

# Protocol Sheet

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## Eppendorf Mastercycler ep<sup>®</sup> *realplex* instrument setup instructions for RT<sup>2</sup> Profiler PCR Arrays

### Important points before starting

- Please read the handbook supplied with the RT<sup>2</sup> Profiler PCR Array, paying careful attention to the “Safety Information” and “Important Notes” sections, before beginning this procedure.
- Make sure the correct thermal block cycler (silver or aluminum) is in place.
- Turn on the Mastercycler instrument; the main switch is located at the back of the instrument. Turn on the computer linked to the instrument and log on to Window XP.
- Start *realplex* software version 2.2.
- Enter Username and Password when the Login dialog box appears and click OK to proceed with the login. The Plate Layout window (Figure 1) will appear.

### Procedure

#### Background calibration

1. **Before setting up an experiment, it is necessary to perform background calibration using an empty, capped A plate. Click on Setup, then Background Calibration. A list of previously performed calibrations will appear. Click New... to open the following dialog box (Figure 2)**
2. **Enter SABiosciences A Plate as the name of the background plate. The default block and lid temperatures (60.0 and 105°C, respectively) do not need to be changed. Insert the background plate into the Mastercycler with well A1 at the upper left corner; slide the heated lid closed, pull the sealing clamp into the closed position and click Calibrate. Click OK to begin calibration.**
3. **After the first reading, the software will prompt you to turn the plate. Open the *realplex* instrument and turn the background plate so that well A1 is in the lower right corner. Then close the instrument and click OK to continue the calibration.**
4. **If background calibration was successful, the plate layout will be highlighted in green and a dialog box will appear with the message Calibration successfully finished. Click OK to continue with the experiment setup.**



5. **Note:** If background calibration fails in some positions, those wells will be highlighted in orange. Failure may be a result of fluorescence contamination on the plate or in some wells of the thermoblock. Retry calibration with a new A Plate; if this also fails, clean the thermoblock as instructed in the instrument manual.

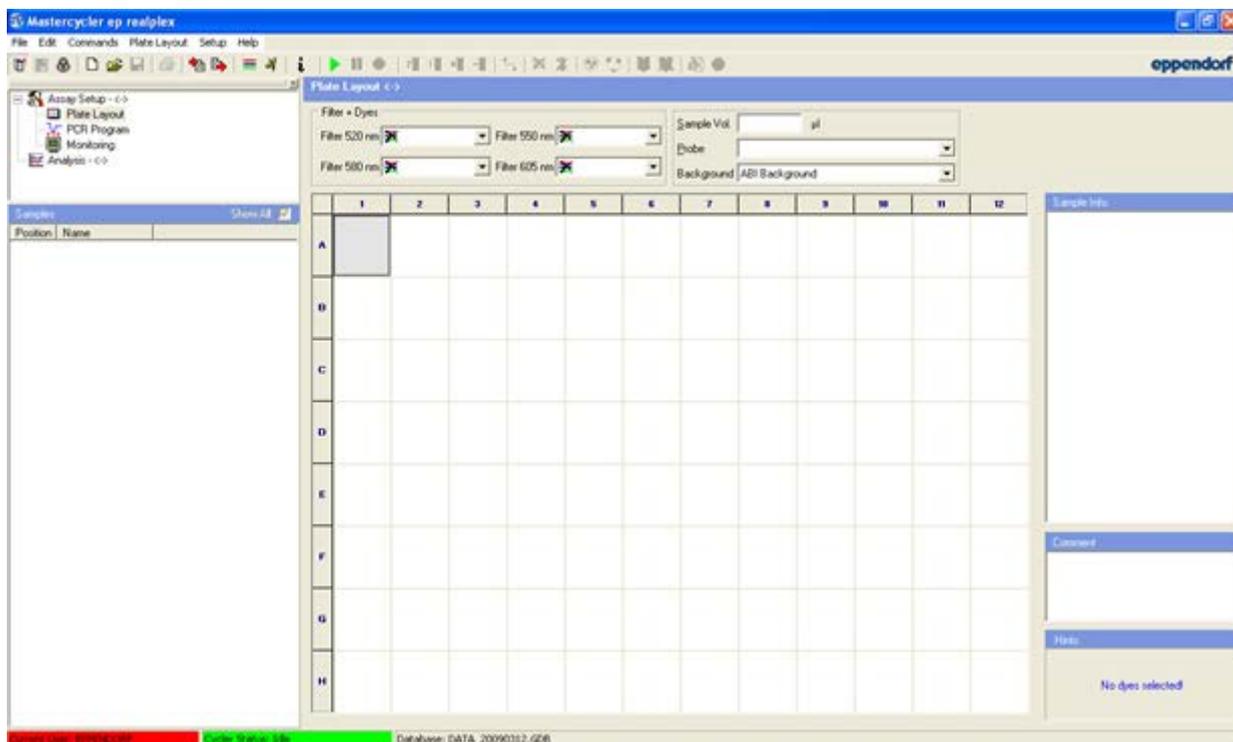


Figure 1. Plate layout window.

