Protocol Sheet

Bio-Rad® iCycler® and iQ® Real-Time PCR Systems instrument setup instructions for EpiTect® ChIP PCR Arrays

Important points before starting

■ Please read the handbook supplied with the EpiTect ChIP PCR Array, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

■ Please make sure that a pure dye calibration has been performed for SYBR® Green I for your system. Please refer to the manufacturer's Instruction Manual for the pure dye calibration procedures if needed.

Procedure

Setting up a PCR protocol template

1. Open the iCycler iQ Optical System Software Version 3.1 on the computer that is connected to the Bio-Rad iCycler iQ Real-time PCR Detection System.

2. Click on the "Library" module on the left panel. Select the "View Protocol" tab. Under "Protocol Files," select "2StepAmp+Melt.tmo." Click "Edit This Protocol" on the right; this will open the "Edit Protocol" tab in the "Workshop" module. Edit the thermal profile in the table at the bottom of the screen as follows (see Figure 1):

    ▪ Click on the "Dwell Time" cell of Cycle 1, Step 1 and change the value to 10:00 minutes with Setpoint 95.0°C.

    ▪ For Cycle 2, Step 1, change the "Dwell Time" value to 0:15 (15 seconds) with Setpoint 95.0°C. For Cycle 2, Step 2, change the "Dwell Time" value to 1:00 minute with Setpoint 60.0°C. Double-check that the value for the "Repeats" cell in "Cycle 2" is 40.

    ▪ The melting curve is performed from Cycle 3 to Cycle 5 with the following default parameters: Cycle 3, 1 Repeat, 1 Step, Dwell Time 1:00 minute at Setpoint 95.0°C; Cycle 4, 1 Repeat, 1 Step, Dwell Time 1:00 minute at Setpoint 55.0°C; Cycle 5, 80 Repeats, 1 Step, Dwell Time 0:10 (10 seconds) at Setpoint 55.0°C, Melt Curve, + Temp 0.5°C.

    ▪ Under "Select Data Collection Step(s)," double-check that real-time detection is selected for Cycle 2, Step 2 and the green camera icon Melt Curve data collection is shown for Cycle 5, Step 1.

- Enter "EpiTect ChIP PCR Array Protocol Template" as the new filename in the field for "Protocol Filename" and then choose "Save this Protocol" to save the template.
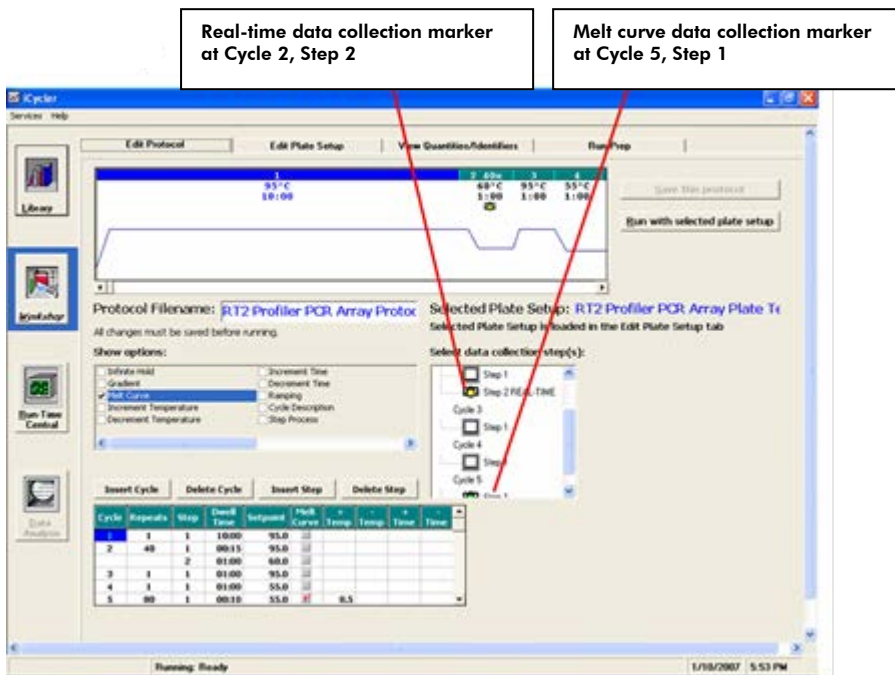

Figure 1. Protocol setup.

