QUANTIFICATION OF DNA EXTRACTS USING THE APPLIED BIOSYSTEMS® QUANTIFILER® TRIO DNA QUANTIFICATION KIT

Background

PCR-based human identification assays such as STR analysis require a defined range of template input. The amount of sample needed to achieve the optimal template range is determined by a human-specific quantification assay. The Applied Biosystems® Quantifiler® Trio DNA Quantification Kit (Trio Kit) is designed for simultaneous quantification of human genomic DNA (small autosomal target) and human male DNA (Y target) using quantitative PCR. There is also a human specific large autosomal target which aids in determining if DNA is degraded. The Trio kit contains an Internal PCR Control (IPC) that is not found in nature. The IPC is used to test successful amplification and functionality of all assay components, and also identify the presence of PCR inhibitors. This internal control is particularly useful to confirm the validity of negative results. Quantification with this assay can be performed on the Applied Biosystems® 7500 Real-Time PCR (RT-PCR) Instrument and must be analyzed using the HID RT-PCR Analysis Software. The Trio Kit uses multicopy target loci for improved detection sensitivity. All target loci of the Trio Kit are human-specific and each consists of multiple copies dispersed on various autosomal chromosomes (for the small autosomal and large autosomal targets) or multiple copies on the Y chromosome (for the male target). The selected targets have conserved primer-binding and probe-binding sites within individual genomes and also have minimal copy number variability between different individuals and population groups. Amplicon length and fluorophore used for detection are listed in the table below.

Target	Amplicon Length	Dye/Quencher
Large Autosomal	214 bases	ABY® /QSY®
Small Autosomal	80 bases	VIC® /MGB
Y (Male)	75 bases	FAM® /MGB
IPC	130 bases	JUN® /QSY®

The human DNA used to generate the DNA quantification standards dilution series consists of pooled human male genomic DNA. One ten-fold dilution series consisting of 5 concentration points ranging from 50 ng/µL to 0.005 ng/µL (of total human DNA) is prepared for each reaction plate. Data collected from the dilution series is used to generate a standard curve and determine DNA concentrations for unknown samples.

The Trio Kit uses PCR primers, dye-labeled TaqMan® probes, and non-fluorescent quenchers for the amplification of the multicopy genomic loci (see the Quantifiler® HP and Trio DNA Quantification Kits User Guide for further description of reaction mechanism). Using the Applied Biosystems® 7500 RT-PCR instrument and HID RT-PCR Analysis Software, the quantification is completed in approximately one hour.

Kit Contents

The Trio Kit contains materials sufficient to perform 400 reactions at a 20 µL volume. The kit components and their quantities are listed in the table below.

Reagent	Volume
Quantifiler® THP PCR Reaction Mix	4 x 1 mL
Quantifiler® Trio Primer Mix	4 x 0.8 mL
Quantifiler® THP DNA Dilution Buffer	2 x 1.8 mL
Quantifiler® THP DNA Standard	1 x 0.12 mL

The kit must be stored at -15 to -25°C upon receipt, and 2 to 8°C after the initial use.

The Primer Mix and PCR Reaction Mix must be kept from direct exposure to light. Excessive exposure to light may affect the fluorescent probes and/or the passive reference dye.

Reagents used for amplification must be kept under sterile conditions.

Reagents will not be used beyond the listed expiration dates. All reagents must be labeled with the date and analyst initials when put into use. Record reagent details in the Reagent Log.

Additional Materials Required

Materials (pipettes, tips, tube opener, etc.) used in amplification set-up are kept in an enclosed workstation and exposed to UV light for approximately 30 minutes after each use.

Approved disposable gloves
Approved lab coat
Kimwipes
Calibrated pipettes with filter-tips to allow pipetting of 1 µL to 1000 µL
Micro-centrifuge tubes
Tube rack
Permanent marker
MicroAmp® Optical 96-Well Reaction Plate or MicroAmp® Optical 8-Tube Strip
MicroAmp® Optical 8-Cap Strips (for use with plates or 8-tube strips)
MicroAmp® Optical Adhesive Seal (for use with plates)
Support base for reaction plate or 8-tube strips
7500 Real-Time PCR Systems Spectral Calibration Kit I
Spectral Calibration Plate with ABY™ Dye
Spectral Calibration Plate with JUN™ Dye
Spectral Calibration Plate with Mustang Purple™ Dye
RNase P Instrument Verification Plate
Micro-centrifuge
Table top centrifuge with 96-well plate adapters
Vortex
7500 Real-Time PCR Instrument
HID Real-Time PCR Analysis Software v1.2

Sample Handling / Disposal

Refer to procedure 05-01-01 Sample Control – General in the MUFSC Quality Assurance Manual for descriptions of methods employed to ensure integrity of evidence samples and assay results. The objectives of the policies are to avoid sample loss, contamination, or any other deleterious alteration of the samples.