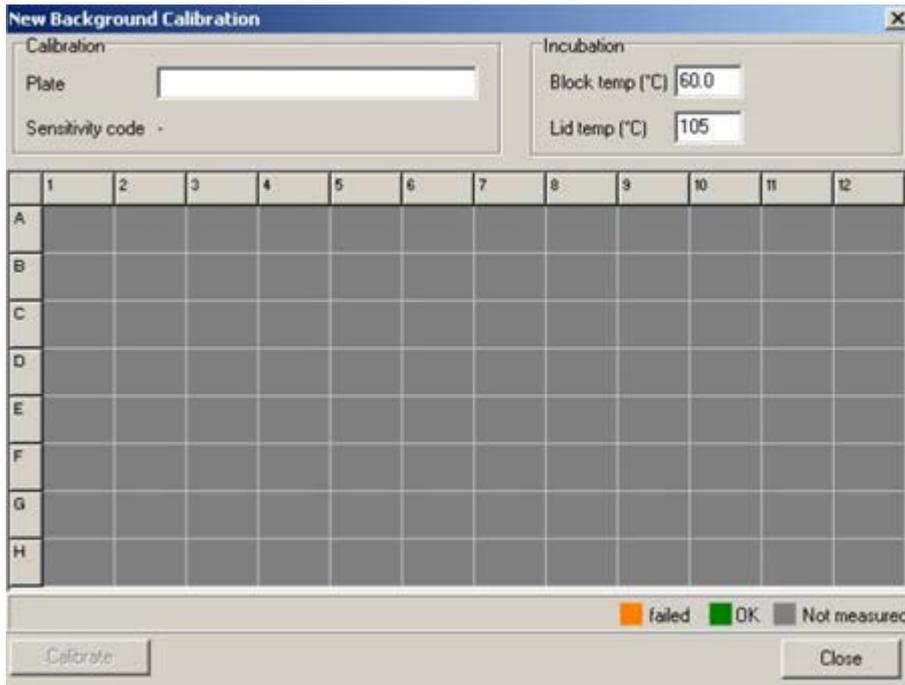


Figure 2. New Background Calibration dialog box.

## RT<sup>2</sup> Profiler PCR Array template setup

6. In the Plate Layout window, specify the following setup parameters:
  - Filter 520nm: SYBR®
  - Sample Vol: 25  $\mu$ l
  - Probe: SYBR Green
  - Background: SABiosciences A plate
7. Select all wells, then right-click in the blue area and select "Unknown" to complete plate layout (Figure 3)

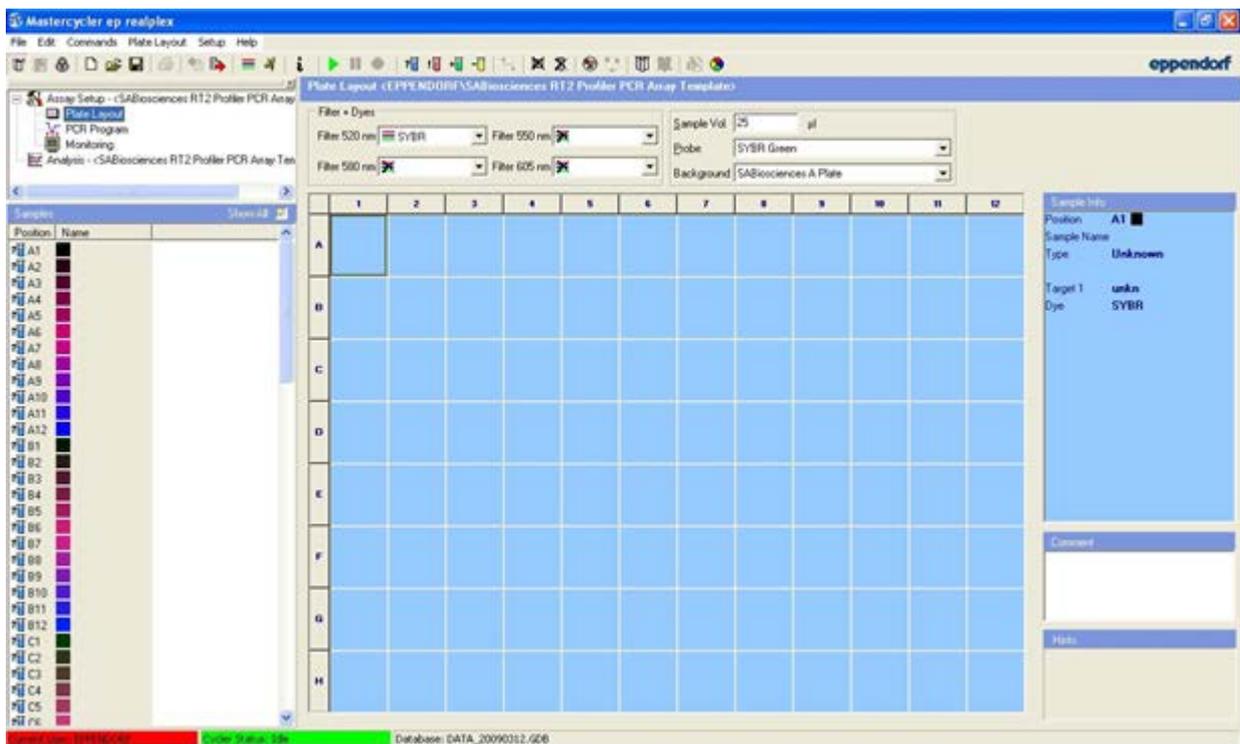


Figure 3. Complete plate layout window.

8. Select PCR Program in the navigator window at the upper left corner of the screen. The default PCR program will appear (Figure 4).

