Protocol Sheet

Rotor-Gene® Q instrument setup instructions for qBiomarker Copy Number PCR Arrays

Important points before starting

- Please read the handbook supplied with the qBiomarker Copy Number 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. **Set "Reaction Volume (µL)" to 20 and click "Next."**
6. **Click "Edit Profile."**
7. **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 times**
        - **95°C, 10 seconds, Not Acquiring**
        - **60°C, 30 seconds, Acquiring to Cycling A on Green**
    - **Click "Insert after…" ➤ "New Melt." Ensure "Optimize gain before melt on all tubes" is checked.**
    - **Click "Ok."**

8. **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."**
9. **Click "Next."**
10. **Click "Save Template" and enter "CopyNum_Rotor_Gene_Q" as the template name.**
11. **Click "Save."**

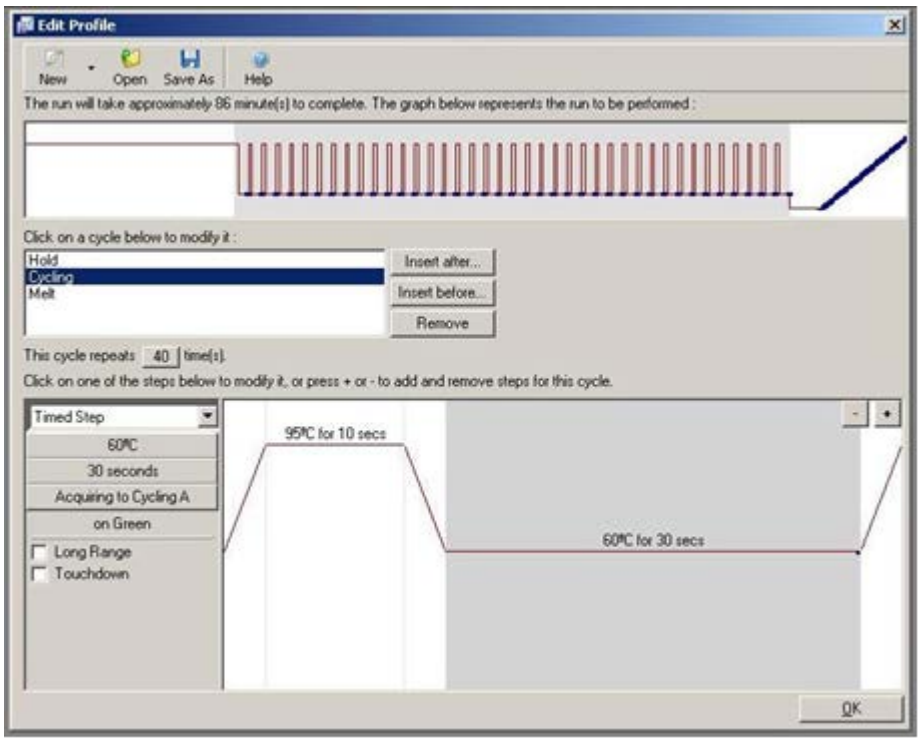

Figure 1. "Edit Profile" tab.

Performing real-time PCR detection

1. **If the Rotor-Gene Q is off, switch on the instrument, and ensure the standby light is lit.**
2. **Open the Rotor-Gene Q Series Software 2.0.**
3. **Under the "New Run" dialog box, click on the "Quick Start" tab, and select "Open a Template In Another Folder."**
4. **Click "New."**
5. **Locate "CopyNum_Rotor_Gene_Q Template" file and click "Open."**

6. **Under the "1. Rotor Selection" tab, select "Rotor-Disc 100."**
7. **Ensure locking ring has been attached to the Rotor-Disc 100, check "Locking Ring Attached" box, and click "Next."**
8. **Verify desired profile.**
9. **Click "Next."**
10. **Click "Start Run."**
11. **Enter name for run and click "Save."**
12. **Rotor-Gene Q run will now commence.**

After the PCR run

13. **Once the PCR run is complete, go to the "Sample Bank."**
14. **Click "Bank On."**
15. **Click "All On."**
16. **Select "Analysis" in program bar.**
17. **Under "Quantitation" tab, select "Cycling A, Green."**
18. **Click "Show."**
19. **Calculate the threshold cycle (C_T) for each well using the instrument's software.**
20. **To define the "Baseline" (Figure 2):**
    - **Observe amplification plots in "Linear View."**
    - **Select "Dynamic Tube" (default analysis setting) to ensure that the average background of each well is determined just before amplification commences.**
    - **(Optional) Select "Ignore First." The fluorescent signal from the initial cycles may not be representative of the remainder of the run. Thus, better results may be achieved if the initial cycles are ignored. Up to 5 cycles can be ignored.**
    - **(Optional) Select "Slope Correction." Selection of this option can improve data for which the baseline (initial cycles) is noticeably sloped. "Noise Slope Correction" improves the data when raw data backgrounds are observed to slope upward or downward before the takeoff point (C_T).**

Note: Ensure that all selections remain consistent across all PCR array runs in the same analysis.