Waste products from this procedure are disposed in regular trash. Tips are disposed in appropriate sharps containers.

Warnings and Precautions

All components of the Trio Kit are not hazardous. All components may cause eye or skin irritation with susceptible persons, may be harmful by inhalation, and may be harmful if swallowed. Each product contains no substances which, at their given concentration, are considered to be hazardous to health. The Quantifiler® THP PCR Reaction Mix contains sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Life Technologies™ recommends handling all chemicals with caution.

Wear suitable personal protective equipment including a lab coat, gloves, and eye/face protection while performing this procedure.

Quality Control

Use of appropriate worksheets from the Analytical Procedures Manual is required when performing this procedure. This includes “Quantifiler Trio Worksheet” and “Quantifiler Trio Workbook MASTER”. Document all information (e.g. lot numbers, sample identifiers) on these worksheets.

Positive Controls

If a positive control was initiated during extraction, it should be added to the quantification plate.

Quantifiler® THP DNA Standard is the kit component with known human DNA concentrations used to make the quantification standards dilution series for quantification of unknown samples. One column of the standard dilution series is set up on the reaction plate.

An internal positive control (IPC) is included in the Quantifiler® Trio Primer Mix. It is added to every sample at the same concentration and therefore has an expected C_T. The IPC provides positive confirmation that all assay components are functioning as expected. It is also useful to identify samples that contain PCR inhibitors.

Negative Controls

All extraction negative controls must be added to the quantification plate.

A quantification negative control will be setup to monitor for contamination in the Trio Kit reagents. This negative control consists of all reagents used in the procedure but contains no DNA sample. Quantifiler® THP DNA Dilution Buffer is added in place of a DNA sample.

Procedures

Before Starting

Ensure the Applied Biosystems® 7500 Real-Time PCR Instrument is calibrated. The following calibration procedures must be performed, in this order:

- Regions of Interest (ROI) Calibration
- Background Calibration
- Optical Calibration
- Dye Calibration for the following dyes: (use 60°C as the default temperature)
    - FAM™ (included in Spectral Calibration Kit I)
    - VIC™ (included in Spectral Calibration Kit I)
    - NED™ (included in Spectral Calibration Kit I)
    - ROX™ (included in Spectral Calibration Kit I)
    - TAMRA™ (included in Spectral Calibration Kit I)
    - ABY™ (follow the custom dye procedure)
    - JUN™ (follow the custom dye procedure)
    - Mustang Purple™ (follow the custom dye procedure)
- RNase P Instrument Verification Plate run

Refer to page 98 of the Quantifiler® HP and Trio DNA Quantification Kit User Guide for more information on calibrations.

Preparing the DNA Quantification Standards

Standard	Quantifiler® THP DNA Standard Total Human [DNA] (ng/µL)	Volume Quantifiler® THP DNA Standard	Volume Quantifiler® THP DNA Dilution Buffer
1	50.00	25 µL	25 µL
2	5.000	10 µL Standard 1	90 µL
3	0.500	10 µL Standard 2	90 µL
4	0.050	10 µL Standard 3	90 µL
5	0.005	10 µL Standard 4	90 µL

After being made, the quantification standards serial dilution series may be used for up to 2 weeks.