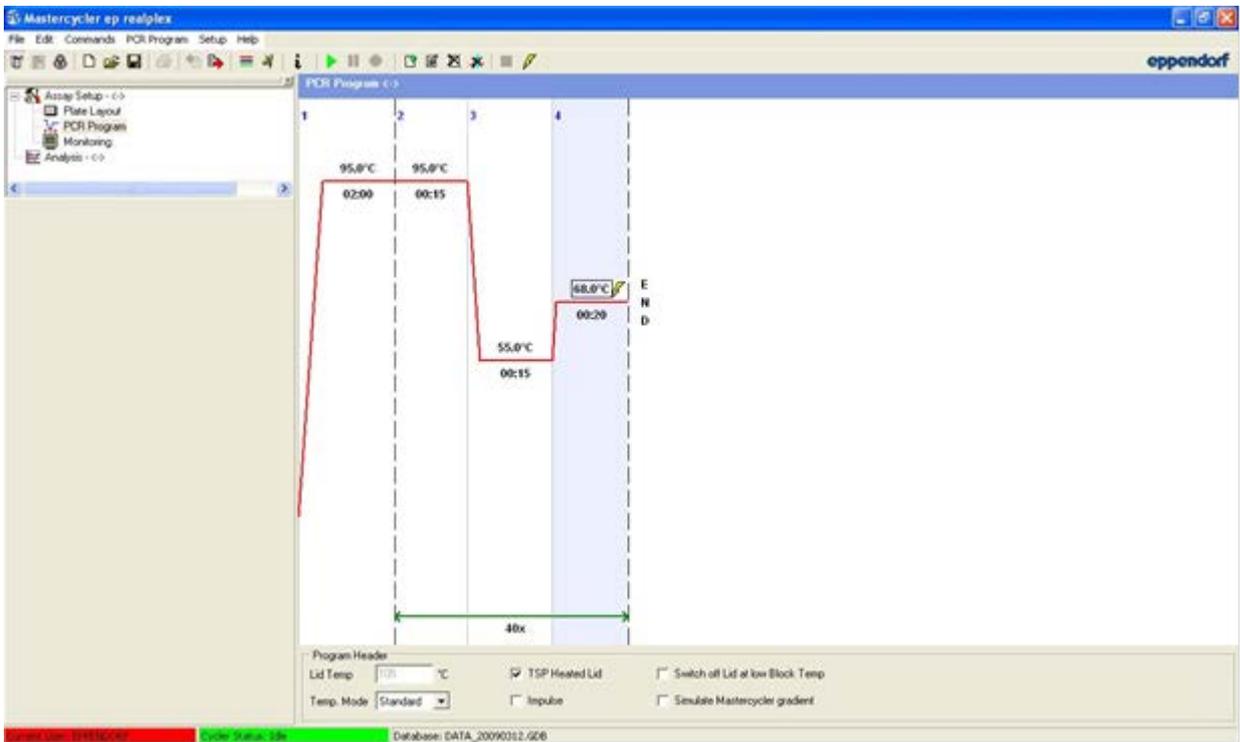


Figure 4. PCR Program window.

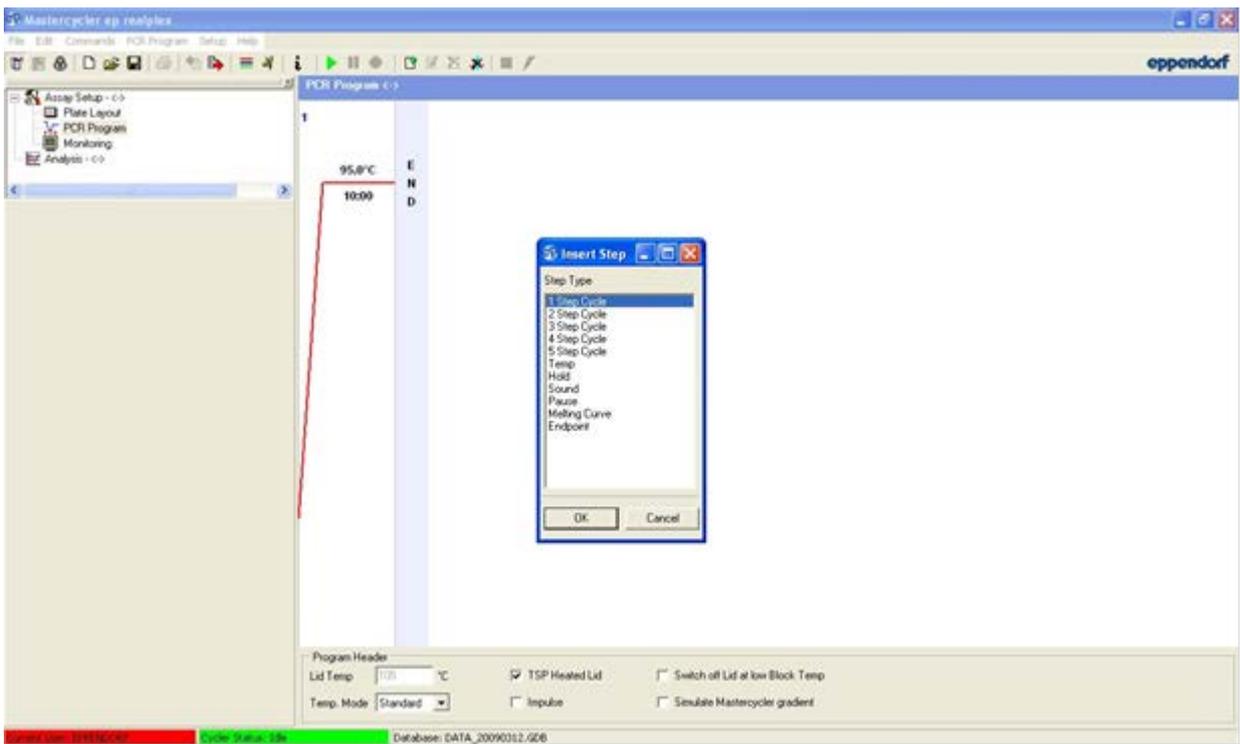


Figure 5. Adding 2-Step Cycle.

