Program of Genomic and Nutrition Laboratory
UCLA Department of Epidemiology
Prepared by Yuko You (yuko@ucla.edu)
01/24/07

7900HT Maintenance Guide

The guide including the routine maintenance procedures of:

I. Performing a Background run

II. Decontaminating the Sample Block

III. Performing a Pure Dye Run

IV. Verifying Instrument Performance Using a TaqMan RNase P Plate

V. Computer Defragment

I. Performing a Background run

When :

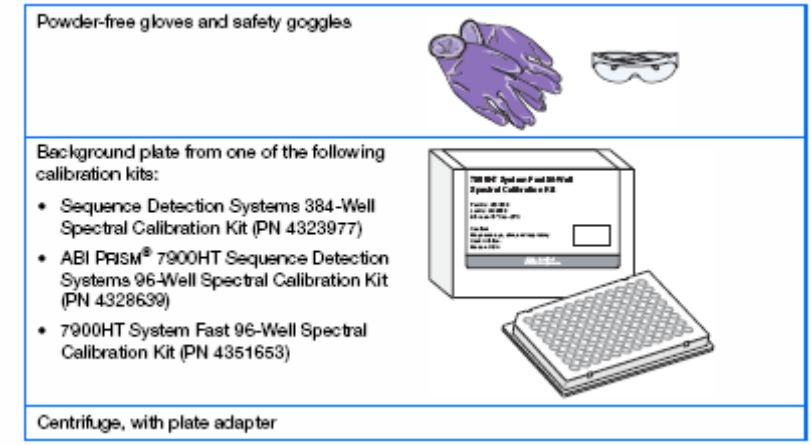
- once a month during heavy use (3-5 runs a day)
- before pure dye calibration, (see part III)
- After installing a new, uncalibrated sample block

Why: Check for dirty wells

Instructions: User Guide 7-16 ~ 19

1. OBTAIN THE TOOLS AND MATERIALS REQUIRED

Material required :



P.S. Background plate (located in the freezer in Warren Hall)

2. PREPARE THE BACKGROUND PLATE.

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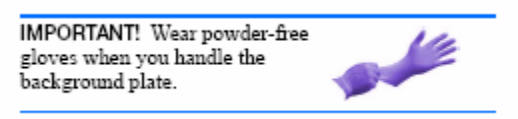
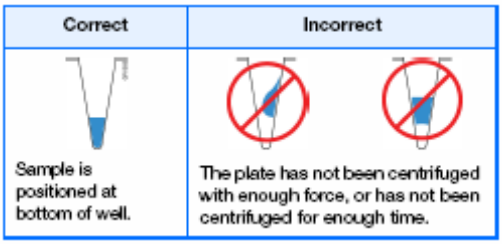


1. Retrieve the calibration kit from the freezer and remove the packaged background plate from within it.
2. Return the calibration kit to the freezer.
3. Allow the background plate to warm to room temperature (approximately 5 min).
4. Remove the background plate from its packaging.
5. Briefly centrifuge the background plate in a centrifuge with plate adapter.

6. Verify that the liquid in each well of the background plate is positioned at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



P.S. Background plate is re-usable, as long as there is fluid in every well.

3. CREATE A PLATE DOCUMENT.

1. Start the SDS software.

2. Click  (or select **File > New**).

3. Complete the New Document dialog box:
   - **a.** Assay – Select **Background** (4a)
   - **b.** Container – Select **384 wells clear plate (4b)**.
   - **c.** Template – Select **Blank Template (4c)**
   - **d. barcode (4e)**

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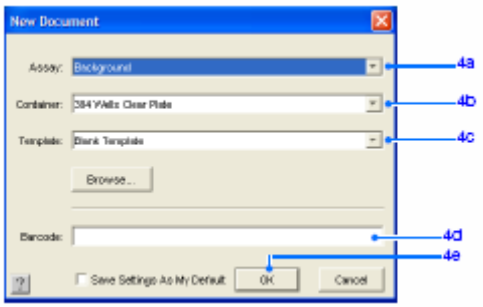


e. Click **OK** (4e). The software creates and opens a plate document with the attributes for a background calibration.

! IMPORTANT! Do not modify the background plate document. The method for a background calibration is coded into the SDS software and consists of a single hold at 60 °C for 2 min. Because the plate contains only PCR buffer or deionized water, the plate document does not require sample or detector labels.

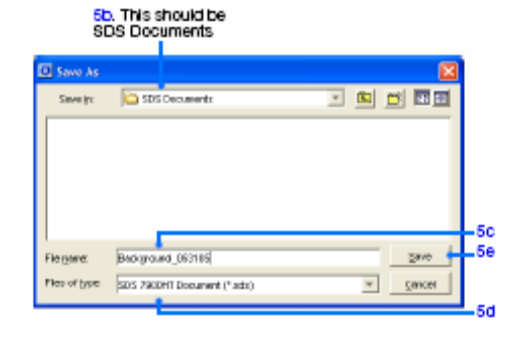
4. Save the plate document:

a. Click  (or select **File > Save**) to open the Save As dialog box.

b. In the File name field **(5c),** type in an appropriate file name, enter:

Background _<date in MMDDYY format>

For example, the file name for a plate run on May 31, 2005, would be: **Background_053105**.



c. Click Save(5e)

