Quickstart Guide
ABI PRISM® 7700 Dissociation Curve Software

Overview

- The ABI PRISM® Dissociation Curve Analysis Software is a Macintosh® application that uses the multicomponent data exported from the SDS software v1.7 to display the dissociation curves for each sample.

- The principle feature of the ABI PRISM Dissociation Curve Analysis Software is to provide melting curve analysis on SYBR® Green dye assays run on the ABI PRISM 7700. However, any dye loaded on the system can be analyzed in the software.

- The ABI PRISM Dissociation Curve Analysis Software is available free of charge from the Applied Biosystems website: www.appliedbiosystems.com/support/software/7700/updates.cfm

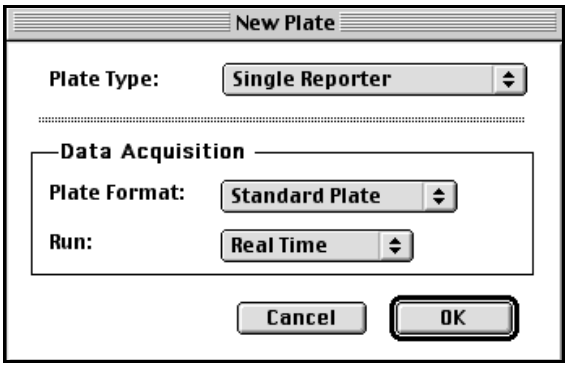
- Pre-requisite: SDS software v1.7 or higher is required for proper data collection. The most current SDS software is available free of charge from the Applied Biosystems website: www.appliedbiosystems.com/support/software/7700/updates.cfm

- Dissociation Curve Analysis can be conducted either at the end of a new real-time run or it can be performed as a separate run of a previously run plate.

Directions

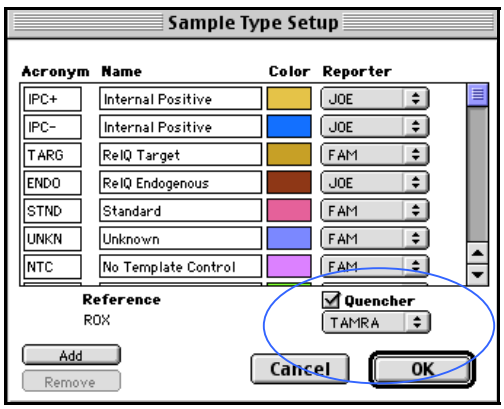
1. Launching the SDS v 1.7:

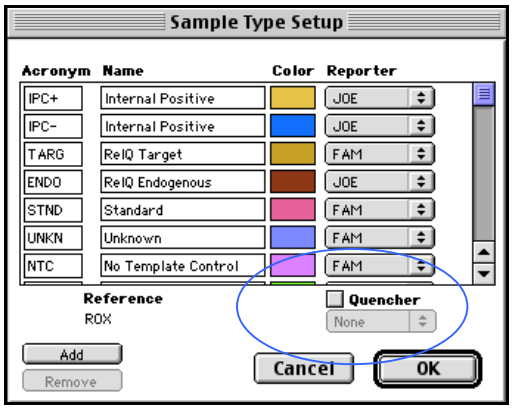
- Double click the **Sequence Detection v1.7** icon and launch the software.
- From the **File** menu select **New Plate**.
- Configure the **New Plate** as shown below.
- When finished with the plate configuration click **OK**.



2. Disabling the Quencher: The quencher dye must be disabled when running SYBR Green dye assays.

- From the Plate Setup screen click on **Sample Type Setup.**
- The **Sample Type Setup** dialog box will appear.



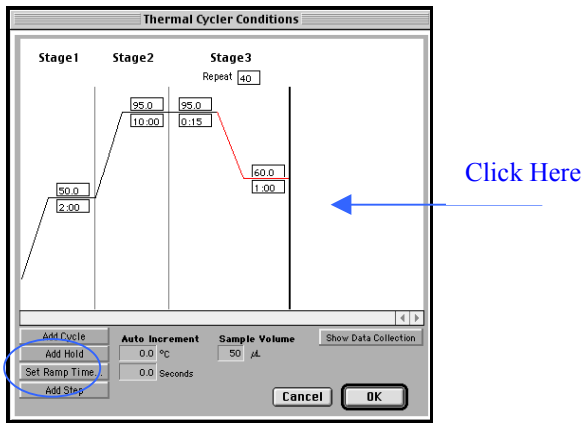


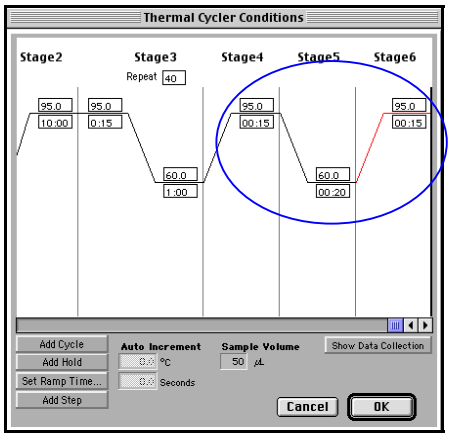
- Click the **Quencher** box and change it to read “none” and unclick the box.
- Click **OK**.

