January 2020

Protocol Sheet

Rotor-Gene® Q real-time PCR run setup instructions for Microbial DNA qPCR Arrays

Important points before starting

- Please read the handbook supplied with the Microbial DNA qPCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.
- Ensure the Rotor-Gene Q is working properly. Refer to the Rotor-Gene Q User Manual if required.

Procedure

PCR protocol template set up

1. Open the Rotor-Gene Q Series Software 2.3.1 on the desktop of the computer that is connected to the Rotor-Gene Q. Select "File"/"New". The "New Run" dialog box will appear.

   Note: the "New Run" dialog box may open automatically.
2. Under the "Advanced" tab, select "Two Step" and click "New".
3. Under the "Welcome to the Advanced Run Wizard!" tab, select "Rotor-Disc 100".
4. 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 as follows:

   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, 2 minutes, Acquiring to Cycling A on Green
   Click "OK".
7. Click "Gain Optimisation".
8. In the "Auto-Gain Optimisation Setup" window, click "Optimise Acquiring" and click "OK". Ensure "Perform Optimisation Before 1st Acquistion" is checked. Click "Close".
9. Click "Next".
10. Click "Save Template" and enter "MicrobialDNAqPCR_Template" as the template name. Click "Save".

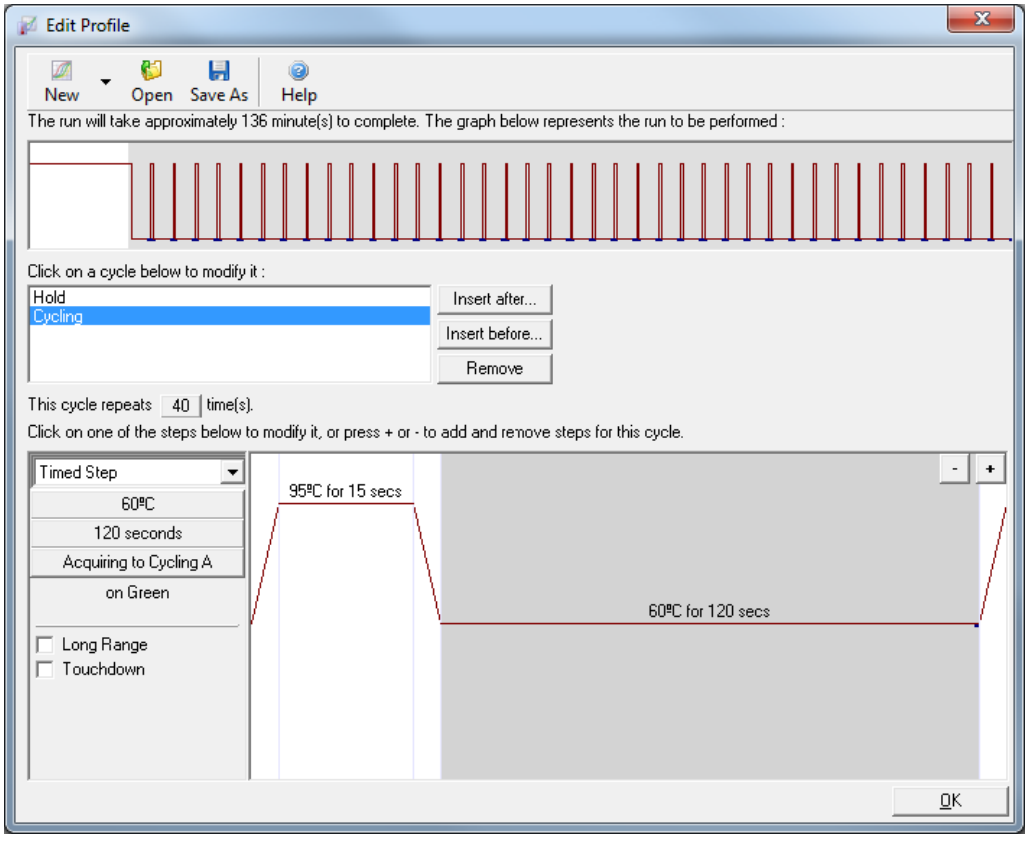

Figure 1. "Edit Profile" Window

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 "MicrobialDNAqPCR_Template" file and click "Open".
6. 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".
7. Under "2. Confirm Profile" tab, verify desired profile.
8. Click "Start Run".
9. Enter name for run and click "Save".
10. Rotor-Gene Q run will start.

After the PCR run

1. Click “Bank On”.
2. Click “All On”.
3. Select “Analysis” in program bar.
4. Under “Quantitation” tab, select “Cycling A. Green”.
5. Click “Show”.
6. 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. Use the following recommended settings:

   Click on “Dynamic Tube”
   Click on “Slope Correct”
   Ignore First 5
   Take off Point Adjustment 15/20
   Outlier removal 0%
   Threshold 0.02
   Eliminate cycle before 10

7. Export the result to an Excel® spreadsheet by placing the mouse in the table of the CT values and clicking “Export to Excel”

Document Revision History

Date	Changes
01/2020	Changed Threshold in Step 6 of “After the PCR run” from 0.01 to 0.02; Layout updates.

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