

Protocol Sheet

Roche® LightCycler® 480 real-time PCR run setup instructions for qBiomarker Somatic Mutation PCR Arrays

Important points before starting

- Please read the handbook supplied with the qBiomarker Somatic Mutation PCR Array, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.
- Make sure the real-time PCR instrument is working properly. Refer to the manufacturer’s Installation and Maintenance manual if needed.
- Make sure the correct thermal block cycler (384-well block format for G plate or 96-well for F plate) is in place.
- Turn on the LightCycler 480 instrument; the main switch is located at the back of the instrument. Turn on the computer linked to the instrument and log on to Windows® XP.

Procedure

Creation of PCR protocol template

1. Start LightCycler 480 software 1.5.0 SP3 (version 1.5.0.39).
2. Enter “Username” and “Password” when the “Login” dialog box appears and click the  button to proceed with the login. The “Overview” window will appear.
3. Wait for the initiation of the instrument to finish and the two status LEDs on the front of the instrument to become stable. The left LED should become a steady green while the right LED should be a steady orange.
4. In the “Overview” window (Figure 1), click the “New Experiment” button on the right-hand side of the window to launch the “Run” module.



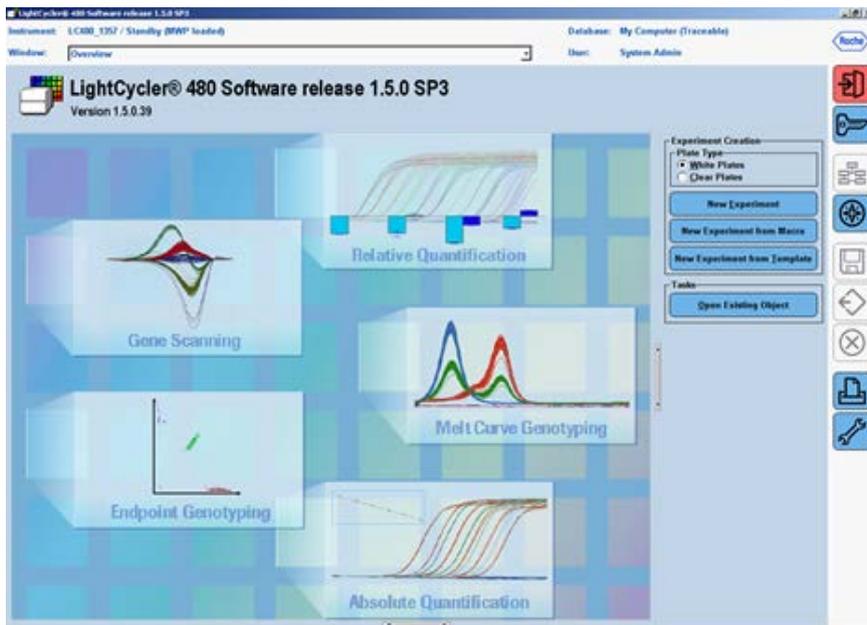


Figure 1. "Overview" window.

5. In the "Setup" area at the top of the "Run Protocol" tab (Figure 2), specify the following setup parameters:
 - "Detection Format": "Monocolor Hydrolysis probe" (click on "Customize" to make sure active filter combination is "FAM(483-533)")
 - "Block Type": should show the correct block format (384 or 96)
 - "Plate ID": (Optional)
 - "Reaction Volume": "10 μ l" for G plate or "25 μ l" for F plate