3. Configuring the Dissociation Curve Profile:
NOTE: There are TWO methods- please choose either method 1 or method 2

Method 1- Linking a dissociation curve profile to the end of a real time run.

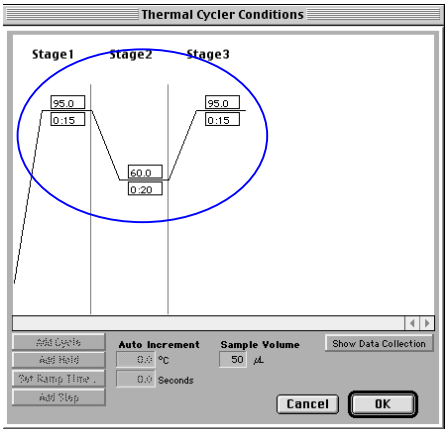
- From the Setup screen click on the **Thermal Cycler Conditions**.
- The **Thermal Cycler Conditions** dialog box will appear.
- Click the vertical bar after the annealing/extension step.
- Click on **Add Hold** three times- adding three distinct stages after the real time profile. .
- Change the temperatures and times as follows: 95°C/15 seconds, 60°C/20 seconds and 95ºC/15 seconds.
- Continue with Step 4.





Method 1: Linked to a real-time run

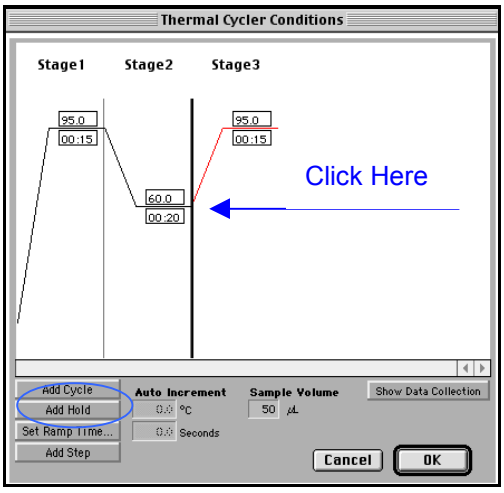
- ***Method 2- Creating a separate dissociation curve profile (after the PCR cycling is completed).***
  - From the Setup screen click on the **Thermal Cycler Conditions**.
  - The **Thermal Cycler Conditions** dialog box will appear.
  - Delete the default thermal cycling profile.
  - Click on **Add Hold** three times- adding three distinct stages.
  - Change the first stage to 95°C/ 15 seconds, the second stage to 60°C / 20 seconds and the third stage to 95°C/ 15 seconds.
  - Continue with Step 4.

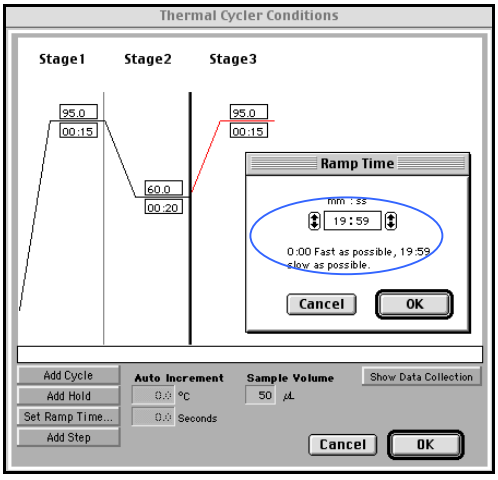


Method 2: After the real time run is complete

4. Setting the ramp time: The ramp should span the longest possible time (19:59). This provides the greatest separation of the derivative peaks during the dissociation curve analysis and therefore maximum resolution for the run.

