Queensland Health
Forensic and Scientific Services

Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

1 Purpose and Scope

This document describes the routine automated and manual methods for the quantification of extracted DNA from casework and reference samples in Forensic DNA Analysis, using the Quantifiler® Trio DNA quantification kit (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA). The procedure for manual methods will be utilised during extended downtimes of the liquid handler platforms. This document applies to all DNA analysis staff performing this procedure.

2 Definitions

AB / ABI	Applied Biosystems
BP	Base pairs
C_T	Cycle threshold
Decapper	LabElite® Integrated I.D. Capper™
DNA	Deoxyribonucleic acid
DI	Degradation index
FR	Forensic Register
IPC	Internal PCR control
LAT	Long autosomal target
MGB	Minor groove binding
PCR	Polymerase chain reaction
QS5	QuantStudio™ 5
RT-PCR	Real time polymerase chain reaction
SAT	Short autosomal target
STARlet	Microlab® STARlet
STR	Short tandem repeats
Y-Target	Y-chromosome target

3 Principle

The Quantifiler® real-time PCR assay measures the relative amount of a DNA target during each amplification cycle of the PCR in real-time. The Quantifiler® Trio DNA quantification kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. It uses multi-copy target loci for improved detection sensitivity. The kit provides DNA quantification results for the following targets:

- SAT (80 bp) – is the primary quantification target for total human genomic DNA, its smaller amplicon size makes it better able to detect degraded DNA samples.
- LAT (214 bp) – is used mainly as an indicator of DNA degradation, by comparing the ratio of its quantification result with that of the SAT.
- Y-Target (75 bp) – allows the quantification of the human male genomic DNA component of samples and can be useful in assessing mixture samples of male and female genomic DNAs.
- IPC (130 bp) – is a synthetic DNA template present in each sample and provides positive confirmation that all assay components are functioning as expected. It confirms the validity of negative results and is useful to identify samples that contain PCR inhibitors.

The results obtained using the Quantifiler® Trio kit can determine the following:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with STR analysis.
- The amount of sample to use in STR analysis.
- The relative quantities of human male and female DNA in a sample that can assist in the selection of an applicable STR kit.
- The DNA quality, with respect to both the levels of DNA degradation and inhibition, which is useful for determining if the STR loci with larger amplicon sizes will likely be recovered in the STR profile.

Two TaqMan® MGB probes labelled with VIC® and FAM™ dye are used to detect amplified SAT, and Y-Target respectively. Also, two TaqMan® QSY® probes labelled with ABY® and JUN® are used to detect amplified LAT and the IPC amplicon respectively.

A set of five prepared DNA standards (in duplicate), reagent blank and samples are added to a 96-well reaction plate and amplified using the AB QS5 real time PCR system (Table 1). At the end of each amplification cycle, each well in the reaction plate is flooded with light from an LED lamp, which excites the fluorescent dyes in each well of the plate. A Complementary Metal Oxide Semiconductor (CMOS) camera collects the differing wavelengths of light emitted. Data analysis algorithms are then applied to the raw data collected using the AB QS5 sequence detection system software.

Table 1 Thermalcycling parameters for the Quantifiler® Trio DNA quantification kit.

STEP	**PARAMETERS**	
Taq Activation	95°C 2 mins	
Denaturation	95°C 9 sec	40 cycles
Primer annealing & template extension	60°C 30 sec	
Reaction volume 20 µL	**9600 Emulation mode**	

Amplification of a sample on the AB QS5 instrument is displayed on the amplification plot. The curves observed in the amplification plot represent the increasing fluorescent signal as the amount of specific amplified product increases. The curve consists of geometric, linear and plateau phases. During the geometric phase, amplification is characterised by a high and constant efficiency. In the linear phase, the slope of the amplification plot decreases steadily as amplification efficiency begins to lower because one or more of the PCR reaction components is below critical concentration. Amplification reaches the plateau phase when the reaction is saturated by product and PCR amplification stops. The Blue (SAT, LAT & Y-Target) and Red (IPC) threshold line should be positioned at approximately the middle of the curve at geometric phase.

On the standard curve of the amplification results, a slope close to -3.3 (SAT), -3.4 (LAT) and -3.3 (Y-Target) indicates optimal, 100 % PCR amplification efficiency. The R^2 value indicates the closeness of fit between the standard curve regression line and the individual cycle threshold (C_T) points.

The Quantifiler® Trio DNA Quantification Kit also uses the ratio of quantification results for the SAT and LAT to give an estimate of degradation in a sample expressed as the DI. According to the manufacturer a DI of 1-10 is considered slightly to moderately degraded and a DI above 10 is considered significantly degraded. DI results may be able to be used to guide sample workflow which may streamline processing. It is calculated by the software using the formula:
[DI = Small autosomal target DNA conc. (ng/µL) / Large autosomal target DNA conc. (ng/µL)]

The Quantifiler® Trio DNA Quantification Kit also includes a Y-Target which provides a quantification concentration for male DNA in a sample including in mixtures of male and female DNA. This will enable the identification of samples suitable for testing with Y-STR analysis. It is calculated by the software using the formula:
[Male DNA:Female DNA Ratio = Quantity of Male DNA/Quantity of Male DNA : (Quantity of Human DNA – Quantity of Male DNA)/Quantity Male DNA]
(All quantities in the equation are ng/µL)

4 Reagents and Equipment

4.1 Reagents

All reaction components are stored at -15 to -25°C and must be stored after initial use at 2 to 8°C. Table 2 outlines all reagents and the storage locations required for quantification.

Table { SEQ Table * ARABIC } Reagents with storage room and location.

Reaction Component	Room	Storage Location (Initial)	Storage Location (after initial use)
Quantifiler® THP PCR Reaction mix	3188	Freezer B	Two-way Fridge
Quantifiler® Trio Primer Mix	3188	Freezer B	Two-way Fridge
Quantifiler® prepared standards	3194	Fridge	Fridge

Prepare fresh Quantifiler® Trio master mix in the biosafety cabinet in room 3188 just prior to commencing quantification.

1. Determine the required volume of reagents by referring to Table 3.

Table { SEQ Table * ARABIC } Quantifiler® Trio master mix volumes

Reaction Component	Equation
Quantifiler® THP PCR Reaction mix	*n* x 10
Quantifiler® Trio Primer Mix	*n* x 8

Note: Where *n* is indicative of the number of samples on a batch.
Note: For each batch prepare *n* + 4 samples.

2. Remove the Quantifiler® THP PCR reaction mix and Quantifiler® Trio primer mix from the fridge / freezer and thaw if required. Vortex and centrifuge before use.
3. Pipette the required amount of Quantifiler® THP PCR reaction mix into a single 2 mL or 2 x 2 mL tubes.

Note: Small quant batches may only require 1 x 2 mL tube for the PCR master mix. For larger batches, please note the STARlet will run faster with 2 master mix tubes.

4. Pipette the required amount of Quantifiler® Trio primer mix to the master mix tube/s containing the Quantifiler® THP PCR reaction mix. Gently vortex and centrifuge.
5. Label with "**QUA**" CW or REF, initial and date.

4.2 Equipment

Table 4 outlines the equipment and the locations required for quantification.

Table { SEQ Table * ARABIC } Location of required equipment.

Equipment	Location
STORstar (B)	3194
Labogene Scanspeed 1248	3191
AB QS5 A RT-PCR	3196
AB QS5 B RT-PCR	3196
Microlab® STARlet with LabElite® Integrated I.D. Capper™ A	3194
Microlab® STARlet with LabElite® Integrated I.D. Capper™ B	3194
Eppendorf Mixmate	3194

4.3 Consumables

Table 5 outlines the consumables and the locations required for quantification.

Table { SEQ Table * ARABIC } Location of required consumables.

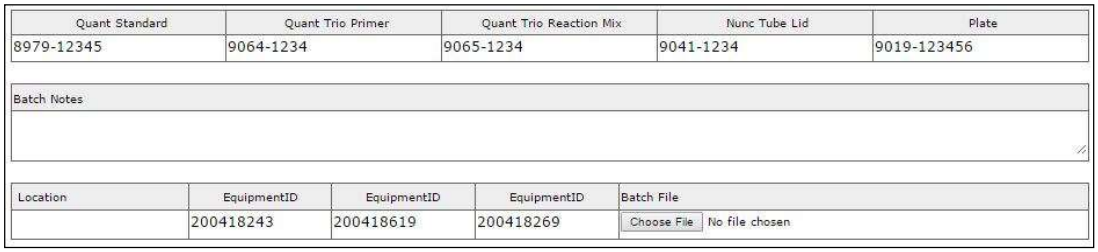
Consumables	Location
50 µL CO-RE tips with filters	3194
96-well optical plate	3194
Nunc™ caps	3194
Optical seal	3194
2 mL QIAGEN tubes	3191

Note: Additional consumables can be found in the Store Room (3184).

4.4 Entering Reagents, Equipment, Consumables and Locations into FR

Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion and entries can be modified after saving. Fields cannot be entered or edited once the batch is completed.

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Using the relevant dropdown menus, select the correct consumables and reagents.
4. Scan the equipment and location barcodes into the required fields (Figure 1).



Quant Standard	Quant Trio Primer	Quant Trio Reaction Mix	Nunc Tube Lid	Plate
8979-12345	9064-1234	9065-1234	9041-1234	9019-123456

Batch Notes

Location	EquipmentID	EquipmentID	EquipmentID	Batch File
	200418243	200418619	200418269	Choose File No file chosen

Figure 1 An example of quantification batch details.

5. Click the **Save** Batch icon.

5 Safety

As per the Anti-Contamination Procedure (22857), PPE is to be worn by all staff when performing this procedure. No part of the body should be placed inside the STARlet while the instrument is performing any procedure.

6 Sample Location and Sample Preparation

6.1 FR Workflow Diary and Electronic Workflow Diary

Batches that require processing can be found in the FR Workflow diary tab (refer to the Forensic DNA Analysis Workflow Procedure (34034).

The electronic Pre-PCR instrument diary (I:\AAA Electronic Workflow Diary\AAA PrePCR Diary) is used to record daily use of the instruments. Maintenance, processed batches and any issues/errors are recorded in the diary.

6.2 Batch Creation

Create or schedule quantification batches according to the Forensic DNA Analysis Workflow Procedure (34034).

6.3 QC Samples

Duplicates of the Quantifiler® standards and a reagent blank will be automatically allocated by the FR when creating the quantification batch according to the Forensic DNA Analysis Workflow Procedure (34034).

6.4 Sample Location and Locating Samples

Samples awaiting quantification are stored in the fridge as described in Table 6.

Table { SEQ Table * ARABIC } Sample storage location.