3. To set up a template file for "Plate Setup," click on the "Library" module on the left panel and then select the "View Plate Setup" tab; click "Create a New Plate Setup" on the right. This will open the "Edit Plate Setup" tab in the "Workshop" module. Choose the "Whole Plate" mode. In the "Samples: Whole Plate Loading" tab (see Figure 2), click to select the icon for "Unknown." In the diagram of the 96-well plate, click on each well one at a time to designate the sample type as "Unknown" and number the well.

4. When finished loading samples, click on "Select and Load Fluorophores" (see Figure 3). Select "SYBR-490" as the fluorophore by clicking the box next to the fluorophore in the "Select or Deselect a Fluorophore" box. Assign a color for SYBR-490 by clicking on one of the crayons. With the "SYBR-490" crayon selected, load fluorophore to all wells by clicking the top left corner of the plate to select all wells at once. Enter "EpiTect ChIP PCR Array Plate Setup Template" as the new filename in the "Plate Setup Filename" box and click "Save this plate setup" to save the file as a *.pts file.
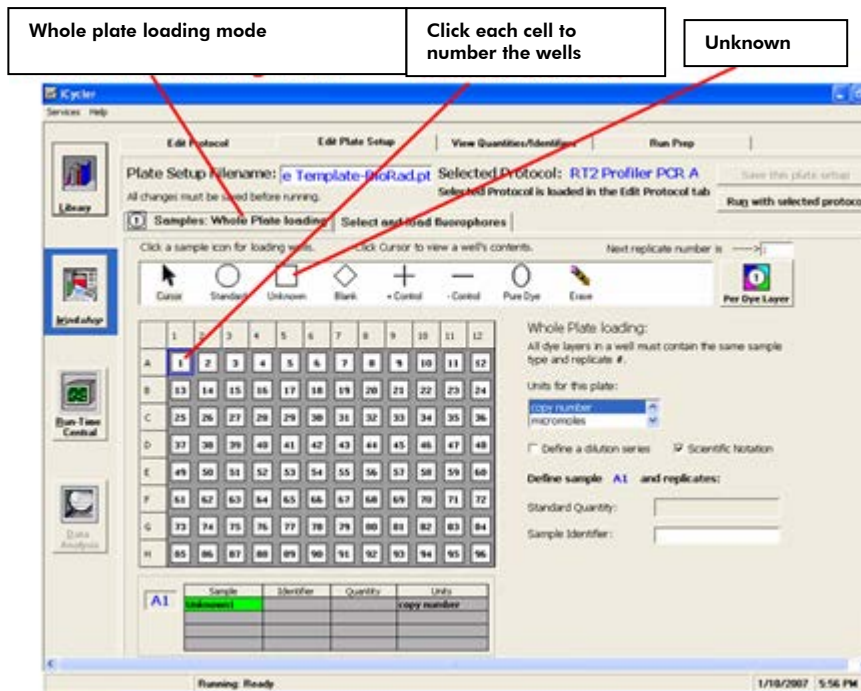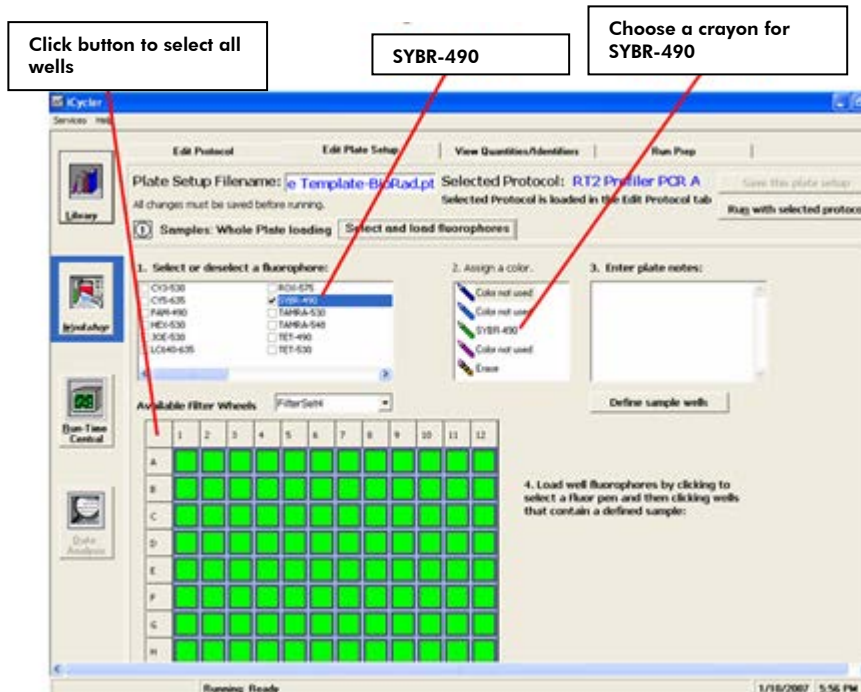
Figure 2. Plate setup — sample loading.

