Procedure for DNA Quantitation with Quantifiler® Trio

1.0 Purpose – This procedure specifies the steps for performing quantification of DNA extracts with the Quantifiler® Trio kit on the ABI QS5Quant Studio 5 instruments (QS5).

2.0 Scope – This procedure applies to casework analysts and trainees in the Forensic Biology Section who are qualified to use the ABI QS5Quant Studio 5 instruments.

3.0 Definitions – See Forensic Biology Section Definition List

4.0 Equipment, Materials and Reagents

- Quantifiler® Trio Kit and associated reagents
- ABI QS5 Quant Studio 5 Real-Time PCR Instrument (QS5QS5) and associated computer
- QIAgility liquid handler
- HID Real-Time Analysis Software v 1.3
- Microsoft Excel
- 96-well optical plates and optical adhesive covers
- Calibrated pipets and tips
- Centrifuge
- Vortex mixer
- Laminar Flow Clean Air Bench
- Amplification grade H_2O (or equivalent qPCR grade)
- DNA CalibratorBase plate
- Extracted DNA
- Various laboratory equipment (microcentrifuge tubes, wipes, etc.)
- Sterile 2 mL tubes
- Strip tubes

5.0 Procedure

5.1 Introduction

5.1.1 The QS5 Real-Time PCR System uses fluorescence-based polymerase chain reaction reagents to provide quantitative detection of nucleic acid (DNA) targets using real-time analysis.

5.1.2 The Quantifiler® Trio kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. The relative quantities of

human male and female DNA in a sample that can assist in the selection of the applicable STR chemistry. Quantifiler® Trio kit uses a multiple-copy target loci.

5.1.3 Interpretation of the results is dependent upon the quality metrics of the standard curves (R^2 value, y-intercept and slope), and the performance of the IPC. If virtual standard curve is being used, these values will be determined during QC of the Quant Trio kit.

5.1.4 Quantifiler® Trio plate setups shall be performed utilizing the QIAgility instrument. If the QIAgility is unable to be used due to maintenance or other issues, manual setup is allowed. The Forensic Biology Technical Leader will be consulted if manual setup is required for other reasons, this consultation shall be documented.

5.2 Manual Preparation of the Standard Curve

5.2.1 All standards shall be prepared by Forensic Scientist(s) as described in the Forensic Biology Procedure for DNA Reagent Preparation and Quality Control. The standards shall be vortexed and centrifuged prior to use by Forensic Scientists.

5.3 Preparation of the Plate Document

5.3.1 Forensic Scientists shall use the Microsoft Excel "Biology Workbook Quant setup tab" for both manual and QIAgility setups to calculate the amount of reagents necessary for the number of samples to be analyzed per plate. This Setup Worksheet takes the standard curve into account for the calculations.

5.3.2 If a batch of samples is in excess of 30 total samples (knowns and unknowns) then witnessing steps shall be added when multiple tubes are open simultaneously during the transfer of tubes (e.g., Qiagility setup). This witnessing will be documented in the case notes. Any additional exemptions to batch processing shall be approved only with written documentation from the Technical Leader.

5.3.3 Each plate shall have a minimum of one Non Template Control (NTC) and DNA calibrator included.

5.3.4 The QS5 import tab of the workbook shall be saved as a .txt file and imported into HID Real-Time Analysis Software v 1.3

5.3.5 The Quant Bank info tab of the workbook shall be saved as a .CSV file. (Select "ok", then "no", then "cancel" in Excel when saving the .csv file.)

5.4 Preparation of the Reaction (Manual Setup)

5.4.1 Vortex the Quantifiler® Trio Primer Mix and then centrifuge before opening the tube. Add the volume as provided in the Setup Worksheet to a sterile 2ml tube.

5.4.2 Gently vortex the Quantifiler® Trio THP Reaction Mix. Add the volume as provided in the Setup Worksheet to the 2ml tube already containing the Primer Mix. This combination of Primer and Reaction Mix is now the Master Mix.

5.4.3 Assemble a 96-well optical plate into a black base. Do not place the optical plate directly onto the benchtop to protect it from scratches and other particulate matter.

5.4.4 Vortex the Master Mix and then centrifuge. Dispense 18 µl of Master Mix into each well of the optical plate, per the Setup Worksheet.

5.4.5 Add 2 µl of Amp grade water to the well designated for the NTC.

5.4.6 Add 2 µl of Calibrator to the well designated for the Calibrator.

5.4.7 Add 2 µl of each sample to the wells as designated by the Setup Worksheet.

5.4.7.1 For samples extracted using the Casework Direct System, samples must be diluted using Amplification Grade water prior to quantitation.

5.4.7.1.1 Pipette 20 µl sample into a new labeled tube.

5.4.7.1.2 Add 5 µl amplification grade water. Vortex briefly.

5.4.7.1.3 Add 2 µl of each diluted sample to the well designated in the Setup Worksheet.

5.4.8 Add 2 µl of each Standard (in duplicate) to the wells as designated by the Setup Worksheet. If virtual standard curve is being used, this may be omitted.

5.4.9 Seal the plate with an optical adhesive cover. Centrifuge the plate at 3000 rpm for 20 seconds. Remove bubbles: While the plate is inside the base, tap the base on the benchtop to bring the bubbles to the liquid surface. Lift the plate, then inspect each well for bubbles; tap each well with gloved fingertip.

IMPORTANT! This step is critical to avoid noise in the fluorescence signal that bubbles can cause.

5.5 Preparation of the Reaction (QIAgility Setup)

5.5.1 Vortex the Quantifiler® Trio Primer Mix and then centrifuge before opening the tube. Add the volume as provided in the Setup Worksheet to a sterile 2 mL tube.