9. Edit the default program to create an RT<sup>2</sup> Profiler PCR Array template. Right-click in segment 2, 3, or 4, then click Delete. All three segments will be deleted.
10. Right-click on END. Select "Add Step", then select "2 Step Cycle" and click OK (Figure 5).
11. Right-click on END. Select "Add Step", then select "Melting Curve" and click OK (Figure 6).

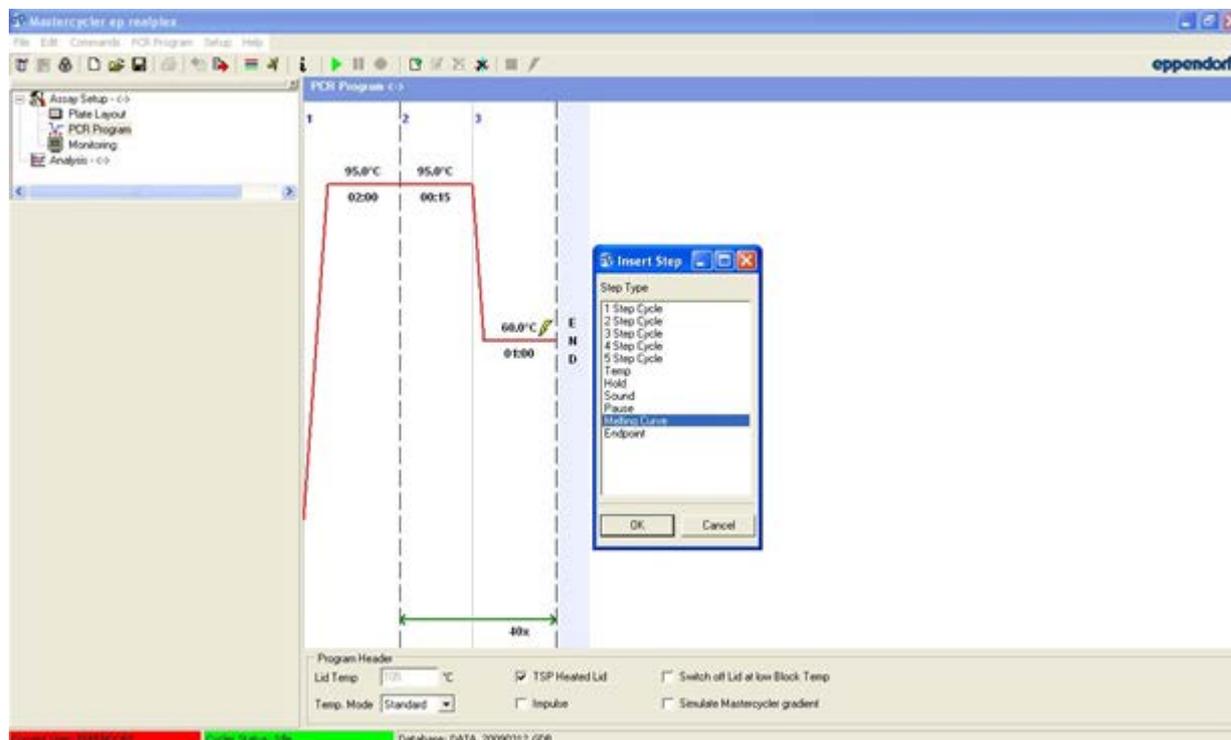


Figure 6. Adding the Melting Curve.

12. Change the length of the heat activation step to 10 minutes. To do so, click on 02:00 in segment 1 and enter 10:00. The final parameters for the PCR program should be as below:

Program	Segment	Temperature (°C)	Hold (mm:ss)	Cycles
Heat activation	1	95.0	10:00	1
PCR cycle	2	95.0	00:15	40
	3	60.0	01:00	
Melt curve	4	95.0	00:15	1
	5	60.0	00:15	
	6		20:00	
	7	95.0	00:15	

## Temperature step parameters

13. Double-click the lightning icon in segment 3. The Temp Step window will appear. Uncheck "Gradient" and change the Ramp [%] to 26.\* The final Temp Step parameters should appear as in Figure 7. Click OK.

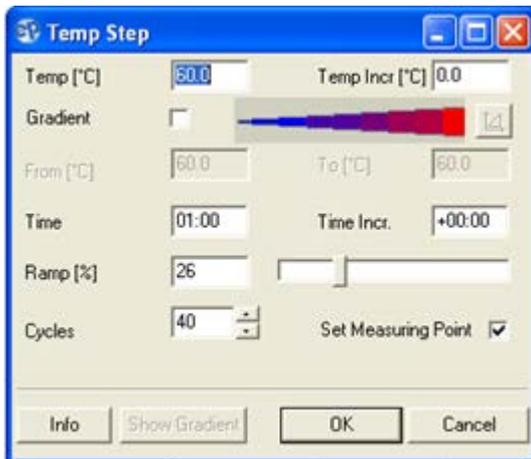


Figure 7. Temp Step parameters.

