

Intermountain Forensics

01

Forensic DNA Technical Leader Approval

Jean Hot

5/21/20

Issue Date

SOP #

Revision #

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

3. Procedure

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

2. Standard Dilution Set

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

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

3. Typical Standard Curve Ranges

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



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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	

Manual Quant Plate Setup

- 4. Prepare the reaction reagents by thawing completely, then vortex 3 to 5 seconds and centrifuge briefly before opening.
- 5. Create a master mix for each sample/reagent blank, including overage, and mix thoroughly by vortexing.

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

- 6. Add 18 µL of the master mix into each reaction well that will be used.
- Add 2 µL of sample or standard (5.00ng/µL DNA standard and NTC) to the applicable wells. NTC and samples are single replicate.

a. TE is used for the NTC.

- 8. Seal the reaction plate with an Optical Adhesive Cover.
- 9. Centrifuge the plate to remove any bubbles.

Quant Studio 5

- 10. Load the plate
 - a. Touch (a) 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 or tube strips. If used, tube strips may fit loosely in the adapter, but the heated cover will apply the appropriate pressure to seat the tube strips securely in the adapter.

c. Touch (\triangleq) 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.

- 11. Software
 - d. For Quant Trio, use HID Real-Time PCR Analysis Software. For Quantiplex, use Quant Studio Design & Analysis Software.

i. HID Real-Time PCR Analysis Software:

- 1. Log in using **IMF** and click **OK**.
- 2. Click the Quantifiler Trio button.
- 3. If a plate layout import file has been created, import the template by clicking File and Import.
- 4. To set up the plate manually, enter plate name in the Experiment Name field.
- 5. Click Plate Setup and add samples.
- 6. 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.
- 7. Start run.
- ii. Quant Studio Design & Analysis Software:
 - 1. Create New Experiment by template by clicking the dropdown arrow next to Create New Experiment and clicking Template. Import "Quantiplex Pro.edt" from the desktop.
 - 2. Import the plate layout by clicking "File" then "Import Plate Setup". Import the plate layout created during setup.
 - 3. Add plate name.
 - 4. Click the Plate tab and assign wells.



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- 5. Start Run.
- 12. When the run ends, unload the plate.
 - e. Touch ⁽⁾ to eject the instrument drawer.
 - f. Remove the plate.
 - g. Touch (a) to close the instrument drawer.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the plate temperature can reach 100°C. Allow it to cool to room temperature before handling. Note: If the instrument does not eject the plate, contact Support

Transfer EDS files

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.

From the laptop:

- 13. Click Export
- 14. Only the Results need to be checked.
- 15. Select Export destination and export the .xls file.

From the instrument home screen:

- 16. Touch Transfer File.
- 17. Select the data destination for the EDS files.
- 18. Navigate to and select a folder.
- 19. Touch OK.
- 20. Touch Transfer.

Note: Touch ⁽ Settings ► Run History to transfer EDS files at any time.

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