5.5.2 Gently vortex the Quantifiler® Trio THP Reaction Mix. Add the volume as provided in the Setup Worksheet to the 2 mL tube already containing the Primer Mix. This combination of Primer and Reaction Mix is now the Master Mix.

5.5.3 Vortex Quantifiler® Trio THP DNA Dilution Buffer and the Quantifiler® THP DNA standard and then centrifuge before opening each tube. Add 10 µl of the DNA standard along with 10 µl of THP Dilution Buffer into the first well of a set of strip tubes (STND 1). Mix reagents by gently pipetting up and down. If using a standard curve already prepared, select the box for premixed standard curve. If a virtual standard curve is being used, this step may be omitted.

5.6 Operation of the QIAgility

5.6.1 Ensure the QIAgility instrument is on. The switch is located on the lower back left corner of the instrument. Ensure that the deck is properly set up for quantitation based on the experiment being run. Forensic Scientists shall open the QIAgility software by double clicking the appropriate shortcut located on the computer desktop.

5.6.1.1 Ensure the "Shortcut to TrioSetup-64QAS" or "Shortcut to Trio Setup-96" Protocol – samples extracted using the DNA Investigator Kit in 1.5 mL or 2 mL tubes.

5.6.1.2 "Shortcut to TrioSetup –plate.QAS" Protocol – samples extracted on the QIAsymphony into a 96-well plate format.

5.6.2 The worktable for Trio setup shall be set up as it appears on the screen. Holding the mouse over any colored well will give information about the contents of that well.

5.6.3 Within the Trio Setup Protocol, left-click on section "A2:Sample." The right hand side of the screen will populate with information for that section. Click the Import button. The "Import Well Data" dialog box will appear.

5.6.4 Under Import File in the top left hand corner, click the browse ··· button. Locate the .CSV file and click open.

5.6.5 Click the Import button at the bottom of the screen to import the samples and then click Finish. On left-hand side under import options, change the Start importing From box to 2.

5.6.6 If Casework Direct samples are being quantitated, left-click on "Reaction block C1". In the upper right hand side of the screen, highlight the first line. Then right-click and choose "Create Sample bank from Target wells." Click the button next to "Existing Bank," highlight "Diluted CWD Samples," click "Add Selection" and then click "Close."

5.6.7 Grasp the handle and pull up to open the instrument lid. Inspect the worktable to ensure that the tip holders and plate holder are in the position which appears on the screen.

5.6.8 Place the following materials on the worktable according to the experiment being run:

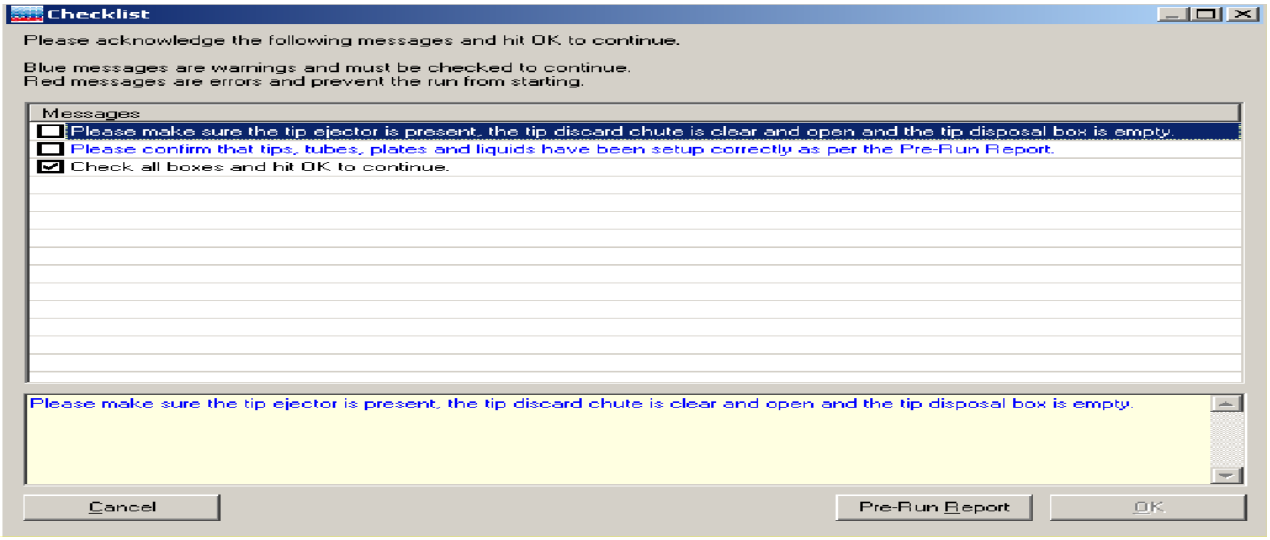
Position	Reagent/Item
F of reagent block R1	2 mL tube of Master Mix (green well in software)
A of Mix Plate block M1	5 mL tube of qPCR grade Water (or equivalent) (large orange well in software)
I of Reagent Block R1	1.5 mL tube of Calibrator (red well in software)
J of Reagent Block R1	1.5 mL tube of qPCR grade Water (or equivalent) (purple well in software)
Reaction block C1	Empty 96 well optical plate
Reaction block B1	Strip tubes into any columns with colored wells (for Casework Direct Samples being quantitated only)

5.6.9 Place the samples into the sample holder(s) according to the Quant Trio setup sheet and deck layout. Place the sample holder(s) onto the Instrument deck.

5.6.10 Check to ensure that there are enough tips to process the run. To do this, left-click on either Tip Holder A1 or B1. On the right hand side of the screen it will indicate if there are enough tips. If there are not enough tips, insert a new set of tips into Tip Holder A1 or B1 (whichever is empty). Right click on the Tip holder that was just filled and choose "Set all tips on current plate to Available." Close the instrument lid.

