



Biopharmaceutical Development Program

Standard Operating Procedure

Title: **ABI Prism® 7900HT SDS Operation and Maintenance**

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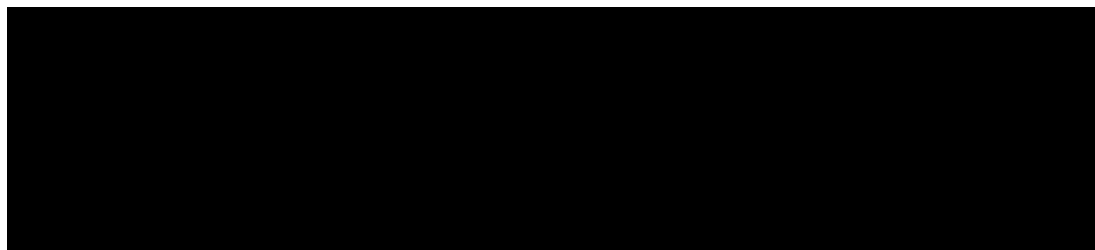


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1.0 Purpose

This SOP describes the basic operation, maintenance, and troubleshooting of the Applied Biosystems, Inc. (ABI) Prism® 7900HT Sequence Detection System (SDS) for quantitative PCR.

2.0 Scope

The ABI Prism® 7900HT SDS for qPCR is a multi-component system consisting of the 7900HT instrument (with 96 or 384 well sample blocks), a hand-held bar code reader, a Windows NT workstation, and a Franek uninterruptible power supply (UPS) and line conditioner. The 7900HT SDS is primarily used by the BDP Process Analytics group for the detection and quantitation of low levels of contaminating DNA or RNA present in test samples.

3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics (PA) has the authority to define this procedure.
- 3.2 PA personnel are responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance.
- 3.3 PA personnel are responsible for the performance of this procedure.
- 3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.
- 3.5 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Materials, Reagents and Equipment

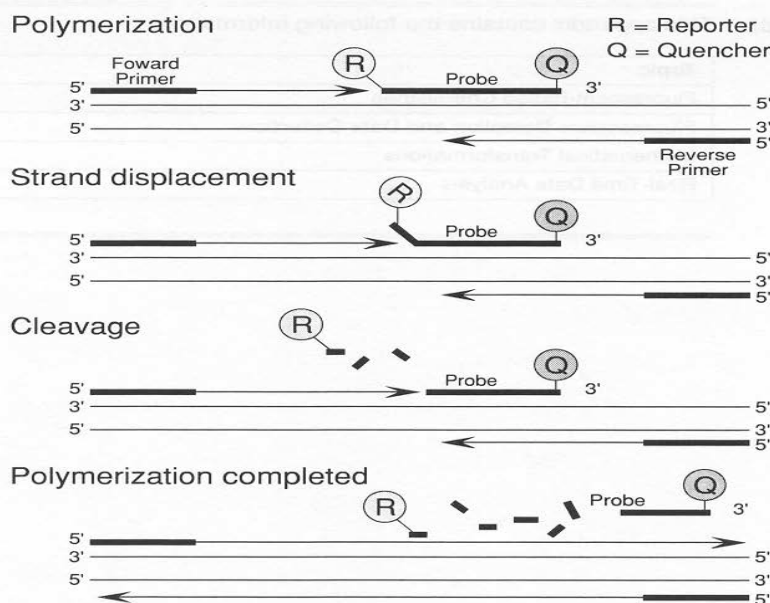
- 4.1 96-well plates, ABI PN-4306737.
- 4.2 Plate optical adhesive covers, ABI PN-4313663.
- 4.3 qPCR Master Mix with UNG, ABI PN-4304437.
- 4.4 TaqMan® EZ (Tth polymerase) RT-qPCR kit, ABI PN-N808-0235.
- 4.5 ABI Exogenous IPC reagent kit, ABI PN-4308323.
- 4.6 Control DNA – see assay specific SOP addendum.
- 4.7 Qiagen DNA or RNA purification system – see assay specific SOP addendum.
- 4.8 DNase Free water, WFI, or PBS dilution buffer – see assay specific SOP addendum.
- 4.9 ABI RNase P Verification Plate, ABI PN-4310982.
- 4.10 ABI Spectral Calibration / Pure Dye Plates, ABI PN-4328639.
- 4.11 Forward and Reverse primers – see assay specific SOP addendum.
- 4.12 Fluorescent labeled Probe – see assay specific SOP addendum.
- 4.13 UV irradiation cabinet for plate setup and negative/sentinel control additions.
- 4.14 Biosafety cabinet for test sample, standards, and positive control additions.

- 4.15 Dedicated “clean” (used for negative controls and test samples) and “dirty” (used for standards and positive controls) Pipette sets.
- 4.16 Aerosol tips as needed.
- 4.17 Powder-free gloves as needed (at least 2 sets per setup needed).

5.0 Real-Time Quantitative (TaqMan™) PCR Overview

Basics of the 5' Nuclease Assay

The PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold® DNA Polymerase to cleave a TaqMan® probe during PCR. The TaqMan probe contains a reporter dye at the 5' end of the probe and a quencher dye at the 3' end of the probe. During the reaction, cleavage of the probe separates the reporter dye and the quencher dye, which results in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. The figure below shows the forklike-structure-dependent, polymerization-associated 5'–3' nuclease activity of AmpliTaq Gold DNA Polymerase during PCR.



When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer (Förster, 1948; Lakowicz, 1983). During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites.

The 5'–3' nucleolytic activity of the AmpliTaq Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product.

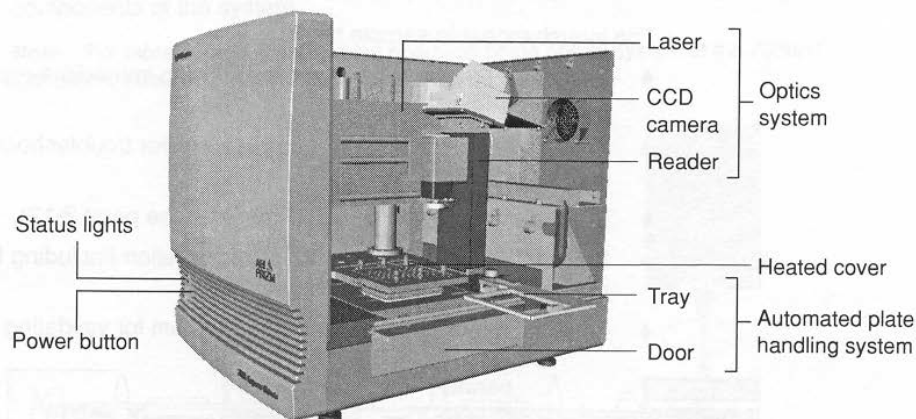
6.0 ABI Prism® 7900HT SDS Overview

The ABI PRISM® 7900HT SDS consists of the qPCR instrument, a hand-held bar code scanner (typically not used), a networked Windows NT computer running the SDS 2.0 qPCR software, and a Franek Dedicated UPS and Power conditioner.