Preparing the Reactions

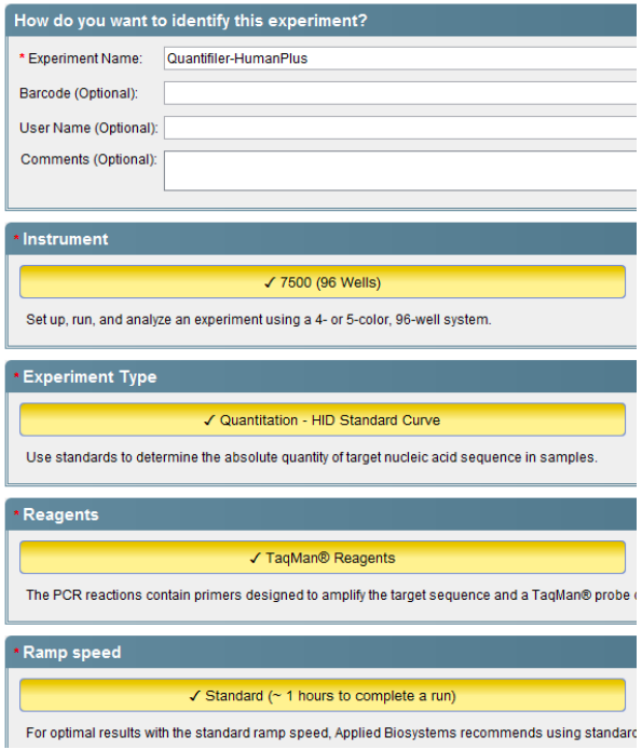
1. Thaw the Quantifiler® Trio Primer Mix thoroughly, then vortex and centrifuge briefly.
2. Gently vortex the Quantifiler® THP PCR Reaction Mix before using. Do not centrifuge the PCR Reaction Mix.
3. Prepare a master mix for n + 10% samples so that each reaction contains the following:
   (NOTE: Ensure that "n" includes samples, controls brought forward from extraction, standards, and the quantification negative control.)

Component	Volume Per Reaction
Quantifiler® Trio Primer Mix	8 µL
Quantifiler® THP PCR Reaction Mix	10 µL

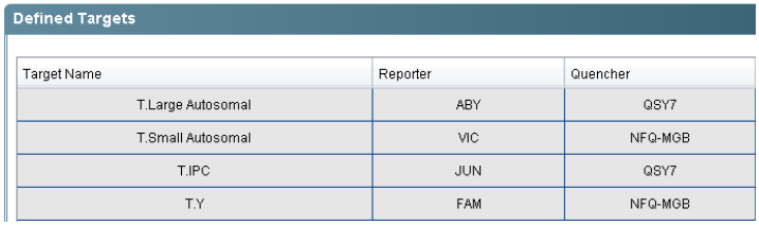
4. Vortex and centrifuge the master mix.
5. Dispense 18 µL of master mix into appropriate wells of a reaction plate.
6. Add 2 µL of the standards into appropriate wells. The use of a multi-channel pipette is recommended.
7. Add 2 µL of Quantifiler® THP DNA Dilution Buffer to quantification negative control wells.
8. Add 2 µL of samples to appropriate wells. A witness must observe sample additions when manually added and initial the appropriate space on the worksheet.
9. Seal the plate.
10. Check for the presence of air bubbles in the reaction mix. If necessary, tap the plate on the bench-top to dislodge bubbles.
11. Centrifuge the plate at 3000 rpm for about 20 seconds to ensure all liquid is at the bottom of each well. Centrifugation may also aid in the removal of bubbles.

Preparing the 7500 instrument and software

12. Power on the 7500 computer and log in. Note: Wait for the computer to finish starting up before powering on the 7500 instrument.
13. Press the power button on the lower left front of the 7500 instrument.
14. Launch the Applied Biosystems® HID Real-Time PCR Analysis Software Version 1.2.
15. On the home screen, click the icon for Quantifiler® Trio, OR if the home screen is not open, click **File→New Experiment→New Quantifiler® Trio Experiment.**
16. In the **Experiment Properties** screen, enter a name for the experiment. All other settings on this screen are automatically set and should match the settings seen below.