5.6.11 Click the green arrow  to start the run. The "Save as" dialog box will appear. Save the run file to the Forensic Scientist's folder under C:\Program Files\QIAgility\Data. This file shall also be imported into the Forensic Scientist's case record repository. The file name shall contain the initials of the operator for the run.

5.6.12 After the Forensic Scientist clicks "Save," the pre-run Checklist screen will appear. If the run has been set up correctly, the checklist will not list any warnings or errors other than the default messages listed below. If other messages are listed, user intervention is required before the run can be started. After completing the listed tasks, select the boxes next to the warnings to continue. Click "OK."



5.6.13 A run can be paused and aborted at any time by clicking the red X.  Pauses will be logged in the post-run report. If the run is aborted, the instrument will discard the tip and return to its resting position. **Note:** A run will also be paused when the instrument lid is opened.

5.6.14 The instrument will complete the current operation and will then pause. A dialog box will appear indicating that the run was paused and whether the run will be continued or aborted. **Note:** The instrument does not stop immediately when the lid is opened. Therefore, wait until the Y-arm has stopped moving and the warning screen is displayed before continuing.

5.6.15 The instrument lid must be closed to abort the run. **Note:** An aborted run cannot be restarted from the point at which it was aborted.

5.6.16 If an error occurs or a warning is detected during a run, a dialog box which contains

information about the error will appear. The Forensic Scientist will have 4 options: "Abort run," "Retry operation," "Ignore error" and "Skip step." Determine the error and the appropriate option.

5.6.17 Upon completion of each run, a support package is constructed and saved automatically to the program. This package contains the log file for the run and is used when reporting a problem to QIAGEN Technical Services. Click "OK." **Note**: The Forensic Scientist will see a message concerning the support package only if an exception occurred during the run.

5.6.18 Upon completion of each run a Post-Run Report will also be displayed. This report shall be saved within the Forensic Scientist's folder on the C drive (C:\ Program Files\QIAgility\Data\) on the computer associated with the QIAgility. Forensic Scientists shall also print a copy of this report using the print to pdf function to add to the case record repository.

5.6.19 Open the instrument lid and remove the optical plate. Seal the plate with an optical adhesive cover. Briefly centrifuge the plate at 3000 rpm to remove any bubbles at the bottom of the wells. Remove bubbles: While the plate is inside the base, tap the base on the benchtop to bring the bubbles to the liquid surface. Lift the plate, then inspect each well for bubbles; tap each well with gloved fingertip.

IMPORTANT! This step is critical to avoid noise in the fluorescence signal that bubbles can cause.

5.6.20 Remove the sample holder(s) and samples, TE and Master Mix tubes from the deck. Empty the biohazard box.

5.6.21 Close the software by clicking the X in the top right corner. Follow the prompts to shut down the instrument. Click "Yes" to quit QIAgility. Click "Move to Safety Position (recommended)." Click "OK."

5.7 Operation of the ABI QS5

5.7.1 Turn on the QS5 instrument. Forensic Scientists shall open the HID Real-Time PCR Analysis Software v. 1.3 Select the username for the analyst operating the instrument.

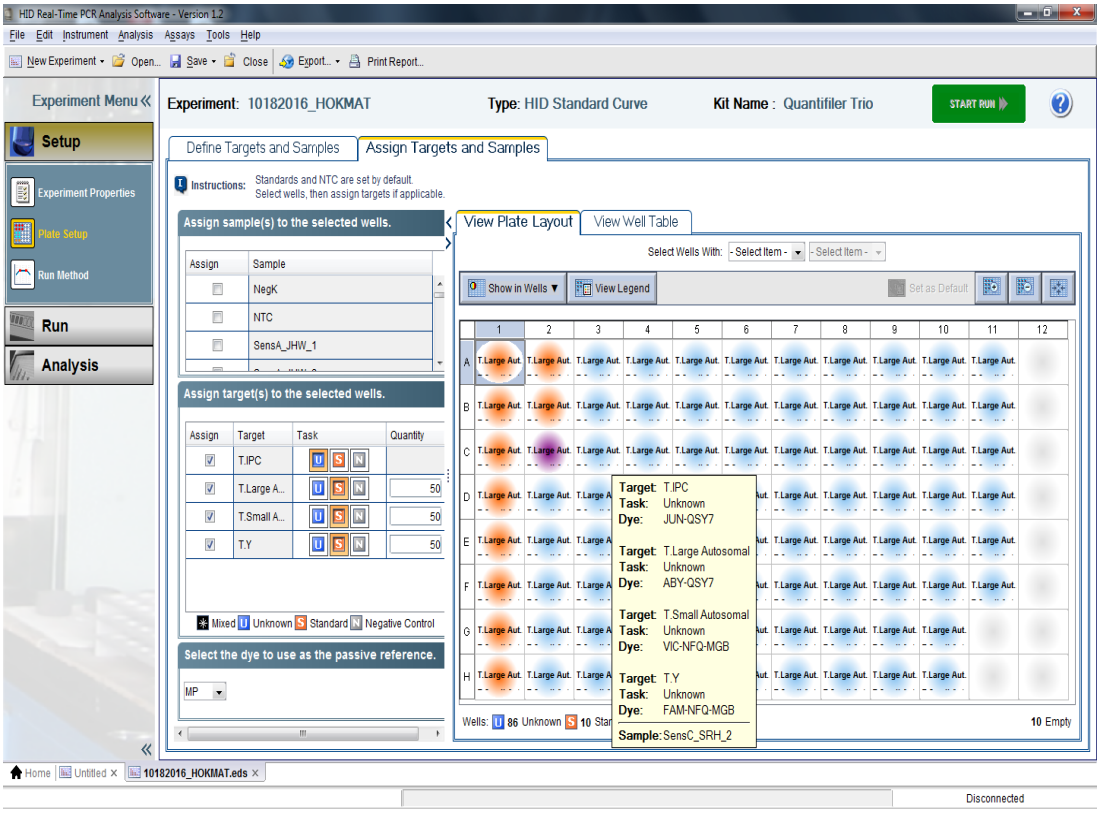
5.7.2 Touch the  button to open the instrument drawer. The plate holder will eject from the instrument. Place the optical plate into the holder with the notch in the top right corner. Well A1 will be in the top left corner.