6.1 7900HT SDS Instrument.

- 6.2 ABI SDS 2.0 Software (Win NT based) controls the 7900HT instrument, allows for creating and saving pre-formatted assay designs, serves as the calibration and maintenance interface and performs post-run qPCR assay analysis with a wide variety of assay types including real-time TaqMan™ assays.

Overview The 7900HT instrument contains the hardware used for thermal cycling and fluorescent detection of SDS chemistries (see page 2-2). The figure below illustrates the major subcomponents of the instrument that are described in further detail below.



IMPORTANT Do not remove the cover to the ABI PRISM 7900HT Sequence Detection System. Only a qualified Applied Biosystems service engineer may repair or adjust the internal components of the 7900HT instrument.

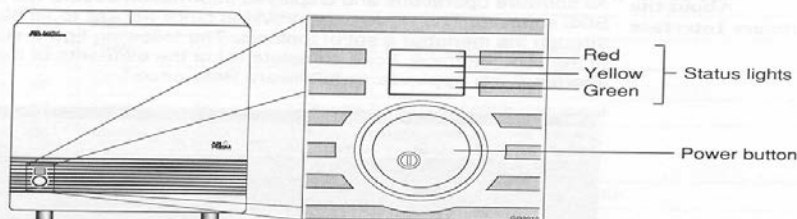
- 6.3 The Franek Dedicated UPS and power conditioner is located next to the computer and protects both the computer and instrument from power surges, brown-outs, and power spikes. The UPS is supplied with a dedicated 20 Amp circuit. In the event of a power failure the UPS will provide only ~15 minutes of instrument operation on battery power. A typical qPCR assay (40-45 cycles) requires ~2 hours of operation, while an RT-qPCR assay typically requires 2.5-3 hours to complete. For further information regarding use and specifications of the Franek UPS, refer to the power supply operator's manual.
- 6.4 Instrument Exhaust System – The 7900HT has a dedicated powered exhaust system to ventilate the waste heat generated by the instrument outside the laboratory. The ceiling-mounted exhaust vent stack should be kept in the open position at all times.

7.0 Operational Procedure

- 7.1 Power On – Turn on the computer, monitor, and log on to the network. Prior to launching the SDS software, turn on the 7900 instrument via the front panel button and check the status lights after 2 minutes. The UPS should always remain on. Typically the instrument and computer can be left on overnight during the week, turn off the instrument if 2 or more days of inactivity are expected. Always log off the network every evening.

Reading the Status Lights

The 7900HT instrument contains three lights located on the lower-left side of the front panel to indicate the status of the instrument.

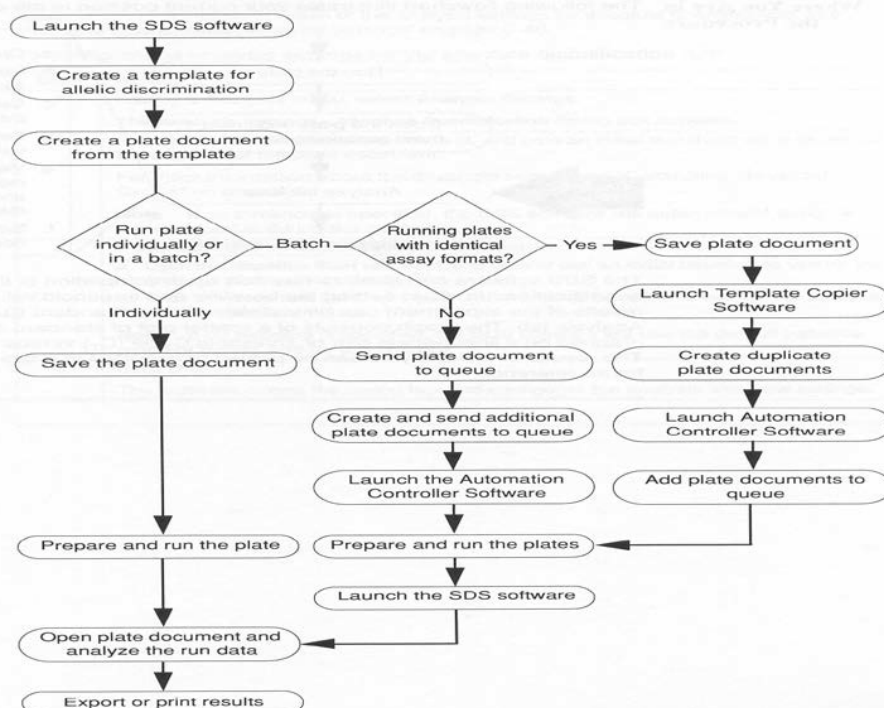


Light/Appearance		Status	Action
Green	Solid	Instrument on and in idle state (ready to run)	None. This state indicates normal instrument function.
Orange	Flashing	The instrument is transmitting/receiving data to/from the computer during a run.	None. This state indicates normal instrument function.
	Solid	If the light remains on during startup for more than 2 min: ♦ the computer is turned off ♦ the instrument did not boot properly or the firmware has locked	Check that the computer is turned on and connected to the instrument. If so, turn off the instrument, wait for 30 sec, and then restart. If the problem persists, check the run log and call technical support.
Red	Solid	Self-tests have detected a serious problem.	Turn off the instrument, wait for 30 sec, and then restart. Check the run log and call technical support.

- 7.2 SDS 2.0 Instrument and Analysis Software – Open the SDS software by double clicking the desktop icon after powering on the instrument. The SDS software resides at “D:/Program Files/Applied Biosystems/SDS 2.0/” in case the icon is deleted. **Open** a previously saved plate template (containing the dye types, reaction types, and plate locations to be used) or select **New Document** if a previously generated plate and/or reaction template does not exist.

