QIAGEN Supplementary Protocol:

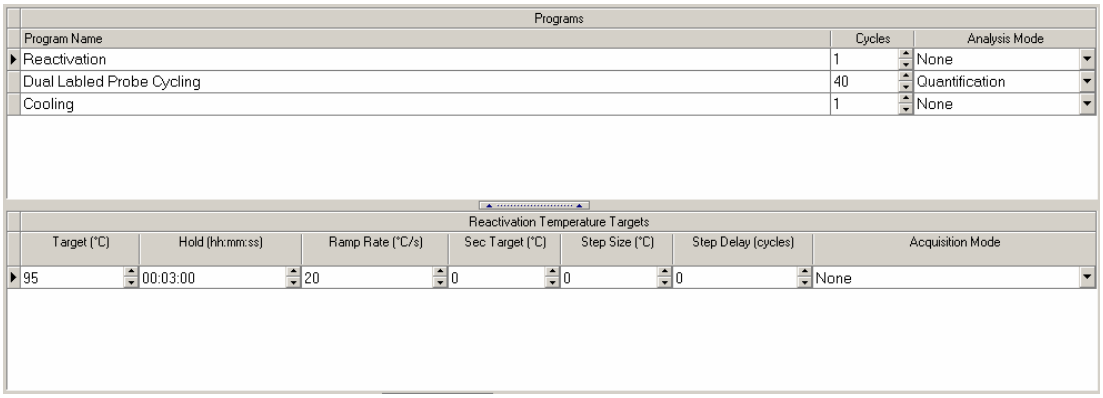
LightCycler® 2.0 Software Setup for the QuantiFast™ Probe PCR +ROX Vial Kit

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 PCR +ROX Vial Kit.

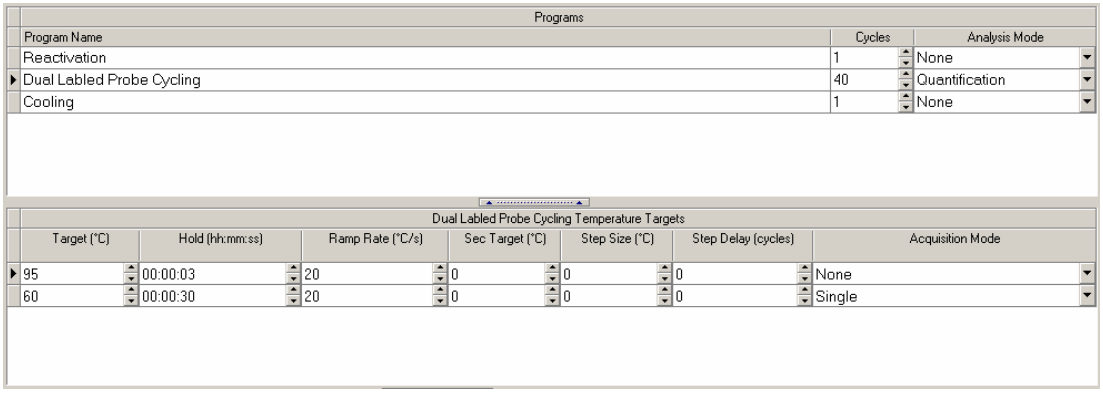
IMPORTANT: Please read the *QuantiFast Probe PCR Handbook*, paying careful attention to the safety information, before beginning this procedure. The QuantiFast Probe PCR +ROX Vial Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Procedure (for dual-labeled probes)

1. **Launch the software and set up the programs as described in the next few steps.**
2. **Create 3 programs and name them, for example, "Reactivation", "Dual-Labeled Probe Cycling", and "Cooling". Select "Reactivation" and set up the parameters for the initial activation step as shown below.**



3. **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.**



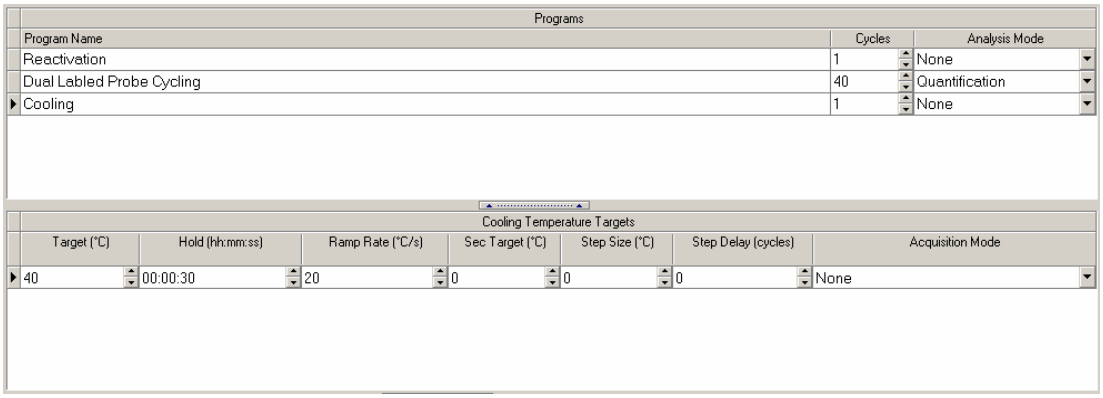
Programs

Program Name	Cycles	Analysis Mode
Reactivation	1	None
Dual Labled Probe Cycling	40	Quantification
Cooling	1	None

Dual Labled Probe Cycling Temperature Targets

Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
95	00:00:03	20	0	0	0	None
60	00:00:30	20	0	0	0	Single

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



Programs

Program Name	Cycles	Analysis Mode
Reactivation	1	None
Dual Labled Probe Cycling	40	Quantification
Cooling	1	None

