Protocol Sheet

Applied Biosystems® 7900 HT (for SDS Software 2.1, 2.3, and 2.4) instrument setup instructions for qBiomarker Copy Number PCR Arrays

Important points before starting

- Please read the handbook supplied with the qBiomarker Copy Number PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.
- Please make sure the system has been properly calibrated for the region of interest (ROI), background, and pure dye. (Refer to the manufacturer's Installation and Maintenance manual for the calibration procedures if needed).
- Please make note of the installed SDS Software version on your instrument.

Procedure

Creation of PCR protocol template

1. Open Fast System SDS Software on the desktop of the computer that is connected to the ABI 7900HT system.

2. Select "File" ➤ "New"; the "New Document" dialog box will appear.

   - "Select Assay" ➤
     - SDS 2.1: "Absolute Quantification (Standard Curve)"
     - SDS 2.3 : "Standard Curve (AQ)"
     - SDS 2.4 : "Standard Curve (AQ)"
   - "Select Container" ➤
     - SDS 2.1: "384-well Clear Plate"
     - SDS 2.3: "96 or 384 wells Clear Plate"
     - SDS 2.4: "96 or 384 wells Clear Plate"
   - "Select Template" ➤
     - SDS 2.1: "Blank Document"
     - SDS 2.3: "Blank Template"
     - SDS 2.4: "Blank Template"
   - **The field for "Barcode" is optional.**
   - **Click "OK."**
   - **Under the "Setup" tab on the center-right, select "Add Detector" button from bottom.**