5.7.3 Within the HID software, select the Quantifiler® Trio option (top left). From the File option in the menu, select Import. Find the .txt file created and saved in **5.3.4**. Select Start Import. A "Confirm Import Plate Setup" message will appear, asking "Are you sure you want to continue with the import?" Select "Yes."

5.7.4 In the Experiment Menu on the left side, under Setup, select Experiment Properties. Enter the file name in Experiment Name in the following format: MMDDYYYY_QS5 instrument name (full date _QS5 Instrument name). If an analyst other than the operator of the QIAgility is starting the run, then add that's analyst's initials to the Experiment name. Verify that the following are selected:

Instrument: QS5 (96 wells)
Experiment Type: Quantitation – HID Standard Curve
Reagents: TaqMan® Reagents
Ramp Speed: Standard (~1 hour to complete a run)

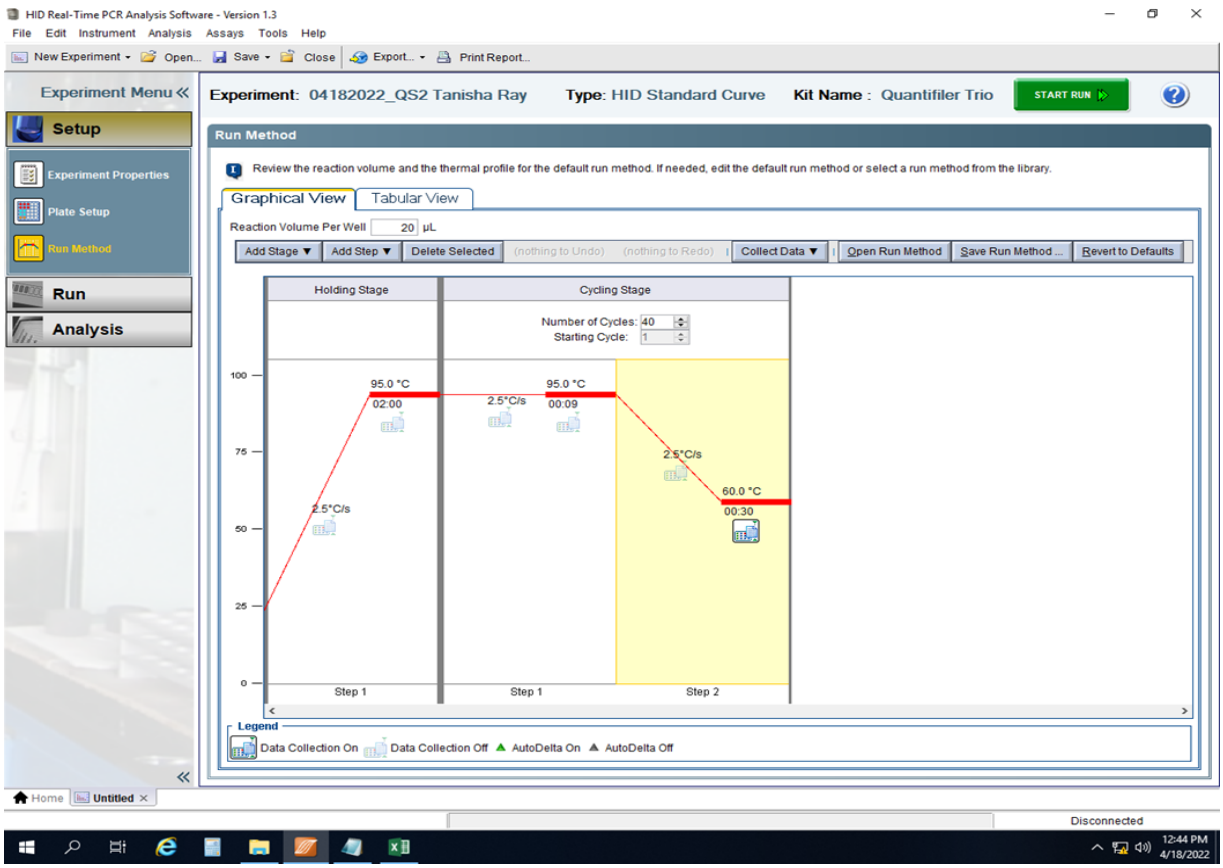
5.7.5 In the Experiment Menu on the left side, under Setup, select Plate Setup. Select the Assign Targets and Samples tab. Verify that the wells contain the correct samples/sample names. Hover the mouse above the well and the sample name will appear at the bottom of the box:



5.7.6 If a sample needs to be added to the plate, double-click on the empty well and select Add Sample from the text box that appears and enter the sample name. Close the box by clicking on the X in the top right corner. Note: these steps can also be used to change the name of a sample.

5.7.7 If a sample needs to be removed from the plate, right click the sample and select clear.

5.7.8 In the Experiment Menu on the left side, under Setup, select Run Method. Verify that reaction volume per well is 20 µl, the number of cycles is set to 40, and that the starting cycle is set to 1 (this can be visualized in either the graphical or tabular views). Also verify that the run method steps are as seen below:



5.7.9 In the top menu bar, select File and Save As. Name the .eds file as described in **5.7.4** i.e., MMDDYYYY_ QS5 instrument name (full date_ QS5 Instrument name). This .eds file shall be saved within the Forensic Scientist's folder on the D drive (D:\AppliedBiosystems\EDS Documents\) on the NC SCL computer associated with the QS5 instrument. Forensic Scientists shall not move or delete the .eds file, until the case files associated with quantitation have been released in the Forensic Advantage software (additional file locations may be identified at the direction of the DNA TL or QCO).