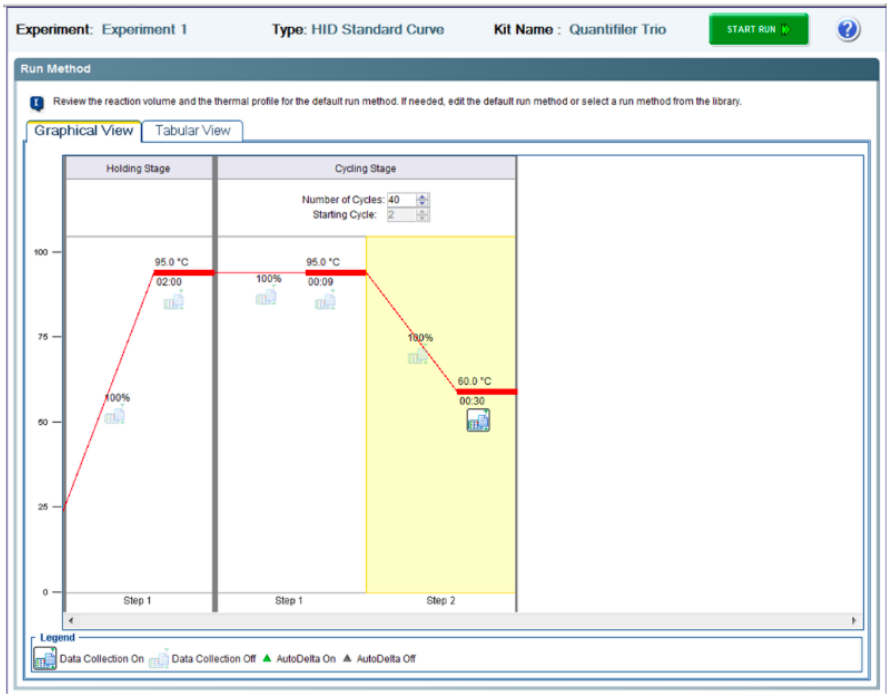
17. If using a template: select **File→Import**, select the text file that has your plate template, click start import, and skip to step 25. If not using a template, continue to step 18.
18. In the left navigational pane, click **Setup** then **Plate Setup**.
19. To add samples, go to the **Define Samples** heading and select **Add New Sample**, then type the name for the sample. Repeat for all samples.
20. Click the **Assign Targets and Samples** tab at the top of the page.
21. The samples added in step 19 must now be added to the plate wells. To select well(s):
    21.1. Single well- click the well
    21.2. Row of wells- click a letter on the side of the layout
    21.3. Column of wells- click a number at the top of a column
    21.4. More than one well, row, or column- drag the pointer over the wells, letters, or columns to select
22. In the **Assign Sample(s) to the Selected Wells** section to the left of the plate layout, locate the desired sample and select the checkbox in the **Assign** column next to the sample name.
23. Repeat steps 21 and 22 for the remaining samples.
24. To change the well a sample is assigned to: click the well, deselect the sample in the **Assign Sample(s) to the Selected Wells** section, click the new well, and select the appropriate sample in the **Assign Sample(s) to the Selected Wells** section.
25. Note: Under the **Define Targets and Samples** heading, targets and their reporter/quenchers are already specified for Trio, and should match the settings specified below.



26. Note: Under the **Assign target(s) to the Selected Wells** heading, targets are automatically assigned and the standard quantities are automatically specified.
26.1. For the samples and negatives, the small autosomal, large autosomal, and Y targets should be specified as Unknowns.
26.2. For the Trio standards, the small autosomal, large autosomal, and Y targets should be specified as Standards. The quantities of each standard for each target should match the table below.

Standard	Quantity (ng/µL)
1	50
2	5
3	0.5
4	0.05
5	0.005

26.3. The IPC target should be set as unknown for all standards, samples, and negatives.
27. In the left navigational pane, click **Setup**, then **Run Method** to view the thermal cycling parameters. The parameters are automatically specified. Ensure the sample volume is 20 µL.



21. Save the plate as an .eds document. **File→Save As**, name appropriately, and save in the proper location.
22. Load the reaction plate on the 7500 RT-PCR Instrument.
22.1. Press the indention on the blue panel on the front of the 7500. The tray should automatically come forward.
22.2. Place the reaction plate into the 7500 SDS instrument tray.
22.3. Press the indention on the blue panel inward to close the instrument.

Note: Position the plate in the instrument thermal block so that well A1 is in the back-left corner and the notched corner of the plate is in the back-right corner.

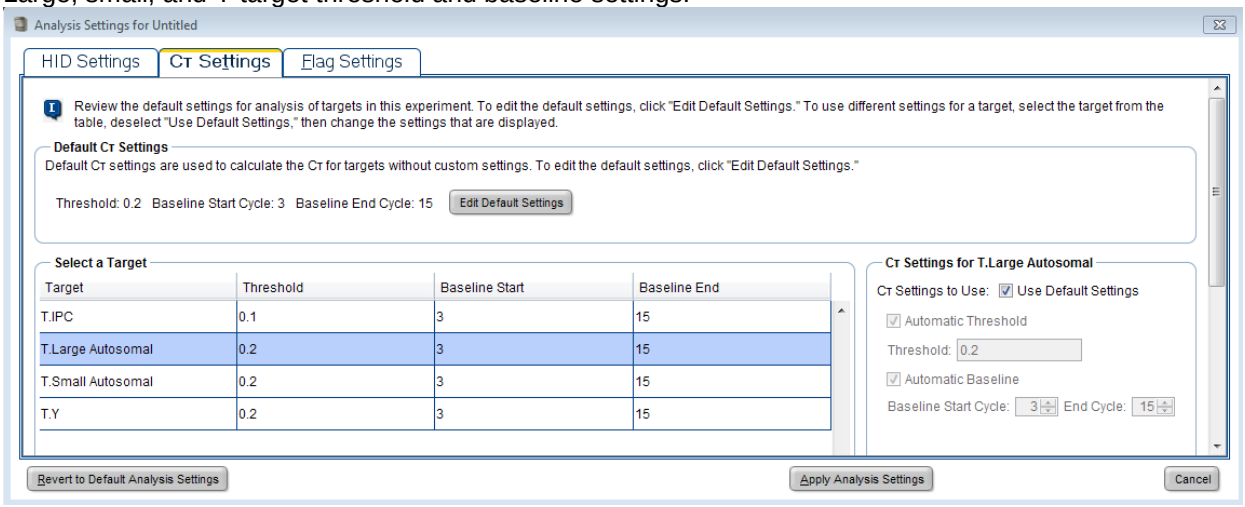
Warning: During instrument operation, the temperature of the heated cover can be as high as 108º C, and the temperature of the sample block can be as high as 100º C. Keep hands away from the instrument until the heated cover and sample block cool to room temperature.

