

Protocol Sheet

Applied Biosystems® 7500 and 7500 FAST (software version 2.0.4) instrument setup instructions for EpiTect® Methyl II PCR Arrays

Important points before starting

- Please read the handbook supplied with the EpiTect Methyl II 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 ABI 7500 v2.0.4 software on the desktop of the computer that is connected to the ABI 7500 (Standard or FAST) system.
2. Select “New Experiment” from the top left of the screen.

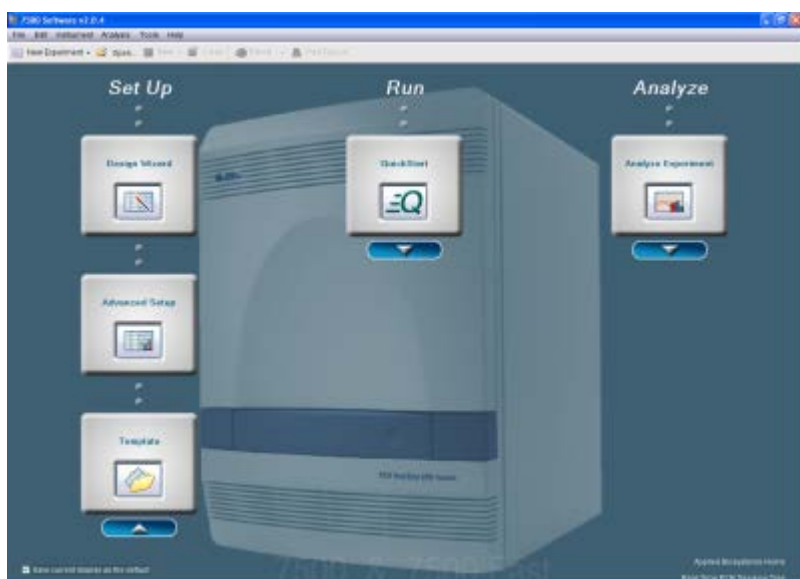


Figure 1. “Set Up” screen.

3. Define “Experimental Properties.”
 - Identify the experiment.



- Type in “Experiment Name” based on the experimental arrangement.
- Type in “Barcode,” “User Name,” and “Comments” (optional).
- Select the instrument that will be used to run the experiment.
 - Select “7500 (96 wells)” or “7500 FAST (96 wells)”.
- Select the type of experiment to be set up.
 - Select “Quantification-Standard Curve.”
- Select the reagent to be used for detection of the target sequence.
 - Select “SYBR® Green Reagents.”
- Select ramp speed to use in the instrument run.
 - Select “Standard (~2 hours to complete a run).”

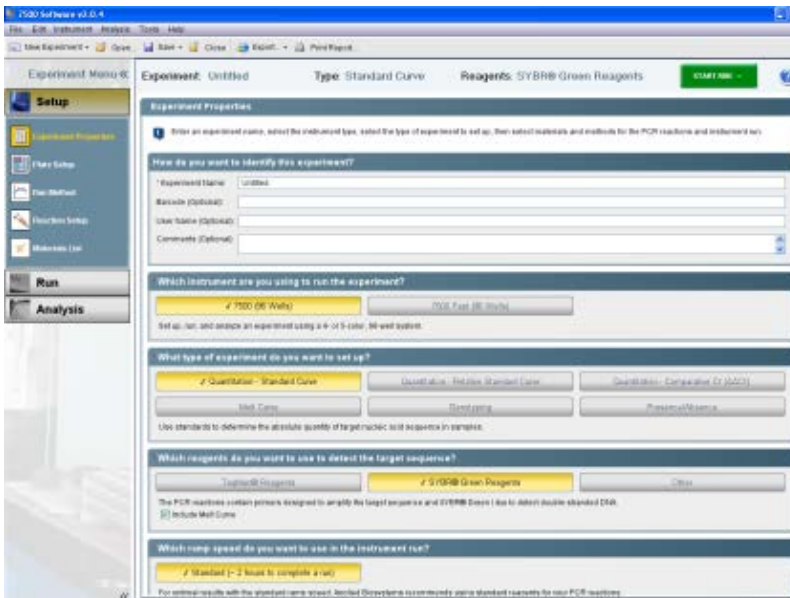


Figure 2. “Experimental Properties” screen.

