Protocol Sheet

Rotor-Gene® Q real-time PCR 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 Rotor-Gene Q Series Software 2.0 on the desktop of the computer that is connected to the Rotor-Gene Q.**
2. **Select "File" ➢ "New." The "New Run" dialog box will appear.**

Note: The "New Run" dialog box may open automatically.

3. **Under the "Advanced" tab, select "Two Step" and click "New."**
4. **Under the "Welcome to the Advanced Run Wizard!" tab, select "Rotor-Disc 100."**
    - **Ensure locking ring has been attached to the Rotor-Disc 100, check "Locking Ring Attached" box, and click "Next."**
5. **Under the "Miscellaneous Options" tab, set "Reaction Volume (µl)" to 20 and click "Next."**
6. **Click "Edit Profile."**
    - **In the "Edit Profile" window (Figure 1), adjust parameters to reflect the following:**
        - **Hold**
            - **Hold Temperature: 95°C**
            - **Hold Time: 10 mins 0 secs**
        - **Cycling**
            - **This cycle repeats 40 time(s)**
            - **95°C, 15 seconds, Not Acquiring**
            - **60°C, 1 minute, Acquiring to Cycling A on Green**

7. **Click "Gain Optimisation."**
    - **In the "Auto-Gain Optimisation Setup" window, click "Optimise Acquiring" and click "Ok."**
    - **Ensure "Perform Optimisation Before 1st Acquisition" is checked.**
    - **Click "Close."**
8. **Click "Next."**
9. **Click "Save Template" and enter "qBiomarker_Mutation_RGQ_Template_M" as the template name.**
10. **Click "Save."**

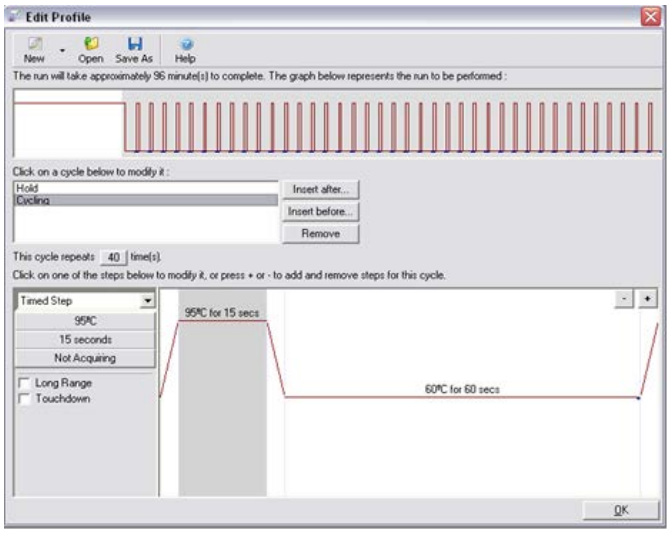

Figure 1. "Edit Profile" tab.

Performing real-time PCR detection

11. **If the Rotor-Gene Q is off, switch on the instrument, and ensure the standby light is lit.**
12. **Open the Rotor-Gene Q Series Software 2.0.**
13. **Under the "New Run" dialog box, click on the "Quick Start" tab, and select "Open a Template In Another Folder."**
14. **Click "New."**
15. **Locate "qBiomarker_Mutation_RGQ_Template_M Template" file and click "Open."**
16. **Under the "1. Rotor Selection" tab, select "Rotor-Disc 100."**
    - **Ensure locking ring has been attached to the Rotor-Disc 100, check "Locking Ring Attached" box, and click "Next."**

17. **Under "2. Confirm Profile" tab, verify desired profile.**
18. **Click "Start Run."**
19. **Enter name for run and click "Save."**
20. **Rotor-Gene Q run will now commence.**

After the PCR run

21. **Click "Bank On."**
22. **Click "All On."**
23. **Select "Analysis" in program bar.**
24. **Under "Quantitation" tab, select "Cycling A. Green."**
25. **Click "Show."**
26. **Determine C_T values. Manually define the threshold value by using the log view of the amplification plots. Select a threshold value above the background signal. The threshold value should be in the lower half of the linear phase of the amplification plot. A threshold setting of 0.03 is recommended as a reference.**
27. **Export the result to an Excel® spreadsheet by placing the mouse in the table of the C_T values and clicking "Export to Excel."**

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.

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