4. RUN THE BACKGROUND PLATE.

1. In the background plate document, select the **Instrument > Real-Time** tabs.

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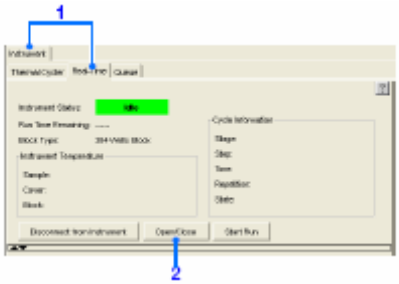
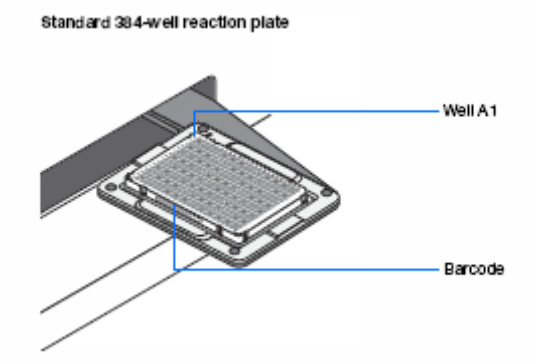


2. Click **OPEN/CLOSE**. The instrument tray rotates to the OUT position.

3. Place the background plate or background TLDA into the instrument tray as shown



4. Click **Start**. The instrument tray rotates to the IN position and performs the background calibration.

Note: Before starting the run, the instrument may pause (up to 15 min) to bring the heated cover to the appropriate temperature.

5. When the background calibration is complete and the Run Complete dialog box appears, click **OK** to close the dialog box.

6. Click **Open/Close** and remove the background plate from the instrument tray.

5. ANALYZE THE BACKGROUND DATA.

Signals exceeding 2500 FSU are considered outside the limit of normal background fluorescence and indicates that the either the background plate or the sample block may be contaminated.

1. Open the plate document for the background calibration.

2. In the toolbar, click  (**Hide/Show System Raw Data Pane)**.

3. Select all wells in the plate document.

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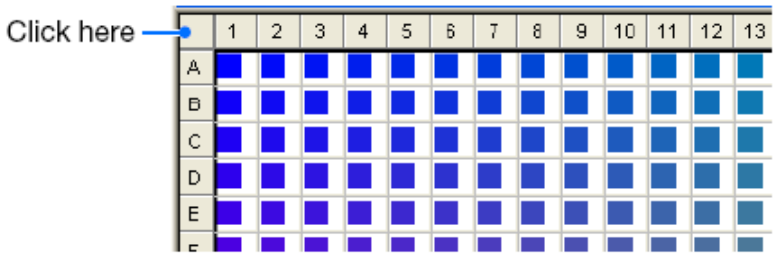
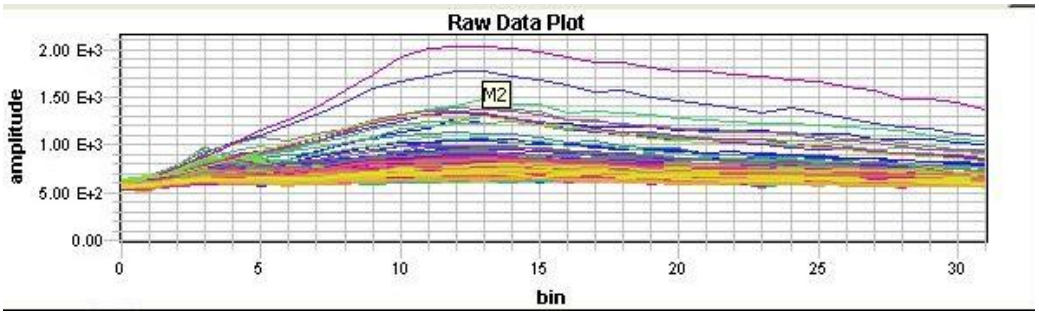


The SDS software highlights the selected wells and displays the raw spectral data.

4. Inspect the raw background data for an aberrant spectral peak or peaks.

Wells producing raw spectra that exceed **2500** FSU (2.5K) is considered irregular and could be contaminated. The following figure illustrates the raw data produced by a run on a sample block module containing a contaminated well.

i.e. Background run at 11/28/2006. All raw spectra were under 2K FSU. If want to know which well was contaminated, simply click on the curve and will tell you which one.



5. Drag the vertical bar in the temperature plot to inspect all the data points. The location(s) of the contaminated well(s) is displayed in tooltip when you move the mouse over the curves in the graph.

Recorded the dirty wells.

6. Decontaminate the sample block as explained on **II. Decontamination**.

7. Run a background plate to confirm that the contaminants have been removed.

If the contamination is present after running the background plate for a second time, the background plate is likely to be the source of contamination.

6. EXTRACT THE BACKGROUND DATA.

! Important! Make sure all dirty wells are decontaminated and re-run the background procedure, repeat the process until all background readings are under 2.5k FSU.

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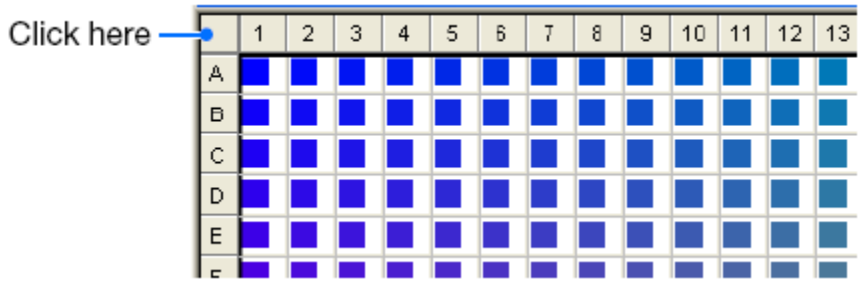
After analyze background data and decontaminated dirty wells, the next step is upgrading the background calibration data. In this procedure you will extract the calibration values from the background plate document. Once extracted, the SDS software stores the data as part of the calibration file located in the Calibration subdirectory of the SDS directory.

