TECHNICAL MANUAL

PowerQuant® System

Instructions for Use of Products
PQ5002 and PQ5008

PowerQuant® System

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1.   Description

The PowerQuant® System[(a–d)] is a five-dye, four-target hydrolysis probe-based qPCR multiplex that amplifies multicopy targets to quantify the total human and human male DNA present in a sample (1). The system also amplifies an additional multicopy target to assess the degree of DNA degradation. Additionally, the PowerQuant® System includes an internal PCR control (IPC) to detect inhibitors in an amplification reaction. The PowerQuant® System achieves levels of sensitivity and inhibitor tolerance comparable to those of newer STR systems (e.g., PowerPlex® Fusion and PowerPlex® Y23 Systems). This Technical Manual describes the PowerQuant® System and provides instructions for use with the Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems® 7500 Software, Version 2.0.6), Applied Biosystems® 7500 Real-Time PCR System for Human Identification (HID Real-Time PCR Analysis Software, Version 1.1 or 1.2) or the QuantStudio™ 5 Real-Time PCR System.

Data generated using the PowerQuant® System can help determine whether an unknown DNA sample is suitable for short tandem repeat (STR) analysis, what is the appropriate STR system to use (e.g., autosomal or Y-STR) and whether the DNA is degraded or PCR inhibitors are present. This information may be used to guide decisions regarding sample repurification, dilution of DNA samples that are inhibited to help ensure accurate quantification and the optimal template volume to add to an autosomal or Y-chromosomal STR amplification.

A standard curve is required to determine the DNA concentration of unknown DNA samples. This standard curve is generated using the amplification results from a dilution series of a male DNA standard of known concentration.

PowerQuant® 20X Primer/Probe/IPC Mix

The PowerQuant® 20X Primer/Probe/IPC Mix includes all primers and probes, the Internal PCR Control and a passive reference dye:

- **Primers and probe for the autosomal DNA target:** The FAM dye-labeled probe of the PowerQuant® System detects a multicopy human autosomal DNA target. The primers are used to amplify an 84-base-pair amplicon. Data from this reaction quantify the total amount of human DNA in a sample. The autosomal target is short, robust to inhibitors and less likely to be affected by degradation events than the longer degradation target.

- **Primers and probes for the Y-chromosomal target:** The CAL Fluor® Gold 540 dye-labeled probes of the PowerQuant® System detect the presence of Y chromosomal DNA. The primers are used to amplify two multicopy loci (amplicons of 81bp and 136bp). The use of two multicopy loci minimizes the effect that variation in copy number of any given marker can have on [Auto]/[Y] ratios and increases sensitivity for male DNA.

- **Primers and probe for the degradation target:** The Quasar® 670 dye-labeled probe of the PowerQuant® System detects the presence of a longer amplicon (294bp) derived from a different region of the same locus as the autosomal target. Due to its greater length, the degradation amplicon is more susceptible to degradation and the presence of inhibitors. The ratio of DNA concentrations determined with the autosomal and degradation targets ([Auto]/[Deg] ratio) can be used to evaluate the degree of degradation (2).

- **Primers, probe and template for the Internal PCR Control:** The TMR dye-labeled probe of the PowerQuant® System detects the Internal PCR Control, a novel DNA template that is included in every amplification reaction. The primers produce an amplified product that is 435bp. Amplification performance of the IPC is used to detect inhibitors in the sample. This is the longest target in the PowerQuant® System, making the IPC more susceptible to inhibitors than the other targets in the multiplex.

- **Passive reference dye:** The CXR dye of the PowerQuant® System is used as a passive reference. The CXR dye is included in each amplification reaction. Data from the other dye channels are normalized to this signal.

PowerQuant® 2X Master Mix

The PowerQuant® 2X Master Mix yields performance (e.g., sensitivity and inhibitor tolerance) comparable to that of newer STR systems and uses hot-start PCR chemistry. The reaction setup should be performed at room temperature and is amenable to automation. Amplification is complete in 1 hour.

PowerQuant® Male gDNA Standard

The PowerQuant® Male gDNA Standard is supplied with the PowerQuant® System. This DNA consists of pooled human male DNA supplied at 50ng/µl. Serial dilutions of this DNA standard are amplified in the same plate as the unknown samples, and the results are used to generate a standard curve for the autosomal, Y and degradation targets. These standard curves are used to determine the DNA concentration for each target. The recommended protocol for the PowerQuant® System uses a four-point standard curve. We recommend performing duplicate amplification reactions with each dilution of the DNA standard and using a dedicated set of pipettes to increase run-to-run consistency. Use the same pipettes to dispense the DNA standard and unknown samples to minimize variability. We do not recommend using other DNA (e.g., DNA isolated from a cell line) as the DNA standard.

PowerQuant® Dilution Buffer

The PowerQuant® Dilution Buffer is supplied as the diluent for serial dilution of the PowerQuant® Male gDNA Standard to create the standard curve.

Instrumentation

The PowerQuant® System is designed and optimized for use with the Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems® 7500 Software, Version 2.0.6), Applied Biosystems® 7500 Real-Time PCR System for Human Identification (HID Real-Time PCR Analysis Software, Version 1.1 or 1.2) or the QuantStudio™ 5 Real-Time PCR System. Calibration with custom dyes is required prior to DNA quantification (see Section 4).

PowerQuant® Analysis Software

The PowerQuant® Analysis Software is available for download to assist with data analysis, data review and DNA normalization prior to STR amplification.

The PowerQuant® Analysis Software is available at: **www.promega.com/resources/software-firmware/**

2.   Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
PowerQuant® System	**200 reactions**	**PQ5002**

Not For Medical Diagnostic Use. This system contains sufficient reagents for 200 reactions. Includes:

- 2 × 1.1ml   PowerQuant® 2X Master Mix
- 1 × 220µl   PowerQuant® 20X Primer/Probe/IPC Mix
- 1 × 150µl   PowerQuant® Male gDNA Standard
- 2 × 1.25ml   Water, Amplification Grade
- 2 × 1.5ml   PowerQuant® Dilution Buffer

PRODUCT	SIZE	CAT.#
PowerQuant® System	**800 reactions**	**PQ5008**

Not For Medical Diagnostic Use. This system contains sufficient reagents for 800 reactions. Includes:

- 8 × 1.1ml   PowerQuant® 2X Master Mix
- 4 × 220µl   PowerQuant® 20X Primer/Probe/IPC Mix
- 3 × 150µl   PowerQuant® Male gDNA Standard
- 5 × 1.25ml   Water, Amplification Grade
- 8 × 1.5ml   PowerQuant® Dilution Buffer

Storage Conditions: Store the PowerQuant® System at −30°C to −10°C in a nonfrost-free freezer. Store the PowerQuant® Male gDNA Standard overnight at 4°C prior to the first use; **do not** refreeze. For short-term storage (less that 1 week), store the other components at +2°C to +10°C. Minimize the number of freeze-thaw cycles. The PowerQuant® 20X Primer/Probe/IPC Mix is light-sensitive and must be stored in the dark. Dilutions of the PowerQuant® Male gDNA Standard in PowerQuant® Dilution Buffer can be stored at 4°C for up to 1 week.

Available Separately

PRODUCT	SIZE	CAT.#
PowerQuant® Calibration Kit	**1 each**	**DS1221**

Not for Medical Diagnostic Use. Includes:

- 60µl   PowerQuant® Calibration Standard, FAM
- 60µl   PowerQuant® Calibration Standard, CFG540
- 60µl   PowerQuant® Calibration Standard, TMR
- 60µl   PowerQuant® Calibration Standard, Q670
- 60µl   PowerQuant® Calibration Standard, CXR
- 30ml   PowerQuant® Calibration Buffer

Storage Conditions: Store the PowerQuant® System Calibration Kit at −30°C to −10°C in a nonfrost-free freezer. For short-term storage (less than 1 week), the kit can be stored at +2°C to +10°C. Minimize the number of freeze-thaw cycles. The PowerQuant® Calibration Kit is light-sensitive and must be stored in the dark.

3. General Considerations

The PowerQuant® System is extremely sensitive; take precautions to minimize contamination. We recommend storing the PowerQuant® reagents separately from DNA samples. We also recommend using clean designated work areas and separate pipettes for pre- and post-amplification steps to minimize the potential for cross-contamination between DNA samples and prevent carryover of nucleic acid from one run to the next. Wear a lab coat and protective eyewear. Wear gloves, and change them often. Prevent contamination by using aerosol-resistant pipette tips. Always include a no-template control (NTC) reaction to detect contamination. We recommend performing NTC reactions in duplicate.

Do not unseal reaction plates after amplification is complete. Unsealing the plates increases the risk of contaminating subsequent reactions with amplified product.

Always check to ensure that the instrument software's analysis settings are set correctly (see Sections 6, 7 and 8).

4. Instrument Calibration

Before using the PowerQuant® System, you must calibrate the instrument for FAM, CAL Fluor® Gold 540, TMR, Quasar® 670 and CXR dyes. To distinguish these dyes from dyes with the same name already calibrated on your instrument, we recommend appending dye names with the prefix "PQ" for the PowerQuant® System: PQ_FAM (for the FAM dye), PQ_CFG540 (for CAL Fluor® Gold 540), PQ_TMR (for TMR), PQ_Q670 (for Quasar® 670) and PQ_CXR (for CXR).

The dyes in the PowerQuant® System Calibration Kit are supplied at 100X. The PowerQuant® Calibration Buffer is supplied as a diluent when preparing a spectral calibration plate for each dye.

Prior to performing PowerQuant® System dye calibration with the Applied Biosystems® 7500 Real-Time PCR System, we recommend performing the Regions of Interest (ROI) calibration, background calibration and optical calibration as described in the *Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide*.

Prior to performing PowerQuant® System dye calibration with the QuantStudio™ 5 Real-Time PCR System, we recommend verifying the factory installed calibrations have been performed and are current with your laboratory instrument maintenance schedule. Refer to the *QuantStudio™ 5 Real-Time PCR Instrument User Guide for Human Identification* for additional information.

Materials to Be Supplied by the User

- PowerQuant® Calibration Kit (Cat.# DS1221)
- Applied Biosystems® 7500 Real-Time PCR System, Applied Biosystems® 7500 Real-Time PCR System for Human Identification or QuantStudio™ 5 Real-Time PCR System
- Applied Biosystems® instrument-related consumables (e.g., MicroAmp® optical 96-well reaction plate and MicroAmp® optical adhesive film)
- sterile, aerosol-resistant pipette tips
- tubes (5ml or larger) for diluting the PowerQuant® Calibration Standards

4.A. Calibration Plate Setup

1. Thaw the five PowerQuant® Calibration Standards (FAM, CFG540, TMR, Q670 and CXR) and PowerQuant® Calibration Buffer.

2. Vortex each PowerQuant® Calibration Standard and PowerQuant® Calibration Buffer for 10 seconds to mix. Do not centrifuge the PowerQuant® Calibration Standards after mixing.

3. Dilute each PowerQuant® Calibration Standard 100-fold in PowerQuant® Calibration Buffer in a separate tube as described below.

Reagent	FAM	CFG540	TMR	Q670	CXR
PowerQuant® Calibration Standard, FAM	22µl	–	–	–	–
PowerQuant® Calibration Standard, CFG540	–	22µl	–	–	–
PowerQuant® Calibration Standard, TMR	–	–	22µl	–	–
PowerQuant® Calibration Standard, Q670	–	–	–	22µl	–
PowerQuant® Calibration Standard, CXR	–	–	–	–	22µl
PowerQuant® Calibration Buffer	2,178µl	2,178µl	2,178µl	2,178µl	2,178µl
Total volume	2,200µl	2,200µl	2,200µl	2,200µl	2,200µl

4. Vortex each diluted PowerQuant® Calibration Standard for 10 seconds to mix. **Do not** centrifuge the diluted standards after mixing.

5. Reserve a separate MicroAmp® optical 96-well reaction plate for each PowerQuant® Calibration Standard. Record the bar code number or label the side of the plate skirt to indicate the calibration standard in the plate.

   Note: Wear gloves at all times when handling the plate, and take care to avoid touching the plate wells and MicroAmp® optical adhesive film unnecessarily. Handle the plate by the edges, and avoid touching the bottom of the plate.

6. Dispense 20µl of diluted PowerQuant® Calibration Standard, FAM, into all 96 wells of the plate reserved for the FAM dye. Repeat this step for each of the other PowerQuant® Calibration Standards by adding 20µl of the diluted calibration standard to each well of the appropriate dye-specific plate.

7. Seal each plate with MicroAmp® optical adhesive film. Protect the plates from exposure to light.

8. Centrifuge plates briefly. To complete the calibration, proceed to Section 4.B for the Applied Biosystems® 7500 Software, Version 2.0.6, Section 4.C for the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2. or Section 4.D for the QuantStudio™ 5.

   Note: After calibration, store the calibration plates protected from light at −20°C for up to 4 months. Reuse the plates when re-analysis is necessary.

4.B. Applied Biosystems® 7500 Real-Time PCR System Calibration Protocol for Applied Biosystems® 7500 Software, Version 2.0.6

The following instructions are for the Applied Biosystems® 7500 Real-Time PCR System instrument using the Applied Biosystems® 7500 Software, Version 2.0.6. This instrument is also compatible with HID Real-Time PCR Analysis Software, Version 1.1 or 1.2; to use this software, see the instructions provided in Section 4.C.

1. Turn on the computer associated with the Applied Biosystems® 7500 Real-Time PCR System.

2. Turn on the Applied Biosystems® 7500 Real-Time PCR System.

3. Open the Applied Biosystems® 7500 Software, Version 2.0.6.

4. From the Instrument menu at the top of the screen, select **Instrument Maintenance Manager**.

5. Select the Dye section from the left panel.

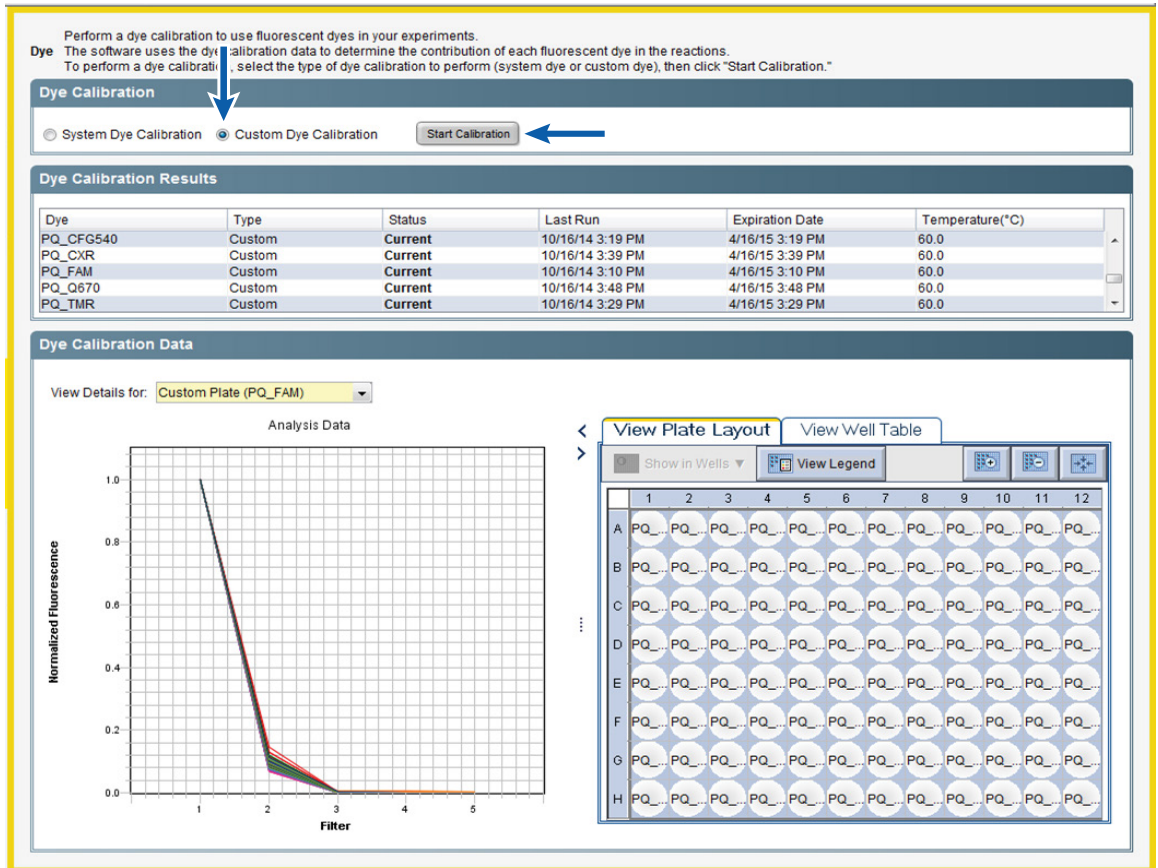6. Choose "Custom Dye Calibration", and select **Start Calibration** (Figure 1).

Figure 1. The Custom Dye Calibration option and Start Calibration button.

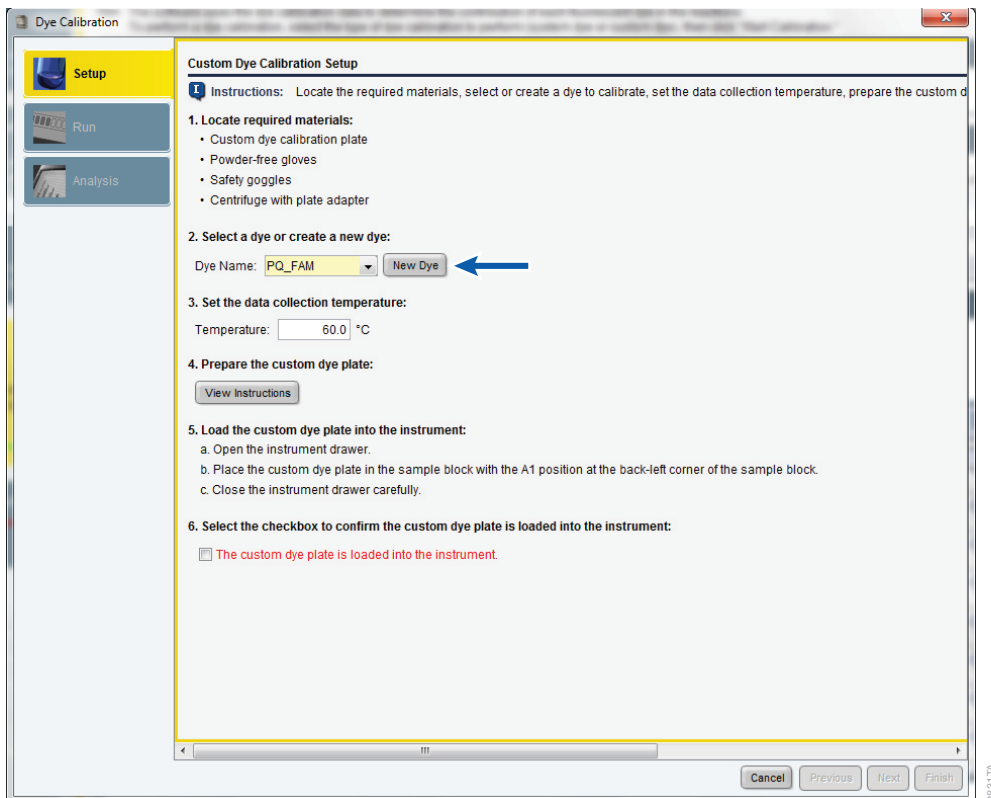7.   In the 'Dye Calibration' window that appears, select the **New Dye** button (Figure 2).

Figure 2. Calibrating a new dye.

8. In the 'Dye Library' window that appears, select the **New** button (Figure 3).
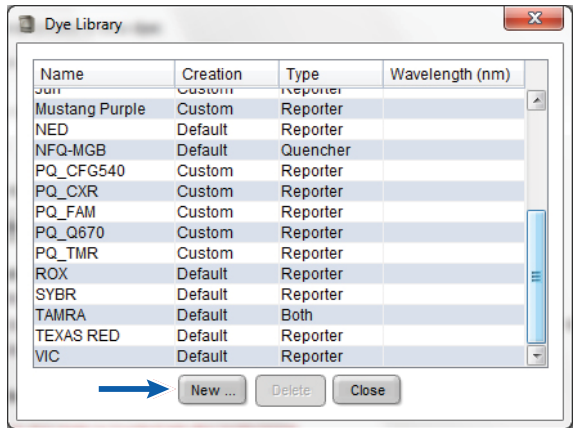

Figure 3. The New button in the 'Dye Library' window.

9. Enter "PQ_FAM" as the new dye name, select the **Reporter** radio button under "Type" and then select **OK** (Figure 4). Repeat this process to define the CFG540 (define as PQ_CFG540), TMR (define as PQ_TMR), Q670 (define as PQ_Q670) and CXR (define as PQ_CXR) dyes.
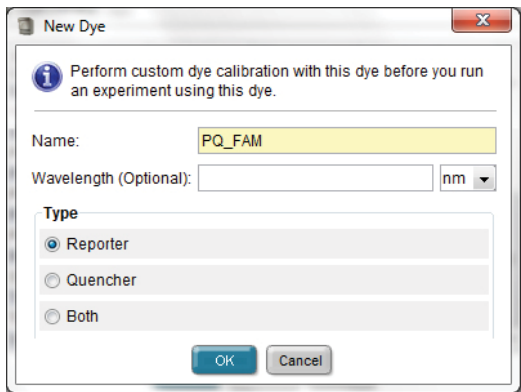

Figure 4. Naming the new dye.

10. In the Custom Dye Calibration Setup window, select **PQ_FAM** from the 'Dye Name' drop-down list (Figure 5).
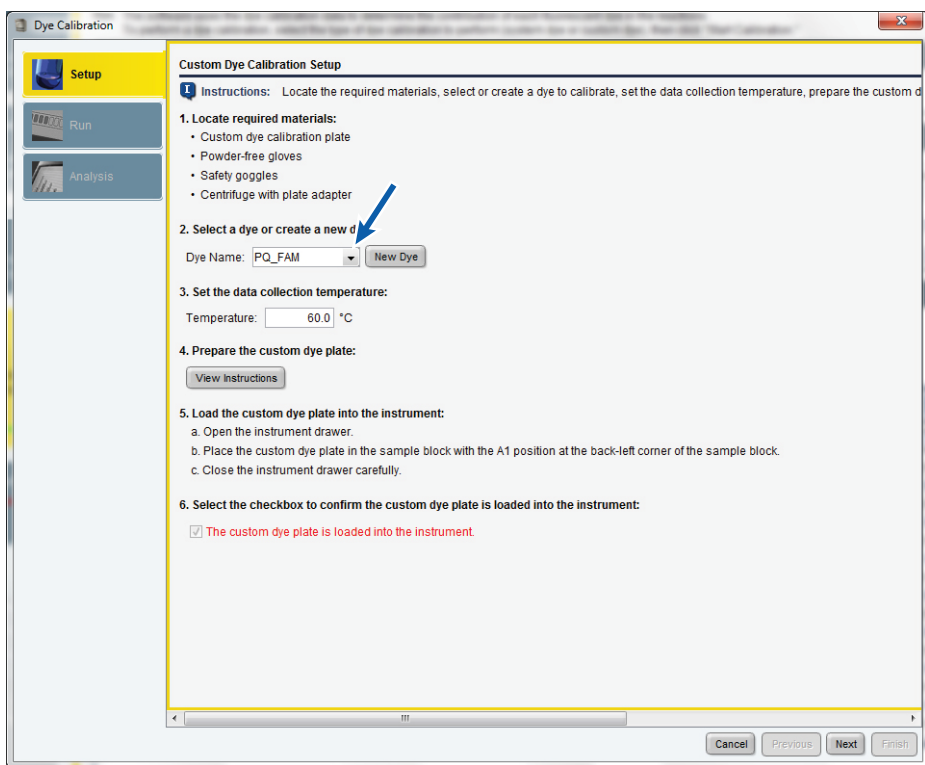

Figure 5. Selecting the dye name from the drop-down list.

11. Load the plate prepared in Section 4.A for the PowerQuant® Calibration Standard, FAM, into the instrument.

12. Check the box labeled "The custom dye plate is loaded into the instrument" (Figure 5).

13. Select **Next**, then **Start Run**.

14. Once the calibration run is complete, select **Next** in the bottom right corner of the window. The software will display the spectra and indicate whether the spectra are acceptable (i.e., which spectra passed) under Status.

15. Select **Finish**, and repeat Steps 10 through 13 with each plate of PowerQuant® Calibration Standards to calibrate the other dyes: CFG540 (PQ_CFG540), TMR (PQ_TMR), Q670 (PQ_Q670) and CXR (PQ_CXR).

    Notes:
    1. FAM should give highest signal in Filter 1, CFG540 should give highest signal in Filter 2, TMR should give highest signal in Filter 3, Q670 should give highest signal in Filter 5 and CXR should give highest signal in Filter 4. Figure 1 shows an example of a dye calibration spectrum (PQ_FAM) across all five filters. Representative images of dye calibration spectra for all the five dyes are shown in Appendix 12.B.
    2. After calibration, store the calibration plates protected from light at −20°C for up to 4 months. Reuse the plates when re-analysis is necessary.

4.C. Applied Biosystems® 7500 Real-Time PCR System Calibration Protocol for HID Real-Time PCR Analysis Software, Version 1.1 or 1.2

The following instructions are for the Applied Biosystems® 7500 Real-Time PCR System instrument using the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2.

1. Turn on the computer associated with the Applied Biosystems® 7500 Real-Time PCR System for Human Identification.

2. Turn on the Applied Biosystems® 7500 Real-Time PCR System for Human Identification.

3. Open the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2.

4. From the Instrument menu at the top of the screen, select **Instrument Maintenance Manager**.

5. Select the Dye section from the left panel.

6. Choose "Custom Dye Calibration", and select **Start Calibration** (Figure 6).
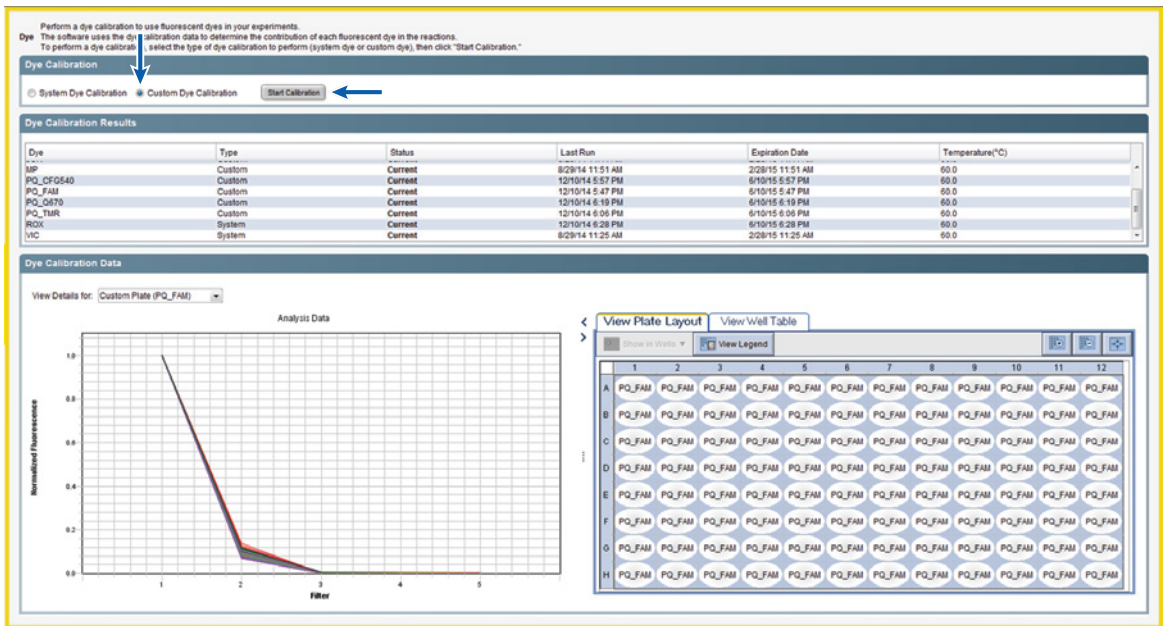

Figure 6. The Custom Dye Calibration option and Start Calibration button.

7. In the 'Dye Calibration' window that appears, select the **New Dye** button (Figure 7).
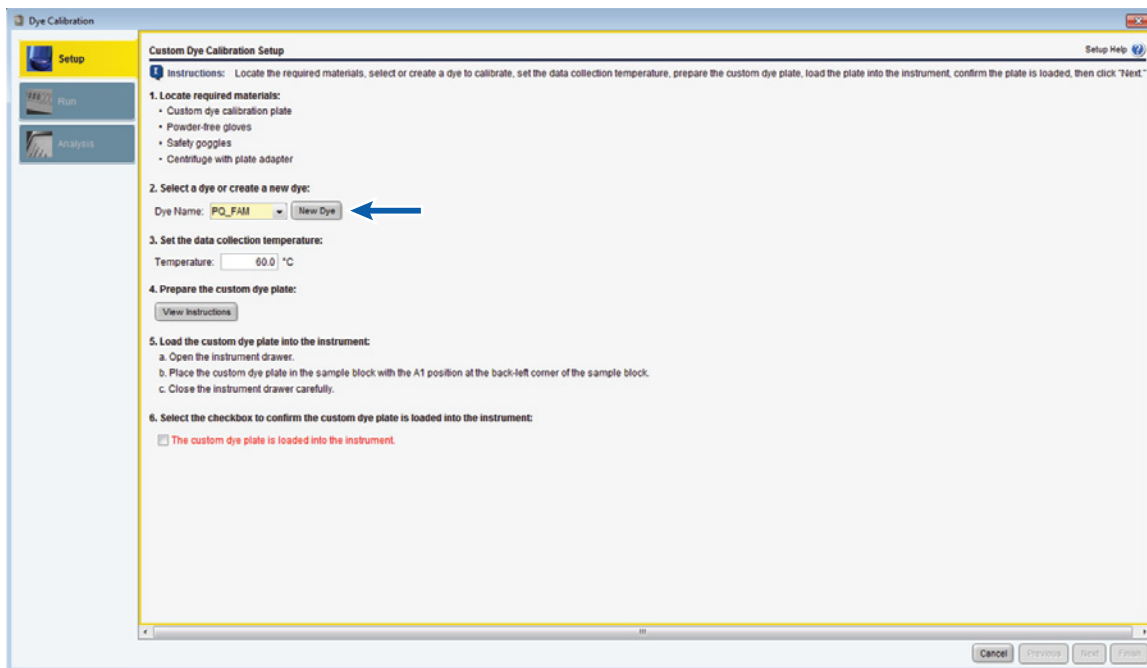

Figure 7. Calibrating a new dye.

8. In the 'Dye Library' window that appears, select the **New** button (Figure 8).
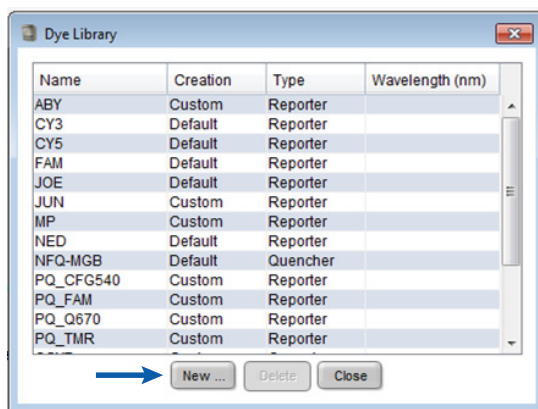

Figure 8. The New button in the 'Dye Library' window.

4.C. **Applied Biosystems® 7500 Real-Time PCR System Calibration Protocol for Applied Biosystems® HID Real-Time PCR Analysis Software, Version 1.1 or 1.2 (continued)**

9. Enter "PQ_FAM" as the new dye name, select the **Reporter** radio button under Type and then select **OK** (Figure 9). Repeat this process to define the CFG540 (define as PQ_CFG540), TMR (define as PQ_TMR) and Q670 (define as PQ_Q670) dyes.
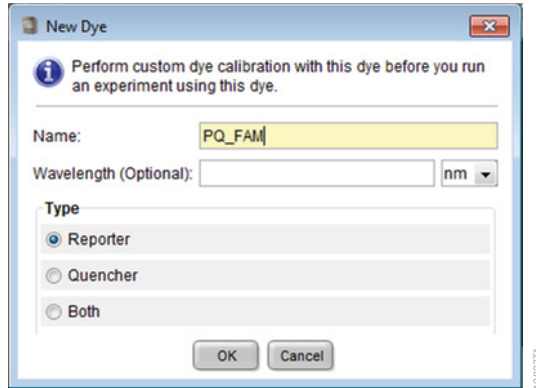

Figure 9. Naming the new dye.

10. In the Custom Dye Calibration Setup window, select **PQ_FAM** from the 'Dye Name' drop-down list (Figure 10).
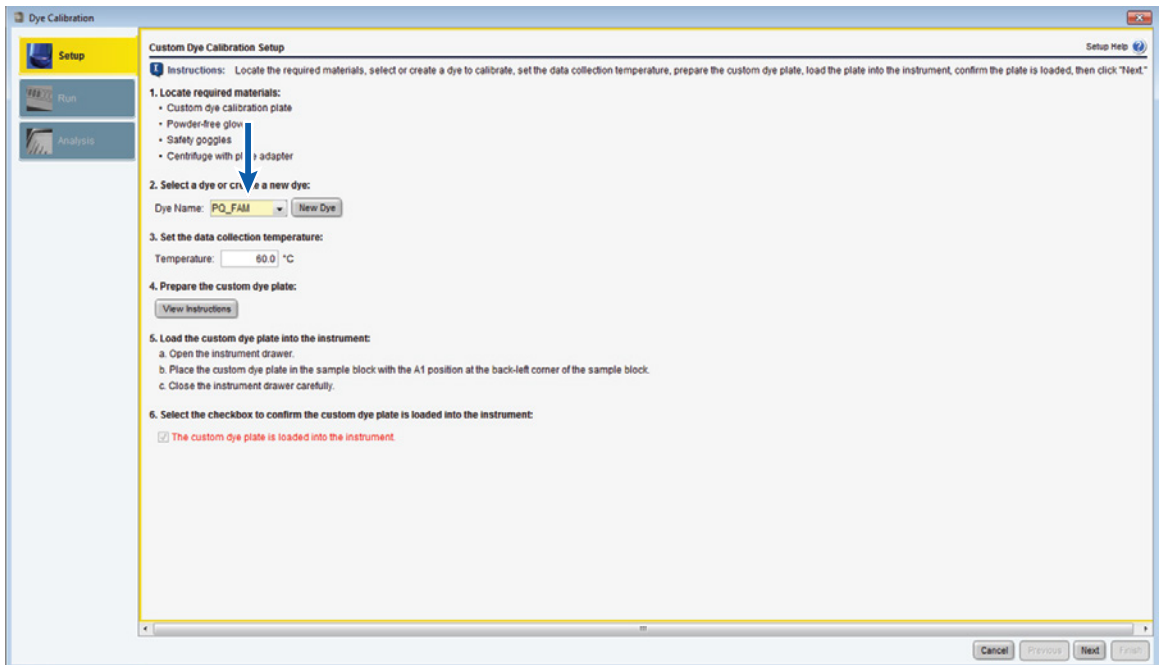

Figure 10. Selecting the dye name from the drop-down list.

11. Load the plate prepared in Section 4.A for the PowerQuant® Calibration Standard, FAM, into the instrument.

12. Check the box labeled "The custom dye plate is loaded into the instrument" (Figure 10).

13. Select **Next**, then **Start Run**.

14. Once the calibration run is complete, select **Next** in the bottom right corner of the window. The software will display the spectra and indicate whether the spectra are acceptable (i.e., which spectra passed) under Status.

15. Select **Finish**, and repeat Steps 10 through 13 with each plate of PowerQuant® Calibration Standards to calibrate the other dyes: CFG540 (PQ_CFG540), TMR (PQ_TMR) and Q670 (PQ_Q670).

    Notes:

    1. FAM should give highest signal in Filter 1, CFG540 should give highest signal in Filter 2, TMR should give highest signal in Filter 3 and Q670 should give highest signal in Filter 5. Figure 6 shows an example of a dye calibration spectrum (PQ_FAM) across all five filters. See Appendix 12.B for representative spectral images of all five dyes.

    2. After calibration, store the calibration plates protected from light at −20°C for up to 4 months. Reuse the plates when re-analysis is necessary.