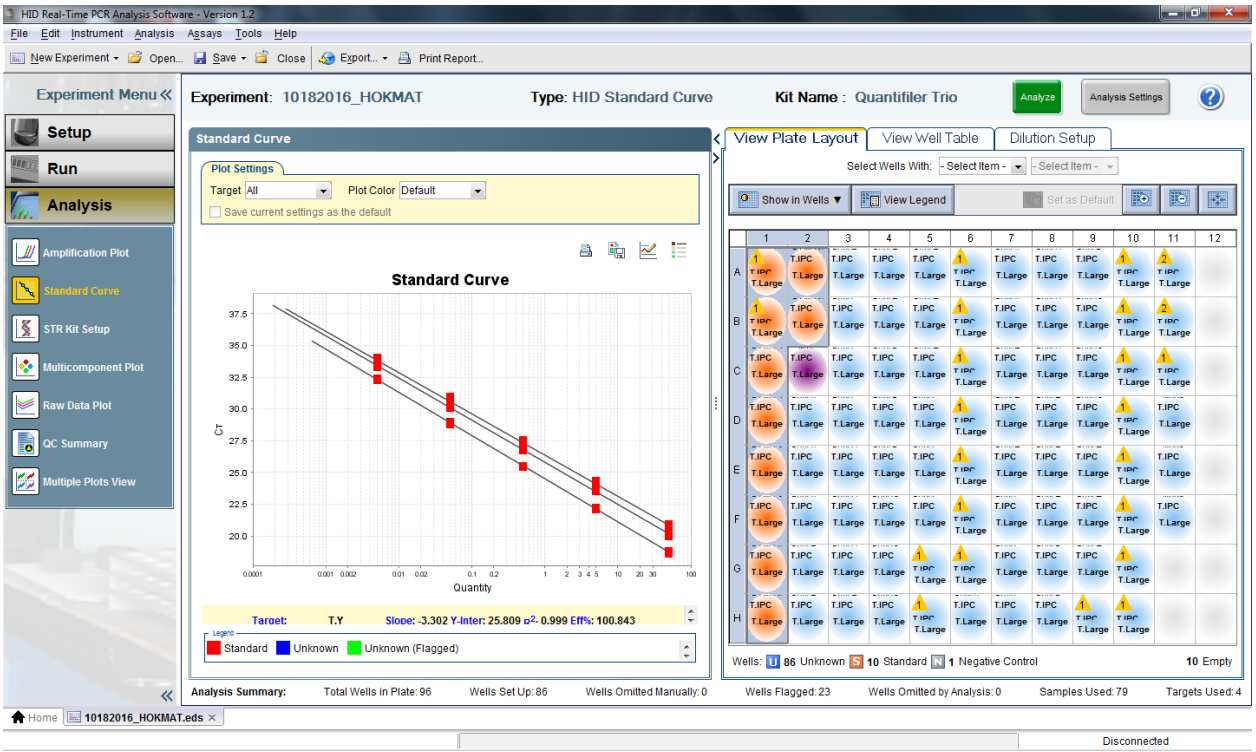
5.7.10 Click on the green Start Run button. Do not click on this button more than once. The assay will take approximately an hour to complete.

5.8 Analysis of Quantifiler Trio Data

5.8.1 Once the assay has finished, click on the green Analyze button in the top right corner of the screen.

5.8.2 **Assessing the Standard Curve (Manual Curve)**: In the Experiment Menu, under Analysis, select Standard Curve. To display only the wells containing the standard curve,

highlight them in the table to the right of the curve. The T.Small Autosomal(human), T.Large Autosomal(degradation) or T.Y(male) or all standard curves can be visualized by selecting all, T.Small Autosomal(human), T.Large Autosomal(degradation) or T.Y(male) from the drop-down menu by Target:



5.8.2.1 The range of the standard curve shall be between the following:

Target	Slope Range
Small Autosomal (SA)	-3.0 to -3.6
Large Autosomal (LA)	-3.1 to -3.7
Y Target (Y)	-3.0 to -3.6

The R^2 shall be ≥ 0.99.

5.8.2.2 Forensic Scientists may drop up to 2 points from each curve in order to achieve the criteria in **5.8.2.1**. The same 2 points may be dropped from the same standard, for both the human and the male curves if necessary, but only for Standard 5.

5.8.2.2.1 The HID software allows the user to select the human, the male, or both when selecting point(s) to drop. Right-click on the well containing the standard to be dropped from the curve; select omit well

and choose which applicable to target to include: Small Autosomal, Large Autosomal or T.Y.

5.8.2.2.2 The data must be reanalyzed with the modified standard curve: select Analyze.

5.8.2.2.3 If a point can be added back to the standard curve, right-click the well and select include. The data must be reanalyzed.

