### **Procedure for Human DNA Quantitation with Quantifiler®**

Version 2

Effective Date: 12/7/2012

- **1.0 Purpose** This procedure specifies the steps for Human Quantification of DNA extracts.
- **2.0 Scope** This procedure applies to Forensic Scientists in the Forensic Biology Section who are qualified to use the ABI Prism 7000 instrument.

#### 3.0 Definitions

- **Inhibition** The total or partial suppression of the PCR process that would result in a partial or no DNA profile being obtained.
- Internal Positive Control (IPC) Each reaction in the Human Quantifiler Kit contains an IPC to help determine whether a sample is a true negative or whether there is inhibition occurring in the PCR reaction. The normal Ct values for the IPC should range from 20 to 30.
- **R**<sup>2</sup> **value** This value measures the closeness of fit between the standard curve line and the Ct values of the DNA standard used in the quantification assay. A value of 1.00 indicates a perfect fit between the standard curve line and the DNA standard data points. In order for the results from a given assay to be valid, the R<sup>2</sup> value shall be greater than or equal to 0.98.
- **Slope** This value indicates the efficiency of the PCR reaction of the quantification assay. A slope value of -3.32 indicates 100 % amplification efficiency.

## 4.0 Equipment, Materials, and Reagents

- ABI Quantitation Kits and Reagents
- ABI Prism 7000 and current software
- Computer
- Disposable 96 well tray for ABI Prism 7000 and optical adhesive cover
- Calibrated Pipets and pipet tips
- Centrifuge
- Vortex mixer
- Various lab equipment (microcentrifuge racks, wipes, etc)
- Biosafety hood or cabinet
- TE
- Extracted DNA
- Human quantitation standards
- Base plate

### 5.0 Procedure

### 5.1 Background

- **5.1.1** The Quantifiler Human Kit uses a real-time PCR reaction with fluorescent dye chemistry to estimate the quantity of amplifiable DNA in each test sample.
- **5.1.2** DNA quantitation results are interpreted by checking the analysis settings of the ABI 7000 machine, the slope of the standard curve of the DNA standards, and the R<sup>2</sup> value of the DNA standards.
- **5.1.3** A maximum of four (4) cases may be analyzed together in a batch.

### 5.2 Setting up the Quantifiler Reactions

**5.2.1** The Forensic Scientist may prepare dilutions from the extracts prior to quantitation due to the possibility of inhibitors in samples (e.g., color of extracts, ability to flow through microcon).

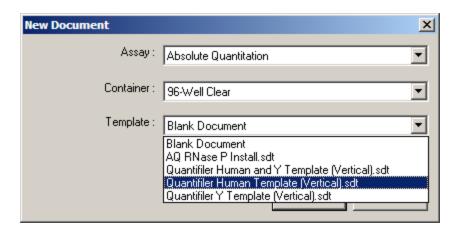
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- **5.2.2** The amount of reagents needed for the number of samples analyzed on a plate shall be calculated by using the ABI Quantifiler Setup Worksheet found on the Section shared drive.
- **5.2.3** Once the ABI Quantifiler Setup Worksheet has been filled out, the data under the "ABI 7000 Sample Setup" tab at the bottom of the screen shall be copied and pasted into a notepad file. This notepad file (i.e., .txt file) shall be saved on the drive associated with the instrument to be used.
- **5.2.4** The Quantifiler Human Primer Mix shall be thawed prior to use. The Forensic Scientist shall vortex the Primer for 5 seconds and then centrifuge briefly before opening the tube.
- **5.2.5** The proper amounts of the reagents (Primer and Reaction Mix) as calculated in **5.2.2** shall be pipetted into a sterile microcentrifuge tube.
- **5.2.6** Vortex the mixture for 5 seconds; then centrifuge briefly.
- **5.2.7** Dispense 23  $\mu$ L of the reagent mixture into each reaction well to be tested in a 96 well reaction plate seated into a base plate.
- **5.2.8** Vortex the DNA samples and Quantitation Standards; then centrifuge briefly.
- **5.2.9** Add 2  $\mu$ L of each of the DNA standards into the corresponding reaction well. The DNA standards shall be run in duplicate.
- **5.2.10** Add 2 μL of each of the DNA samples into the corresponding reaction well. For the NTC (negative control), add 2 μL of TE buffer (use same TE as before when making dilutions).
- **5.2.11** Seal the reaction plate with the optical adhesive cover. Use plastic tool to seal optical cover in place. Tear off white edges.
- **5.2.12** Centrifuge the plate at 2000 rpm for 2 minutes and check for bubbles at the bottom of each well.

