QIAGEN Supplementary Protocol

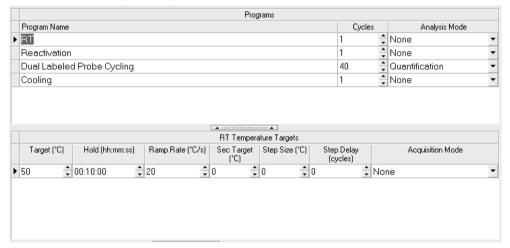
LightCycler[®] 2.0 software setup for the QuantiFast[®] Probe RT-PCR +ROX Vial Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 2.0 software (version 4.0) 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.

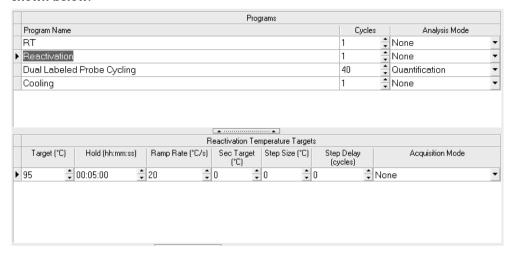
Procedure (for dual-labeled probes)

- 1. Launch the software and set up the programs as described in the next few steps.
- Create 4 programs and name them, for example, "RT", "Reactivation", "Dual-Labeled Probe Cycling", and "Cooling". Select "RT" and set up the parameters for the reverse-transcription step as shown below.

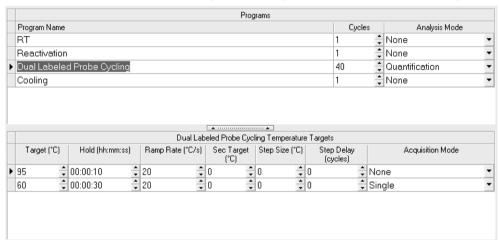




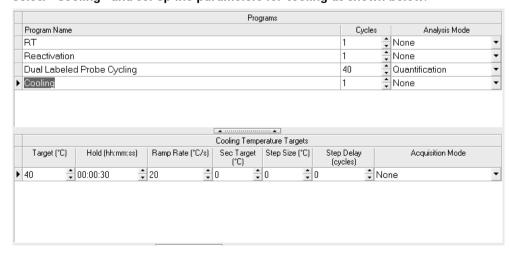
3. Select "Reactivation" and set up the parameters for the initial activation step as shown below.



4. Select "Dual-Labeld Probe Cycling" and set up the parameters for PCR cycling as shown below. Be sure to select "Single" for "Acquisition Mode" at the 60°C step.



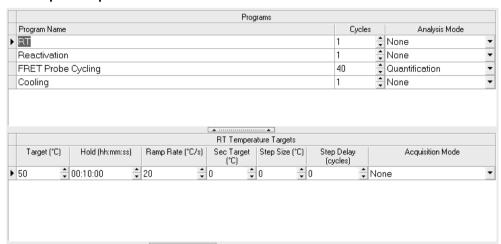
5. Select "Cooling" and set up the parameters for cooling as shown below.



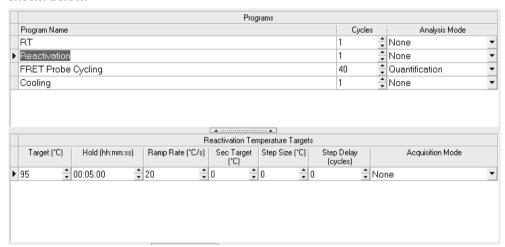
6. Load your PCR capillaries and start the program.

Procedure (for FRET probes)

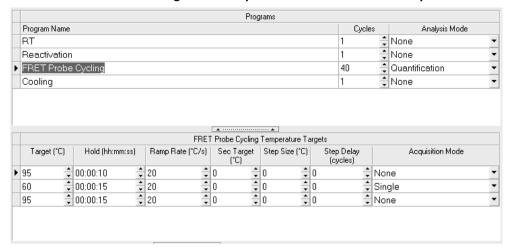
- 1. Launch the software and set up the programs as described in the next few steps.
- 2. Create 4 programs and name them, for example, "RT", "Reactivation", "FRET Probe Cycling", and "Cooling". Select "RT" and set up the parameters for the reverse-transcription step as shown below.



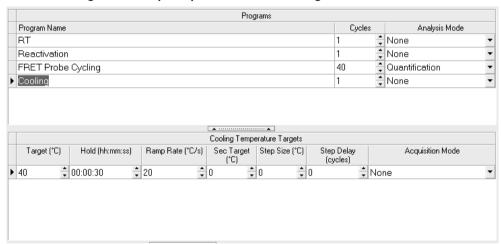
Select "Reactivation" and set up the parameters for the initial activation step as shown below.



4. Select "FRET Probe Cycling" and set up the parameters for PCR cycling as shown below. Be sure to select "Single" for "Acquisition Mode" at the 60°C step.



5. Select "Cooling" 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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