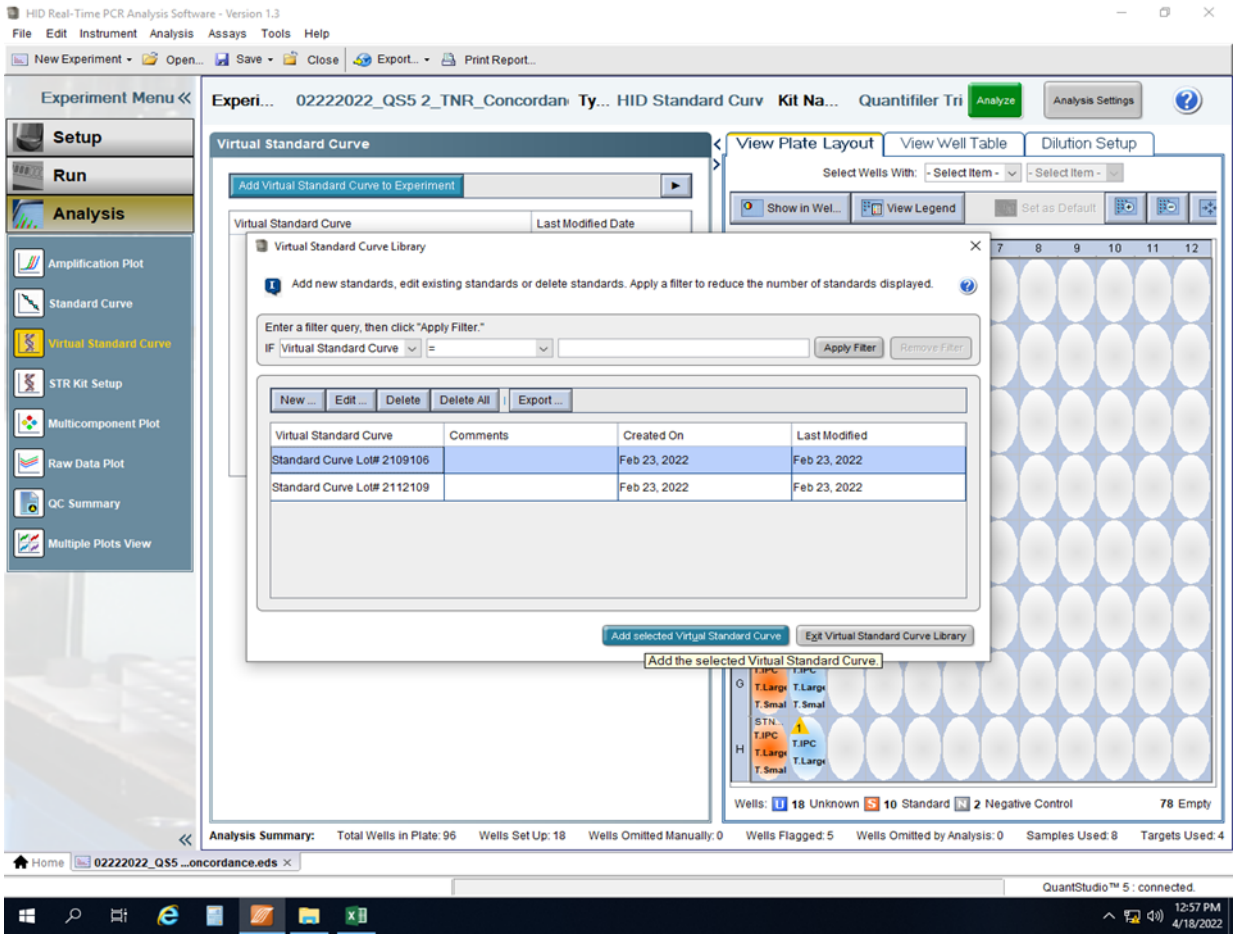
5.8.2.3 If either standard curve cannot be adjusted to meet the criteria in **5.8.2.1**, the Forensic Scientist shall repeat the assay (i.e., both the human and the male curves must pass criteria). If limited sample exists, the Forensic Scientist shall consult with the DNA Technical Leader on how to proceed.

5.8.2.3.1 If an entire assay is not being used for analysis (i.e., the standard curve does not meet quality metric criteria in **5.8.2.1**), the data shall still be printed to PDF format (refer to the section standardization for file naming). This PDF shall include: the Setup Worksheet, the standard curve(s) and the results. A Dilution Worksheet shall not be required in this instance.

5.8.2.3.2 If an entire assay is not being used due to instrument or computer failure, no data may be generated; if no data is generated; only the Setup Worksheet is required (in PDF format) along with the instrument file.

5.8.2.4 If the standard curve is modified to meet the quality criteria, both the modified and unmodified standard curve(s) must be included in the assay PDF document.