4. Click “Plate Setup” (from tab list on left).

- Click on “Define Targets and Samples.”
 - “Target Name”: Use “Default Target 1”, select “SYBR” for “Reporter” and “None” for “Quencher.” Select “Green” (or any other) for “Color.”
 - “Define Samples”: select sample such as “Sample 1” or your defined sample name.
 - For “Define Biological Replicate Group”, choose “None.”
 - Click “Assign Targets and Samples.”

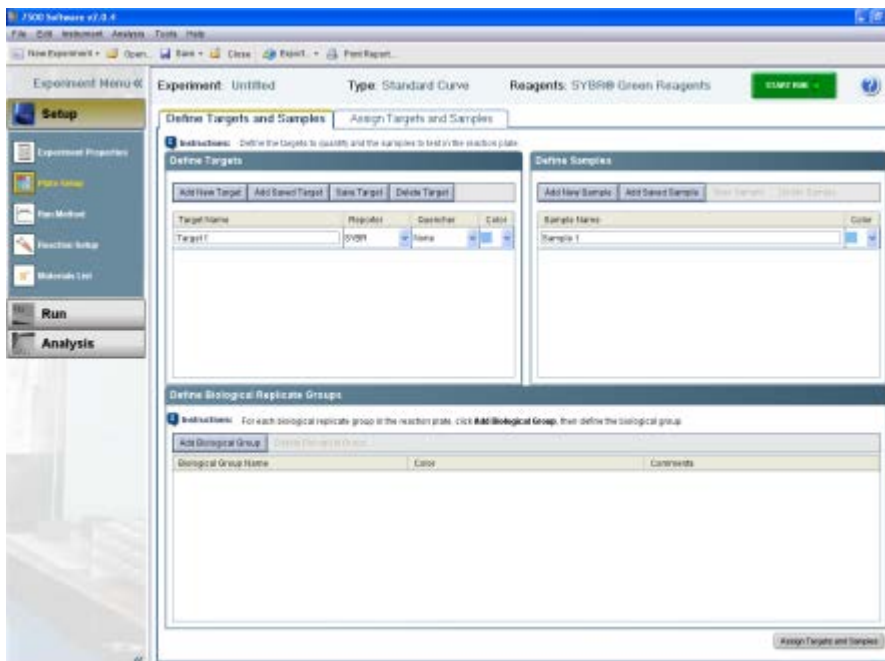


Figure 3. "Define Targets and Samples" tab.

- In "Assign Targets and Samples" tab:
 - Highlight the entire plate by clicking the square between A and 1 in "View Plate Layout."
 - Check the box next to "Target 1" under "Assign target(s) to the selected wells" (if different wells are set for different target bases on the array format, wells can be checked individually).
 - For "Assign samples to the selected wells", select "Sample 1" or the name as previously defined.
 - Verify that all wells in the "Plate Layout" view have the "U" symbol ("U" = unknown) and "Sample 1."
 - Select "ROX" as the passive reference dye.