16. To calibrate CXR, in the Dye screen select "System Dye Calibration", and select the **Start Calibration** button (Figure 11).
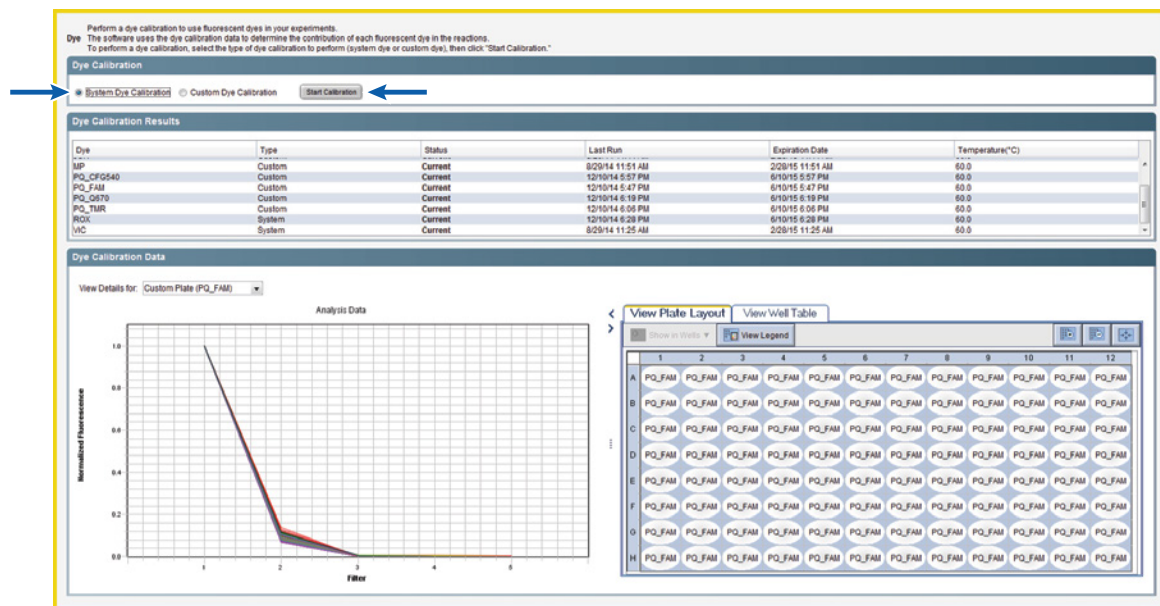

Figure 11. Selecting "System Dye Calibration" and the Start Calibration button.

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17. The Overview window opens. Select **Next**.

18. The Materials Required window opens. Check only the box for "ROX", and select **Next** (Figure 12).
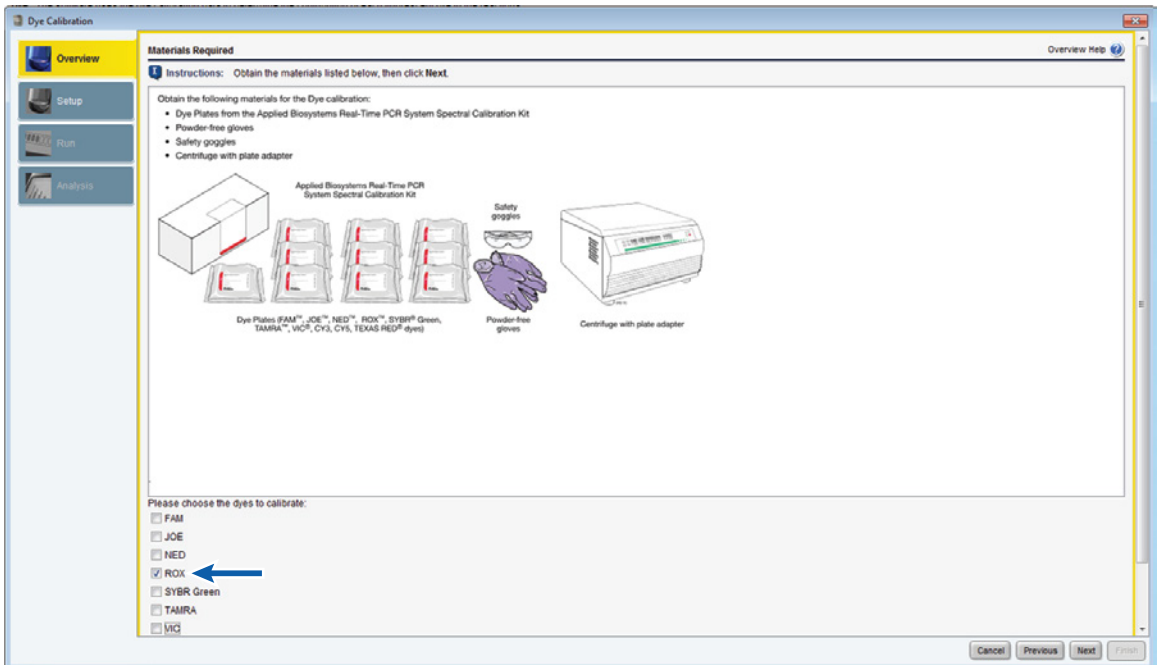
Figure 12. Choosing the dye to calibrate.

19. The 'Preparing the Plate' window opens. Select **Next**.

20. The 'Loading the Plate' window opens. Select **Next**. Load the plate with the PowerQuant® Calibration Standard, CXR, into the instrument.

21. Select **Start Run**.

22. Once the calibration run is complete, select **Next** in the bottom right corner of the window. The software will display the spectrum and indicate whether the spectrum is acceptable (i.e., spectrum passed) under Status.

23. Select **Next**.

24. Select **Finish Calibrating ROX**.

4.D. QuantStudio™ 5 Real-Time PCR System Calibration Protocol

1. Enter the Settings menu on the QuantStudio™ 5 home screen. Select the Maintenance and Service option on the subsequent screen.

2. Select **Calibrations>Custom>Custom Dye**.

3. Select **Add Custom Dye**.

4. Enter and save the following dye names: "PQ_FAM", "PQ_CFG540", "PQ_TMR", "PQ_Q670" and "PQ_CXR".

5. Confirm that "Reporter" is selected as the Type for each dye.

6. Load the appropriate dye calibration plate onto the instrument. You can open and close the tray door by touching the **Eject** icon on the home screen.

7. Select the corresponding dye you wish to calibrate in the Custom Dye menu. Enter "60°C" for the calibration temperature.

8. Select the **Start** button. Each dye calibration will require approximately 3 minutes to complete.

9. "Calibration Complete" and "View Results" will display at the end of each calibration run. Refer to the next section for information on reviewing and evaluating the dye calibration results. Unload the plate and repeat the calibration process for each of the PowerQuant® calibration standard dye plates.

Evaluating the PowerQuant® Dye Calibration Spectra

1. Select **View Results>Details**.

   Note: The calibration spectra will be displayed on the QuantStudio™ 5 instrument screen.

2. Review the dye spectrum plot for each calibration run. Examples of passing calibration spectra for each of the PowerQuant® dyes are provided in Appendix 12.B.

3. Select **Accept Results** to confirm that the calibration result is acceptable. A second confirmation will appear in which you will have to **Accept Results** again. This action will save the calibration data in the instrument.

   You can select **Reject Results** if the results are unacceptable.

   Note: You can test the calibration plate again. For further calibration troubleshooting please refer to the Troubleshoot Calibration Failure section in Appendix A of the *QuantStudio™ 5 Real-Time PCR Instrument User Guide for Human Identification*.

5. Reaction Plate Setup

When using the PowerQuant® System for the first time, we recommend completing the steps in Section 6, 7 or 8 prior to assembling the reactions. Once you are familiar with instrument setup, the instrument can be programmed after reaction setup.

Materials to Be Supplied By the User

- sterile, aerosol-resistant pipette tips
- pipettes dedicated to pre-amplification work
- Applied Biosystems® instrument-related consumables (e.g., MicroAmp® optical 96-well reaction plate and MicroAmp® optical adhesive film)
- PowerQuant® Dilution Buffer or TE⁻⁴ buffer (pH 8.0)
- tubes to prepare the DNA standard dilution series

This protocol uses 2µl of template DNA per reaction. The template volume can be increased as long as the final reaction volume remains constant. The template DNA volume and final reaction volume must be the same for both the DNA standards and unknown DNA samples. This allows the DNA standards to be considered as a concentration (in ng/µl) instead of input amount in ng (concentration in ng/µl × volume). We recommend performing duplicate amplifications of each DNA standard and each unknown DNA sample. Performing duplicate analysis of each sample DNA and averaging the quantification results can reduce variability.

The four-point serial dilutions prepared in this section include DNA standards in the range of 3.2pg/µl to 50ng/µl. These values can be modified if desired. Section 12.C describes alternative serial dilutions of the PowerQuant® Male gDNA Standard to prepare five-, six- and seven-point standard curves.

When diluting the PowerQuant® Male gDNA Standard or unknown DNA samples, use the PowerQuant® Dilution Buffer or TE⁻⁴ buffer (pH 8.0); do not use water as a diluent.

Note: We recommend changing gloves often, especially after handling high-concentration DNA.

5.A. Serial Dilution of the PowerQuant® Male gDNA Standard

Multiple freeze-thaw cycles of the PowerQuant® Male gDNA Standard can increase variability in the standard curve. Store the PowerQuant® Male gDNA Standard at 4°C overnight before the first use, and vortex thoroughly prior to each use. We recommend long-term storage at 4°C.

Perform serial 25-fold dilutions of the PowerQuant® Male gDNA Standard, and then amplify these dilutions to create four-point standard curves to determine the concentration of autosomal, Y and degradation targets in the unknown DNA samples. Accurate serial dilution of the PowerQuant® Male gDNA Standard is essential to accurately quantify unknown DNA samples; carefully mix and pipet each DNA standard dilution.

The PowerQuant® Dilution Buffer or TE⁻⁴ buffer (pH 8.0) can be used as the diluent for the PowerQuant® Male gDNA Standard. Serial dilutions of the PowerQuant® Male gDNA Standard prepared with PowerQuant® Dilution Buffer can be stored for up to 1 week at 4°C. Serial dilutions prepared with TE⁻⁴ buffer (pH 8.0) should be prepared fresh daily.

1. Ensure that the PowerQuant® Male gDNA Standard was stored at 4°C overnight. If necessary, thaw the PowerQuant® Dilution Buffer completely. Vortex the PowerQuant® Male gDNA Standard three times at high speed for 10 seconds each time.

   Note: After the initial thaw, store the PowerQuant® Male gDNA Standard and PowerQuant® Dilution Buffer at 4°C.

2. Label three tubes with the following concentrations: 2ng/µl, 0.08ng/µl and 0.0032ng/µl.

3. Prepare fresh serial dilutions of the PowerQuant® Male gDNA Standard as indicated in Table 1. Vortex each dilution for 10 seconds prior to removing an aliquot for the next dilution. Change pipette tips between dilutions.

Table 1. Serial Dilution of the PowerQuant® Male gDNA Standard (25-Fold Dilutions).

DNA Concentration	Volume of PowerQuant® Male gDNA Standard	Volume of PowerQuant® Dilution Buffer
50ng/µl	Use undiluted PowerQuant® Male gDNA Standard	0µl
2ng/µl	4µl of undiluted PowerQuant® Male gDNA Standard	96µl
0.08ng/µl	4µl of 2ng/µl dilution	96µl
0.0032ng/µl	4µl of 0.08ng/µl dilution	96µl

Notes:

1. We recommend performing duplicate amplifications of each DNA standard.

2. Change gloves after handling high-concentration DNA such as the PowerQuant® Male gDNA Standard.

3. Serial dilutions of PowerQuant® Male gDNA Standard prepared with the PowerQuant® Dilution Buffer can be stored for up to 1 week at 4°C. Do not store dilutions prepared with TE⁻⁴ buffer (pH 8.0).

5.B. Reaction Setup

Include a no-template control reaction for each set of reactions. Add 2µl of TE^{-4} buffer (pH 8.0) or Water, Amplification Grade, to these reactions instead of template DNA. No amplification product (i.e., DNA concentration <1.0pg per 2µl input volume) should be detected in the NTC reaction. A DNA concentration of >1.0pg per 2µl input volume for the NTC reaction indicates the presence of contaminating DNA.

Sample DNA may be diluted in TE^{-4} buffer (pH 8.0) if desired.

Note: The PowerQuant® System is extremely sensitive. The NTC reaction may yield amplification products in the subpicogram range. We recommend that you perform duplicate amplifications of the NTC reaction.

1. Thaw the PowerQuant® 2X Master Mix, PowerQuant® 20X Primer/Probe/IPC Mix and Water, Amplification Grade, completely at room temperature.

2. Vortex the PowerQuant® 2X Master Mix and PowerQuant® 20X Primer/Probe/IPC Mix for 10 seconds to mix. Do not centrifuge after mixing as this may cause the primers and probes to be concentrated at the bottom of the tube.

3. Determine the number of reactions to be set up, including NTC reactions. Increase this number by 10–15% to compensate for pipetting error and reagent loss on sides of pipette tips. While this approach requires using a small amount of extra reagent, it ensures that enough reaction mix is prepared for all amplifications. Amplification of the unknown DNA samples and DNA standards using the same reaction mix is critical.

4. Use Table 2 to calculate the volume of each component required to prepare sufficient reaction mix for the number of reactions determined in Step 3.

Table 2. Preparation of Reaction Mix for DNA Quantification Using the PowerQuant® System.

Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
Water, Amplification Grade[1]	7µl	×		=	
PowerQuant® 2X Master Mix	10µl	×		=	
PowerQuant® 20X Primer/Probe/IPC Mix	1µl	×		=	
Final volume	18µl				

[1]The volume of water given here assumes 2µl of template per 20µl reaction. If the volume of template is different, adjust the volume of water accordingly.

5. Prepare the reaction mix by combining the volumes of Water, Amplification Grade, PowerQuant® 2X Master Mix and PowerQuant® 20X Primer/Probe/IPC Mix calculated in Step 4.

6. Vortex for 10 seconds to mix. Do not centrifuge after mixing.

7. Add 18µl of reaction mix to the reaction wells of a MicroAmp® optical 96-well reaction plate.

   Note: Wear gloves at all times when handling the plate, and take care to avoid touching the plate wells and MicroAmp® optical adhesive film unnecessarily. Handle the plate by the edges, and avoid touching the bottom of the plate.

8. Add 2µl of the PowerQuant® Male gDNA Standards prepared in Section 5.A or unknown DNA sample to the wells as shown in Figure 13.

	1	2	3	4	5	6	7	8	9	10	11	12
A	50ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
B	50ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
C	2ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
D	2ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
E	0.08ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
F	0.08ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
G	0.0032ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	NTC
H	0.0032ng/µl	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	NTC

Figure 13. An example of a 96-well plate that shows locations of the DNA standards (yellow), NTC reactions (blue) and unknown samples.

9. Add 2µl of TE^{-4} buffer (pH 8.0) or Water, Amplification Grade, to the NTC reactions.

10. Seal the plate with MicroAmp® optical adhesive film.

   Note: Ensure that all wells are adequately sealed to prevent evaporation during thermal cycling.

11. Centrifuge the plate briefly to collect the contents of each well at the bottom. The plate is ready for thermal cycling. Protect the plate from extended light exposure or elevated temperatures prior to thermal cycling.

   Note: Artifacts may occur if the time between amplification setup and the start of thermal cycling exceeds 2 hours.

6. Run Setup and Thermal Cycling Using the Applied Biosystems® 7500 Real-Time PCR System and Applied Biosystems® 7500 Software, Version 2.0.6

The following instructions are for the Applied Biosystems® 7500 Real-Time PCR System instrument using the Applied Biosystems® 7500 Software, Version 2.0.6. This instrument is also compatible with HID Real-Time PCR Analysis Software, Version 1.1 or 1.2; to use this software, use the instructions provided in Section 7.

A template file (.edt file) can be used to store the dye information, target names and tasks; well locations and DNA concentrations for the DNA standards; run method and analysis settings. A plate setup import file may be used to add sample names, well position and standard information to a run template. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

Amplification is complete in 1 hour.

6.A. Creating a Run Template

1. Turn on the computer associated with the Applied Biosystems® 7500 Real-Time PCR System.

2. Turn on the Applied Biosystems® 7500 Real-Time PCR System.

3. Open the Applied Biosystems® 7500 Software, Version 2.0.6.

4. Before using the PowerQuant® System, check that the instrument was calibrated using the PowerQuant® Calibration Kit (Section 4).

5. On the 'Home' screen under Set-Up, select the **Advanced Setup** icon. Alternatively, select the **New Experiment** button, and choose "Advanced Setup" from the drop-down menu.

6. In the Experiment Properties form (Figure 14), specify the following parameters:

    • Enter a name for the template in the Experiment Name field.

    • Select the instrument you are using to run the experiment by selecting **7500 (96 Wells)**.

    • Select the type of experiment you want to set up by selecting **Quantitation-Standard Curve**.

    • Select the reagents you want to use to detect the target sequence by selecting **TaqMan® Reagents**.

    • Select the ramp speed you want to use in the instrument run by selecting **Standard (~2 hours to complete run)**.
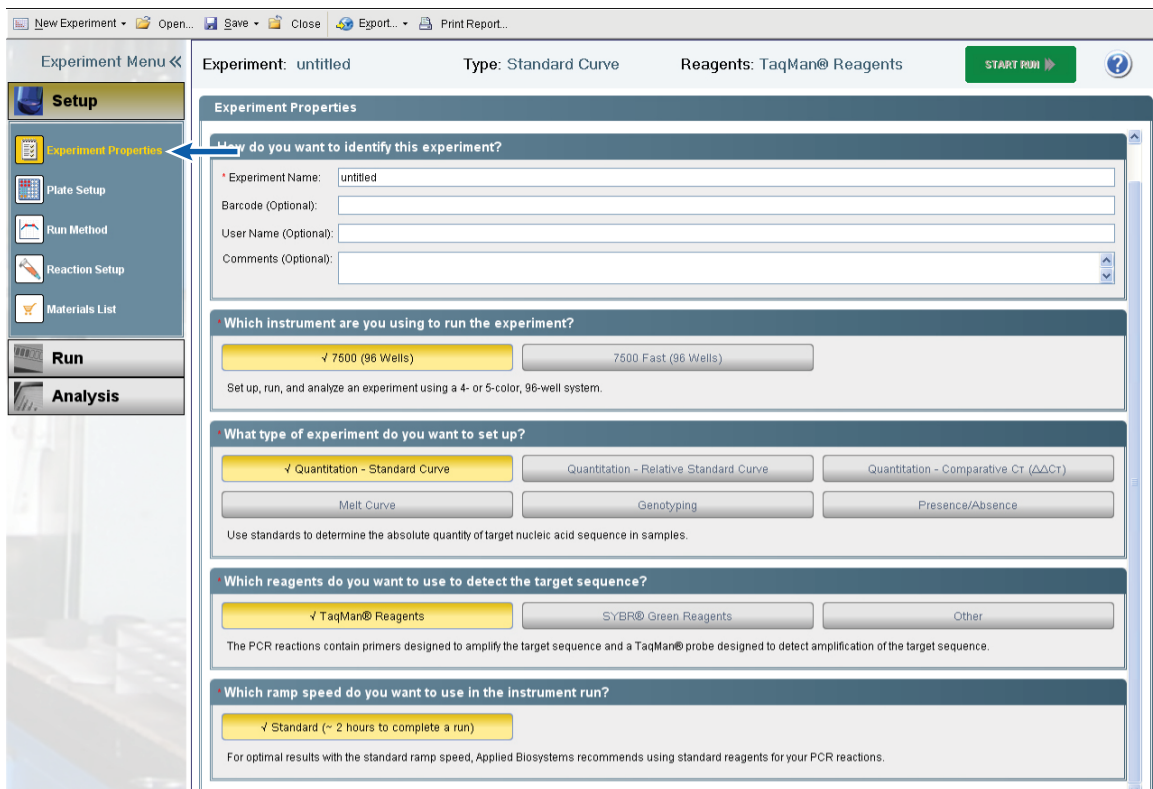
Figure 14. The Experiment Properties screen.

6.A. Creating a Run Template (continued)

7. Select "Plate Setup" from the Setup menu in the left panel. Select the 'Define Targets and Samples' tab. Select **Add New Target** (Figure 15) three additional times, and enter the following information to specify the targets:

Target Name	Reporter	Quencher
Autosomal	PQ_FAM	NFQ-MGB
Y	PQ_CFG540	NFQ-MGB
Degradation	PQ_Q670	NFQ-MGB
IPC	PQ_TMR	NFQ-MGB

Note: Target name identifiers are necessary for the PowerQuant® Analysis Software to recognize these targets.
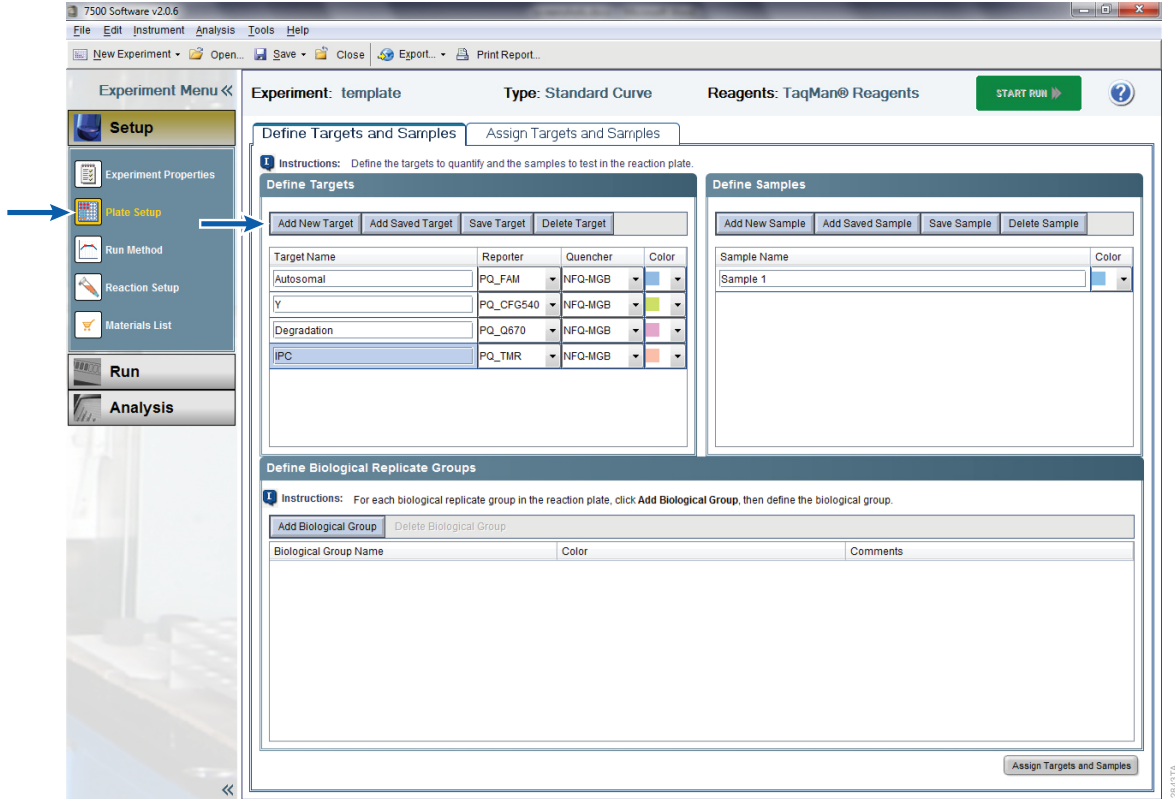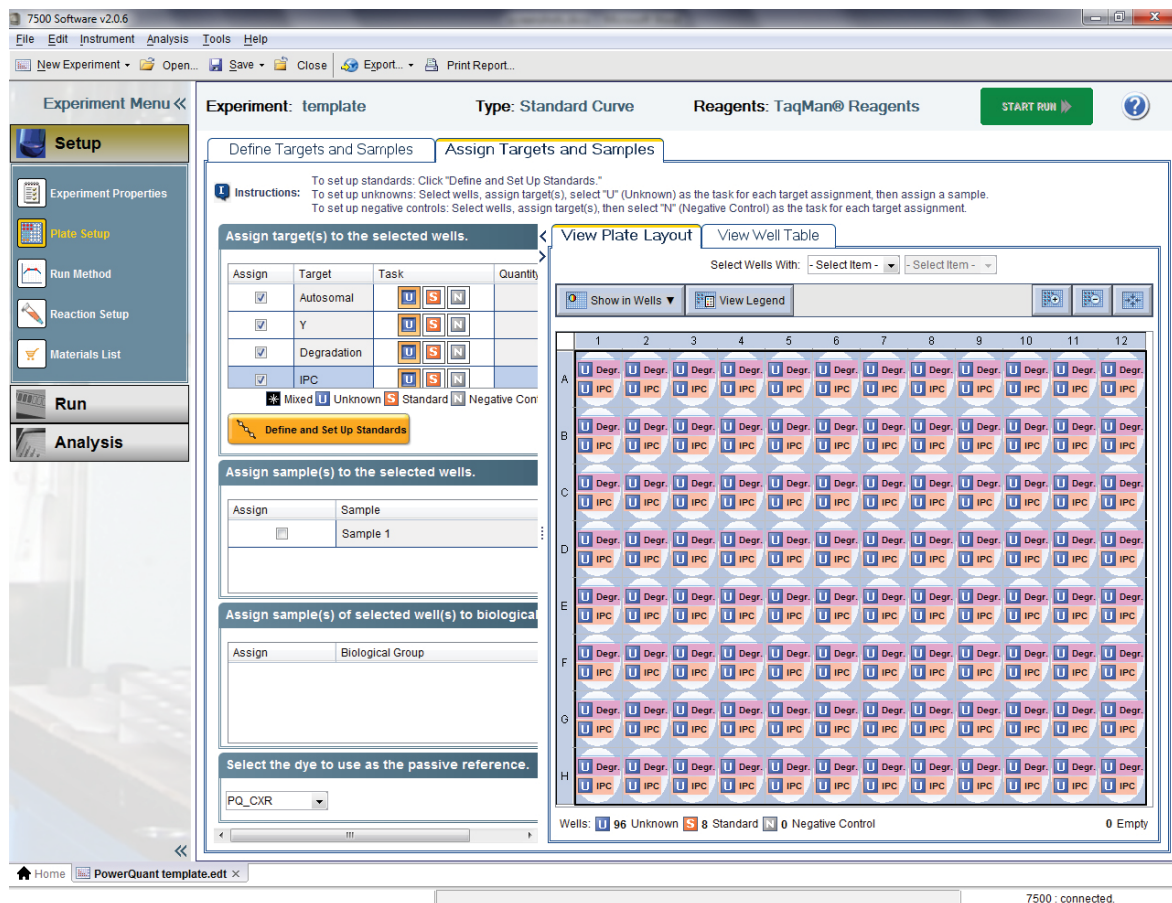
Figure 15. The Plate Setup Screen.

8. Select the 'Assign Targets and Samples' tab (Figure 16), and highlight the wells in use on the 'View Plate Layout' tab by dragging the pointer over the plate wells. Assign all four targets to the selected wells by selecting the boxes under "Assign".

Figure 16. Assigning targets to wells.

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6.A. Creating a Run Template (continued)

9. Highlight the wells containing the DNA standards on the 'View Plate Layout' tab, and select **S** as the Task for the autosomal, Y and degradation targets (Figure 17).

   Note: The Task for the IPC should be "U".

Figure 17. Assigning DNA concentrations to wells with DNA standards.

10. Enter the concentration for each DNA standard in the Quantity field without the unit of measure (i.e., enter 50 for 50ng/µl).

   Notes:

   1. Multiple wells with DNA standards at the same DNA concentration can be highlighted at the same time so that values need to be entered only once.

   2. The Define and Setup Standards tool will not work for assigning a standard curve to multiple targets in the same sample.

11. Select **PQ_CXR** from the drop-down menu labeled "Select the dye to use as the passive reference" (Figure 18).

Figure 18. The Assign Targets and Samples screen. Select **PQ_CXR** as the passive reference.

6.A. Creating a Run Template (continued)

12. From the Analysis menu at the top of the screen, select **Analysis Settings**.

13. On the 'CT Settings' tab (Figure 19) in the Select a Target panel, select the Autosomal target.

14. In the CT Settings for Autosomal section, uncheck the "Use Default Settings" box and uncheck the "Automatic Threshold" box. Enter "0.2" for the threshold. Leave the "Automatic Baseline" box checked.

15. Repeat this process for each target listed in the Select a Target panel using the following threshold values:
    - Autosomal: 0.2
    - Degradation: 0.2
    - IPC: 0.03
    - Y: 0.2

    Select **Apply Analysis Settings**.
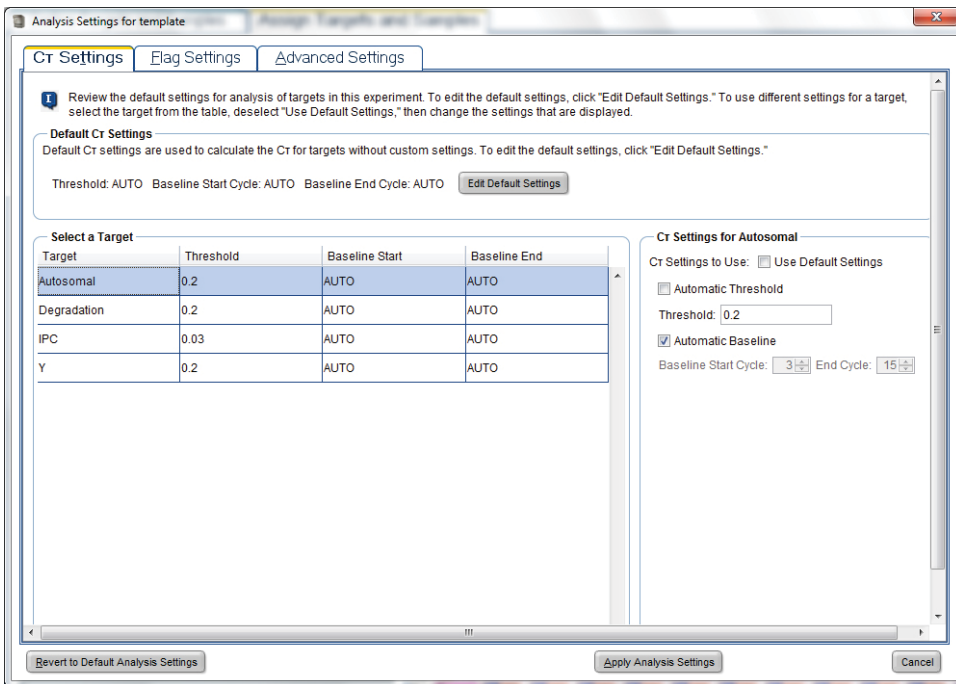
Figure 19. The 'Analysis Settings' window.

16. Select **Run Method** under Setup in the left panel. Modify the default run method as directed below:

- Enter "20" for "Reaction Volume per Well".
- Select the first Holding Stage, and click with the right button on the computer's mouse to bring up a menu. Select **Delete Selected** to delete this stage.
- Change the second Holding Stage to 2 minutes at 98°C.
- Enter "39" for "Number of Cycles" under Cycling Stage.
- Change Step 1 to 98°C for 15 seconds and Step 2 to 62°C for 35 seconds.
- Ensure that the **Data Collection On** icon is active for Step 2 of the Cycling Stage.
  Note: The **Data Collection On** icon is shown in the legend at the bottom of the screen.

The completed PowerQuant® System run method is shown in Figure 20.

Figure 20. The completed PowerQuant® System run method.

6.A. Creating a Run Template (continued)

17. From the File menu at the top of the screen, select **Save as Template**, and choose a location to save the file. The instrument setup and thermal cycling program can be saved as a template for future use. Use this .edt file as a template to create the experiment document as described in Section 6.B.

    Note: The template is saved as an .edt file.

6.B. Starting a Run

1. To create the experiment document, open the template file created in Section 6.A by selecting **Open** from the File menu at the top of the screen. Navigate to the .edt file, and select **Open**.

   Note: If the template file was just created, the .edt file will still be open.

2. Save the .edt template as a .eds file by selecting **Save As…** from the File menu at the top of the screen. Choose a location to save the file.

3. Highlight the unused wells, and deselect all of the targets.

4. To add sample names, select **Plate Setup** from the Setup menu in the left panel, and select the 'Define Targets and Samples' tab (Figure 15). Select **Add New Sample**, and enter the sample name in the section provided. Repeat for all samples.

5. When all sample names are entered, navigate to the 'Assign Targets and Samples' tab (Figure 16). Highlight the well or wells that contain replicates of the same sample, and check the Assign box adjacent to the corresponding sample name in the "Assign sample(s) to the selected wells" section of the 'Assign Targets and Samples' tab. Repeat until all samples are assigned.

   Note: Alternatively, you can use a plate setup import file by selecting **Import** from the File menu at the top of the screen. Navigate to the .txt file, select the file and select **Start Import**. Select **Yes** and continue with the import when prompted. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

6. If a sample was assigned to the incorrect well, select the well and uncheck the sample under "Assign sample(s) to the selected wells", and then assign the correct sample name.

7. Check that "PQ_CXR" is selected as the passive reference.

8. Save the .eds file.

9. Press the tray door.

10. Place the plate prepared in Section 5.B in the open tray door, and ensure that the plate sits correctly in the plate holder, with well A1 in the top left corner. Push the tray door to close, and immediately select **Start Run**. Run time is 1 hour.

6.C. Evaluating Standard Curves Using the Applied Biosystems® 7500 Software, Version 2.0.6

1. Select **Analysis** in the left side panel, and ensure that all wells to be analyzed are highlighted on the 'View Plate Layout' tab and that no targets are selected for unused wells.

2. Select **Analyze**.

3. To display standard curves, select **Standard Curve** from the Analysis menu in the left side panel. Display the standard curve for each target by selecting that target from the 'Target' drop-down menu (Figure 21). The standard curve parameters are located below the standard curve plot.

    Note: For more information about how the slope and R^2 values can be used to evaluate the standard curve, see Section 10.
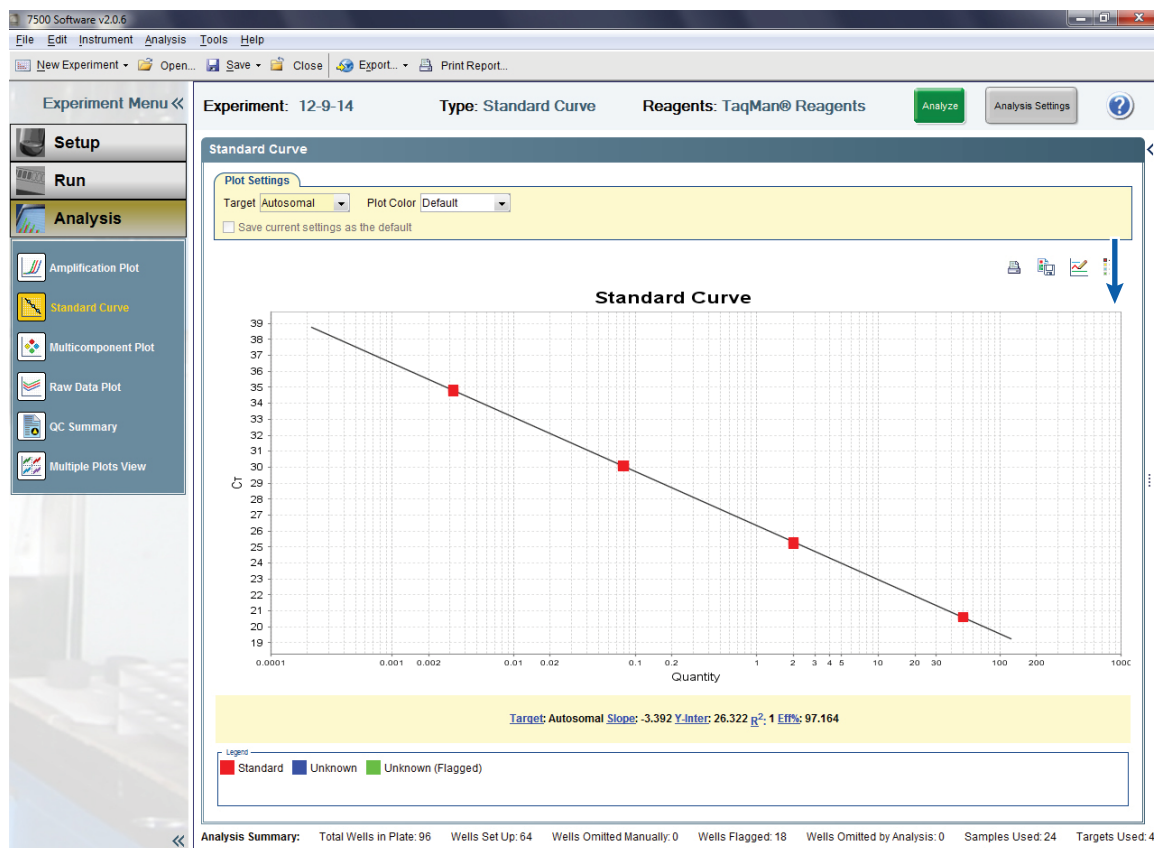
Figure 21. Displaying the standard curve for the autosomal target.