23. Click Start Run.

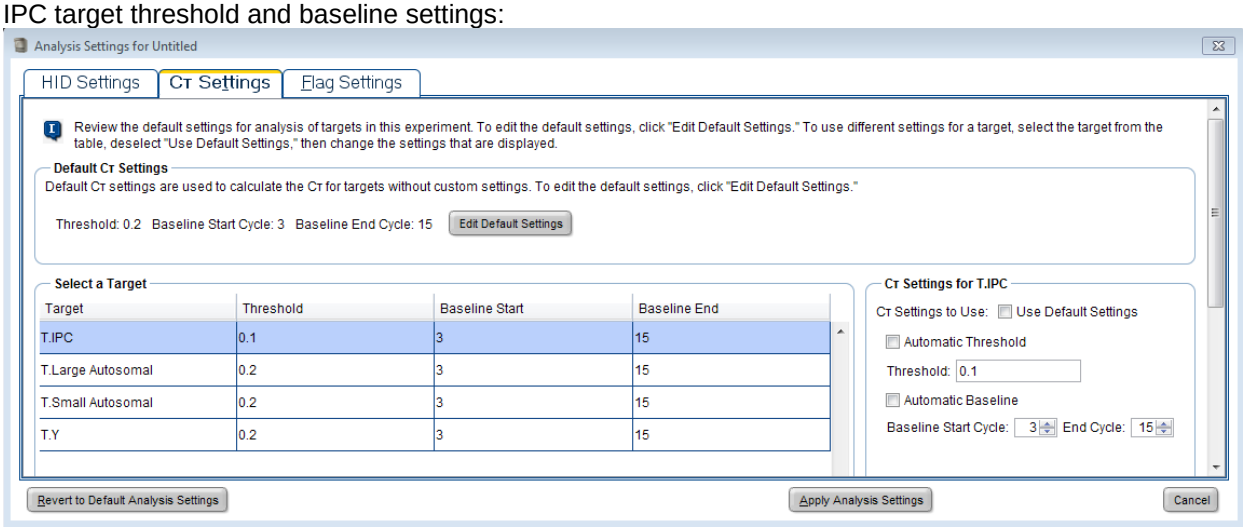
Analyzing and viewing results

24. Open the .eds plate document to analyze.
25. Verify the analysis settings by clicking the **Analysis Settings** button next to the green **Analyze** button.
    25.1. Click the **C_T Settings** tab.
    25.2. If the analysis settings match those shown below, click **Apply Analysis Settings**.
    25.3. If analysis settings differ from those show below, change them to match the settings, then click **Apply Analysis Settings.**

Large, small, and Y target threshold and baseline settings:



IPC target threshold and baseline settings:



Note: The IPC threshold should be set at 0.1. Trio Kits have been validated using the Manual Baseline method. Studies were also performed applying the Automatic Baseline method and the Manual Baseline method to evaluated potential differences between the methods for concentrations from 5-0.005 ng/µL. No statistically significant differences were observed within this range for C_T values generated using the Automatic Baseline and Manual analysis methods. A value of 0.1 was used for the IPC Threshold during the

developmental validation studies. Before using alternative baseline methods (automatic) or thresholds, perform the appropriate internal validation studies.

