Intermountain Forensics	SOP #	QTY-200
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Quantification Setup

1. Purpose

This document describes the procedure for setting up a manual quantification plate using Quant Trio (ThermoFisher) and Quantiplex Pro (Qiagen) and running it on the Quant Studio 5 (ThermoFisher) instrument.

2. Summary

Directions for making Standard Dilution Sets, Virtual Standard Curves, and manually setting up a quantification plate are given for Quant Trio and Quantiplex Pro. Additionally, includes instructions for starting a run and exporting data on the Quant Studio 5 instrument.

3. Procedure

Creation of a Virtual Curve

1. Each newly received lot of quantification standard requires Quality Control assessment of a standard curve. Create a dilution series and quant in triplicate following the directions below. Upon obtaining a passing standard curve, save the dilution set as a Virtual Curve as the **(Kit)(Lot #)-(Expiration Date)**.

2. **Standard Dilution Sets**

Quant Trio

Standard	Concentration (ng/µL)	Volumes	Dilution factor
Std. 1	50.000	10µL [100ng/µL stock] + 10µL Quantifiler™ THP DNA dilution buffer	2X
Std. 2	5.000	10µL [Std. 1] + 90µL Quantifiler™ THP DNA dilution buffer	10X
Std. 3	0.500	10µL [Std. 2] + 90µL Quantifiler™ THP DNA dilution buffer	10X
Std. 4	0.050	10µL [Std. 3] + 90µL Quantifiler™ THP DNA dilution buffer	10X
Std. 5	0.005	10µL [Std. 4] + 90µL Quantifiler™ THP DNA dilution buffer	10X

Quantiplex Pro

Standard	Concentration (ng/µL)	Volumes	Dilution factor
Std. 1	50.000	Undiluted DNA	
Std. 2	5.000	5µL [Std. 1] + 45µL QuantiTect Nucleic Acid Dilution Buffer	10X
Std. 3	0.500	5µL [Std. 2] + 45µL QuantiTect Nucleic Acid Dilution Buffer	10X
Std. 4	0.050	5µL [Std. 3] + 45µL QuantiTect Nucleic Acid Dilution Buffer	10X
Std. 5	0.005	5µL [Std. 4] + 45µL QuantiTect Nucleic Acid Dilution Buffer	10X

3. **Passing Standard Curve Requirements**

Quantifiler™ Trio Targets	Typical Slope (range)	Average Slope
Small Autosomal (SA)	−3.0 to −3.6	−3.3
Large Autosomal (LA)	−3.1 to −3.7	−3.4
Y Target (Y)	−3.0 to −3.6	−3.3

Quantiplex Pro Targets	Typical Slope (range)	Average Slope
Human, Y, Degradation	−3.0 to −3.6	−3.3

Documentation

1. Open a DOC-307 Case Chronicle document and save a copy to the Batches in Progress folder on the Shared Drive as the Batch ID with the following naming format: YYMMDDAnalyst Initials (Example: 230215SW)
    a. If previous extractions were performed for this batch, then open the previously saved document.
2. Click on the Quant tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
    a. Batch ID
    b. Performed by
    c. Date of quantification
    d. Reagent Lot Numbers
4. Ensure the correct kit is selected from the drop-down list.
5. Add the number of Samples to the Sample field of the header.
    a. This information is used by the DOC-307 Case Chronicle to calculate the volume of master mix components and should include the total number of samples, reagent blanks, calibrator, and NonTemplate Control, An additional 15% is automatically added for overage to account for pipetting error.
6. Enter the Calibrator and NonTemplate Control into field 1A and 1B, respectively.
7. Enter the sample identification numbers for all samples and reagent blanks that will be quantitated in the batch layout in the subsequent fields.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos Control											
B	Neg Control											
C	IMF-23-00001-01-01-A											
D	IMF-23-00001-02-01-A											
E	IMF-23-00001-RB											
F	IMF-23-00002-01-01-A											
G	IMF-23-00002-RB											
H												
	Notes:											

8. Enter a note into the Notes field, for any extracts combined prior to quantification.
    a. The note must include the sample identification numbers for the combined samples.
9. To create an import file:
    a. Click on the Import Tab for the kit being utilized.
    b. Click on File > Save As
    c. Save the file as a .txt file with the batch ID as the file name.

Manual Quant Plate Setup

1. Prepare a fresh 5.00ng/µL DNA standard for use as a calibrator for the quantification plate according to the table below.