6.D. Exporting Analyzed Data from the Applied Biosystems® 7500 Software, Version 2.0.6

1. Ensure that all wells with data for export are highlighted in the plate map. Select **Export** from the toolbar.

2. On the 'Export Properties' tab of the 'Export Data' window (Figure 22), select the following Export Properties:
    - Select **Results** in the "Select data to export" section.
    - Select **One File** from the "Select one file or separate files" drop-down menu.
    - Select **.xls** as the File Type.
    - Specify the appropriate export file name.
    - Use the **Browse** button to select the file location.

    Select **Start Export**.
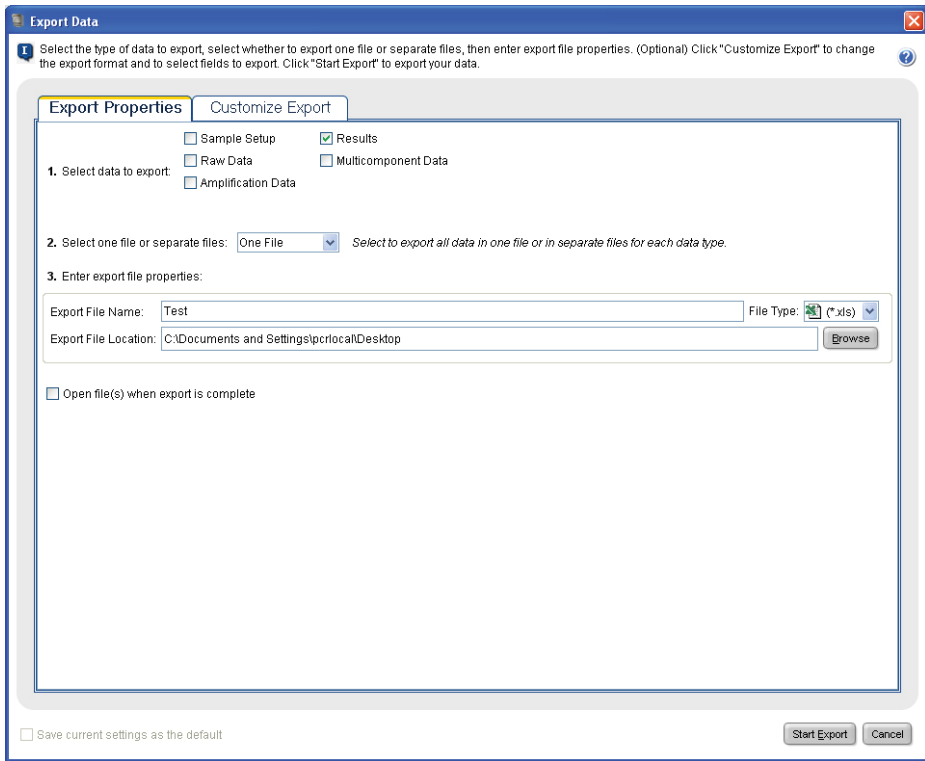
Figure 22. The 'Export Data' window.

3. In the 'Export Completed' window that appears, select **Close Export Tool**.

7.   **Run Setup and Thermal Cycling Using the Applied Biosystems® 7500 Real-Time PCR System for Human Identification and HID Real-Time PCR Analysis Software, Version 1.1 or 1.2**

The following instructions are for the Applied Biosystems® 7500 Real-Time PCR System for Human Identification with HID Real-Time PCR Analysis Software, Version 1.1 or 1.2.

A template file (.edt file) can be used to store the dye information, target names and tasks; well locations and concentrations for the DNA standards; run method and analysis settings. A plate setup import file may be used to add sample names, well position and standard information to a run template. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

Amplification is complete in 1 hour.

7.A.   Creating a Run Template

1.   Turn on the computer associated with the Applied Biosystems® 7500 Real-Time PCR System for Human Identification.

2.   Turn on the Applied Biosystems® 7500 Real-Time PCR System for Human Identification.

3.   Open the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2.

4.   Before using the PowerQuant® System, check that the instrument was calibrated with the PowerQuant® Calibration Kit (Section 4).

5.   On the 'Home' screen, select the **Custom Assays** button, or from the Assays menu select **Custom Assays**.

6.   Select **New Experiment** from the toolbar. Select "Advanced Setup" from the drop-down menu.

7.   In the Experiment Properties form, specify the following parameters (Figure 23):
   •   Enter a name for the template in the Experiment Name field.
   •   Select the instrument you are using to run the experiment by selecting **7500 (96 Wells)**.
   •   Select the type of experiment you want to set up by selecting **Quantitation-Standard Curve**.
   •   Select the reagents you want to use to detect the target sequence by selecting **TaqMan® Reagents**.
   •   Select the ramp speed you want to use in the instrument run by selecting **Standard (~2 hours to complete run)**.

7.A. Creating a Run Template (continued)
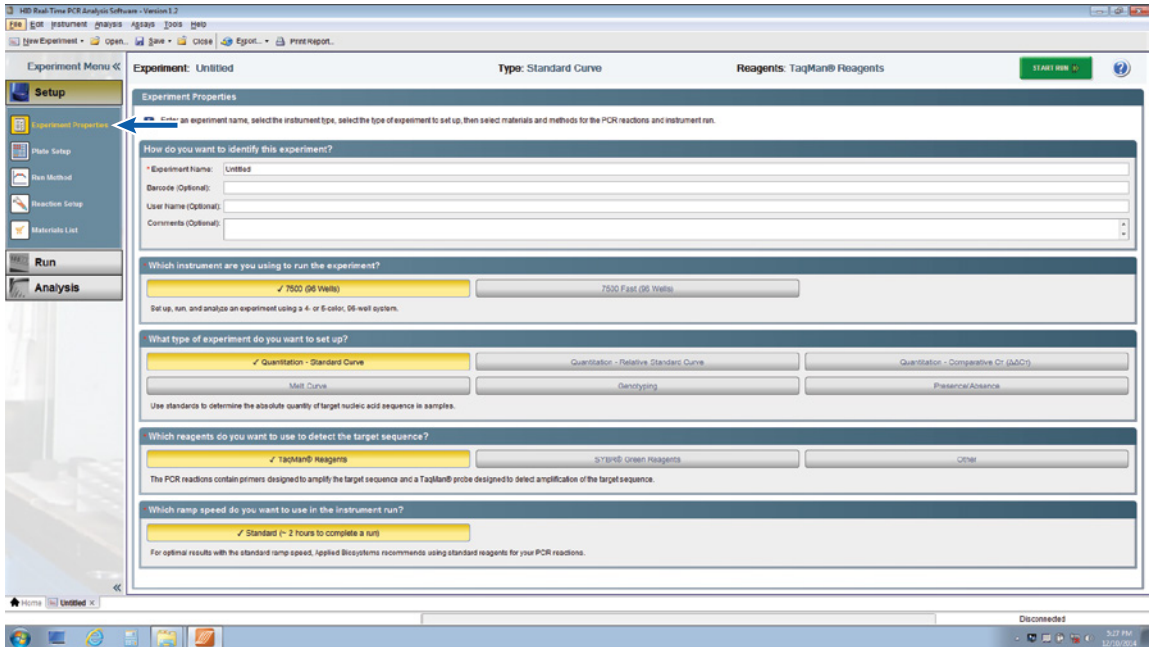
Figure 23. The Experiment Properties screen.

8. Select **Plate Setup** from the Setup menu in the left panel. Select the 'Define Targets and Samples' tab. Select **Add New Target** (Figure 24) three additional times, and enter the following information to specify the targets:

Target Name	Reporter	Quencher
Autosomal	PQ_FAM	NFQ-MGB
Y	PQ_CFG540	NFQ-MGB
Degradation	PQ_Q670	NFQ-MGB
IPC	PQ_TMR	NFQ-MGB

Note: Target name identifiers are necessary for the PowerQuant® Analysis Software to recognize these targets.
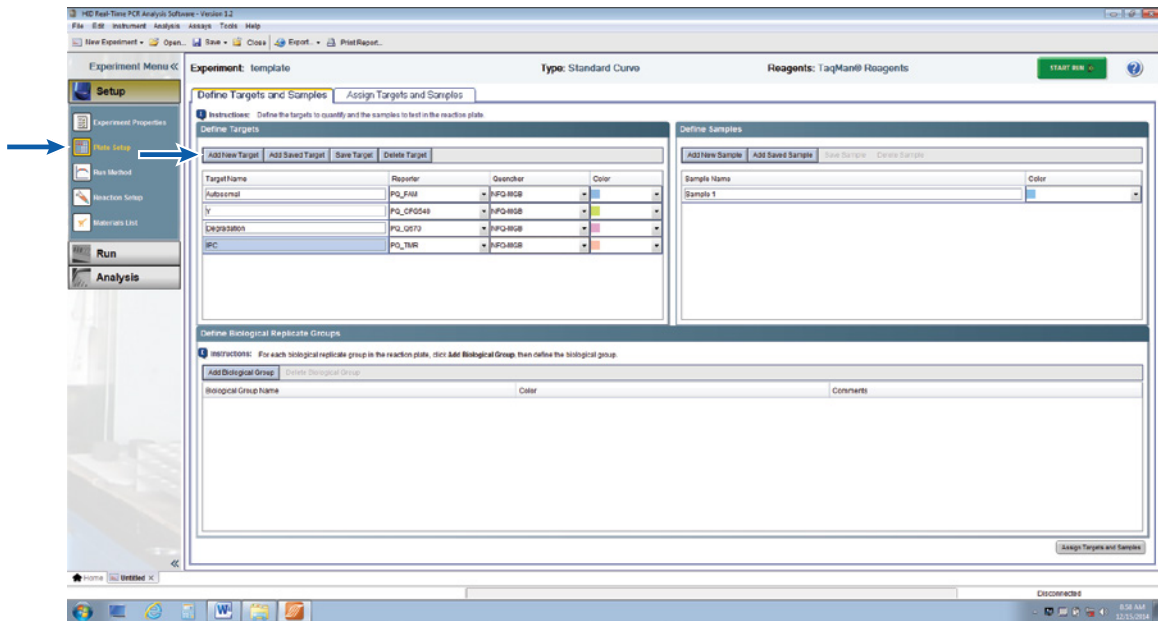
Figure 24. The Plate Setup screen.

9.   Select the 'Assign Targets and Samples' tab (Figure 25), and highlight the wells in use on the 'View Plate Layout' tab by dragging the pointer over the plate wells. Assign all four targets to the selected wells by selecting the boxes under "Assign".
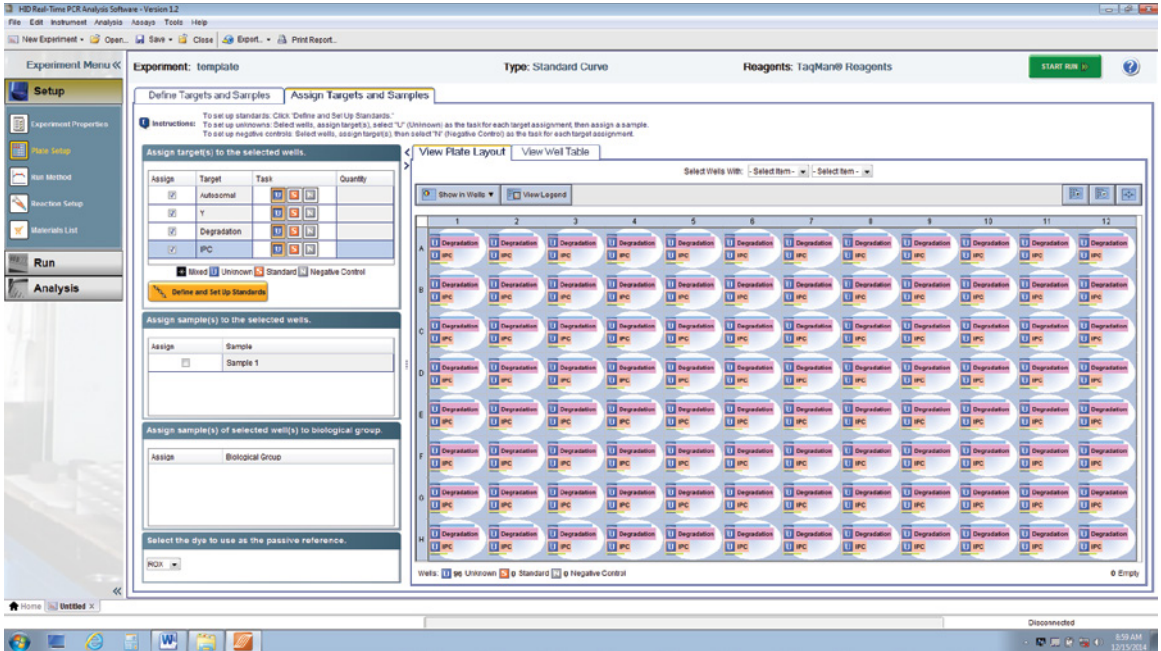
Figure 25. Assigning targets to wells.

10. Highlight the wells containing the DNA standards on the 'View Plate Layout' tab, and select **S** as the Task for the autosomal, Y and degradation targets (Figure 26).

    Note: The Task for the IPC should be "U".
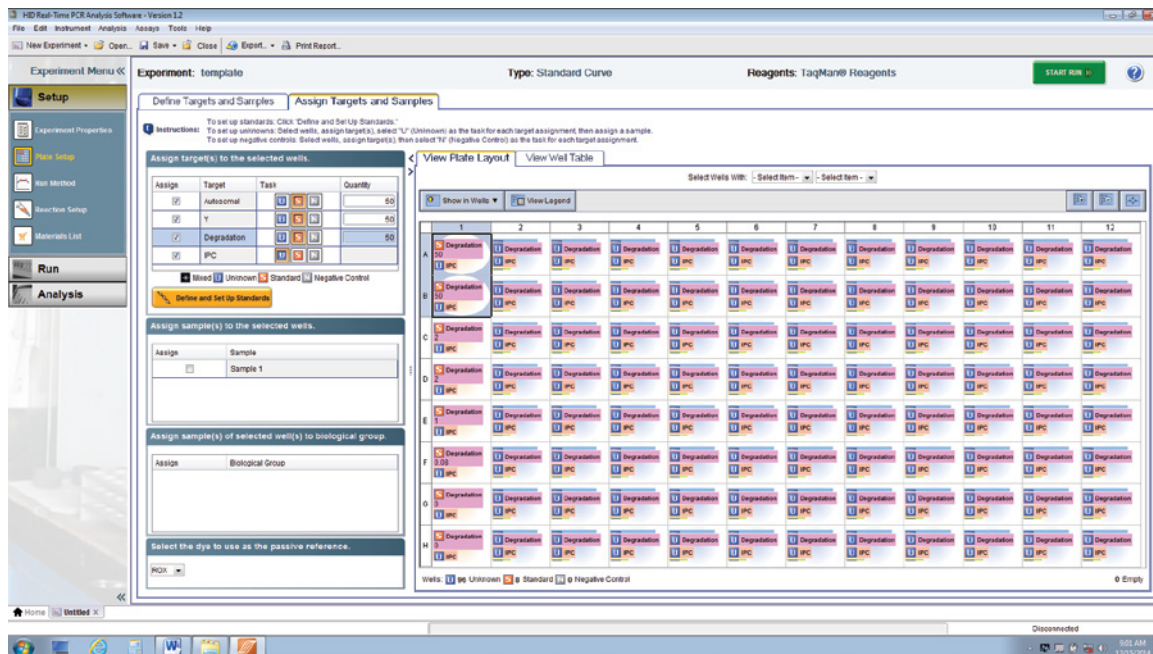
Figure 26. Assigning DNA concentrations to wells with DNA standards.

11. Enter the DNA concentration for each DNA standard in the Quantity field without the unit of measure (i.e., enter 50 for 50ng/µl).

    Notes:

    1. Multiple wells with DNA standards at the same DNA concentration can be highlighted at the same time so that values need to be entered only once.

    2. The Define and Setup Standards tool will not work for assigning a standard curve to multiple targets in the same sample.

7.A. Creating a Run Template (continued)

12. Select **ROX** from the "Select the Dye to use as the passive reference" drop-down menu (Figure 27).
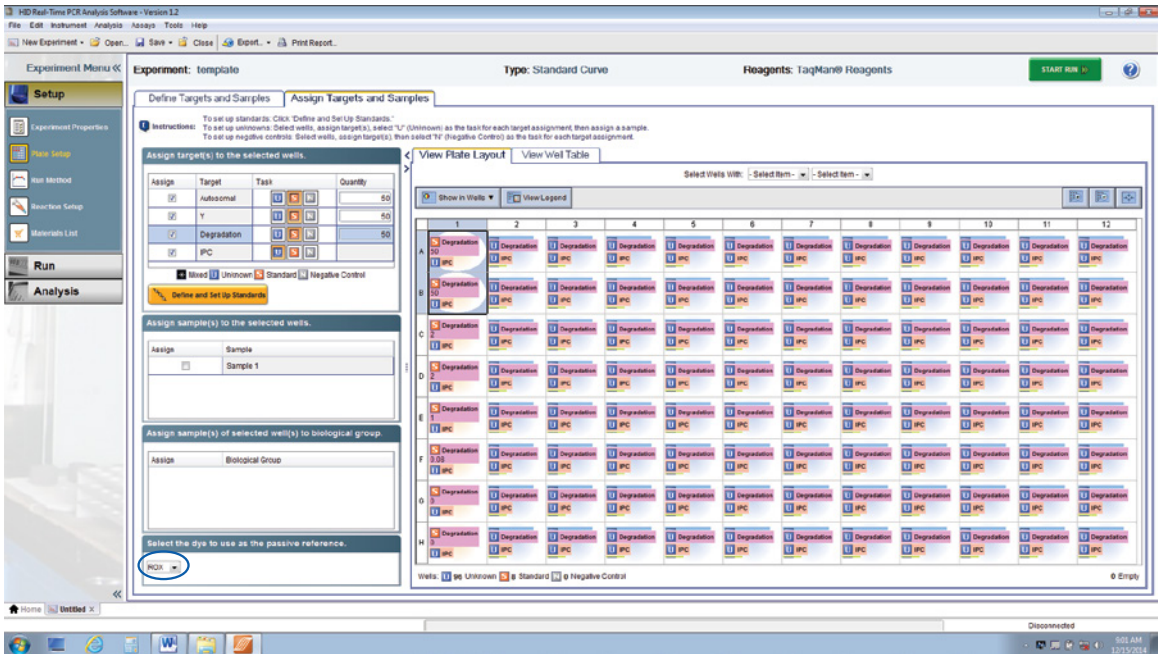
Figure 27. The 'Assign Targets and Samples' screen. Select **ROX** as the passive reference.

13. From the Analysis menu at the top of the screen, select **Analysis Settings**.

14. On the 'CT Settings' tab (Figure 28) in the Select a Target panel, select the Autosomal target.

15. In the CT Settings for Autosomal section, uncheck the "Use Default Settings" box, and uncheck the "Automatic Threshold" box. Enter "0.2" for the threshold. Leave the "Automatic Baseline" box checked.

16. Repeat this process for each of the targets listed in the Select a Target panel using the following threshold values:

    • Autosomal: 0.2
    • Degradation: 0.2
    • IPC: 0.03
    • Y: 0.2

    Select **Apply Analysis Settings**.
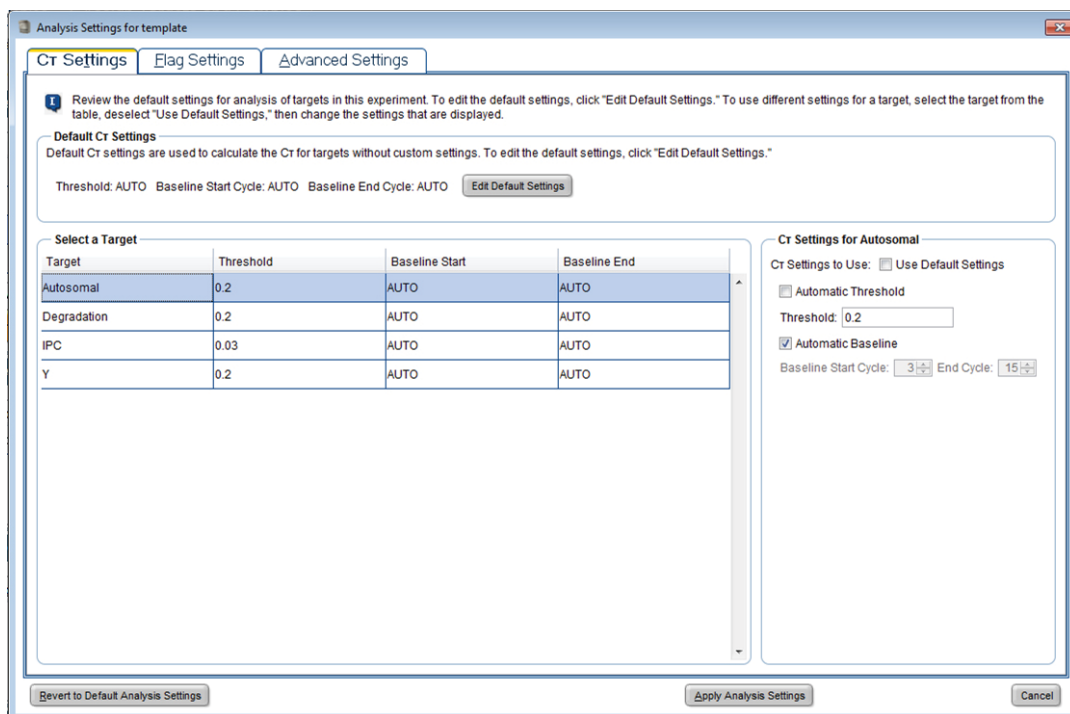
Figure 28. The 'Analysis Settings' window.

7.A. Creating a Run Template (continued)

17. Select **Run Method** under Setup from the left panel. Modify the default run method as directed below:

    • Enter "20" for "Reaction Volume per Well".

    • Select the first Holding Stage, and click with the right button on the computer's mouse to bring up a menu. Select **Delete Selected** to delete this stage.

    • Change the second Holding Stage to 2 minutes at 98°C.

    • Enter "39" for "Number of Cycles" under Cycling Stage.

    • Change Step 1 to 98°C for 15 seconds and Step 2 to 62°C for 35 seconds.

    • Ensure that the **Data Collection On** icon is active for Step 2 of the Cycling Stage.

      Note: The **Data Collection On** icon is shown in the legend at the bottom of the screen.

    The completed PowerQuant® System run method is shown in Figure 29.

Figure 29. The completed PowerQuant® System run method.

18. From the File menu at the top of the screen, select **Save as Template**, and choose a location to save the file. The instrument setup and thermal cycling program can be saved as a template for future use. Use this .edt file as a template to create the experiment document as described in Section 7.B.

    Note: The template will be saved as an .edt file.

7.B.   Starting a Run

1.   On the Home screen select the **Custom Assays** button, or from the Assays menu at the top of the screen select **Custom Assays**.

2.   To create the experiment document, open the template file created in Section 7.A by selecting **Open** from the File menu at the top of the screen. Navigate to the .edt file, and select **Open**.

   Note: If the template file was just created, the .edt file will still be open.

3.   Save the .edt template as an .eds file by selecting **Save As…** from the File menu at the top of the screen. Choose a location to save the file.

4.   Highlight the unused wells, and deselect all of the targets.

5.   To add sample names, select **Plate Setup** from the Setup menu in the left panel, and select the 'Define Targets and Samples' tab (Figure 24). Select **Add New Sample**, and enter the sample name in the section provided. Repeat for all samples.

6.   When all sample names are entered, navigate to the 'Assign Targets and Samples' tab (Figure 25). Highlight the well or wells that contain replicates of the same sample, and check the Assign box adjacent to the corresponding sample name in the "Assign sample(s) to the selected wells" section of the 'Assign Targets and Samples' tab. Repeat until all samples are assigned.

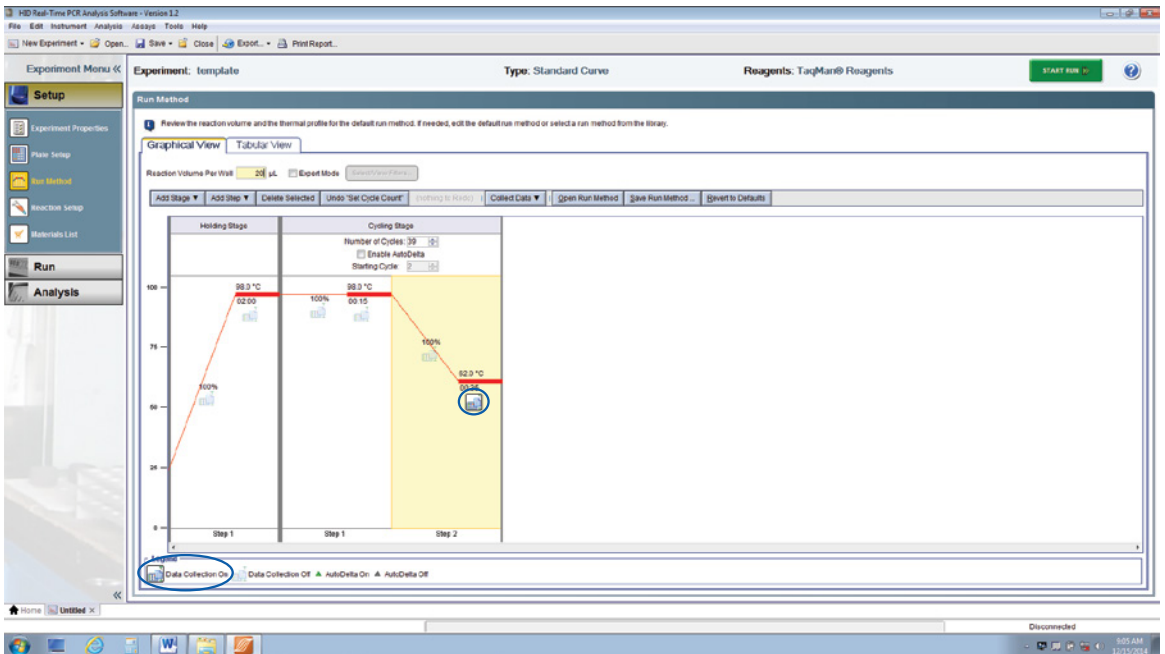   **Note:** Alternatively, you can use a plate setup import file by selecting **Import** from the File menu at the top of the screen. Navigate to the .txt file, select the file and select **Start Import**. Select **Yes** and continue with the import when prompted. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

7.   If a sample was assigned to the incorrect well, select the well and uncheck the sample under "Assign Samples", and then assign the correct sample name.

8.   Check that "ROX" is selected as the passive reference.

9.   Save the .eds file.

10.   Press the tray door.

11.   Place the plate prepared in Section 5.B into the open tray door, and ensure that the plate sits correctly in the plate holder, with well A1 in the top left corner. Push the tray door to close, and immediately select **Start Run**. Run time is 1 hour.

7.C. Evaluating Standard Curves Using the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2

1. Select **Analysis** in the left side panel, and ensure that all wells to be analyzed are highlighted on the 'View Plate Layout' tab and that no targets are selected for unused wells.

2. Select **Analyze**.

3. To display standard curves, select **Standard Curve** from the Analysis menu in the left side panel. Display the standard curves for all targets by selecting "All" from the 'Target' drop-down menu (Figure 30). The standard curve parameters are located below the standard curve plot.

    Note: For more information about how the slope and R^2 values can be used to evaluate the standard curve, see Section 10.
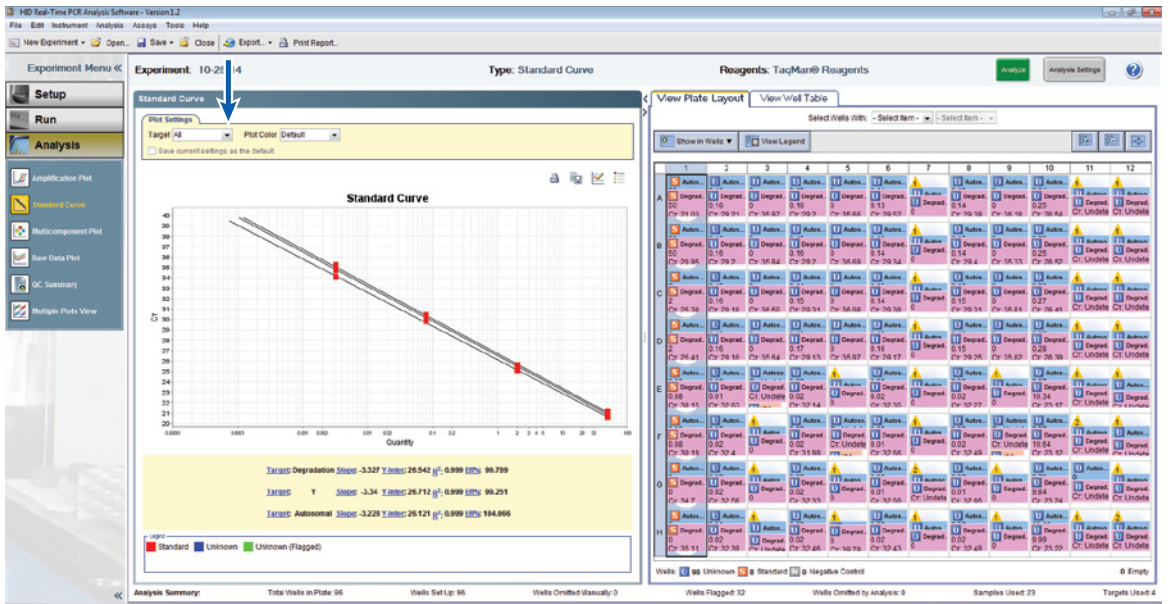
Figure 30. Displaying standard curves for all targets.

7.D. Exporting Analyzed Data from the HID Real-Time PCR Analysis Software, Version 1.1 or 1.2

1. Ensure that all wells with data for export are highlighted in the plate map. Select **Export** from the toolbar.

2. On the 'Export Properties' tab of the 'Export Data' window (Figure 31), select the following Export Properties:

   • Select **Results** in the "Select data to export" section.

   • Select **One File** from the "Select one file or separate files" drop-down menu.

   • Select **.xls** as the File Type.

   • Specify the appropriate export file name.

   • Use the **Browse** button to select the file location.

   Select **Start Export**.
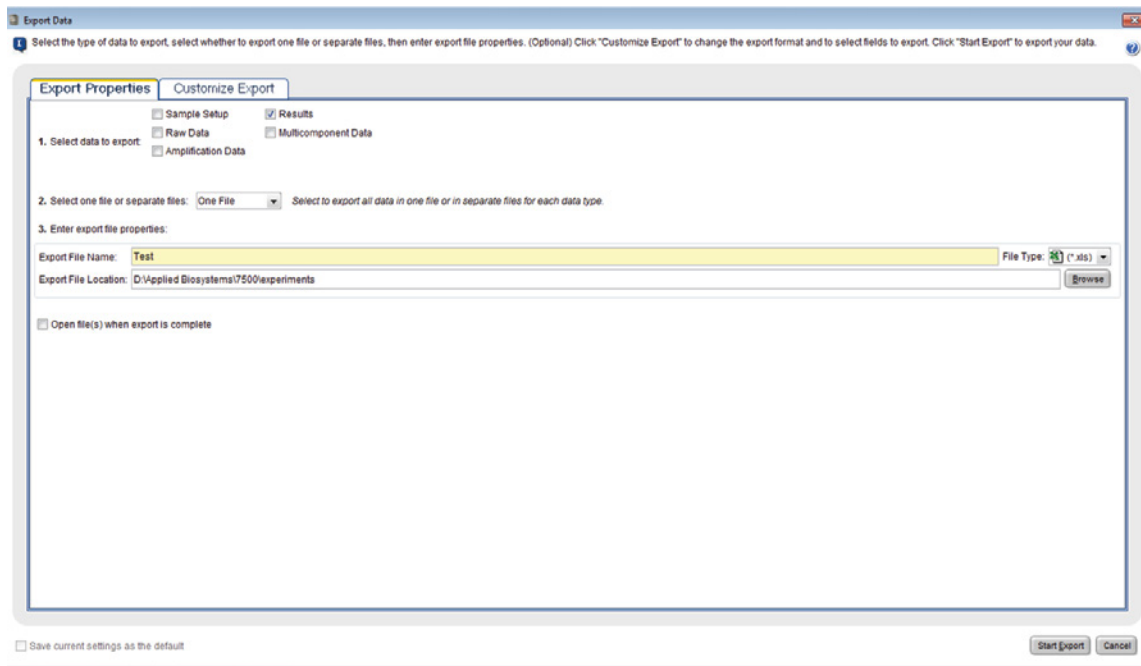
Figure 31. The 'Export Data' window.

3. In the 'Export Completed' window that appears, select **Close Export Tool**.

8. Run Setup and Thermal Cycling Using the QuantStudio™ 5 Real-Time PCR System

The following instructions are for the QuantStudio™ 5 Real-Time PCR System.

A Test Document Template file (.edt file) can be used to store the dye information, target names and tasks, well locations and concentrations for the DNA standards, run method and analysis settings. A plate setup import file may be used to add sample names, well position and standard information to a run template. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

Amplification is complete in 1 hour.

8.A. Setting up the QuantStudio™ Design and Analysis Desktop Software

The following instructions are for use with the PowerQuant® System and the QuantStudio™ Design and Analysis Desktop Software, Version 1.5 and 1.5.1.

Adding the PowerQuant® Dyes

1.   Select **Tools** from the toolbar. Select **Dye Library** from the drop-down menu (Figure 32).
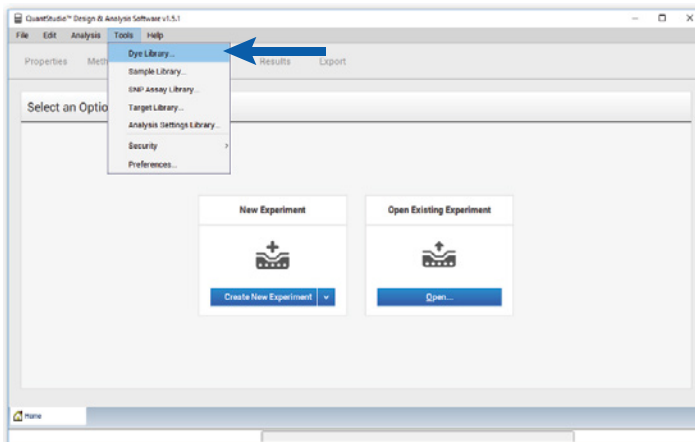
Figure 32. The QuantStudio™ Design and Analysis Software Tools menu.

2.   Add the five PowerQuant® custom dye names by selecting the **New** button (Figure 33).

3.   Enter and save the following dye names: "PQ_FAM", "PQ_CFG540", "PQ_TMR", "PQ_Q670" and "PQ_CXR".

4. Confirm that "Reporter" is selected as the Type for each dye (Figure 33).

    Note: The dye names must match those entered in the Custom Dye section when the dye calibrations were performed in Section 4.D.
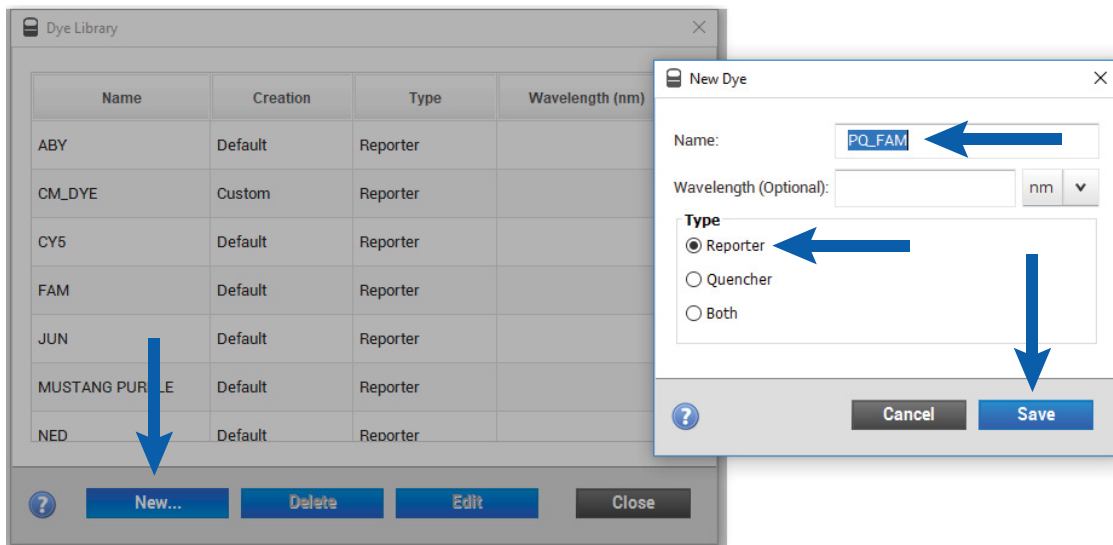
Figure 33. Adding a new custom dye to the Dye Library.