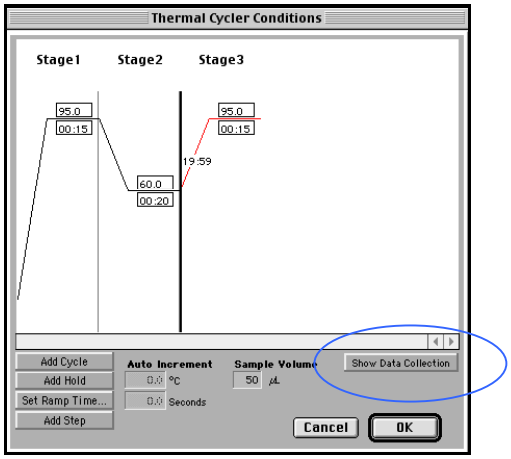
- Click or select the ramp line connecting the annealing stage with the final denaturation stage. The software will highlight the selected stage in red.
- Click on **Set Ramp Time** box.
- The **Ramp Time** dialog box will appear.
- Set the ramp time for **19:59**.
- Click **OK.**

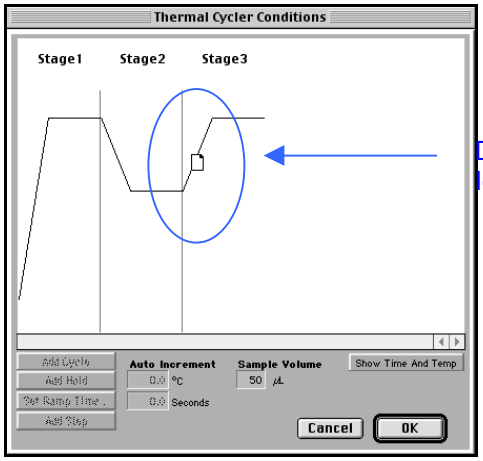




5. Setting the Data Collection Points

- Click **Show Data Collection.**
- The Data Point Collection icons will appear on the Thermal Cycler Conditions dialog box.
- Add a **Data Collection** icon to the ramp by clicking on the slope.
- Click **OK.**

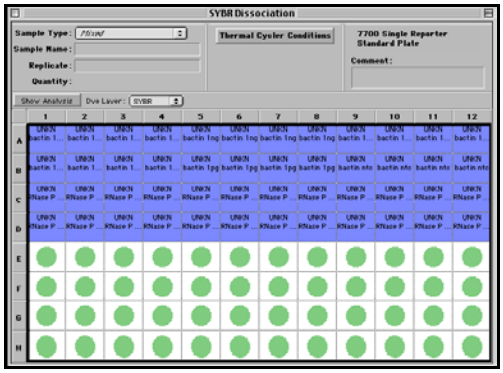




NOTE: When linking a dissociation curve profile to the end of a real-time run make sure there are data collection icons located on the annealing step during the cycling.

6. Labeling plate:

- Configure the plate document in the SYBR dye layer with the appropriate sample information.



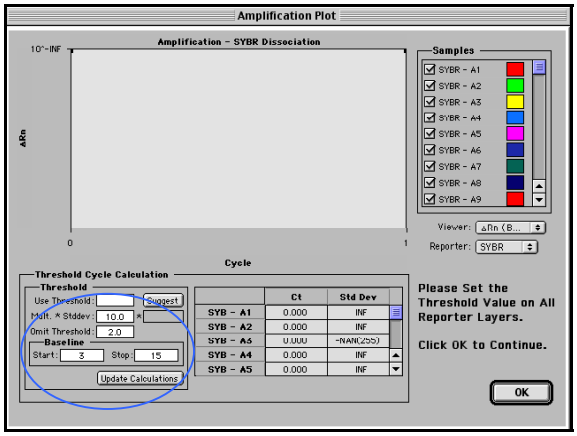
7. Initiating the run:

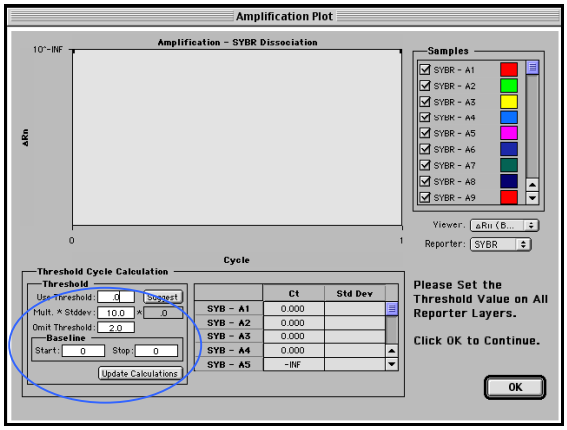
- Load the sample plate into the ABI PRISM 7700 Sequence Detection System.
- Click on **Show Analysis**.
- Click **Run** to start the run.

8. Preparing the Data:

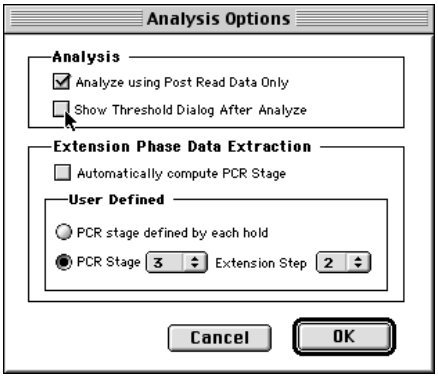
- After the run is completed, go to the top tool bar and click on **Analysis.**
- Scroll down to **Analyze** and analyze the data.
- Click **OK** and close the Amplification Plot.