- ▪ **In the "Detector Manager" window:**
  - • **SDS2.1**
    - o **Select "SYBR®" for the reporter dye.**
    - o **Click "Copy To Plate Document" button.**
    - o **Click "Done" button (See Figure 1).**
    - o **Choose "ROX™" for the "Passive Reference" box.**
  - • **SDS2.3**
    - o **Click "New"**
    - o **In the "Add Detector" dialog box:**
      - ▪ **Enter "SYBR" in the "Name" field.**
      - ▪ **Select "SYBR" for the "Reporter."**
      - ▪ **Click "OK."**
    - o **Click once on the newly added "Detector."**
    - o **Click "Copy To Plate Document" button.**
    - o **Click "Done" button.**
    - o **Choose "ROX" for the "Passive Reference" box.**
  - • **SDS2.4**
    - o **Click "New"**
    - o **In the "Add Detector" dialog box:**
      - ▪ **Enter "SYBR" in the "Name" field.**
      - ▪ **Keep "Group" field as "Default."**
      - ▪ **Keep "Description" and "AIF Assay ID" empty.**
      - ▪ **Select "SYBR" for the "Reporter."**
      - ▪ **Select "Non Fluorescent" for "Quencher."**
      - ▪ **Click the square with color beside "Color", then select preferred color in the "Select Detector Color/Swatches" page (usually green for SYBR). Click "OK" to close the page.**
      - ▪ **Click "OK."**
    - o **Click once on the newly added "Detector."**
    - o **Click "Copy To Plate Document" button.**
    - o **Click "Done" button.**
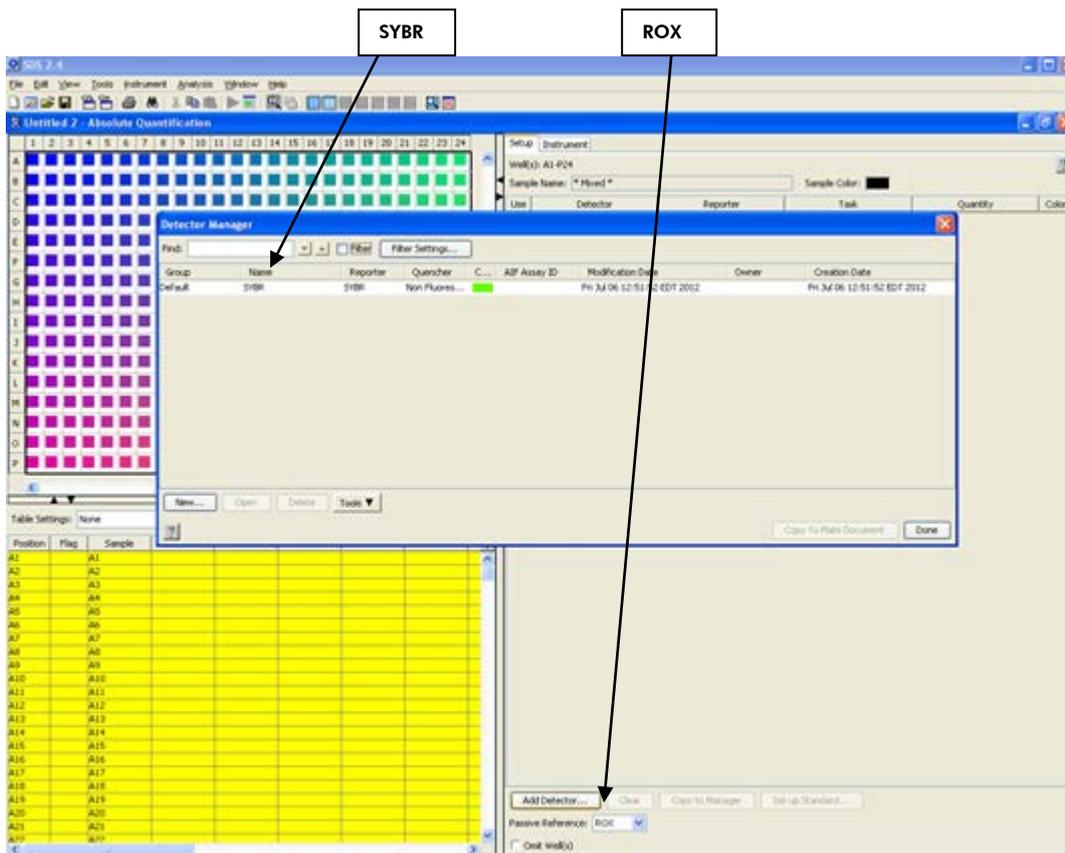   - o **Choose "ROX" for the "Passive Reference" box.**