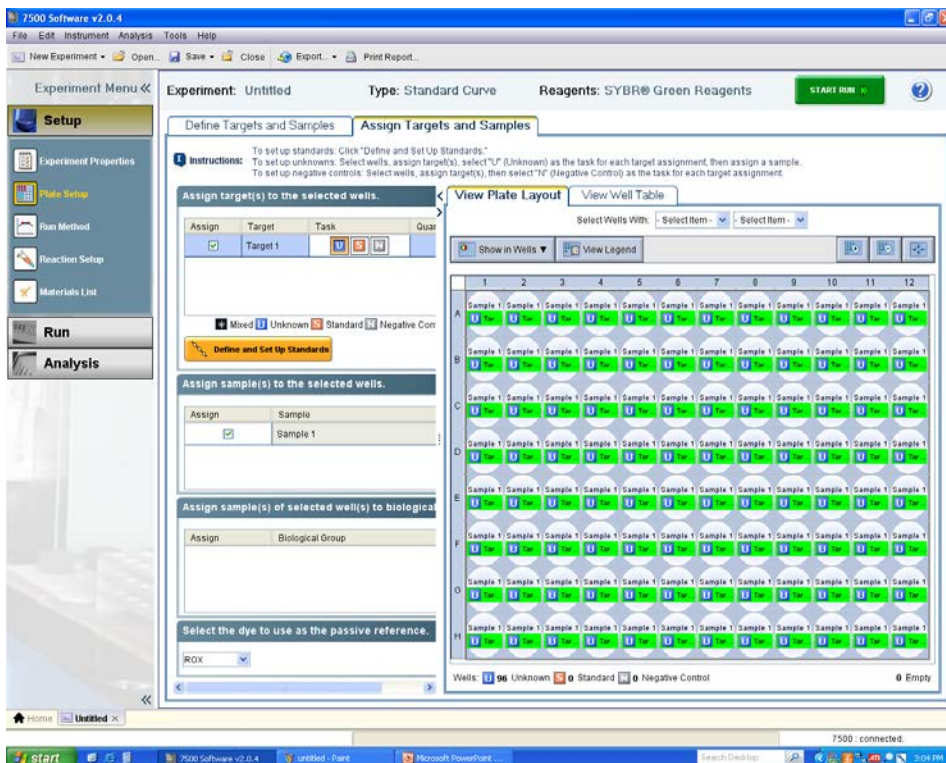


Figure 4. “Assign Targets and Samples” and “View Plate Layout” tabs.

5. Click “Run Method” menu (this setting should default to run protocol with melting curve). Change settings in the “Graphical View”:
 - Set “Reaction Volume Per Well” to 25 µl.
 - “Holding Stage”: 95°C for 10 minutes
 - “Pre-amp Stage”:
 - “Number of Cycles”: 3
 - “Temperature”: 99°C 00:30, 72°C 01:00
 - “Cycling Stage”
 - “Number of Cycles”: 40
 - 97°C for 00:15; 72°C for 01:00
 - “Melt Curve Stage”: Use default setting from 60°C to 97°C.

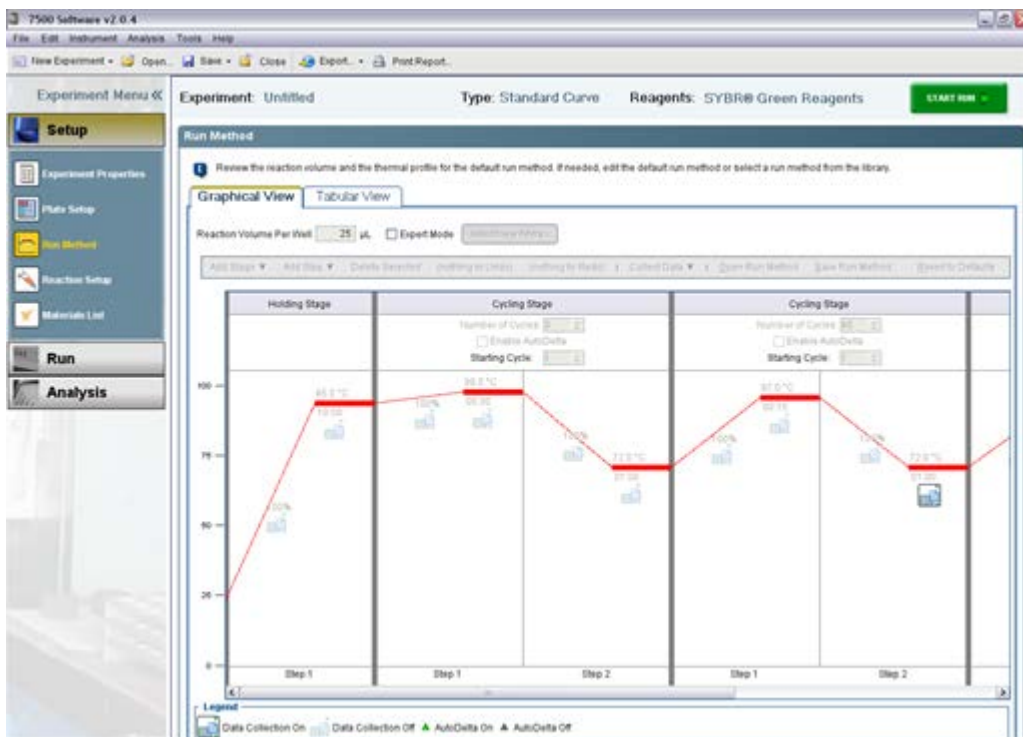


Figure 5. "Graphical View" tab on "Run Method" screen.

6. Click "Start Run."
7. Save the template file for further use by clicking "Select File" > "Save As Template". Alternatively, download the template from http://sabiosciences.com/dna_methylation_data_analysis.php.