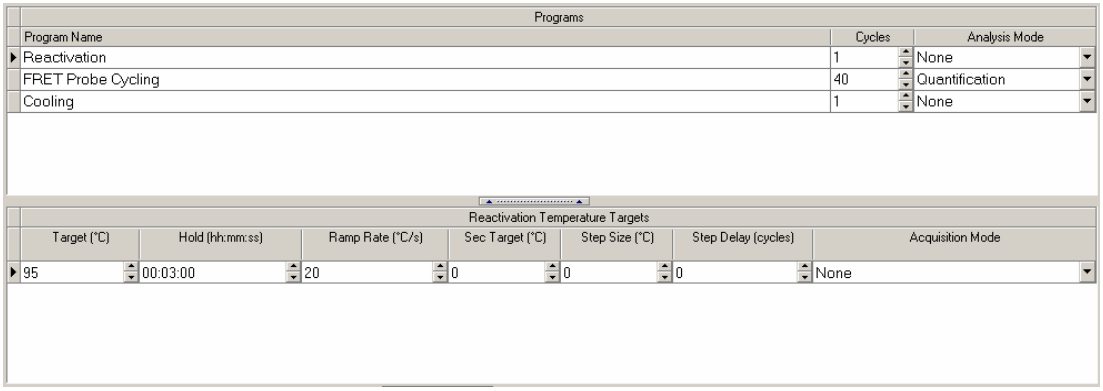
Cooling Temperature Targets

Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
40	00:00:30	20	0	0	0	None

5. **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 3 programs and name them, for example, "Reactivation", "FRET Probe Cycling", and "Cooling". Select "Reactivation" and set up the parameters for the initial activation step as shown below.**



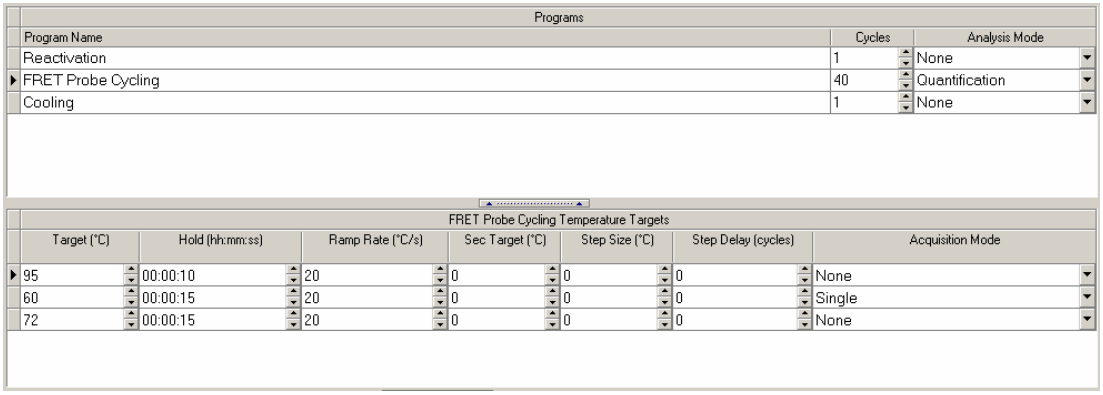
Programs

Program Name	Cycles	Analysis Mode
Reactivation	1	None
FRET Probe Cycling	40	Quantification
Cooling	1	None

Reactivation Temperature Targets

Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
95	00:03:00	20	0	0	0	None

3. **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.**



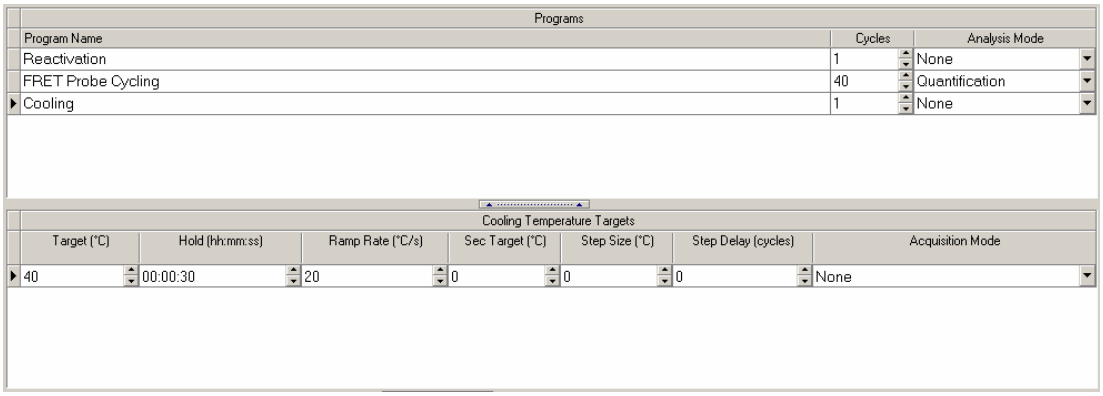
Programs

Program Name	Cycles	Analysis Mode
Reactivation	1	None
FRET Probe Cycling	40	Quantification
Cooling	1	None

FRET Probe Cycling Temperature Targets

Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
95	00:00:10	20	0	0	0	None
60	00:00:15	20	0	0	0	Single
72	00:00:15	20	0	0	0	None

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



Programs

Program Name	Cycles	Analysis Mode
Reactivation	1	None
FRET Probe Cycling	40	Quantification
Cooling	1	None

Cooling Temperature Targets

Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)	Acquisition Mode
40	00:00:30	20	0	0	0	None

5. **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/handbooks/default.aspx .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .

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