Sample type	Storage Device	Storage Location
DNA Extracts	Fridge or Freezer	3194

Locate samples according to Forensic DNA Analysis Workflow Procedure (34034).

6.5 Analytical Notes

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. On the batch **Exhibit Analysis** page, if any samples are coloured half orange, hover the cursor over it to check for analytical notes that request for specific processing comments (e.g. Quant and hold) (Figure 2).

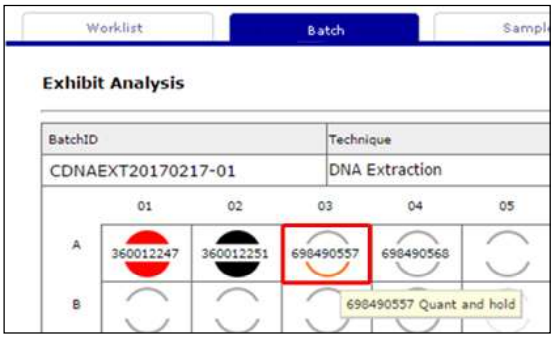


Figure { SEQ Figure * ARABIC } An example of an Analytical Note displayed with an orange semi-circle.

6.6 Uploading Files

1. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
2. Click **Edit/Update Batch** icon.
3. Click the **Choose File** button in the **Batch File** field (Figure 1).
4. Browse the I:\ drive folders for the required file and click **Open.**

Note: If the file cannot be seen (e.g. .trc files) change the drop down box “All files”.

5. Click the **Save Batch** icon

7 Procedure

7.1 Quantification Set up

1. In the Clean Reagent Room (3188) prepare Quantifiler® Trio master mix. Refer to Section 4.1 for preparation of reagents.
2. Set up of quantification is performed using the dedicated Microlab® STARlets A and B located in Room 3194.
3. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
4. Check for Analytical notes as per Section 6.5 and action as required.
5. In the **Batch File** field, click the **BatchID_Trio_QS5_Map.txt** file and save to I:\ABI Quantifiler.
6. In the **Batch File** field click the **BatchID_QUANT_MAP.xls** file and save file to I:\Pre PCR STARlet\All Plate Maps. Check for duplicate samples as the STARlet is **NOT** programmed to have the same sample on a batch twice.

Note: If duplicate samples are required, please refer to Section 14.2.4 Duplicate Sample/s on Batch.

7. Ensure STARlet Daily Start-up has been performed as described in Operation and Maintenance of the Microlab® STARlet and LabElite® Integrated I.D. Capper™ (34050).
8. Launch the Method Manager software via the desktop icon (Figure 3).

Figure 2 The Method Manager desktop icon.

9. Select the '**QUANTS & AMPS**' button on the home page (Figure 4).

Figure { SEQ Figure * ARABIC } Method Manager home page.

10. Click on the **Layout** button to open the layout for the Quantifiler Trio method.
11. Centrifuge the Nunc™ rack of samples for 1 min at 2000 rpm (657 *xg*).
12. Using the Mixmate, vortex the Nunc™ rack of samples for 1 min at 1000 rpm.
13. Check the first and last barcodes of the extracts against the FR plate map and place the Nunc™ rack of samples onto the platform of the Decapper.
14. Decontaminate and place all the required labware onto the autoload tray in the designated track positions as outlined in the layout (Figure 5).

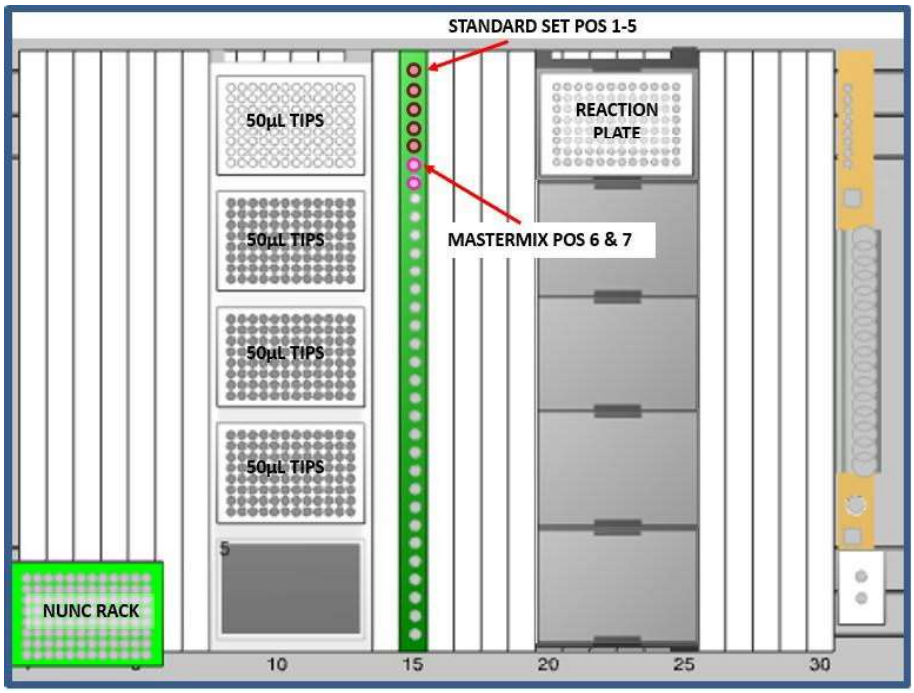


Figure { SEQ Figure * ARABIC } Quantifiler Trio deck layout. Note that the Nunc rack of samples is placed on the decapper platform at the beginning of the method.

15. Ensure there are at least one and a half full racks of 50 µL CO-RE filter tips in the tip carrier (TIP-CAR) in **Tracks 8-13** of the autoload tray.
16. Briefly vortex and centrifuge the Quantifiler® prepared standards and place in positions **1-5** of the sample carrier (SMP-CAR) in **Track 15** (Figure 5).

Note: Ensure the Quantifiler® prepared standards are within the expiry date and there is sufficient volume remaining (>20 µL).

17. Briefly vortex and centrifuge the master mix tube/s and place in **position 6** (& **7** if using two tubes) of the sample carrier in **Track 15** (Figure 5).
18. Label a skirted 96-well optical plate with the Batch ID on the front and the Batch ID barcode on the right side and place into position **1** of the multiflex carrier (APE-CAR) in **Tracks 20-25**.
19. After ensuring all the necessary labware has been positioned on the autoload tray, close the deck layout and click **Run** on the Quantifiler Trio screen of the Method Manager.
20. A prompt will appear to check the deck layout. Select **Continue** once the carriers on the autoload tray match the deck layout.
21. A prompt will appear to load the plate and sample carriers onto the deck. Select **OK** to load the carriers.
22. A prompt will appear **Edit Tip Count** asking for the first and last position of the tips in the tip carrier (Figure 6).

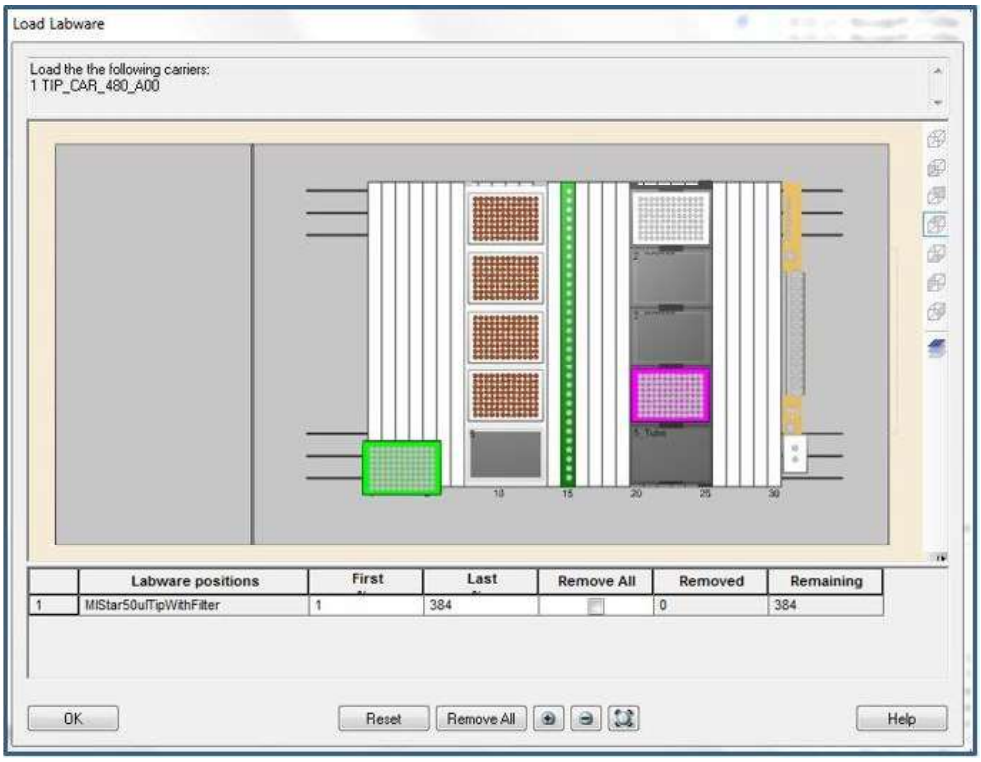


Figure { SEQ Figure * ARABIC } Edit tip count dialogue box.

23. To edit the tip counter, highlight individual tips are present by clicking on the individual positions or click and drag to highlight multiple positions. These methods will also remove already highlighted tips. If it is necessary to remove all highlighted tips, check the **Remove All** box and then highlight the positions of present tips. When the tip counter matches the tips in the carrier (TIP-CAR), click **OK**.

24. A prompt will appear to enter the quantification Batch ID, link the platemap and select the number of MasterMix tubes to be loaded (Figure 7).

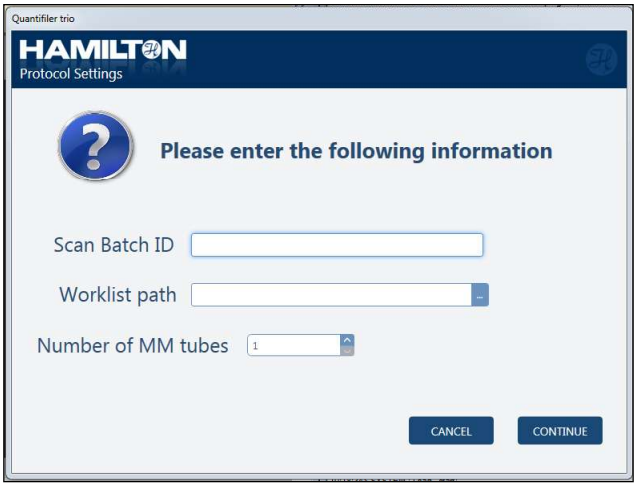


Figure { SEQ Figure * ARABIC } Dialogue box prompt.