Kit	Volumes
Quant Trio	10µL [Std. 1] + 90µL Quantifiler™ THP DNA dilution buffer
Quantiplex Pro	5µL [Std. 1] + 45µL QuantiTect Nucleic Acid Dilution Buffer

    a. The calibrator will have a 1-week expiry date and must be tracked in the Prepared Reagent Log.

2. Prepare the reaction reagents by thawing completely, then vortex 3 to 5 seconds and centrifuge briefly before opening.
3. Create a master mix for each sample/reagent blank/calibrator/non-template control, including overage, and mix thoroughly by vortexing.
    a. Refer to the header of the Quant tab of DOC-307 Case Chronicle for exact volumes suitable for the number of samples included in the batch.

Kit	Reaction Mix Volume	Primer Mix Volume
Quantifiler™ Trio	10 µL	8 µL
Investigator® Quantiplex® Pro	9 µL	9 µL

4. Pipette 18 µL of the master mix into each reaction well that will be used.
5. Add 2 µL of sample/calibrator/non-template control (NTC) to the appropriate wells, according to the layout in the DOC-307 Case Chronicle on the Quant tab.
    a. TE is used for the NTC.
6. Seal the reaction plate with an Optical Adhesive Cover.
7. Centrifuge the plate to remove any bubbles.

Quant Studio 5 Run

1. Load the plate
    a. Touch ⏏ to eject the instrument drawer.
    b. Load the plate onto the plate adapter so that well A1 of the plate is in the top-left corner of the plate adapter.
    Note: Do not remove the black plate adapter before loading a plate.
    c. Touch ⏏ to close the instrument drawer.
    CAUTION! PHYSICAL INJURY HAZARD. The instrument does not have a sensitive stopping function while closing the drawer. Be sure plate is loaded properly and keep hands and lab coats clear.
2. Software
    a. For Quant Trio, use the HID Real-Time PCR Analysis Software.
        i. Log in using *IMF* and click **OK**
        ii. Click the **Quantifiler Trio button**.
        iii. Create plate layout
            1. If a plate layout import file has been created, import the template by clicking **File** and selecting **Import**.
            2. To enter the plate layout manually:
                a. Enter plate name in the Experiment Name field.
                b. Click Plate Setup and add samples.
                c. Click Assign targets and sample locations and assign well locations by clicking on the well and then checking the box next to the desired sample.
        iv. Click **Start Run**.
    b. For Quantiplex, use the Quant Studio Design & Analysis Software.
        i. Create a New Experiment by template by clicking the dropdown arrow next to Create New Experiment and clicking Template.
        ii. Import “Quantiplex Pro.edt” from the desktop.
        iii. Import the plate layout by clicking “File” then “Import Plate Setup”.
        iv. Select the plate layout created during setup.
        v. Add the plate name
        vi. Click the Plate tab and assign wells.
        vii. Click **Start Run**.
3. When the run ends, unload the plate.
    d. Touch ⏏ to eject the instrument drawer.
        i. Note: If the instrument does not eject the plate, contact Support.
    e. Remove the plate.

i. **CAUTION! PHYSICAL INJURY HAZARD**. During instrument operation, the plate temperature can reach 100°C. Allow it to cool to room temperature before handling.

f. Touch ⏏ to close the instrument drawer.

Transfer EDS files

1. EDS files are saved automatically upon completion of the run but can be manually exported if changes are made within the software after the run.
   a. To transfer manually from the laptop:
      i. Click Export
      ii. Ensure the Results button is checked, and all other options are unchecked
      iii. Selected the Export Destination
      iv. Name the file with the batch ID.
      v. Choose the file type as a .xls file.
      vi. Click the Export button.
   b. To transfer manually from the instrument home screen:
      i. Touch ⚙ **Settings►Run History**
      ii. Touch **Transfer File**.
      iii. Select the data destination for the EDS files.
      iv. Navigate to and select a folder.
      v. Touch **OK**.
      vi. Touch **Transfer**.

4. References

Investigator® Quantiplex® Pro Handbook
Quantifiler™ HP and Trio DNA Quantification Kits USER GUIDE
QuantStudio™ 5 Real-Time PCR Instrument (for Human Identification) USER GUIDE
QuantStudio™ Design and Analysis Software USER GUIDE

5. Definitions

EDT File: "Template" file. This houses the template for the assay (use caution to not overwrite template files)
EDS File: "Result" file. This is the output of a run and is considered the raw data file to be utilized by interpretation software.
Import File / Plate Layout: A .txt file that identifies the sample/control locations within a plate