Protocol Sheet

Stratagene® Mx3000P® qPCR System 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 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 Stratagene MxPro QPCR Software on the computer that is connected to the Stratagene Mx3000P instrument. The "New Options" dialog box for an experiment will appear.

2. Select "Real-time: SYBR® Green (with Dissociation Curve)" and click "OK." The new plate document will appear.

3. On the top panel of the plate document, select Mx3000P (4 filter set plate).

4. On the "Plate Setup" tab (see Figure 1), click the square button with the word "All" on the top left corner of the diagram of the 96-well plate to select all wells. The selected wells will be highlighted in green.
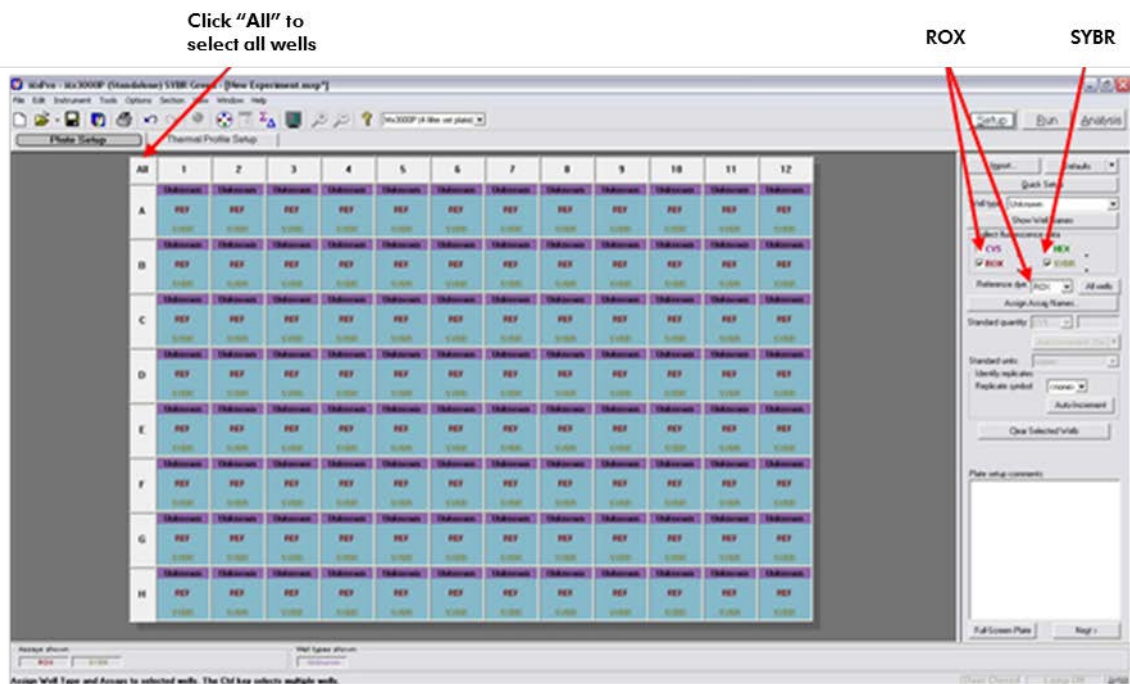
Figure 1. Plate setup.

5. On the right panel:

    - Select "Well-type" ➤ "Unknown."

    - Choose "SYBR" and "ROX" for "Collect Fluorescence Data."

    - Select "Reference Dye" ➤ "ROX."

6. Click "Next" to go to the "Thermal Profile Setup" tab (see Figure 2). In the "Application Segment" panel to the right, click "Normal 2 Step." To change the default setting for the thermal profile, click directly on the number that needs to be changed. Adjust the parameters to reflect the following:

    - Segment 1

        - Temperature: 95°C

        - Time: 10:00

    - Segment 2 (3 Steps, 40 cycles)

        - Step 1: 94°C, 00:15

        - Step 2: 60°C, 01:00, "Endpoints Data Collection Marker"

    - Segment 3 (default melt curve)

Note: "All Points Data Collect Marker" must be present between Step 2 and Step 3.