Procedure Flowchart

The following figure illustrates the general procedures that comprise the setup and execution of an absolute quantification run.



7.3 Creating a 96-Well Plate Document

- 7.3.1 From the **File Menu** select **New**. The “New Document” dialog box will appear.
- 7.3.2 In the dialog box, select **Assay – Absolute Quantification** and select **Container – 96-Well Clear Plate** for all TaqMan assays. Select the appropriate **Template** document or **Blank Template** for new assay designs.

7.4 Creating and/or Running a Plate Template – Selecting Detectors

From the **Tools** menu, select **Detector Manager**. In the “Detector Manager” dialog box, select **New** from the pop-up menu to open the “Add Detector” dialog box. In most cases the fluorescent dyes and assays that will be used in qPCR will be pre-loaded in the SDS software and using **Detector Manager** will be unnecessary unless a new assay is being used for the first time.

- 7.4.1 Under the **Name** field type the name of the new assay and/or dye (for example: Human Beta-Actin FAM/TAMRA), the name must be unique to be saved.
- 7.4.2 Under the **Description** field type a short but detailed description of the assay and amplicon target.
- 7.4.3 Select the reporter and quencher dyes that will be used in the assay. If a custom dye or quencher is being used that is not preloaded in the SDS software, a pure dye plate must be defined and run before using it in qPCR assays. See the ABI 7900HT User Guide for details.
- 7.4.4 Non-fluorescent quencher dyes (Black Hole quenchers) and SYBR Green assays should set the **Quencher** to **Non Fluorescent**.
- 7.4.5 Select the **Color** that will represent the detector for this specific assay (useful if more than one assay is run on a plate).
- 7.4.6 Comments can be added to the **Notes** file as desired.
- 7.4.7 Click **OK** to save the detector and close the “Add Detector” menu.

7.5 Creating and/or Running a Plate Template – Adding Detectors to a Plate Document.

- 7.5.1 In the Detector Manager dialog box, press and hold the **Ctrl** key while clicking the detectors to be applied to the Plate Document.
- 7.5.2 Once the selected detectors are highlighted, click **Copy to Plate Document** to add the detectors to the well inspector of the Plate Document. Click **Done**.
- 7.5.3 Apply the detectors to the plate grid by selecting the wells for each detector to be used (typically only one detector per assay will be used). Apply the detector to the selected wells by clicking the appropriate check box in the Plate Document **Setup** menu's **Use** column.

7.6 Creating and/or Running a Plate Template – Applying Detector Tasks

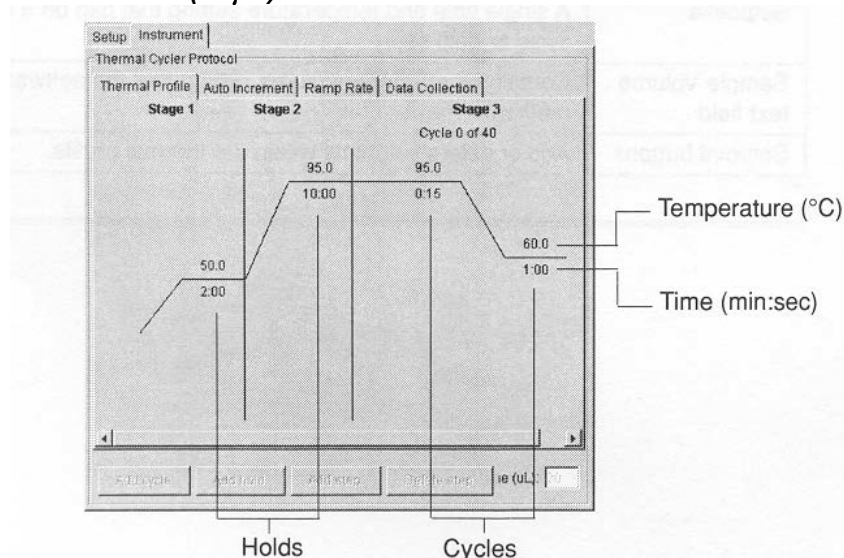
- 7.6.1 Once the plate document contains detector information, the Detector Tasks must be set for selected wells. Tasks include: NTC, Standard, and Unknown wells.
- 7.6.2 On the plate Plate Grid select the wells that will be used as Standards, from the Well Inspector (the left-hand side of the plate window) click the **Task** column for the detector entry and select **Standard**. The SDS software will label each selected well with the chosen task initial (**N**, **S**, or **U**).
- 7.6.3 For standard wells, highlight the standards that will contain the same amount of DNA (or RNA) and click the **Quantity** column in the well inspector window. Enter the mass or copy number of DNA/RNA amplicon that will be used in the chosen standard, use the same unit of measure for all standards in the assay.
- 7.6.4 Repeat the steps in 7.6 for identifying NTC and Unknown sample wells. Quantity information will not be required for non-standard wells. Unknown (test) wells should be named and identified according to what test or spiked sample will be loaded in that well.

7.7 Setting the Passive Reference Dye in the Plate Document – For each plate document, select the passive reference dye in the menu bar. Nearly all qPCR Master Mixes from ABI use ROX as the passive reference dye.

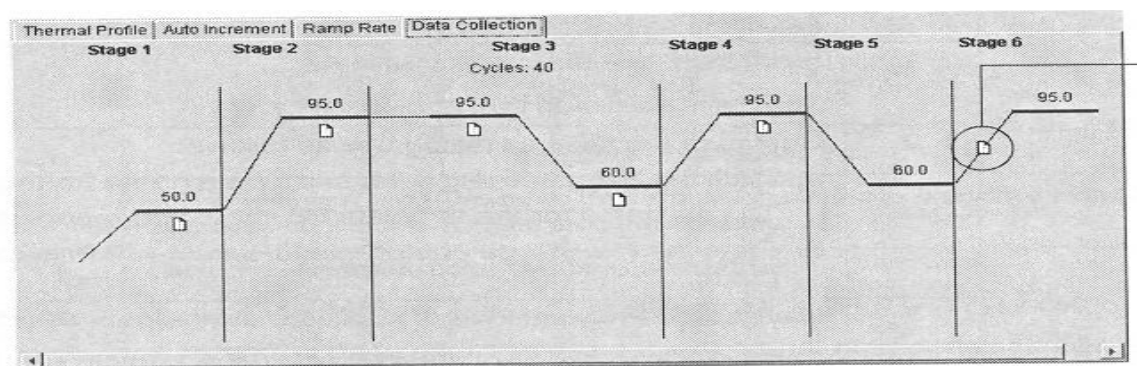
7.8 Programming the Method Thermal Profile

- 7.8.1 Click the “Instrument” tab and select the “Thermal Profile” tab to create a new temperature profile.
- 7.8.2 Enter the desired cycle temperatures and cycle number (in Stage 3 for TaqMan assays), and sample volume in the Sample Volume text field.