26. Click the green **Analyze** button.

To view the standard curve:

27. In the left navigational panel, click **Analysis,** then **Standard Curve**
28. In the **Target** drop-down list, select **All**.
29. View the C_T values for the quantification standard reactions and the calculated regression line, slope, Y-intercept, and R^2 values.
30. Note: The gap between the small autosomal, large autosomal, and male C_T values may vary depending on relative slopes of the targets and the instrument.

To view the amplification plot:

31. Note: The amplification plot can display one of the following: C_T versus well position view, or plot of normalized reporter signal (Rn) versus cycle (linear view).
32. In the left navigational panel, click **Analysis,** then **Amplification Plot.**
33. Select a plot color in the drop-down list: Well, Sample, Target, or Flag Status.
34. Select the target(s) to view in drop-down list located under the amplification plots. Select **All** to view all targets simultaneously or select a single target.
35. Select the applicable samples in the plate layout.

To export the results:

36. Select the wells you wish to export in the **View Plate Layout** tab of any screen under the **Analysis** navigational panel.
37. In the toolbar, click **File→Export**
38. Select **Results** as the type of data to export.
39. Select **Separate Files** or **One File** in the drop down list.
40. Enter a file name and export location.
41. Make sure the file type is .xls.
42. The results fields included in the export can be specified under the **Customize Export** tab.
43. Click **Start Export** to export the data to the file(s) that you selected.
44. Note: to export data for the Quantifiler® Trio Workbook MASTER, make sure you select One File, and that **All Results Fields** is selected under the **Customize Export** tab.
45. When the export is complete, click either **Export More Data** or **Close the Export Tool.**

Results Interpretation

Examining the Standard Curve

The standard curve is a graph of C_T of quantification standard reactions plotted against the starting quantity of the standards. The software calculates the regression line by calculating the best fit with the quantification standard data points. The regression line formula has the form: $C_T = m\ [\log (Qty)] + b$. Where m is slope, b is the Y-intercept, and Qty is the starting DNA quantity. There are values associated with the regression analysis, including an R^2 value, slope, and Y-intercept.

The manufacturer recommends running duplicates or two rows of each sample of the DNA quantification standards for each reaction plate. The MUFSC has decided to use just one row of standards, which contains one of each quantification standard concentration for each reaction plate. This decision was based on an assessment of the standard curve quality and cost of the Quantiplex HYres kit. In this assessment, the quality and cost of one row of standards per plate was compared to the quality and cost of two rows of standards. The results suggest that one column of standards compared to two does not significantly lower the quality of the standard curves analyzed, and the cost analysis described a large amount of reagents that would be conserved, reducing the cost per plate. The standard curves of the Trio Kit were assessed as well. During the Trio Kit validation, 19 rows of standards were set up, and each was evaluated as its own standard curve. Of the 19 standard curves, 17 passed

and two failed. The slope and R^2 values of all targets (large autosomal, small autosomal, and male) were out of range for one of the failing standard curves, and just the small autosomal target's R^2 value was out of range (0.979) for the second failing standard curve. It is understood that reducing each plate to just one row of standards may result in a higher failure rate, and more reagents may be consumed if plates must be re-setup. However, the net savings would still be substantial.

R^2 Value

The R^2 values are measures of closeness of fit between the standard curve regression line and the individual C_T data points of quantification standard reactions. Possible values are 0 to 1. A value of 1.00 indicates a perfect fit between the regression line and the data points. **The R^2 value should be ≥ 0.99**.

Note: Round the R^2 value to the nearest 100th. For example 0.9852341 can be rounded to 0.99.

Slope

The slope is an indicator of PCR amplification efficiency for the assay. A slope of -3.3 indicates 100% PCR efficiency (i.e. the number of copies of amplification product is doubled at each cycle during the exponential phase of PCR). **An acceptable range for the slope is -3.0 to -3.6 for the small autosomal target and the male target, and -3.1 to -3.7 for the large autosomal target.**

Note: Round the slope value to the nearest 10th. For example -3.164891 can be rounded to -3.2.

For **known samples only**, it is permitted to omit one standard in order to adjust the R^2 and slope to acceptable values. If the standard curve cannot be adjusted to acceptable values, the quantification reaction needs to be repeated from set up and new standards should be made. **The DNA Technical Leader must be informed of a failed plate**.