Figure 1. Plate setup — selecting dye.

- ▪ Select the "Instrument" tab, then the "Thermal Profile" tab. Highlight "Stage 1" (50°C for 2:00 minutes) by right-clicking the mouse and dragging it across "Stage 1." Delete "Stage 1" by clicking the "Delete" button. Then continue to edit the "Thermal Profile" as follows: Enter 95.0°C for 10:00 minutes for "Stage 1"; for "Stage 2," enter 95.0°C for 0:15 (15 seconds) followed by 60.0°C for 1:00 minute with "Repeats 40" (40 cycles). Click the "Add Dissociation Stage" button; the pre-set dissociation stage will be added as "Stage 3."

  - • For SDS Software 2.1: Uncheck the box next to "9600 Emulation".
  - • For SDS Software 2.3/2.4: select "Standard" mode.

- ▪ Click "Add Dissociation Stage" for dissociation curve detection.
- ▪ Put 10 µl for "Sample Volume."
- ▪ Then select "File" ➤ "Save As" to save the template file. Save the file as SDS 7900HT Template Document (*.sdt) with the filename "CopyNum PCR Array Protocol 7900HT Template" (click "Save").
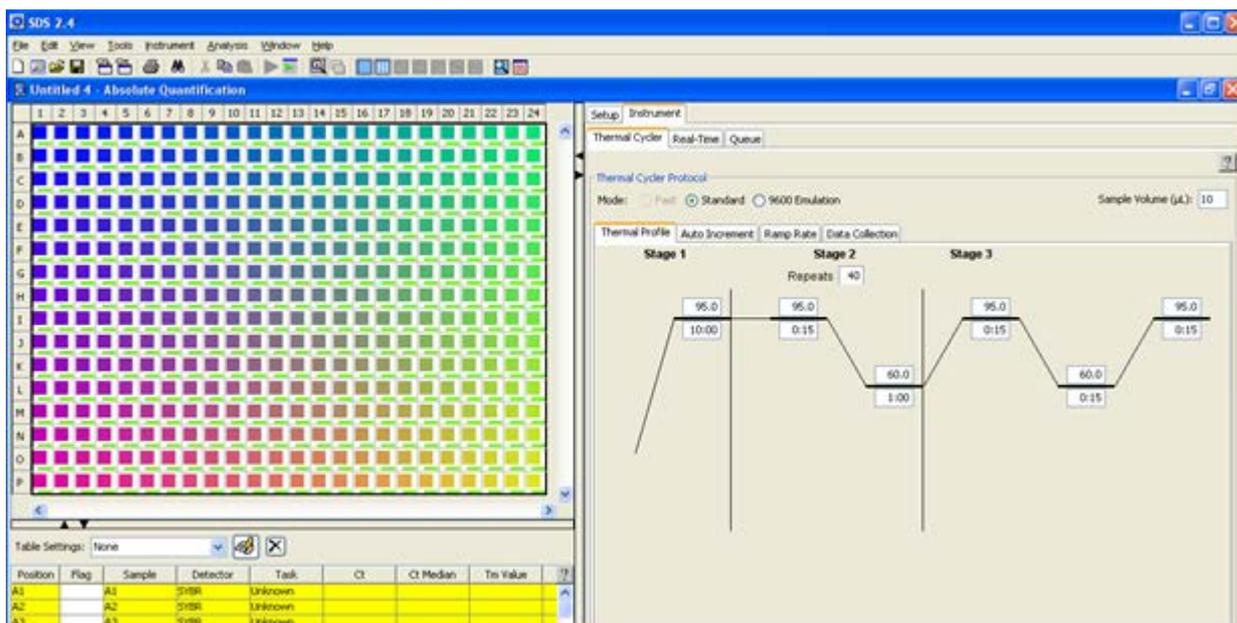
Figure 2. Plate setup — setting thermal profile.

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. Make sure that there are no bubbles in any well, and that the reaction mix is positioned at the bottom of each well. If not, centrifuge the plate at ~1000 x g for 60 seconds.

5. Open the ABI 7900HT Fast System SDS Software.

6. Select "File" ➤ "New." In the "New Document Wizard" dialog box, select "Browse" to load the "CopyNum PCR Array Template" file. Then click "Finish." This will load the previously saved setup to the new plate document.

    ▪ To set up a sample plate (See Figure 2), click the square button in the upper left corner of the full plate layout panel (between the letter "A" and "1") to select all wells. Once selected, the lower full plate table panel will be highlighted in yellow and the box next to "SYBR" on the left panel will be checked.

7. Save the new document under a new filename as "SDS 7900HT Documents" (*sds).

8. Select the "Instrument/Real-Time" tab. Click "Connect/Connect to Instrument" to connect the computer to the thermal cycler. Click "Open" to open the plate tray and place your plate in the precision plate holder with A1 in the top left corner. Click "Close" to load the plate. Click "Start/Start Run" to begin the PCR run. Wait 30