Note: Duplicate or triplicate reactions on a plate MUST contain the same final volume (in μL).



- 7.8.3 Use the mouse pointer to highlight text or graphical portions of the method that are to be changed. Use the **Add Cycle** and **Add Hold** buttons to add new method segments. The reaction volumes and complete method parameters will be detailed in the relevant assay SOP if appropriate.
- 7.9 Programming the method Ramp Rate. Click the “Ramp Rate” tab. Always select the ABI 9600/7700 emulation mode with the 96-well block.
- 7.10 Programming the method Data Collection. Click the “Data Collection” tab and click on the profile lines (or page icons) to add or delete data collection points during the run. Always include data points at all temperatures for the entire method profile.



- 7.10.1 Save the completed template using the **File / Save As / ABI PRISM SDS Template Document (.sdt)** commands. Save the appropriately named template in the “C:/Program Files/Applied Biosystems/SDS 2.0/ Templates” directory so that it can be automatically accessed by the SDS software using the “Template” dropdown menu.
- 7.11 Loading and Running a Plate
- 7.11.1 Prepare and load the master mix for all samples, followed by the sentinel, NTC, standards and unknown (test) samples (that are not expected to have a high DNA/RNA concentration) into the plate in the described order.
- 7.11.2 Prepare and load into a 96 well qPCR plate, the negative controls (sentinel and NTC samples) and load the master mix with any necessary additional water for the standards and unknowns in a PCR setup (UV irradiated) hood using barrier pipette tips, dedicated pipettes, and dedicated “clean” reagents. Use new Nitrile gloves at all times. UV irradiate assay materials and water reaction components and reagents, with the exception of the Master Mix, and standard DNA/RNA and test sample prior to use for 0.5 to 2.5 hours.
- 7.11.3 In a laminar flow Biosafety hood, prepare the standard dilution series and load the standards, and spike samples in the 96 well plate. Use dedicated reagents, barrier tips, and “dirty” pipettes for the standards. Always use new Nitrile gloves.
- 7.11.4 Purify (using Qiagen or Phenol-Chloroform) high protein and/or high salt samples and/or prepare any necessary dilutions of test (unknown) samples that are expected to contain significant levels of DNA/RNA on the bench-top using dedicated “dirty” pipettes and barrier tips. Alternatively, test samples expected to be relatively free of DNA/RNA and that don’t require purification prior to

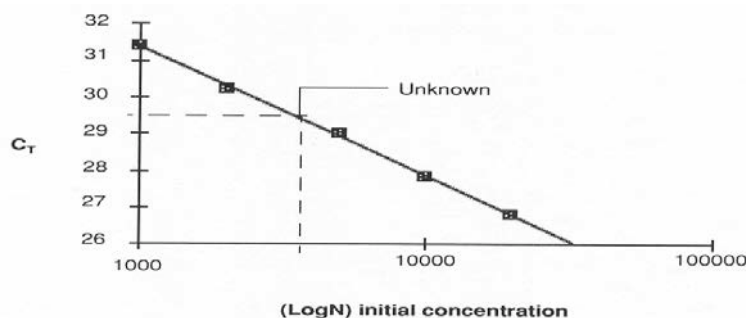
loading should be prepared and loaded in the laminar flow hood. Refer to assay specific documentation for unknown sample purification, dilution, and DNA/RNA spike requirements and conditions.

- 7.11.5 The final volume of all samples, including standards, should be identical to within 20% of the standard volume used.
- 7.11.6 Once all volumes have been added to a 96-well plate, cover the plate with an optically clear adhesive cover and seal the cover using the ABI provided “spreader.”
- 7.11.7 In the SDS software Plate Document, click the **Instrument** tab. Under the **Real-Time** tab click **Open** to expose the 7900 plate tray.
- 7.11.8 Place the 96-well plate on the tray with the A1 well position towards the pivot bar – align the beveled edge of the plate with the beveled edge of the tray.
- 7.11.9 Click **Start** in the Plate Document. Alternatively if additional changes need to be made to the run set-up in the SDS software, select **Close** and make the necessary changes prior to selecting **Start**.
- 7.11.10 The 7900 will close the tray and perform the run, data may be analyzed immediately following completion of the run. Do not stop a run once it has been started – refer to the 7900HT Users Guide for more information.
- 7.11.11 The SDS software will display the cycle number, time remaining and current block and cover temperatures during the run under the **Real Time** tab.
- 7.11.12 Fill out the instrument log book indicating the run date, start time, assay details (amplicon name, etc.), QC request number (if appropriate), and operator name.
- 7.12 Post-Run Analysis (refer to section 8.0).
- 7.13 Saving the Run, Plate Removal, and Power Down
 - 7.13.1 Save the run data (prior to analysis) by selecting **Save** or **Save As** from the SDS main menu bar. The run should be saved to “D:/7900 results/<run name>.sds”. The file name should include the PA requisition number (if appropriate), and amplicon tested for.
 - 7.13.2 Once a run has been completed and the run data saved, open the instrument tray and remove the 96-well plate. Inspect the plate to ensure that no portions of the optical adhesive cover delaminated and inspect the volumes and conditions of sample in each loaded well. Note any unusual observations in the run log.
 - 7.13.3 Discard the plate in the biohazardous trash. DO NOT OPEN the plate.
 - 7.13.4 Ensure that the run data has been saved and close out the SDS-Software. Close the network connection if no further runs are to be performed later in the day. If no further runs are to be performed within the next 48 hours, turn off the 7900HT instrument by pressing the front panel power switch. Once the 7900HT

instrument is off, shut down the instrument computer and monitor using the NT “Start” menu button. Do not turn off the Franek UPS Power Supply.

8.0 Post-Run Real-Time qPCR Analysis Using the SDS Software

- 8.1 The ABI 7900HT and SDS software can determine the absolute and/or relative level of DNA or RNA present in an unknown (test) sample by comparing the test C_T score to the C_T standard curve generated with standard controls in the same run.