25. Scan the barcode to enter the quantification Batch ID. Select the correct platemap by browsing to **I:\Pre PCR STARlet\All Plate Maps\BatchID_QUANT_MAP.xls** and select the number of master mix tubes (1 or 2).
26. Select **Continue** to begin the method.
27. A prompt will appear to load samples and an empty Nunc™ rack onto the Decapper (Figure 8).

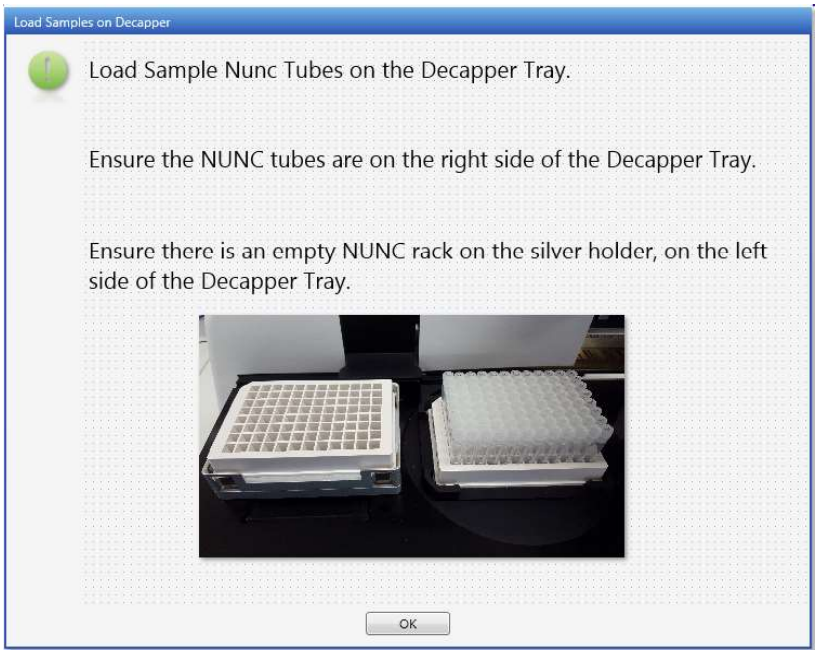


Figure { SEQ Figure * ARABIC } Prompt to load samples and empty Nunc™ rack to Decapper.

28. Ensure that the samples and empty Nunc™ rack have been loaded. Select **OK** to continue the method.
29. A prompt will appear to unload the plate carrier and check volumes and bubbles of master mix within the reaction plate (Figure 9).

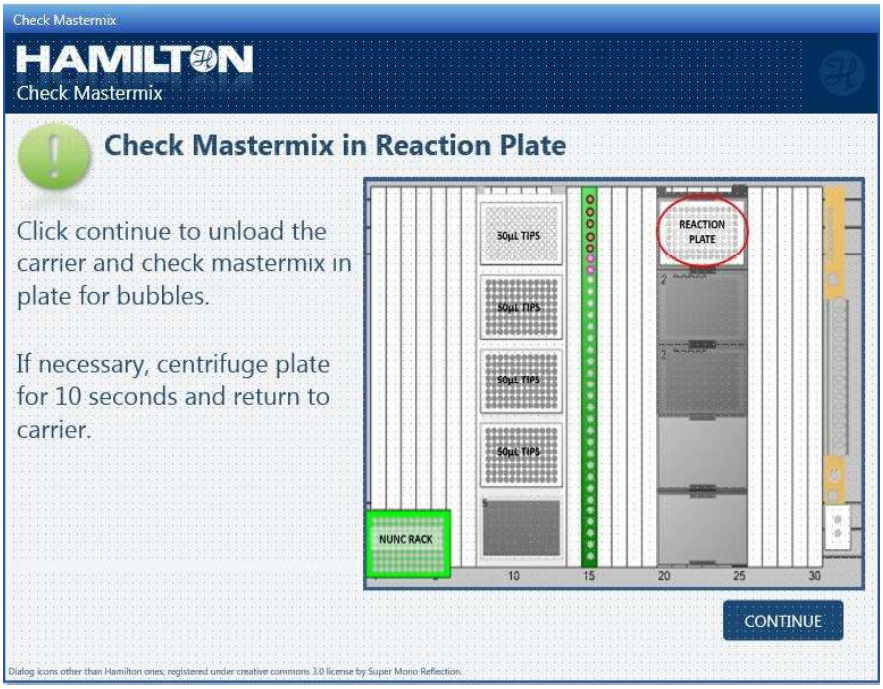


Figure { SEQ Figure * ARABIC } Prompt to check master mix in the reaction plate.

30. Select continue to unload the multiflex carrier (APE-CAR) in **Track 20-25**.
31. Check that the liquid within the plate is sitting at the bottom of the wells. If the master mix liquid is not at the bottom of the well, seal the plate and centrifuge for 10 sec at 2000 rpm (657 g).
32. A prompt will appear to load the reaction plate on the multiflex carrier (APE-CAR) (Figure 10).

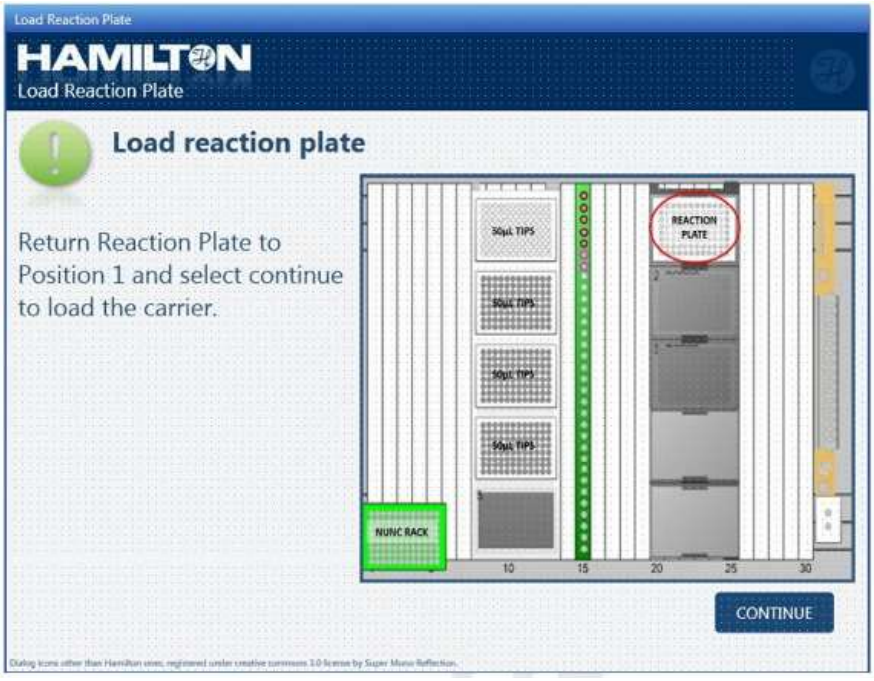


Figure { SEQ Figure * ARABIC } Prompt to reload the reaction plate.

33. Remove the seal and replace plate in position **1** of the multiflex carrier (APE-CAR).
34. Select "**Continue**" to load the multiflex carrier (APE-CAR) and continue the method.
35. A prompt will appear to discard old Nunc™ caps and load new Nunc™ caps onto the Decapper (Figure 11).

Figure { SEQ Figure * ARABIC } Prompt to load new Nunc™ caps on the Decapper.

36. Discard the old Nunc™ caps and clean the tray with 5% TriGene™, followed by 70% Ethanol. Load new caps onto the Decapper. Click "**OK**".
37. A prompt will appear to unload the carriers (Figure 12). Click OK and the carriers will automatically unload and the DNA extracts will be recapped.

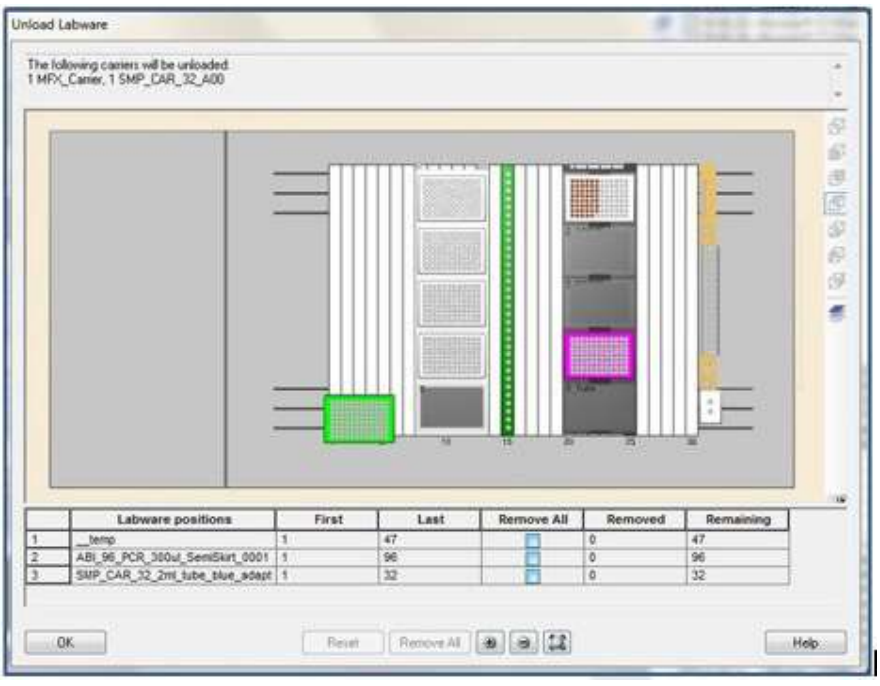


Figure { SEQ Figure * ARABIC } Prompt to unload carriers at the end of the run.

38. Check the plate to ensure all wells contain the required volume and that no bubbles are present. Seal the 96-well plate with an optical adhesive seal.

Note: Excessive bubbles in the wells are critical and may affect the accuracy of the quantification results.

39. Centrifuge the optical plate for 1 minute at 2000 rpm. Place the plate in the pass-through hatch to the PCR/CE Room (3194) and notify the CE Operators.

40. Check the plate again to ensure all wells contain the required volume and that no bubbles are present.

41. Refer to Section 7.2 for performing the quantification on the QS5 instrument and Section 7.3 for Quantifiler® results analysis.

42. The STARlet should have begun re-capping the open tubes. Re-cap and return Quantifiler® prepared standards to the fridge. Discard empty master mix tubes into the biohazard waste bin.

