Protocol Sheet

Applied Biosystems® ViiA™ 7 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 real-time PCR instrument is working properly. Refer to the manufacturer's installation and maintenance manual if needed.

Procedure

PCR protocol template set up

1. **Open the ViiA 7 v1.1 software on the desktop of the computer that is connected to the ViiA 7 instrument. Select "File"/"New Experiment"/"Experiment".**
2. **Under "Setup", select the "Experimental Properties" menu (Figure 1).**
3. **Select "384-Well Block", "Standard Curve", "TaqMan® Reagents", and "Standard".**

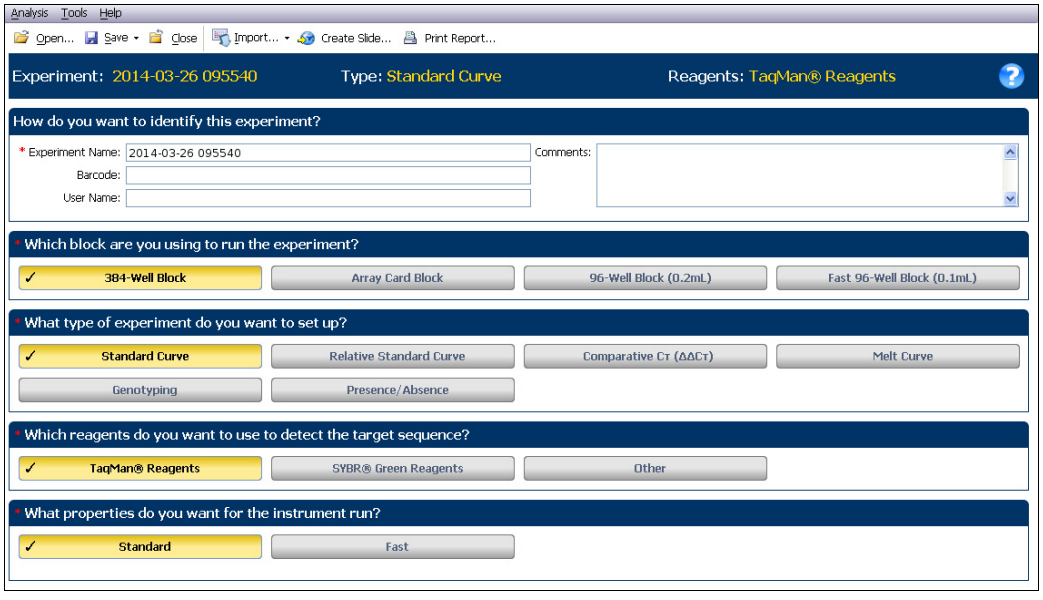

Figure 1. Experimental properties.

4. **Under "Setup", select "Define" menu. In "Define Targets and Samples" tab, ensure that "FAM" is listed beside "Target 1". For "Quencher", select "none".**

5. **Under "Setup", select "Assign" menu. Highlight the entire plate in the "Plate Layout" window. Check the box next to "Target 1" under "Targets" and verify that all wells in "Plate Layout" view display the "U" symbol ("U" = unknown).**

6. **Under "Setup", select "Run Method" menu (Figure 2). Enter "10 µL" for "Reaction Volume Per Well". Adjust parameters as follows:**

   Holding Stage:

   Temperature: 95°C

   Time: 10:00

   Cycling Stage:

   Number of Cycles: 40

   Step 1: 95°C, 00:15

   Step 2: 60°C, 02:00

   Change Ramp Rate to 1°C/s

7. **Detect and record FAM™ fluorescence from every well during the annealing step of each cycle.**

8. **Select "File", then "Save As Template". Save the file as "Experimental Template files" (*.edt) with the filename "MicrobialDNAqPCR_Template" and click "Save".**

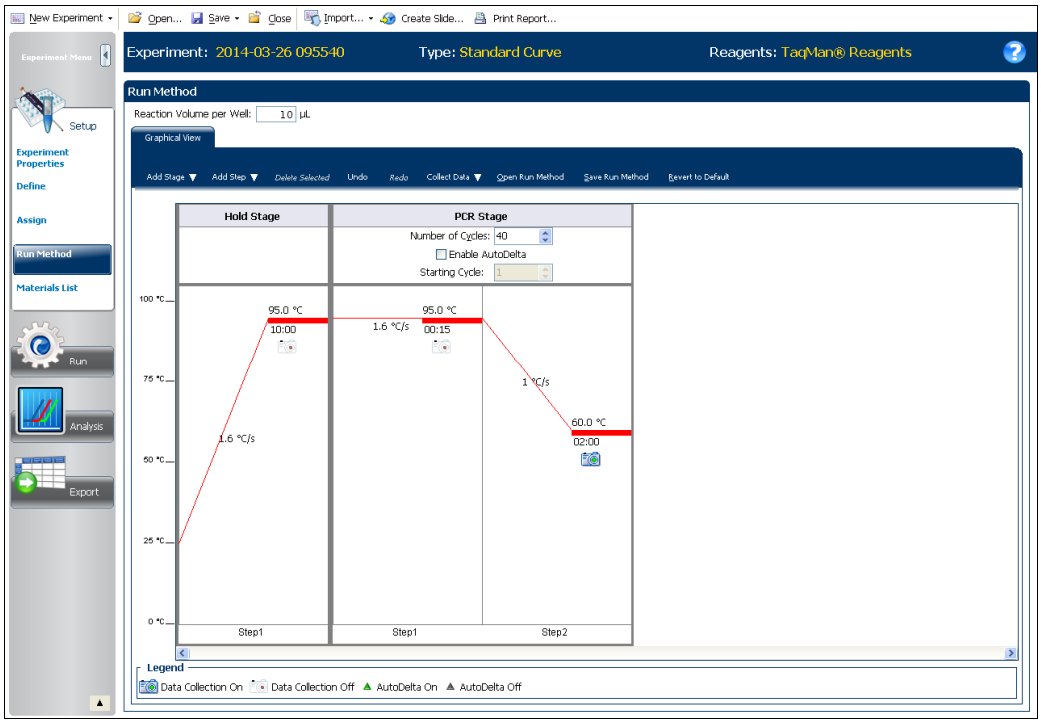

Figure 2. Run method.

Protocol Sheet

Real-time PCR detection

1. **If the thermocycler is off, press the power button to switch on the instrument. Wait for the instrument to boot and display the Power status light. Switch on the computer connected to the thermocycler.**
2. **Ensure that the plate has been centrifuged for 1 min at 1000 *g* to remove any bubbles.**
3. **Open the tray and place the plate in the precision plate holder with the last row (row H) facing the front. Make sure the plate is properly aligned in the holder, well A1 should be positioned at the top-left corner of the tray. To close the tray door, press the tray to move it into the instrument while applying pressure to the right side of the tray at an angle.**
4. **Open the ViiA 7 v1.1 software.**
5. **Select "File"/"New Experiment"/"From Template". Select the "MicrobialDNAqPCR_Template" file and click "Open".**
6. **Verify that run method is correct.**
7. **Select "Run Menu".**
8. **Click "Start Run" in "Run Status" window.**

After the PCR run

1. **To determine C_T values, Set "Baseline" at 8 to 20 cycles and "Threshold" at 0.2.**
2. **Export C_T values.**