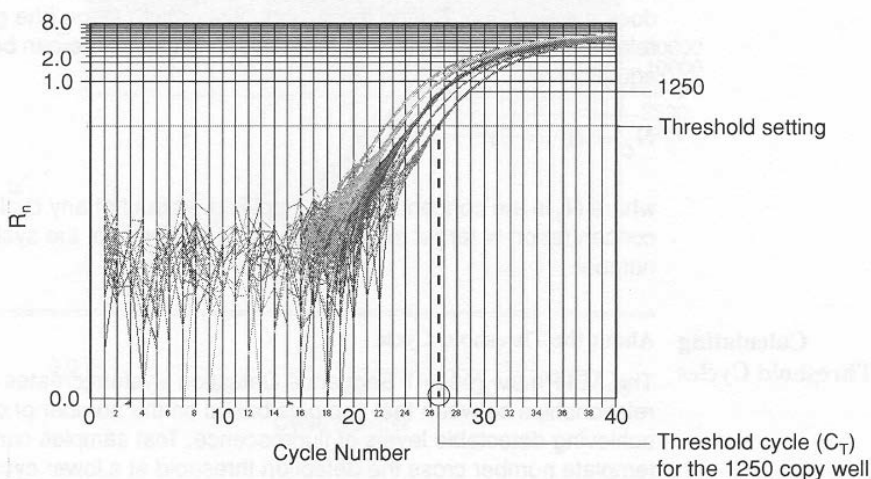


How the SDS Software Determines C_T s

To determine the C_T for an amplification curve, the SDS software collects data from a predefined range of PCR cycles called the ‘baseline’ (the default baseline occurs between cycles 3 and 15). The software then calculates a mathematical trend based on the baseline cycles’ R_n values and the standard deviation for the R_n values.

Next, the SDS software applies a threshold setting value to the amplification plot. The software applies the manual threshold setting specified in the Analysis Settings (see page C-40) for the real-time run. The amplification plot is viewed and the threshold value adjusted manually.

Finally, the algorithm searches for the point on the amplification curve at which the R_n value crosses the limit defined by the threshold setting. The cycle at which this point occurs is defined as the threshold cycle (C_T). For example, on the graph of R_n vs. Cycle Number below, the C_T value for the 1250 copy standard wells is 26.853.



Significance of Threshold Cycles

Begin with the equation describing the exponential amplification of the PCR:

$$X_n = X_m(1 + E_x)^{n-m}$$

where:

- X_n = number of target molecules at cycle n (so that $n \geq m$)
- X_m = number of target molecules at cycle m (so that $m \leq n$)
- E_x = efficiency of target amplification (between 0–1)
- $n - m$ = number of cycles elapsed between cycle m and cycle n

Amplicons designed and optimized according to Applied Biosystems guidelines (amplicon size <150 bp) have amplification efficiencies that approach 100 percent. Therefore $E_x=1$ so that:

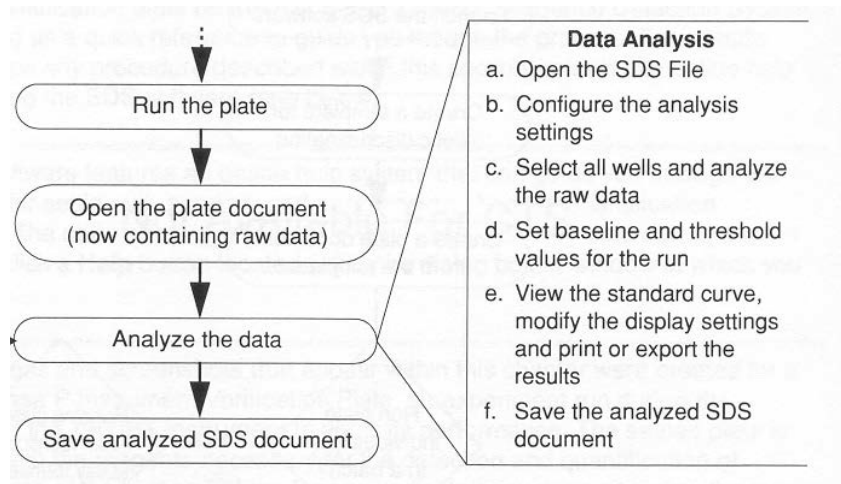
$$\begin{aligned} X_n &= X_m(1 + 1)^{n-m} \\ &= X_m(2)^{n-m} \end{aligned}$$

To define the significance in amplified product of one thermal cycle, set $n - m = 1$ so that:

$$\begin{aligned} X_1 &= X_0(2)^1 \\ &= 2X_0 \end{aligned}$$

Thus, each cycle in the PCR reaction corresponds to a two-fold increase in product. Likewise, a change in threshold cycle number of one must equate to a two-fold difference in initial template concentration.

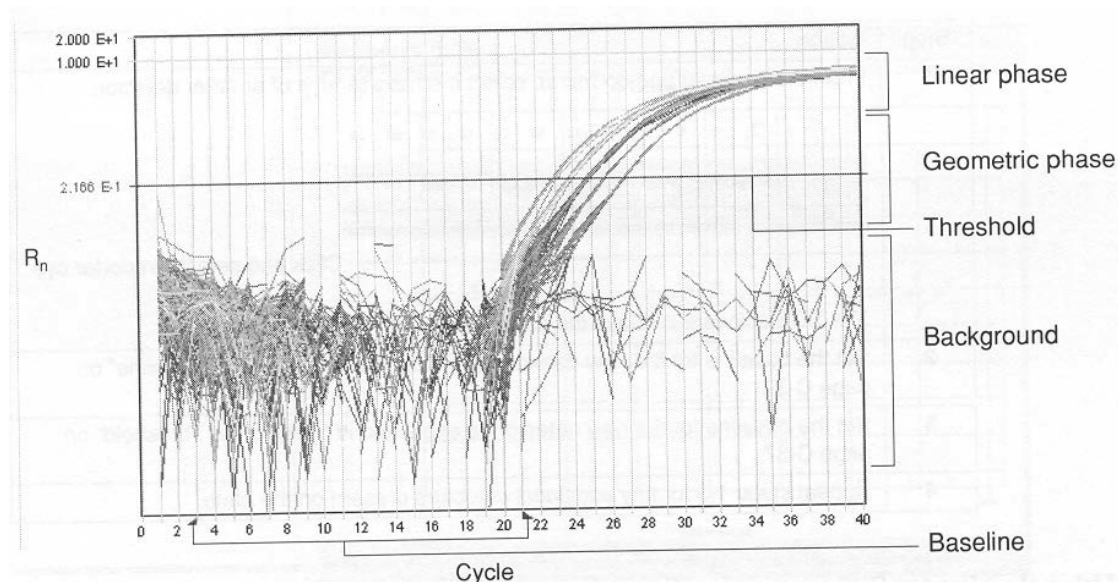
8.2 Data Analysis Flow Chart



Refer to the relevant assay or product specific SOP the correct threshold level, background range (typically 3-15 cycles), and approximate expected Ct scores of standards.