43. Store the re-capped Nunc™ tube extracts in the upright freezer in the Pre-PCR sorting room (Room 3194 A).

44. On the PC, navigate to **C:\Program Files (x86)\HAMILTON\Log Files**, sort by date modified and locate the most recent Quantilifer Trio trace file, e.g. “Quantifiler_Trio_Setup_v2_4c08086aa87e4a829cccb8438c349283_Trace.trc”.

45. Open the trace file, check that it belongs to the batch and then close. Rename the file as the Batch ID, e.g. “CDNAQUA20160525-04.trc”.

46. Copy the renamed trace file into **I:\Pre PCR STARlet\Trc Files**

47. Upload the trace file to FR as outlined in Section 6.6.

48. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

49. Once uploaded, electronically archive the trace file within the appropriate month folder within **I:\Pre PCR STARlet\Trc Files\01 – Jan**

50. If not performing another run remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol.

Note: When changing between casework and reference batch preparations on the STARlet, the sample carriers and modules are required to be cleaned with 5% Trigene and 70% Ethanol (not the deck).

7.2 Performing Quantification on the QS5 Instrument

1. In the PCR/CE room (3196) retrieve the optical plate from pass through hatch.

2. Check the plate to ensure all wells contain the required volume and that no bubbles are present. If required centrifuge the plate for 1 minute at 2000 rpm.

3. Turn on the AB QS5 and login to the PC using the “**INSTR-ADMIN**” username.

4. Once the desktop has loaded, log into Novell using the "**biology**" username and the current password.
5. Launch the QS5 system software (Figure 13).

Figure { SEQ Figure * ARABIC } HID Real-Time PCR analysis software v1.3 desktop icon.

6. Login using the "**Robotics**" username and click **OK** (Figure 14).

Figure { SEQ Figure * ARABIC } HID Real-Time PCR analysis software login screen.

7. From the home screen click on **Quantifiler® Trio** button.
8. From the main screen, click **File** → **Import**.
9. Select **Browse** and navigate to I:\ABI Quantifiler and select the relevant platemap file.
10. Click **Start Import**. When prompted click **Yes**.
11. Enter the Batch ID and user initials on the **Experiment Properties** page.
12. View the plate map by selecting **Setup → Plate Setup → Assign Targets and Samples** tab **→ View Plate Layout** tab.
13. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (34034).
14. Check the last barcode of the FR platemap against the imported platemap on the QS5. (Use cursor to hover over well position on the plate layout screen for barcode to appear).
15. Using the QS5 touchscreen, click on the eject button on the top righthand corner to eject the loading tray. Place the 96-well plate with the Batch ID label on the front facing out onto the loading tray. Click on the eject button again to close the loading tray.
16. Click **START RUN**.
17. A prompt will appear to save the file. Ensure the filename is the Batch ID and click **Save**.

18. Ensure the progress bar appears indicating the run has started.

19. Ensure all equipment is selected against the batch in FR as per Section 4.4.

7.3 Quantifiler® Trio Results Analysis

Upon completion of the Quantification the QuantStudio®5 HID Software analyses the data and prepares the report.

Note: In the CT Settings tab of the Analysis settings, the CT Settings for the LAT, SAT and Y standard curves must be set to Automatic. This is a hard setting for all runs that may only need to be checked during troubleshooting.

1. On the left navigational panel of the screen click on the **Analysis** tab → **Amplification Plot** to observe the morphology of the amplification (Figure 15).

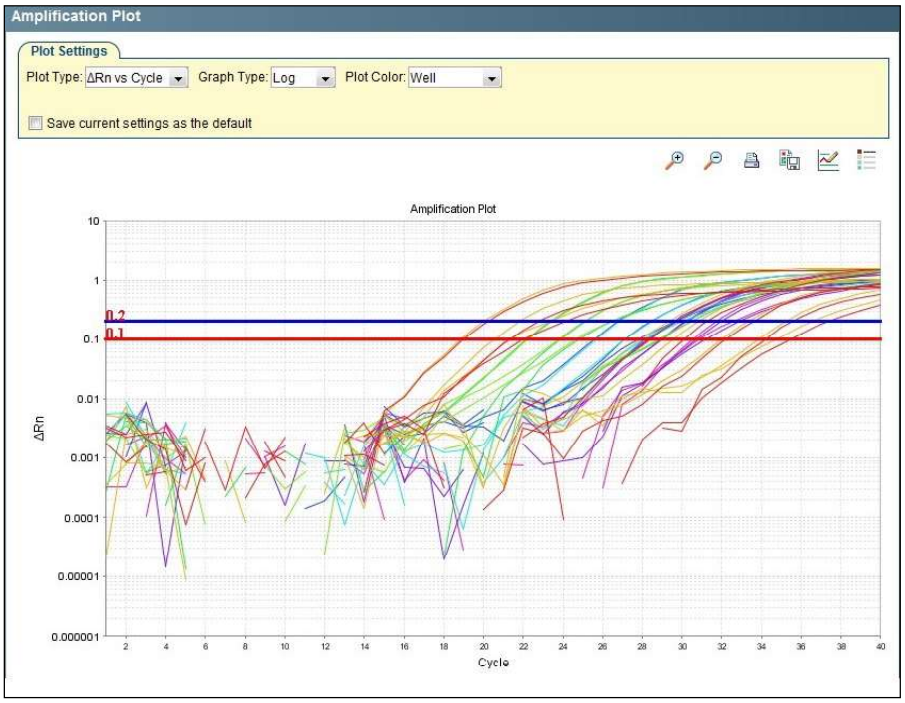


Figure 3 Quantifiler® Trio amplification plot.

Note: If jagged peaks, blips or spikes are observed (Figure 16), this is an indication of an electrical interruption. See the Analytical HP5 to determine if the quantification plate is to be repeated.

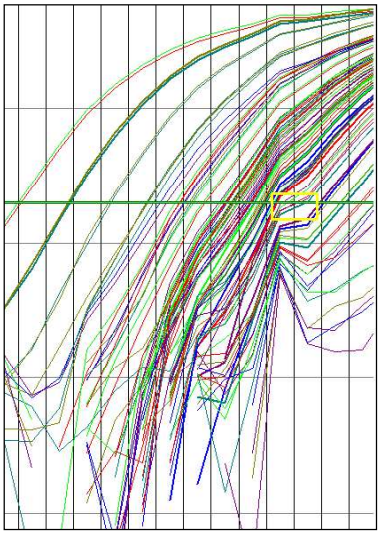


Figure { SEQ Figure * ARABIC } Amplification plot displaying abnormal amplification morphology.

2. Click on **Multicomponent Plot** to observe the amount of fluorescence of all targets. Take note of any spike or blips in the plot between cycles 3-15 (Figure 17).

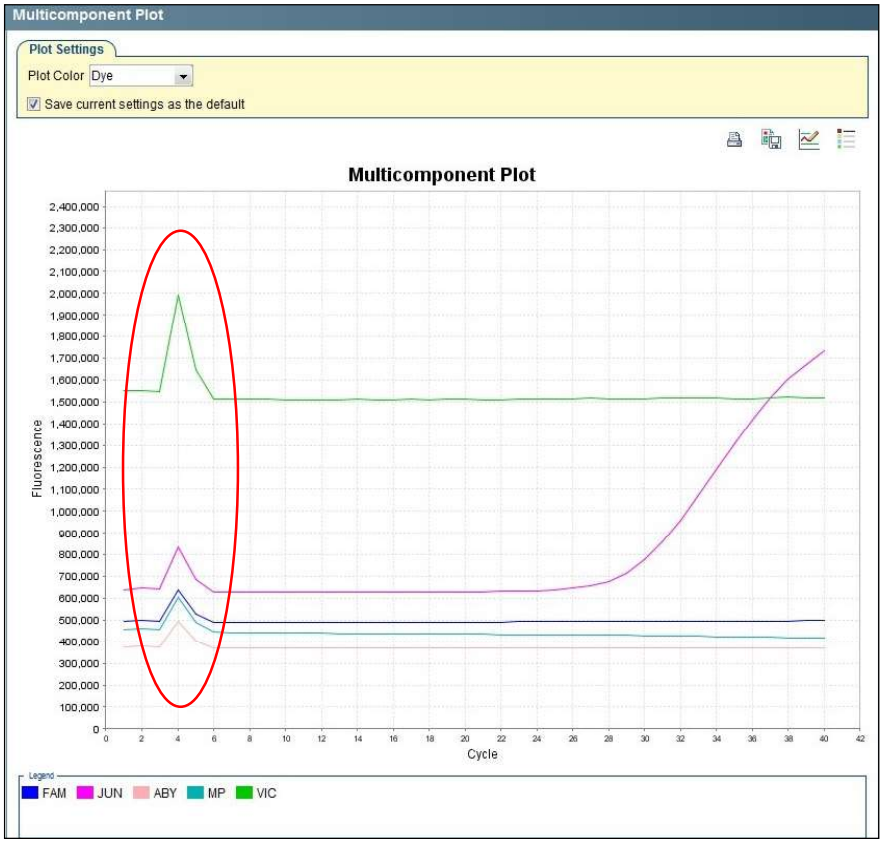


Figure { SEQ Figure * ARABIC } Quantifiler® Trio multicomponent plot displaying the fluorescence of one well.

Note: An abnormal plot that displays any spikes or short / long blips in the fluorescence readings between cycles 3-15 may indicate signs of bubbles in the wells or well

evaporation. This in turn can affect the C_T value calculated for the DNA targets and sample/s should be re-quantified.

3. If a sample has excessive noise in the baseline between cycles 3-15 on the Multicomponent Plot, flag the sample/s so it appears red on the Results PDF by performing the following:

   a) Change the well colour on the platemap for that sample by selecting **Setup → Plate Setup → Define Samples and Targets**.

   b) On the **Define Samples** window on the right, find the sample that requires the flag and change the colour to Red by clicking on the drop-down option under the **Color** column.