**Note:** \* 26% is the recommended ramp rate for the silver block. The recommended ramp rate for the aluminum block is 35%.

14. When the PCR Program setup is complete, the window should appear as in Figure 8.

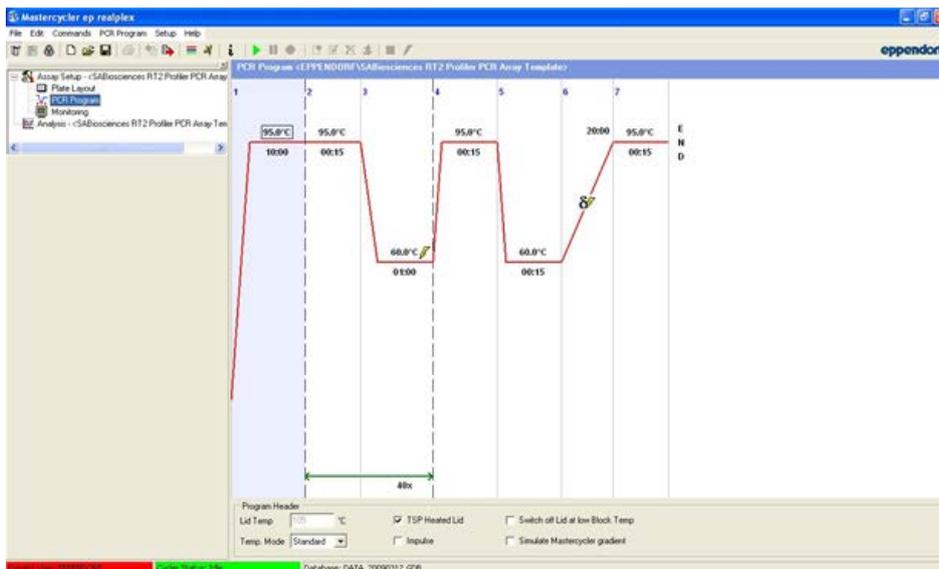


Figure 8. Final PCR Program screen.

15. Click **File** and select **Save As**. In the **Assay Name** field, enter the filename **RT2ProfilerPCRArrySilver** or **RT2ProfilerPCRArryAluminum**, depending on which sample block is being used. From the drop-down list for **Assay Type**, select **Template**. Click **OK**.

## Performing real-time PCR detection

To load the PCR array plate and start the run:

16. When the PCR array is prepared and centrifuged, bring the plate to the *realplex* instrument to load.
17. Make sure the correct thermal block cycler (silver or aluminum) is in place.
18. If the *realplex* instrument is not on, turn it on using the main switch at the back of the instrument. Turn on the computer linked to the instrument and log on to Windows XP.
19. Start *realplex* software version 2.2.
20. Enter **Username** and **Password** when the **Login** dialog box appears and click **OK** to proceed with the login. The **Plate Layout** window will appear.
21. Wait for the **LED** on the top of the instrument to turn green. Make sure the instrument has been warmed up for at least 15 minutes before starting an assay.
22. Push the sealing clamp back into the open position, then slide the heated lid back. Place the PCR array plate on the thermoblock with well **A1** in the upper left corner.
23. Slide the heated lid forward and pull the clamp down.
24. In the **Plate Layout** window, click **File** then **Open**. Select the previously saved **RT2ProfilerPCRArrySilver** or **RT2ProfilerPCRArryAluminum** run template as appropriate, then click **OK**.
25. Click **File**, then **Save as**. Save the file under a new filename as an **Assay**.
26. Click the green arrow button to start the PCR program.
27. The dialog box **Start PCR** will appear. Ensure that **SABiosciences A Plate** is selected as the background plate, then click **OK**. Realtime data acquisition can be viewed in the **Monitoring** window.

## Data analysis

28. Click **File**, then **Open Assay** (Figure 9) and find the run file to be analyzed. Alternatively, upon completion of a PCR experiment, a dialog box will appear prompting you to start analysis. Click **Yes**.
29. **Note:** It is possible to open a data file for analysis during an ongoing assay. A message will appear alerting you that the file will be opened for data analysis only. Click **OK** to view the assay in the analysis window. You will not be able to view plate layout or PCR program information for the completed assay in this mode.