8.B. Creating a Run Template

1. Open the QuantStudio™ Design and Analysis Software and select the **Create New Experiment** icon on the home screen (Figure 34).
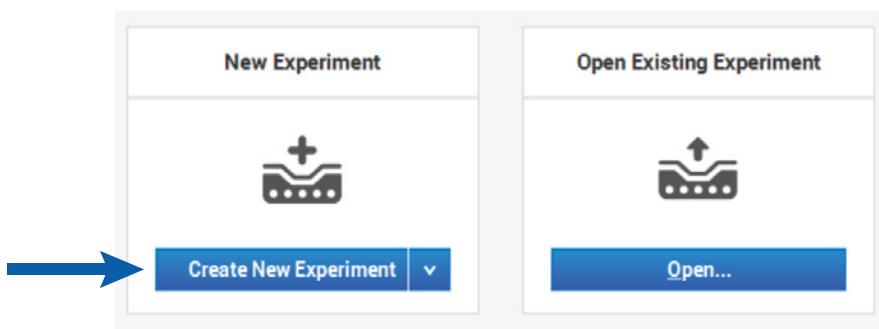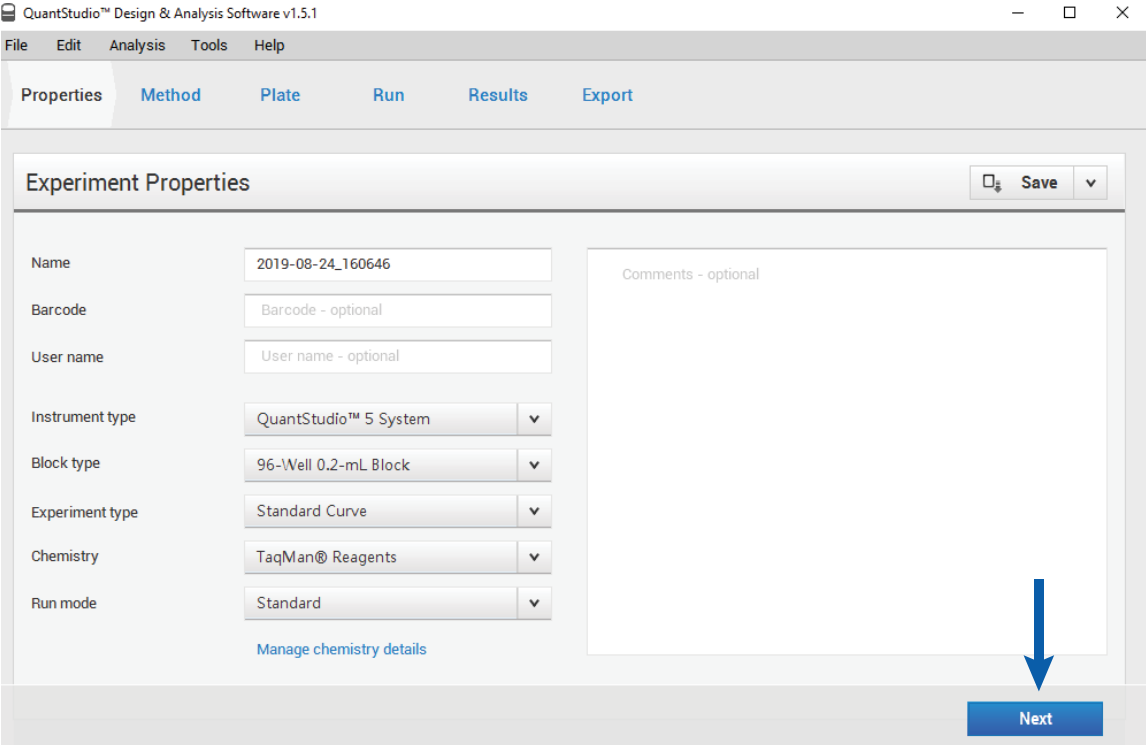
Figure 34. The Create New Experiment icon on the 'Home' screen.

8.B. Creating a Run Template (continued)

2. Specify the following parameters in the Experiment Properties form (Figure 35):

   - Enter a name for the template in the Name field.

   - Select the instrument you are using to run the experiment by selecting **QuantStudio™ 5 System**.

   - Select the block type by selecting **96-Well 0.2-mL Block**.

   - Select the type of experiment you want to set up by selecting **Standard Curve**.

   - Select the chemistry you want to use to detect the target sequence by selecting **TaqMan® Reagents**.

   - Select the run mode you want to use by selecting **Standard**.

Figure 35. The 'Experiment Properties' screen.

3. Select **Next**.

4. Select the 'Experiment Method' tab and modify the default run method as directed below:

    •   Enter "20" in the "Volume" box.

    •   Select the first Holding stage and select the **[-]** icon to delete it.

    •   Change the remaining Hold Stage to 98°C for 2 minutes.

    •   Change Step 1 of the PCR Stage to 98°C for 15 seconds.

    •   Change Step 2 of the PCR Stage to 62°C for 35 seconds.

    •   Ensure that the **Data Collection On** icon is active for Step 2 of the PCR Stage.
        Note: The icon legends are shown at the bottom of the screen.

    •   Enter "39" for the number of cycles in the box below the PCR Stage.

    •   Change the ramp rates for all three steps to "2.44°C/s".

    Select **Next**.

    The completed PowerQuant® System run method is shown in Figure 36.
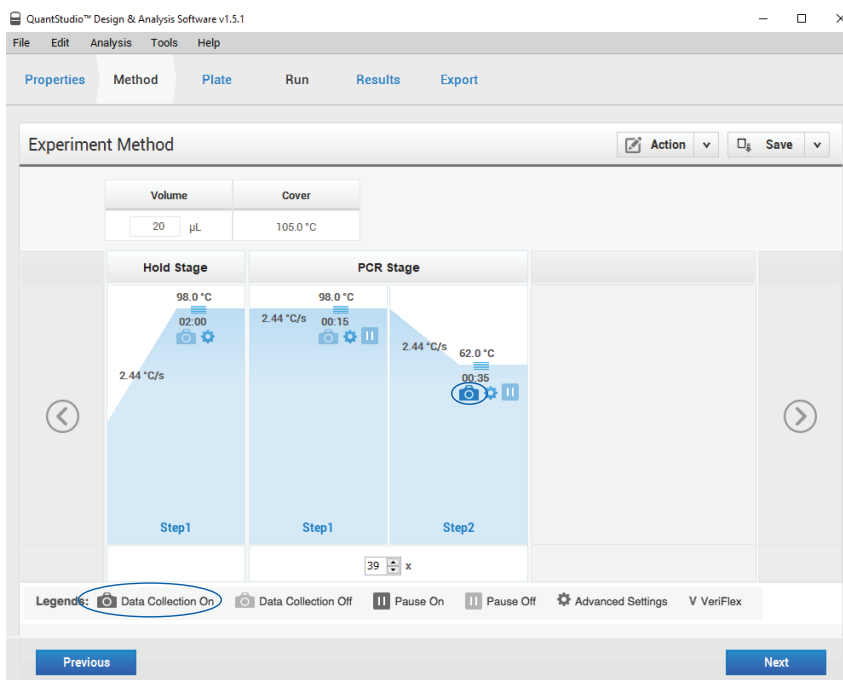
Figure 36. The completed PowerQuant® System run method.

8.B. Creating a Run Template (continued)

5. Enter the PowerQuant® targets in the Targets section of the 'Advanced Setup' tab in the Assign Targets and Samples section (Figure 37). Select **[+] Add** three additional times and enter the following target-specific information:

Target Name	Reporter	Quencher
Autosomal	PQ_FAM	NFQ-MGB
Y	PQ_CFG540	NFQ-MGB
Degradation	PQ_Q670	NFQ-MGB
IPC	PQ_TMR	NFQ-MGB

Note: Target name identifiers are necessary for the PowerQuant® Analysis Software to recognize these targets.
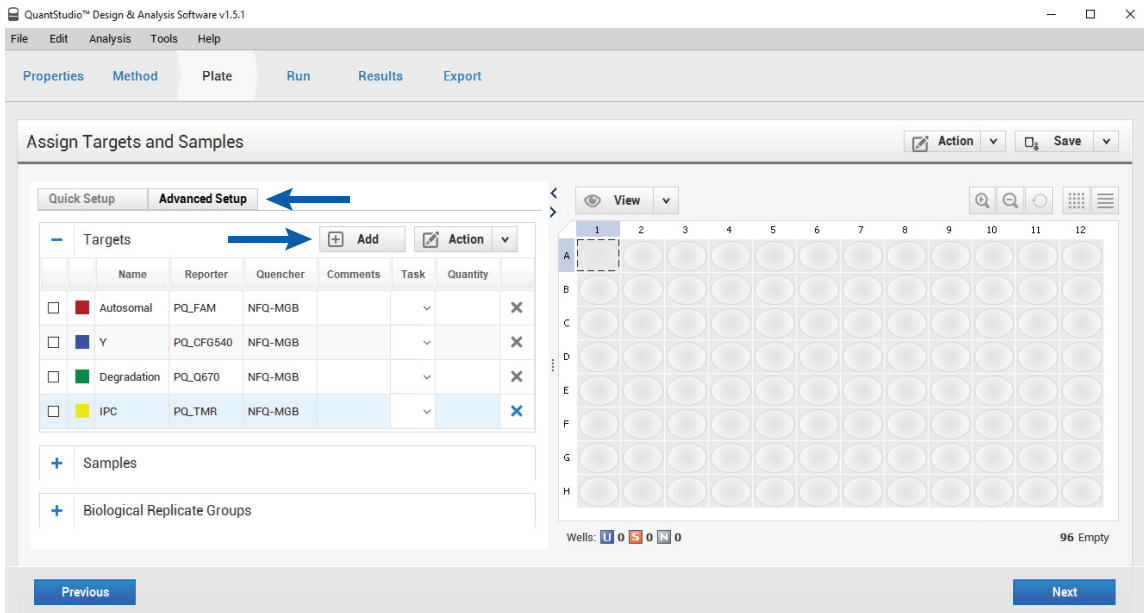
Figure 37. The 'Assign Targets and Samples' tab.

6. Highlight all wells in the plate map by dragging the pointer over the plate wells. Assign all four targets to all wells by selecting the box next to each target name (Figure 38).
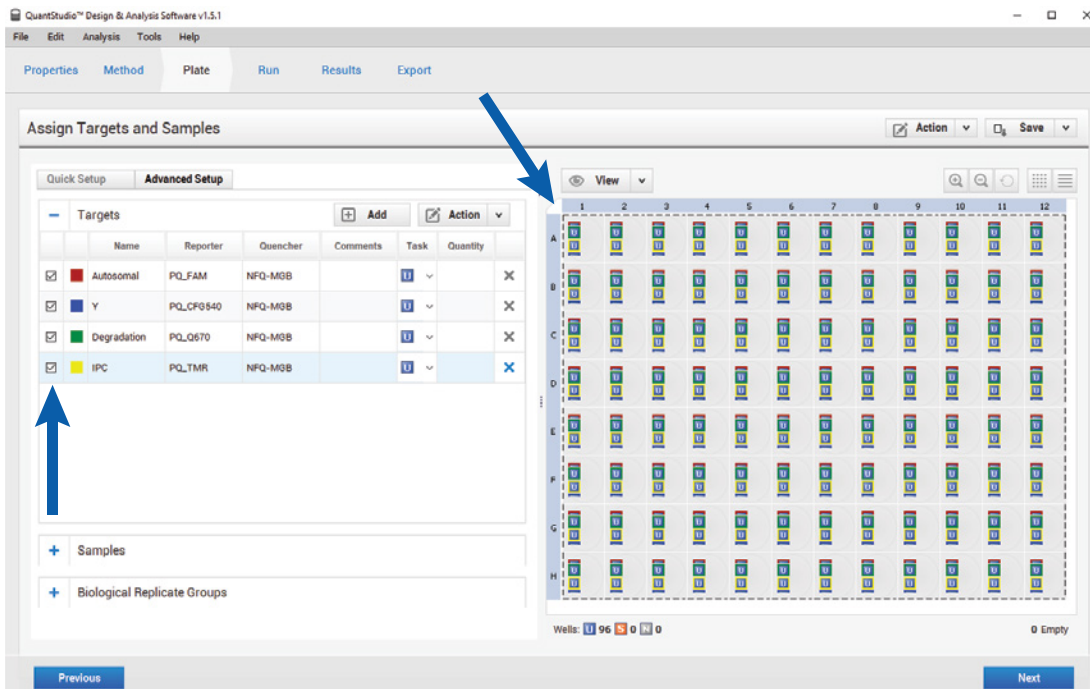
Figure 38. Assigning targets to wells.

8.B. Creating a Run Template (continued)

7. Highlight the wells containing DNA standards and select **S** as the Task for the autosomal, Y and degradation targets (Figure 39).

   Note: The Task for the IPC target should be "U".
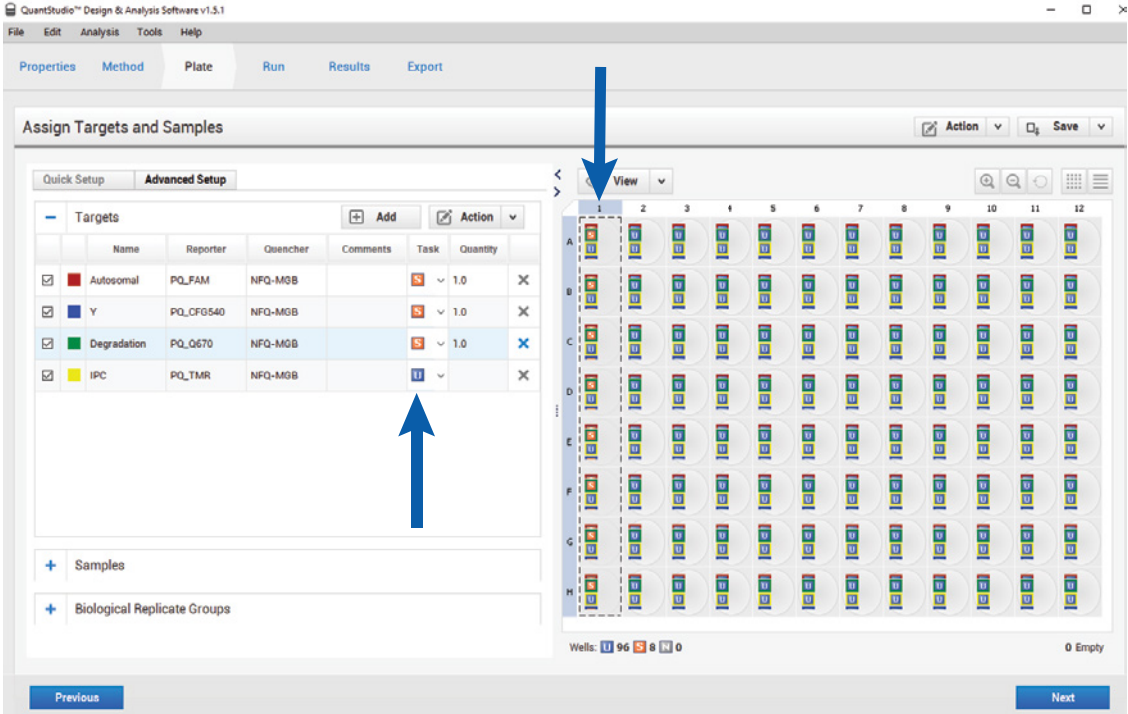
Figure 39. Designating the Task for each target in wells containing DNA standards.

8. Enter the concentration of each DNA standard in the Quantity field without a unit of measure (Figure 40).

   Example: Enter "50" for 50ng/µl, "2" for 2ng/µl, "0.08" for 0.08ng/µl and "0.0032" for 0.0032 ng/µl. Highlight wells with DNA standards of the same concentration simultaneously, then enter the value. Repeat for each DNA standard concentration.
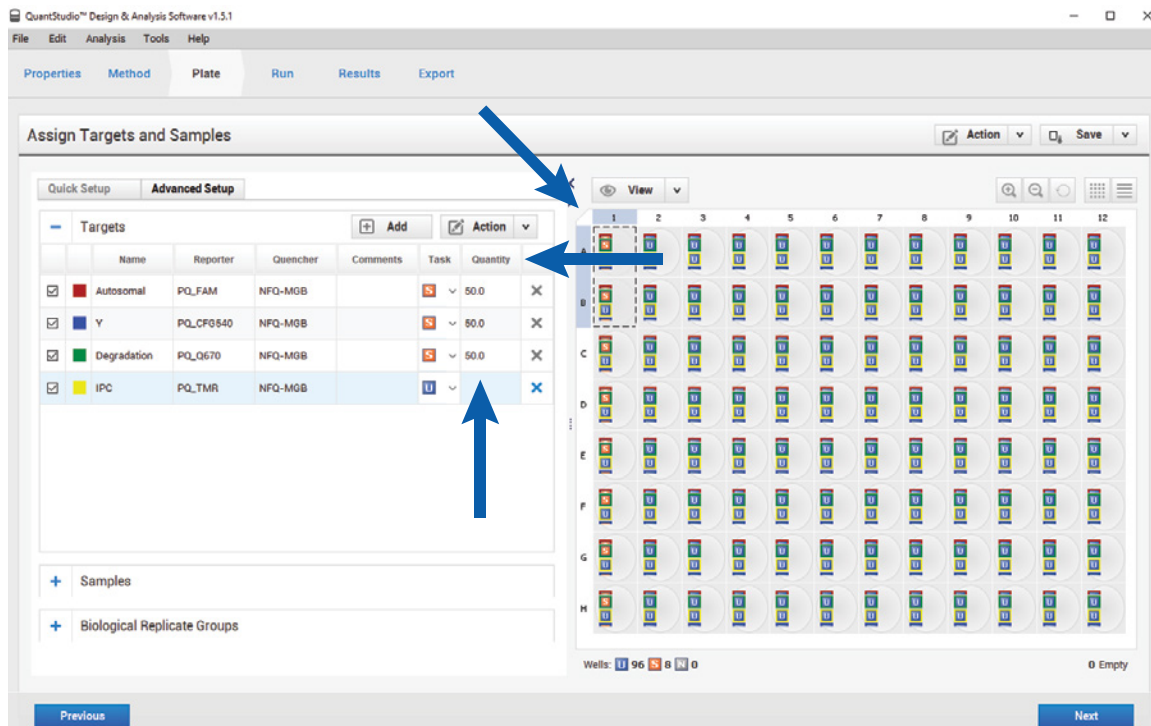
Figure 40. Assigning DNA concentrations to wells with DNA standards.

8.B. Creating a Run Template (continued)

9. In the Samples section of the 'Advanced Setup' tab, select **[+] Add** three times and enter a sample name for each concentration of the DNA standards (one name for each concentration; Figure 41).

Figure 41. Entering sample names for the DNA standards.

10. Highlight all wells with DNA standards of the same name. Assign the DNA standard name to the selected wells by selecting the box adjacent to the corresponding DNA standard name. Repeat for each DNA standard concentration (Figure 42).
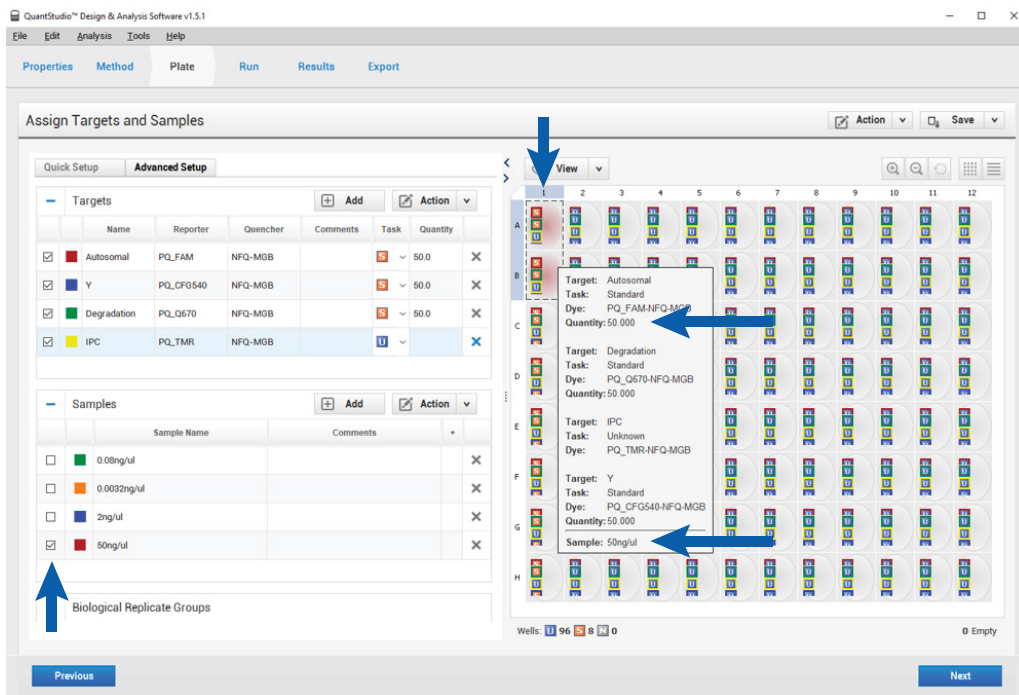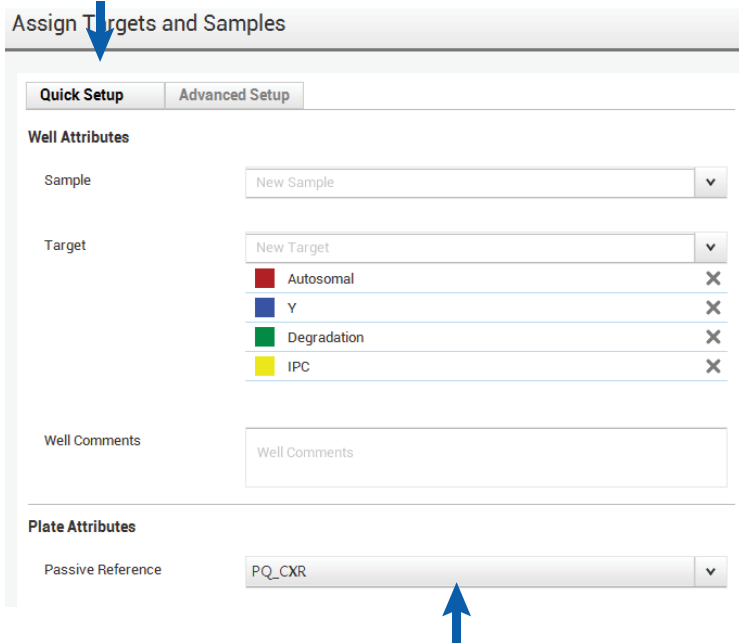
Figure 42. Assigning sample names for the DNA standards.

8.B. Creating a Run Template (continued)

11. Select the 'Quick Setup' tab. In the Plate Attributes section, select **PQ_CXR** as the Passive Reference (Figure 43).
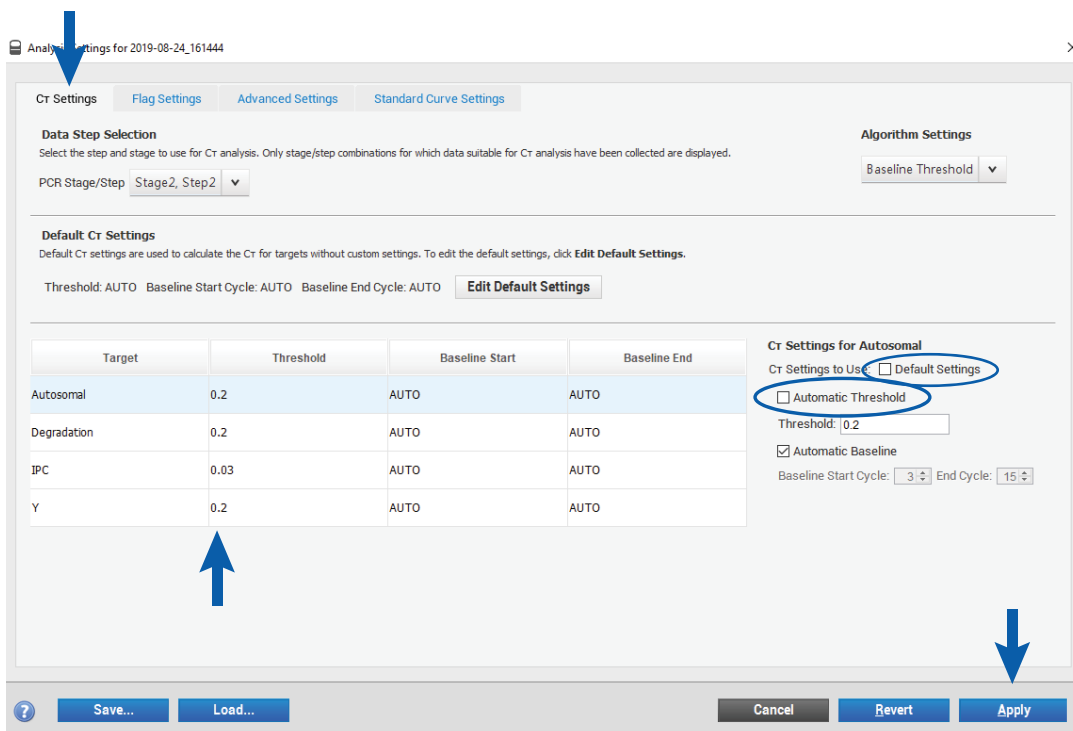
Figure 43. The 'Quick Setup' tab. Select **PQ_CXR** as the passive reference.

12. Select **Analysis Settings** from the 'Analysis' drop-down menu.

13. On the 'CT Settings' tab (Figure 44), select the Autosomal Target.

14. In the CT Settings for Autosomal section, uncheck the "Default Settings" box and uncheck the "Automatic Threshold" box. Enter "0.2" for the threshold. The "Automatic Baseline" box should be selected.

15. Repeat this process for each of the targets using the following threshold values:

    - Autosomal: 0.2
    - Degradation: 0.2
    - IPC: 0.03
    - Y: 0.2

    Select **Apply**.

Figure 44. The 'CT Settings' tab.

8.B. Creating a Run Template (continued)

16. Select the 'Export' tab. Review the following parameters and adjust as needed (Figure 45).

   • Set the File Type to "QuantStudio" and ".xls".

   • The "Open exported files when complete" box should be selected.

   • The "Results" box should be selected in the Content section. Deselect the "Sample Setup" and "Amplification Data" boxes.

   • "Unify the above content into one file" should be selected under the Options section.

   • Select the **Customize** button and confirm that the following options shown in the Select Content list of the 'Customize' screen (Figure 46) are deselected: Well, Omit, Y-Intercept, R^2, Slope, Efficiency, Amp Status, C_q Conf, Rn (last cycle) and Delta Rn (last cycle).

   • The boxes for "Skip Empty Wells" and "Skip Omitted Wells" at the top of the 'Customize' screen should remain selected if your software version displays them.
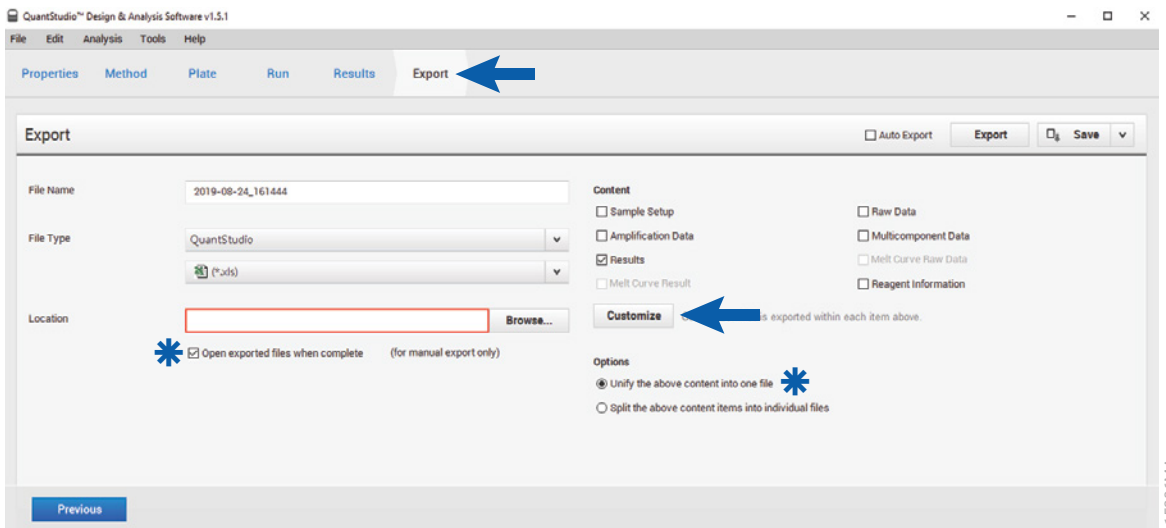
Figure 45. The 'Export' tab.

Figure 46. The 'Customize' screen and Select Content list.

17.   From the File menu at the top of the screen, select **Save As**. Choose a location to save the template file as an .edt file (Figure 47). The template file can now be used to create future PowerQuant® experiment documents for the QuantStudio™ 5 Real-Time PCR System.

8.B. Creating a Run Template (continued)
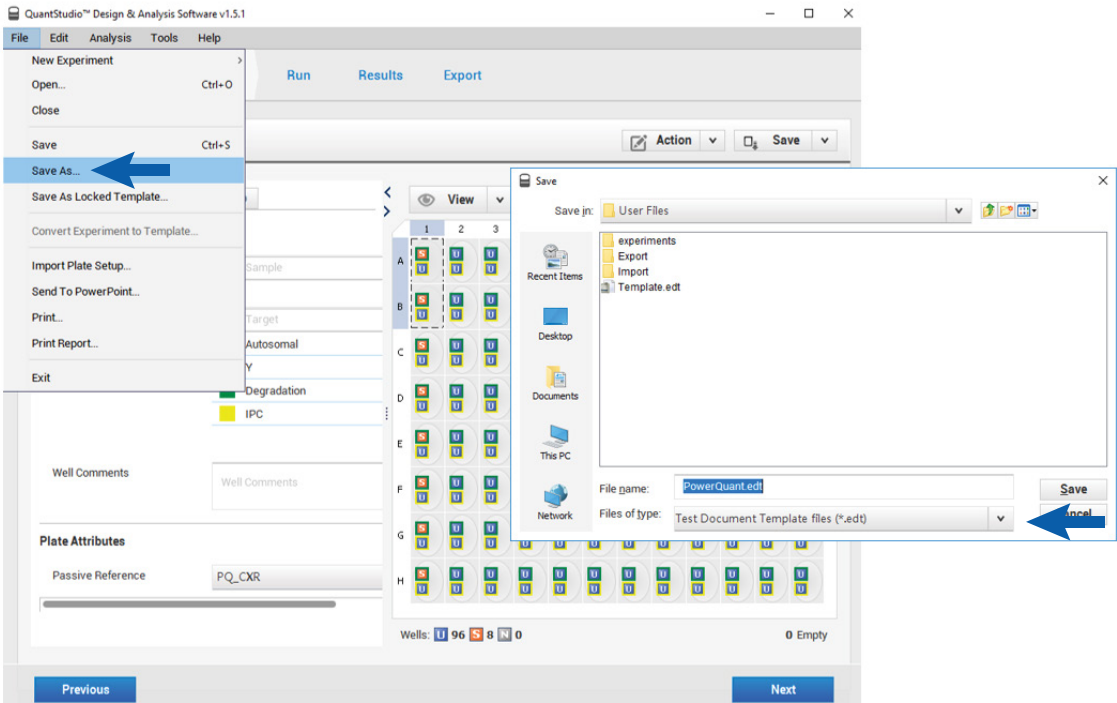
Figure 47. Saving a Test Document Template (.edt) file.

8.C. Starting a Run

1. Open the QuantStudio™ Design and Analysis Software and select the **Open Existing Experiment** icon from the home screen (Figure 48).
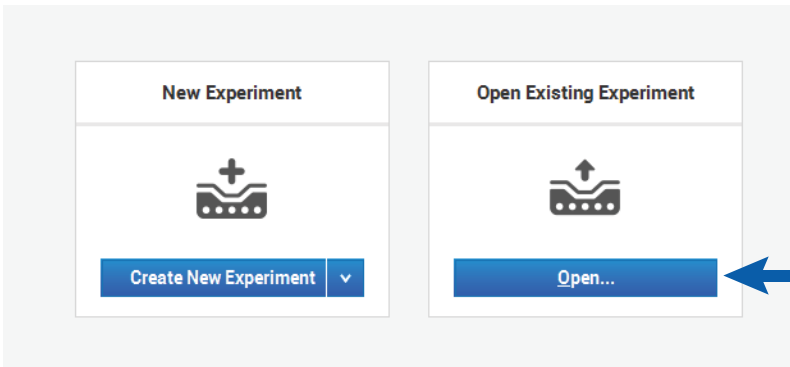
Figure 48. The QuantStudio™ Design and Analysis Software, Open Existing Experiment icon.

2. Open the template file (.edt) created in Section 8.B and navigate to the 'Plate' tab.

3. Enter the names of the DNA samples in the Samples section of the 'Advanced Setup' tab (Figure 49) by selecting **[+] Add** until the appropriate number of sample name lines are present. Type the name of each sample only once.
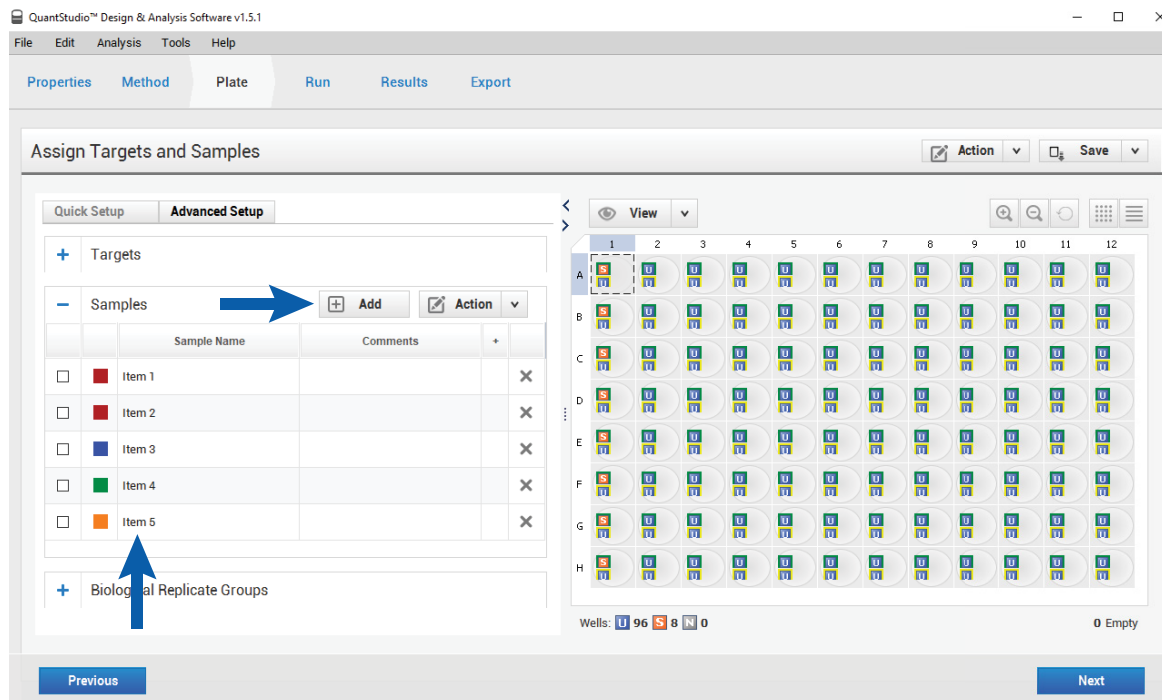
Figure 49. The 'Advanced Setup' tab in the 'Assign Targets and Samples' screen.

8.C. Starting a Run (continued)

4. Highlight the well or well(s) on the plate map that contain replicates of the same sample and select the box adjacent to the corresponding sample name from the Samples list. Repeat until all sample names have been assigned to a well on the plate map.

   Notes:

   1. If a sample name is inadvertently assigned to the wrong well, reselect that well and deselect the box next to the wrong sample name. Locate and assign the correct sample name.

   2. Alternatively, you can use a plate setup import file by selecting **File>Import Plate Setup** (Figure 50). Browse to the location where your plate .txt file is stored. Select the file and select the **Apply** button. Select **Yes** and continue with the import when prompted. The software will display an "Import Successful" box. Select **OK** to move forward with starting the run. See Section 9.H for instructions on creating a plate setup import file with the PowerQuant® Analysis Software.