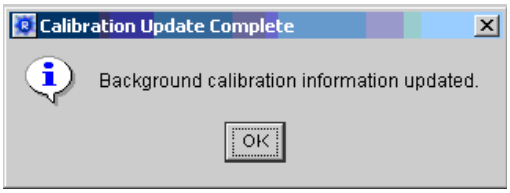
1. In the Plate Grid of the background plate document, click the box above the A label to select all wells.



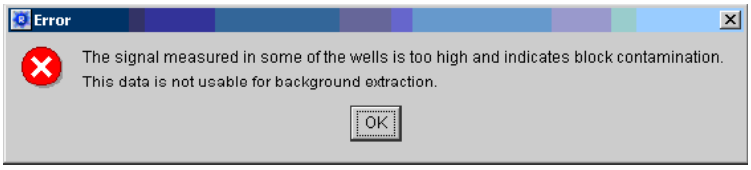
2. Select **Analysis** > **Extract Background**.

The software attempts to extract the background signal and displays the success of the extraction in a dialog box.

3. If the software displays:



Calibration Update Complete – The analysis is successful. The raw spectra read from the background plate conform to acceptable limits. Click OK, then go to step 4.



Error – The run is unsuccessful. The software stopped the extraction because one or more raw spectra exceed 2500 FSU.

Click OK, then troubleshoot the failed run as explained in "Troubleshooting Background Calibrations" on page 8-9 of 7900 HT user guide .

4. Click  (or select **File**>**Save**) to save the background plate document.

5. Select **File**>**Close** to close the background plate document.

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II. **Decontaminating the Sample Block**

When : Identified dirty wells from the background run procedure.
Why: Eliminate residual PCR related products, including fluorescent labeled TaqMan® probes.
Instructions : User Guide 7-14 ~ 15

1. MATERIAL REQUIRED:

Powder-free gloves
Pipettors and pipet tips
ddH2O
10% Sodium hypochlorite (bleach) solution
Cotton swabs (better be the special q-tip from electronic stores with very fine tip. Can use the toothpicks with cotton to form a fine tip cotton swab)

2. REMOVE THE SAMPLE BLOCK FROM THE 7900HT INSTRUMENT

1. Double-click on 

the desktop (or select **Start > All Programs > Applied Biosystems > SDS 2.2.2 > SDS Automation Controller 2.2.2**) to start the Automation Controller Software.

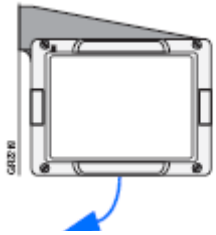
2. Select the **Run Status** tab. The module is operating normally if the software is receiving a temperature reading.

3. Click **Open/Close Door** to rotate the instrument tray to the **OUT** position.

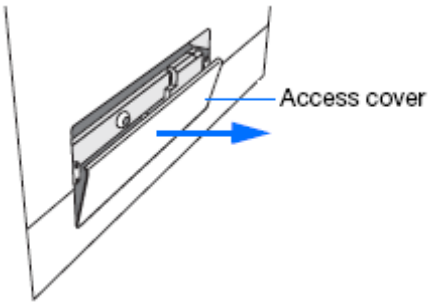
4. Select **File** > **Exit** to close the SDS.

5. Power off and unplug the 7900HT instrument. Wait 20 to 30 min for the heated cover to cool.(If after run)

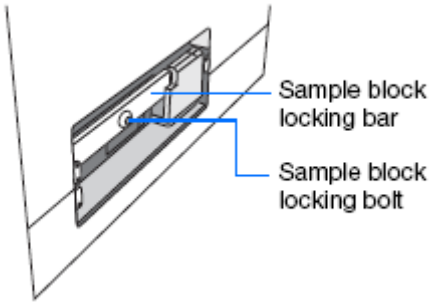
6. If the instrument tray is in the OUT position (outside of the instrument), push it into the instrument to provide an open workspace.



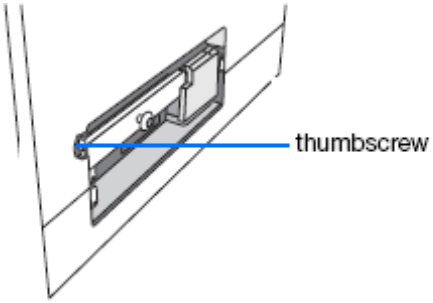
7. Push the instrument tray inside the instrument, and then remove the thermal cycler access cover to permit access to the sample block.



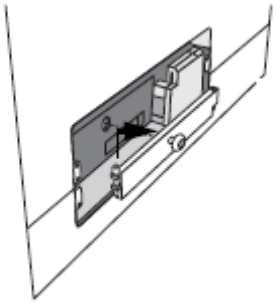
8. Using a 5/16-inch hex key, turn the sample block locking bolt counter-clockwise until it is very loose but still attached to the sample block locking bar.



9. Loosen the thumbscrew securing the sample block locking bar to the instrument chassis (may be a 5/32-inch hex bolt on some instruments).

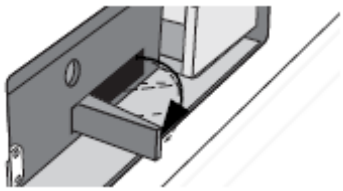


10. Lift the sample block locking bar up and out of the instrument.

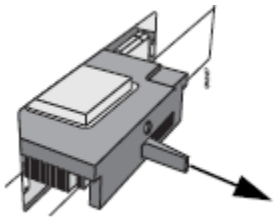


11. Remove the sample block from the instrument:

a. Rotate the release lever at the base of the sample block 90 degrees.

