Protocol Sheet

Bio-Rad iCycler® and iQ™ 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.

■ Please make sure the real-time PCR instrument is working properly. Refer to the manufacturer's Installation and Maintenance manual if needed.

Procedure

Creation of PCR protocol template

1. Open the iCycler iQ Optical System software v3.1 on the computer that is connected to the Bio-Rad iCycler iQ Real-time PCR Detection system.

2. Click on the "Library" module on the left panel. Select the "View Protocol" tab. Under "Protocol Files," select "2Step.tmo." Click "Edit This Protocol" on the right; this will open the "Edit Protocol" tab in the "Workshop" module. Edit the thermal profile in the table at the bottom of the screen as follows (see Figure 1):

   ▪ Click on the "Dwell Time" cell of Cycle 1, Step 1 and change the value to 10:00 minutes with Setpoint 95.0°C.

   ▪ For Cycle 2, Step 1, change the "Dwell Time" value to 0:15 (15 seconds).

      • For Cycle 2, Step 2, change the "Dwell Time" value to 1:00 (1 minute) with Setpoint 60.0°C.

      • Verify that the value in the "Repeats" cell in Cycle 2 is 40.

   ▪ Under "Select data collection step(s)," double-check that "REAL-TIME" detection is selected for Cycle 2, Step 2.

   ▪ Enter "qBiomarker_Mutation_PCR_Array_Template" as the new filename in the field for "Protocol Filename." Choose "Save this Protocol" to save the template.
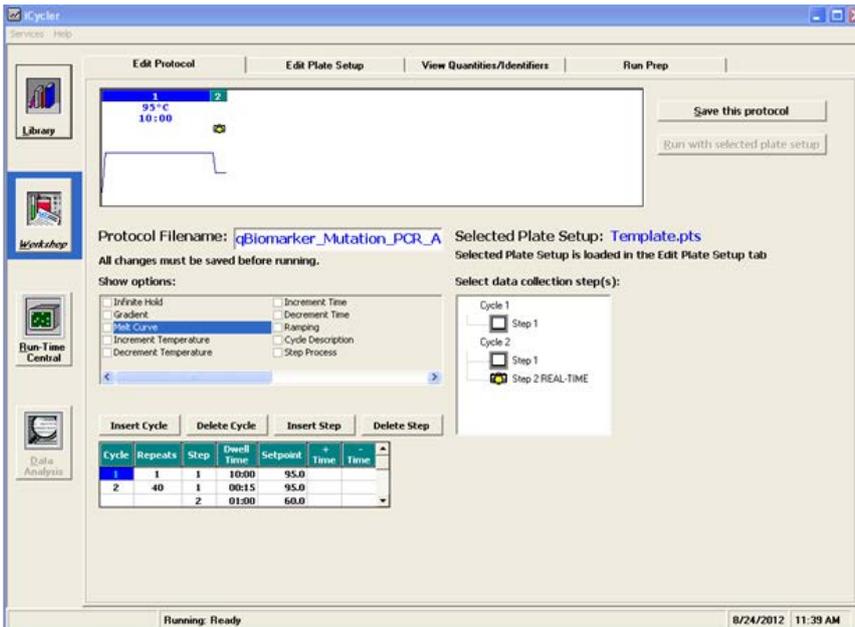
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Figure 1. Protocol setup.

3. To set up a template file for "Plate Setup," click on the "Library" module on the left panel and then select the "View Plate Setup" tab; click "Create a New Plate Setup" on the right. This will open the "Edit Plate Setup" tab in the "Workshop" module. Choose the "Whole Plate" mode. In the "Samples: Whole Plate loading" tab (see Figure 2), click to select the icon for "Unknown." In the diagram of the 96-well plate, click on each well one at a time to designate the sample type as "Unknown" and number the well.
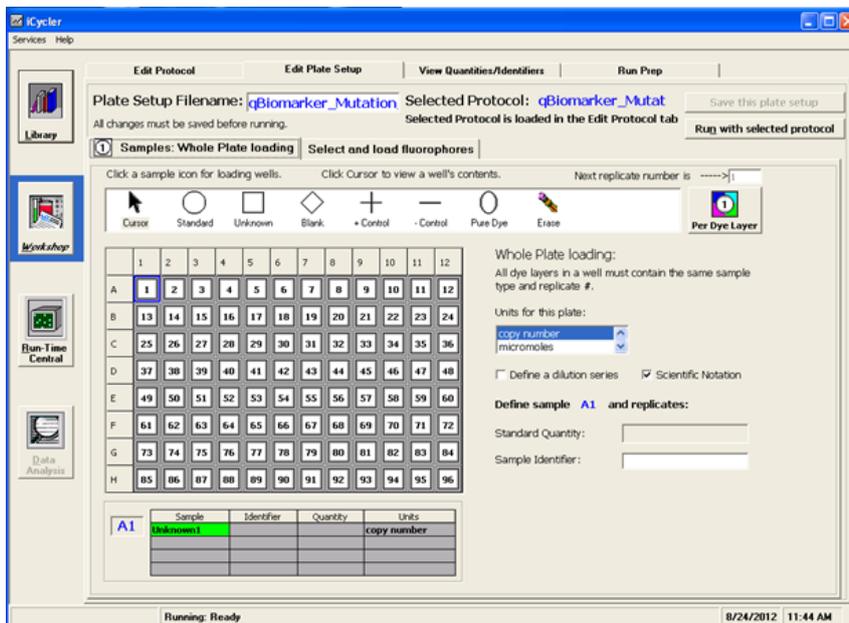

Figure 2. Plate setup (1).