Performing real-time PCR detection

1. If the thermocycler is off, press the power button to switch on the instrument. Wait for the instrument to boot and display the "Power" status light. Switch on the computer connected to the thermocycler.
2. Make sure that there are no bubbles in any well, and that the reaction mix is positioned at the bottom of each well. If this is not the case, centrifuge the plate at about 2000 x g for 2 minutes.
3. Open ABI 7500 Software v2.0.4 (or similar version).
4. Select "File" > "New Experiment" > "From template." Then select the right template for your experiment. Click "Open." This will load the previously saved setup to the new plate document.
5. Add an "Experiment Name" in "Setup" > "Experiment Properties."
6. Save the new document under a new filename, as "Experiment Documents Single File" (*.sds).
7. Load the plate in the plate tray with A1 in the top left corner.

8. Select the "Run" tab on the left panel. Click "Start Run" to begin the PCR run. Wait 30 seconds to 1 minute for the initial priming, and then the run should start. The estimated run time will then appear on the screen.

After the PCR run

1. Select "Analysis" on the left panel. On the lower panel, "Options," select the radio button next to "Auto Baseline" and deselect the radio button next to "Threshold: Auto." Add a desired threshold (0.05 is usually fine; please refer to step 19 for manual selection of the threshold). Click the green "Analyze" button on the top right to perform the data analysis.
2. Selecting the radio button on lower panel next to "Threshold" will show the threshold in the figure, and similarly for "Baseline."
3. Under "Amplification Plot/Plot Setting," the default setting for "Plot Type" is " ΔR_n vs. Cycle." Other plot types may also be selected. The default "Graph Type" appears as log format, but linear is also available.
4. For exporting results, choose "Select File" ► "Export." The "Export Data" window will appear. Under "Export Properties":
 - Select data to export. Use the radio button to select the data to export (usually "Results").
 - Select one file or separate files by selecting the radio button.
 - Enter export file properties for name and location. Also select the "File Type" (choose *.xls for Excel[®] file and *.txt for regular text file).
 - Select the radio button beside "Open file(s) when export is complete" to view the file immediately after exporting.

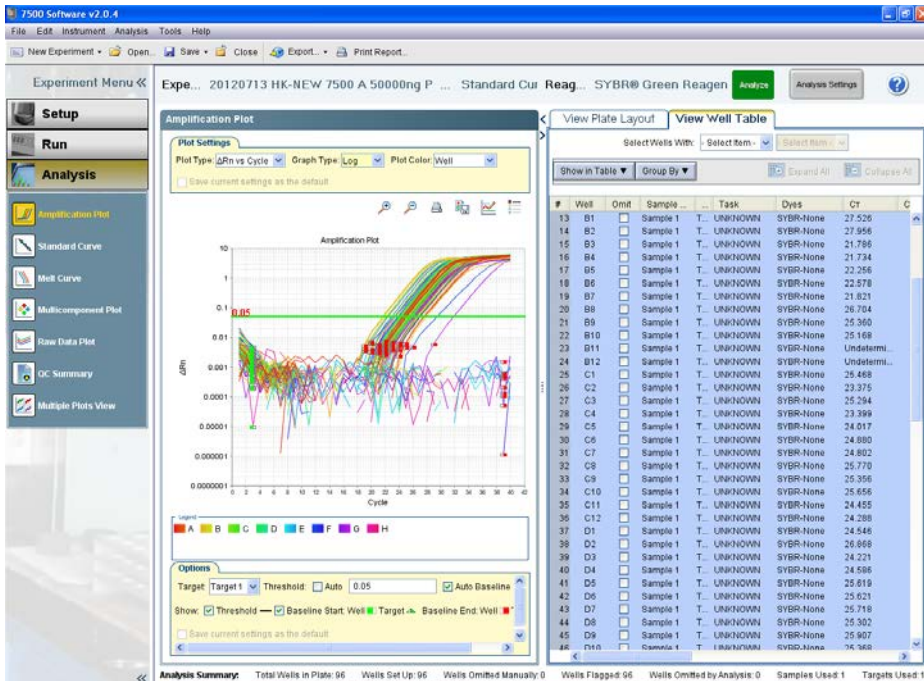


Figure 6. Data analysis screen.

5. Follow the procedures below to select the baseline and threshold manually if needed. (Please use the same threshold for all plates in one study).

- To define the baseline, use the “Linear View” of the “Amplification Plot/Plot Settings.” With the linear plots, determine the cycle number at which the earliest amplification can be seen. Use the red sliding bars on the X-axis to set the manual 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 Plot/Plot Settings.” 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 (please remember the threshold that is set the first time and use it for all subsequent analysis in same study).

The EpiTect Methyl II 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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