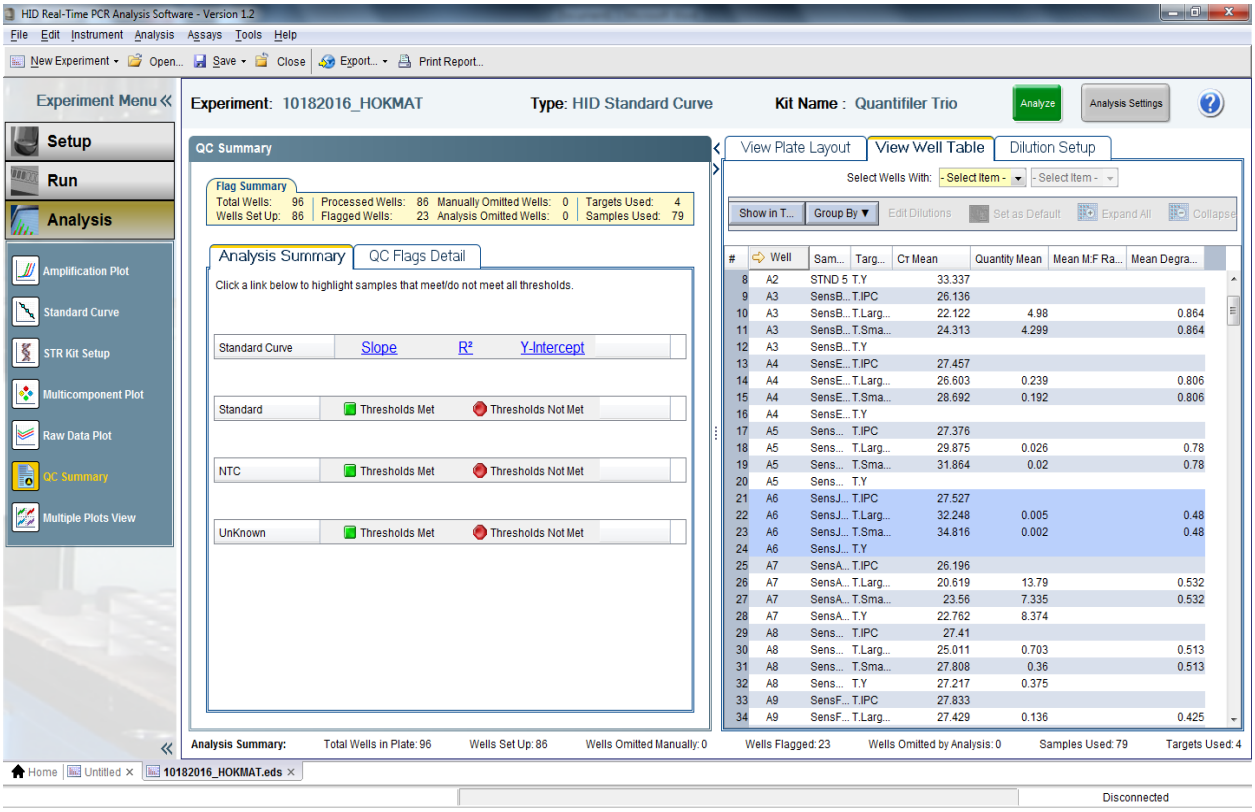
5.8.3 **Analysis using the Virtual Standard Curve:** Once the assay has finished, apply the standard curve to the assay. Select Virtual Standard Curve from the left menu under "Analysis". Click on "Add Virtual Standard Curve to Experiment" and highlight the appropriate standard curve. Once highlighted click "Add virtual standard curve" and analyze samples.



5.8.3.1 The concentration of the quant calibrator shall be approximately 5 ng/µl for the small autosomal target. If the quant calibrator is significantly different from this amount, the Forensic Scientist will evaluate the results obtained for the samples to determine is additional analysis steps are required. Consultation with the TL may be done to determine processing of the samples.

5.8.4 **Assessing the Small Autosomal Target**: In the Experiment Menu, go to Analysis and QC Summary. This page provides a snapshot of the data and automatically flags possible sample issues. In the Analysis Summary tab, any such samples are hyperlinked to the View Plate Layout on the right hand side of the screen. Within the View Plate Layout, flagged wells have a yellow triangle with an exclamation point.

5.8.4.1 Highlight the well(s) of interest in the View Plate Layout tab, and then select the View Well Table tab. The wells will be highlighted a darker blue in this tab (scroll through):



5.8.4.2 Forensic Scientists shall assess the quality of the data of the samples and controls by evaluating the IPC and Trio (Large and Small Autosomal Target), and Trio Male Target results. A general guide:

T. Human S.A. Target (VIC® Dye) Trio Human L.A. Target (ABY Dye) Trio Human Male Target (FAM™ dye)	Trio IPC (JUN™ Dye)	**Interpretation**
No amplification	Amplification	Negative result negative (no human DNA detected)
No amplification	No amplification	Invalid result
Amplification, Sample quantity >5 ng/ul	Amplification appears reduced relative to average IPC C_T value for standards	High sample concentration may suppress IPC amplification →IPC result inconclusive
Amplification (high CT and low delta R_n); more pronounced in T. Large Autosomal	No amplification or amplification appears slightly reduced relative to the average IPC Ct values for Standards.	PCR inhibitor present

5.8.4.3 **NTC** - Due to the sensitivity of the real-time PCR method, extremely low levels of DNA (levels that do not affect downstream applications such as STR analysis) can be detected. "It is possible to detect C_T values <40 for an extraction blank and negative control samples while performing a real-time PCR reaction." The NTC sample **may** be repeated based on the training and experience of the Forensic Scientist. If the other negative controls within the same assay show CT values greater than 40, the Forensic Scientist may proceed with the amplification of the samples. Positive amplification occurs when the Ct value for the target is <40.

5.8.4.4 **Negative Controls** – If the CT value of a negative extraction control (for either the Trio Human or Trio Male detectors) is less than 40, the Forensic Scientist shall continue processing the samples, realizing that DNA may be observed in those controls when amplified.

5.8.4.5 Samples – Samples shall be evaluated by taking the IPC results into consideration.

5.8.4.5.1 Invalid IPC: if both the IPC and the autosomal/male targets fail to amplify (undet.), then it is not possible to distinguish between the absence of DNA, PCR reaction failure, or inhibition. Such samples shall be re-quantitated with dilutions (unless a dilution of the sample has been quantitated and valid IPC results were obtained). There is a potential for invalid (undet) IPC values with Casework Direct samples. Scientists need to ensure proper dilution of samples is being performed prior to quantification in order to avoid. Additionally undet IPCs may be detected in Casework Direct samples if 1-thioglycerol is improperly pipetted during setup.

5.8.4.5.2 Inconclusive IPC: if extremely high concentrations of DNA are present in a sample, competition between the human/male targets and the IPC for PCR reagents may suppress the IPC, which results in a low C_T for the human/male targets and a C_T higher or undet. for the IPC. (Note: this situation is not necessarily inhibition; therefore, no notations shall be made as such.) Alternatively, if the C_T is high for the human/male target as well as the IPC, then inhibition may be present. Inhibition may also be indicated when the C_T is undet. for the IPC and the C_T is high for the human/male targets.

