Prepare the Reactions

1 Prepare the experimental reactions by combining the components of the reagent mixture in the order listed in the table below. Prepare a single reagent mixture for replicate reactions (plus at least one reaction volume excess) using multiples of each component. Keep the reagent mixture on ice.

<table>
<thead>
<tr>
<th>Reagent Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free PCR-grade water to bring final volume to 20 µl (including RNA)</td>
</tr>
<tr>
<td>10 µl of 2× SYBR Green QRT-PCR Master Mix</td>
</tr>
<tr>
<td>x µl of upstream primer at optimized concentration (150–500 nM)</td>
</tr>
<tr>
<td>x µl of downstream primer at optimized concentration (150–500 nM)</td>
</tr>
<tr>
<td>0.2 µl of 100 mM DTT</td>
</tr>
<tr>
<td>1 µl of RT/RNase Block</td>
</tr>
</tbody>
</table>

2 Gently mix the reagent mixture without creating bubbles, then distribute the mixture to the experimental reaction tubes. Keep the reactions on ice.

3 Add x µl of experimental RNA to each reaction to bring the final reaction volume to 20 µl. The table below lists a suggested quantity range for different RNA templates.

<table>
<thead>
<tr>
<th>RNA</th>
<th>Quantity per reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA</td>
<td>0.1 pg – 100 ng</td>
</tr>
<tr>
<td>mRNA</td>
<td>0.1 pg – 1 ng</td>
</tr>
</tbody>
</table>

4 Mix the reactions without creating bubbles, then centrifuge briefly.
Set Up the QPCR Plate and Thermal Profile

1. From the New Run screen, click the **Advanced** tab to access the **Advanced Wizard** options.
2. Select the **Two Step with Melt** template and click **New**.
3. Use the boxes of the wizard to make selections appropriate for your experiment.

   *In the Temperature Profile box, click Edit to open the Profile Editor. Adjust the cycling protocol according to the table below.*

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Duration of Cycle</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 minutes</td>
<td>50°C</td>
</tr>
<tr>
<td>1</td>
<td>3 minutes</td>
<td>95°C</td>
</tr>
<tr>
<td>40</td>
<td>10 seconds</td>
<td>95°C</td>
</tr>
<tr>
<td></td>
<td>10 –20 seconds*</td>
<td>60°C</td>
</tr>
</tbody>
</table>

* The exact annealing/extension time needs to be optimized for each target.

Run the PCR Program

1. Place the reactions in the Rotor-Gene Q instrument.
2. On the last screen of the wizard click **Start Run**.

Analyze Data

1. Analyze the results of the run as needed for your experiment.

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