   3. If the QuantStudio™ Real-Time PCR System is being operated as a standalone instrument without a laptop or desktop, then the sample names must be saved and imported as part of the previously created .edt template file.
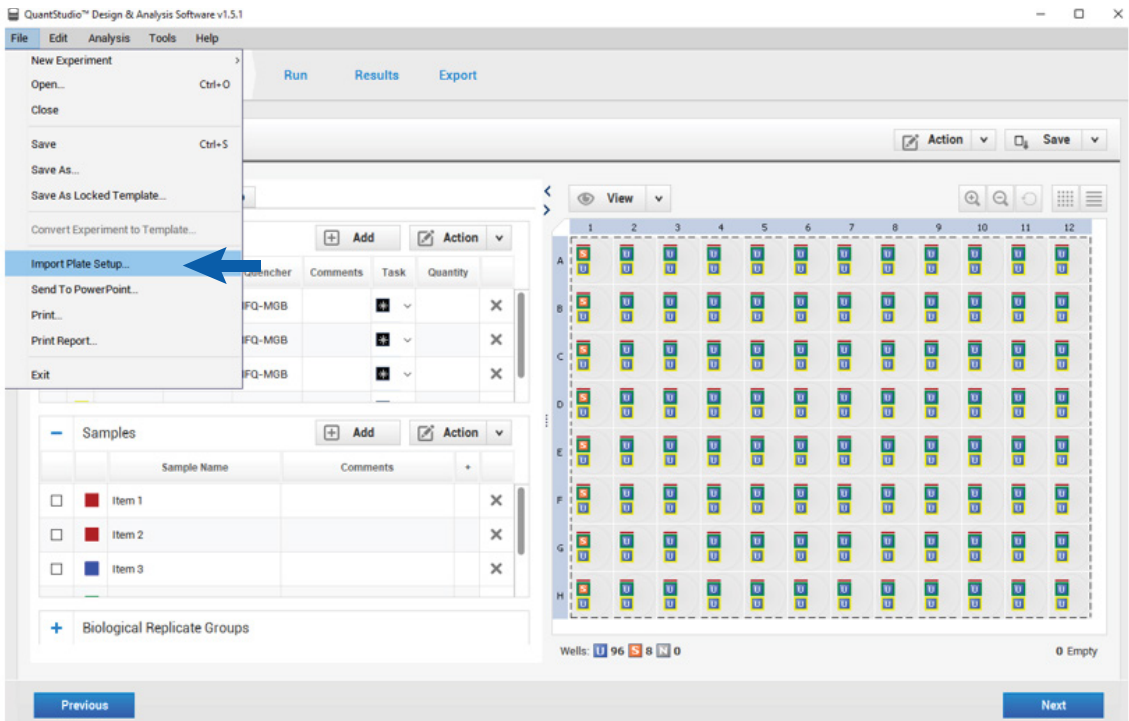
Figure 50. The Import Plate Setup option in the File menu.

5. Highlight all unused wells on the plate map and deselect all targets by unchecking the boxes next to the target names.

6. Open the instrument tray door by selecting the **Eject** icon on the home screen.

7. Place the plate prepared in Section 5.B in the open tray door, and ensure that the plate sits correctly in the plate holder, with well A1 in the top left corner. Select the **Eject** icon again to close.

8. Navigate to the 'Run' tab. Select **Start Run** (Figure 51). The software will prompt you to save your experiment as an .eds file. Run time is approximately 1 hour.

9. Following completion of the run, the instrument screen will ask you to select a destination for your completed .eds file. Select **Transfer File** to navigate to your chosen file location (USB, network, instrument folder), then select **OK**.

   Note: All completed .eds files can be found in the Run History folder under instrument Settings at any time.
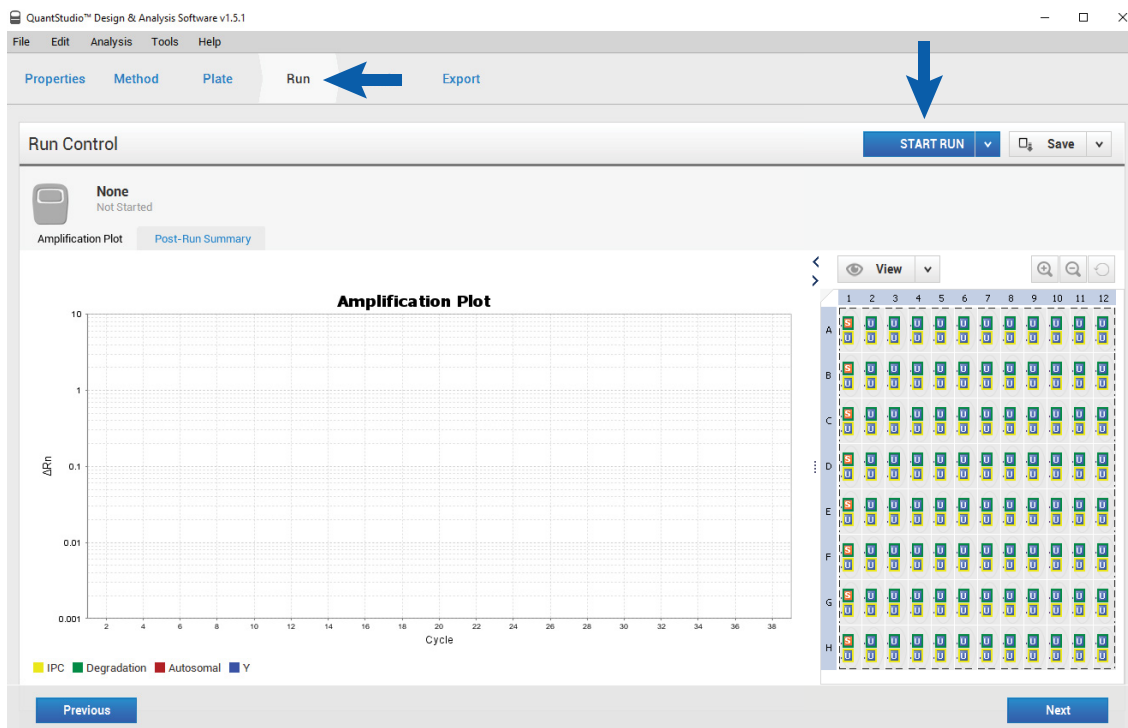
Figure 51. The 'Run' tab.

8.D. Evaluating Standard Curves Using the QuantStudio™ Design and Analysis Software

1. Select the 'Results' tab. Ensure that all wells to be analyzed are highlighted in the 'View' tab and that no targets are selected for unused wells. Select **Analyze**.
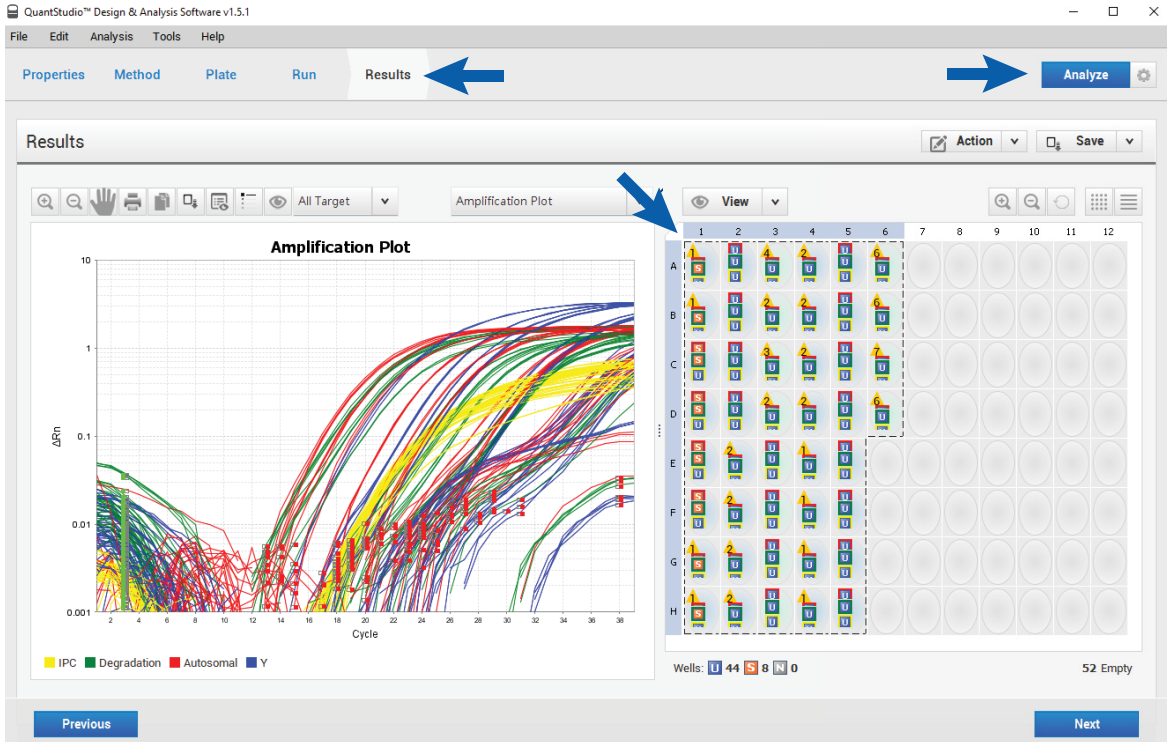
Figure 52. The Amplification Plot and plate map on the 'Results' tab.

2. To display all standard curves, select **Standard Curve** from the drop-down menu located above the Amplification Plot section (Figure 53).
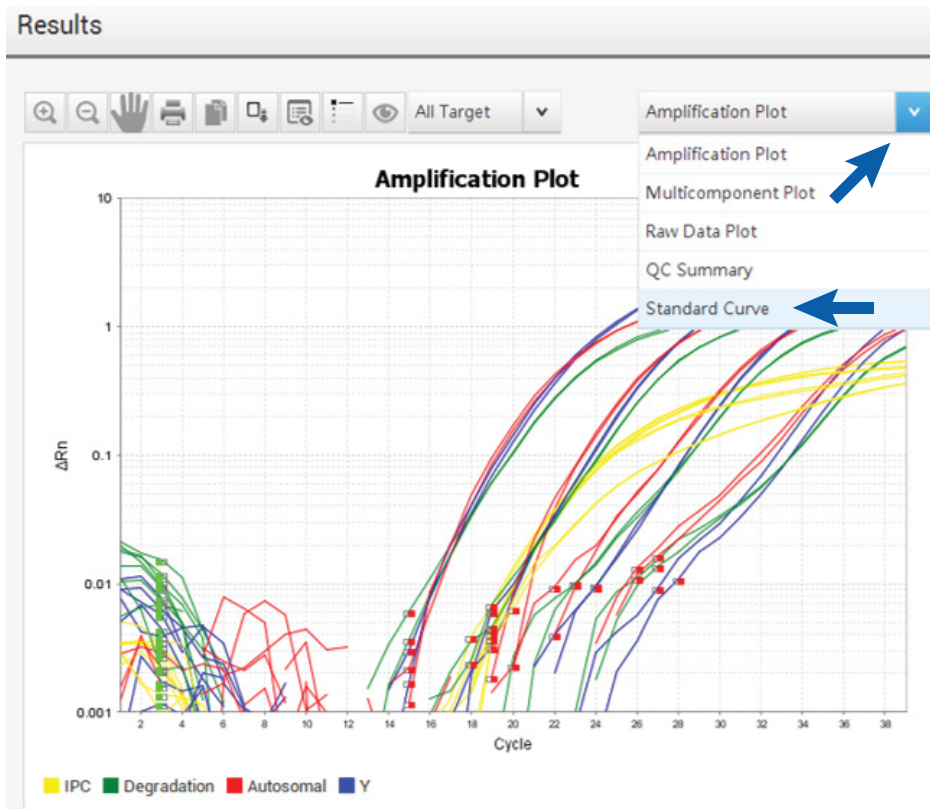
Figure 53. Displaying the standard curves for all targets.

3. Display the standard curve for each individual target by selecting the Eye icon (Figure 54). The standard curve for each target may be viewed by choosing the appropriate target from the 'All Target' drop-down menu (Figure 54). The standard curve parameters are located below the standard curve plot.

    Note: For more information on how the slope and R² values can be used to evaluate the standard curve, see Section 10.
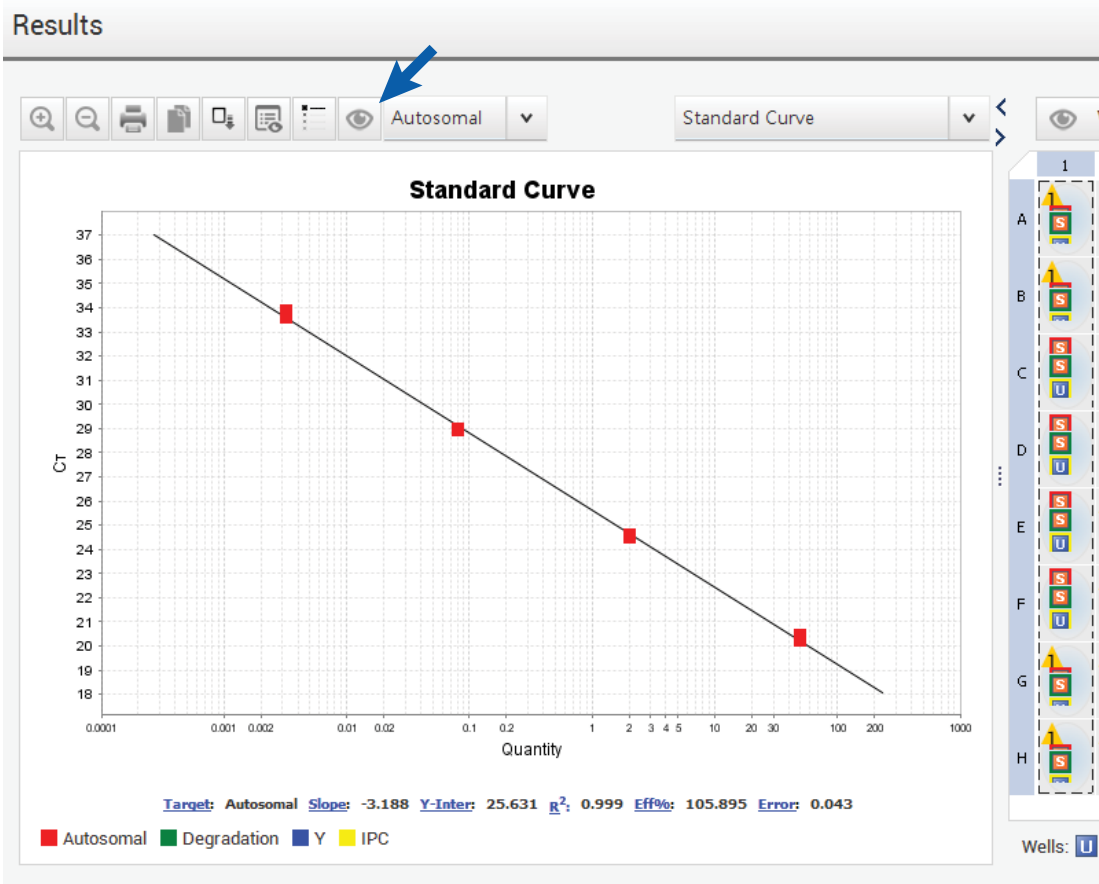
Figure 54. Displaying the standard curve for the autosomal target.

4. Save any changes made to the .eds file.

8.E. Exporting Analyzed Data from QuantStudio™ Design and Analysis Software

1.   Confirm that all wells containing data for export are highlighted in the plate map and select the 'Export' tab.

2.   Specify an appropriate export file name. Select **Browse** and choose a file location. Select **Export** (Figure 55).
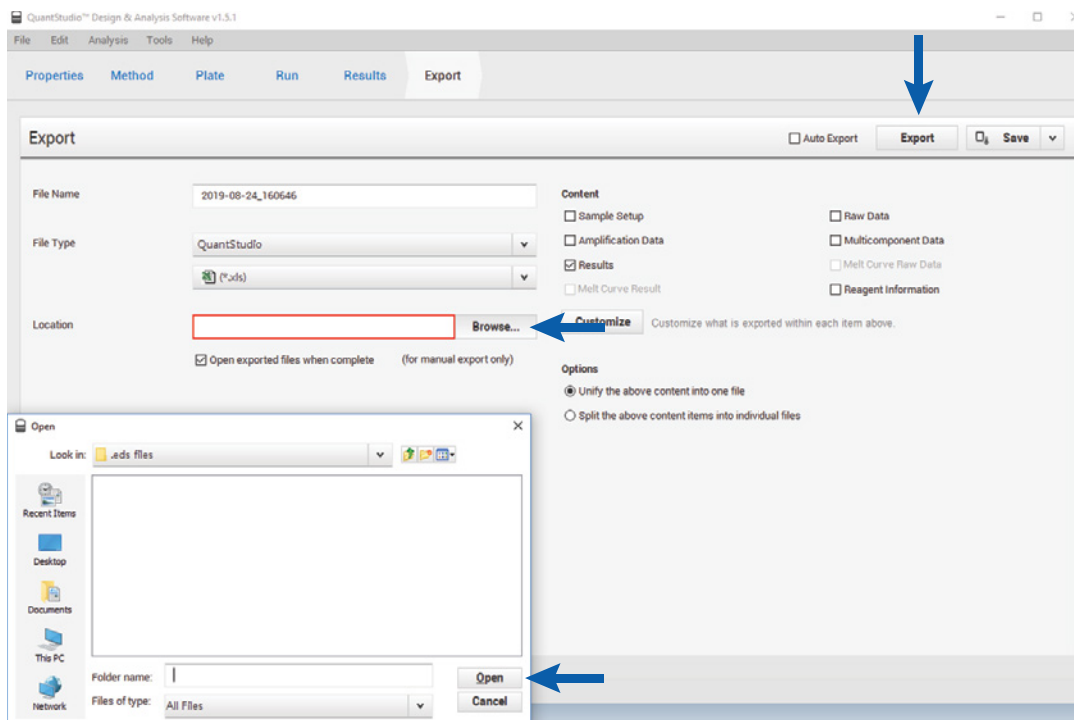
Figure 55. Exporting Results from the QuantStudio™ Design and Analysis Software.

Promega

9. PowerQuant® Analysis Software

9.A. Overview

The two main functions of the PowerQuant® Analysis Software are creating sample sheets for import into the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System, and analyzing data exported from these instruments. The PowerQuant® Analysis Software performs calculations and evaluations on data exported from the 7500 and QuantStudio instruments for the following parameters:

- Standard curve generation and quality (acceptable R^2, slope and Y-intercept values)
- Sample quality (possible inhibition, mixture or degradation)
- DNA normalization for autosomal or Y-STR amplification based on DNA target mass, pipetting volumes and diluent volumes
- Analysis with a virtual standard curve

The PowerQuant® Analysis Software Plate Designer can be used to input sample names in the qPCR instrument software through the creation of a .txt import file and to calculate the volumes of PowerQuant® reaction components.

Once the Results Excel® file is exported from the instrument software and imported into the PowerQuant® Analysis Software, the PowerQuant® Analysis Software calculates standard curves for each of the three targets (autosomal, Y and degradation). The sample quantity is calculated for all unknown samples using the standard curves, as well as ratios between the autosomal and Y targets ([Auto]/[Y]), and the autosomal and degradation targets ([Auto]/[Deg]). The software calculates the difference in C_q values for the IPC in an unknown sample and the IPC in the closest DNA standard of the standard curve (to monitor PCR inhibition). In addition, this software performs calculations for normalizing the input amounts of DNA based on the autosomal or Y target quantification values.

The PowerQuant® Analysis Software is compatible with data exported from the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System. For questions regarding instrument compatibility, contact Promega Technical Services for more information. E-mail: **genetic@promega.com.**

Notes:

1. C_q value (quantification cycle) is used rather than C_T (cycle threshold) per MIQE guidelines (3).
2. Worksheets generated by this software have been locked to prevent editing of the formulas, linked cells and named ranges in the worksheets.
3. For assessment of possible male/female DNA mixtures, DNA degradation and PCR inhibition, individual laboratories should determine and validate acceptable threshold values relevant to the success of downstream applications. The diversity of sample types, purifications and potential PCR inhibitors does not allow for specific threshold value recommendations. Default threshold settings in the PowerQuant® Analysis Software are values intended to be customized by the user.

9.B. Downloading the PowerQuant® Analysis Software

The PowerQuant® Analysis Software can be downloaded from the Promega web site **www.promega.com/resources/software-firmware/**. There is no cost for this download, but registration is required. The PowerQuant® Analysis Software part number is 7002673. A single file downloads: the PowerQuant Assays Installer.exe application.

System Requirements
- Operating system: Microsoft Windows 7, 8.1 Pro, 10 (x86 and x64)
- Installed memory: 2GB RAM
- External resource: Microsoft .NET Framework 4.8 (available at no charge from www.microsoft.com)
- Processor requirement: dual-core processor

9.C. Installing the PowerQuant® Analysis Software

The software installs in the Promega\PowerQuant folder automatically. You cannot change the install location. The installation must be performed with administrator permissions on the computer.

1. Double-click the PowerQuant Assays Installer.exe application.

2. The software installer displays the 'License Agreement' window. Read the agreement and select the radio button labeled "I accept the agreement", and select **Next** to continue with the installation.

3. The software installer displays the 'Information' (Folder Permissions) window. Select **Next** to continue.

4. The software installer displays the 'Database Installation' window. Select **Yes, Replace (Overwrite) The Existing Database** or **No, Keep The Existing Database**. Select **Next** to continue.
   Note: For the first installation of the PowerQuant® Analysis Software, either database option can be used.

5. The software installer displays the 'Ready to Install' window. Select **Install** to continue.

6. The 'Unconfirmed Set Permissions Success' window is displayed. Select **OK**.

7. After installation, the software displays the 'Completing the PowerQuant Assays Setup Wizard' window. Select **Finish**.

Notes:

The PowerQuant® Analysis Software is configurable for several user-access models. The software installer creates a directory in the Program Files folder or the Program Files (x86) folder containing all required files to run the program. This directory allows all users of the computer to access the same program and data. However, by copying the contents of the application folder to other locations, different methods of access can be achieved.

Single-User (Private) Deployment

If several individuals use the same computer and each user requires an individual database, copy the contents of the Promega\PowerQuant folder to another folder and create a shortcut to this folder on the desktop. If the folder is copied to an individual user's C:\Users\UserName folder, then only that user can access these data. This deployment requires each user to log into the computer and perform this step.

Multi-User/Multi-Computer Deployment

If several users need to access a common database, copy the contents of the Promega\PowerQuant Analysis Software folder to a shared drive on the network. The software automatically defaults to the shared network location.

9.D. Removing the PowerQuant® Analysis Software

1. In the Promega\PowerQuant Analysis Software folder, double-click the unins000.exe file.

2. The "PowerQuant Assays Uninstall" dialog box is displayed. Select **Yes** to remove the software.

3. The software is uninstalled. The 'PowerQuant Assays Uninstall' window displays the message "PowerQuant Assays was successfully uninstalled from your computer". Select the **OK** button.

If you subsequently need to reinstall the software, follow the installation instructions in Section 9.C.

9.E. The Main Menu

Installation of the PowerQuant® Analysis Software automatically places a shortcut to the software on the desktop. Select the **PowerQuant Assays** icon on the desktop to launch the software. Launching the PowerQuant® Analysis Software grants Standard user access. To access the Admin functions, users must log in as an administrator with a password. (See Section 9.F for information about User Accounts).

The main menu is located at the top of the software window, comprising five tabs:

- The 'Import' Tab
- The 'Configuration Tools' Tab
- The 'Edit' Tab
- The 'Print' Tab
- The 'Help' Tab

'Import' Tab

On the *Import* tab, selections are made for the data file source, sample assessment settings, standard curve assessment settings, normalization settings and plate map (Figure 56). Once selections are complete, the user selects a PowerQuant® Results Excel® file for analysis. Analyzed data are displayed and can be saved as an Excel® or .pdf file. See Section 9.I for additional information about the 'Import' tab.
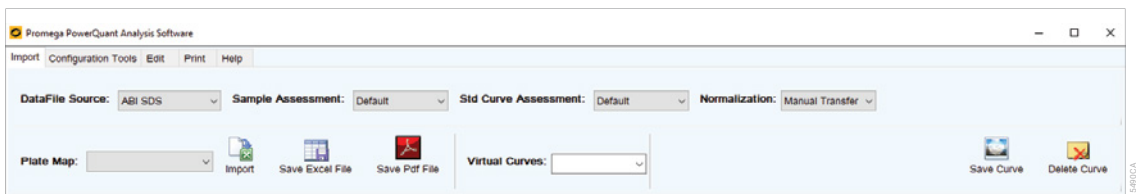
Figure 56. The 'Import' tab.

'Configuration Tools' Tab

The 'Configuration Tools' tab provides access to the 'PowerQuant Administrative Tools' window and the 'Promega PowerQuant Plate Designer' window (Figure 57).

The **Configuration Settings** icon opens the 'PowerQuant Administrative Tools' window. The PowerQuant Administrative Tools allow the user to:

- Set default parameters used in the report
- Set values for the PowerQuant® dye names
- Set values for standard curve and sample assessment
- Set values used for normalization
- Manage user accounts

The software includes default settings for most parameters based on the results of testing during product development. These values are intended to be customized by the user based on the results of your laboratory's internal validation. Additional information about these settings is provided in Section 9.G.

The **Design Plate Map** icon opens the 'Promega PowerQuant Plate Designer' window. The Promega PowerQuant Plate Designer is used to create and edit plate maps. Additional information about plate maps is provided in Section 9.H.
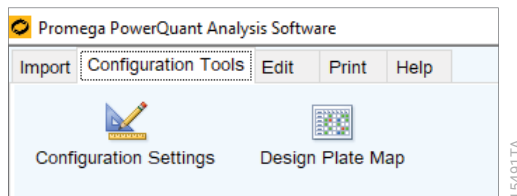
Figure 57. The 'Configuration Tools' tab.

'Edit' Tab

The 'Edit' tab contains the basic functions for editing cells (Figure 58).
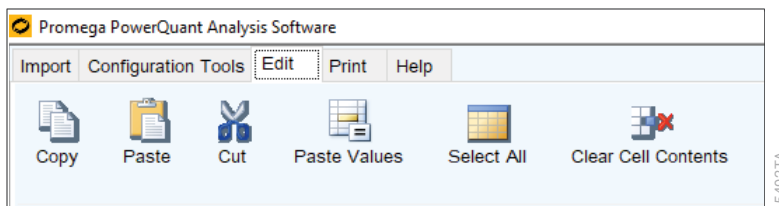
Figure 58. The 'Edit' tab.

9.E. The Main Menu (continued)

'Print' Tab

The 'Print' tab contains print functions, including print preview and printer selection options (Figure 59).
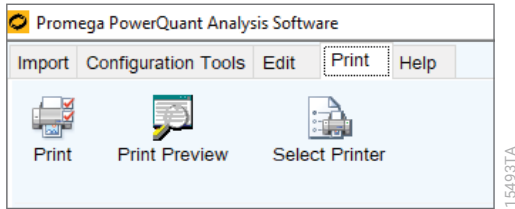
Figure 59. The 'Print' tab.

'Help' Tab

Videos providing information on software features and functions are located in the 'Help' tab (Figure 60).

The Import video describes how to import a PowerQuant® Results Excel® file into the PowerQuant® Analysis Software.

The Configuration Settings video demonstrates how to create or modify values for configuration settings, dye names, standard curve assessment, sample assessment and normalization.

The Design Plate Map video describes how to create a plate map.

Note: The videos found on the 'Help' tab include sound. Check sound and volume settings in Microsoft® Windows and the video player.

The Technical Manual icon opens a link to the *PowerQuant® System Technical Manual* #TMD047.

The Zoom icon opens a window with instructions on how to zoom in or out in the spreadsheet.

The Read Me icon opens a window containing information about the software.
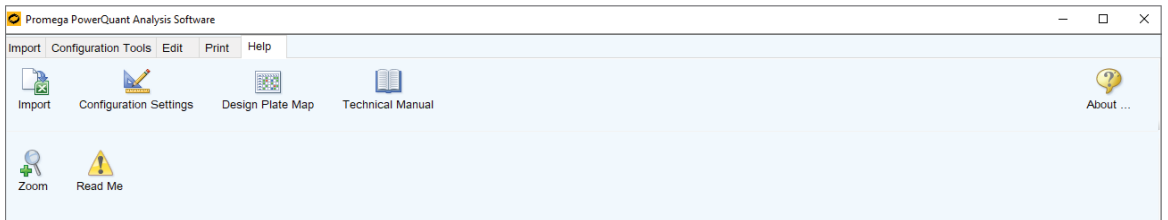
Figure 60. The 'Help' tab.

9.F. User Accounts

A Standard user can:

* View configuration settings, dye names, standard curve, sample assessment and normalization settings created by an Admin user

* Create, edit and delete plate maps

* Use the 'Import' tab to analyze data

An Admin user can:

* Edit configuration settings

* Create, edit and delete calibrator assessment, standard curve, sample assessment and normalization settings

* Create, edit and delete dye set names

* Create, edit and delete plate maps

* Use the 'Import' tab to analyze data

* Perform user-managements tasks (create, edit and delete new users)

Initial Login and User Management

1. Select the 'Configuration Tools' tab from the main menu and select the **Configuration Settings** icon (Figure 57).

2. The 'Administrator Login' window opens (Figure 61).
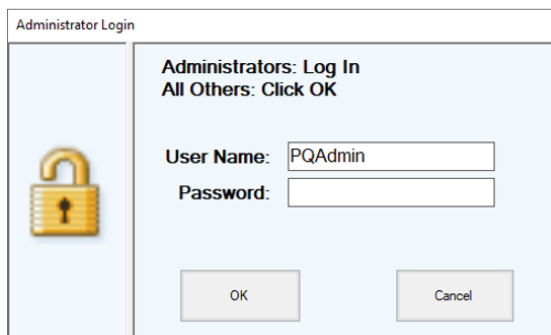
Figure 61. The 'Administrator Login' window.

9.F. User Accounts (continued)

3. To log in as an Administrator for the first time, enter the following information:

   User Name: PQAdmin

   Default Password: Promega

   Notes:

   1. The User Name field is not case-sensitive. The Password field is case-sensitive.

   2. The default User Name appearing in the 'Administrator Login' window is the Microsoft® Windows user name.

4. Select the **OK** button.

Adding a New Admin User

1. To create a new Admin user, select the 'Login Manager' tab in the 'PowerQuant Administrative Tools' window (Figure 62).
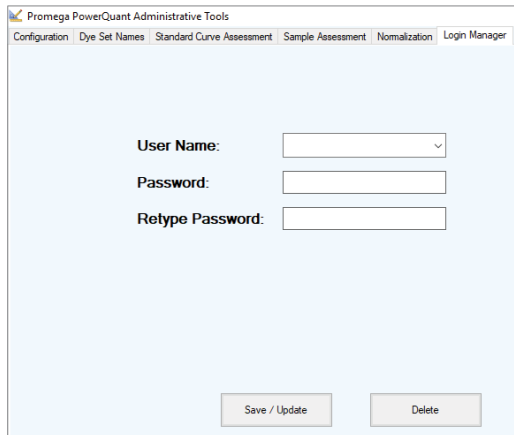
Figure 62. The 'Login Manager' tab.

2. Enter a new user name in the User Name field.

3. Type a new password in the Password field.
   Note: The new password does not have complexity requirements and characters are not restricted.

4. Retype the password in the Retype Password field. The password entries must be identical.

5. Select the **Save/Update** button.

6. Select the **OK** button in the "User Role Created" dialog box.

Deleting an Admin User

1. To delete an Admin user, select the 'Login Manager' tab.

2. Select a user from the 'User Name' drop-down menu.

3. Select the **Delete** button to remove the user.

4. Select **Yes** to confirm deletion.

5. Select the **OK** button in the 'User Role Deleted' dialog box.

Notes:

1. Incorrectly entered passwords result in a Standard user login. The 'Administrator Login' window closes and a "ReadOnly Access Granted" dialog box opens (Figure 63). To attempt the login again, close the 'Administrative Tools' window. Select the **Configuration Settings** icon and follow the instructions for an Admin user login.

2. Information stored in the software database is independent of the computer's Microsoft® Windows login. Therefore, any user can see all database entries. See Section 9.C for more information about multi-user installation.
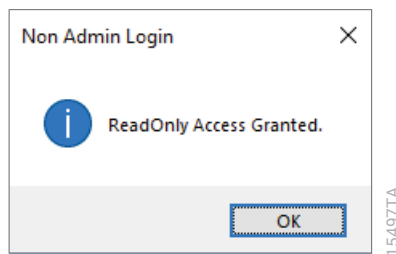
Figure 63. Non Admin Login notification.

9.G.   Getting Started

Before data analysis, settings for analysis and reporting must be defined. These settings include: default units for DNA concentration; well-naming convention; calibrator assessment (for use with virtual standard curves); selection of worksheets to omit from the final report; definitions for the PowerQuant® System dye names; standard curve assessment settings; sample assessment settings; and normalization settings for STR analysis. The Admin user defines these parameters in the 'PowerQuant Administrative Tools' window, accessed by opening the Configuration Settings in the 'Configuration Tools' tab (Figure 57).

Set Configuration Defaults

Selections made in the 'Configuration' tab of the 'Promega PowerQuant Administrative Tools' window are the settings used in the analysis and the final report (Figure 64). The 'Configuration' tab is used to set units for DNA concentration, well-naming convention (A1, A2, A3. . . or A01, A02, A03. . .), calibrator assessment values (for use with a virtual standard curve only, see Section 9.L) and selection of worksheets to omit from the final report.

1.   Select the 'Configuration Tools' tab in the main menu and press the **Configuration Settings** icon (Figure 57). The 'PowerQuant Administrative Tools' window opens along with the 'Administrator Login' window.

2.   Enter a valid Admin User Name and password (Figure 61). See Section 9.F for additional information about user accounts. (Default Admin User Name: PQAdmin; Default Password: Promega).

   Note: An Administrator login is required to save configuration settings.

3.   Select the 'Configuration' tab (Figure 64).
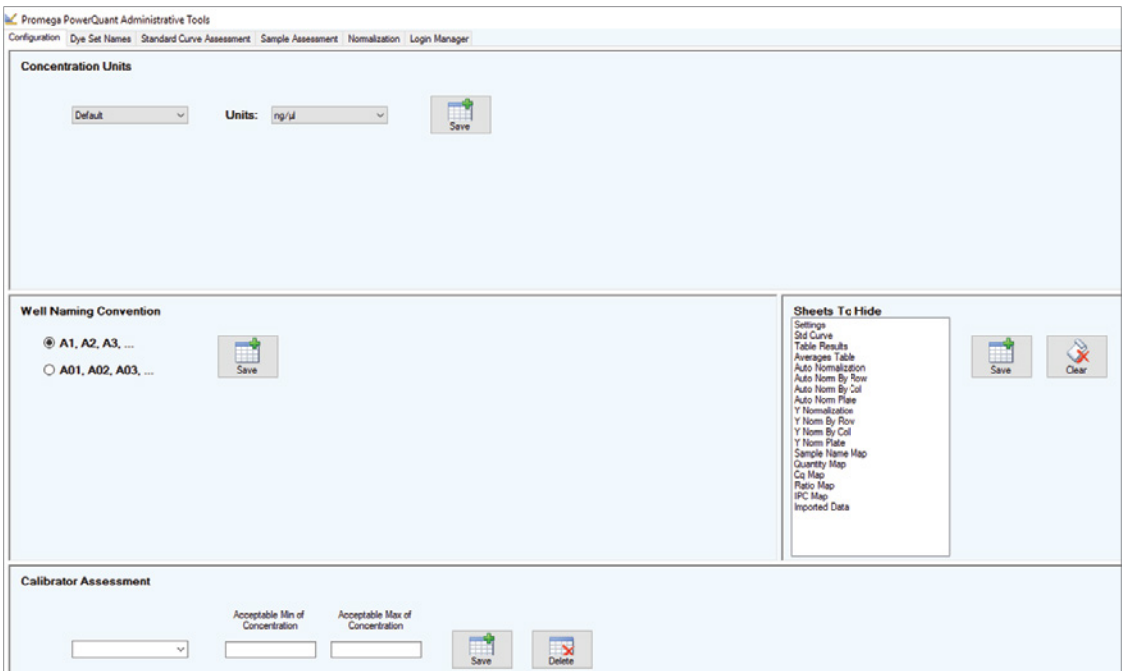
Figure 64. The 'Configuration' tab.

4.   Set definitions for the following:

Concentration Units: Select the Default units for concentration from the 'Units' drop-down menu and select the **Save** icon. See the drop-down menu in the software to view options for concentration units and well-naming.

Well-Naming Convention: Select the well-naming convention from the two radio button options and select the **Save** icon.

Calibrator Assessment: Calibrator assessment definitions are required for use with a virtual standard curve. Definitions for a minimum and maximum concentration are required for assessment. These definitions are expressed as a ratio. See Section 9.L for additional information about virtual standard curves.

Sheets to Hide: The PowerQuant® Analysis Software generates multiple worksheets for a final report. This section allows the user to select worksheets that will not be viewable in the final report. Select the sheets to hide from the list and select the **Save** icon. See Section 9.J for more information about the contents of each of the worksheets.

Create or Retrieve Dye Set Names

Selections made in the 'Dye Set Names' tab of the 'PowerQuant Administrative Tools' window are used to create a sample plate map for import into the real-time qPCR instrument software. Dye sets can be created, retrieved, edited and deleted.