4. Click on **Standard Curve** in the Analysis tab to observe the standard curve of all targets (Figure 18).

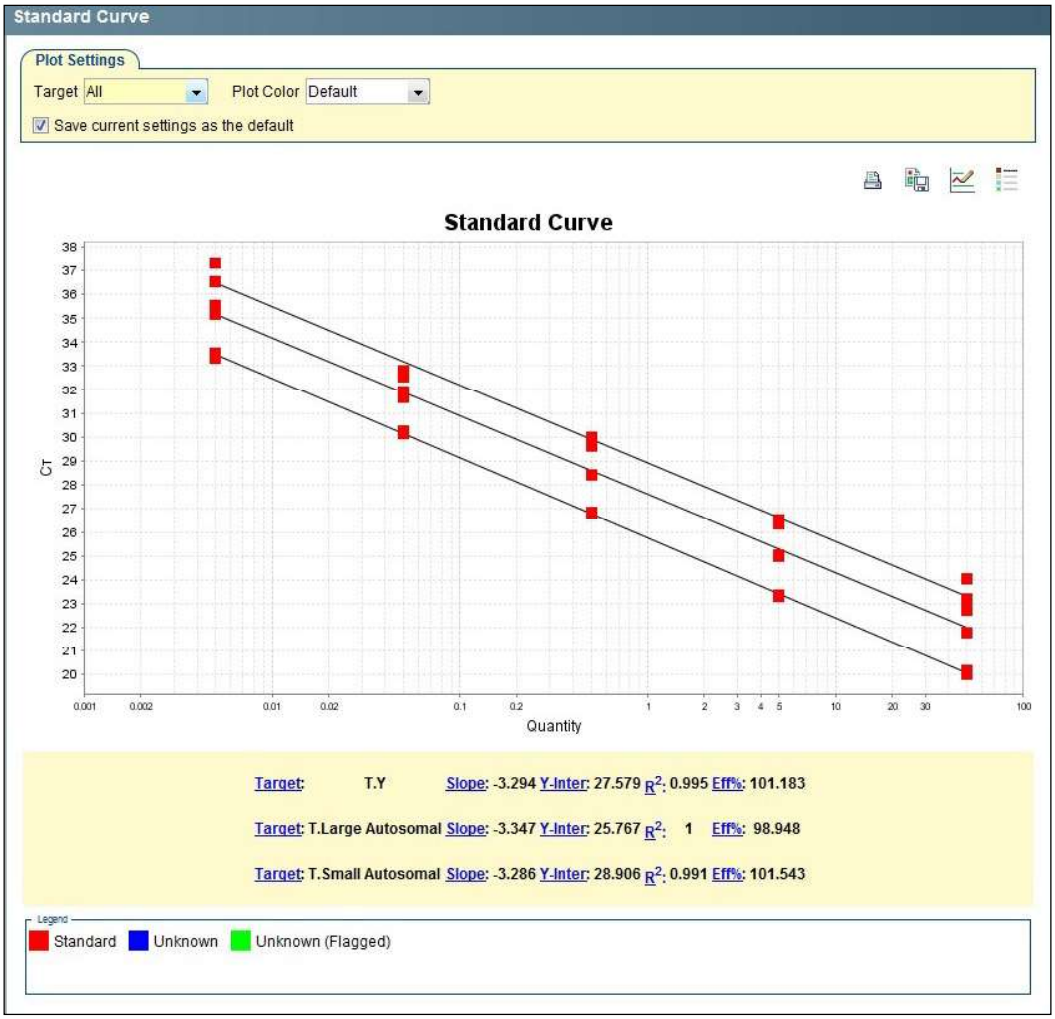


Figure { SEQ Figure * ARABIC } Standard Curve Results in the Analysis Tab, the standard at the top left is STD#5.

5. Assess the standard curve results and ensure that the slope, Y-intercept and R^2 values fall within the allowable ranges for all targets (Table 7).

Table { SEQ Table * ARABIC } Criteria thresholds for the Quantifiler® Trio standard curve.

Target-Y Criteria	**Allowable Thresholds**
Slope	-3.0 to -3.6
Y-Intercept	25.64 – 26.58 (3SD)
R^2	≥0.98000
SAT Criteria	**Allowable Thresholds**
Slope	-3.0 to -3.6
Y-Intercept	25.35 – 28.94 (3SD)
R^2	≥0.98000
LAT Criteria	**Allowable Thresholds**
Slope	-3.1 to -3.7
Y-Intercept	19.71 – 30.47 (3SD)
R^2	≥0.98000

6. To improve the Slope, Y-Intercept and R^2 thresholds, up to 2 standard curve data points (not from the same standard) can be omitted by performing the following:
   a) Select the **View Plate Layout** tab in the right navigational panel.
   b) Highlight the relevant well/s, right click and select **Omit → Well**.
   c) Click **Analyze** on the main screen, this will re-analyse the data without the selected standard replicate.
   d) If the well needs to be added back for analysis, right click and select **Include.**
   e) Note in the FR batch notes which standards have been omitted (e.g. single replicate of Std#1 omitted from the standard curve).
   f) If more than two individual data points from the standard curve are to be omitted, notify Analytical Senior Scientist (HP5).
7. If the SAT Y-Intercept values fall outside the range, notify the Pre-PCR scientists and proceed to step 10.
8. If either of the Target-Y or LAT values for Y-Intercept, Slope or R^2 are outside the ranges, notify the Analytical Senior Scientist (HP5).
9. Check the reagent blank by moving the cursor over the reagent blank well position on the **View Plate Layout** tab and verify that no DNA quantity is present. If amplification did result, take note of the quantification value and notify the Pre-PCR scientists.
10. Using the QS5 touchscreen, click on the eject button on the top righthand corner to eject the loading tray.
11. Remove the optical plate from the QS5 and check each well for evaporation before discarding the plate into a biohazard bin. Click on the eject button again to close the loading tray.

12. Evaporated wells should be noted by adding a comment in the quantification batch in FR and by changing the colour of the sample well to red in the Result PDF (as per step 3 of Section 7.3). These samples are also repeated by ordering another quantification as per step 10 of Section 8.

13. **Ensure all wells** are selected and click **Export…** from the top toolbar (Figure 19).

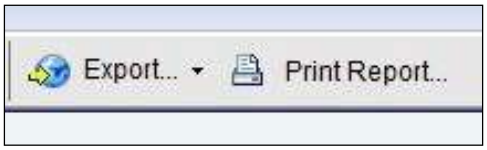


Figure { SEQ Figure * ARABIC } Export and Print Report buttons.

14. On the export data window remove the "_data" from the Batch ID in the **Export File Name** field (Figure 20). Ensure that the **File Type** extension is (*.xls) and the **Export File Location** is I:\Results\Qres.

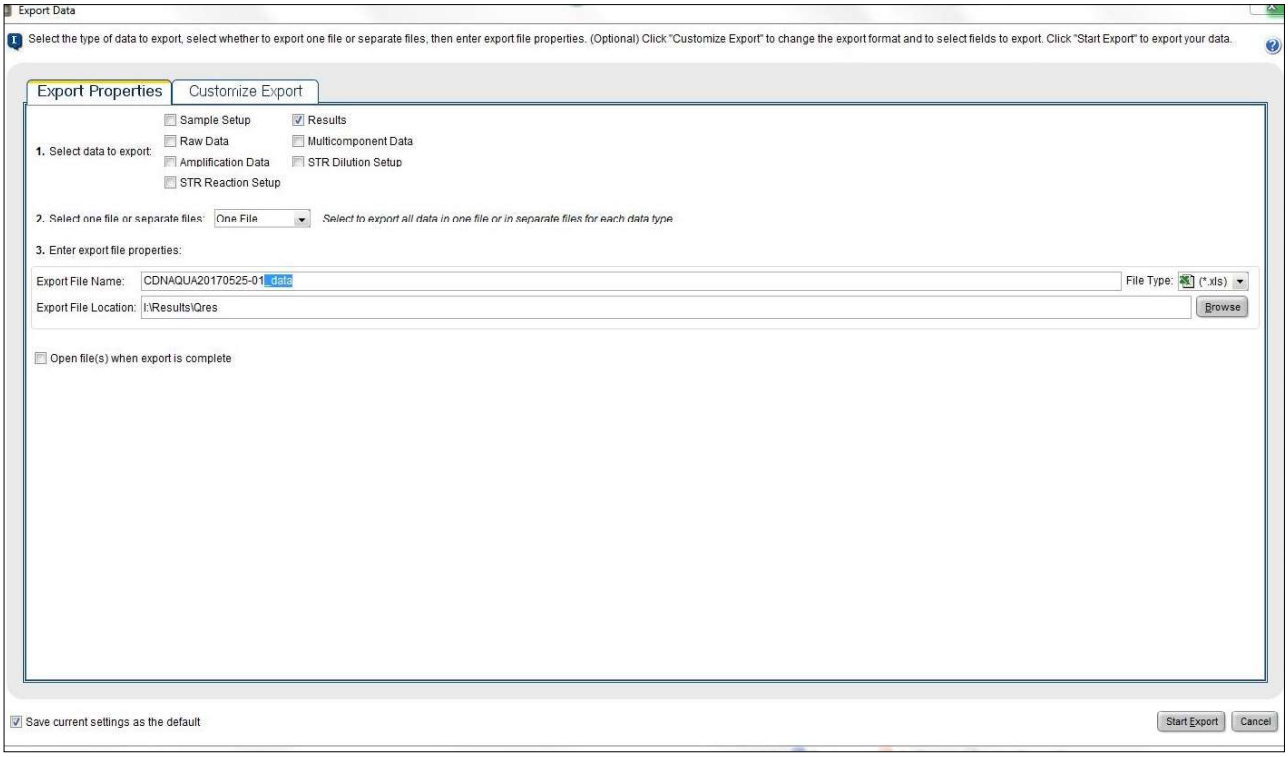


Figure { SEQ Figure * ARABIC } Export data window.

15. Click **Start Export** and close Export Tool when complete.

Note: The DI and the Male:Female ratio results can both be accessed in the exported file.

16. **Ensure all wells** are selected and click **Print Report…** on the top tool bar (Figure 21).

17. Ensure that the boxes shown in Figure 21 are checked and click **Print Report**.

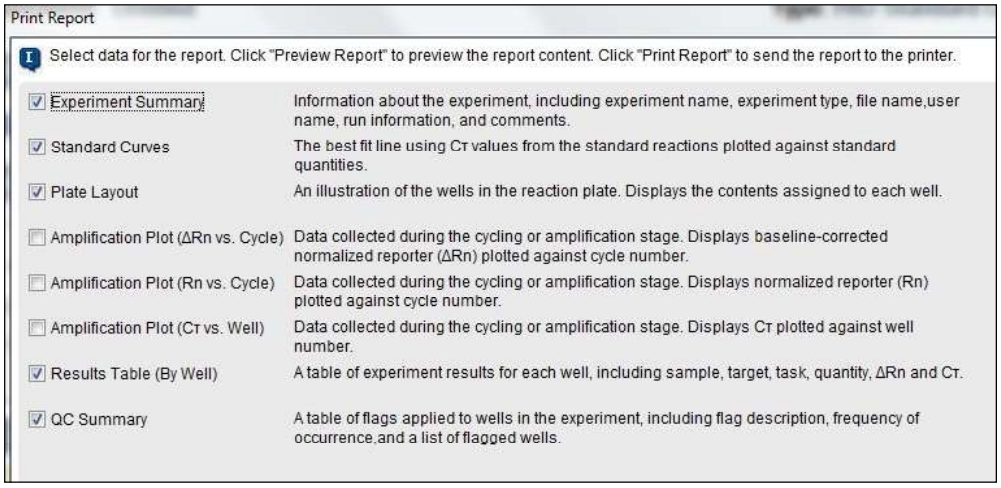


Figure { SEQ Figure * ARABIC } Print Report selection window to export.