7. Select "File" ➤ "Save As" to save the template file. Save the file under the filename "CopyNum_PCR_Array_ Mx3000P.mxp".
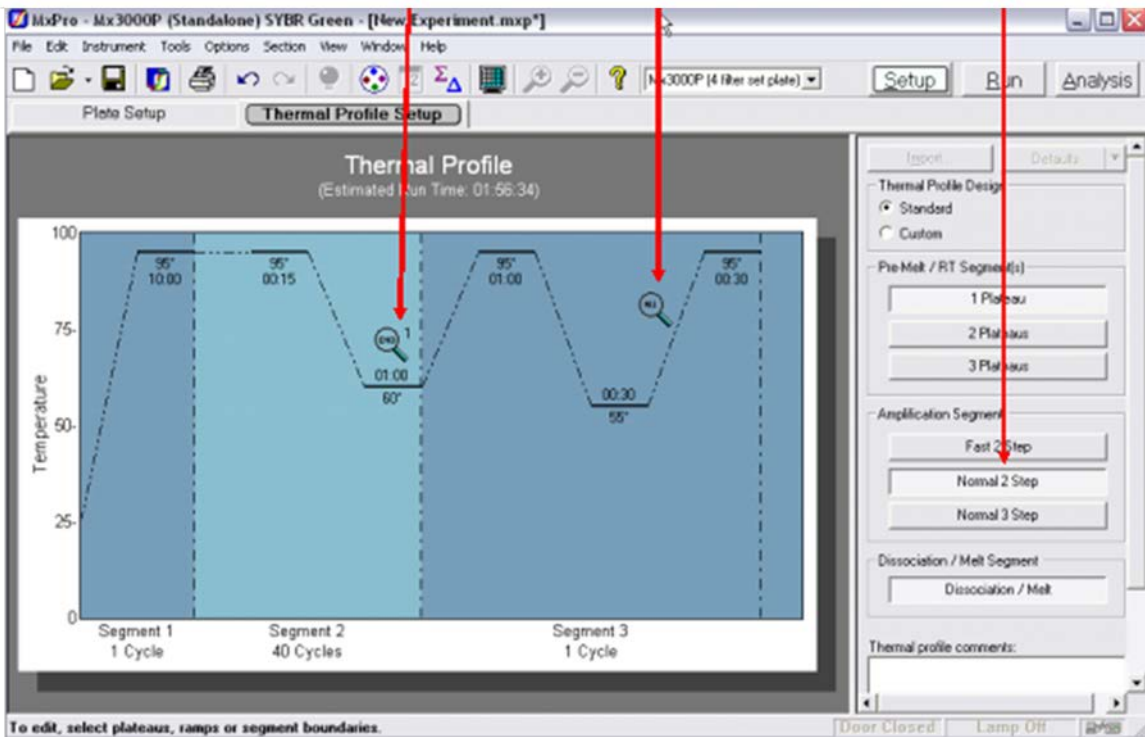
Figure 2. "Thermal Profile Setup" screen.

Performing real-time PCR detection

1. Check to verify that the power status indicator (the lower LED on the front of the instrument) is lit and the "Ready" status indicator (the upper LED) is continuously lit (glowing green). A blinking "Ready" status indicator indicates an experiment is already in process; if the "Ready" status indicator is off, the instrument is not available or ready to run an experiment.

2. Ensure the reaction mix in each well of your reaction plate is free of any bubbles and positioned at the bottom of the well. If not, centrifuge the plate at ~1000 g for 1 min.

3. Open the door located on the front of the instrument by sliding it all the way to the top. To expose the thermal block, pull forward on the hot-top handle and lift the hot-top up and away from the thermal block. Place your plate in the plate holder with the last row (row H) facing front. Well A1 should be positioned at the top-left corner of the thermal block. Make sure the plate is properly aligned in the holder. Close the hot-top assembly by pressing down the hot-top and pushing the handle back into its original place. Slide down the door to close.

4. Open the Stratagene MxPro QPCR Software. Click "Cancel" when the "New Experiment Options" dialog box appears.

5. Select "File" ➤ "Open." Load the CopyNum_PCR_Array_ Mx3000P.mxp file. This will load the previously saved setup to the new plate document. Save the new document under a new filename.