1.   Select the 'Dye Set Names' tab (Figure 65).
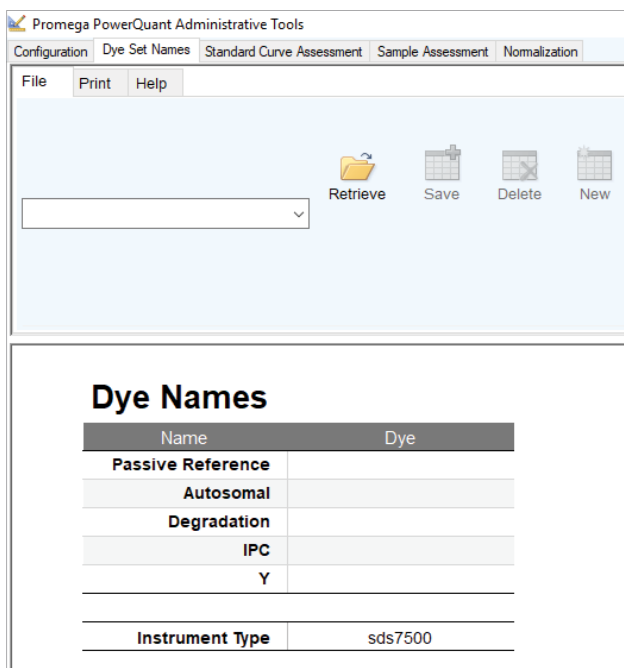
Figure 65. The 'Dye Set Names' tab.

9.G.   Getting Started (continued)

Creating a New Dye Set

1.   Select the **New** icon.

2.   Select **Yes** in the 'Confirm Data Delete' dialog box to clear the form.

3.   Enter a name for the dye set in the drop-down menu field.

4.   Enter the dye names in the fields provided for Dye associated with target Name and select the **Save** icon (Figure 66).

     Note: The dye names must match those used for instrument calibration (see Section 4). A preloaded dye set containing PowerQuant dyes is available as a default option. For the Applied Biosystems® 7500 Real-Time PCR System for Human Identification and HID Real-Time PCR Analysis Software, Version 1.1 or 1.2, enter "ROX" in the Passive Reference field.

5.   Ensure "sds7500" is entered in the Instrument Type field.

     Note: Plate map import using the default entry "sds7500" is compatible with current versions of Applied Biosystems® 7500, 7500 HID and QuantStudio™ 5 Real-Time PCR System software (Figure 66).
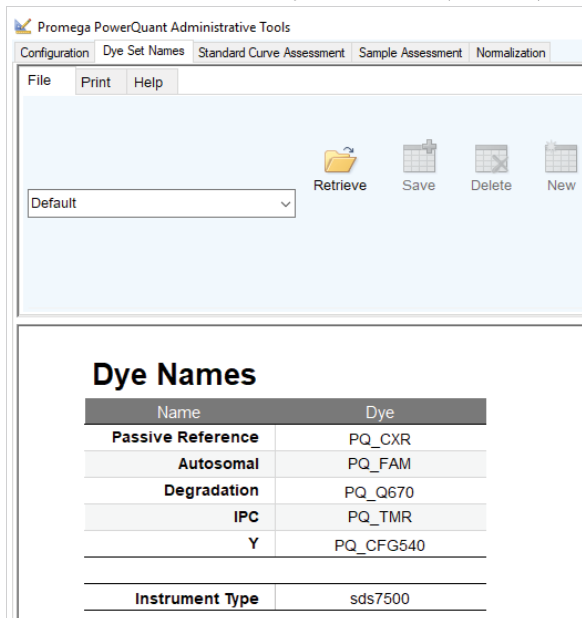
Figure 66. The 'Dye Set Names' tab with the default dye names for each target.

6.   Select the **Save** icon.

7.   Select **Yes** in the 'Save Confirm' dialog box.

     Note: The dye set name appears in the dialog box. Confirm it is the correct dye set.

8.   Select **OK** in the 'Save Complete' dialog box.

9.   The newly created dye set is available for selection in the drop-down menu indicating addition to the database.

Viewing or Editing an Existing Dye Set

1. Select a dye set from the drop-down menu and select the **Retrieve** icon.

2. The stored dye names for the dye set appear in the Dye fields associated with each target name.

3. Edit the dye names in the Dye fields or Instrument Type field as needed.

4. Select the **Save** icon.

5. Select **No** in the 'Set Already Exists' dialog box and enter a new name in the dye set drop-down menu field; or

6. Select **Yes** in the 'Set Already Exists' dialog box to overwrite the existing dye set.
   Note: The dye set name appears in the dialog box. Confirm it is the correct dye set.

7. Select **OK** in the 'Save Complete' dialog box.

Deleting a Dye Set

1. Select a dye set from the drop-down menu and select the **Delete** icon.

2. A 'Delete Confirm' dialog box appears.
   Note: The dye set name appears in the dialog box. Confirm it is the correct dye set.

3. Select **No** to cancel the deletion; or

4. Select **Yes** to confirm the deletion. Select **OK** in the 'Delete Complete' dialog box.
   Note: The dye set is removed from the drop-down menu.

Create/Save Standard Curve Assessment Settings

Selections made on the 'Standard Curve Assessment' tab of the 'PowerQuant Administrative Tools' window provide the software with criteria used to evaluate the standard curve.

Creating a New Standard Curve Assessment

1. Select the 'Standard Curve Assessment' tab and select the **New** icon (Figure 67).

2. Select **Yes** in the 'Confirm Data Delete' dialog box to clear the form.

3. Enter the values for Minimum Acceptable R-Squared (R^2) and Minimum and Maximum Acceptable Slope. (See Section 10 for additional information about interpretation of PowerQuant® System data). Additionally, Minimum and Maximum Acceptable values for the Y-intercept may be entered; selections for Y-intercept values are optional and not required for analysis with the PowerQuant® Analysis Software. All entries in this form must be numeric.

   a. Specify the minimum passing R-Squared (R^2) value that is acceptable to your laboratory for the standard curve for each quantification target (autosomal, Y and degradation). The software flags a standard curve with an R^2 value that falls below the minimum passing value.

   b. Specify the upper and lower limits that are acceptable for the slope of the standard curve for each quantification target. The software flags a standard curve with slope values outside the upper and lower limits.

   c. Specify the upper and lower numerical values that are acceptable for the Y-intercept of the standard curve for each quantification target. The software flags a standard curve with Y-intercept values outside the upper and lower limits.
   Note: Specifying values for upper and lower limits are optional for the Y-intercept.

4. To save the entries, enter a name for the standard curve assessment settings in the drop-down menu field and select the **Save** icon. A 'Save Complete' message box appears when the standard curve assessment settings have been saved.

    Note: The standard curve settings name appears in the dialog box. Confirm it is the correct name.

5. Select **OK** in the 'Save Complete' dialog box.

6. The newly created standard curve assessment settings are available for selection in the drop-down menu indicating addition to the database.
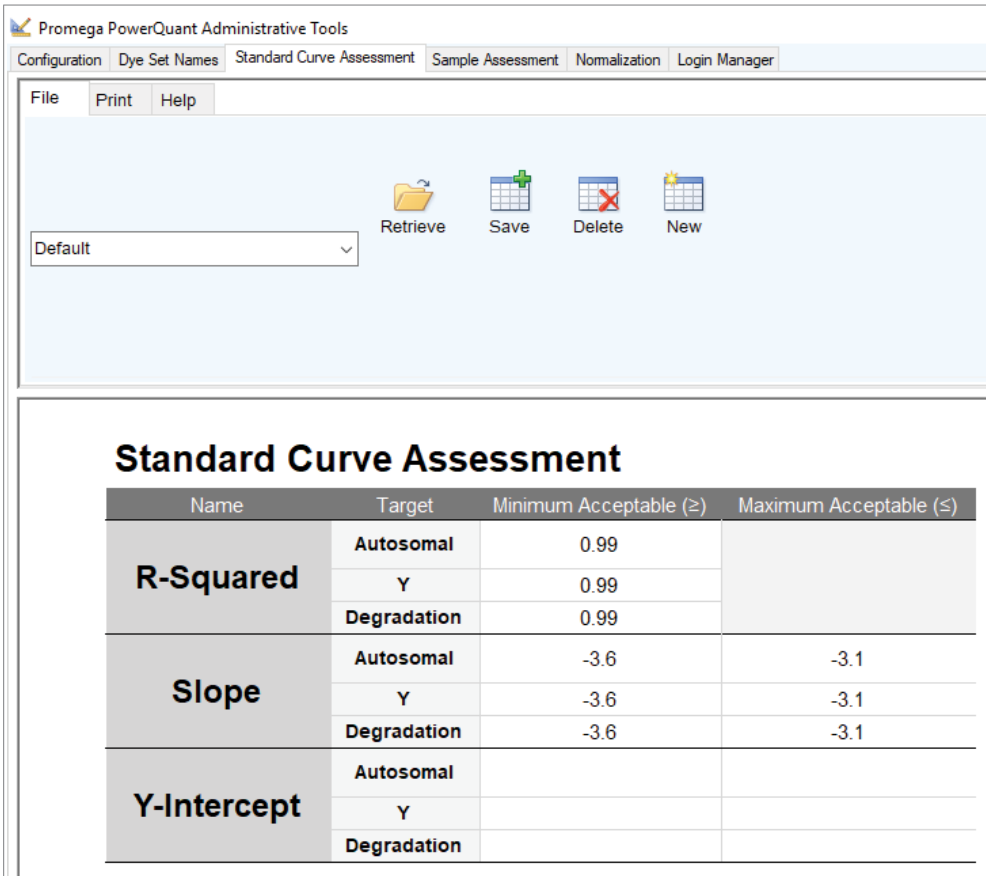
Figure 67. The 'Standard Curve Assessment' tab.

Note: The standard curve assessment values displayed in the image above are default values. Default values should be customized to reflect results from internal validation studies conducted by individual laboratories.

Viewing or Editing an Existing Standard Curve Assessment

1. Select a standard curve assessment from the drop-down menu and select the **Retrieve** icon.

2. The stored standard curve assessment values for each parameter and target appear.

3. Edit the parameters as needed.

4. Select the **Save** icon.

5. Select **No** in the 'Set Already Exists' dialog box and enter a new name in the standard curve assessment drop-down menu field; or

6. Select **Yes** in the 'Set Already Exists' dialog box to overwrite the existing standard curve assessment.
   Note: The standard curve assessment name appears in the dialog box. Confirm it is the correct name.

7. Select **OK** in the 'Save Complete' dialog box.

Deleting an Existing Standard Curve Assessment

1. Select a standard curve assessment from the drop-down menu and select the **Delete** icon.

2. A 'Confirm Delete' dialog box appears.
   Note: The standard curve assessment name appears in the dialog box. Confirm it is the correct name.

3. Select **No** to cancel the deletion; or

4. Select **Yes** to confirm the deletion. Select **OK** in the 'Delete Complete' dialog box.
   Note: The standard curve assessment is removed from the drop-down menu.

Create/Save Sample Assessment Settings

Creating a New Sample Assessment Setting

1. Select the 'Sample Assessment' tab and select the **New** icon (Figure 68).

2. A 'Confirm Data Delete' dialog box appears. Select **Yes** to clear the form.

3. Enter Threshold Values and Sample Assessment Messages. The threshold values should be customized to reflect results from internal validation studies conducted by individual laboratories (See Section 10 for additional information about interpretation of PowerQuant® System data).

   a. In the Inhibitor threshold value field, specify the minimum IPC shift value at which you may expect to encounter inhibition with amplification of STRs. The IPC shift is the calculated difference in C_q values for the IPC in an unknown sample and the IPC of the closest DNA concentration in the standard curve.

   b. In the Male/Female threshold value field, specify the minimum [Auto]/[Y] ratio indicative of a potential male/female mixture.

   c. In the Degradation threshold value field, specify the minimum [Auto]/[Deg] ratio indicative of a potentially degraded DNA sample.
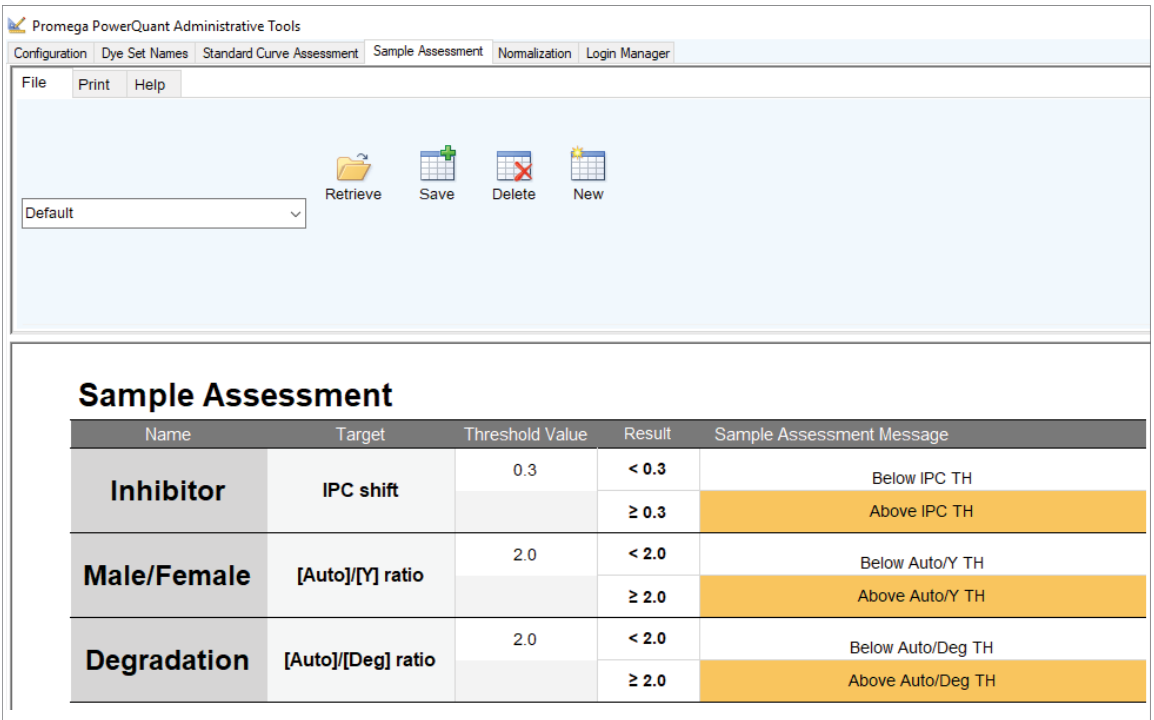
9.G. Getting Started (continued)

4. To save the entries, type a name into the drop-down menu and select the **Save** icon. A 'Save Complete' dialog box appears when the sample assessment settings have been saved.

   Note: The sample assessment settings name appears in the dialog box. Confirm it is the correct name.

5. Select **OK** in the 'Save Complete' dialog box.

6. The newly created sample assessment settings are available for selection in the drop-down menu indicating addition to the database.

Figure 68. The 'Sample Assessment' tab.

Notes:

1. The sample assessment settings displayed in the image above are the default values. These values should be customized to reflect results from internal validation studies.

2. For assessment of possible male/female DNA mixtures, DNA degradation and PCR inhibition, individual laboratories should determine and validate acceptable threshold values relevant to the success of downstream applications. The diversity of sample types, purifications and potential PCR inhibitors does not allow for specific threshold value recommendations. Default threshold settings in the PowerQuant® Analysis Software are values intended to be customized by the user based on results from their own internal validation studies.

Viewing or Editing an Existing Sample Assessment

1. Select a sample assessment from the drop-down menu and select **Retrieve**.

2. The sample assessment values appear in the form. Edit the sample assessment settings as desired and select the **Save** icon.

3. Select **No** in the 'Set Already Exists' dialog box and enter a new name in the sample assessment drop-down menu field; or

4. Select **Yes** in the 'Set Already Exists' dialog box to overwrite the existing sample assessment.
   Note: The sample assessment settings name appears in the dialog box. Confirm it is the correct name.

5. Select **OK** in the 'Save Complete' dialog box.

Deleting an Existing Sample Assessment

1. Select a sample assessment name from the drop-down menu and select the **Delete** icon.

2. A 'Confirm Delete' dialog box appears.
   Note: The sample assessment settings name appears in the dialog box. Confirm it is the correct name.

3. Select **No** to cancel; or

4. Select **Yes** to confirm. Select **OK** in 'Delete Complete' dialog box.
   Note: The sample assessment is removed from the drop-down menu.
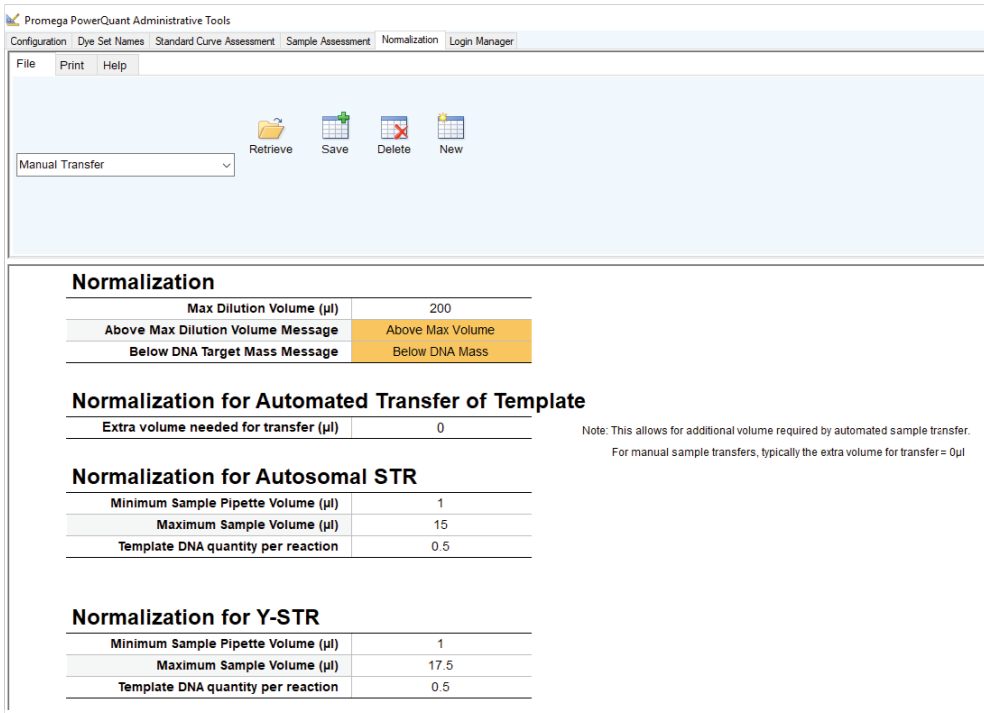
Create/Save Normalization Settings

Parameters defined in the 'Normalization' tab of the 'PowerQuant Administrative Tools' window are used in calculations for normalizing the DNA target mass for autosomal or Y-STR amplification, as well as pipetting volumes and diluent volumes. Additionally, user-defined message fields are used to flag samples that may need additional attention based on the normalization parameters. See Section 9.J for additional information about the autosomal and Y-STR normalization worksheets.

Creating a New Normalization Setting

1. Select the 'Normalization' tab and select the **New** icon (Figure 69).

2. A 'Confirm Data Delete' dialog box appears. Select **Yes** to clear the form.

3. Enter Normalization values.

Figure 69. The 'Normalization' tab.

Normalization

In the field for Max Dilution Volume (µl), specify the largest allowable volume of diluent to add to a concentrated DNA sample to try and achieve the desired final DNA concentration (e.g., the maximum tube volume or maximum well volume on a 96-well plate).

In the field for Above Max Dilution Volume Message, enter text used to indicate that a volume greater than the specified Max Dilution Volume is required to dilute the DNA sample to the desired final DNA concentration.

In the field for Below DNA Target Mass Message, enter text used to indicate that the DNA concentration determined using the autosomal or Y target (for the Autosomal or Y Normalization worksheets) is too low to achieve the specified target DNA mass (the units displayed for DNA mass are based on the default selection made in the 'Configuration' tab), even when using the maximum volume of DNA sample.

Normalization for Automated Transfer of Template

In this section, the user can define additional volume to ensure adequate volume for liquid detection and automated transfer of normalized samples. For example, if a liquid handler requires a minimum volume of 16.5µl for liquid detection, then enter "1.5µl" for a 15µl Maximum Sample Volume (µl). Extra pipetting volume is not necessary for automated protocols developed by Promega.

For manual sample transfer, enter the required extra volume needed for transfer. Enter "0µl" if no additional volume is needed.

Normalization for Autosomal STR

The PowerQuant® Analysis Software uses the values defined in this section and the autosomal DNA concentration of a DNA sample to determine the volume of undiluted DNA required to achieve the target mass in an autosomal STR amplification reaction. If a DNA sample is too concentrated, the user-defined values are used to calculate the sample dilution needed to achieve the target mass of DNA. The software flags any sample that does not reach the target mass of DNA based on the maximum sample volume.

Specify the minimum volume of DNA sample that can be pipetted in the Minimum Sample Pipette Volume (µl) field. The volume of a DNA sample used in the Auto Normalization worksheet is not lower than this specified value for preparation of the normalized dilution.

In the Maximum Sample Volume (µl) field, specify the maximum volume of DNA sample or the normalized dilution that can be added to the autosomal STR amplification reaction.

In the Template DNA quantity per reaction field, specify the desired mass of DNA to be amplified. (The units for DNA mass are based on the concentration units selected in the 'Configuration' tab.)

Normalization for Y-STR

The PowerQuant® Analysis Software uses the values defined in this section and the male DNA concentration (Y target) of a DNA sample to determine the volume of undiluted DNA required to achieve the target mass in a Y-STR amplification reaction. If a DNA sample is too concentrated, the user-defined values are used to calculate the sample dilution needed to achieve the target mass of DNA. The software flags any sample that does not reach the target mass of DNA based on the maximum sample volume.

Specify the minimum volume of DNA sample that can be pipetted in the Minimum Sample Pipette Volume (µl) field. The volume of a DNA sample used in the Y Normalization worksheet is not below this specified value for preparation of the normalized dilution.

In the Maximum Sample Volume (µl) field, specify the maximum volume of DNA sample or the normalized dilution that can be added to the Y- STR amplification reaction.

In the Template DNA quantity per reaction field, specify the desired mass of DNA to be amplified. (The units for DNA mass are based on the concentration units selected in the 'Configuration' tab.)

4. To save the entries, type a name into the drop-down menu and select the **Save** icon. A 'Save Complete' dialog box appears when the normalization settings have been saved.

   Note: The normalization settings name appears in the dialog box. Confirm it is the correct name.

5. Select **OK** in the 'Save Complete' dialog box.

6. The newly created sample settings are available for selection in the drop-down menu indicating addition to the database.

9.G. Getting Started (continued)

Viewing or Editing Normalization Settings

1. Select a normalization settings name from the drop-down menu and select the **Retrieve** icon.

2. The normalization settings values appear in the form. Edit the settings as desired and select the **Save** icon.

3. A 'Set Already Exists' dialog box prompts you to confirm the save action.

   Note: The normalization settings name appears in the dialog box. Confirm it is the correct name.

4. Select **No** in the 'Set Already Exists' dialog box and enter a new name in the normalization drop-down menu field; or

5. Select **Yes** to overwrite the normalization settings.

6. Select **OK** in the 'Save Complete' dialog box.

Deleting Normalization Settings

1. Select a normalization settings name from the drop-down menu and select **Delete**.

2. A 'Confirm Delete' dialog box appears.

   Note: The normalization settings name appears in the dialog box. Confirm it is the correct name.

3. Select **No** to cancel; or

4. Select **Yes** to confirm. Select **OK** in 'Delete Complete' dialog box.

   Note: The normalization settings name is removed from the drop-down menu.

9.H. Designing a Plate Map

The PowerQuant® Analysis Software uses the plate map to identify wells containing standards, unknowns, negatives and calibrators. The software uses the concentrations of the DNA standards defined in the plate map to generate the standard curve. The PowerQuant® Analysis Software Plate Designer also includes features for creation of an import file for the instrument software and calculations for the individual components for the PowerQuant® amplification reaction setup.

General Considerations on Creating a Plate Map

- Plate maps are required for analysis of data exported from the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System to be compatible with the PowerQuant® Analysis Software.

- Sample names entered in a plate map are used to create a .txt file for import in the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System software; sample names are not stored as part of a plate map in the PowerQuant® Analysis Software database.

- Any user can create or save a plate map.

- Wells can be assigned either Standard, Negative, Unknown and Calibrator (Calibrator assignments are available for use with virtual standard curves only. See Section 9.L for additional information about virtual standard curves).

    Standard: Data from sample wells designated as standards are used to generate the standard curve and determine the DNA concentration for all remaining sample types. No sample assessments are made for this sample type.

    Negative: Results from sample wells designated as negatives are compared to the sample assessment settings entered in the Sample Assessment Settings (see Section 9.G). No additional assessment is performed on negatives.

    Unknown: Results from sample wells designated as unknowns are compared to the sample assessment settings entered in the Sample Assessment Settings (see Section 9.G).
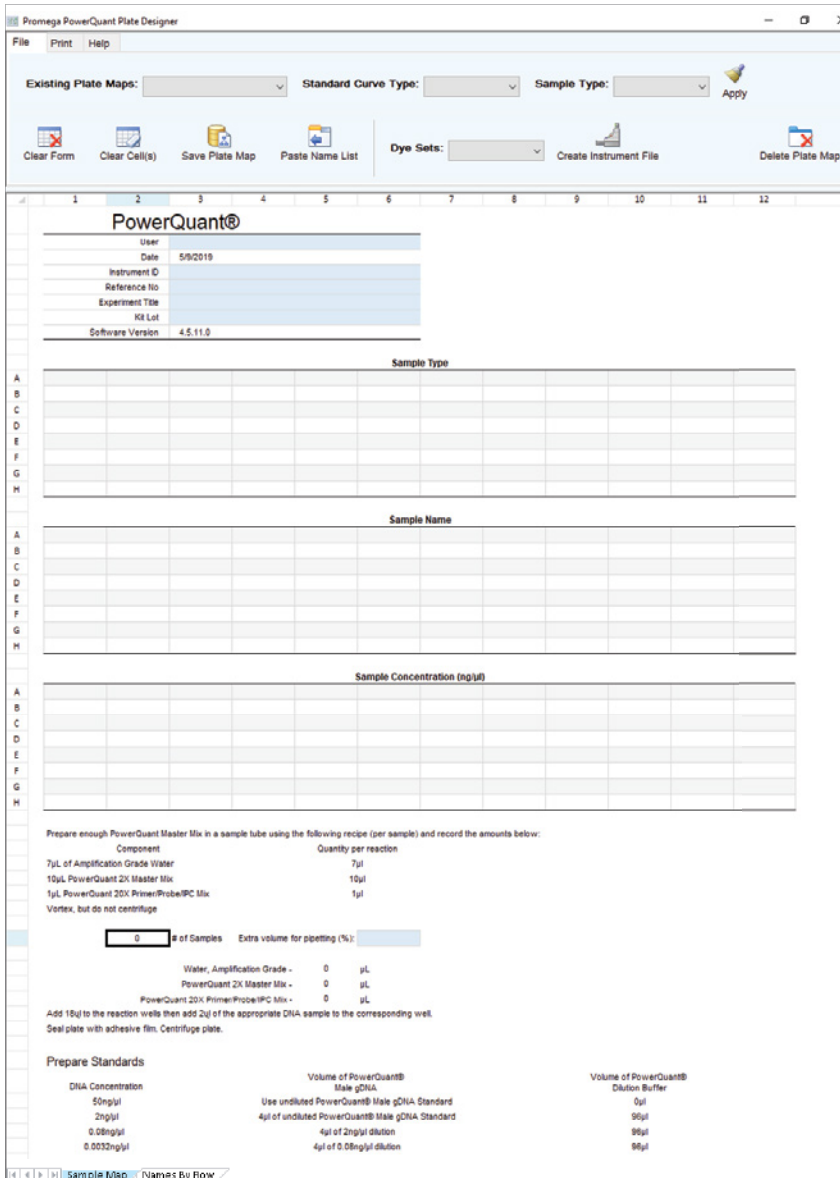
    Calibrator: Results from sample wells designated as calibrators are compared to the Sample Assessment Settings and the Calibrator Assessment Settings (for use with virtual standard curves only). See Section 9.L for additional information about virtual standard curves.

- The PowerQuant® Analysis Software uses the plate map to identify wells containing standards when importing a PowerQuant® Results Excel® file. With the positions of the standards defined, the software then analyzes the Results file to generate the standard curve and determine sample concentration.

- A number of user-defined fields are available to record information (e.g., user, date, instrument ID). Information entered in these fields are not stored as part of a plate map in the PowerQuant® Analysis Software database.

9.H. Designing a Plate Map (continued)

Creating a New Plate Map

1. In the 'Configuration Tools' tab (Figure 57), select the **Design Plate Map** icon to open the Promega PowerQuant® Plate Designer (Figure 70).

Figure 70. The 'Promega PowerQuant Plate Designer' window.

2. Select a standard curve type from the 'Standard Curve Type' drop-down menu. Select **Yes** in the 'New Standard Curve Origin' dialog box to clear the form.

The **Imported** Standard Type selection indicates data for a standard curve will be imported into the PowerQuant® Analysis Software from the Results Excel® file exported from the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System software.

The **Virtual** Standard Type selection indicates data for a standard curve are stored from a previous run in the PowerQuant® Analysis Software and not contained in the file exported from the instrument software. See Section 9.L for additional information about virtual standard curves.

3. Select **Yes** in the 'New Standard Curve Origin' dialog box.

4. In the Sample Type plate map, highlight the wells of interest and select the sample type from the 'Sample Type' drop-down menu and select the **Apply** icon. The plate map displays the assigned sample type.

   a. Select the **Standard** sample type for sample wells containing the PowerQuant® Male gDNA standard dilutions used to generate the standard curve.
      Notes:
      1. Standard wells must be defined when standard curve data are included in the imported file for analysis.
      2. Standard is not a sample type option when a virtual standard curve is used for analysis. See Section 9.L for additional information about virtual standard curves.

   b. Select **Unknown** for wells containing unknown samples.

   c. Select **Negative** or **Unknown** for wells containing negative controls.
      Note: The negative designation is for use inside the PowerQuant® Analysis Software only and will not be transferred to the .txt files for import into the real-time PCR instrument software.

   d. Select **Calibrator** for wells containing calibrator samples of known concentration (for use with virtual standard curves only, see Section 9.L for additional information).
      Notes:
      1. Calibrator is not a sample type option when an imported standard curve is used for analysis.
      2. The calibrator designation is for use inside the PowerQuant® Analysis Software only and will not be transferred to the .txt files for import into the real-time PCR instrument software.

   e. Select **Unknown** for all remaining wells in the 96-well plate (Figure 74).

5. In the Sample Concentration plate map, enter the concentrations for wells containing standards. This is required for any wells designated as standards in the Sample Type plate map. The concentration units displayed reflect the default selected on the 'Configuration' tab (see Section 9.G). Only numerical values are accepted in this plate map.

6. To store the locations of the Sample Types and the Sample Concentrations of the DNA standards, select the **Save Plate Map** icon.
   Note: The Save function does not apply to the Sample Names portion of the plate map; sample name information is not stored in the plate map database. Sample Names are used to create .txt files for import into the real-time PCR instrument software.

9.H. Designing a Plate Map (continued)

7. Enter a name for the plate map in the 'Plate Map Name' dialog box and select the **OK** button (Figure 71).
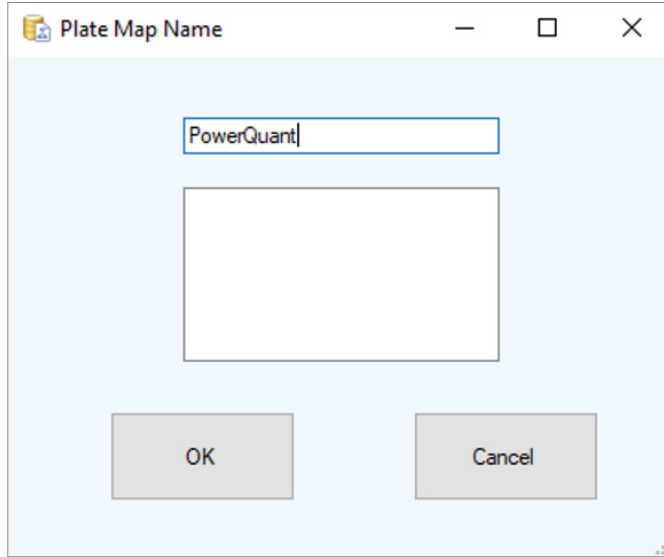
Figure 71. 'Plate Map Name' dialog box.

8. The newly created plate map is now available for selection in the 'Existing Plate Maps' drop-down menu indicating addition to the database (Figure 72).

Editing an Existing Plate Map

1. Select a plate map from the 'Existing Plate Maps' drop-down menu (Figure 70).

2. An "Apply Plate Map" message appears.

3. Select **No** to cancel clearing the form and importing the template; or

4. Select **Yes** to clear the form and fill it with the selected template information.

5. In the Sample Type plate map, highlight the wells of interest and select the sample type from the drop-down menu and select the **Apply** icon. The plate map displays the assigned sample type.

6. To clear wells in any of the plate maps, highlight the cells and select the **Clear Cell(s)** icon.

7. To store the locations of the Sample Types and the Sample Concentrations of the DNA standards, select the **Save Plate Map** icon.

    Note: The Save function does not apply to the Sample Names portion of the plate map; sample name information is not stored in the plate map database. Sample Names are used to create .txt files for import into the real-time PCR instrument software.
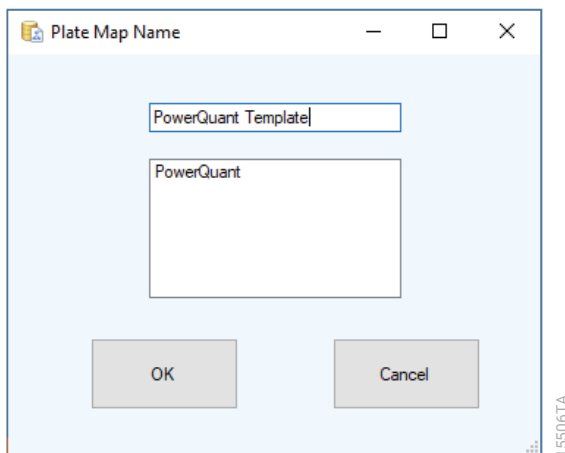
Figure 72. 'Plate Map Name' dialog box with a previously saved plate map.

8. Either enter a new name for the plate map in the 'Plate Map Name' dialog box and select the **OK** button; or

9. Select the existing template name from the list in the 'Plate Map Name' dialog box (Figure 72).

    a. A 'Plate Map Already Exists' dialog box appears with the existing plate name.

    b. Select **No** to cancel overwriting the template; or

    c. Select **Yes** to overwrite the template. Select the **OK** button.

Deleting a Plate Map

1. Select a plate map from the "Existing Plate Maps" drop-down menu.

2. Select the **Delete** icon.

3. A 'Confirm Delete' dialog box appears with the plate name.

    a. Select **No** to cancel deleting the plate map; or

    b. Select **Yes** to delete the plate map.

4. Select the **OK** button in the 'Delete Complete' dialog box.

    Note: The plate map is removed from the drop-down menu.

Creating a .txt file for Import into the Real-Time PCR Instrument

1. To create a .txt for import in the Applied Biosystems® 7500, 7500 HID or QuantStudio™ 5 Real-Time PCR System software, follow the instructions in "Creating a Plate Map or Editing an Existing Plate Map".

2. Select a dye set from the 'Dye Sets' drop-down menu of the 'Promega PowerQuant Plate Designer' window (Figure 70).

9.H. Designing a Plate Map (continued)

3. Enter sample names in the Sample Name plate map.

   Note: Some symbols are not compatible with .txt file import. Limit the use of symbols in sample names.

   a. Sample names can be entered manually on the 'Sample Map' tab or copied and pasted into the plate map in a 96-well format (Figure 70).

   b. Alternatively, the 'Names by Row' tab (located at the bottom of the screen) can be used to arrange sample names and well positions listed by row. Use the **Paste Name List** icon to transfer the sample name information entered on this tab to the 'Sample Map' tab (Figure 73).