5.8.4.5.3 Negative Result: If the C_T for the IPC is within an acceptable range in comparison to the C_T for the IPC observed for the DNA Standards within the run and the autosomal/male targets are undet., then this is a valid negative result (i.e., no DNA is detected).

5.8.5 To print the standard curves, click on the Standard Curve option under the Analysis menu, highlight the standards in the View Plate Layout map on the right hand side of the screen.

5.8.5.1 In the Standard Curve window, make sure that the Target dropdown is set to All and click the Print icon. Print the standards curves in PDF format.

5.8.6 If using a manual curve, standards shall be printed. If using the virtual curve, no printing is required. To print the results, in any option under the Analysis menu, highlight the NTC, standards, and all samples in the View plate Layout map on the right hand side of the screen.

5.8.6.1 At the top of the screen in the menu bar, select Print Report. Select the Results Table (By Well). Print the report in PDF format.

5.8.7 To export the data, in the Experiment Menu, go to Analysis. In the View Plate Layout tab, select the entire plate.

5.8.7.1 From the top of the screen in the menu bar, select Export.

5.8.7.2 Within the Export Properties tab, only the Results box should be selected under line 1. Under line 2, only one file should be selected. In line 3, the Forensic Scientist shall choose a location to export the data for use in Excel. Note: the correct file type for the exported data is .xls.

5.8.7.3 Select Start Export. When the export has been completed, the HID software will ask "What do you want to do next?" Select Close Export Tool if finished or, Export More Data if additional files require exporting.

5.8.8 Close the HID software. Open the QS5 instrument door and remove the optical plate. Dispose of the plate in the biohazard waste. Close the instrument door and shut off the instrument.

5.9 Analysis of Quantifiler Trio Data in Microsoft Excel

5.9.1 Open the exported results from **5.8.5** in Excel.

5.9.2 Open the Biology workbook and copy the data from **5.9.1** into the Quant Raw Data tab of the Excel Workbook.

5.9.3 In the Dilution Worksheet tab, the data is now present.

5.9.4 The Dilution Worksheet automatically calculates the amount of DNA to add to a 1ng PowerPlex® 6C Fusion reaction or 0.5 ng PowerPlex® Y23 reaction. For samples which require dilution due to large quantities, the Dilution Worksheet provides the amounts necessary to create a 1ng/µl or 0.5ng/ul dilution. Note: the Dilution Worksheet allows the Forensic Scientist to adjust the raw extract volume to be used in making the dilution, but is set to default with 5 µl.

5.9.5 Samples with possible sperm extracted using the Casework Direct kit that have a male:female mixture ratio greater than 1:10 shall be re-extracted using a differential extraction.

5.9.6 Samples extracted using the DNA Investigator Kit that have a male:female mixture ratio of greater than 1:100 and meet the criteria set forth in the Procedure for

Analysis and Interpretation of Y-STR Amplification shall be amplified with PowerPlex® Y23. Samples with a male:female ratio greater that 1:2000 shall not be amplified, unless the male quant value is above 0.002 ng/ul or to show transfer of the female portion onto the item, the extract will be conserved for possible future testing.

5.9.7 The CW Direct quantitation values will be analyzed to determine the sample selection for amplification using the below chart. For DNA Investigator extractions, samples below the quantitation value of 0.002 ng/ul; shall not be amplified; extracts will be preserved for possible future testing. For samples extracted using the DNA Investigator kit where the extract volume can be concentrated, the samples may be amplified after concentration. Exemptions to the limits shall be approved only with written documentation from the Technical Leader.

Male Quant Value	Male: Female Ratio Value	Action
	>1:25000	Stop Analysis
≤0.001 ng/ul		Stop Analysis
0.001< Sample <0.002	>1:7500	Stop Analysis
>0.001	>1:10	Re-extract if Differential Sample
>0.001	>1:10	Stop Analysis if Questioned Sample**
>0.0005	<1:10	Clean-up Lysate
>0.002	<1:10	Direct Amp lysate

** If more swabs are present that cutting a larger sample size could affect results, then further processing may occur (e.g., ¼ of 1 swab cut vs ¼ total of 8 swabs)

5.9.8 Samples should be evaluated to ensure that evidence showing transfer is amplified. For example, if the case is a single donor case and an intimate sample sperm fraction is of sufficient quantity to amplify, it may not be necessary to amplify the non-sperm fraction.

5.9.9 Print the Dilution Worksheet as a PDF and add it to the PDF file(s) generated with the Setup Worksheet, standard curve, and results. The Dilution Worksheet provides the information necessary to perform PowerPlex® 6C Fusion reaction or PowerPlex® Y23 reaction. This PDF, along with the instrument file, shall be added to the case object repository for documentation.

5.10 Maintenance of the ABI QS5 and QIAgility

5.10.1 Refer to the Section Procedure for Calibration and Equipment Maintenance.

5.10.2 If a Forensic Scientist attempts to use an instrument and receives a warning that spectral calibrations are overdue, the Section QCO or designee shall be notified immediately by the Forensic Scientist and that instrument shall not be used until the calibrations are performed.

5.11 Quality Control of the Quantifiler Trio Kit - Refer to the Procedure for DNA Reagent Preparation and Quality Control.

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 Calibration and Equipment Maintenance

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control

Quantifiler® Trio DNA Quantification Kit User's Manual (PN 4485354, Rev E), 2015 Applied Biosystems

Applied Biosystems QS5/QS5 Fast Real-Time PCR Systems System Maintenance (PN 4387777 Rev. D), 2010 Applied Biosystems

9.0 Records – N/A

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
05/24/2024	5	5.3.2 updated sample limit wording and added witnessing step; 5.6.1, 5.6.6 – section updated for naming conventions; 5.6.5 – update import; 5.6.8 – table updated; 5.9.6 – updated CW direct result analysis