18. Select **CutePDF Writer** in the print window and click **OK**.
19. Save the report to **I:\Results\Qres** using the Batch ID (eg.CDNAQUA20161118-01.pdf).
20. Close the experiment tab and click **Yes** when prompt appears to save changes.

8 Batch finalisation

1. Open the **Results PDF** for the batch saved in **I:\Results\Qres**
2. Ensure the standard curve slopes, Y-intercept and R^2 values are within the allowable ranges for all targets.
3. If the SAT Y-Intercept values are outside the range, the batch is to be repeated. Fail and re-create the quantification batch as follows and upload **Results PDF** only (step 5).
   a) In FR, click the **Edit/Update Batch** icon, change the batch status to FAIL (Figure 22), add a suitable batch note, select the PDF result file only and click the **Save** Batch icon.

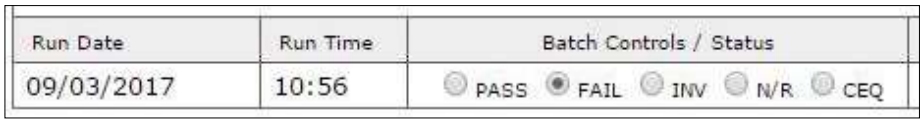


Figure { SEQ Figure * ARABIC } Batch status.

   b) Create a quantification batch in FR using the failed batch as a template as shown in the Forensic DNA Analysis Workflow Procedure (34034).
   c) Use the **Batch / SBox / File** sample source option.
   d) Use the Batch ID of the quantification batch that is to be repeated as the **Batch Source / Storage Box**.
   e) Click the **Save** icon.

4. Ensure the Reagent Blank (located in well C2) has an undetermined quantification value for the LAT, SAT and Target-Y. If the Reagent Blank has a value, refer to Table 8 for actions required.

Table 2 Criteria threshold for the Quantifiler® reagent blank control.

Reagent Blank (ng/µL)	**Action Required**
>0 to ≤ 0.001	Note the quantification value in the FR as a batch note (e.g. reagent blank 0.00081 ng/µL < acceptable threshold).
> 0.001	Contamination may have occurred. Note the quantification value in the FR as a batch note entry (e.g. reagent blank 0.0052 ng/µL > acceptable threshold). Notify analytical senior scientist before further action. Actions required will either be to monitor the reagent blank on the following quantification batch for contamination issues or clean the block on the QS5 instrument as per Operation and Maintenance of the AB QuantStudio 5 RT-PCR Instrument (35028) then fail and repeat quantification batch as per step 3 of Section 8.

5. Upload the **Results PDF** to FR as outlined in Section 6.6.

6. Upload the **Results Excel** file to FR as outlined in Section 6.6. This will take some time to upload and once finished the Quant Results page will open (Figure 23).

BatchID	Technique	Method	Plate / Rack ID
CDNAQUA20170519-01	DNA Quantification	Quantifiler Trio	

Well	SampleID	T.SA (Qty)	Priority / Analytical Note	µL	Technique	Method	Diln
A1	STD 1 (50ng/uL)	50					
A2	STD 5 (0.005ng/uL)	0.005					
A3	360002469	0.000000	P1		STR Amplification	PowerPlex21 3130xl	
B1	STD 1 (50ng/uL)	50					
B2	STD 5 (0.005ng/uL)	0.005					
B3	360002378	0.000000	P2		STR Amplification	PowerPlex21 3130xl	
C1	STD 2 (5ng/uL)	5					
C2	Reagent Blank	0.000000					
C3	360002367	0.225500	P2		STR Amplification	PowerPlex21 3130xl	
D1	STD 2 (5ng/uL)	5					
D2	360000702	0.001000	P1 Micron 32.0 uL	32.0	STR Amplification	PowerPlex21 3130xl	
D3	360000276	0.000000	P2		No DNA Detected		
E1	STD 3 (0.5ng/uL)	0.5					
E2	360002431	0.000000	P1		STR Amplification	PowerPlex21 3130xl	
E3	690149580	0.555000	P3		STR Amplification	Profiler Plus 3130xl	
F1	STD 3 (0.5ng/uL)	0.5					
F2	360002420	0.248700	P1 EXTPB 360001639 used as positi ...		STR Amplification	PowerPlex21 3130xl	
F3	360000347	0.012000	P3		STR Amplification	Profiler Plus 3130xl	
G1	STD 4 (0.05ng/uL)	0.05					
G2	360001031	0.457000	P1		STR Amplification	PowerPlex21 3130xl	
H1	STD 4 (0.05ng/uL)	0.05					
H2	360002475	0.412000	P1		STR Amplification	PowerPlex21 3130xl	

Figure 4 Quant results page.

7. Ensure samples have correct **Technique** and **Method** selected as per sample type and quantification value as shown in Table 9.

Table { SEQ Table * ARABIC } Default values for Quant Results page.

Type	Quantification Value	Priority	Technique	Method
CW	<0.001 ng/µL	P1	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	P1	Post-Extraction	Microcon PowerPlex21
	>0.0088 ng/µL	P1	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P1	Post-Extraction	Dilution
	<0.001 ng/µL	P2 or P3	No DNA Detected	Blank
	≥ 0.001 – 0.0088 ng/µL	P2 or P3	DNA Insufficient	Blank
	>0.0088 ng/µL	P2 or P3	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	P2 or P3	Post-Extraction	Dilution
	<0.001 ng/µL	QPS ENVM*	No DNA Detected	Blank
	≥0.001 – ≤0.0088 ng/µL	QPS ENVM*	DNA Insufficient	Blank
	>0.0088 ng/µL	QPS ENVM*	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	QPS ENVM*	Post-Extraction	Dilution
Ref	<0.0088 ng/µL	All	On Hold	Reference Sample Review
	≥0.0088 – ≤0.0176 ng/µL	All	Post-Extraction	Microcon PowerPlex21
	>0.0176 ng/µL	All	STR Amplification	PowerPlex21 3500xl
	>5 ng/µL	All	Post-Extraction	Dilution

Note: QPS ENVM samples will have the well designation highlighted blue.

8. Check for any samples with a **Quant & hold** or **NWQPS** analytical note in the Priority / Analytical Note column and:
   - if the **Technique** is no DNA detected or DNA Insufficient, no action is required.
   - If the **Technique** is anything else (e.g. STR Ampflication or Post-Extraction) change the **Technique** and **Method** fields to blank on the Quant Results page in FR.

Note: Ensure truncated analytical comments ending in "…" are expanded and checked as Quant and Hold samples must not progress to further processing.

9. Check for any Microcon® samples which will have a volume in the **µL** column. Ensure that the sample has defaulted to the correct STR Amplification Method, which is PowerPlex21 3500xL for volumes ≥ 23 µL and PowerPlex21 3500xL Manual for volumes < 23 µL.

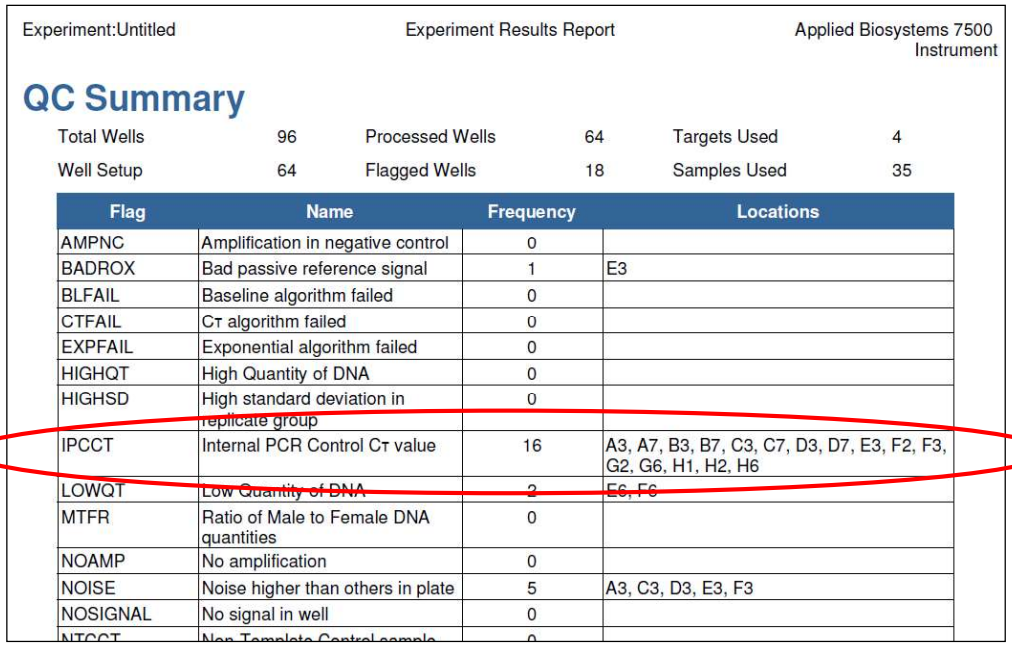
10. Check the plate layout page of the **Results PDF** for any samples flagged red. For these samples, change the **Technique** and **Method** fields to blank on the Quant Results page in FR. If required, add an exhibit testing for another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064), include an appropriate sample notation (e.g. "Excessive noise in the Multi-component plot which may have affected quant results – sample to be re-quanted" or "well evaporation").

11. For any concentrated samples (> 5 ng/µL), the default **Technique** will be a "Post Extraction" and **Method** a "Dilution" on the Quant Results page in FR. Calculate the dilution factor required (aiming for a C_T value of approximately 0.5 ng/µL) and enter into the **Diln** column. **Do not** press **Enter** after entering dilution factors.

12. For any samples that are displayed on the QC summary page of the **Results PDF** (Figure 24), open the exhibit detail page for each of the samples in a new tab in FR.

- If a sample has come from either a Microcon® batch or a Nucleospin® batch, contact the case scientist before changing the **Technique** and **Method**.
- If a sample has come from any other batch type change to "Post-Extraction" **Technique** and "Nucleospin" **Method** on the Quant Results page in FR.

Note: ***Inhibited samples*** are those that have an IPC C_T value of undetermined or values 2 units above the average IPC C_T from the standards.