   Note: Using the 'Names by Row' tab to add sample names to the plate map will overwrite any entries made to the plate map on the 'Sample Map' tab.
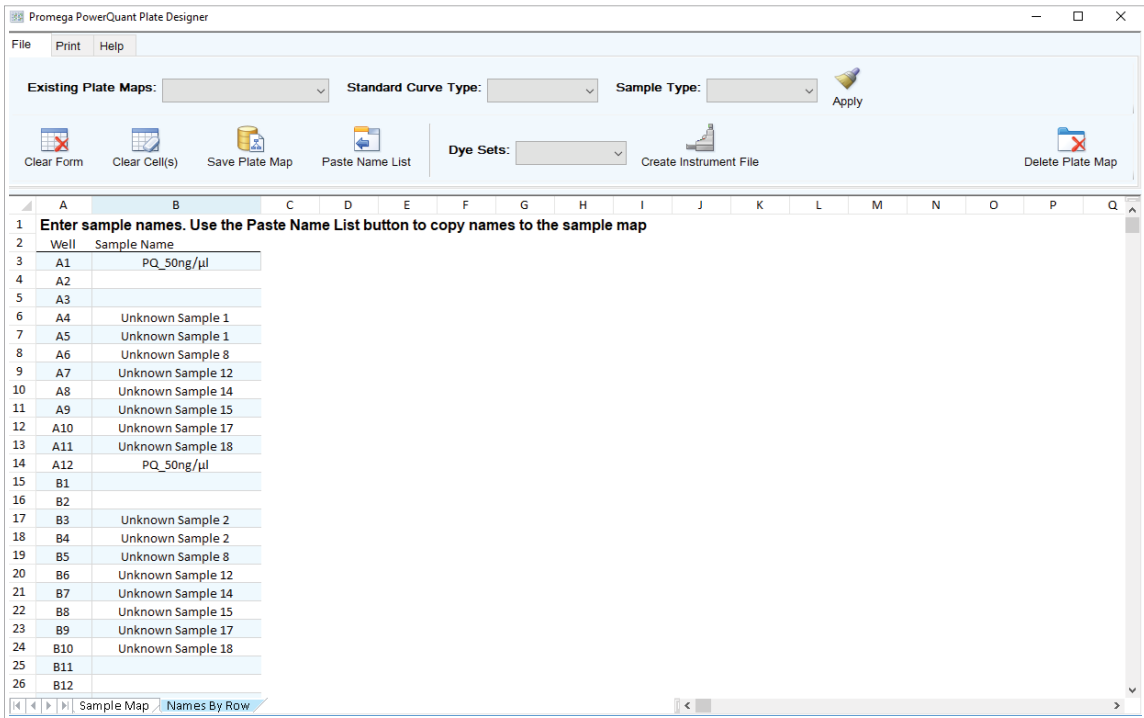
Figure 73. The 'Names by Row' tab in the Promega PowerQuant Plate Designer.

Figure 74. The 'Sample Map' tab in the 'Promega PowerQuant Plate Designer File' window with Sample Type, Sample Names and Standard Sample Concentration.

9.H. Designing a Plate Map (continued)

4. In the Sample Type plate map, highlight all unused wells and select the **Clear Cells** button.

5. Select the **Create Instrument File** icon to create the file.

6. The 'Excel Data Bucket' window opens. Select the **Save Instrument File** button (Image not shown).

7. A file explorer opens.

8. Navigate to the location of interest, enter a file name and select **Save**.

9. Select the **Return** button to close the 'Excel Data Bucket' window.

10. Use the Import function in the instrument software to add the information in the plate setup import file to a previously created run template. See Sections 6.B, 7.B or 8.C for additional information.

    Note: Confirm that the C_T Settings are set correctly in the instrument software. See Sections 6.A, 7.A or 8.B for additional information.

Preparation of PowerQuant® Amplification Reaction Mix

1. The number of sample names entered in the Sample Name plate map on the 'Sample Map' tab of the Promega PowerQuant Plate Designer determines the number of reactions used to calculate the preparation of PowerQuant® Amplification Reaction Mix (Figure 74). (See Section 5.B for information on reaction setup.)

2. Use the 'Extra Volume for pipetting (%)' field to compensate for pipetting error and reagent loss on sides of pipette tips (an extra 10–15% extra volume is recommended; see Section 5.B). Enter the percentage converted to a decimal value (e.g., enter "0.10" for 10%).

9.I. Importing the PowerQuant® Results Excel® File

The 'Import' tab of the PowerQuant® Analysis Software is used to process qPCR data generated with the PowerQuant® System (Figure 75).

The PowerQuant® Analysis Software **does not** store analyzed data. Analyzed data must be saved as an Excel® workbook or as a .pdf file.

Note: Successful analysis requires the inclusion of sample names in the PowerQuant® Results Excel® file exported from the instrument software, as well as the designation of a Sample Type for all used sample positions in the PowerQuant® Analysis Software Plate Map (see Section 9.H).

1. Use the drop-down menus to make the following selections: data file source; sample assessment, standard curve assessment and normalization settings; plate map; and virtual curve (for use with virtual standard curves only; see Section 9.L for additional information on virtual standard curves) (Figure 75). See Section 9.G for information about settings and Section 9.H for information about plate maps.

    Note: Selecting the correct plate map from the menu is critical for analysis with the PowerQuant® Analysis Software. Failure to do so may lead to incomplete or erroneous results.
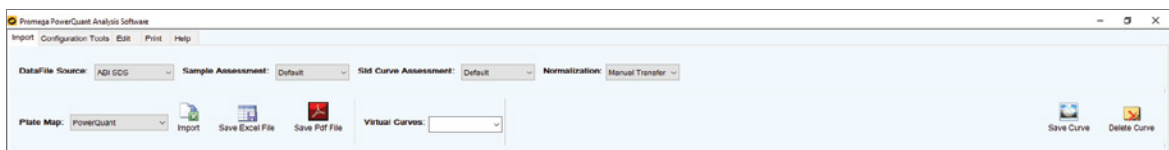
Figure 75. The 'Import' tab.

2. Select the **Import** icon. A dialog box labeled "Make Sure You Have Saved Your Work Before Proceeding" will appear. Select **No** to abort the import, or **Yes** to clear the form.

3. Use the file explorer to navigate to the file of interest, select the file and select **Open**. The analysis begins.

4. When the import and analysis are complete, multiple worksheets are created. The software displays the Settings worksheet.
   Note: Once the file is imported, the **Import** icon is renamed **Open Import File**.

9.J. The PowerQuant® Analysis Software Report

Worksheets General Information

The following worksheets are created by the PowerQuant® Analysis Software after analysis is complete. These worksheets are locked to prevent editing of the formulas, linked cells and named ranges.

Review the following worksheets created in Section 9.I in the PowerQuant® Analysis Software.

Settings Worksheet

The Settings worksheet displays general information about the analysis, standard curve and standard curve assessment settings, sample assessment settings and normalization settings (Figure 76). See Section 9.G for additional information about these settings. See Section 9.L for information about the Settings worksheet when a virtual standard curve is used.

a.   In the PowerQuant® header section on the Settings worksheet, there are fields shaded white or light blue.

   i.   The white cells display information populated by the PowerQuant® Analysis Software that cannot be modified:

- The Date field is populated with the date the PowerQuant® Analysis Software analysis was completed.

- The Data File Source field is populated with the source of the imported data file (i.e., ABI 7500 SDS).

- The Plate Map Name field is populated with the name of the plate map selected in section 9.I.

- The Imported File Name field is populated with the name of the imported PowerQuant® Results Excel® file.

- The Software Version field is populated with the version of the PowerQuant® Analysis Software used for analysis.

   ii.   The following light blue cells can be defined by the user:

- The User field is automatically populated with the Microsoft® Windows user name.
  Note: Although, this field is automatically populated, it is editable.

- Instrument ID

- Reference Number

- Experiment Title

- Kit Lot
  Note: The information displayed and entered in the header on the Settings worksheet is displayed on all PowerQuant® Analysis Software generated worksheets.

PowerQuant®

User	
Date	07/03/19
Data File Source	ABI 7500 HID
Plate Map Name	PowerQuant
Imported File Name	PowerQuant data.xlsx
Instrument ID	
Reference Number	
Experiment Title	
Kit Lot	
Software Version	4.5.11

Standard Curve Assessment

Name	Target	Min Value	Max Value
R-Squared	Autosomal	0.99	
	Y	0.99	
	Degradation	0.99	
Slope	Autosomal	-3.6	-3.1
	Y	-3.6	-3.1
	Degradation	-3.6	-3.1
Y-Intercept	Autosomal		
	Y		
	Degradation		

Sample Assessment

Name	Target	Threshold	Result	Sample Assessment Message
Inhibitor	IPC shift	0.3	< 0.3	Below IPC TH
			≥ 0.3	Above IPC TH
Male/Female	[Auto]/[Y] ratio	2.0	< 2.0	Below Auto/Y TH
			≥ 2.0	Above Auto/Y TH
Degradation	[Auto]/[Deg] ratio	2.0	< 2.0	Below Auto/Deg TH
			≥ 2.0	Above Auto/Deg TH

Normalization

Max Dilution Volume (µl)	200
Above Max Dilution Volume Message	Above Max Volume
Below DNA Target Mass Message	Below DNA Mass

Normalization for Automated Transfer of Template

Extra volume needed for transfer (µl)	0

Normalization for Autosomal STR

Minimum Sample Pipette Volume (µl)	1
Maximum Template Volume (µl)	15
Template DNA per reaction (ng)	0.5

Normalization for Y-STR

Minimum Sample Pipette Volume (µl)	1
Maximum Template Volume (µl)	17.5
Template DNA per reaction (ng)	0.5

15510TA

Figure 76. The Settings worksheet.

9.J.   The PowerQuant® Analysis Software Report (continued)

Std Curve Worksheet

The Std Curve (Standard Curve) worksheet displays a table of the C_q values for each of the standard samples (Figure 77). It also displays the calculated R^2, Slope, Y-Intercept and Amplification Efficiency for each target, along with a comparison to the R^2 thresholds, slope limits, and Y-intercept thresholds. The R^2 thresholds, slope limits and Y-intercept thresholds used are displayed on the Settings worksheet.
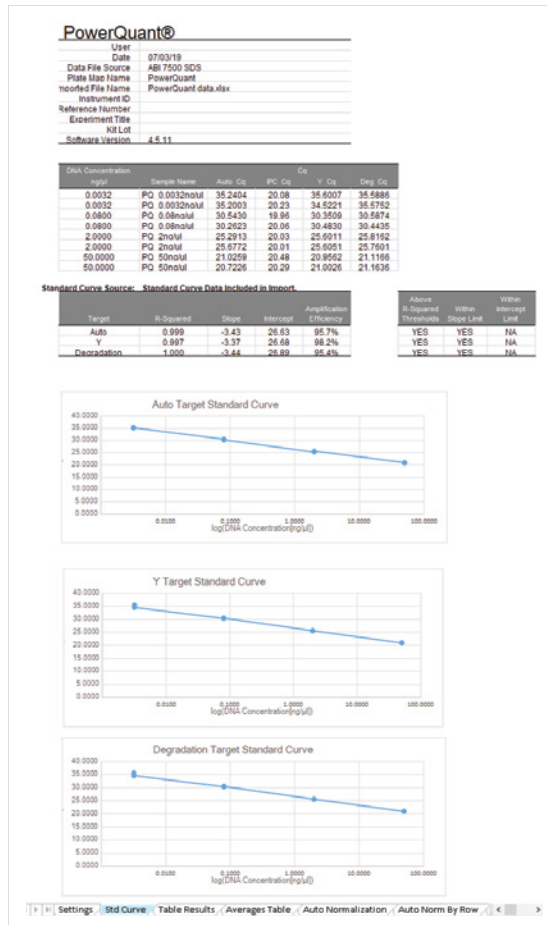
Figure 77. The Std Curve worksheet.

Table Results Worksheet

The Table Results worksheet displays data output sorted by well position (Figure 78). The DNA concentration and C_q value for each target are displayed in respective columns.

The IPC C_q value for the DNA standard with the closest DNA concentration is displayed for each sample in the Closest IPC column. The difference in C_q values for the IPC of the closest DNA standard and the IPC of the DNA sample is displayed in the IPC Shift column. The IPC Threshold Result column displays the message defined in the Sample Assessment Message fields based on the IPC Shift value for the sample and the IPC Shift threshold value.

The ratio of autosomal DNA concentration and male DNA concentration is calculated and displayed in the [Auto]/[Y] column. The [Auto]/[Y] Threshold column displays the message defined in the Sample Assessment Message fields based on the [Auto]/[Y] value for the sample and the [Auto]/[Y] threshold value.

The ratio of autosomal DNA concentration and degradation target DNA concentration is calculated and displayed in the [Auto]/[Deg] column. The [Auto]/[Deg] Threshold column displays the message defined in the Sample Assessment Message fields based on the [Auto]/[Deg] value for the sample and the [Auto]/[Deg] threshold value.

Standard curve data and comparisons to the thresholds and slope limits, defined in the Configuration Settings and selected for analysis, are provided in a table. Thresholds and messages selected for the Sample Assessment settings are also displayed in a table.

The worksheet does not display calculated sample concentrations or sample assessments for samples designated with the Standard sample type.

Note: Filter and sort functions are provided in the Table Results. These functions allow a user to select, exclude and sort data within the table. The filter and sort functions are accessed by selecting the arrow button in the column header. Use the Text Filter function to select data (checked) or exclude data (unchecked). Use the Sort function to reorder data smallest to largest or largest to smallest. Application of these functions may take a few minutes to complete while the software updates data links to other pages in the workbook.

9.J.   The PowerQuant® Analysis Software Report (continued)

Figure 78. The Table Results worksheet.

Averages Table

The Averages Table worksheet contains output results sorted alphabetically by sample name, with averages calculated for replicate samples (i.e., samples with the same sample name) (Figure 79). The DNA concentration and C_q value for each target are displayed in respective columns.

Data for the standard samples are not displayed.

The IPC C_q value for the sample is displayed. The difference in C_q values for the IPC of the closest DNA standard and the IPC of the DNA sample is displayed in the IPC Shift column. The IPC Threshold column displays the message defined in the Sample Assessment Message fields based on the IPC Shift value for the sample and the IPC Shift threshold value.

The IPC C_q value for the DNA standard with the closest DNA concentration is displayed for each sample in the Closest IPC column. The difference in C_q values for the IPC of the closest DNA standard and the IPC of the DNA sample is displayed in the IPC Shift column. The IPC Threshold Result column displays the message defined in the Sample Assessment Message fields based on the IPC Shift value for the sample and the IPC Shift threshold value.

The ratio of autosomal DNA concentration and male DNA concentration is calculated and displayed in the [Auto]/[Y] column. The [Auto]/[Y] Threshold column displays the message defined in the Sample Assessment Message fields based on the [Auto]/[Y] value for the sample and the [Auto]/[Y] threshold value. For samples with no C_q value detected for the Y target, the [Auto]/[Y] column displays "No Y C_q". For samples with no C_q value detected for the autosomal target, the column displays "No Auto C_q".

The ratio of autosomal DNA concentration and degradation target DNA concentration is calculated and displayed in the [Auto]/[Deg] column. For samples with no C_q value detected for the Deg target, the [Auto]/[Deg] column displays "No Deg C_q". For samples with no C_q value detected for the autosomal target, the column displays "No Auto C_q". The [Auto]/[Deg] Threshold column displays the message defined in the Sample Assessment Message fields based on the [Auto]/[Deg] value for the sample and the [Auto]/[Deg] threshold value.

All values for individual wells displayed on the Averages Table worksheet are identical to those on the Table Results worksheet.

Note: Filter and sort functions are provided in the Averages Table. These functions allow a user to select, exclude and sort data within the table. The filter and sort functions are accessed by selecting the arrow button in the column header. Use the Text Filter function to select data (checked) or exclude data (unchecked). Use the Sort function to reorder data smallest to largest or largest to smallest. Application of these functions may take a few minutes to complete while the software updates data links to other pages in the workbook.

Figure 79. The Averages Table worksheet.

9.J. **The PowerQuant® Analysis Software Report (continued)**

Autosomal Normalization Worksheets

The autosomal normalization worksheets contain the output normalization values for each DNA sample based on the DNA concentration determined using the autosomal target for that sample and the autosomal normalization parameters set on 'Normalization' tab of the Configuration Settings (See Section 9.G for more information about Configuration Settings). The Normalization parameters are displayed at the top each normalization worksheet (Figure 80).

Worksheets for DNA template normalization based on the autosomal target are provided in four different formats:
- Samples sorted by sample name (Auto Normalization);
- Samples arranged by row (Auto Norm by Row);
- Samples arranged by column (Auto Norm by Col); and
- Samples arranged in a 96-well format (Auto Norm Plate)

All normalization worksheets display the well position for the DNA sample used in the qPCR amplification plate.

The Auto Normalization worksheet, the Auto Norm by Row worksheet and the Auto Norm by Col worksheet display the following columns of values:

Sample Name: Displays the sample name assigned to the well position.

Sample Concentration: Displays the concentration in the units defined on the 'Configuration' tab of the *Configuration Settings* based on the quantification results from the autosomal target.

Template Volume (µl): If the DNA template requires dilution, the value displayed in this column is the volume of template DNA to add to the diluent to achieve the desired target DNA mass. If the DNA template does not require dilution, the value displayed is the volume of undiluted DNA to add directly to the autosomal STR amplification reaction. This volume will not be less than the Minimum Sample Pipette Volume (µl) nor greater than the Maximum Sample Volume (µl).

Diluent to Add (µl): Displays the volume of diluent required to normalize the sample DNA concentration to the target concentration. This volume is the difference between the value entered for the Template Volume (µl) and the value displayed in the Volume of Normalization (µl) column.

Volume of Normalization (µl): Displays the total volume of the normalized dilution.

Volume for Rxn (µl): Displays the volume of undiluted template or normalized template to add to the amplification reaction.

DNA Template: Displays the target mass of DNA for the autosomal STR amplification reaction based on the volumes and dilution steps.

Note: The units displayed for the DNA template mass reflect the units selected for DNA concentration in the 'Configuration' tab of the Configuration Settings (see Section 9.G).

Message: Displays messages to indicate either a volume greater than the specified Max Dilution Volume (µl) is required to dilute the specified volume of DNA sample to the desired final concentration or that the DNA concentration determined using the autosomal target is too low to achieve the specified target DNA mass with the Maximum Sample Volume (µl).

Note: If multiple DNA quantification results are available for a sample, these values are displayed for individual replicates and for the average autosomal quantification result for each set of replicates in the Auto Normalization worksheet.

The Auto Norm by Plate worksheet simply displays the normalization values in three 96-well plates: Diluent Required (μl), Template to Add (μl) and Total Volume (μl).

Note: Filter and sort functions are provided in the Auto Normalization worksheet, the Auto Norm by Row worksheet, and the Auto Norm by Col worksheet. These functions allow a user to select, exclude and sort data within the table. The filter and sort functions are accessed by selecting the arrow button in the column header. Use the Text Filter function to select data (checked) or exclude data (unchecked). Use the Sort function to reorder data smallest to largest or largest to smallest. Application of these functions may take a few minutes to complete while the software updates data links to other pages in the workbook.

Figure 80. The Auto Normalization worksheet.

Y-STR Normalization Worksheets

The Y-STR normalization worksheets contain the output normalization values for each DNA sample based on the DNA concentration determined using the Y target for that sample and the Y-STR normalization parameters set on the 'Normalization' tab of the Configuration Settings (See Section 9.G for more information about Configuration Settings). The Normalization parameters are displayed at the top each normalization worksheet (Figure 81).

Worksheets for DNA template normalization based on the Y target are provided in four different formats:

- Samples sorted by sample name (Y Normalization);
- Samples arranged by row (Y Norm by Row);
- Samples arranged by column (Y Norm by Col); and
- Samples arranged in a 96-well format (Y Norm Plate)

All normalization worksheets display the well position for the DNA sample used in the qPCR amplification plate.

The Y Normalization worksheet, the Y Norm by Row worksheet and the Y Norm by Col worksheet display the following columns of values:

Sample Name: Displays the sample name assigned to the well position.

Sample Concentration: Displays the concentration in the units defined on the 'Configuration' tab of the Configuration Settings based on the quantification results from the Y target.

Template Volume (µl): If the DNA template requires dilution, the value displayed in this column is the volume of template DNA to add to the diluent to achieve the desired target DNA mass. If the DNA template does not require dilution, the value displayed is the volume of undiluted DNA to add directly to the Y-STR amplification reaction. This volume will not be less than the Minimum Sample Pipette Volume (µl) nor greater than the Maximum Sample Volume (µl).

Diluent to Add (µl): Displays the volume of diluent required to normalize the sample DNA concentration to the target concentration. This volume is the difference between the value entered for the Template Volume (µl) and the value displayed in the Volume of Normalization (µl) column.

Volume of Normalization (µl): Displays the total volume of the normalized dilution.

Volume for Rxn (µl): Displays the volume of undiluted template or normalized template to add to the amplification reaction.

DNA Template: Displays the target mass of DNA for the Y-STR amplification reaction based on the volumes and dilution steps.

Note: The units displayed for the DNA template mass reflect the units selected for DNA concentration in the 'Configuration' tab of the Configuration Settings (see Section 9.G).

Message: Displays messages to indicate either a volume greater than the specified Max Dilution Volume (µl) is required to dilute the specified volume of DNA sample to the desired final concentration or that the DNA concentration determined using the Y target is too low to achieve the specified target DNA mass with the Maximum Sample Volume (µl).

Note: If multiple DNA quantification results are available for a sample, these values are displayed for individual replicates and for the average Y quantification result for each set of replicates in the Y Normalization worksheet.

The Y Norm by Plate worksheet simply displays the normalization values in three 96-well plates: Diluent Required (µl), Template to Add (µl) and Total Volume (µl).

Note: Filter and sort functions are provided in the Y Normalization worksheet, the Y Norm by Row worksheet and the Y Norm by Col worksheet. These functions allow a user to select, exclude and sort data within the table. The filter and sort functions are accessed by selecting the arrow button in the column header. Use the Text Filter function to select data (checked) or exclude data (unchecked). Use the Sort function to reorder data smallest to largest or largest to smallest. Application of these functions may take a few minutes to complete while the software updates data links to other pages in the workbook.

Figure 81. The Y Normalization worksheet.

9.J. The PowerQuant® Analysis Software Report (continued)

Sample Name Map Worksheet

The Sample Name Map worksheet displays the sample type and sample name in a 96-well plate layout (Figure 82).

Figure 82. The Sample Name Map worksheet.

Quantity Map Worksheet

The Quantity Map worksheet displays DNA concentrations calculated for all targets by the PowerQuant® Analysis Software for all samples in a 96-well plate layout format (Figure 83).

PowerQuant®

User	
Date	07/03/19
Data File Source	ABI 7500 SDS
Plate Map Name	PowerQuant
Imported File Name	PowerQuant data.xlsx
Instrument ID	
Reference Number	
Experiment Title	
Kit Lot	
Software Version	4.5.11

DNA Concentration (ng/µl)

		1	2	3	4	5	6	7	8	9	10	11	12
A	Auto				0.1385	0.1464	223.6954	0.1773	0.0018	0.1911	0.0031	1.2771	
	Y				0.1190	0.1216	0.3227	0.1693	0.0021	0.1504	0.0009	1.4329	
	Degradation				0.1254	0.1275	170.3068	0.1499	0.0016	0.1691	0.0023	1.2691	
B	Auto				0.1653	0.1391	231.4677	0.1985	0.0031	0.2277	0.0032	1.3475	
	Y				0.1284	0.1286	0.3468	0.1772	0.0015	0.1601	0.0022	1.4945	
	Degradation				0.1487	0.1450	188.7106	0.1607	0.0029	0.1768	0.0023	1.3139	
C	Auto				0.1696	0.1809	202.9504	0.2014	0.0018	0.2056	0.0020	1.2646	
	Y				0.1146	0.1036	0.1148	0.1659	0.0026	0.1713	0.0025	1.5006	
	Degradation				0.1470	0.1418	162.3977	0.1443	0.0019	0.2107	0.0020	1.4456	
D	Auto				0.1647	0.1519	193.0264	0.1857	0.0020	0.1929	0.0024	1.2852	
	Y				0.0662	0.0613	0.0935	0.1655	0.0013	0.1768	0.0020	1.4687	
	Degradation				0.1368	0.1152	154.0200	0.1697	0.0015	0.2041	0.0020	1.4460	
E	Auto				0.1665	0.1807	205.9583	0.0188		0.0272		1.4615	
	Y				0.0226	0.0219	0.0250	0.0173		0.0206		1.6481	
	Degradation				0.1378	0.1279	163.1195	0.0196		0.0240		1.6769	
F	Auto				0.1536	0.1827	218.2628	0.0188		0.0211		1.3090	
	Y				0.0122	0.0162	0.0267	0.0189		0.0164		1.4064	
	Degradation				0.1261	0.1289	168.3560	0.0199		0.0203		1.3467	
G	Auto				0.1813	0.1716	134.4264	0.0214		0.0196		1.2809	Undetermined
	Y				0.0062	0.0097	0.0027	0.0200		0.0182		1.4373	Undetermined
	Degradation				0.1397	0.1240	109.0401	0.0155		0.0211		1.2483	Undetermined
H	Auto						136.8834	0.0173		0.0214		1.1016	Undetermined
	Y						0.0024	0.0183		0.0150		1.4031	Undetermined
	Degradation						112.8890	0.0158		0.0181		1.1729	Undetermined

NEGATIVE
STANDARD

Auto Norm By Col / Auto Norm Plate / Y Normalization / Y Norm By Row / Y Norm By Col / Y Norm Plate / Sample Name Map / **Quantity Map** / Cq Map

Figure 83. The Quantity Map worksheet.

9.J. The PowerQuant® Analysis Software Report (continued)

C_q Map Worksheet

The C_q Map worksheet displays the C_q values for all targets detected for each well position (Figure 84).

PowerQuant®

User	
Date	07/03/19
Data File Source	ABI 7500 SDS
Plate Map Name	PowerQuant
Imported File Name	PowerQuant data.xlsx
Instrument ID	
Reference Number	
Experiment Title	
Kit Lot	
Software Version	4.5.11

Cq

		1	2	3	4	5	6	7	8	9	10	11	12
A	Auto	21.03			29.58	29.49	18.57	29.21	36.04	29.10	35.25	26.27	
	Y	20.96			29.79	29.76	28.33	29.27	35.68	29.45	36.87	26.15	
	Degradation	21.12			29.99	29.96	19.22	29.72	36.54	29.54	35.94	26.53	
B	Auto	20.72			29.31	29.57	18.52	29.04	35.23	28.83	35.19	26.19	
	Y	21.00			29.68	29.67	28.22	29.21	36.13	29.35	35.64	26.09	
	Degradation	21.16			29.73	29.77	19.07	29.62	35.61	29.47	35.97	26.48	
C	Auto	25.29			29.27	29.18	18.71	29.02	36.08	28.99	35.90	26.28	
	Y	25.60			29.84	29.99	29.84	29.30	35.40	29.26	35.44	26.08	
	Degradation	25.82			29.75	29.80	19.29	29.78	36.27	29.21	36.18	26.34	
D	Auto	25.68			29.32	29.44	18.79	29.14	35.93	29.08	35.61	26.26	
	Y	25.61			30.65	30.76	30.14	29.31	36.34	29.21	35.74	26.11	
	Degradation	25.76			29.86	30.12	19.37	29.54	36.64	29.26	36.15	26.34	
E	Auto	30.54			29.30	29.18	18.69	32.55		32.00		26.06	
	Y	30.35			32.22	32.26	32.07	32.61		32.35		25.95	
	Degradation	30.59			29.85	29.96	19.28	32.76		32.46		26.12	
F	Auto	30.26			29.42	29.16	18.61	32.55		32.38		26.23	
	Y	30.48			33.11	32.71	31.97	32.48		32.68		26.18	
	Degradation	30.44			29.98	29.95	19.24	32.74		32.71		26.44	
G	Auto	35.24			29.17	29.26	19.33	32.36		32.49		26.26	Undetermined
	Y	35.60			34.11	33.45	35.32	32.40		32.54		26.15	Undetermined
	Degradation	35.59			29.83	30.01	19.88	33.11		32.65		26.56	Undetermined
H	Auto	35.20					19.30	32.67		32.36		26.49	Undetermined
	Y	34.52					35.50	32.53		32.82		26.18	Undetermined
	Degradation	35.58					19.83	33.08		32.88		26.65	Undetermined

NEGATIVE
STANDARD

Y Normalization / Y Norm By Row / Y Norm By Col / Y Norm Plate / Sample Name Map / Quantity Map / Cq Map / Ratio Map / IPC Map

Figure 84. The C_q Map worksheet.

Ratio Map Worksheet

The Ratio Map worksheet displays the [Auto]/[Deg] ratios, the [Auto]/[Y] ratios and IPC sample assessment messages by well position (Figure 85). Additionally, well positions with values exceeding threshold values set for the Male/Female and Degradation sample assessments are flagged.

Figure 85. The Ratio Map worksheet.

9.J. The PowerQuant® Analysis Software Report (continued)

IPC Map Worksheet

The IPC Map worksheet displays the IPC shift and the IPC shift message by well position (Figure 86).

PowerQuant®

User	
Date	07/03/19
Data File Source	ABI 7500 SDS
Plate Map Name	PowerQuant
Imported File Name	PowerQuant data.xlsx
Instrument ID	
Reference Number	
Experiment Title	
Kit Lot	
Software Version	4.5.11

IPC Shift Plate Map

Name	Target	Threshold Value	Result	Sample Assessment Message
Inhibitor	IPC shift	0.3	< 0.3	Below IPC TH
			≥ 0.3	Above IPC TH

IPC Shift

	1	2	3	4	5	6	7	8	9	10	11	12
A				0.07	-0.24	-0.41	-0.11	-0.17	-0.02	-0.15	0.01	
B				-0.10	-0.25	-0.50	-0.36	-0.54	-0.07	-0.12	-0.35	
C				-0.16	-0.07	-0.82	-0.15	-0.60	-0.16	-0.45	0.02	
D				-0.16	-0.25	-0.62	-0.23	-0.15	-0.24	-0.19	0.01	
E				-0.35	-0.09	-0.75	-0.30		-0.29		0.05	
F				-0.10	-0.15	-0.79	-0.46		-0.22		0.08	
G				-0.10	-0.21	-0.68	-0.29		-0.21		0.01	-0.40
H						-0.47	-0.14		-0.17		0.37	-0.26

IPC Shift Message

	1	2	3	4	5	6	7	8	9	10	11	12
A				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	
B				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	
C				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	
D				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH	
E				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH		Below IPC TH		Below IPC TH	
F				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH		Below IPC TH		Below IPC TH	
G				Below IPC TH	Below IPC TH	Below IPC TH	Below IPC TH		Below IPC TH		Below IPC TH	Below IPC TH
H						Below IPC TH	Below IPC TH		Below IPC TH		Above IPC TH	Below IPC TH

	NEGATIVE
	STANDARD

Y Normalization | Y Norm By Row | Y Norm By Col | Y Norm Plate | Sample Name Map | Quantity Map | Cq Map | Ratio Map | **IPC Map** | Imported Data

Figure 86. The IPC Map worksheet.

Imported Data Worksheet

The Imported Data worksheet displays the imported raw data spreadsheet (image not shown).

9.K. Print and Save the PowerQuant® Analysis Software Report

After data review in the PowerQuant® Analysis Software, the report worksheets can be printed or saved.

Note: These data are not stored in the PowerQuant® Analysis Software database.

Printing and saving the report are performed in the 'Import' tab.

Printing a Report

1. To preview pages for a printed report, select the **Print Preview** icon in the 'Print' tab (Figure 87).
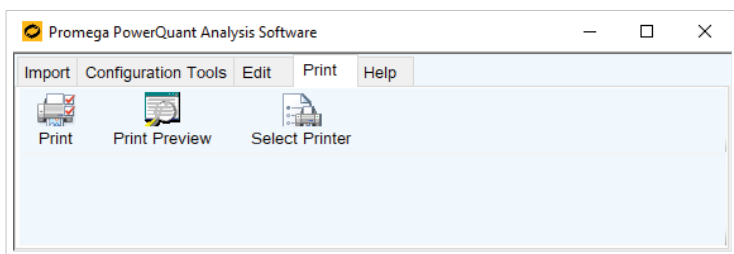
Figure 87. Printing options in the *Print* tab.

    a. A 'Select Sheet(s) to Preview' dialog box appears.

    b. Highlight the worksheets to preview.
      Note: Use the **Ctrl** or **Shift** keys on the keyboard to select multiple sheets for preview.

    c. Select **OK** to view the first Print Preview worksheet.

    d. To print previewed pages, select the **Print** icon in the toolbar.

    e. Select the **Close** button to close the Print Preview worksheet and display the next preview.
      Note: The generation of each Print Preview worksheet may take a few seconds.

2. Print pages using the print functions on the 'Print' tab. Select **Print** to choose the sheets to print.

    a. A 'Select Sheet(s) to Print' dialog box appears (Figure 88).
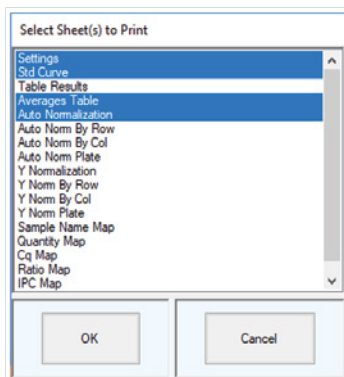
Figure 88. The 'Select Sheet(s) to Print' dialog box.

Promega

9.K. Print and Save the PowerQuant® Analysis Software Report (continued)

    b. Highlight the worksheets to print.

       Note: Use the **Ctrl** or **Shift** keys on the keyboard to select and print multiple sheets.

    c. Select **OK** to send the print job to the default printer.

       Note: Once a file is saved and closed, these print options are no longer available within the PowerQuant® Analysis Software.

Saving a Report

1. To save the file as an Excel® workbook, select the **Save Excel File** icon on the 'Import' tab (Figure 75).

    a. A file explorer opens.

    b. Navigate to the location of interest, enter a file name and select **Save**.

2. To save the file as a .pdf, select the **Save Pdf File** icon on the 'Import' tab.

    a. A file explorer opens.

    b. Navigate to the location of interest, enter a file name and select **Save**.

    c. A "PDF Save Complete" message appears when the save is complete.

3. If finished, close the PowerQuant® Analysis Software.

9.L. Analysis with a Virtual Standard Curve

This section describes how to analyze PowerQuant® System data using a virtual standard curve to determine sample concentration. The virtual curve comprises standard curve data stored in the PowerQuant® Analysis Software from a previous analysis. When a virtual standard curve is used, the stored standard curve data is accessed by the software and used to calculate the sample concentration for all unknown samples. When a virtual standard curve is used, calibrators with known sample concentration act as positive controls for qPCR with the PowerQuant® System.

Individual laboratories should validate the acceptable use of virtual standard curves and calibrators relevant to the success of downstream applications. Default threshold settings in the PowerQuant® Analysis Software are values intended to be customized by the user.

Note: The IPC C_q shift will not be calculated when virtual standard curves are used for qualitative and quantitative assessment of samples. Labs should perform a detailed internal validation to determine downstream paths for samples containing possible inhibitors.

Creating a Virtual Curve

The first step in performing analysis with a virtual standard curve is adding standard curve data to the PowerQuant® Analysis Software database.

1. Follow the steps in Section 9.I, Importing the PowerQuant® Results Excel® File.

    Note: To create the virtual curve, be sure to select a plate map with **Imported** as the Standard Type selection.

2. Review standard curve data and evaluate whether the standard curves for each target meet acceptance criteria. Once standard curve data are determined to be acceptable, enter a name for the standard curve in the 'Virtual Curves' drop-down menu (Figure 89).
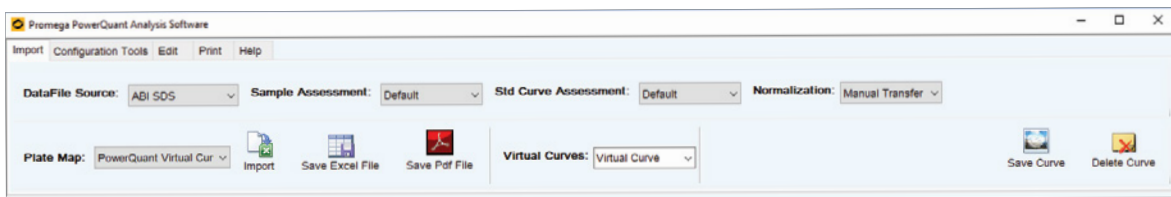
Figure 89. Naming a virtual curve.

3. Select the **Save Curve** icon.

4. Select **Yes** in the 'Save Confirmation' dialog box to store standard curve data.

Setting Calibrator Assessment Parameters

Calibrators are used with the PowerQuant® System as positive controls for amplification when DNA standards are not included on the amplification plate. Numerical values are defined to evaluate the DNA concentrations detected for the calibrators.

1. On the 'Configuration Tools' tab, select the **Configuration Settings** icon to open the 'PowerQuant® Administrative Tools' window (Figure 57).

   Note: The Calibrator Assessment limits are set using an Admin user account (see Section 9.F. for more information about user accounts).

2. On the 'Configuration' tab, in the Calibrator Assessment section, select **Default** from the drop-down menu. See Section 9.G for additional information on the 'Configuration' tab (Figure 64).