Y-Intercept

The Y-intercept indicates the expected C_T value for a sample with Qty=1 (for example, 1 ng/µL). Some variation in the Y-intercept value is expected, and therefore the manufacturer cannot provide a meaningful Y-intercept specification that will apply to all laboratories. Labs should monitor the Y-intercept values over time. Y-intercept variations can be caused from pipetting of standards or minor lot-to-lot variations of kits. Target-to-target variation is also expected. For example, the Y-intercept for the large autosomal target is typically lower than the Y-intercept for the small autosomal target or the male target. This is because of the higher copy number of the large autosomal target relative to the copy number of the small autosomal and male targets. Additionally, differences between 7500 instruments results in small differences in Y-intercept values for each of the targets, but minor differences do not affect assay performance or quantification accuracy.

Internal Positive Control

The IPC is intended to report chemistry or instrument failure, errors in assay setup, and the presence of inhibition in the sample. The IPC of the Trio Kit has been developed with increased inhibitor tolerance to better correlate with more recently introduced STR amplification kits. A large range of C_T values is possible since samples contain unknown amounts of DNA and inhibitors. The IPC system template DNA is present at a consistent concentration across all reactions on a plate. This means that the IPC C_T should be relatively consistent in typical reactions. The presence of PCR inhibitors and/or high DNA concentrations can increase the IPC C_T relative to the average IPC C_T of the quantification standards on the same plate. Based on validation studies, the IPC is expected to generate a C_T of approximately 27 (this may vary per instrument), but can range from 24.185 to 30.166. In addition to the C_T value, "positive amplification" can be an interpretation factor. Positive amplification occurs when the C_T value for the target is less than 40. Viewing the Amplification Plot of a detector, positive amplification has occurred if the amplification curve continues to rise to a higher fluorescence level after it crosses the threshold, before eventually plateauing. Situations such as those in the following table may arise.

Large, Small, or Male Target	Internal Positive Control	Possible Interpretation
No amplification	Positive Amplification and C_T approximately 27**	No detectable DNA
No amplification	No amplification	Inconclusive (inhibition, no detectable DNA and/or PCR failure are all possibilities)
Positive amplification with low C_T	No amplification or high C_T value**	Inconclusive (inhibition and/or high quantity of DNA in sample out-competes the IC target for PCR components)
Positive amplification with high C_T	No amplification or high C_T value**	PCR Inhibition

**According to validation study results, the IPC C_T should be approximately 27, but can range from 24.185 and 30.166.

For more information regarding the interpretation of the IPC results, please see page 44 in the Quantifiler HP and Trio DNA Quantification Kits User Guide.

Quantification Negative Control

The quantification negative control is expected to have no amplification of the small autosomal, large autosomal, and male targets. The IPC is expected to generate a C_T of approximately 27, but can range from 24.185 to 30.166. Positive amplification of the large autosomal, small autosomal, or male target with a C_T of approximately 27 may be an indication of contamination in the kit components. This type of result will be evaluated on a case-by-case basis to determine impact on the unknown sample results. The DNA Technical Leader will be informed of any such occurrence.

Samples Moving forward to STR PCR Amplification

One (1) reagent blank per extraction must be amplified with the samples. If one has a quant value > zero then that one must be chosen for amp (at the same sensitivity conditions as required by the sample(s) containing the least amount of DNA [e.g. at the same condition and volume as the sample with the largest volume amp load])

Globalfiler and 24plex QS Amplification Guidelines

***Stop at Quant (SAQ)**:*

Total Small Autosomal DNA: **<0.0380ng**
Total Human Male DNA: **<0.0266ng**

- ANY fraction/sample whose maximum possible small autosomal target is below 0.0380ng stops at quant regardless of the male target of the other fraction.
- Ecell fractions do NOT SAQ if the sperm fraction is going forward, unless the Small Autosomal quant falls below 0.0380ng.
- Sperm fractions do not SAQ alone unless the Small Autosomal quant falls below 0.0380ng ***OR*** the male quant falls below 0.0266ng ***AND*** the ecell fraction is going forward with maximum possible male target >0.0266ng.

***Amplification using Full Reaction Volume:** (with exception noted below)*

- *Single Source Male Samples (% Male >100%)*
  - Human MALE Target ~DI for samples with DI < 1.
  - Human MALE Target ~1ng for single source samples with DI between 1 and 3.
  - Human MALE Target ~2ng for ALL samples with DI >3.

- *Single Source and Mix with Major Component Samples (% Male <10% or between 70% and 100%)*
  - Small Autosomal Target ~DI for single source samples with DI < 1.
  - Small Autosomal Target ~1ng for single source samples with DI between 1 and 3.
  - Small Autosomal Target ~2ng for ALL samples with DI >3.

