Protocol Sheet

Rotor-Gene® Q real-time PCR run setup instructions for RT² Profiler PCR Arrays

Important points before starting

- Please read the handbook supplied with the RT² Profiler 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.**
5. **Ensure locking ring has been attached to the Rotor-Disc 100, check Locking Ring Attached box, and click Next.**
6. **Set Reaction Volume (µl) to 20 and click Next.**
7. **Click Edit Profile. In the Edit Profile window (Figure 1), adjust parameters to reflect the following:**

- **Hold**
  - **Hold Temperature: 95°C**
  - **Hold Time: 10 min 0 sec**
- **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 Optimization. In the Auto-Gain Optimization Setup window, click Optimize Acquiring and click OK. Ensure Perform Optimization Before 1st Acquisition is checked. Click Close.**
9. **Click Next.**
10. **Click Save Template and enter RT2_Rotor_Gene_Q as the template name.**
11. **Click Save.**

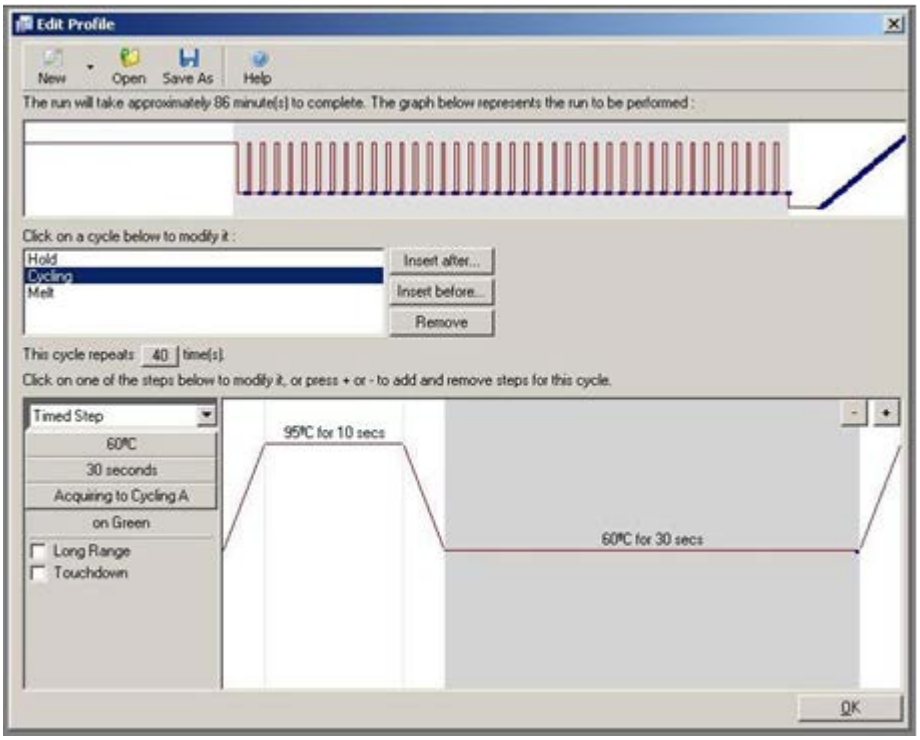

Figure 1. Screenshot of the Edit Profile tab.

Performing real-time PCR detection

12. **If the Rotor-Gene Q is off, switch on the instrument, and ensure the standby light is lit.**
13. **Open the Rotor-Gene Q Series Software 2.0.**
14. **Under the New Run dialog box, click on the Quick Start tab, and select Open a Template In Another Folder.**
15. **Click New.**
16. **Locate RT2_Rotor_Gene_Q Template file and click Open.**
17. **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.**
18. **Verify desired profile.**
19. **Click Next.**
20. **Click Start Run.**
21. **Enter name for run and click Save.**
22. **Rotor-Gene Q run will now commence.**

After the PCR run

23. **Once the PCR run is complete, observe the Sample Bank.**
24. **Click Bank On.**
25. **Click All On.**
26. **Select Analysis in program bar.**
27. **Under Quantitation tab, select Cycling A. Green.**
28. **Click Show.**
29. **Calculate the threshold cycle (C_T) for each well using the instrument's software.**
    - **To define the Baseline:**
      - **Observe amplification plots in Linear View**
      - **Select Dynamic Tube (default analysis setting) to ensure the average background of each well is determined just before amplification commences.**
      - **(Optional) Select Ignore First.** 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 whose 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.