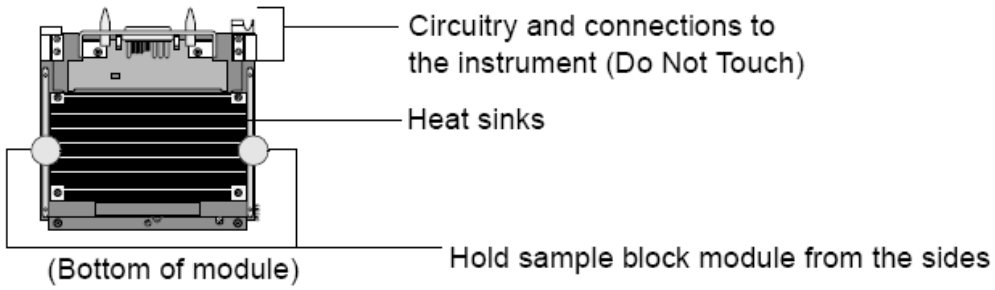


b. Being careful not to damage the heat sinks on the bottom of the sample block, slide the sample block out of the instrument and place it on a clean, level surface.



c. Circuitry and connections facing you with the sample block facing up, the upper left well is **A1**.

!Important! The interchangeable sample blocks are delicate pieces of equipment containing several fragile components that can break if handled improperly. The figure below illustrates the correct locations for handling the interchangeable sample block module.



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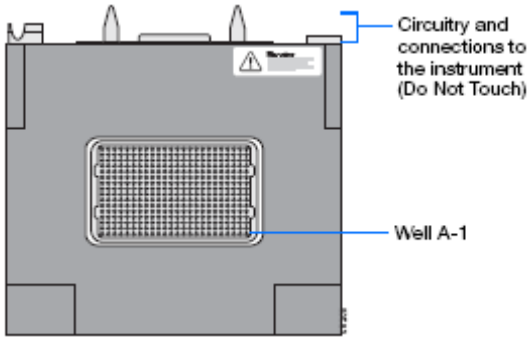
3. CLEANING THE SAMPLE BLOCK WELLS

1. Locate the suspected contaminated wells on the sample block, using the figure at below as a guide. (i.e. put q-tip in the dirty wells)



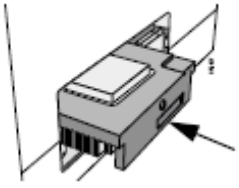
2. Use ddH2O first. Rinse (pipet and remove) each contaminated well with three treatments of deionized water at the appropriate volume for the sample block (40uL per well for a Standard 384-Well Block)
3. Allow the sample block to sit for 3-5 min.
4. Using a pipet, remove the solution from the wells of the sample block.
5. Rinse (pipet and remove) each contaminated well with 3 treatments of deionized water at the appropriate volume for the sample block. (For a 384-well sample block, rinse affected wells with 40 uL deionized water)
6. Using a q-tip, scrub inside of each contaminated well.
7. Replace the sample block as explained in "**4. REPLACING THE SAMPLE BLOCK**"
8. Run a background plate to confirm that the contamination has been removed. If not, use 10% Bleach solution or Absolute isopropanol instead of ddH2O to re-perform the decontamination steps above.

4. REPLACING THE SAMPLE BLOCK

1. Load the sample block into the instrument compartment:

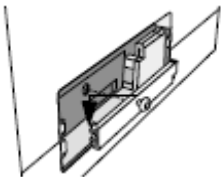
a. Being careful not to damage the heat sinks on the bottom of the sample block, rest the sample block on the metal runners on either side of the instrument bay.

b. Carefully slide the sample block into the instrument until the front of the block is flush with the rear of the locking bar.



c. Once seated, firmly press on the sample block to ensure a good connection.

2. Replace the sample block locking bar.



3. Tighten the thumb screw (from step 9 on page 7-6) to secure the sample block locking bar to the instrument chassis (may be a 5/32 Hex bolt).

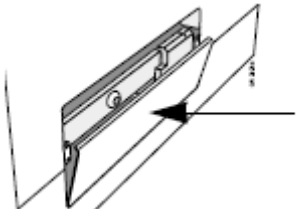
4. Using the 5/16 Hex key, turn the sample block locking bolt clockwise until it is flush with the locking bar.

5. Again, press on the right and left sides of the front surface of the sample block to ensure that it is seated securely.

6. Replace the thermal cycler access cover as follows:

a. Fit the lip at the bottom of the access cover over the lower edge of the bay.

b. Push the cover towards the instrument until it snaps into place.



7. Plug in and power on the 7900HT instrument.

8. Confirm the function of the installed sample block:

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III. Performing a Pure Dye Run

When:

- Every 6 months depending on instrument use
- Before performing an instrument verification run (see part IV)
- After changing sample block formats (see page 7-4)

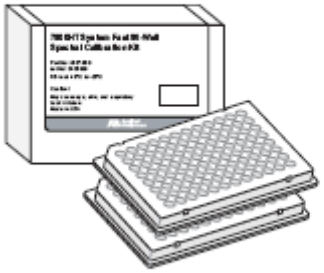
IMPORTANT! Always run a background plate before performing a Pure Dye calibration.

Why: Calibrate signals

Instructions : User Guide 7-20 ~ 29

1. OBTAIN THE TOOLS AND MATERIALS REQUIRED

Material required :

Powder-free gloves and safety goggles	
Pure dye plate(s) from one of the following calibration kits: • Sequence Detection Systems 384-Well Spectral Calibration Kit (PN 4323977) • ABI PRISM® 7900HT Sequence Detection Systems 96-Well Spectral Calibration Kit (PN 4328639) • 7900HT System Fast 96-Well Spectral Calibration Kit (PN 4351653) Note: Both the standard 96-well and Fast 96-well calibration kits contain two pure dye plates.	
Centrifuge, with plate adapter	

P.S. Pure Dye plate (located in the freezer in Warren Hall)

2. PREPARE THE PURE DYE PLATE

1. Retrieve the calibration kit from the freezer, then remove the packaged pure dye plate(s).
2. Return the calibration kit to the freezer.
3. Allow the pure dye plate(s) to warm to room temperature (approximately 5 min).
4. Remove a pure dye plate from its packaging. The fluorescent dye contained in the wells of each pure dye plate is photosensitive. Prolonged exposure to light can diminish the fluorescent signal strength of the plate.