- *Suspected Mixtures (% Male between 10% and 70%)*
  - Small Autosomal Target ~2x the DI when DI < 1
  - Small Autosomal Target ~2ng for mixtures with ≥ 10% MALE DNA and DI ≥1.

    NOTE: *If case is type 1 and the ecell fraction is a mix in this category, the ecell fraction may be amped following the single source rules if the sperm fraction has a clear single source or major component male.*
  - Small Autosomal Target ~1ng for mixtures with < 10% MALE DNA and DI between 1 & 3.
  - Small Autosomal Target ~2ng for ALL samples with DI > 3 (including mixtures with < 10% MALE DNA).

- *Male Victim Cases- almost always have potential for a mix of more than one male (unless female suspect)*
  - *Follow guidelines for Suspected Mixtures*
    - *% Male < 100% : use Small Autosomal for target*
    - *% Male > 100% : use Human Male for target*

Amplification using Half Reaction Volume (Globalfiler ONLY):

- Optimal DNA targets range from 0.25ng to 1ng depending on the DI.
  - Dilute samples to 0.25ng instead of 0.5ng
  - DI <3 Target 0.25ng
  - DI between 3 & 5 Target up to 0.75ng
  - DI above 5 Target up to 1ng
  - SAQ thresholds do NOT apply to samples amped at half reaction volume

NOTES:

- The master mix volume is cut in half, so the amount of targeted DNA must be decreased as well. *[Cutting the validated DNA target for GF in half was producing grossly off scale samples that did NOT improve when diluting the amp product and re-running]*
- The expected PHR established in the GF validations do *NOT* apply to samples amped at half reaction volume. Severe PHR imbalances in several loci within a sample are common therefore, re-amping with FULL reaction volume may be necessary for some samples.

Fusion 6C Amplification Guidelines

Stop at Quant (SAQ)*:*

Total Small Autosomal DNA: **<0.0500ng**
Total Human Male DNA: **<0.0200ng**

- ANY fraction/sample whose maximum possible small autosomal target is below 0.0500ng stops at quant regardless of the male target of the other fraction.
- Ecell fractions do NOT SAQ if the sperm fraction is going forward, unless the Small Autosomal quant falls below 0.0500ng.
- Sperm fractions do not SAQ alone unless the Small Autosomal quant falls below 0.0500ng ***OR*** the male quant falls below 0.0200ng ***AND*** the ecell fraction is going forward with maximum possible male target >0.0200ng.

Amplification:

- *Single Source Male Samples (% Male >100%)*
  - Human MALE Target ~1ng for samples with DI < 1.
  - Human MALE Target ~1.25ng for single source samples with DI between 1 and 3.
  - Human MALE Target ~2.5ng for ALL samples with DI >3.

- *Single Source and Mix with Major Component Samples (% Male <10% or between 70% and 100%)*
  - Small Autosomal Target ~1ng for single source samples with DI < 1.
  - Small Autosomal Target ~1.25ng for single source samples with DI between 1 and 3.
  - Small Autosomal Target ~2.5ng for ALL samples with DI >3.

- *Suspected Mixtures (% Male between 10% and 70%)*
  - Target ~2x the DI for mixtures with ≥ 10% MALE DNA and DI < 1
  - Target ~2.5ng for mixtures with ≥ 10% MALE DNA and DI ≥1
    NOTE: *If case is type 1 and the ecell fraction is a mix in this category, the ecell fraction may be amped following the single source rules if the sperm fraction has a clear single source or major component male.*
  - Target ~1.25ng for mixtures with < 10% MALE DNA and DI between 1 & 3.
  - Target ~2.5ng for ALL samples with DI > 3 (including mixtures with < 10% MALE DNA).

- *Male Victim Cases- almost always have potential for a mix of more than one male (unless female suspect)*
  - *Follow guidelines under Suspected Mixtures*
    - *% Male < 100% : use Small Autosomal for target*
    - *% Male > 100% : use Human Male for target*

Comments on Storage

The reaction plate is not stored. It may be discarded within the PCR Laboratory after analysis is complete.

Technical Assistance

For support, visit lifetechnologies.com/support or email techsupport@lifetech.

References

Qiagen® Investigator® QuantiPlex HYres Quantitation Kit- Reduction from Two Columns to a Single Column of Standards. MUFSC DNA Laboratory Analytical Procedure Manual. December 2014.

Quantifiler® HP and Trio DNA Quantification Kits User Guide. Applied Biosystems by Life Technologies. Publication Number 4485354, Revision C. August 2014.

MUFSC Internal Validations for Globalfiler, Investigator 24plex QS, and Fusion 6C.