

1. Briefly centrifuge tubes containing primers to ensure that the pellet is located at the bottom of the tube.
2. Dissolve primers to a convenient stock concentration in nuclease-free water or 0.1X TE buffer (1 mM Tris, 0.1 mM EDTA).
3. Mix the primers by pipetting or vortexing briefly and spin down.
4. Store at -20°C in small aliquots and avoid repeated freeze and thaw cycles.

Real-time PCR Protocol

• This recommended protocol can be modified to get optimal results, according to real-time PCR instrument and target DNA sequences.

1

Preparation of reaction mixture

1. Add template DNA, primers, and nuclease-free water into *AccuPower*® GreenStar™ qPCR PreMix (K-6210, not provided) tubes to a total volume of 20 µl or 50 µl. Do not calculate the dried pellet.

- Preparation of reaction mixture

Components	20 µl reaction	50 µl reaction
Template DNA (40-200 ng)	Variable	Variable
Forward primer (10 pmol/µl)	1-2 µl	2-5 µl
Reverse primer (10 pmol/µl)	1-2 µl	2-5 µl
Nuclease-free water	Variable	Variable
Total volume	20 µl	50 µl

2. Seal real-time PCR tubes with an optical adhesive film (3111-4110, not provided).
3. Mix the reaction mixture by vortexing and briefly spin down.

2

Real-time PCR

4. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	1-5 min	1 cycle
Denaturation	95°C	5-20 sec	40-45 cycles
Annealing/Extension	55-60°C	40-45 sec	
Detection (Scan)			
Melting	-		1 cycle

*** Note:** For maximum yield and specificity, temperatures and cycling times should be optimized for each new template DNA or primers.