- When finished loading samples, click on "Select and load fluorophores" (see Figure 3). Select "FAM" as the fluorophore by clicking the box next to the fluorophore in the "Select or Deselect a Fluorophore" box. "Assign a color" for FAM by clicking on one of the crayons. With the "FAM" crayon selected, load fluorophore to all wells by clicking the top left corner of the plate to select all wells at once. Enter "qBiomarker_Mutation_PCR_Array_Plate_Setup_Template" as the new filename in the "Plate Setup Filename" box.
- Click "Save this plate setup" to save the file as a *.pts file.
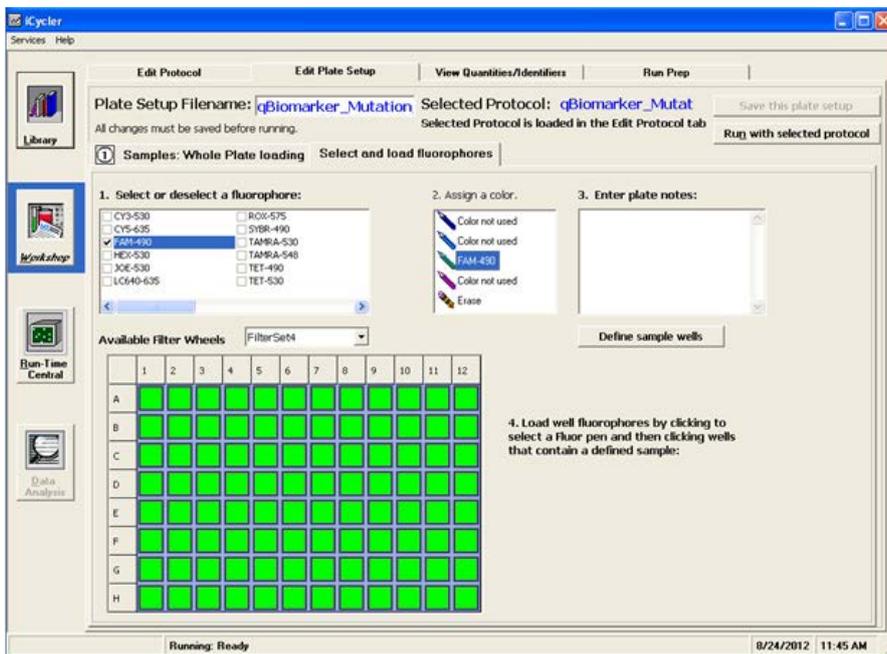

Figure 3. Plate setup (2).

Real-time PCR detection

4. If the thermocycler is off, turn on the iCycler and log onto the instrument. Allow the camera to warm up for 30 minutes, then switch on the computer connected to the thermocycler.

5. Ensure that the plate has been centrifuged for 1 minute at 1000 *g* to remove any bubbles.

6. Open the thermocycler cover to expose the thermal block. Place your plate in the plate holder with the last row (row H) facing front. Well A1 should be positioned at the top-left corner of the holder. Close the cover.

7.   Open the iCycler iQ Optical System software.

8.   In the "Library" module, click to open the "View Protocol" tab.

- Under "Protocol Files," click to highlight and load the previously saved "qBiomarker_Mutation_PCR_Array_Template" file.

- Click to open the "View Plate Setup" tab; under "Plate Setup Files," click to highlight and load the "qBiomarker_Mutation_PCR_Array_Plate_Setup_Template" file.

- Click "Run with Selected protocol" to initiate the run with the selected plate setup and indicated protocol.

- This will lead you to the "Run Prep" tab in the "Workshop" module.

9.   In the "Run Prep" tab:

- Enter the reaction volume (25 μL) for the "Sample volume."

- Select "Well Factor Plate" under "Select Well Factor Source."

  - If using the iCyler iQ, iQ5, or MyiQ, well factors must be collected at the beginning of each experiment. Well factors are used to compensate for any system or pipetting nonuniformity.

- When ready, click on "Begin Run." A "Save" dialog box will open. Enter a name for the optical data file (*.opd).

After the PCR run

10. Select the "Library" module and click on the "View Post-Run Data" tab. Select a data file and click "Analyze Data" on the right under "Data Analysis Operations." The data file will open in the "PCR Quantification" tab of the "Data Analysis" module. Select "PCR Base Line Subtracted Curve Fit" in the field for "Select analysis mode."

11. Choose "FAM" in the "Select a Reporter" box.

12. To determine C_T values, set Baseline at 3 to 18 cycles. To define the Threshold value, use the "Log View" of the amplification plot and place it above the background signal but within the lower half to one-third of the linear phase of the amplification plot.

13. Click on the "Reports" button to obtain the customized reports of the PCR quantification data; the window for "Report Viewer" will open. On the left panel under "Select Report," select to view "Melt and Amp Data." To save the report, select "Save to File," and enter a filename for the report to be saved in the Rich Text Files format.

14. Close the "Report Viewer" window, and check the "Open/Save Settings" and select "Save OPD File" to save the data file with the current analysis parameters.

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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