W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University

Microarray Resource

Real-time ABI 7900HT Protocol

A. Equipment and supplies:

Pre-assembled 384-well plate, sealed, kept in the dark
Optically clear-adhesive plate cover*
Plate sealer*
Plate centrifuge*
*denotes availability at the Keck Affymetrix Facility

NOTE: PLEASE REFER TO THE ABI 7900 USERS GUIDE AND USER BULLETIN #2 FOR MORE DETAILS ON EXPERIMENTAL SET UP AND DATA ANALYSIS.

B. Method:

1. Spin plate(s) down in centrifuge @ 3500 rpm for 3 min.
2. Seal with optically clear plate cover and plate sealer, ensuring not to touch any area over a well.
3. Turn on ABI 7900 using the power button on the front bottom left. Wait 1 min for instrument to power up.
4. Launch SDS 2.1 software.
5. Select File → New. Select appropriate protocol, e.g.'Absolute Quantification'.
6. Select the **Instrument** tab.
7. Adjust thermocycling parameters, add dissociation stage, and volume of reaction, if needed.
8. Select 'Connect' to the instrument.
9. Select 'Open/Close' to open instrument and load plate in the correct orientation, with well A1 aligned appropriately. Shut door.
10. Select **Set Up** tab. Select 'Add Detector'.
11. Either (1) select preexisting detector from list and hit 'Copy to plate document' or (2) **New** to create new detector. For a new detector, enter name and detector type. Follow instruction 1 in step 11.
12. Repeat process until all detectors have been listed in the set up page.
13. For each well on the plate that has a reaction in it, assign appropriate detector and task, i.e. unknown, standard (for wells in standard curves, add quantity of template in ng), or no template control (NTC).
14. Select 'Save as' and save as an .sds file and/or a .sdt (template file). If you save as an .sdt file, the plate layout will be reusable. In order to begin a run, ensure the software is viewing an .sds file (not an .sdt file).
15. Select 'Start' on the **Instrument** tab.
16. At the completion of the run, remove plate from instrument and shut the 7900HT off.

Data analysis:

1. Open .sds file.
2. Hit green play button (triangle) on tool bar to analyze data.
3. To view analyzed data, the wells selected on the plate layout must match selected detector in viewing pane.
4. The file will need to be reanalyzed each time it is either modified or opened in SDS. Select the **Save** option when closing a modified .sds file.
5. To export data, select 'Export' from the file menu in the tool bar. Browse to folder to save to. Name and save .txt file.
6. To view in Excel, launch Excel from the **Start** menu. Select 'Open'. Select 'All files' in the dialog box. Select 'Finish' and view exported results.