seconds to 1 min for the initial priming, then the run should start. The estimated run time will then appear on the screen.
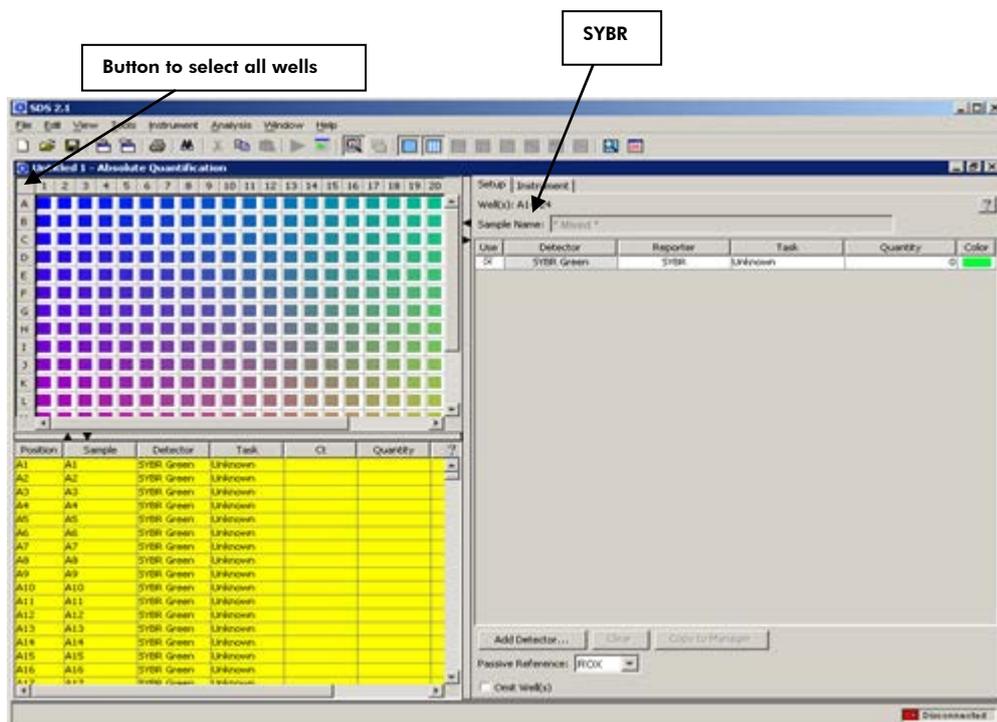

Figure 3. Plate setup — selecting sample.

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. Select "Analysis" ➤ "Analysis Settings"; the "Analysis Settings" dialog box will appear. Select the radio buttons next to "Automatic Baseline" and "Manual C_T" (0.2 is usually fine; please refer to step 13 for manual selection of the threshold), and click "OK."

11. Click the "Result" tab. Display "Data" as "ΔRn vs. Cycle." If all wells are not yet selected, click the square button in the upper left corner of the diagram of the plate (between the letter "A" and "1") to select all wells. The selected wells will be highlighted in yellow in the lower left panel.

12. Select "Analysis" ➤ "Analyze." The software will calculate the data and generate the C_T (lower-left panel).

13. Follow the procedures below to select the baseline and threshold manually (see figures 4 and 5):

Note: Please use the same threshold for all your plates in one study.

   ▪ **To define the baseline, use the "Linear View" of the amplification plots. Double-click on the Y-axis. The window for "Display Settings" will appear. For "Amplification Plot Properties," select**

ABI 7900 real-time PCR setup instructions for qBiomarker Copy Number PCR Arrays

"Auto Scale" for both the Y and X-Axes. Select "Linear View" for "Y-Axis," then click "OK." With the linear plots, determine the cycle number at which the earliest amplification can be seen. Use the red sliding bars on the X-axis to set the manual baseline to start from cycle number 2 through two cycle values before the earliest visible amplification.

- To define the threshold value, use the "Log View" of the amplification plots. Double-click on the Y-axis. The window for "Display Settings" will appear. For "Amplification Plot Properties," select "Auto Scale" for both the Y and X-Axes. Select "Log View" for "Y-Axis" and click "OK." With the log plots, place the threshold line above the background signal but within the lower third of the linear phase of the amplification plot.