NOTE: If the Amplification Plot is blank and does not close, change the **Baseline Start**, **Stop** and **Threshold** to 0 (zero). Click **Update Calculations** then click **Okay** and the Amplification Plot will close.



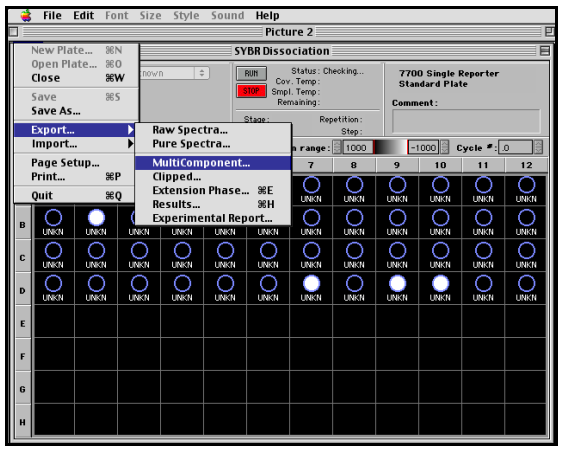


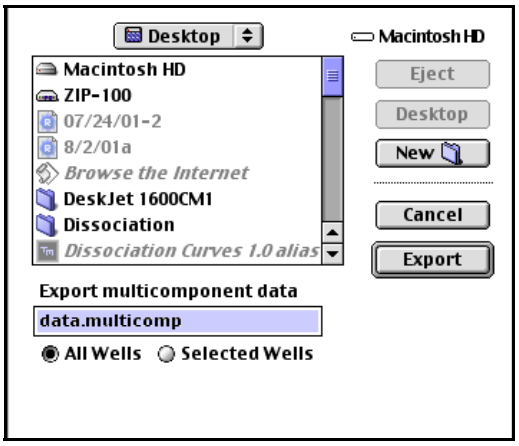
Alternatively, the Amplification Plot can be prevented from appearing by going to **Analysis** and clicking **Options** and then Deselect **Show Threshold Dialog After Analysis.**



9. Exporting the Multicomponent Data

- From the top tool bar go to **File.**
- Scroll down to **Export.**
- Scroll down to **Multicomponent.**
- The Export Dialog box will appear.
- **Name** and **Export** the Multicomponent Data- In this example the file was named data.multicomp and saved to the Desktop. The file **MUST** contain the suffix **.multicomp**.

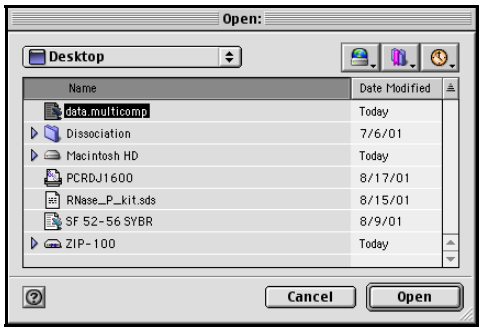




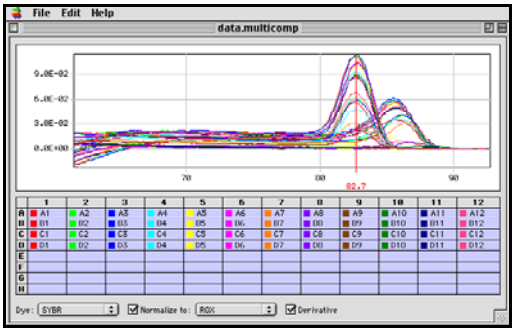
- After exporting the data, save the run and quit the SDS software.

10. Dissociation Curve data analysis:

- Launch the **Dissociation Curve Software** by double clicking on the icon.
- From the top tool bar, go to **File**, scroll to **Open**.
- Navigate to the saved Multicomponent data.
- Open the saved Multicomponent data.



- When the multicomponent data is opened, the software will automatically generate a dissociation curve graph.



The Red Bar can be moved horizontally and will display the temperature for the point indicated. The X-axis is temperature and the Y-axis is Derivative or Fluorescence.

11. Saving the Dissociation Curve Data: The data can be exported and saved either as a graph image or as a report.

- From the top tool bar scroll down to export **Graph Image** or **Report**.
- **Name** and **Export** the data.

- Graph Image exports a PICT file of the dissociation curves.
- Report exports a tab-delimited text file of the dissociation data.

12. Additional features of the software can be found within the Help Folder contained within the Dissociation Curve Analysis 1.0 *ƒ* folder that was downloaded from the web. Double click on the start.html file to open the Help document.