Figure 3. Plate setup — fluorophore loading.

Performing real-time PCR detection

1. If the thermocycler is off, turn on the iCycler and log onto the instrument. Allow the camera to warm up for 30 minutes, and then switch on the computer connected to the thermocycler.

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 x g for 45 seconds.

3. Open the thermocycler cover to expose 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 holder. Close the cover.

4. Open the iCycler iQ Optical System Software.

5. In the "Library" module, click to open the "View Protocol" tab. Under "Protocol Files," click to highlight and load the previously saved "EpiTect ChiP PCR Array Protocol Template" file. Then click to open the "View Plate Setup" tab; under "Plate Setup Files," click to highlight and load the "EpiTect ChIP PCR Array Plate Setup Template" file. Click "Run with Selected Protocol" to initiate the run with the selected plate setup and indicated protocol. This will lead you to the "Run Prep" tab in the "Workshop" module (see Figure 4). Enter the reaction volume (25 μl) for the "Sample volume." Select "Experimental Plate" under "Select Well Factor Source." When ready, click on "Begin Run." A "Save" dialog box will open. Enter a name for the optical data file (*.opd).
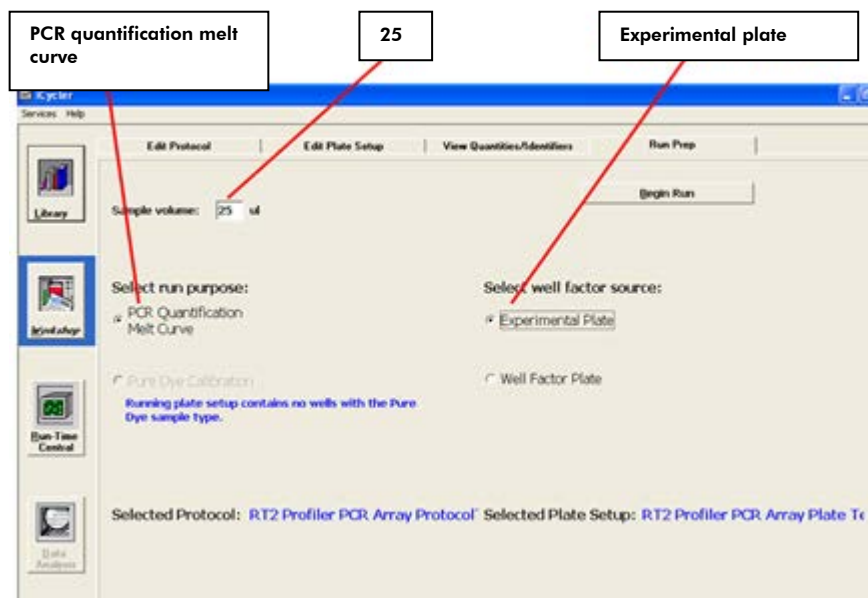

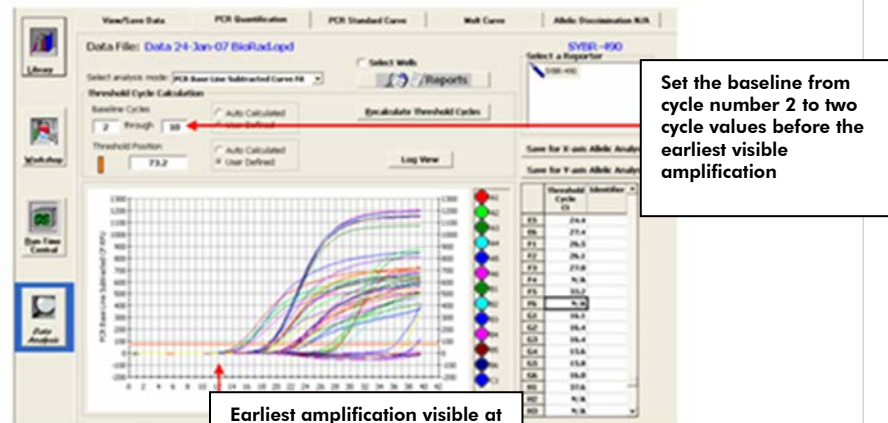Figure 4. Plate run — running plate. .