Experiment:Untitled — Experiment Results Report — Applied Biosystems 7500 Instrument

QC Summary

Total Wells	96	Processed Wells	64	Targets Used	4
Well Setup	64	Flagged Wells	18	Samples Used	35

Flag	Name	Frequency	Locations
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	1	E3
BLFAIL	Baseline algorithm failed	0	
CTFAIL	Cᴛ algorithm failed	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHQT	High Quantity of DNA	0	
HIGHSD	High standard deviation in replicate group	0	
IPCCT	Internal PCR Control Cᴛ value	16	A3, A7, B3, B7, C3, C7, D3, D7, E3, F2, F3, G2, G6, H1, H2, H6
LOWQT	Low Quantity of DNA	2	E6, F6
MTFR	Ratio of Male to Female DNA quantities	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	5	A3, C3, D3, E3, F3
NOSIGNAL	No signal in well	0	
NTCCT	Non-Template Control sample	0	

Figure 5 QC report summary page.

13. Click the **Save** icon. This will automatically fill the **Run date / time** and select **PASS** as the batch status for the quantification batch.

Note: Once uploaded to FR the Results PDF can be deleted from I:\Results\Qres after one week.

14. Review the Quant Results page after saving to check the **negative extraction controls** quantification values. Refer to Table 10 for appropriate actions.

Table { SEQ Table * ARABIC } Actions for extraction negative controls.

Extraction Negative Controls Quantification Value	**Action Required**
0.000000 ng/µL	Nil
≤ 0.001 ng/µL	Note the quantification value in the **extraction batch notes** (e.g. extraction negative control 0.00081ng/µL < acceptable threshold) and allow sample to be amplified.
> 0.001 ng/µL	Contamination may have occurred. Note the quantification value in the **extraction batch notes** (e.g. extraction negative control 0.0052 ng/µL > acceptable threshold). Order another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064), allow the sample to progress to amp and notify the Analytical Senior Scientist (HP5).

15. Review the Quant Results page after saving to check the **positive extraction controls** quantification values. If the value is undetermined or lower than expected, order another quantification as per the Miscellaneous Analytical Procedures and Tasks (34064) and allow the sample to progress to amp. Enter a batch note in the **extraction** batch.

9 Validation

- Acedo, P., Mathieson, M., Ryan, L., Allen, C. 2015 Validation of Quantifiler® Trio.
- Dwyer, T., Darmanin, A., Ryan, L. and Allen, C. (2016). Project Proposal #173: Validation of Hamilton STARlet A for Quantification and Amplification Assay Setup.
- Kaity, A., Ryan, L., Mathieson, M., Allen, C. 2019 Validation of two QuantStudio™ 5 Real-Time PCR Systems.

10 Quality assurance/acceptance criteria

Refer to Section 7.3 for the quality assurance/acceptance criteria for quantification.

11 References

- Applied Biosystems (2015). QuantStudio 3 and 5 Real-Time PCR Systems. Installation, use and maintenance. [PN MAN0010407, Revision C.0].
- Applied Biosystems (2017). HID Real-Time PCR Analysis Software. Version 1.3. [PN MAN0009819, Revision D.0].
- Applied Biosystems (2017). QuantStudio 5 Real-Time PCR System for Human Identification. Site preparation guide. [PN MAN0016701, Revision A.0].
- Thermo Fisher Scientific, Quantifiler® HP and Trio DNA Quantification Kits User Guide, Publication Number 4485354, Revision A. Publication Number 4485354, Revision A ed2014.
- J.Y. Liu, Direct qPCR quantification using the Quantifiler® Trio DNA quantification kit. Forensic Science International: Genetics 13 (2014) 10-19

- [D.T. Chung, J. Drabek, K.L. Opel, J.M. Butler, B.R. McCord, A study of the effects of degradation and template concentration on the amplification efficiency of the miniplex primer sets. J. Forensic Sci. 49 (2004) 733–740
- S. Vernarecci, E. Ottaviani, A. Agnostino, E. Mei, L. Calandro, P. Montagna, Quantifiler®Trio Kit and forensic samples management: A matter of degradation. Forensic Science International: Genetics 16 (2015) 77-85.
- J.M. Roberston, S.M. Dineen, K.A. Scott, J. Lucyshyn, M. Saeed, D.L. Murphy, A.J. Schweighardt, K.A. Meiklejohn, Assessing PreCRTM repair enzymes for restoration of STR profiles from artificially degraded DNA for human identification. J Forensic Science International: Genetics 12 (2014) 168-180.
- Thermo Fisher Scientific, Introduction of Improvements to the Quantifiler™ HP and Quantifiler® Trio, Customer Notification Letter (May 2015).
- Bright, J.-A., Cockerton, S., Harbison, S., Russell, A., Samson, O. and Stevenson, K. (2011), The Effect of Cleaning Agents on the Ability to Obtain DNA Profiles Using the Identifiler™ and PowerPlex® Y Multiplex Kits. Journal of Forensic Sciences, 56: 181–185.

12 Associated documents

QIS: 17195 Spill Control
QIS: 22857 Anti-Contamination Procedure
QIS: 35028 Operation and Maintenance of the AB QS5 RT-PCR Instrument
QIS: 34034 Forensic DNA Analysis Workflow Procedure
QIS: 34042 Procedure for the use of the STORstar unit for automated sequence checking
QIS: 34050 Operation and Maintenance of the Microlab® STARlet and LabElite® Integrated I.D. Capper™
QIS: 34063 Preparation of DNA Quantification Standards and In-house Quality Controls
QIS: 34064 Miscellaneous Analytical Procedures and Tasks
QIS: 34103 Receipt, Storage and Preparation of Chemicals, Reagents & Kits
QIS: 34132 DNA Extraction and Quantification of Samples using the QIAsymphony SP and AS - FR

13 Amendment history

Version	Date	Author/s	Amendments
1	18 April 2017	T Dwyer, A Kaity, L Farrelly, M Mathieson	FR changeover from AUSLAB to Forensic Register. Liquid handler changeover from MPII to STARlet. Minor editing.
2	08 Nov 2017	T Dwyer	Update Y-Intercepts for all Standard Curve thresholds. Remove re-quant of negative controls with a quant value <acceptable threshold. Change negative control and reagent blank threshold to 0.001ng/uL to align with CW samples and as per the recommendation in Validation. Update hyperlinks to link to FR SOPs. Enter actions for QPS ENVM samples.

Version	Date	Author/s	Amendments
3	06 June 2019	A Kaity	Replacement of 7500 RT-PCR instruments with QS5 RT-PCR. Update transition table. Remove references to Profiler Plus.
4	July 2020	B Micic T Prowse	Minor formatting. Removed figures 16-18 not required, updated Fig 14, Section 6.1, Tables 7 & 9, moved checking evaporated wells to prior to result export, removed ordering Ref dilutions manually, added check for DILN samples. Update Section 7.3 to specify Automatic Baseline used following Minor Change July 2020.
5	January 2021	A Darmanin	Updated 3130xl to 3500xL.
6	April 2021	A Darmanin	Changed STARlet MasterMix position from the reagent module to the sample carrier. Updated images. Removed the 2nd centrifugation of the Nunc tube rack prior to quant setup. Corrected the referencing of all Figures and Tables.
7	Mar 2022	B Micic	Minor formatting. Removed Fig5. Updated Table 7 with new Y-intercept values. Table 9- corrected Ref On hold value. Re-arranged Appendix section, added note to (now) 14.4 section, added sections 14.2 STARlet Troubleshooting programs & 14.5 Locked batch troubleshooting. Updated to new template, amended figures, removed dNTO and NTC from Definition section.

14 Appendices

14.1 Appendix 1 - Manual Quantifiler® Trio Quantification Setup Procedure

The following procedure describes the method for the manual quantification of extracted DNA when the liquid handler is expected to be unavailable for ≥48 hours or if the DNA extract volume is <20 µL.

1. In the Clean Reagent Room (3188) prepare Quantifiler® Trio master mix. Refer to Section 4.1 for preparation of reagents.
2. Manual DNA quantification is to be performed in room 3194 in a biosafety cabinet.
3. Access the batch in FR and check for Analytical notes as per Section 6.5 and action as required.
4. Print the platemap from the **BatchID_QUANT_MAP.xls** file ensuring lab numbers and volumes are visible.
5. Centrifuge the Nunc™ rack of samples for 1 min at 2000 rpm (657 g).
6. Using the Mixmate, vortex the Nunc™ rack of samples for 1 min at 1000 rpm.
7. Centrifuge the Nunc™ rack of samples again for 1 min at 2000 rpm (657 g).
8. Check the first and last barcodes of the extracts against the FR platemap.
9. Briefly vortex and centrifuge the Quantifiler® prepared standards.

Note: Ensure the Quantifiler® prepared standards are within the expiry date and there is sufficient volume remaining (> 20 µL).

10. Briefly vortex and centrifuge the master mix tube/s.
11. Label a skirted 96-well plate with the Batch ID on the front and the Batch ID barcode on the right side.
12. Dispense 18µL of master mix to each required well of the optical reaction plate (as per the platemap).
13. Apply a breathable sealing film to surface of labelled 96-well plate.

Note: Well designations may be written on the surface of the breathable sealing film for ease of reference.

14. For the following steps, check that the well position being pipetted into matches the standard or barcode according to the FR platemap.
15. Pipette 2µL of Quantifiler® prepared standard or DNA extract into each of the wells of the labelled 96-well plate as per the platemap.
16. ***CAREFULLY*** remove the breathable sealing film and seal the 96-well plate with an optical adhesive seal.
17. Centrifuge the optical plate for 1 minute at 2000 rpm. Place the plate in the pass-through hatch to the PCR/CE Room (3194).

18. Check the plate to ensure all wells contain the required volume and that no bubbles are present.

Note: Excessive bubbles in the wells are critical and may affect the accuracy of the quantification results.

19. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

20. Proceed to Section 7.2.

14.2 Appendix 2 - Troubleshooting: STARlet Troubleshooting Programs

If a method is aborted part-way through a run, there are troubleshooting methods to allow the operator to restart the method from the last completed step. Which troubleshooting program used is dependent on what stage of the set up the run was aborted. The operator should be able to identify where the method has aborted by reading the trace file. (**Note:** Mastermix is added to the plate first, followed by Standards and then DNA extracts). Once this has been determined, the operator can then choose the appropriate troubleshooting method to continue the run.

The Quantifiler Trio program cannot process batches with duplicate samples. If duplicate samples are required there is a troubleshooting program that can prepare batches with duplicate samples.