- 8.3 On the Plate Document well inspector window, highlight the entire plate and click **Analyze** to initiate the automated plate analysis. A new Analysis tab will appear in the Plate Document window once the analysis is completed.

- 8.4 From the Plate Document **Analysis** menu select **Analysis Settings**. The “Analysis Settings-Absolute Quantification” dialog box will open. Set the **Threshold** value according to the assay/product specific document. The threshold value should fall within the geometric (exponential) phase of the reaction as seen on the **R_n vs. Cycle** graph window. The Baseline determines the average background used in the analysis calculations, ensure that the baseline cycle range selected does not show continuous amplification in either the standard or unknown wells as this will artificially increase the background average and reduce assay sensitivity. An assay may be considered invalid if the required threshold or baseline values are outside of their established ranges.



- 8.5 Using the Plate Document **Standard Curve** window review the r-squared value of the curve, the slope (typically near -3.3), and the Y-intercept Ct. Compare the results with the relevant product specifications or assay SOP.
- 8.6 As many as 1 non-identical standards may be dropped from a 6-8 point standard curve performed in duplicate, as many as 2 non-identical points may be dropped in a 6-8 point standard curve performed in triplicate.
- 8.7 Dropping standard curve points may only be performed when the deviation from the expected Ct score (based on the standard curve with all points included) exceeds 2.5 Ct.
- 8.8 Dropped standard points require that the standard well to be dropped is deselected in the Plate Document inspector window as a standard (S) and assigned as not in use. The plate must then be reanalyzed for a new standard curve to be generated.
- 8.9 Re-save the analyzed run using the same run/file name.
- 8.10 Under the **Print Setup** menu, set the font size to 9 and print the results including the “AQ” (analyzed qPCR results), thermal cycler conditions, document information, standard curve, and amplification plot. Ensure that the threshold value is printed on the amplification plot page and the standard curve data is printed with the standard curve plot.

- 8.11 Raw and analyzed data may be exported in tab-delimited text (.txt) format for Excel or Origin based automated spreadsheet analysis using the **Export** menu.

9.0 Data Archival and Storage

- 9.1 On a weekly basis any SDS run data saved to "D:/7900 results/" should be backed-up to the network H: drive. Copy the ".sds" files to H:/BDP_Public/QC files/Molecular Biology/7900 Results. This also allows remote viewing and analysis of data with networked computers running the SDS software.
- 9.2 Data from runs greater than 12 months (1 year) old may be removed from the local computer (D: drive) as necessary if an archived copy is on the H: drive.
- 9.3 CD-R, CD-RW, or other digital copies of sds files may be made as necessary but may not be used as an official archival storage medium.

10.0 Routine Maintenance

- 10.1 Refer to chapter 7 of the ABI 7900HT Users Guide for greater in-depth coverage of routine and general instrument maintenance.
- 10.2 Annually the ABI 7900HT instrument should be inspected and maintained by the ABI field service engineer (or as updates are released) according to the BDP contract. ABI maintains a record and schedule of field service and maintenance appointments but may be contacted by phone as necessary at: 800.831.6844.
- 10.2.1 All ABI service calls and scheduled visits should be logged in the instrument logbook, including a description of work performed.
- 10.2.2 Repair and inspection receipts as well as performance and upgrade records will be maintained in the specific MEF and PA instrument files.
- 10.3 Bi-annually (every 6 months) the ABI 7900HT instrument should have the performance verified by BDP-PA by performing a Background Run and an ABI RNaseP Plate or Pure Dye Plate. These performance verification tests are not required if they have been performed by an ABI service engineer with the previous 6 months. Background runs should always be performed immediately following block cleaning and instrument maintenance.
- 10.3.1 Refer to the ABI 7900HT Users Guide, chapter 7, for details regarding the set-up and analysis of Background, Pure Dye, and RNase P Plates. Save performance verification Background, Pure Dye, RNase P run data to D:/7900 results/<file name.sds>. Include the type of assay and the run date as part of the file name as indicated in the 7900HT Users Guide.
- 10.3.2 Analyze and compare the data obtained with the Background, Pure Dye, and RNase P Plates with prior saved results according to the ABI 7900HT Users Guide. Print the results and place in the PA instrument folder. Note any significant changes from prior results and note any inconsistencies or unexpected results.
- 10.3.3 Log the performance verification tests and a summary of the results in the instrument logbook.

10.4 General External Cleaning

- 10.4.1 The ABI 7900HT, computer, and power supply should be externally wiped down once every 2 weeks to remove excess dust and particulates that may otherwise become embedded in the sample block or obstruct the instrument and computer exhaust fans.

10.5 Decontaminating the Sample Block

- 10.5.1 Refer to the ABI 7900HT Users Guide, chapter 7, for details regarding the removal, cleaning, replacement and re-installation, of the 96-well sample block.
- 10.5.2 The sample block may become contaminated with reaction mixes leaking from a poorly sealed or defective 96-well plate. The presence of fluorophores and nucleic acids on the block surface will interfere with subsequent runs and must be removed. Always check the integrity of plates after each run to ensure the block has not been contaminated.
- 10.5.3 Do not use any chemical solution, cleaning agent, or abrasive, other than those listed in the Users Guide or as directed by an ABI service engineer, on the sample block or the block may be irreparably damaged.