- The values for C_T will be displayed in the lower left panel for each well. To export the result to an Excel® spreadsheet, select "File" ➤ "Export" ➤ "Results Table" and save the file as a "Tab-delimited Text file" (*txt). This file format can be opened in Microsoft® Excel.
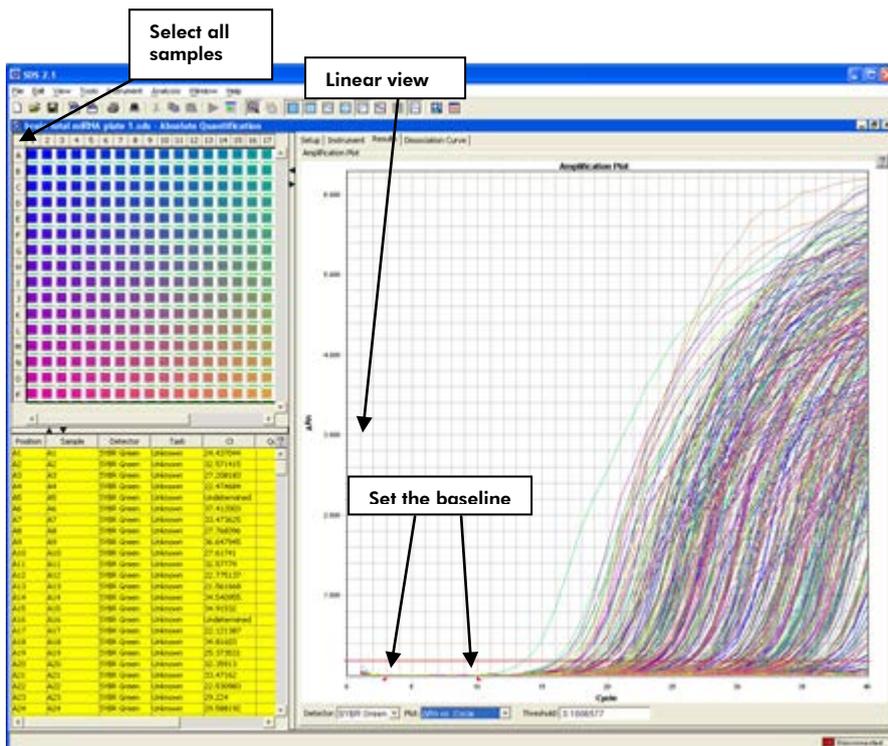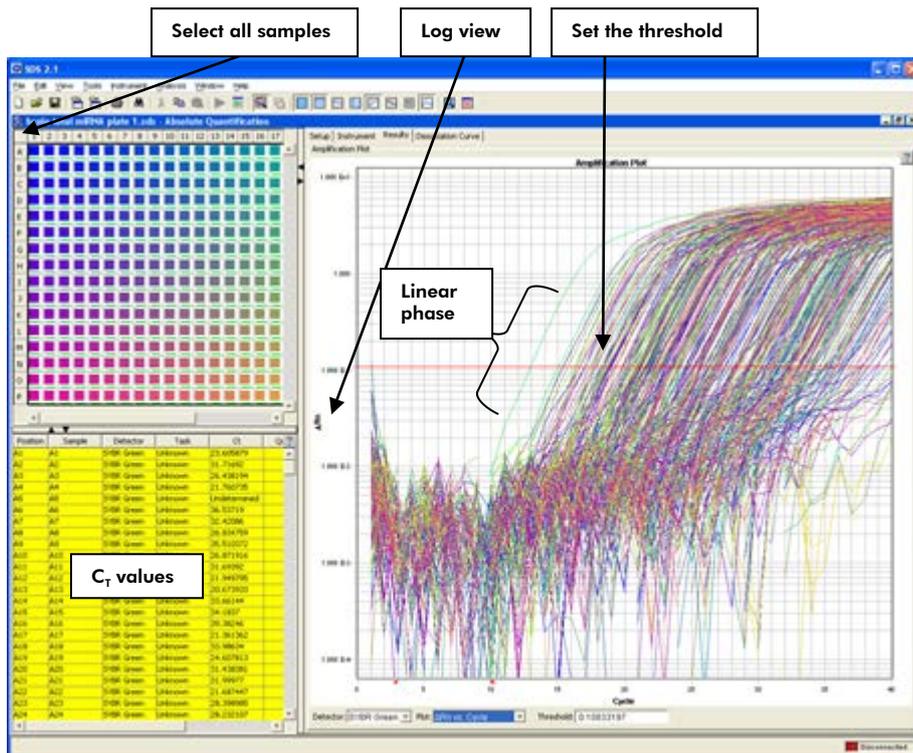

Figure 4. Setting the baseline.

Figure 5. Setting the threshold.

The qBiomarker Copy Number 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.

Sample & Assay Technologies