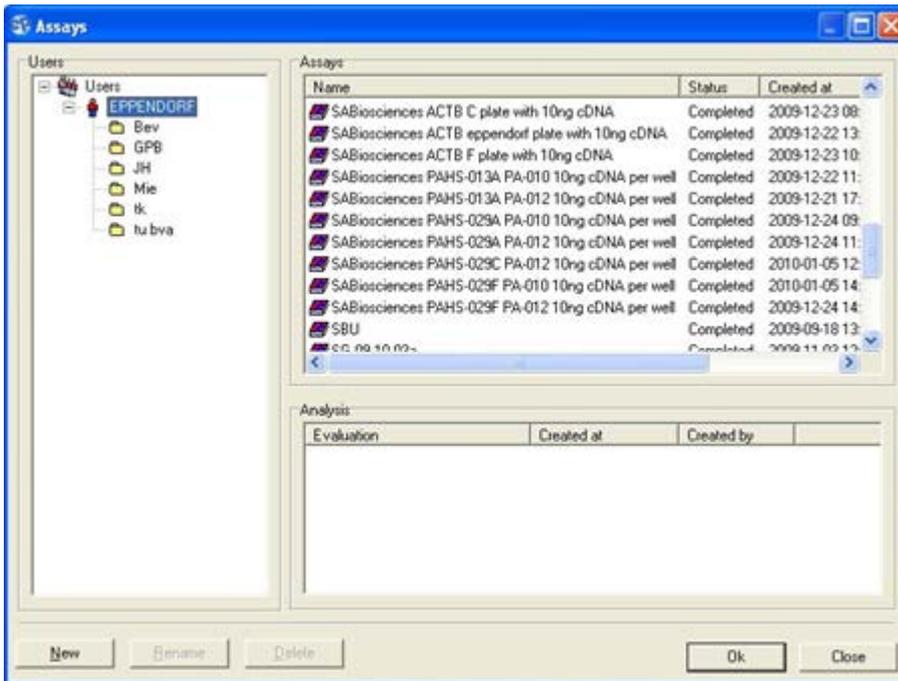


Figure 9. Open the Run File.



Figure 10. Analysis only.

30. In the navigator window at the upper left corner, click on Analysis. The top panel (Figure 11) features a Type of Application combo box. Click on the button to select an analysis module.



Figure 11. Analysis panel.

31. Select Quantification (Figure 12) from as the type of application to obtain  $C_T$  values. Numerical values are listed in the Sample/Analysis window to the left of the screen while amplification curves are shown in the main work area as a function of fluorescence and cycle number. As a default, the curves are shown in linear scale; to view the curves in log scale, check the Log Scale box in the top panel. Click on the button to save the file with results. To view a selection of samples, highlight the desired wells in the Sample/Analysis window or in the MiniPlate Layout at the lower left part of the screen.

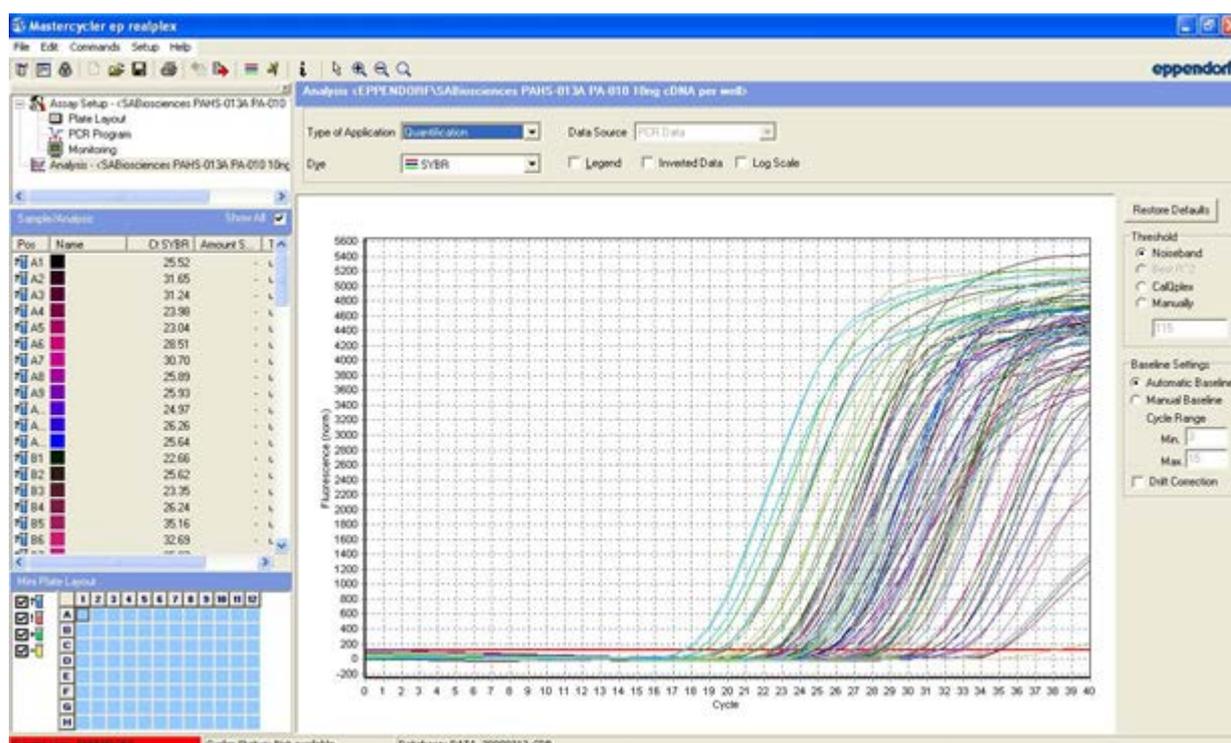


Figure 12. Quantification Analysis window.

32. The Threshold should be set at the same level for all of the PCR arrays in your experiment. The threshold should be set using the log view of your amplification data and set somewhere in the lower 1/3 of your amplification curves. A good indication that the threshold is set in the proper location is that the values of the PPC assays on catalogued arrays should be between 18  $C_T$  and 22  $C_T$ .
33. To set the Baseline, use the Automatic Baseline setting.