IMPORTANT! Do not discard the packaging for the pure dye plate. The pure dye plate can be used repeatedly if it is stored in its original packaging sleeve.

5. Briefly centrifuge the pure dye plate in a centrifuge with plate adapter.

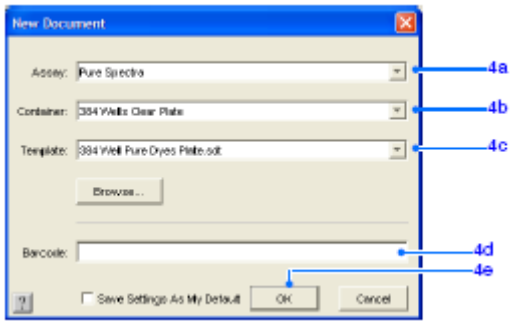
6. Verify that the pure dye standard in each well of the pure dye plate is positioned at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

3. CREATING A PURE-DYE PLATE DOCUMENT

1. Double-click  on the desktop (or select **Start> All Programs > Applied Biosystems > SDS 2.2.2 > SDS 2.2,2**) to start the SDS software.

2. Click  (or select **File**>**New**).

3. Complete the New Document dialog box:



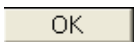
a. Assay (4a) – Select **Pure Spectra**.

b. Container (4b)– Select the appropriate format.

c. Template (4c)– Select the appropriate template, as described in the table below

For Container....	Select Template.....
384 Wells Clear Plate	384 Well Pure Dyes Plate.sdt
96 Wells Clear Plate (for a Standard 96-Well Sample Block)	• 96 Well Pure Dyes Plate 1.sdt to run Plate 1 (containing FAM, JOE,NED, and ROX dyes) • 96 Well Pure Dyes Plate 2.sdt to run Plate 2 (containing SYBR Green, TAMRA, TET, and VIC dyes)

d. If the pure dye plate or pure dye TLDA is labeled with a barcode, click the **Barcode** field, then type in or scan the barcode number (4d).

e. Click  (4e). The software creates and opens a plate document with the attributes for a pure dye calibration.

IMPORTANT! Do not modify the pure dye plate document.

5. Save the plate document:

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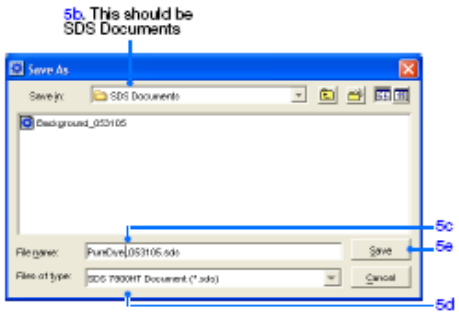


a. Click (or select **File** **Save**) to open the Save As dialog box.

b. If the Save in field does not display SDS Documents, navigate to **Applied Biosystems >SDS Documents**.

c. In the File name field, type in an appropriate file name, as described in the table below.

For Container…	**Type…**
384 Wells Clear Plate	**PureDye**_<*date in MMDDYY format*> For example, the file name for a plate run on May 31, 2005, would be: **PureDye_053105**.

d. Select **SDS 7900HT Document (*.sds)** from the Files of type drop-down list.

e. Click Save. The software saves the plate document.

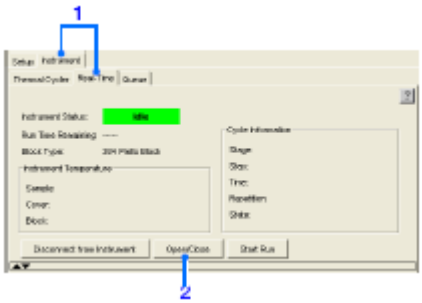
4. RUNNING A PURE DYE PLATE

1. Briefly centrifuge the pure dye plate

2. Load the pure dye plate into the 7900HT instrument as follows:

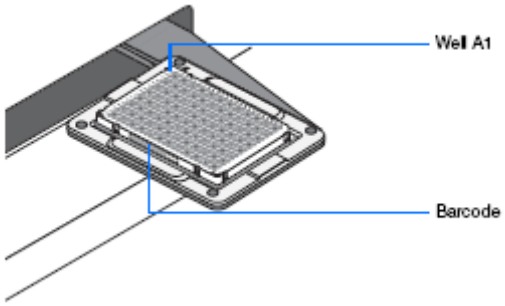
a. From the plate document in the SDS software, click the **Instrument** tab.

b. From the **REAL-TIME** tab in the **Instrument** tabbed page, click **Open/Close**. The instrument tray rotates to the OUT position.



c. Place the pure dye plate into the instrument tray.

Note The A1 position is located in the top-left side of the instrument tray.



3. Click **Start**.

The 7900HT instrument begins the pure dye run. The method for a pure dye run is hard-coded into the software and consists of a single 2-min hold at 60 °C.

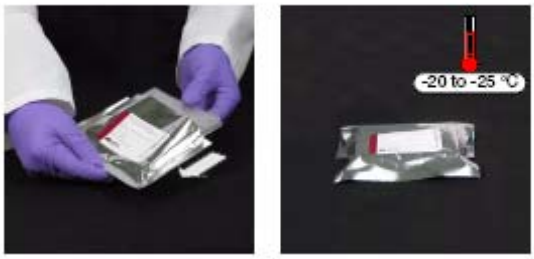
Note Before starting the run, the instrument may pause (up to 15 min) to heat the heated cover to the appropriate temperature.