# 5.3 Setting up a Plate Document

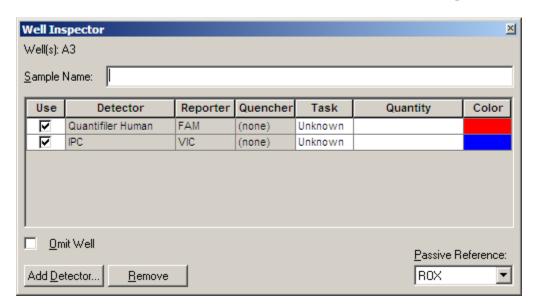
- **5.3.1** Restart the computer.
- **5.3.2** Turn on the ABI 7000.
- **5.3.3** Log on to the computer and open the ABI PRISM 7000 SDS Software.
- **5.3.4** Click *File* and choose *New*.
  - **5.3.4.1** The box below appears in the middle of the screen:



- **5.3.4.2** Select the appropriate template for the reactions.
- **5.3.4.3** Click on *File* in the menu bar and click on *Import Sample Setup*.
- **5.3.4.4** Choose the .txt file created and saved on that computer from step **5.2.3**.
- **5.3.4.5** (Optional) Click on *View* in the menu bar and click on *Well Inspector*. Highlight all the wells that are not to be used and click the *Omit Well* box in the Well Inspector.

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**5.3.4.6** After completing the plate setup, click on *Instrument tab* and check the settings. The settings shall be as follows:

	Stage 1	Stage 2	
# of cycles			
(Reps)	1	40	
Temp.			
(Celsius)	95	95	60
	10	15	1
Time	minutes	seconds	minute

**5.3.4.7** Ensure that the sample volume is set to 25  $\mu$ L and that the box next to "9600 Emulation" is checked.

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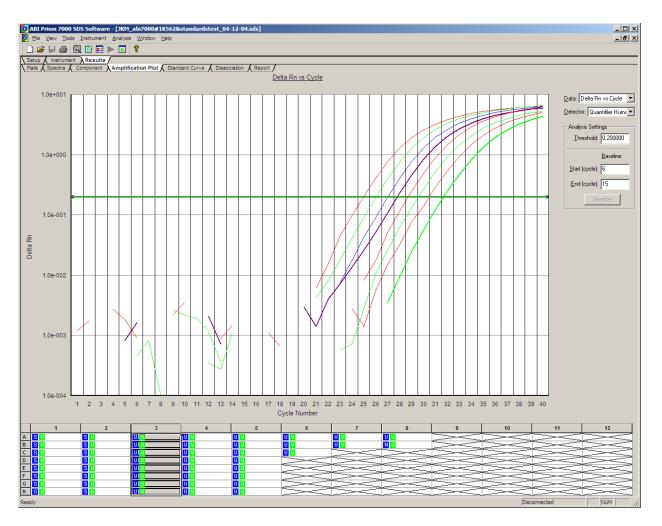
**5.3.4.8** Prior to starting the run, the Forensic Scientist shall save and name the document (i.e., .sds file). From *File* on the menu bar, select *Save As* and name the document with the case number in the file name if it contains a single case, or save the document with the date if multiple cases are present on the plate.