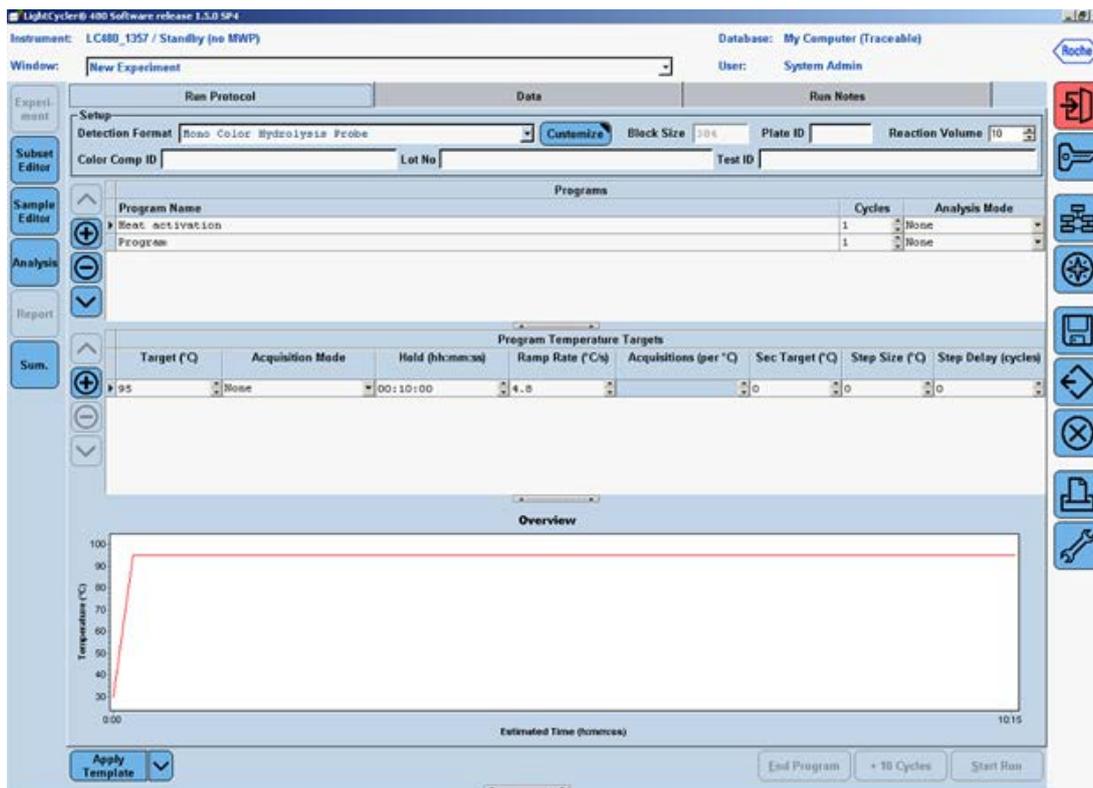


Figure 2. Initial "Run Protocol" window.

6. In the "Programs" section, click the  button twice to add two more program lines.
7. Click to change the "Program Name" of the first program to "Heat Activation". Leave the number of "Cycles" at "1" and leave the "Analysis Mode" as "None". In the "Program Temperature Targets" section, enter the values for the following parameters (see Figure 3):

Target (°C)	Acquisition mode	Hold (hh:mm:ss)	Ramp rate (°C/s)	Acquisition (per °C)	Sec target (°C)	Step size (°C)	Step delay (cycles)
95	None	00:10:00	4.8*		0	0	0

