Protocol Sheet

Stratagene Mx3000P® qPCR System real-time PCR run setup instructions for RT² Profiler PCR Arrays

Important points before starting

- Please read the handbook supplied with the RT² Profiler 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.**

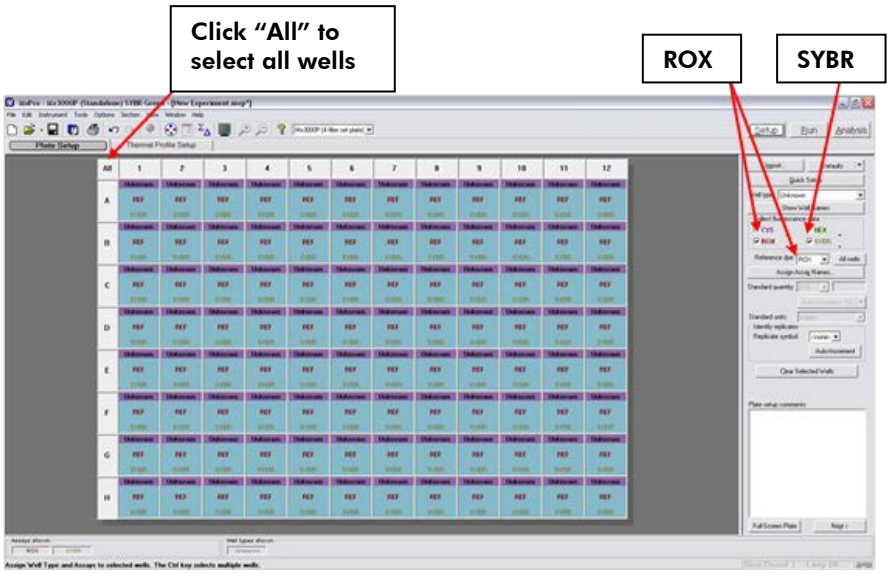

Figure 1. Plate setup.

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)**

     All Data Points Collect Marker must be present between steps 2 and 3.
7. **Select File ➤Save As to save the template file. Save the file under the filename "RT2_Profiler_PCR_Array_ Mx3000P.mxp".**

Note: Alternatively, download the PCR protocol template file (RT2_Profiler_PCR_Array_Template_Mx3000P_M.mxp) from the SABiosciences website at http://www.sabiosciences.com/home.php.

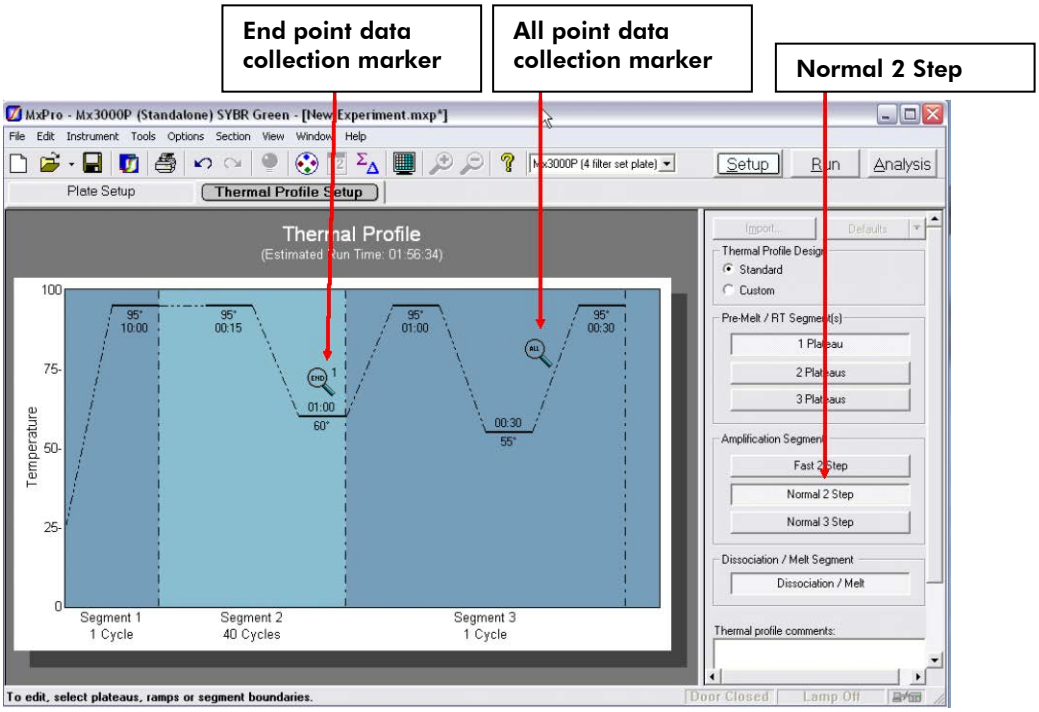

Figure 2. Thermal profile setup.