The four troubleshooting programs are:

1. Q1. Quant Start from Add Master Mix
2. Q2. Quant Start from Add Standards
3. Q3. Quant Start from Add Samples
4. Quantifiler Trio DUPLICATE SAMPLES

14.2.1 Quant Start from Add Master Mix

This troubleshooting program is used when:

- samples have been decapped
- some or no mastermix has been added to the plate
- no Standards have been added to plate
- no samples have been added to plate

1. If some mastermix has been added to the plate, edit the platemap to change the mastermix volume (MMVOL column) to 0 for the samples that already have mastermix.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q1. Quant Start from Add Master Mix**'.
6. A dialogue box will appear (Figure 25). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be on the decapper stage, inside the STARlet

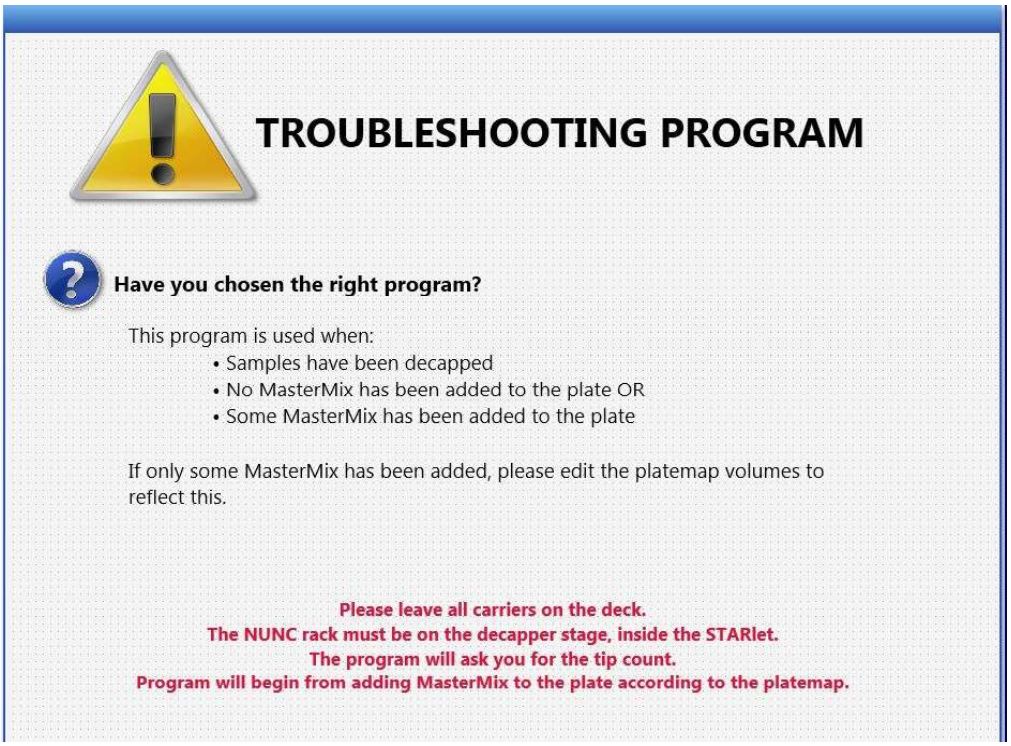


Figure { SEQ Figure * ARABIC } Troubleshooting Program Dialog box- starting at the addition of MasterMix.

7. Continue the program, the program will start from adding mastermix to the plate as per the platemap.

14.2.2 Quant Start from Add Standards

This troubleshooting program is used when:

- samples have been decapped
- mastermix has been added to the plate
- some or no Standards have been added to plate
- no samples have been added to plate

1. If some Standards have been added to the plate, edit the platemap to change the sample volume (SVOL column) to 0 for the Standards that have already been added to the plate.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q2. Quant Start from Add Standards**'.
6. A dialogue box will appear (Figure 26). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be in position 4 of the Multifelx carrier in Tracks 20-25

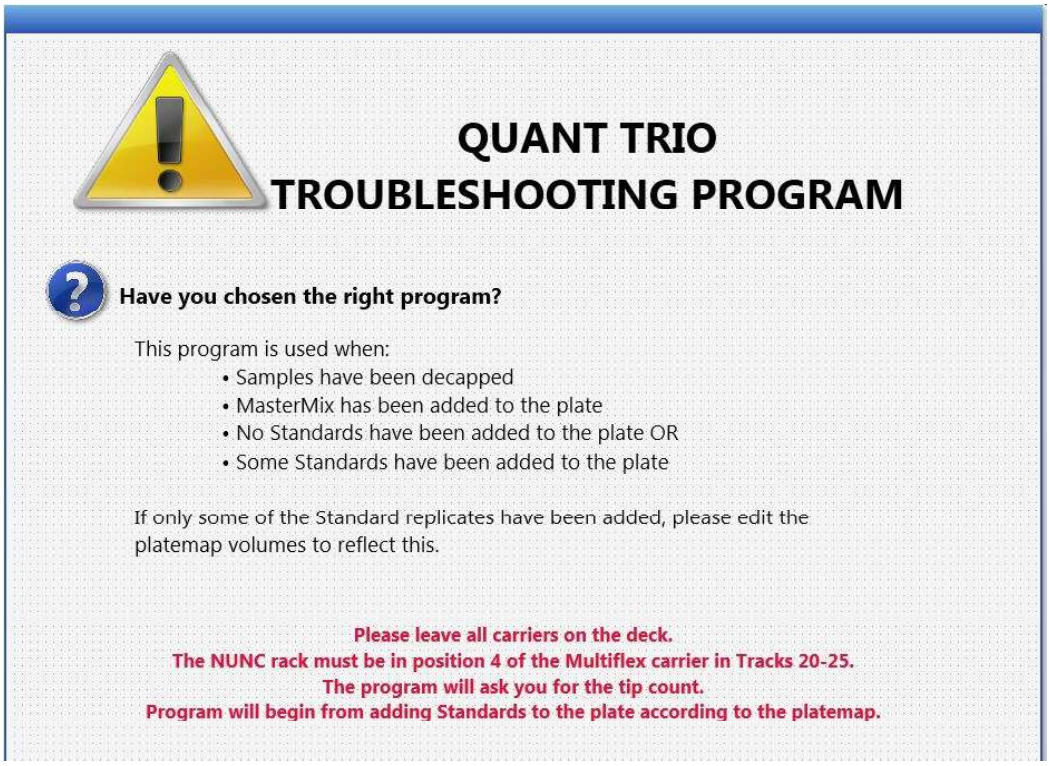


Figure { SEQ Figure * ARABIC } Troubleshooting Program- starting at the addition of Standards.

7. Continue the program, the program will start from adding the Standards to the plate as per the platemap.

14.2.3 Quant Start from Add Samples

This troubleshooting program is used when:

- samples have been decapped
- mastermix has been added to the plate
- Standards have been added to plate
- Some or no samples have been added to plate

1. If some samples have been added to the plate, edit the platemap to change the sample volume (SVOL column) to 0 for the samples that have already been added to the plate.
2. Upload the edited platemap to FR.
3. If required, launch the Method Manager software via the desktop icon.
4. Select the '**Troubleshooting**' button on the home page (Figure 4).
5. Select '**Q3. Quant Start from Add Samples**'.
6. A dialogue box will appear (Figure 27). Read and follow the prompts:
    a. check that you have selected the correct program
    b. check that all required carriers are loaded onto the STARlet deck
    c. NUNC rack must be in position 4 of the Multifelx carrier in Tracks 20-25

Figure { SEQ Figure * ARABIC } Troubleshooting Program- starting at the addition of samples.

7. Continue the program, the program will start from adding the samples to the plate as per the platemap.

14.2.4 Duplicate Sample/s on Batch

1. Complete steps 1-8 of Section 7.1 Quantification Set up.
2. Open the STARlet quant platemap and the duplicate quant template (I:\Pre PCR STARlet\All Plate Maps\Duplicate Quant Template) (alternatively any amp platemap can also be used). Copy the '**Source Pos**' column and paste it into the quant platemap into **column G** (to the right of the 'SVOL' column).
3. Locate the duplicate sample/s in the platemap and edit the '**Source Pos**' column to reflect the correct position for the physical extract. Save the platemap.
4. Upload the edited platemap to FR.
5. If required, launch the Method Manager software via the desktop icon.
6. Select the '**Troubleshooting**' button on the home page (Figure 4).
7. Select '**Quantifiler Trio DUPLICATE SAMPLES**'.
8. Continue as per step 10 of Section 7.1 Quantification Set up.

14.3 Appendix 3 - Troubleshooting: Batch Completed prior to Results Upload

If the quantification batch is completed in FR prior to the upload if the result file (.xls), the samples do not transition to the next required step of processing. The result file (.xls) cannot then be uploaded to a completed batch to transition the samples. The following procedure describes the method used to progress samples when the quantification batch has been completed prior to the import of the result file (.xls).

1. For the original batch add “See Batch” to the batch comments field.
2. In the Batch Notes field add: “Batch completed prior to results being uploaded. Results have been uploaded under (New Batch ID)”.
3. Change the batch status to “Pass”.
4. Upload the PDF results file to this original batch.
5. Create a new quantification batch of the same type using the original batch as the source batch.
6. Sequence check and lock the batch.

Note: Check if the samples from the quantification batch have been stored. If the samples have been stored, locking the newly created quantification batch will remove all the samples from the storage location in FR. If this occurs, you must re-store all the samples back into FR storage.

7. Add a “See Batch” comment to the new batch.
8. In the Batch Notes field add: “Samples processed on (Original Batch ID). This batch was created solely to upload the results for processing to continue.”
9. Copy and rename the .xls results file to match the new Batch ID in the results folder.
10. Open the new .xls file and edit the Batch ID inside the file to match the new Batch ID. Save and close the file.
11. Upload the .xls results file to the new batch and complete as per routine procedure.

14.4 Appendix 4 - Troubleshooting: Locked Batch that needs samples to be removed

Once the quantification batch is locked in FR, samples cannot be removed from the batch. If there are samples that are required to removed (duplicate samples, on hold, etc), ideally the quantification batch would be re-created in FR (using the original batch). The samples removed (that require removal) and the (new) batch re-STORstarred. Sometimes due to time constraints this is not possible. The following procedure describes the method used for processing a quantification batch that contains samples that do not require quantification.

1. For samples that do not require quantification, edit the quantification batch platemap for the STARlet and change the sample volume (in SVOL column) to zero. Upload the edited platemap into FR.
2. In the Batch Notes field add a comment to note each sample that required to be removed (but could not be removed in FR as the batch was locked) and that no sample was physically used/added to the quantification reaction plate. Also add that these samples/positions are not to be transitioned from the quant result page.
3. Add a notation to each sample noted in the Batch notes to explain that the sample was not physically used/added to the quantification reaction plate.
4. Prepare the quantification batch as per normal procedure.

Note: When running the batch on the QS5, the instrument platemap must match FR. **Do not remove/omit any samples from the QS5 platemap**. If the samples that were not added to the quantification plate are removed, then the QS5 result file cannot be uploaded into FR.

5. Upload the result file and blank out the Technique and Method for the samples / positions that did not require quantification and then complete the normal result finalisation steps.