4. When the pure dye run is complete and the **Run Complete** dialog box appears:

a. Click **OK** to close the dialog box.

b. Click **Open/Close**, and remove the pure dye plate from the instrument tray.

c. Place the pure dye plate or each pure dye TLDA inside its packaging, then return it to the calibration kit in the freezer.



d. Analyze and extract the pure dye calibration information as explained in the following sections.

5. EXTRACTING PURE DYE INFORMATION FROM THE ANALYZED RUN

1. In the pure dye plate document, select **Analysis > Extract Pure Dye Wizard**.
2. Follow the instructions as explained in the Pure Dye Wizard to extract the pure dye spectra. When presented with each screen:

a. Inspect the spectra for shifts in peak location.

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Prepared by Yuko You (yuko@ucla.edu)
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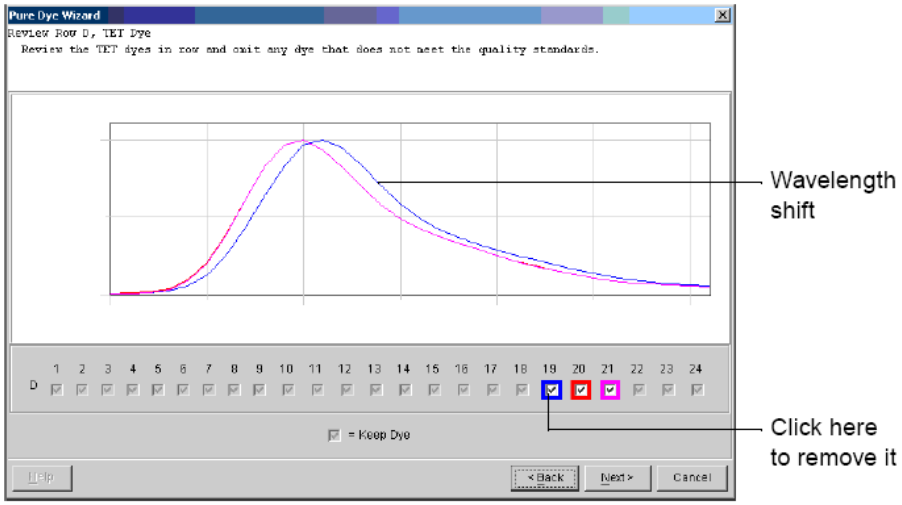

b. If the data set contains an outlying peak, eliminate it by clicking the check box of the associated well.

Note: Dye spectra are generally acceptable if they peak at the same location as their group but diverge slightly at other wavelengths.

c. Click when finished.

d. Repeat steps a through c for all remaining wells until prompted with a message reporting the extraction of the pure dyes. The software extracts the pure spectra and stores the data as a component of the calibration file.

3. Click  (or select **File > Save**) to save the pure dye plate document

4. Select **File > Close** to close the pure dye plate document.

5. If you are performing the pure dye calibration for a:

• Standard 384-well sample block – The pure dye calibration is complete.

IMPORTANT! You must calibrate the instrument for all pure dye plates or pure dye TLDAs provided in your calibration kit.

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UCLA Department of Epidemiology
Prepared by Yuko You (yuko@ucla.edu)
01/24/07

IV. Verifying Instrument Performance Using a TaqMan RNase P Plate

When :

- When changing sample block formats for the first time
- As needed to verify the function of the 7900HT instrument

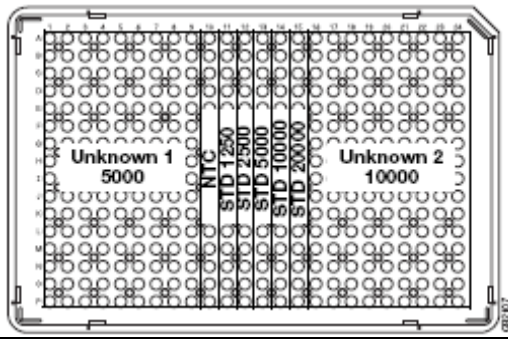
Why: Verify the performance of the 7900HT instrument for a sample run.

Instructions: User Guide 7-24 ~ 26

IMPORTANT! Perform a pure dye calibration before performing the instrument verification run.

About RNase P Plates

RNase P plates are pre-loaded with the reagents required to detect and quantify genomic copies of the human RNase P gene. Each well contains pre-loaded reaction mix (TaqMan® Universal PCR Master Mix, RNase P primers, and FAM™ dye-labeled probe) and a known concentration of human genomic DNA template.
The table below illustrates the arrangement of standards and samples on each type of RNase P plate that is available for the 7900HT Fast System. The RNase P plates contain five replicate groups of standards (1250, 2500, 5000, 10,000, and 20,000 copies), two unknown populations (5000 and 10,000 copies), and template control (NTC) wells.



1. OBTAIN THE TOOLS AND MATERIALS REQUIRED

Material required:

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Powder-free gloves and safety goggles	
Appropriate RNase P plate for your sample block: • TaqMan® RNase P 384-Well Instrument Verification Plate (PN 4323306) • TaqMan® RNase P Instrument Verification Plate (PN 4310982, standard 96-well reaction plate) • TaqMan® RNase P Fast 96-Well Instrument Verification Plate (PN 4351979)	
Centrifuge, with plate adapter	

P.S. RNase P plate is not reusable. Need to place order before perform the run.

