

Protocol Sheet

Stratagene Mx3000P[®] qPCR System real-time PCR run setup instructions for miScript[®] miRNA PCR Arrays

Important points before starting

- Please read the handbook supplied with the miScript miRNA 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.



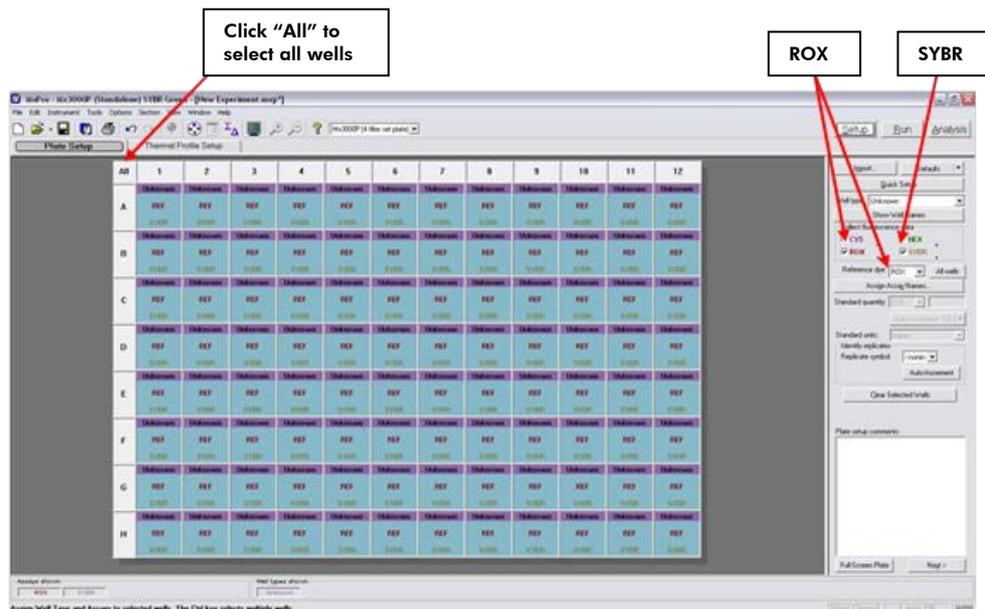


Figure 1. Plate setup.

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). 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: 15:00
- Segment 2 (3 Steps, 40 cycles)
 - Step 1: 94°C, 00:15
 - Step 2: 55°C, 00:30
 - Step 3: 70°C, 00:30, Endpoints Data Collection Marker
- Segment 3 (default melt curve)

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

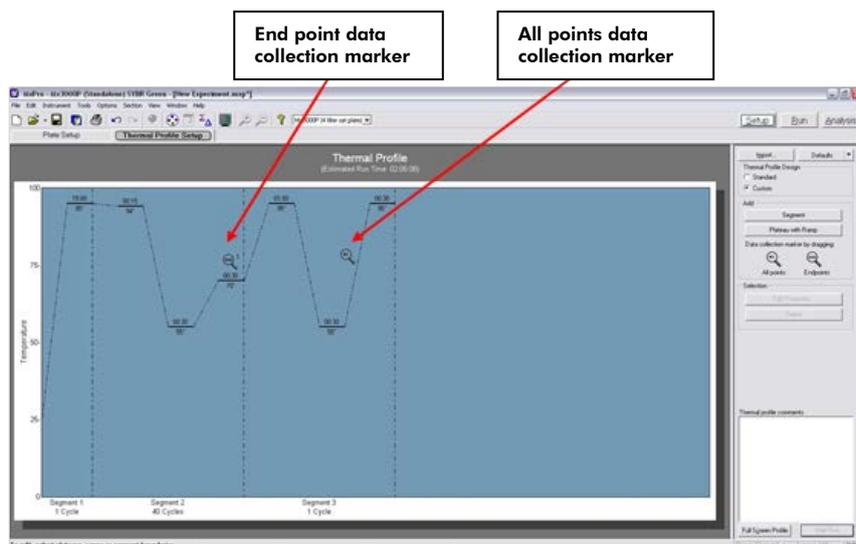


Figure 2. Thermal profile setup.

7. Then select "File" > Save As to save the template file. Save the file under the filename "miScript_miRNA_PCR_Array_Template".

Note: Alternatively, download the PCR protocol template file

(miScript_miRNA_Template_Mx3000P_M.mxp) from the QIAGEN website, www.qiagen.com.

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 that the plate has been centrifuged for 1 minute at 1000 g to remove any bubbles.
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 "miScript_miRNA_PCR_Array_Template" 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" > "dR_n."
16. To determine C_T values, set Baseline and Threshold as described in the *miScript miRNA PCR Array Handbook*.
17. 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.
18. 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.

The miScript miRNA PCR Array is intended for molecular biology applications. This product is 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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Australia ■ 1-800-243-800
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