Protocol Sheet

Applied Biosystems® 7900HT 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 7900HT SDS Version 2.4 software on the desktop of the computer that is connected to the ABI 7900HT instrument.**
2. **Select "File" ➤ "New." The "New Document Wizard" dialog box will appear.**
    - **Select "Assay": "Standard Curve (AQ)."**
    - **Select "Container": "384-well Clear Plate."**
    - **Select "Template": "Blank Template."**
    - **The field for "Barcode" is optional.**
    - **Click "OK."**
    - **Under the "Setup" tab, click the "Add Detector…" button at the bottom of the tab.**
    - **In the "Detector Manager" window, select "FAM" for the reporter dye, click the "Copy To Plate Document" button, and click the "Done" button (see Figure 1). Ensure that "ROX" is selected for "Passive Reference."**

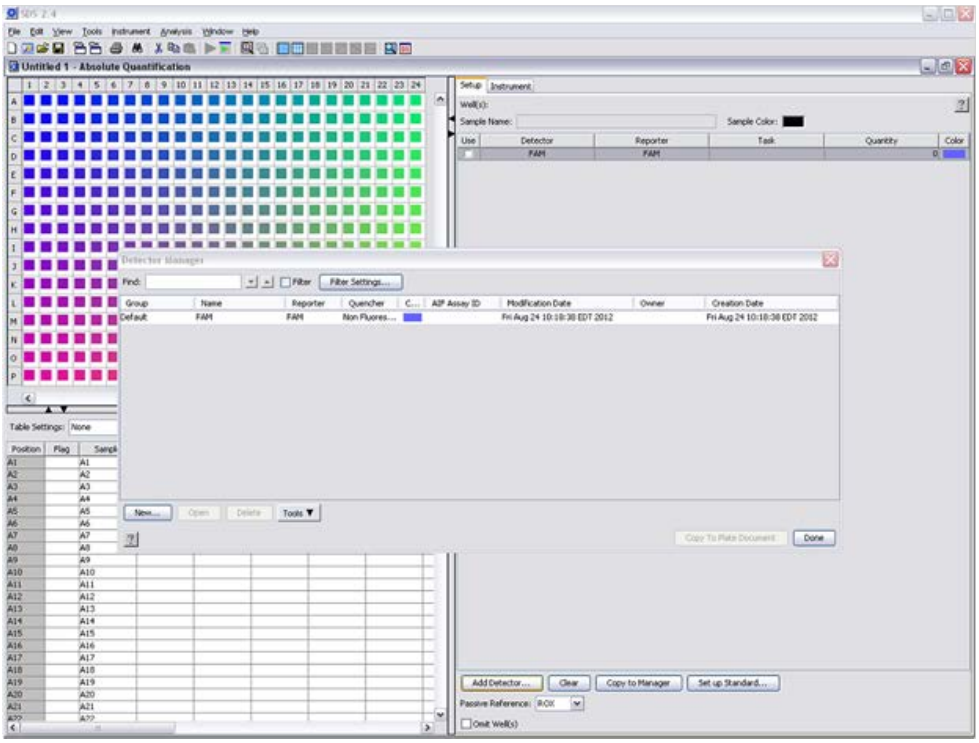

Figure 1. "Detector Manager" window.

- **Click the square button in the upper left corner of the 384-well layout panel (between the letter 'A' and '1') to select all wells. Once selected, the 384-well table will be highlighted in yellow. Click the box next to "FAM." This selects FAM as the detector for all wells.**

