Quickstart Guide ABI PRISM[®] 7700 Dissociation Curve Software

<u>Overview</u>

- 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: <u>www.appliedbiosystems.com/support/software/7700/updates.cfm</u>
- 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**.

New Plate				
Plate Type:	Single Reporter 🔷			
Data Acquisi	tion			
Plate Format:	Standard Plate 😫			
Run:	Real Time 💠			
	Cancel 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.



Sample Type Setup						
Acrony	n Name	Color	Reporter			
IPC+	Internal Positive		JOE 🔹 📃			
IPC-	Internal Positive		JOE 🜻			
TARG	RelQ Target		FAM 单			
ENDO	RelQ Endogenous		JOE 单			
STND	Standard		FAM 🜻			
UNKN	Unknown		FAM 单			
NTC	No Template Control		FAM			
Reference ROX			Quencher None			
Add Cancel OK						

- 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 95°C/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**.

Analysis Options
Analysis
Show Threshold Dialog After Analyze
Extension Phase Data Extraction Automatically compute PCR Stage
User Defined
Cancel OK

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.

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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.

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Desktop 🗘	 , \ , \ ,
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🕨 🛥 Macintosh HD	Today
PCRDJ1600	8/17/01
RNase_P_kit.sds	8/15/01
SF 52-56 SYBR	8/9/01
ZIP-100	Today
	v
0	Cancel Open

• 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.

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Im	жI	
Import Well Names		ЖM
Export Graph Image		ЖG
Ex	ЖE	
Qu	it	жQ

- 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 f folder that was downloaded from the web. Double click on the start.html file to open the Help document.

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