### 5.4 Operating the ABI PRISM 7000

- **5.4.1** Lift the handle at the bottom of the door and push the door all the way to the back until it locks into place. (If door does not lock, hold in place).
- **5.4.2** Place compression pad over the optical adhesive cover with the brown side facing up and the holes in the compression pad corresponding to the wells in the reaction plate.
- **5.4.3** Place the reaction plate into the plate holder inside the instrument with well A1 in the top left corner.
- **5.4.4** Close the instrument door.
- **5.4.5** In the *Instrument* tab of the software, click the "start" button on the right-hand side of the screen. It takes approximately one hour and forty-six minutes for the PCR to run and give the results.

### 5.5 Analyzing Data in SDS Software

- **5.5.1** Once the ABI 7000 is finished running, under the *Setup* tab, click on the well containing the NTC, and then click on the *Amplification Plot* tab in the SDS software. Confirm all 40 cycles were completed. If all cycles have not been completed but the "analyze" button is green, notify the DNA Quality Control officer and do not close the SDS software as the information present on the screen is needed for placing a service call to the manufacturer. The Forensic Scientist shall rerun the plate again. If all 40 cycles are completed, click the "analyze" button (green arrow icon).
- **5.5.2** Click the *Results* tab and highlight quantity results in a plate format.



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- **5.5.3** Click on the *Amplification Plot* tab (see above screen) and review the data for any unacceptable activity in the negative control (as defined in Section **5.5.6.1.1**) and to determine that the internal positive control (IPC) reaction worked in each well.
- 5.5.4 Click on the *Standard Curve* tab and check the values of the slope and R<sup>2</sup>. The slope shall be between -2.90 and -3.30, while the R<sup>2</sup> value shall be greater than or equal to 0.98. A maximum of two points of the slope may be dropped to account for pipetting variations. It should be noted that if a Forensic Scientist determines two points need to be dropped, the points cannot come from the same concentration (i.e., a Forensic Scientist cannot drop both 50 ng/uL concentration points). If a point or points are dropped, then both the original and adjusted slopes shall be printed and placed in the case notes. If after dropping two points, the slope and R<sup>2</sup>values are still not in range, the Forensic Scientist shall notify both the DNA QC Officer and the DNA Technical Leader as well as re-quantitate the samples unless there is limited sample present. If limited sample exists, the Forensic Scientist shall see the DNA Technical leader for discussion and approval on how to proceed. The intercept indicates what the Ct value of a 1 ng/μL sample would be for that run. "Print to PDF" the standard curve(s); refer to the Documentation and Review Procedure for naming convention of the PDF file.
- **5.5.5** Click on the *Report* tab and interpret results by selecting the NTC and wells containing samples for each case (if batched).

# **5.5.6** Interpreting IPC amplification results:

Quantifiler Human (FAM Dye)	IPC (VIC Dye)	<u>Interpretation</u>
No amplification	Amplification	True negative
No amplification	No amplification	Invalid result
Amplification (low Ct and high delta Rn)	No amplification	Disregard IPC result
Amplification (high Ct and low delta Rn)	No amplification	Partial PCR inhibition

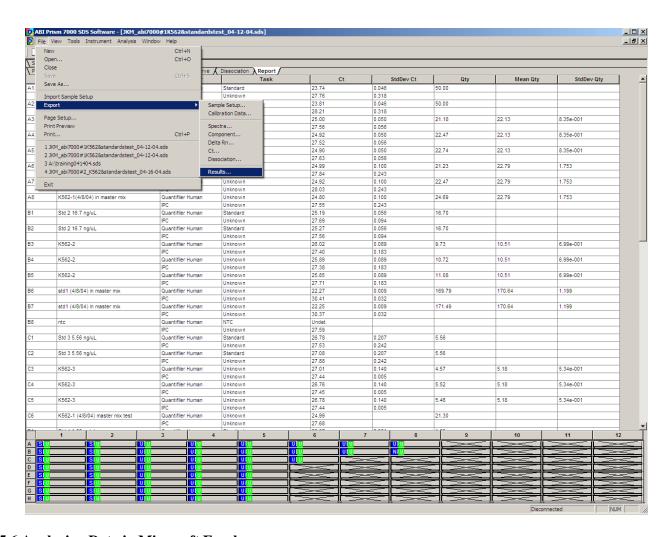
Note: The IPC may show inhibition if the Ct value in the sample is greater than the IPC value of the NTC or if the sample reads "Undetermined." In most cases, dilutions should be performed or the samples should be cleaned up using the "Clean up of Extracted DNA" from the Forensic Biology Section Procedure for Performing Organic DNA Extractions.