2. PREPARE THE RNase P PLATE

1. Retrieve the RNase P package from the freezer, then remove the packaged RNase P plate.

2. Allow the RNase P plate to warm to room temperature (approximately 5 min).

3. Remove the RNase P plate from its packaging.

4. Briefly centrifuge the RNase P plate in a centrifuge with plate adapter.

5. Verify that the liquid in each well of the RNase P plate is positioned at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

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Prepared by Yuko You (yuko@ucla.edu)
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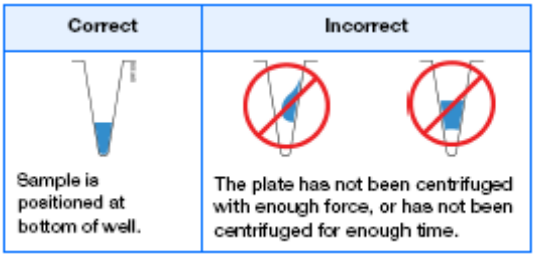
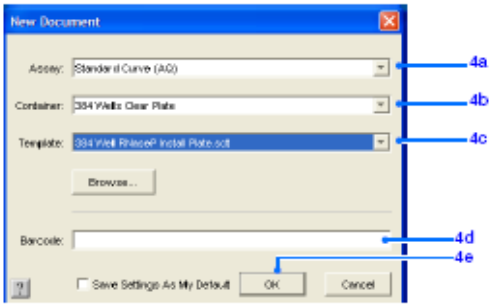


3. CREATING A PLATE DOCUMENT FOR THE VERIFICATION RUN

1. Launch the SDS software.

2. From the **File** menu, select **New**. The **New Document** dialog appears.

3. Configure the **New Document** dialog box as follows.



Drop-Down List	Select
Assay	Absolute Quantification
Container	<the appropriate plate format>
Template	<the template file of the appropriate plate format>

P.S. Container=384 well clear well plate

Template=384 Well RNaseP Install Plate.sdt

4. If desired, enter the bar code information into the plate document as follows:

a. Click the **Barcode** text field.

b. Remove the RNase P plate from the packaging and scan its bar code using the hand-held bar code reader.

5. Click **OK**. The software creates a plate document.

Note Do not modify the RNase P plate document. The template is pre-programmed with detector and method information for the run.

6. Save the plate document as follows:

a. From the **File** menu, select **Save**. The **Save** dialog appears.

b. Click the **Barcode** text field and either:

– Type a name or bar code number for the plate, and click **Save**.

– Using the hand-held bar code reader, scan the bar code number.

c. From the **Files of type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**.

d. Click **Save**. The software saves the plate document. The software is now configured for the RNase P run.

Verification_<date in MMDDYYformat>

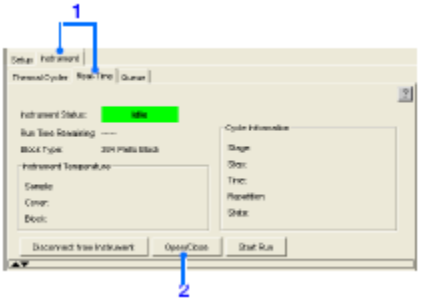
For example, the file name for a plate run on May 31, 2005, would be: Verification_053105.

4. PREPARING AND RUNNING AN RNase P PLATE

1. Briefly centrifuge the TaqMan RNase P Instrument Verification Plate.

2. From the plate document in the SDS software, click the **Instrument** tab. The software displays the Instrument tabbed page.

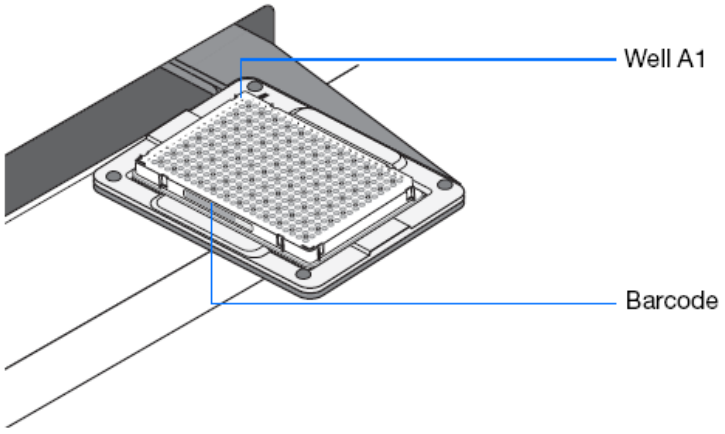
3. From the lower portion of the **Instrument** tab, click the **Real-Time** tab. The software displays the Real-Time tabbed page.



4. If the instrument tray is within the 7900HT instrument, click **Open/Close**. The instrument tray rotates to the OUT position.

5. Place the RNase P plate into the instrument tray.

Note The A1 position is located in the top-left corner of the instrument tray.



6. Click **Start**. The 7900HT instrument begins the run.

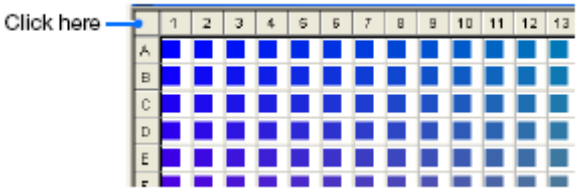
Note Before starting the PCR run, the instrument may pause (up to 15 min) to heat the heated cover to the appropriate temperature.

5. ANALYZING THE INSTRUMENT VERIFICATION RUN

1. Click (or select **Analysis** > **Analyze**). The SDS software analyzes the run data. A status bar at the bottom of the plate document window indicates progress; the status bar disappears when the analysis is complete.

2. View the results:

a. In the Plate Grid, click the box above the A label to select all wells.



b. Click the **Results** tab to view the plots.

3. Choose from the following:

• **Automatic Ct** – The SDS software automatically generates baseline values for each well and threshold values for each detector.