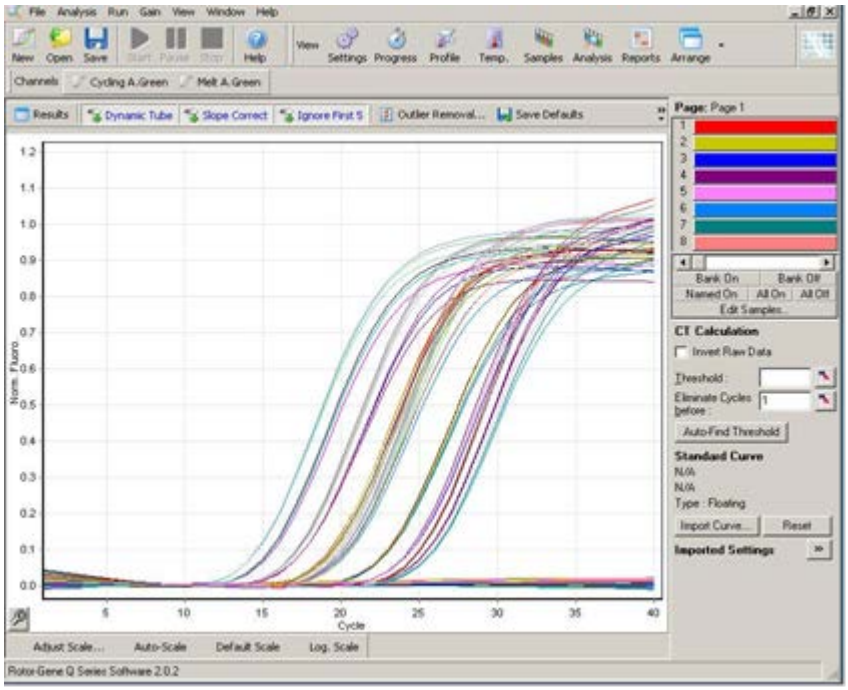

Figure 2. Setting the baseline.

- **To 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.**

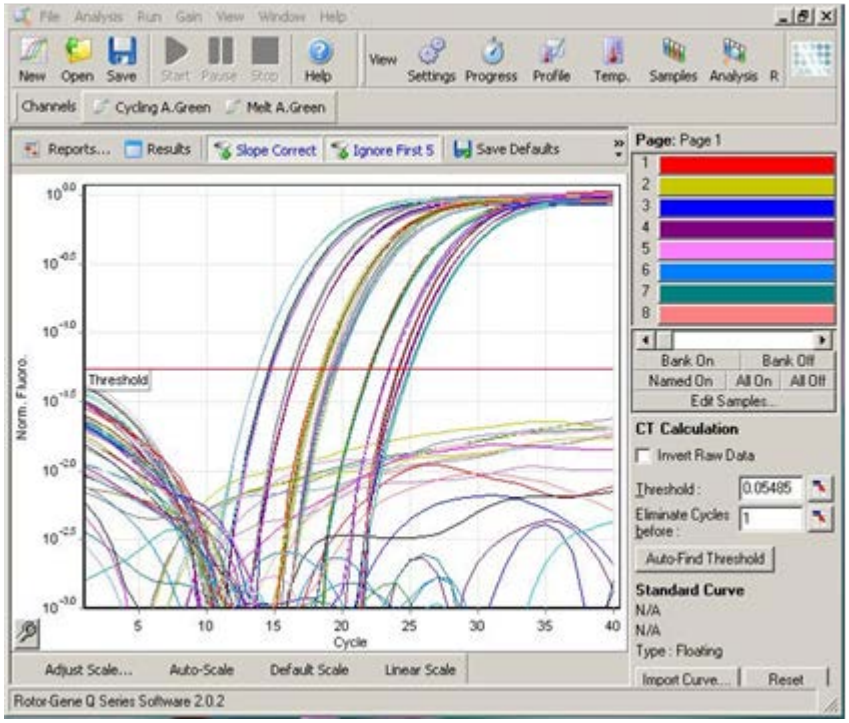

Figure 3. Setting the threshold.

The RT^2 Profiler PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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