Materials Required The cleaning procedure requires the following materials:

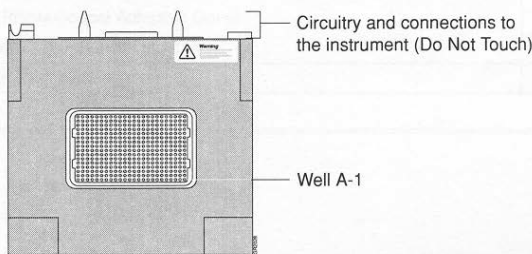
Material	Part Number
Cotton swabs	—
10% Sodium hypochlorite (bleach) solution	—
Isopropanol, 100 percent pure	—
5/32 in Hex key	—

⚠ WARNING CHEMICAL HAZARD. Sodium hypochlorite (bleach) is a liquid disinfectant that can be corrosive to the skin and can cause skin depigmentation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ WARNING CHEMICAL HAZARD. Isopropanol is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Cleaning the Sample Block Wells

To clean the sample block wells:

Step	Action
1	Run a background plate as explained in "Performing a Background Run" on page 7-13 to isolate the contaminated sample block wells.
2	Remove the sample block as explained in "Replacing the Sample Block Module" on page 7-6.
3	Using the following figure as a guide, locate the suspected contaminated wells on the sample block. 
4	Pipet 40 µL 10% bleach solution into each suspected contaminated well.
5	Let the sample block sit for 3-5 min.

To clean the sample block wells: *(continued)*

Step	Action
6	Using a pipet, remove the bleach solution from the wells of the sample block.
7	Rinse each contaminated well with 3 treatments of 40 µL deionized water. Note Absolute isopropanol can be substituted for water in the third treatment.
8	Remove any remaining isopropanol or water from the sample block.
9	Replace the sample block as explained in "Replacing the Sample Block Module" on page 7-6.
10	Run a background plate to confirm that the contamination has been removed.

11.0 Troubleshooting Diagnosis and Procedures

- 11.1 Instrument hardware and SDS software maintenance and troubleshooting should be formed by or in conjunction with an ABI service representative only.
- 11.2 Refer to the ABI 7900HT Users Guide for further information regarding troubleshooting unexpected assay results, instrument and assay setup, and run analysis.
- 11.3 Refer to the following table for initial troubleshooting diagnosis of Real-Time qPCR assays.

Real-Time Troubleshooting	Observation	Possible Cause	Recommended Action
	Run will not start	No calibration files	See if the calibration files are in the correct folder. Perform a background and pure dye run.
	Raw Data view		
	Strange R _n plot	Wrong detector setup, incorrect dyes selected	Correct the software setup by reassigning the correct dyes to the detectors.
	Raw spectra signals from replicate groups or controls do not exhibit similar spectral 'humps'	Contamination	
	Samples do not fluoresce at the correct wavelength (for example, samples containing only FAM-labeled TaqMan™ probes should not exhibit a VIC™ dye component)	♦ Contamination ♦ Pipetting error ♦	
	Signals plateau (saturation)	♦ reaction contains too much template ♦ ♦	
	Multicomponent view		
	ROX signal fluoresces below the reporter dyes	♦ Pipetting error (well does not contain probe)	♦
	Signals do not fluoresce above the background	♦ Pipetting error (well does not contain probe, PCR master mix, or both)	
	Reporter dyes do not conform to the fit	♦	
	Amplification Plot view		
	More than 10 outliers in each population	Evaporation	Check the the adhesive cover seal for leaks.
	Standard Curve view		

Observation	Possible Cause	Recommended Action
Standard curve is off	<ul style="list-style-type: none">◆ Poor threshold setting◆ Poor baseline setting◆ Run data contains outliers	<ul style="list-style-type: none">◆ Check data for outliers.◆ Check for evaporation in wells.◆ Adjust threshold and baseline.

12.0 ABI Service, Sales, and Technical Support Contact Information

Applied Biosystems, Inc. general and technical support contact information:

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13.0 Abbreviations and Definitions

- 13.1 **5' Nuclease Assay** – The TaqMan qPCR assay relies on the ability of the DNA polymerase to displace and cleave the nascent 5' end nucleotides of a fluorescently labeled probe annealed to the DNA being amplified. The probe cleavage allows for an increase in fluorescence of the released 5' dye.
- 13.2 **ABI** – Applied Biosystems, Inc. - The 7900HT SDS instrument and software manufacturer and major reagent supplier for TaqMan® qPCR assays.
- 13.3 **Amplification Plot** – A graphic display in the ABI SDS analysis software that shows the normalized signal level vs. the cycle number of a completed qPCR reaction. The shape of the resulting amplification curve for a given reaction (well) can be used to determine the appropriate threshold level and whether true amplification has occurred.
- 13.4 **Background Signal** – Also see Baseline. The SDS software determines the amount of ROX dye present in each reaction and normalizes all reactions to a fixed level of ROX, once this is done the SDS software determines the average level of signal of all non-ROX dyes present in the reactions within the baseline settings. Only signal levels exceeding this averaged background level are reported by the software.
- 13.5 **Baseline** – The selectable range of PCR cycles where the SDS software determines the average background signal level of all reactions containing Master Mix. The default baseline is cycles 3-15. The presence of detectable amplification product at cycles <18 may require that the baseline be narrowed to prevent skewing the average background signal level.
- 13.6 **Black Hole Quencher (BHQ)** – A type of quencher molecule that totally absorbs excitation energy the Reporter moiety with little or no fluorescence of the Black Hole molecule itself. BH quenchers are useful for maximal reduction of signal background in sensitive TaqMan assays.
- 13.7 **Clean** – also see "Dirty," Reagents and Materials that have not been exposed to DNA/RNA.