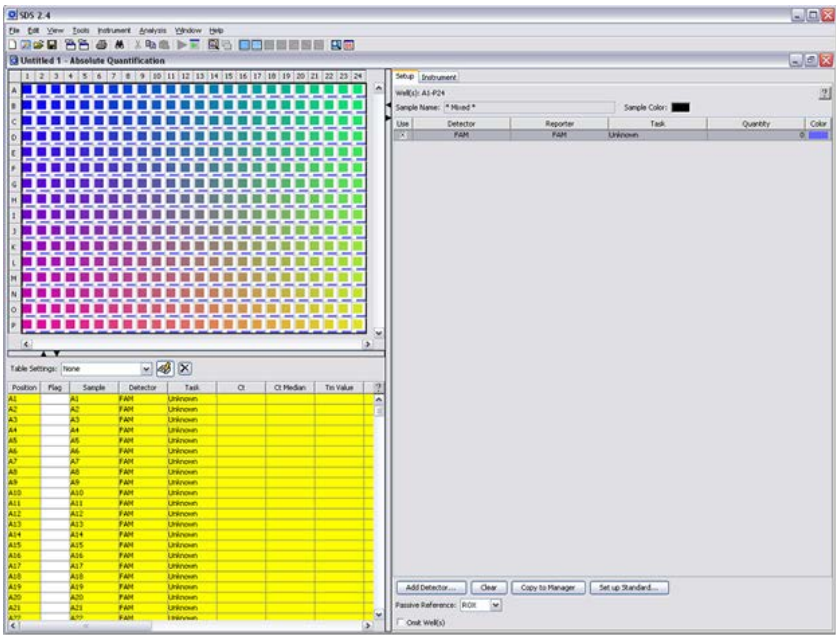

Figure 2. "Absolute Quantification" screen.

- **Click the "Instrument" tab, then the "Thermal Cycler" ➤ "Thermal Profile" tab (see Figure 3).**
    - **Highlight and delete "Stage 1 (50°C for 2:00 minutes)" by clicking the mouse, dragging it across Stage 1, and clicking the "Delete Step" button.**
    - **Stage 1: Enter 95.0°C for 10:00 minutes.**
    - **Stage 2 (2 Steps)**
        - **Step 1: 95.0°C for 0:15 (15 seconds)**
        - **Step 2: 60.0°C for 1:00 (1 minute)**
        - **Enter 40 for Repeats.**
    - **Detect and record FAM fluorescence from every well during the annealing step of each cycle.**
    - **Ensure that "Standard" is checked for "Mode."**
    - **Type 10 for "Sample Volume (µL)."**

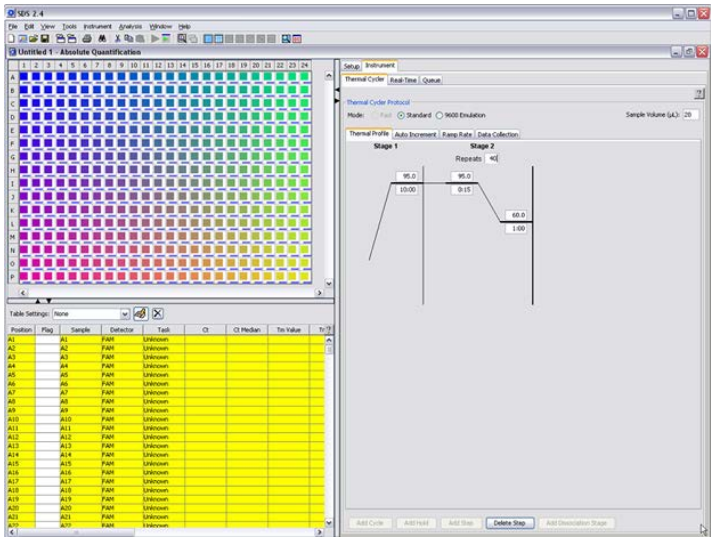

Figure 3. Thermal profile.

- **Select "File" ➤ "Save As" to save the template file. Save the file as an SDS Template file (*.sdt) with the filename "qBiomarker_Mutation_PCR_Array_Template" (click "Save").**

Performing real-time PCR detection

3. **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.**
4. **Ensure that the plate has been centrifuged for 1 minute at 1000 *g* to remove any bubbles.**
5. **Open the ABI 7900HT SDS Version 2.4 software.**
6. **Select "File" ➤ "New." In the "New Document Wizard" dialog box, select "Browse" to load the "qBiomarker_Mutation_PCR_Array_Template" file. Then click "Finish." This will load the previously saved setup to the new plate document.**
7. **Save the new document under a new filename as an SDS Document (*sds).**
8. **Click the "Instrument" tab. Click "Real-Time" tab, then click "Connect to Instrument" to connect the computer to the thermal cycler. Click "Open/Close" to open the plate tray and place your plate in the precision plate holder with A1 in the top left corner. Click "Start Run" to begin the PCR run. Wait 30 seconds to 1 minute for the initial priming, then the run should start. The estimated run time will then appear on the screen.**

After the PCR run

9. **When the PCR run is complete, a small dialog box stating, "The run completed successfully" will appear on the screen. Click "OK"; this will close the box.**
10. **To determine C_T values, set Baseline at 8 to 20 cycles and Threshold at 0.1.**
11. **The C_T values will be displayed in the lower left panel for each well. To export the result to an Excel spreadsheet, select "File" ➤ "Export" ➤ "Results Table." The file will be saved as a tab-delimited text file, and can be opened using Microsoft® Excel®.**

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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