Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td colspan="3">Resuspension Protocol</td></tr>
<tr><td>1</td><td> 
Resuspension of primer</td><td>1. Briefly centrifuge tubes containing primers to ensure that the pellet is located at the bottom of the tube.

2. Dissolve primers in nuclease-free water or 0.1X TE buffer (1 mM Tris, 0.1 mM EDTA) as follows: for 100 reaction products, add 250 µl, and for 200 reaction products, add 500 µl, respectively.

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.</td></tr>
<tr><td colspan="3">Example of Real-time PCR Protocol
• AccuTarget™ Real-Time PCR Primer Library is verified with the following protocol using BIONEER's AccuPower® GreenStar™ qPCR PreMix.</td></tr>
<tr><td>2</td><td> 
Preparation of reaction mixture</td><td>1. Add template DNA, primers, nuclease-free water, and 50X ROX dye (optional, not provided) into AccuPower® GreenStar™ qPCR PreMix (Cat. No. K-6200, not provided) tubes to make a total volume of 50 µl. Do not calculate the dried pellet.

• Preparation of reaction mixture
<table><tr><th>Components</th><th>50 µl reaction</th></tr><tr><td>Template DNA (40-200 ng)</td><td>Variable</td></tr><tr><td>Forward primer</td><td>2.5 µl</td></tr><tr><td>Reverse primer</td><td>2.5 µl</td></tr><tr><td>(Optional) 50X ROX dye*</td><td>1 µl</td></tr><tr><td>Nuclease-free water</td><td>Variable</td></tr><tr><td>Total volume</td><td>50 µl</td></tr></table>* Note: ROX dye is used for normalization of intensity by background subtraction. The use of ROX dye is recommended for Applied Biosystems 7500 Real-Time PCR System, but not required for BIONEER Exicycler™ 96 Real-Time PCR System.

2. Seal real-time PCR tubes with an optical adhesive film (Cat. No. 3111-4110).

3. Mix the reaction mixture by vortexing and briefly spin down.</td></tr>
<tr><td>3</td><td> 
Real-time PCR</td><td>4. Perform the reaction under the following conditions.
<table><tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr><tr><td>Pre-denaturation</td><td>94°C</td><td>5 min</td><td>1 cycle</td></tr><tr><td>Denaturation</td><td>95°C</td><td>10 sec</td><td rowspan="3">40 cycles</td></tr><tr><td>Annealing</td><td>58°C</td><td>25 sec</td></tr><tr><td>Extension</td><td>72°C</td><td>30 sec</td></tr><tr><td>Detection (Scan)</td><td></td><td></td><td></td></tr><tr><td>Melting</td><td>-</td><td></td><td>1 cycle</td></tr></table>5. After the reaction is completed, analyze the results.</td></tr>
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