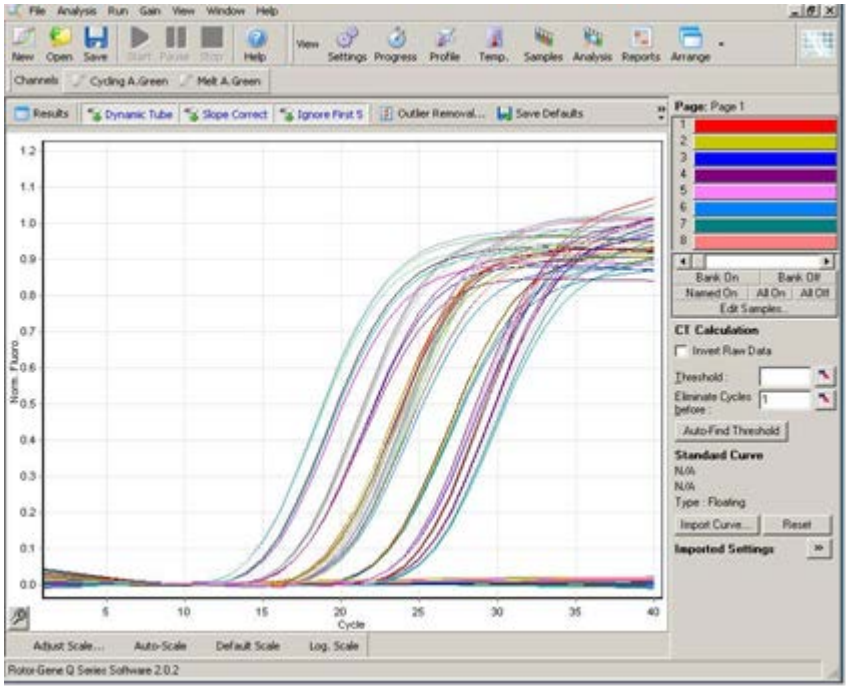

Figure 2. Setting the baseline.

21. Manually define the threshold value (Figure 3):

- **Observe the "Log View" of the amplification plots.**
- **In the "C_T Calculation" box (under "Sample Bank"), click the button beside the "Threshold" box.**
- **Move mouse to amplification plot and click mouse to place threshold above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.**
- **Right-click on "Quant. Results" window.**
- **Click "Export to Excel®."**
- **This file format can be opened in the Microsoft® Excel program.**

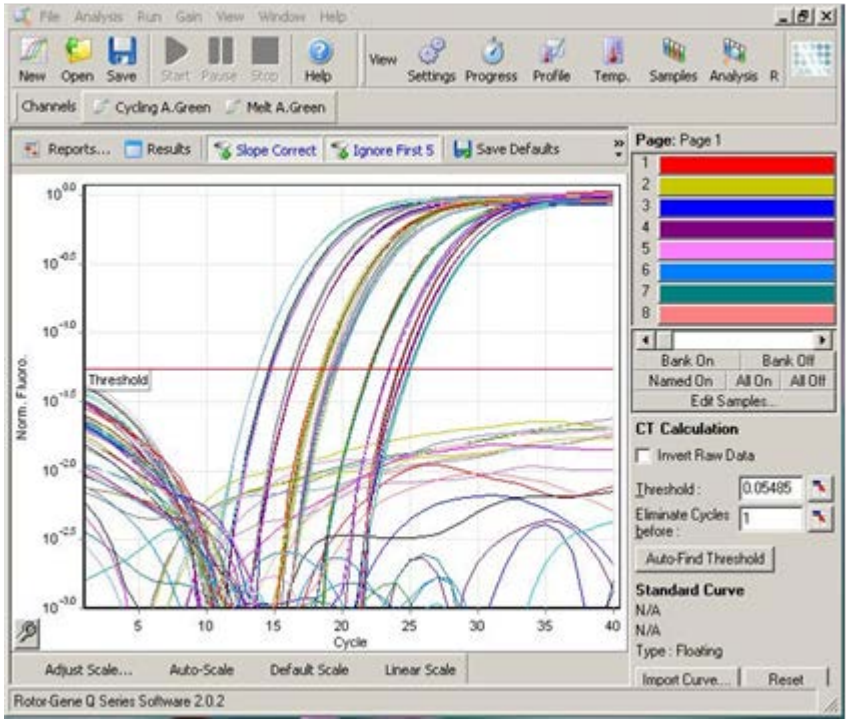

Figure 3. Setting the threshold.

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