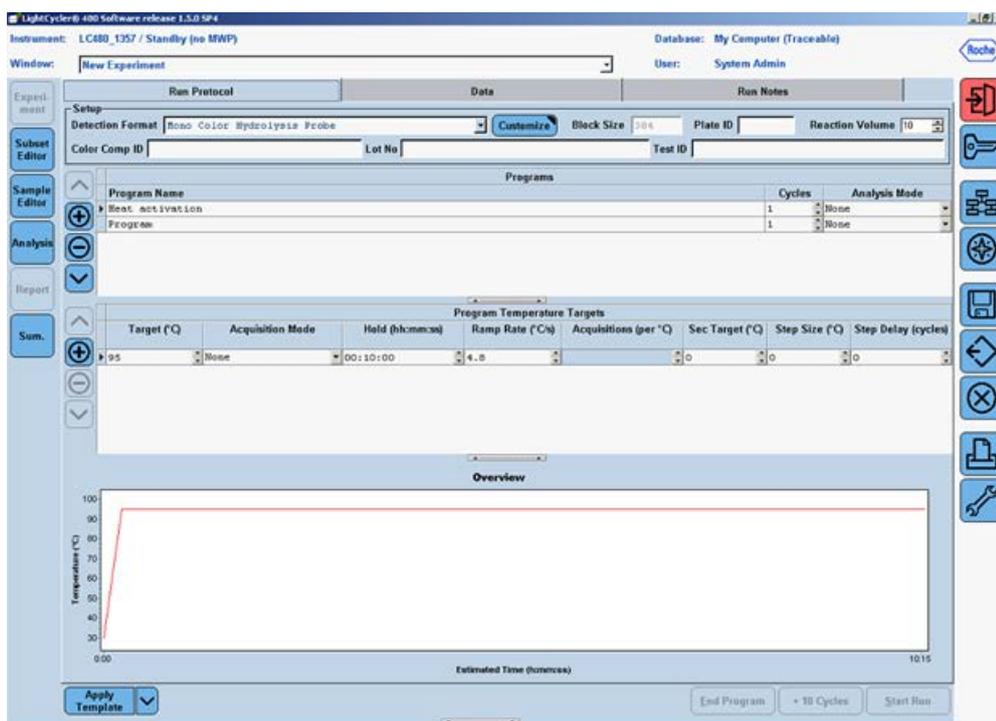


Figure 3. Setup for the first program — “Heat Activation.”

- Click to change the “Program Name” of the 2nd program to “PCR Cycling”. Change the number of “Cycles” to “45”[†] and change the “Analysis Mode” to “Quantification”. In the “Temperature Targets” section, click the  button twice to add two more temperature target lines. Enter the values for the following parameters (Figure 4):

Target (°C)	Acquisition mode	Hold (hh:mm:ss)	Ramp rate (°C/s)	Acquisition (per °C)	Sec target (°C)	Step size (°C)	Step delay (cycles)
95	None	00:00:15	1.0		0	0	0
60	None	00:01:00	1.0		0	0	0
70	Single	00:00:30	1.0		0	0	0

[†] The PCR is run for extra five cycles (i.e. 45 cycles) rather than the typical 40 cycles, as this will allow the use of the Second Derivative Maximum method available with the LightCycler 480 software for data analysis. See Data Analysis section for further details.