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### **5.5.6.1** Controls

- **5.5.6.1.1 NTC:** For each set of samples analyzed, a "No Template Control" (NTC) shall be run to show that there was no contamination during the setup of the assay. Due to the sensitivity of the real-time PCR method, extremely low levels of DNA (levels that do not effect downstream applications such as STR analysis) can be detected. Therefore, if a Ct value of 36 or greater is observed in the NTC, it is still considered to be a negative sample and the results of the assay are still valid. However, if the Ct value of a NTC is less than 36, the NTC sample **may** be repeated. However, if the other negative controls show Ct values greater than 36, the Forensic Scientist may proceed with the amplification of the samples.
- **5.5.6.1.2 Negative Extraction Controls:** If the Ct value of a negative extraction control is less than 36, the Forensic Scientist may continue processing the samples, realizing that activity may be observed in those controls in the post amplification product.
- **5.5.7** Click the *File* button, go to *Export*, and click *Results*.
- **5.5.8** Print the results (separately if batched) to PDF and save the file on the instrument computer; refer to the Documentation and Review Procedure. Note: up to 13 samples (or wells) can fit to a printed page.
- **5.5.9** If cases are batched on the same run, create separate SDS files for each (refer to the Forensic Biology Section Procedure for Documentation and Review).



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# 5.6 Analyzing Data in Microsoft Excel

- **5.6.1** Open the exported results document in Excel.
- **5.6.2** Open the "ABI 7000 Dilution Calculation Worksheet" in Excel.
- **5.6.3** Select all the data from **5.6.1** by pressing Ctrl + A.
- **5.6.4** Click on cell A1 of the *Results* section of the ABI 7000 Dilution Calculation Worksheet and paste the results.
- **5.6.5** Click on the ABI 7000 Dilution Calculation Worksheet tab to see the results in plate format.
- **5.6.6** Confirm the volume of the sample necessary to dilute the sample to 1 ng/μL. (There needs to be approximately 2 ng of DNA in the Identifiler<sup>TM</sup> reaction, therefore samples that are ~10 ng/μL and lower do not need to be diluted.) There are two charts, one for dilutions (> 10 ng/μL) and the second for samples not requiring dilutions. The spreadsheet automatically calculates how much TE is necessary to prepare the dilution for samples requiring dilution; it also provides the amount of DNA necessary for the Identifiler<sup>TM</sup> amplification reaction for samples which do not require dilution.

5.6.7 Select samples (one column at a time) that are >10 ng/ $\mu$ L, copy and paste special (values) into dilution chart. Next select sample in same column that are <10 ng/ $\mu$ L, copy and paste special (values) into second chart.

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**5.6.8** Forensic Scientists shall save this document and print to PDF using the proper name to transfer to the FA object repository (see the Forensic Biology Section Procedure for Documentation and Review).

#### **6.0 Limitations-** N/A

### 7.0 Safety-N/A

### 8.0 References

Forensic Biology Section Procedure for Documentation and Review

Forensic Biology Section Procedure for Performing Organic DNA Extractions

Forensic Biology Section Procedure for DNA Casework Training

Forensic Biology Section Procedure for Performance Check and Equipment Maintenance

## 9.0 Records - N/A

### 10.0 Attachments - N/A

Revision History				
Effective Date	Version Number	Reason		
09/17/2012	1	Original Document		
12/7/2012	2	5.6.6 - changed dilution from 2 ng/ul to 1 ng/ul		