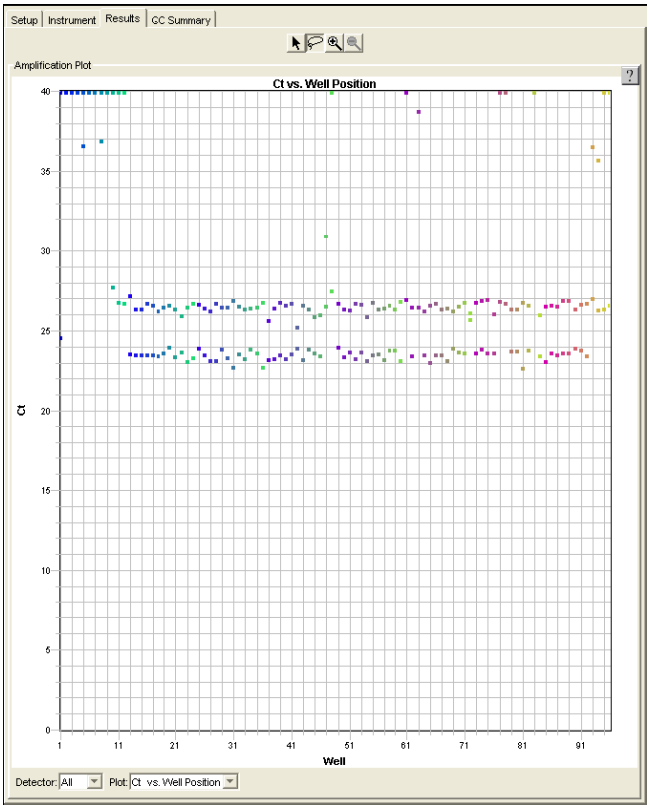
• **Manual Ct** – The SDS software calculates baseline and threshold values for a detector based on the assumption that the data exhibits typical amplification curves. If you are setting the baseline manually, specify the Start and End cycles.

IMPORTANT! After analysis, you must verify that the baseline and threshold were called correctly for each well by clicking on the well in the Plate Grid and viewing the resulting plots. For more information about manually adjusting the baseline and threshold settings, refer to the *Sequence Detection Systems Software version 2.3 Online Help (SDS Online Help).*

4. If necessary, remove outliers:

a. Determine the outlier well positions.

Note: The C_T vs. Well Position view in the Results tab can help you determine the outlier well positions. To access this view, click the **Plot** drop-down list at the bottom of the amplification Plot.



b. In the Plate Grid, select the outlier wells.

Note: To select more than one well at a time, hold down the **ctrl** key while selecting the wells. The number of wells that can be removed to pass specifications is shown in the table below.

Format	Maximum No. of Wells that Can be Removed from Each Set	
	Unknowns	Standards
Standard 96-well RNase P Plate	6	1
Fast 96-well RNase P Plate	6	1
Standard 384-well RNase P Plate	10	2
RNase P TLDA	4	4

c. Click the **Setup** tab, then select the **Omit Wells(s)** check box. A red ✕ appears in each of the selected wells in the Plate Grid.

5. Select **Analysis** > **Analyze**) to reanalyze the data.

6. VERIFYING INSTRUMENT PERFORMANCE

Program of Genomic and Nutrition Laboratory
UCLA Department of Epidemiology
Prepared by Yuko You (yuko@ucla.edu)
01/24/07

During analysis, the SDS software generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards, then calculates the concentration of the two unknown populations using the standard curve. To complete the verification, the average copy number (mean quantity) and standard deviation for the unknown populations are entered into the following formula to assess the instrument performance:

The install specification of the ABI PRISM 7900HT Sequence Detection System demonstrates the ability to distinguish between 5,000 and 10,000 genome equivalents with a 99.7% confidence level for a subsequent sample run in a single well. The following equation verifies the 7900HT install specifications:

$$(\text{Qty Unk}_{(\text{high copy})}) - 3\,(\text{Qty }\sigma_{(\text{high copy})}) > (\text{Qty Unk}_{(\text{low copy})}) + 3\,(\text{Qty }\sigma_{(\text{low copy})})$$

Calculation Term	Value	Unknown Populations			
		Standard 96-well RNase P Plate	Fast 96-well RNase P Plate	Standard 384-well RNase P Plate	RNase P TLDA
Qty Unk $_{(\text{high copy})}$	Average quantity of high copy number	10,000	10,000	10,000	1600
Qty $\sigma_{(\text{high copy})}$	Quantity standard deviation of high copy number				
Qty Unk $_{(\text{low copy})}$	Average quantity of low copy number	5000	5000	5000	800
Qty $\sigma_{(\text{high copy})}$	Quantity standard deviation of low copy number				

The calculation states that the average copy number of the larger (10,000 for reaction plates or 1600 for TLDAs) unknown population minus three standard deviations is greater than the average copy number of the smaller (5000 for reaction plates or 800 for TLDAs) unknown population plus three standard deviations. If the calculation is true, then the instrument passes the validation specification, verifying instrument performance.

Note Up to 6 wells from each replicate group in a 96-well TaqMan RNase P Instrument Verification Plate can be ignored to meet specification.

Note Up to 10 wells from each replicate group in a 384-well TaqMan RNase P Instrument Verification Plate can be ignored to meet specification.

Program of Genomic and Nutrition Laboratory
UCLA Department of Epidemiology
Prepared by Yuko You (yuko@ucla.edu)
01/24/07

V. Computer Defragment

When: Once a month

Purpose: Maintain the optimal computer operation

How:

1. Select **Start** > **All Programs** > **Accessories** > **System Tools** > **Disk Defragmenter**
2. Wait until the work done