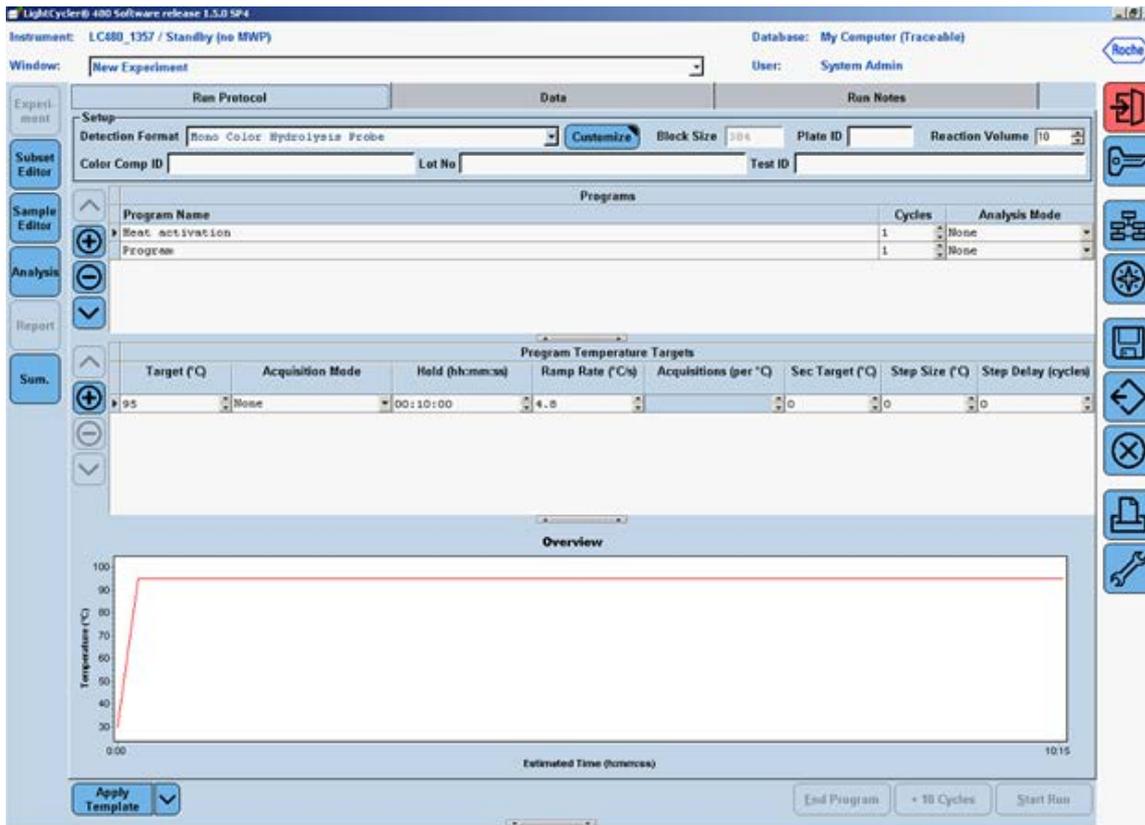


Figure 4. Setup of the second program — “PCR Cycling.”

- Click the down arrow button (▼) right next to the “Apply Template” button on the lower left corner of the screen; select the “Save As Template” button (Figure 6) to save the run template under the filename “qBiomarker_Mutation_PCR_Array_384_LC” if using the 384-well format or “qBiomarker_Mutation_PCR_Array_96_LC” if using the 96-well format.

