

Protocol Sheet

Applied Biosystems® ViiA™ 7 real-time PCR run 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 ViiA 7 v1.1 software on the desktop of the computer that is connected to the ViiA 7 instrument.
2. Select “File” > “New Experiment” > “Experiment.”
3. Under “Setup” > “Experimental Properties” menu (see Figure 1):
 - Select “384-Well Block.”
 - Select “Standard Curve.”
 - Select “TaqMan® Reagents.”
 - Select “Standard.”

Figure 1. Experimental properties.



4. Under "Setup" > "Define" menu:
 - In "Define Targets and Samples" tab, ensure that "FAM" is listed beside "Target 1." For "Quencher," select "none."
5. Under "Setup" > "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 have the "U" symbol ("U" = unknown).
6. Under "Setup" > "Run Method" menu (see Figure 2):
 - Enter "10 μ l" for "Reaction Volume Per Well."
 - Adjust parameters to reflect the following:
 - Holding Stage
 - Temperature: 95°C
 - Time: 10:00
 - Cycling Stage
 - Number of Cycles: 40
 - Step 1: 94°C, 00:15
 - Step 2: 60°C, 01:00

Note: Detect and record FAM fluorescence from every well during the annealing step of each cycle.

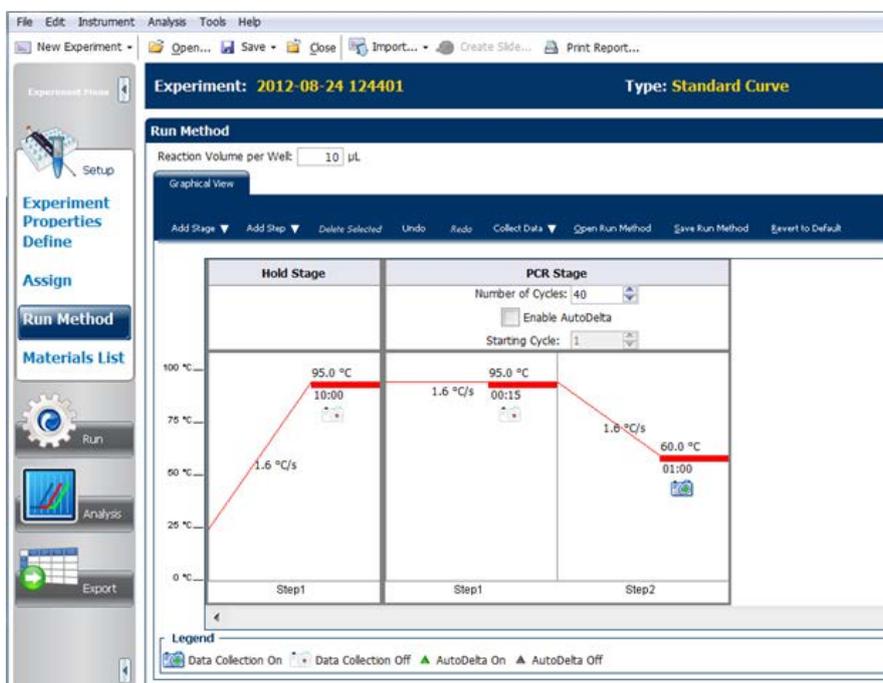


Figure 2. "Run Method" screen.

7. Select "File" > "Save As Template." Save the file as an Experimental Template file (*.edt) with the filename "qBiomarker_Mutation_PCR_Array_Template" (click "Save").

Performing real-time PCR detection

8. 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.
9. Ensure that the plate has been centrifuged for 1 minute at 1000 g to remove any bubbles.
10. Open the tray and place the plate in the precision plate holder with the last row (row H) facing 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.
11. Open the ViiA 7 v1.1 software.
12. Select "File" > "New Experiment" > "From Template." Select the "qBiomarker_Mutation_PCR_Array_Template" file and click "Open."
13. Verify that run method is correct.
14. Select "Run Menu."
15. Click "Start Run" in "Run Status" window.

After the PCR run

16. To determine C_T values, set Baseline at 5 to 18 cycles and Threshold setting at 0.1.

17. Export C_T values.

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.

For up-to-date licensing and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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