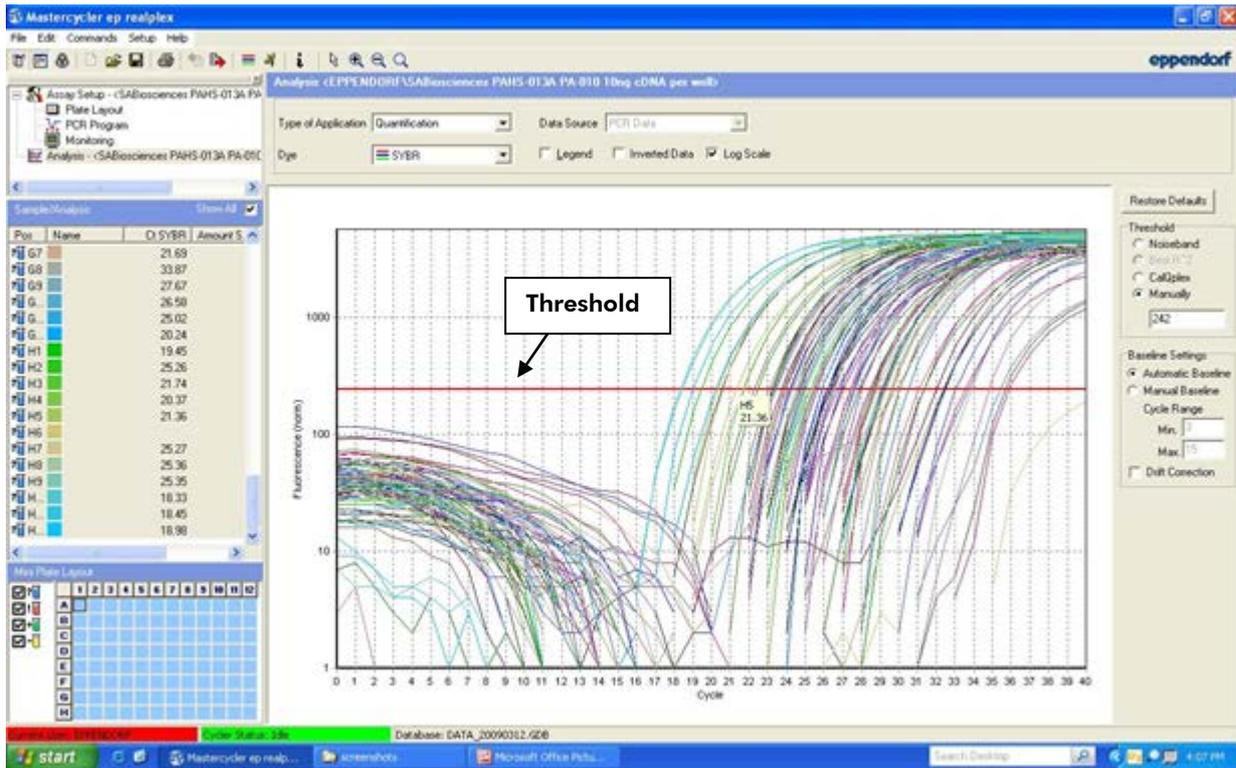


Figure 13. Log view of raw data.

34. To export  $C_T$  values, right click in the Sample/Analysis window and select Save Results As. Export results in text format to view results as a spreadsheet. Click OK. When prompted, choose the file destination, filename, and type. Click Save to export.
35. To export results from a selection of wells, highlight the desired wells in the Sample/Analysis window before right-clicking to save results. Under Include, click the radio button for Selected Samples before proceeding with the export.
36. Note: The entire run file, containing all setup information, raw data, and analyses, can be exported as an \*.asy file. This will allow you to open the file using the *realplex* software on another computer. To do this, go to File and Export. The dialog box Export assay to file will appear. Select the destination for the file and enter a filename. The file type should be realplex-Exchange (\*.asy). Click Save to finish.
37. To import a file, open the *realplex* software and create a new document by clicking on the button (or go to File and New Assay). You will not be able to import a file if an assay is currently in progress. Go to File, then Import. In the Import Assay from file dialog box, find the file you wish to import. Click Open.

38. To proceed with the melt curve analysis, select **Melting Curve** as the type of application in the top panel. The **Sample/Analysis** window will show the wells and corresponding melting point values. Peak curves are shown in the main working window and below that is a plot of fluorescence vs. temperature. Again, a selection of curves may be viewed by selecting the desired wells in either the **Sample/Analysis** or **MiniPlate Layout** windows. Melting points will be analyzed only for those peaks that are above the threshold. The default threshold is 33%, but this can be changed by editing the value at the right of the screen. Alternatively, the threshold can be manually adjusted by moving the horizontal red line. Below the default value, the temperature that is displayed represents the temperature at the vertical green line. This line can be moved to any peak maximum to determine the melting point.
39. To export T<sub>m</sub> values, right click in the **Sample/Analysis** window and select **Save Results As**. Export results in text format to view results as a spreadsheet. Click **OK**. When prompted, choose the file destination, filename, and type. Click **Save** to export.