Performing real-time PCR detection

8. **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.**
9. **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 mins.**
10. **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.**
11. **Open the Stratagene MxPro QPCR Software. Click Cancel when the New Experiment Options dialog box appears.**
12. **Select File ➤ Open. Load the RT2_Profiler_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.**
13. **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

14. **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.**
15. **Select the Results page. On the right panel, choose Amplification plots for Area to analyze. Select 40 for Last cycle. Select Fluorescence ➤ dRn. 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 values.

- **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.**
- **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.**

16. **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.**
17. **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.**

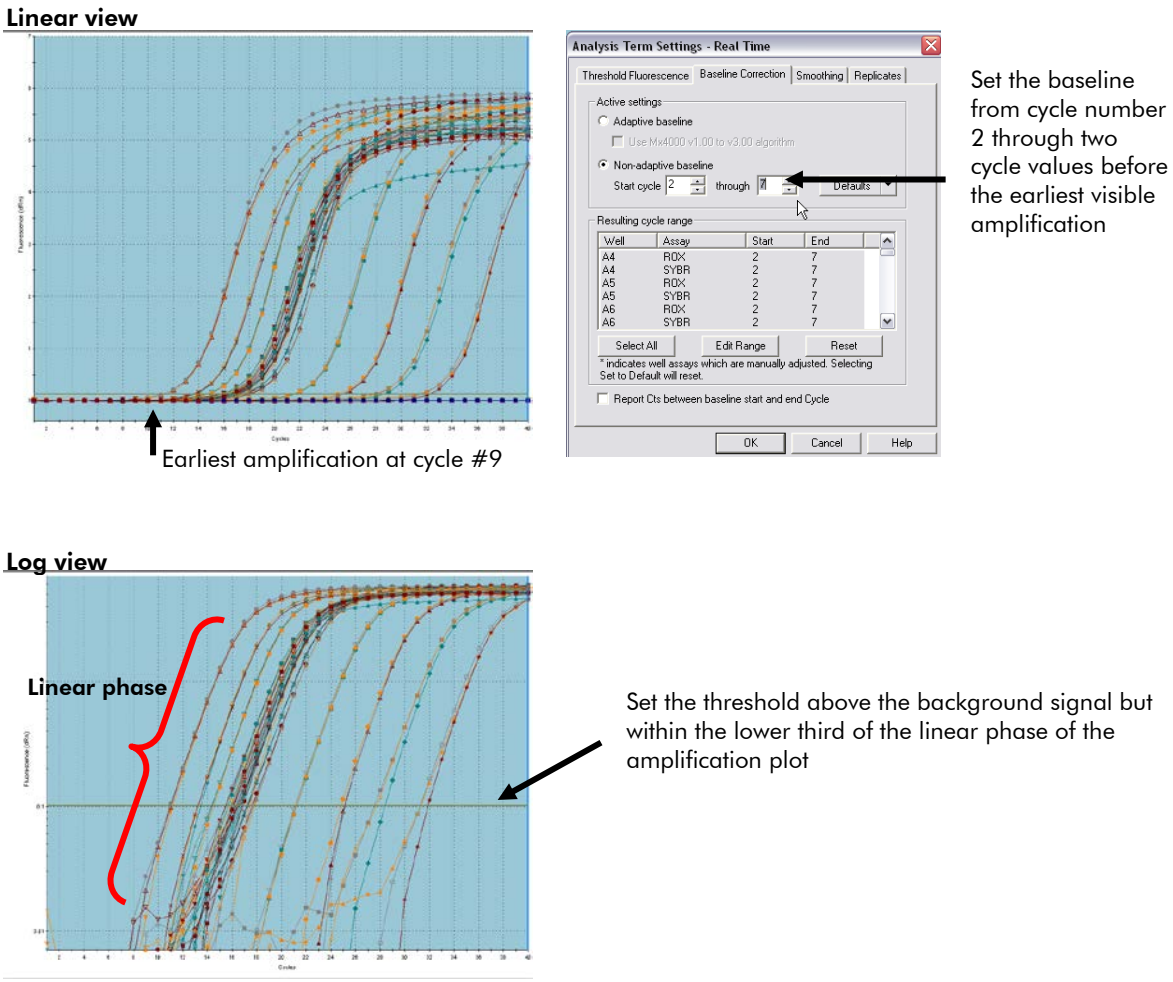

Figure 3. Setting the baseline and threshold.

The RT^2 Profiler PCR Array is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature . Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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