Protocol Sheet

Applied Biosystems® 7500 (Fast block) 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 ABI 7500 v2.0.4 software on the desktop of the computer that is connected to the ABI 7500 instrument.**
2. **Select "File" ➤ "New Experiment" ➤ "Advanced Setup."**
3. **Under "Setup" ➤ "Experiment Properties" menu (see Figure 1):**
    - **Select "7500 Fast (96 Wells)."**
    - **Select "Quantitation – Standard Curve."**
    - **Select "TaqMan® Reagents."**
    - **Select "Standard (~ 2 hours to complete a run)."**

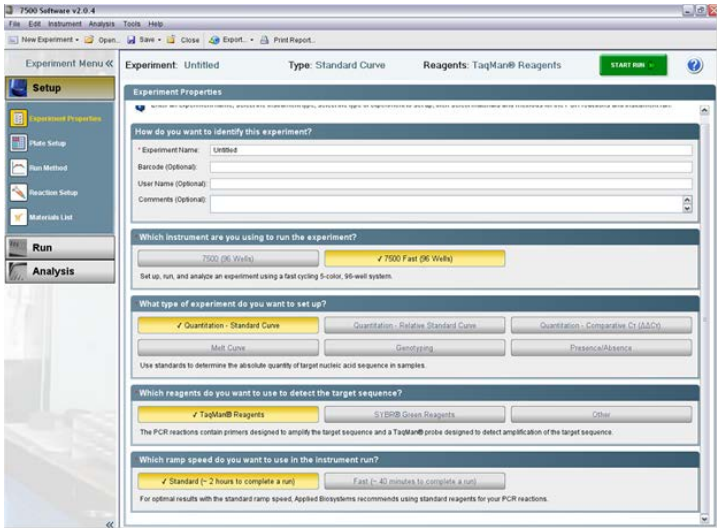

Figure 1. Experiment properties.

4. **Under "Setup" ➤ "Plate Setup" menu:**
    - **In "Define Targets and Samples" tab, ensure that "FAM" is listed beside "Target 1." For "Quencher," select "None."**
    - **In "Assign Targets and Samples" tab:**
        - **Highlight the entire plate in the "View Plate Layout" window.**
        - **Check the "Assign" box next to "Target 1" under "Assign targets to the selected wells" and verify that all wells in "Plate Layout" view have the "U" symbol ("U" = unknown).**
5. **Under "Setup" ➤ "Run Method" menu (see Figure 2):**
    - **Enter 25 µl for "Reaction Volume Per Well."**
    - **Adjust parameters to reflect the following:**
        - **Holding Stage**
            - **Temperature: 95°C**
            - **Time: 10:00**
        - **Cycling Stage (2 Steps)**
            - **Number of Cycles: 40**
            - **Step 1: 95°C, 00:15**
            - **Step 2: 60°C, 01:00**
            - **Step 3: 70°C, 00:34, Data Collection On**

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

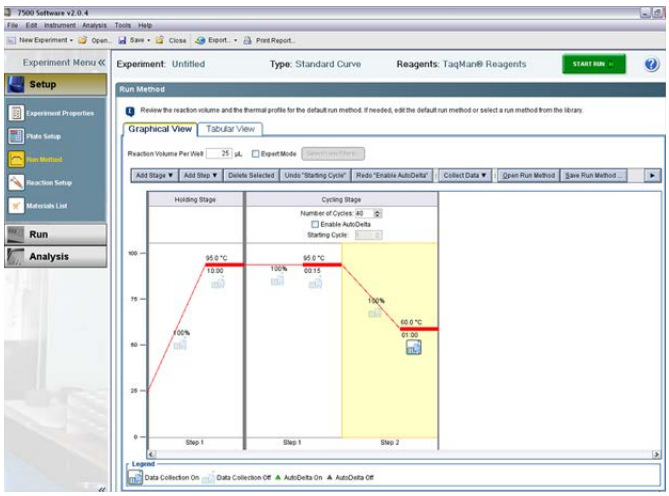

Figure 2. "Run Method" screen.

6. **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

7. **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.**
8. **Ensure that the plate has been centrifuged for 1 minute at 1000 g to remove any bubbles.**
9. **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.**
10. **Open the ABI 7500 v2.0.4 software.**
11. **Select "File" ➤ "New Experiment" ➤ "From Template." Select the "qBiomarker_Mutation_PCR_Array_Template" file and click "Open."**
12. **Verify that run method is correct.**
13. **Select "Run Menu."**
14. **Click "Start Run" in "Run Status" window.**

After the PCR run

15. To determine C_T values, set Baseline at 3 to 15 cycles and Threshold at 0.1.

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.

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