6. Click "Start Run" to begin the PCR run. Wait for about 30 seconds for the initial priming. The estimated run time will then appear on the screen.
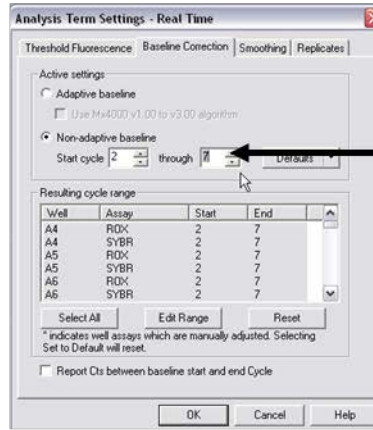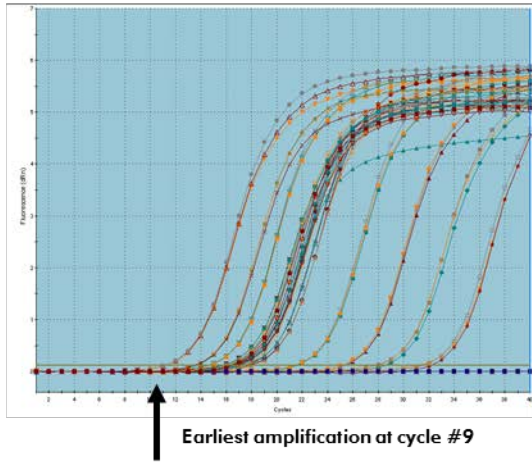
After the PCR run

1. Select "Analysis" on the top panel of the plate document page and choose the "Analysis Selection/Setup" page. Click the square button with the word "All" on the top left corner of the diagram of the 96-well plate to select all wells. The selected wells will be colored in green.

2. Select the "Results" page. On the right panel, choose "Amplification plots" for "Area to analyze." Select "40" for "Last cycle." Select "Fluorescence" ➤ "dR_n". Then follow the procedures below to calculate the threshold cycle (C_T) for each well (see Figure 3):

**Note:** We highly recommend manually setting the baseline and threshold value.

3. To determine the baseline, use the "Linear View" of the amplification plots. Double-click one of the axes. The window for "Graph Properties" will appear. For both "Y" and "X-Axes", select "Lo -> Hi" for Orientation and "Use Automatic Limits." Select "Linear Scale" for "Y-Axis" and also for "X-Axis." Then click "OK." With the linear plots, determine the cycle number at which the earliest amplification can be seen. Select in the top menu "Options" ➤ "Analysis Term Settings." Set the "Non-adaptive baseline" to start from cycle number 2 through two cycle values before the earliest visible amplification. Click "OK."

4. To define the threshold value, use the "Log View" of the amplification plots. Open the "Graph Properties" window by double-clicking one of the axes as above. Select "Log Scale" for "Y-Axis." Then 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.

5. Once C_T values have been determined, select "Text Report" in "Area to analyze" to display the results. On the right panel, select to display the "Column" for "Well," "Well type," "Threshold," "C_T" and "Tm Product 1" for each well. In "Assays Shown" box at the bottom of the screen, deselect the "ROX" button and make sure the "SYBR" button is selected so that only the data for SYBR Green will be displayed in the text report.

6. To export the result to an Excel spreadsheet, select "File" ➤ "Export Text Report" ➤ "Export Text Report to Excel" and save the file as a Microsoft Excel file.

Linear View

Earliest amplification at cycle #9

Analysis Term Settings - Real Time

Threshold Fluorescence | Baseline Correction | Smoothing | Replicates

Active settings
○ Adaptive baseline
☐ Use Mx4000 v1.00 to v3.00 algorithm
● Non-adaptive baseline
Start cycle 2 through 7   Defaults

Resulting cycle range

Well	Assay	Start	End
A4	ROX	2	7
A4	SYBR	2	7
A5	ROX	2	7
A5	SYBR	2	7
A6	ROX	2	7
A6	SYBR	2	7

Select All   Edit Range   Reset

* indicates well assays which are manually adjusted. Selecting Set to Default will reset.

☐ Report Cts between baseline start and end Cycle

OK   Cancel   Help

Set the baseline from cycle number 2 through two cycle values before the earliest visible amplification.

Log View
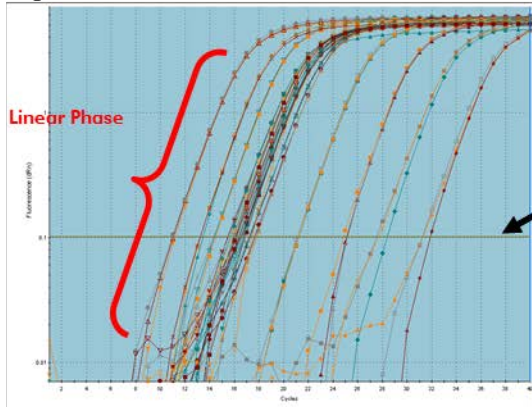
Linear Phase

Set the threshold above the background signal but within the lower third of the linear phase of the amplification plot.

Figure 3. Setting the baseline and 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.

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