- 13.8 **Ct** – Threshold cycle, the interpolated PCR amplification cycle at which the normalized signal response of a fluorescent qPCR reaction exceeds an arbitrarily chosen intensity level (the threshold value). Ct is measured to 1/100ths of a cycle.
- 13.9 **Cycle / Cycle Number** – The use of controlled repetitive temperature changes to allow for denaturation and amplification of DNA. One repetition is one cycle of amplification.
- 13.10 **Dirty** – Materials and supplies that may have been contaminated with DNA/RNA. (also see "Clean").
- 13.11 **dNTP** – deoxy-(Any)nucleotide triphosphate, usually denoting the presence of all 4 nucleotide bases required for DNA amplification or Reverse Transcription.
- 13.12 **dUTP** – a non-natural base replacing dTTP in the ABI TaqMan® Master Mix that allows for the enzymatic degradation of carry-over contaminating DNA (containing UMP) by the enzyme UDG/UNG in the Master Mix.
- 13.13 **FAM Dye** – A common reporter dye with peak fluorescence at ~520 nm.
- 13.14 **FWD Primer** – Forward primer, one of two primers required for PCR amplification of a sequence defined DNA segment.
- 13.15 **Geometric Phase** – The initial stage of PCR amplification where the number (mass) of DNA amplicon increases geometrically, doubling with every cycle. The last several cycles of the geometric phase of amplification is detected by the instrument as being above the average background level and is used to most accurately locate the threshold level.
- 13.16 **IPC** – Internal Positive Control, an exogenous DNA segment (and associated primers and probe) included in qPCR reactions to assess PCR inhibition caused by a test sample.
- 13.17 **Linear Phase** – The second of three phases in PCR amplification where the rate of amplicon copy number increase per cycle changes to a arithmetic (linear) function due to the rate limiting availability of one or more reaction components. This can be used to set the threshold level if necessary, although it is not optimal.
- 13.18 **Master Mix** – A pre-mixed solution containing all the required reagents for qPCR except the target DNA, primers, probe, and diluent as needed.
- 13.19 **MSE** – Mean Squared Error of the dye multicomponent de-convolution performed by the 7900HT SDS software (residual error). See Multicomponent graph.
- 13.20 **Multicomponent Graph** – A view of the un-normalized fluorescent signal from a selected reaction (well) on the plate. The graph shows the response level for each dye present in the reaction, including the reference dye ROX and the MSE residual error resulting from the de-convolution calculations used to determine the contribution from each individual dye to the combined total fluorescence in the reaction over time. The Multicomponent graph is typically used to determine if a low questionable amplification response in a well is due to true product amplification or to a spurious increase in total fluorescence. True amplification will show a characteristic increase in reporter dye signal and a loss in quencher dye signal level.

- 13.21 **NTC** – No Test Control, the negative control of a PCR reaction that does not contain test sample (diluent is used in its place) but does contain all other reaction components.
- 13.22 **Plateau Phase** – The third and final phase of a PCR amplification where one or more components of the reaction is exhausted preventing further increase in copy number (mass) or fluorescent signal increase. The threshold level should never be placed on or near the plateau signal level.
- 13.23 **Probe** – The crucial 3rd “primer” in a TaqMan assay, the probe anneals to a region near the center of the PCR amplicon and is covalently labeled with a 5’ reported dye and a 3’ quencher molecule (often another dye). The labeled probe allow for the 5’ nuclease assay (TaqMan).
- 13.24 **Pure Dye Run** – A plate containing individual dyes that is used in 7900HT validation, troubleshooting, and calibration maintenance.
- 13.25 **qPCR** - quantitative Polymerase Chain Reaction, unlike routine PCR allows for the direct comparative quantification of target sequence in a PCR reaction.
- 13.26 **Quencher** – The chemical moiety attached to the 3’ end of a TaqMan probe that partially absorbs the excitation energy from the Reporter dye (5’) labeled nucleotide and shifts / reduces (quenches) the fluorescence of the probe until the Reported nucleotide is cleaved from the probe.
- 13.27 **Real-Time Analysis** – The ability of the ABI 7900 SDS to continuously and in “real-time” to read the change in fluorescence of a 5’ nuclease-based qPCR reaction as the PCR thermal program is executing.
- 13.28 **Reporter** – The fluorescent dye covalently attached to the 5’ end of a nucleotide probe that is released during amplification (see 5’ nuclease) and subsequently detected via the increase in total fluorescence.
- 13.29 **REV Primer** – Reverse primer, one of two primers required for PCR amplification of a sequence defined DNA segment.
- 13.30 **Rn (and delta Rn)** – Normalized (fluorescent) Response, the critical measurement of PCR reaction (in each well) fluorescence normalized to the ROX reference dye response per reaction. Rn (or delta Rn – the change in Rn per cycle) is used in all further software calculations and in the amplification curve.
- 13.31 **RNase-P** – A pre-loaded plate containing Master Mix with primers, probe, and RNase-P DNA target sequence that is used in 7900HT validation, troubleshooting, and calibration maintenance.
- 13.32 **ROX Dye** – The passive reference dye present in Master Mix formulations and is required for cross-well normalization of fluorescent signals. Peak fluorescence is at ~610 nm.
- 13.33 **RT-qPCR** – Reverse Transcription – Quantitative Polymerase Chain Reaction.
- 13.34 **SDS** – Sequence Detection System, the use of nucleotides labeled with fluorogenic dyes allows the detection and quantitation of DNA in solution.

- 13.35 **Sentinel Control** – Similar to the NTC control, but prepared prior to all other reactions, the sentinel control is used to assess the possibility of accidental reaction contamination during sample and plate preparation.
- 13.36 **STD / Standard curve** – Absolute quantification of test samples is achieved through the use of a 5 or greater log dilution series of a known mass DNA standard; the Ct values of the DNA standard dilution series is used by the SDS software to generate a standard response curve against which the test samples' Ct is compared for determining the samples' initial DNA mass. Assay acceptance criteria specify allowable ranges for the standard curve R², slope, and Y-intercept values.
- 13.37 **TAMRA Quencher** – A common quencher dye with peak fluorescence at ~580nm.
- 13.38 **TaqMan™ Chemistry** – The use of a doubly fluorescent labeled DNA probe cleaved by the 5' nuclease activity of a DNA polymerase to detect and quantitate a PCR amplicon in real-time.
- 13.39 **Threshold** – An arbitrary normalized reporter signal level that is set within the geometric (or linear) phase of the qPCR reactions in order to determine the reaction's Ct value. Specific assays will require the use of predetermined threshold levels.
- 13.40 **UNG (UDG)** – Uracil-N-Glycosylase (also known as Uracil-Deglycosylase), an optional enzyme present in the ABI TaqMan Master Mix that prevents carry-over contamination from prior qPCR reactions by rendering dUMP containing DNA abasic and thus unable to amplify.
- 13.41 **VIC Dye** – A common reporter dye with a peak fluorescence at ~550nm.

14.0 References and Related Documents

- 14.1 ABI Prism® 7900HT Sequence Detection System User Guide CTS Manual, also referred to in this SOP as the "7900HT Users Guide", copyright 2001, Applied Biosystems, Inc., Foster City, CA.
- 14.2 ABI SDS 2.0 Software, Applied Biosystems, Inc. Foster City, CA.
- 14.3 ABI Primer Express Software, Applied Biosystems, Inc. Foster City, CA.
- 14.4 ABI online reagent and consumables catalog at www.appliedbiosystems.com