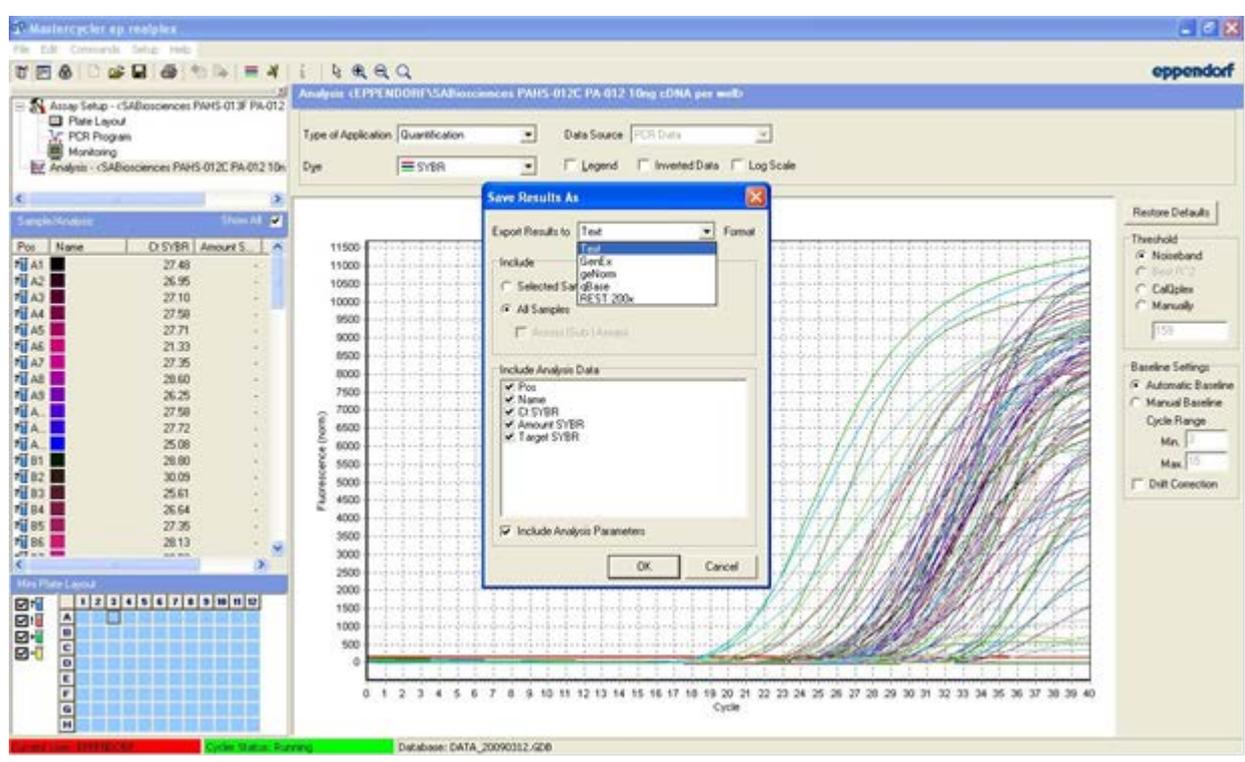


Figure 14. Exporting the C<sub>T</sub> results.

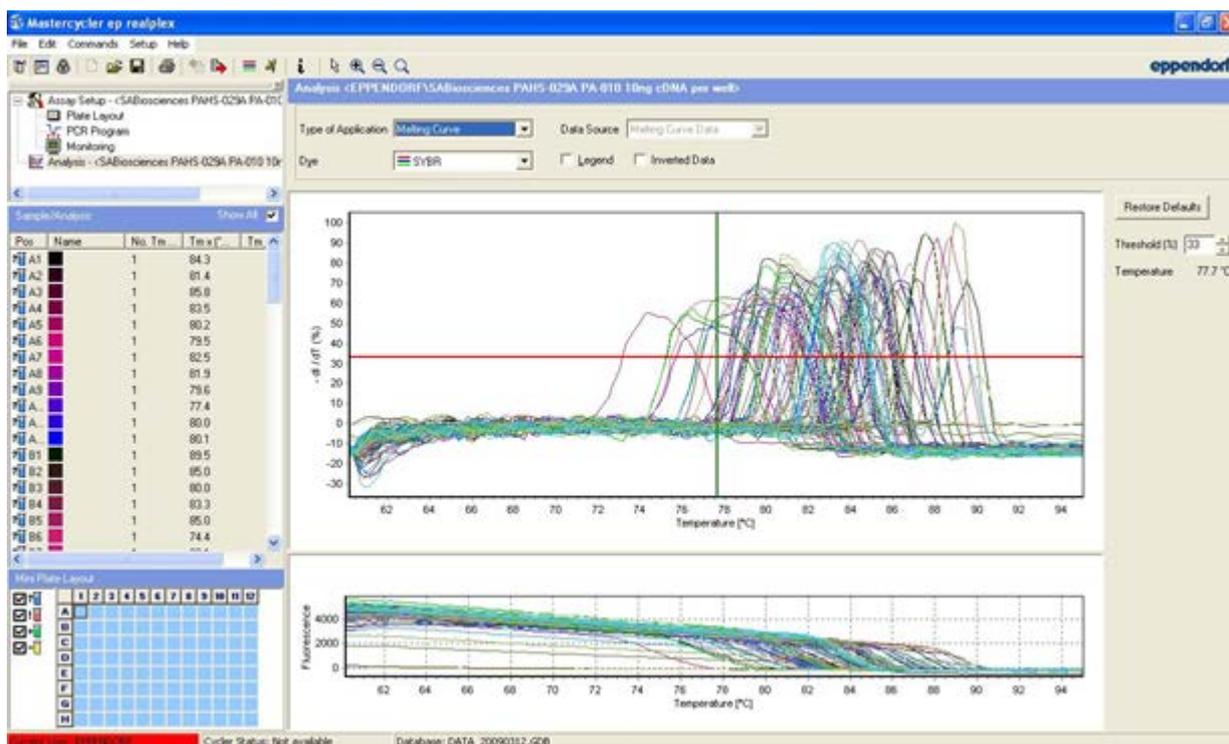


Figure 15. Melting curve analysis.

The RT<sup>2</sup> 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](http://www.qiagen.com/literature). Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx).

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