

QIAGEN Supplementary Protocol

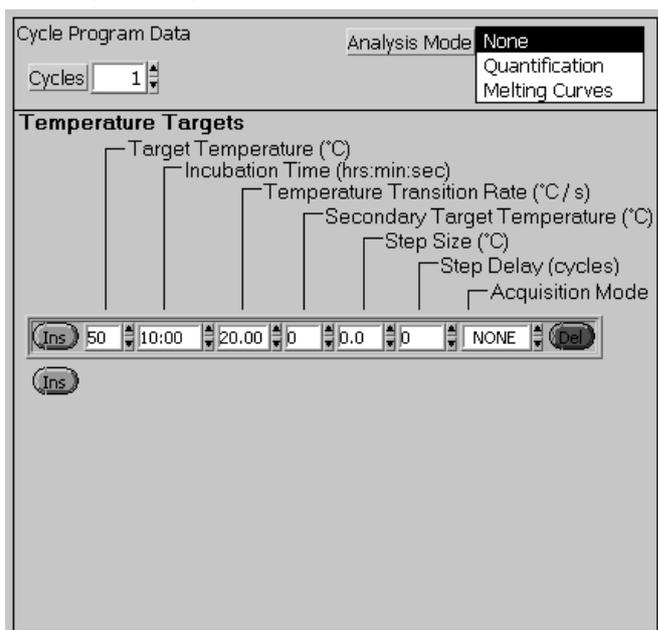
LightCycler® 1.x software setup for the QuantiFast® Probe RT-PCR + ROX Vial Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 1.x software (version 3.5) when using the QuantiFast Probe RT-PCR + ROX Vial Kit.

IMPORTANT: Please read the *QuantiFast Probe RT-PCR Handbook*, paying careful attention to the “Safety Information” section, before beginning this procedure.

Procedure

1. Launch the software and set up the cycle program data as described in the next few steps.
2. Select “None” in “Analysis Mode” and set up the parameters for the reverse-transcription step as shown below.



Cycle Program Data

Analysis Mode: None

Cycles: 1

Quantification
Melting Curves

Temperature Targets

Target Temperature (°C): 50

Incubation Time (hrs:min:sec): 10:00

Temperature Transition Rate (°C/s): 20.00

Secondary Target Temperature (°C): 0

Step Size (°C): 0.0

Step Delay (cycles): 0

Acquisition Mode: NONE

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3. Select "None" in "Analysis Mode" and set up the parameters for the initial activation step as shown below.

Cycle Program Data

Analysis Mode: **None**
 Quantification
 Melting Curves

Cycles: 1

Temperature Targets

Target Temperature (°C): 95
 Incubation Time (hrs:min:sec): 5:00
 Temperature Transition Rate (°C/s): 20.00
 Secondary Target Temperature (°C): 0
 Step Size (°C): 0.0
 Step Delay (cycles): 0
 Acquisition Mode: NONE

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4. If using dual-labeled probes, select "Quantification" and set up the parameters for PCR cycling as shown below. Be sure to select "SINGLE" for "Acquisition Mode" at the 60°C step.

Cycle Program Data

Analysis Mode: **Quantification**
 None
 Melting Curves

Cycles: 40

Temperature Targets

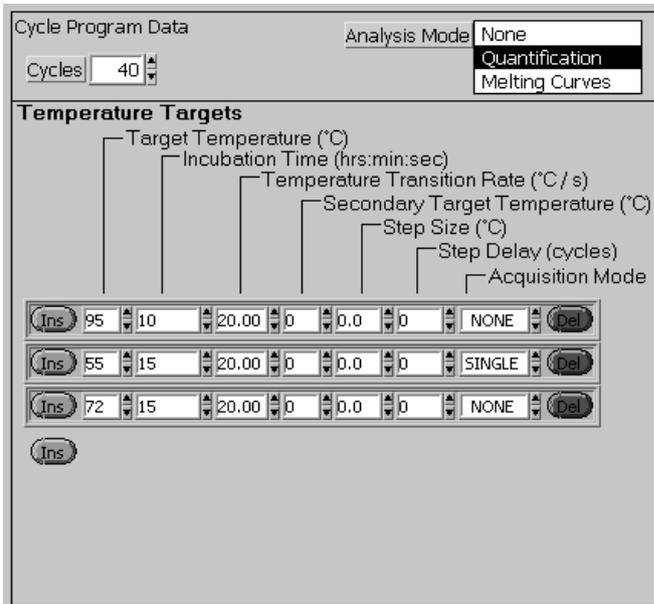
Target Temperature (°C): 95
 Incubation Time (hrs:min:sec): 10
 Temperature Transition Rate (°C/s): 20.00
 Secondary Target Temperature (°C): 0
 Step Size (°C): 0.0
 Step Delay (cycles): 0
 Acquisition Mode: NONE

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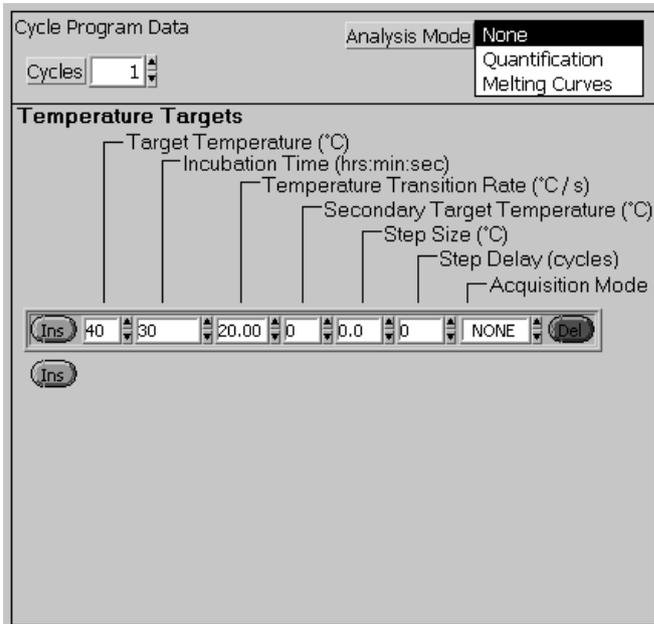
Target Temperature (°C): 60
 Incubation Time (hrs:min:sec): 30
 Temperature Transition Rate (°C/s): 20.00
 Secondary Target Temperature (°C): 0
 Step Size (°C): 0.0
 Step Delay (cycles): 0
 Acquisition Mode: SINGLE

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If using FRET probes, select “Quantification” and set up the parameters for PCR cycling as shown below. Be sure to select “SINGLE” for “Acquisition Mode” at the 55°C step.



5. Select “None” and set up the parameters for cooling as shown below.



6. Load your PCR capillaries and start the program.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

The QuantiFast Probe RT-PCR +ROX Vial Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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