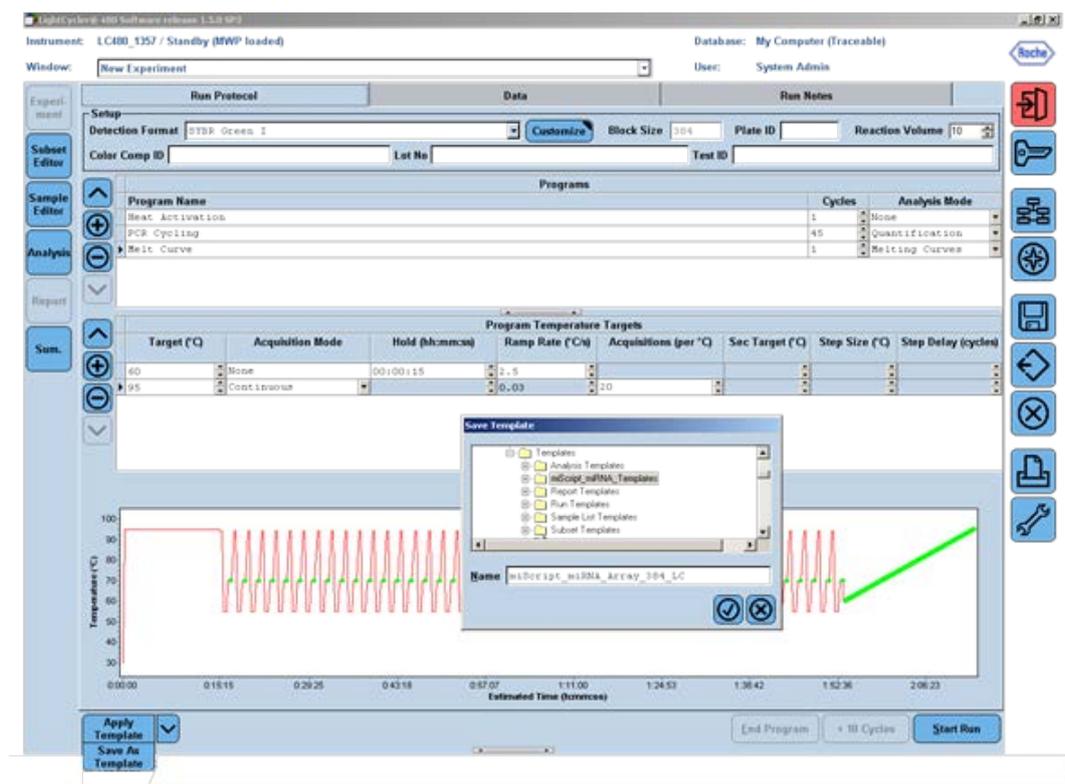


Figure 5. Saving the “Run Template” file.

Real-time PCR detection

To load the PCR array plate and start the run:

10. Prepare the PCR array following the *qBiomarker Somatic Mutation PCR Array Handbook*.
11. Make sure the correct thermal block (384-well block format for G plate or 96-well for F plate) is in place.
12. If the instrument is not on, turn it on using the main switch at the back of the instrument. Turn on the computer linked to the instrument and log on to Windows XP.
13. Start LightCycler 480 software (version 1.5.0.39).
14. Enter “Username” and “Password” when the “Login” dialog box pops up and click the  button to proceed with the login. The “Overview” window will appear.
15. Wait for the two status LEDs on the front of the instrument to become stable. The left LED should become a steady green while the right LED should be a steady orange.
16. Press the \leftrightarrow open/close button on the front of the instrument (located next to the instrument status LEDs). The plate loading arm will slide out of the right side of the instrument. Place the PCR array plate into the frame of the loading arm with the flat

edge of the array closest to the instrument and the edge with a beveled corner pointing away from the instrument.

17. Press the \leftrightarrow open/close button again to retract the loading arm. Wait for both of the status LEDs to turn steady green.
18. In the "Overview" window, click the "New Experiment" button on the right-hand side of the screen.
19. In the "Run Protocol" tab, click the "Apply Template" button on the lower left corner of the screen. Select and double-click to open the appropriate previously saved "qBiomarker_Mutation_PCR_Array_384_LC" or "qBiomarker_Mutation_PCR_Array_96_LC" run template file.
20. Press the "Start Run" button on the lower right corner of the screen to initiate the run. When prompted, save the file under a new filename after which the run will begin.

Data analysis

21. In the "Navigator" window, find and open the run file to be analyzed.
 - For data analysis using the LightCycler 480, we highly recommend the use of the Second Derivative Maximum analysis method available in this software. This is an algorithm based on the kinetics of a PCR reaction. This method identifies the crossing point (Cp) of a PCR reaction as the point where the reaction's fluorescence reaches the maximum of the second derivative of the amplification curve, which corresponds to the point where the acceleration of the fluorescence signal is at its maximum. Hence, this crossing point should always be located in the middle of the log-linear portion of the PCR amplification plot. The advantage of this analysis method is that it requires little user input and produces consistent results.
22. Obtaining the Crossing Point (Cp) Cycle values: Once the selected run file is open, click the "Analysis button" on the left-hand side of the window. In the "Create new analysis" list, double-click to select "Abs Quant/2nd Derivative Max". In the "Create new analysis" window that opens, make sure the "Analysis Type" is "Abs Quant/2nd Derivative Max". Click  to confirm the selection.
23. The "Analysis Window" with the amplification curves will appear. To proceed, click the down arrow button () on the lower right corner of the screen to set the analysis to "High Confidence." Next click the "Calculate" button to generate Cp values. The calculation may take a few minutes.

24. Once the Cp values are generated, move the mouse over to the "Samples" window and click to highlight the Samples result table. Right click the "Export" button to export the data. A "Save table data" dialog box will appear (Figure 10). Enter a filename to save the data as a text file. Then click the  button on the right panel to save the Second Derivative Maximum analysis and the Cp values as part of the run file.

The qBiomarker Somatic Mutation PCR Arrays are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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