After the PCR run

1. Select the "Library" module and click on the "View Post-Run Data" tab. Select a data file and click "Analyze Data" on the right under "Data Analysis Operations." The data file will open in the "PCR Quantification" tab of the "Data Analysis" module. Select "PCR Base Line Subtracted Curve Fit" in the field for "Select analysis mode."

2. Choose "SYBR-490" in the "Select a Reporter" box.

3. Follow the procedures below to calculate the threshold cycle (C_T) for each well (see Figure 5):

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

- To define the "Baseline," use the linear view of the amplification plots by clicking the "Normal View" button right above the graph. If the graph is already in the linear scale, the "Log View" button will appear instead. With the linear plots, determine the cycle number at which the earliest amplification can be seen. Under "Threshold Cycle Calculation," select "User Defined" for "Baseline Cycles" and set the 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. Select "User Defined" for "Threshold Position." 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. Click "Recalculate Threshold Cycles" to update threshold cycle values.

4. Click on the "Melt Curve" tab of the "Data Analysis" module to view the melting curves.

5. Click on the "Reports" button to obtain the customized reports of the PCR quantification data; the window for "Report Viewer" will open. On the left panel under "Select Report," select to view "Melt and Amp Data." To save the report, select "Save to File," and enter a filename for the report to be saved in the Rich Text Files format.

6. Close the "Report Viewer" window, and check the "Open/Save Settings" and select "Save OPD File" to save the data file with the current analysis parameters.

Linear view

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

Earliest amplification visible at cycle 12.

Log view

Linear phase

Set the threshold above the background and within the lower third of the linear phase of the amplification plot
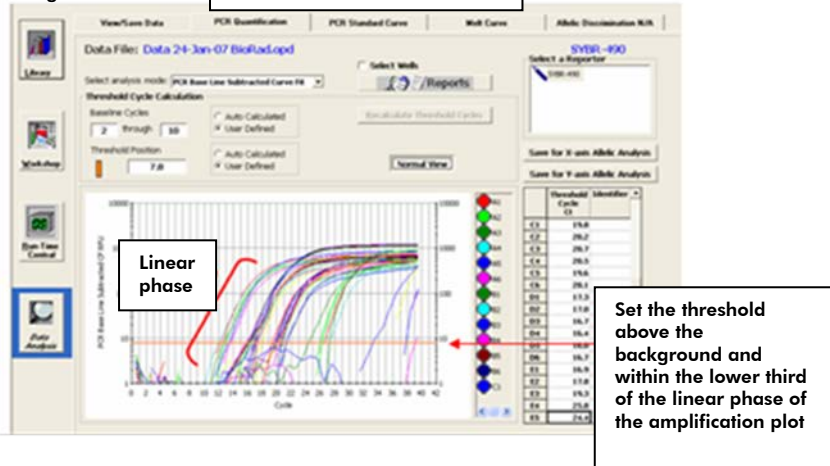
Figure 5. Setting the baseline and threshold values.

The EpiTect ChIP 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.

www.qiagen.com

Australia ▪ 1-800-243-800	**France** ▪ 01-60-920-930	**The Netherlands** ▪ 0800 0229592
Austria ▪ 0800/281010	**Germany** ▪ 02103-29-12000	**Norway** ▪ 800-18859
Belgium ▪ 0800-79612	**Hong Kong** ▪ 800 933 965	**Singapore** ▪ 65-67775366
Canada ▪ 800-572-9613	**Ireland** ▪ 1800 555 049	**Spain** ▪ 91-630-7050
China ▪ 021-51345678	**Italy** ▪ 800 787980	**Sweden** ▪ 020-790282
Denmark ▪ 80-885945	**Japan** ▪ 03-6890-7300	**Switzerland** ▪ 055-254-22-11
	Korea (South) ▪ 1544 7145	**UK** ▪ 01293-422-911

QIAGEN

Sample & Assay Technologies