3. Enter values for the "Acceptable Min of Concentration" and the "Acceptable Max of Concentration" (Figure 90). These values are expressed as a ratio and must be numerical (e.g., set "0.90" as Acceptable Min of Concentration and "1.10" as the Acceptable Max of Concentration).
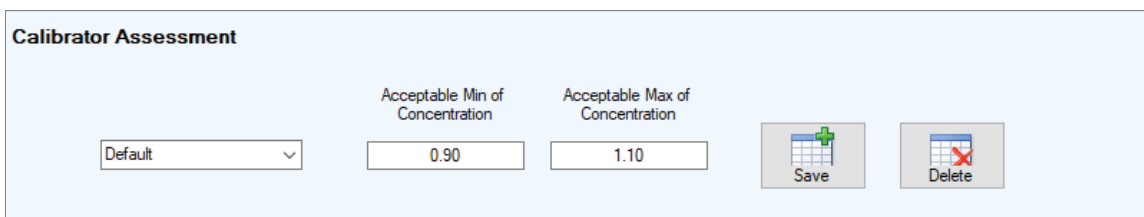
Figure 90. Setting Calibrator Assessment values on the 'Configuration' tab.

4. Enter a name for the calibrator assessment in the 'Calibrator Assessment' drop-down menu.

5. Select the **Save** button in the Calibrator Assessment section.

9.L.   Analysis with a Virtual Standard Curve (continued)

Creating a Plate Map for Use with a Virtual Curve

Successful analysis with the PowerQuant® Analysis Software requires a plate map with the virtual curve format.

1.   In the 'Configuration Tools' tab (Figure 57), select the **Design Plate Map** icon to open the 'Promega PowerQuant Plate Designer' window (Figure 91).

2.   From the 'Standard Curve Type' drop-down menu, select **Virtual**.

3.   Select **Yes** in the 'New Standard Curve Origin' dialog box.

4.   Select **Default** in the 'Calibrator Flags' drop-down menu.
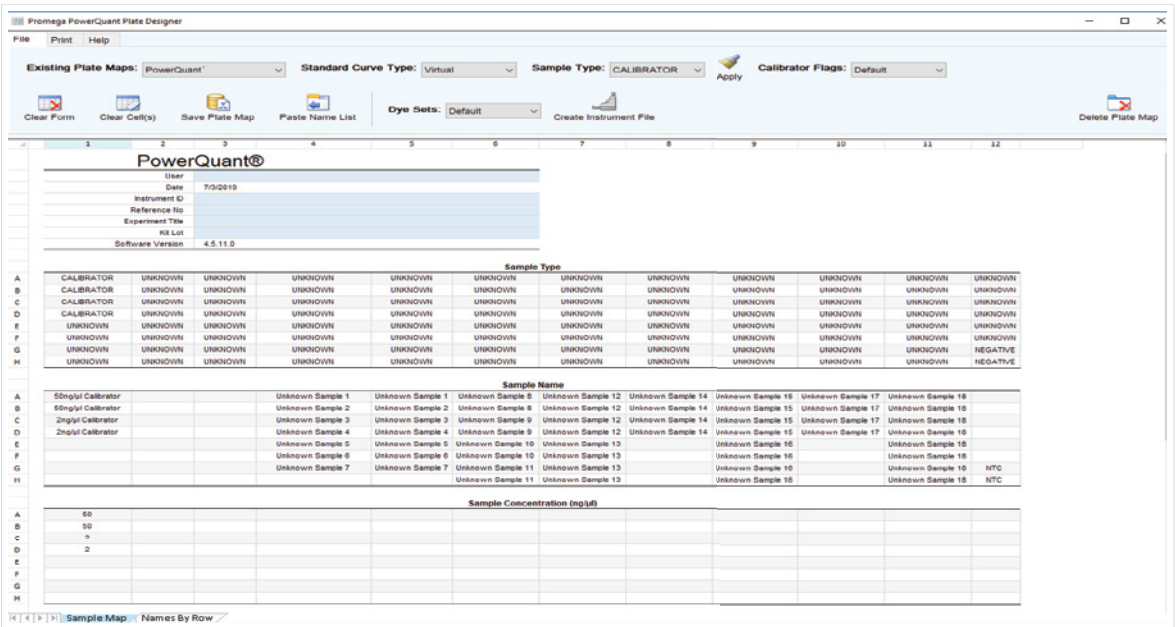
Figure 91. Plate Designer menu options for a virtual standard curve.

5. In the Sample Type plate map, highlight the wells of interest and select the sample type from the 'Sample Type' drop-down menu and select the **Apply** icon. The plate map displays the assigned sample type.

   a. Select the **Calibrator** sample type for sample wells containing samples with known concentration run concurrently with unknown samples.

      Notes:

      1. Calibrator wells must be selected when a virtual standard curve is used.
      2. The software limits the number of wells designated as a Calibrator to four.
      3. Standard is not a sample type option when a virtual standard curve is used for analysis. See Section 9.H for additional information about standard curves included in the imported data file.

   b. Select **Unknown** for wells containing unknown samples.

   c. Select **Negative** or **Unknown** for wells containing negative controls.

      Note: The negative designation is for use inside the PowerQuant® Analysis Software only and will not be transferred to the .txt files for import into the real-time PCR instrument software.

6. In the Sample Concentration plate map, enter the concentrations for wells containing calibrators. The concentration units displayed reflect the default selected in the 'Configuration' tab (see Section 9.G).

7. To store the locations of the Sample Types and the Sample Concentrations of the DNA standards, select the **Save Plate Map** icon.

   Note: The Save function does not apply the Sample Names portion of the plate map; sample name information is not stored in the plate map database.

8. Enter a name for the plate map in the 'Plate Map Name' dialog box and select the **OK** button.

9.L.   Analysis with a Virtual Standard Curve (continued)

Importing the PowerQuant® Results Excel® File for Analysis with a Virtual Standard Curve

The 'Import' tab of the PowerQuant® Analysis Software is used to process qPCR data generated with the PowerQuant® System (See Section 9.I for additional information about importing the PowerQuant® Results® Excel file).

The PowerQuant® Analysis Software does not store analyzed sample data. Analyzed data must be saved as an Excel® workbook or as a .pdf file.

Successful analysis requires the inclusion of sample names in the PowerQuant® Results Excel® file exported from the instrument software, as well as the designation of a Sample Type for all used sample positions in the PowerQuant® Analysis Software Plate Map (see Section 9.H).

1.   Use the drop-down menus to make the following selections: data file source; sample assessment, standard curve assessment and normalization settings; and plate map (Figure 92). See Section 9.G for information about settings and Section 9.H for information about plate maps.

   Note: Select the plate map saved in Step 8 of "Creating a Plate Map for Use with a Virtual Standard Curve".
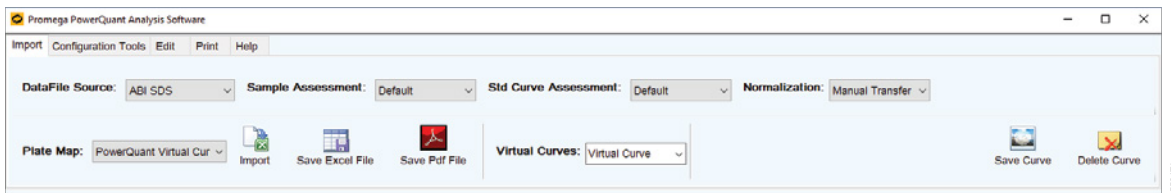
Figure 92. Menu options for a virtual standard curve on the 'Import' tab.

2.   Select the **Import** icon. A dialog box labeled "Make Sure You Have Saved Your Work Before Proceeding" will appear. Select **No** to abort the import, or **Yes** to clear the form.

3.   Use the file explorer to navigate to the file of interest, select the file and select **Open**.

4.   When the import is complete, multiple worksheets are created. The software displays the Settings worksheet.

5.   After file import, select a virtual standard curve from the 'Virtual Curves' drop-down menu.

6.   Select the **Apply Curve** icon (Figure 93).

   Note: Analysis is not complete until the virtual standard curve is applied. Once the file is imported, the **Import** icon is renamed **Open Import File**.
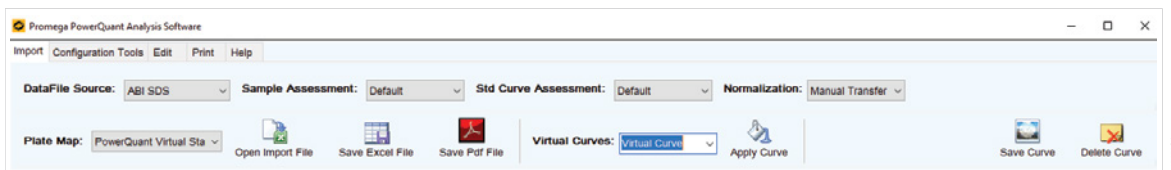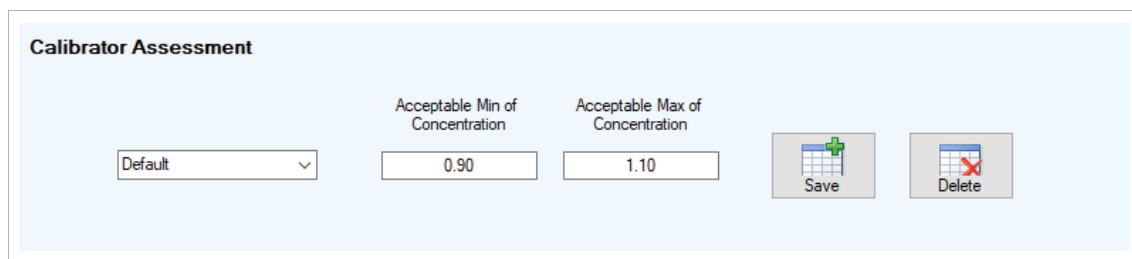
Figure 93. The Apply Curve icon in the 'Import' tab.

7. Select **Yes** in the 'Apply Confirmation' dialog box.

8. The report worksheets are populated with data from calculations performed with the virtual standard curve.

9. See Section 9.I for additional information about saving and/or printing the analysis worksheets.

PowerQuant® Software Export File

When a virtual standard curve is used to calculate sample concentration, the PowerQuant® Analysis Software creates the same worksheets described in Section 9.J.

The Settings worksheet displays the minimum and maximum acceptable concentration values of the calibrators when a virtual standard curve is used (Figure 94).

Figure 94. Calibrator assessment settings on the Settings worksheet.

The Std Curve, Table Results and Averages Table worksheets display some additional information when a virtual standard curve is used to determine sample concentration. The standard curve source selected during data import is displayed above the standard curve parameters. The Calibrator Data and Analysis section displays the concentration of the calibrators entered on the plate map, the C_q values, the calculated concentration and comparison to the acceptable min and max concentration (Figure 95).

These values should be determined experimentally as part of internal validation studies and should be evaluated in accordance with a laboratory's standard operating procedures.

Calibrator Designated Concentration (ng/µl)	SampleName	Auto Cq	Y Cq	Deg Cq	[Auto] (ng/µl)	[Y] (ng/µl)	[Deg] (ng/µl)	Acceptable Min	Acceptable Max	Auto Calibrator	Y Calibrator Within	Deg Calibrator
0.0800	Calibrator 2	30.5430	30.3509	30.5874	0.0700	0.0800	0.0800	0.0720	0.0880	NO	YES	YES
2.0000	Calibrator 1	25.6772	25.6051	25.7601	1.9000	2.0800	2.1300	1.8000	2.2000	YES	YES	YES

Figure 95. The Calibrator Data and Analysis Section of the Std Curve worksheet.

When analysis is performed with a virtual standard curve, no values are displayed in the columns for Closest IPC, IPC Shift and IPC Threshold Result on the Table Results worksheet and the Averages Table worksheet.

10. Interpretation of PowerQuant® Data

In addition to the concentrations of autosomal and male DNA used to normalize DNA samples for STR amplification, the PowerQuant® System yields information that can be used to evaluate amplification performance, detect PCR inhibitors in the amplification reaction, detect the presence of male and female DNA mixtures and evaluate the degree of DNA degradation.

Note: [Auto]/[Y] and [Auto]/[Deg] ratios may not be reliable in samples with low DNA concentrations (i.e., less than 1pg/µl) due to stochastic effects.

The 7500 Applied Biosystems® Real-Time PCR System Software, QuantStudio® Design and Analysis Software (see Section 6.C, 7.C and 8.D) and the PowerQuant® Analysis Software (see Section 9) perform a linear regression to the standard dilution series data and calculate the equation for the line of best fit (the standard curve). The equation is in the form of $y = mx + b$, where x = log concentration and $y = C_q$. The R^2 value is a measure of the fit of the data points to the regressed line. The slope (m in the equation) is an indication of the PCR efficiency. A slope of -3.3 indicates 100% PCR efficiency (i.e., the number of copies of amplification product is doubled at each cycle). The Y-intercept (b in the equation) is defined as the y value (C_q) when x (log concentration) equals 0. Therefore, b corresponds to the C_q value for a sample with a concentration of 1ng/µl [$\log_{10}(1) = 0$].

In general, the standard curve for each target (autosomal, Y and degradation) has an average slope (m) in the range of -3.1 to -3.6 and an R^2 value >0.990. See Section 11 for more information about troubleshooting standard curves that fall out of these ranges. We recommend tracking and evaluating Y-intercept values for any significant changes from run to run.

10.A. Internal PCR Control

Amplification performance for the IPC can be used to evaluate overall performance of a PowerQuant® amplification reaction or detect PCR inhibitors in the DNA sample. IPC data analyzed using the PowerQuant® Analysis Software can be interpreted as follows:

- If a sample yields no detectable amplification for the autosomal, Y and degradation targets but has an IPC amplification curve that crossed the amplification threshold without an IPC shift greater than that specified in the 'Sample Assessment' tab, then the absence of a quantification result is not likely due to PCR inhibition, but rather insufficient amplifiable human DNA.

- If a sample has an IPC Shift value greater than the value specified in the Sample Assessment tab, or if the C_q value for the IPC is undetermined (regardless of detectable amplification for the autosomal, Y and degradation quantification targets), then this indicates that a PCR inhibitor may be present in the amplification reaction. Repeat the DNA purification if necessary.

- If a sample has an IPC Shift value greater than the value specified in the 'Sample Assessment' tab and the [Auto]/[Deg] ratio is less than the ratio specified on the 'Sample Assessment' tab, then the sample likely contains a PCR inhibitor but the DNA is not likely to be degraded. Repeat the DNA purification if necessary.

- If a sample has an IPC Shift value greater than the value specified in the 'Sample Assessment' tab and the [Auto]/[Deg] ratio is greater than the ratio specified on the 'Sample Assessment' tab, then the sample likely contains a PCR inhibitor and the DNA is possibly degraded. Repeat the DNA purification if necessary.

  Note: The IPC is the longest target in the PowerQuant® System and therefore is more susceptible to inhibition relative to the shorter autosomal and Y targets. The degradation target is long, and amplification performance also may be affected by inhibitors.

10.B. [Auto]/[Y] Ratio

The [Auto]/[Y] ratio can be used to evaluate whether a sample includes a male/female DNA mixture. The results from the [Auto]/[Y] calculations performed by the PowerQuant® Analysis Software may be interpreted as follows:

- If the message "No Y C_q" is displayed for a sample, then no male DNA was detected.

- If a sample has an [Auto]/[Y] ratio less than the value specified in the 'Sample Assessment' tab, then the sample may contain male DNA only or low levels of female DNA.

- If a sample has an [Auto]/[Y] ratio greater than the value specified in the 'Sample Assessment' tab, then the sample contains a possible mixture of male and female DNA.

10.C. [Auto]/[Deg] Ratio

The [Auto]/[Deg] ratio can be used to evaluate whether a DNA sample was degraded. The results from the PowerQuant® Analysis Software may be interpreted as follows:

- If a sample has an [Auto]/[Deg] ratio less than the value specified in the 'Sample Assessment' tab, then the DNA in the sample is not likely degraded, regardless of the value in the IPC Shift column.

- If a sample has an [Auto]/[Deg] ratio greater than the value specified in the 'Sample Assessment' tab and the value for the IPC Shift is less than the value specified in the 'Sample Assessment' tab, then the DNA in the sample is likely degraded but the sample does not contain PCR inhibitors.

- If a sample has an [Auto]/[Deg] ratio and IPC Shift value greater than the values specified in the 'Sample Assessment' tab, then this sample is likely to contain PCR inhibitors and may or may not contain degraded DNA.

- If the message "No Deg C_q" is displayed for a sample and the IPC Shift value is less than the value specified in the 'Sample Assessment' tab, then the DNA in the sample is likely severely degraded but the sample does not contain PCR inhibitors.

- If the message "No Deg C_q" is displayed for a sample and the IPC Shift value is greater than the value specified in the 'Sample Assessment' tab, then the sample likely contains PCR inhibitors and the DNA may be degraded.

11. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com** E-mail: **genetic@promega.com**

11.A. The PowerQuant® System

Symptoms	Causes and Comments
Custom dye calibration failed	Prepare new standard calibration plate(s), and recalibrate as described in Section 4.
Flat amplification curves and no C_q values detected for any of the targets, including the IPC, for a subset of samples in the 96-well plate	An amplification inhibitor was present in the DNA template. Dilute the template DNA, and requantify using 2µl of the diluted template. Repeat the DNA purification if necessary.
	Verify that reaction mix was added to the failed amplification reactions.
	Examine the volume of liquid in each well of the reaction plate to verify that evaporation did not occur during cycling. Ensure that all wells were adequately sealed to prevent evaporation during thermal cycling.
Flat amplification curves and no C_q values detected for any of the targets, including the IPC, for all samples in the 96-well plate	Verify that the thermal cycler was programmed correctly (see Section 6, 7 or 8).
	The PowerQuant® 2X Master Mix lost activity, or the PowerQuant® 20X Primer/Probe/IPC mix was degraded. Minimize the number of freeze-thaw cycles. Store the PowerQuant® 20X Primer/Probe/IPC Mix protected from light.
	From the Experiment menu in the Applied Biosystems® 7500 software, select the Analysis section, and then select the Multicomponent Plot to determine if fluorescence was detected for any of the system dyes, including the passive reference dye. If no fluorescence was detected, check to be sure that the PowerQuant® 20X Primer/Probe/IPC Mix was added to the amplification reactions.
Nonlinear standard curve (R^2 values less than 0.98) or slope outside of the specified range	Be sure that the PowerQuant® Male gDNA Standard was thawed completely and well mixed prior to use. Store the DNA standard at 4°C overnight prior to use to improve sampling consistency.
	Be sure that each dilution of the PowerQuant® Male gDNA Standard was well mixed before removing an aliquot for the next serial dilution. Use the same pipette to dispense each aliquot. Change the pipette tip between each dilution step. Change the pipette tip between each addition of DNA standard to the 96-well plate.

Symptoms	Causes and Comments
Nonlinear standard curve (R^2 values less than 0.98) or slope outside of specified range (continued)	Calibrate pipettes to minimize variability when pipetting. Amplify each DNA standard in duplicate to minimize the effects of variation on the standard curve.
	An error was made during dilution of the PowerQuant® Male gDNA Standard. Verify all calculations, and repeat the dilution. Avoid pipetting volumes less than 1µl.
	Incorrect DNA concentrations were entered into the Applied Biosystems® 7500 Real-Time PCR System Software or the QuantStudio™ Design and Analysis Software. Verify that all DNA standard concentrations were entered correctly.
	Ensure that the correct passive reference was selected (e.g., CXR or ROX™ dye).
	Examine the volume of liquid in each well of the reaction plate to verify that evaporation did not occur during cycling. Ensure that all wells were adequately sealed to prevent evaporation during thermal cycling.
	Analysis settings in the Applied Biosystems® 7500 Real-Time PCR System Software or the QuantStudio™ Design and Analysis Software were not set correctly. Verify that the analysis settings were set as described in Section 6, 7 or 8.
	Discrepancy in the well location of standards between the plate and sample map. Verify that the standard wells were identified correctly in the sample map.
Inconsistency between replicates of the same DNA sample	Examine the volume of liquid in each well of the reaction plate to verify that evaporation did not occur during cycling. Evaporation of the reaction mix affects amplification of the degradation and IPC targets (late C_q values).
	DNA concentration was low. Differences in amplification results for replicate samples with low levels of DNA template can be due to stochastic effects.
DNA detected in no-template controls	Detection of >1.0pg per 2µl input volume DNA in the no-template controls can indicate the presence of contaminating DNA. Refer to Section 3 for guidelines to prevent DNA contamination.
ΔRn of amplification plots (ΔRn vs Cycle) not normalized (greater than 1,000) with poor standard curves	Ensure that a passive reference is selected (e.g., CXR or ROX™ dye).
Noisy amplification plots with no distinct amplification curve and no IPC amplification plots detected	Wells with no reaction mix were analyzed. Be sure that only wells that contain amplification reactions are highlighted and analyzed in Section 6, 7 or 8.

11.B. The PowerQuant® Analysis Software

Symptoms	Causes and Comments
Installation	
Software download hangs, is terminated before finishing, or is corrupted	Slow internet connection or internet service interruption. Attempt a new download. If the problem persists, contact Promega Technical Services.
"Access restricted" warning during installation	Local computer permission not granted. Contact your IT/IS department to grant administrator access to the user profile in which you are attempting to install the software.
Problem installing MS .NET framework 4.8	Contact your IT/IS department for support, or see: https://support.microsoft.com/en-us
Selecting Promega PowerQuant® Assays in the Start Menu results in operating system looking for the software installer, or software fails to launch	Installation completed by a non-administrator user. Contact your IT/IS department to install the software with an administrator account.
Functionality	
Wrong threshold comparisons made	Incorrect selections made in the plate map in the Promega PowerQuant Plate Designer or in the 'Import' tab. • Confirm that the Sample Type is set correctly in the plate map. • Confirm that the correct Plate Map is selected during import. • Confirm selections made for the thresholds in the 'PowerQuant Administrative Tools' window.
User Access	
Missing or forgotten password	Incorrect user permissions. Log in as PQAdmin and replace the original user's password or delete and recreate the user profile. Contact Promega Technical Services for additional assistance.
Read-only access granted	Incorrect user permissions. To attempt the login again, close the 'PowerQuant Administrative Tools' window. Select the 'Configuration Tools' tab, then select the **Configuration Settings** icon and low the instructions for an Admin user login.
	Incorrect password entered. To attempt the login again, close the 'PowerQuant Administrative Tools' window. Select the 'Configuration Tools' tab, then select the **Configuration Settings** icon and follow the instructions for an Admin user login.
Design Parameters will not launch	Promega.Administrator.Toolz.exe was removed from the Promega\PowerQuant install folder. Uninstall and reinstall the PowerQuant® Analysis Software (see Section 9.D and 9.C).
Plate Designer will not launch	PowerQuant.Plate.Designer.exe was removed from the Promega\PowerQuant install folder. Uninstall and reinstall PowerQuant® Analysis Software.

Symptoms	Causes and Comments
Design Parameters	
Unable to create, edit or save Configuration Settings	Incorrect user permissions. Close the 'PowerQuant Administrative Tools' window and log in with an administrator account.
Error message occurs on attempted save of Configuration Settings or a plate map in the Promega PowerQuant Plate Designer	PowerQuant® Analysis Software database missing or corrupted. Contact Promega Technical Services.
Save/Delete/New icons grayed out or disabled in the tabs of the 'PowerQuant Administrative Tools' window	Read-only access permission granted to user. Close the 'PowerQuant Administrative Tools' window and log in with an administrator account.
Dye set name does not appear in the 'Dye Sets' drop-down menu in the Promega PowerQuant Plate Designer	Confirm that the dye set was saved in 'Dye Set Names' tab of the 'PowerQuant Administrative Tools' window. Close the 'Promega PowerQuant Plate Designer' window and log in with an administrator account.
Drop-down menus are empty in the 'Configuration Settings' tab	Read-only access permission granted to user. Close the Design Parameters window and log in with an administrator account.
Drop-down menus are empty in the Import tab of the software and/or the PowerQuant Plate Designer	Missing or corrupted database. Contact Promega Technical Services.
Unexpected results from the Standard Curve samples	Error in setting up the Plate. • Confirm the correct Sample Type is selected for all used wells in the plate map using the Promega PowerQuant Plate Designer. • Confirm that the correct values were entered for Sample Concentration section of the PowerQuant Plate Designer. • Confirm that the CT Settings are set correctly in the instrument software. See Sections 6.A, 7.A or 8.B for additional information. • Confirm the plate map has the correct Standard Curve Type selected in the Promega PowerQuant Plate Designer. • Confirm that the correct Plate Map is selected during import. • If a virtual standard curve is used, confirm that the curve is applied to the completed analysis.
Help Tab	
Help videos will not launch	Missing software components. • Confirm that Windows Media Player is installed on the computer. • DesigningHelp.wmv, ImportingHelp.wmv or PlateMapsHelps.wmv was removed from the Promega\PowerQuant install folder. Uninstall and reinstall the PowerQuant® Analysis Software.
Technical Manual will not open from software button	No internet connection. The *PowerQuant® System Technical Manual* #TMD047 is available online at: **www.promega.com**

11.B. The PowerQuant® Analysis Software (continued)

Symptoms	Causes and Comments
System Errors	
Message: "Could Not Find a Base Path"	The database is missing from the Promega\PowerQuant folder. Contact Promega Technical Services. E-mail: **genetic@promega.com**
Exception Error: System.IO.FileNotFoundException	One or more required files for the PowerQuant® Analysis Software are missing. • Uninstall and reinstall the PowerQuant® Analysis Software. • Contact Promega Technical Services. E-mail: **genetic@promega.com**
Import	
Exception Error: Error opening file	File in use by another program. Confirm that the file to be imported is not open in another program. Inside the error message, the text will include "because it is being used by another process".
Message: "Unrecognized File Extension" or "File Retrieve Abandoned"	Wrong or unexpected file type. • Confirm that the correct export file type is selected in the qPCR software (.txt, .csv, .xls, .xlsx) when exporting the qPCR results file. • Confirm that the correct column export options are chosen from the qPCR software. • Confirm that the correct DataFile Source is selected in the 'Import' tab.
File does not import	Wrong or unexpected file type. • Confirm that the correct export file type is selected in the qPCR software (.txt, .csv, .xls, .xlsx) when exporting the qPCR results file. • Confirm that the correct column export options are chosen from the qPCR software. • Confirm that the correct DataFile Source is selected in the 'Import' tab.
No sample concentration is calculated	Error in sample type or plate map parameters. • Confirm that the correct Sample Type is selected in the plate map using the Promega PowerQuant Plate Designer. • Confirm that the plate map has the correct Standard Curve Type selected in the Promega PowerQuant Plate Designer. • Confirm that the correct Plate Map is selected during import. • If a virtual standard curve is used, confirm that the curve is applied to the completed analysis.

For support of other instruments with similar fluorescent dye capabilities, contact Promega Technical Services for more information. E-mail: **genetic@promega.com**

12. Appendix

12.A. References

1. Holland, P.M. *et al.* (1991) Detection of specific polymerase chain reaction product by utilizing the 5´→3´ exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc. Natl. Acad. Sci. USA* **88**, 7276–80.

2. Hudlow, W.R. *et al.* (2008) A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A diagnostic tool for STR typing. F*orensic Sci. Int. Genet.* **2**, 108–25.

3. Bustin, S.A. *et al.* (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611–22.

12.B. Spectral Calibration Results
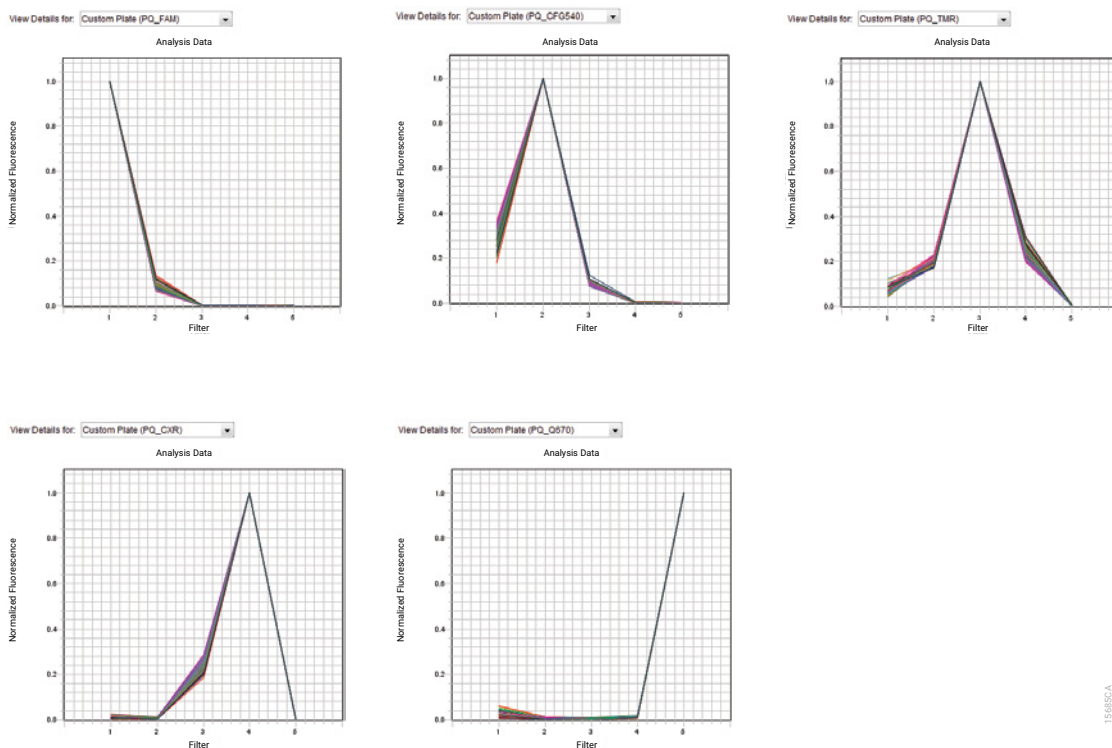
Figure 96. Example dye calibration results for the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. See Sections 4.B and 4.C for instrument calibration instructions.

12.B. Spectral Calibration Results (continued)
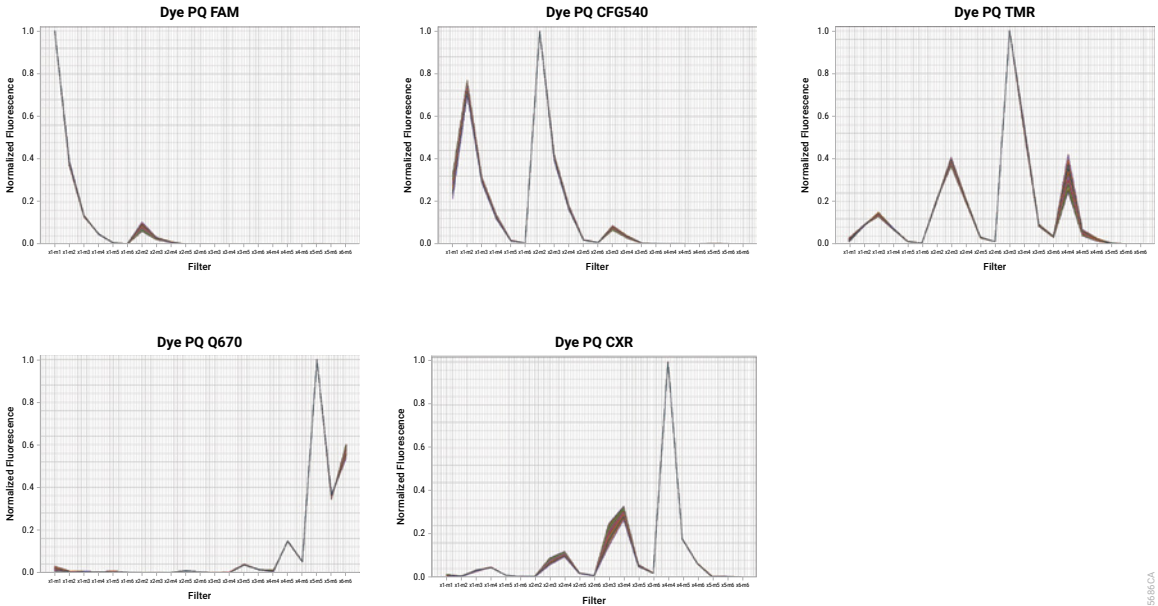
Figure 97. Example dye calibration results for the PowerQuant® System on the QuantStudio™ 5 Real-Time PCR System.
See Section 4.D for instrument calibration instructions.

12.C. Preparation of Alternative Standard Curves

Although we recommend performing a four-point standard curve, you also can prepare standard curves with additional points. See Tables 3–5 for the recommended dilution schemes for the PowerQuant® Male gDNA Standard to prepare five-point, six-point and seven-point standard curves.

Table 3. Preparation of a 5-Point Standard Curve (Tenfold Dilutions).

DNA Concentration	Volume of PowerQuant® Male gDNA Standard	Volume of PowerQuant® Dilution Buffer
50ng/µl	Use undiluted PowerQuant® Male gDNA Standard	0µl
5ng/µl	10µl of undiluted PowerQuant® Male gDNA Standard	90µl
0.5ng/µl	10µl of 5ng/µl dilution	90µl
0.05ng/µl	10µl of 0.5ng/µl dilution	90ul
0.005ng/µl	10µl of 0.05ng/µl dilution	90µl

Table 4. Preparation of a 6-Point Standard Curve (Eightfold Dilutions).

DNA Concentration	Volume of PowerQuant® Male gDNA Standard	Volume of PowerQuant® Dilution Buffer
50ng/µl	Use undiluted PowerQuant® Male gDNA Standard	0µl
6.25ng/µl	10µl of undiluted PowerQuant® Male gDNA Standard	70µl
0.781ng/µl	10µl of 6.25ng/µl dilution	70µl
0.0977ng/µl	10µl of 0.781ng/µl dilution	70µl
0.0122ng/µl	10µl of 0.0977ng/µl dilution	70µl
0.0015ng/µl	10µl of 0.0122ng/µl dilution	70µl

Table 5. Preparation of a 7-Point Standard Curve (Fivefold Dilutions).

DNA Concentration	Volume of PowerQuant® Male gDNA Standard	Volume of PowerQuant® Dilution Buffer
50ng/µl	Use undiluted PowerQuant® Male gDNA Standard	0µl
10ng/µl	10µl of undiluted PowerQuant® Male gDNA Standard	40µl
2ng/µl	10µl of 10ng/µl dilution	40µl
0.4ng/µl	10µl of 2ng/µl dilution	40µl
0.08ng/µl	10µl of 0.4ng/µl dilution	40µl
0.016ng/µl	10µl of 0.08ng/µl dilution	40µl
0.0032ng/µl	10µl of 0.016ng/µl dilution	40µl

12.D. Using the PowerQuant® Analysis Tool

The PowerQuant® Analysis Tool is a Microsoft Excel® macro-enabled template. The template initially contains two visible worksheet tabs: Home and Admin Settings. The Home worksheet includes the version number and directions to save the template. The Admin Settings worksheet includes sections to define parameters used in calculations by the PowerQuant® Analysis Tool and sections to define values for evaluating or calculating the following:

- standard curves (acceptable R^2 and slope values)

- sample quality (possible inhibition, mixture or degradation)

- DNA normalization for autosomal or Y-STR amplification based on DNA target mass, pipetting volumes and diluent volumes

- samples that may need additional attention based on the user-defined normalization parameters

Once the Results Excel® file is exported from the Applied Biosystems® 7500 Software (Version 2.0.6 or HID Version 1.1 or 1.2) and imported into the PowerQuant® Analysis Tool, the tool calculates the [Auto]/[Y] and [Auto]/[Deg] ratios along with the difference in C_q values (ΔC_q) for the IPC in an unknown sample and the IPC in the closest DNA standard of the standard curve (to monitor PCR inhibition). In addition, this tool performs calculations for normalizing the input amounts of DNA based on the autosomal or Y target quantification values.

Notes:

1. C_q value (quantification cycle) is used rather than C_T (cycle threshold) as per MIQE guidelines (3).

2. The PowerQuant® Analysis Tool requires the inclusion of sample names in the Results Excel® file exported from the Applied Biosystems® 7500 Software for these calculations.

3. Use of the PowerQuant® Analysis Software rather than the PowerQuant® Analysis Tool is recommended for data files exported from the QuantStudio™ Design and Analysis Software.

4. In this section, references are made to both [Auto]/[Deg] and [Auto]/[D]. [Auto]/[D] is used in reference to the names of the column headers in the analysis tool.

The PowerQuant® Analysis Tool can be downloaded free of charge from the Promega web site at:
www.promega.com/resources/tools/powerquant-analysis-tool/

All worksheets, except for the Results worksheet, are locked and password-protected. The Results worksheet is unlocked and sortable.

A password is required to change values within the Admin Settings worksheet. The default password is "Admin". For instructions to change the password, see Section 12.E.

Note: You may print data from each worksheet.

1. Navigate to the downloaded PowerQuant® Analysis Tool, and select the icon.

   Note: Opening the Microsoft Excel® software first then opening the PowerQuant® Analysis Tool allows the PowerQuant® Analysis Tool template to be modified and overwritten. If this occurs inadvertently, download a new copy of the PowerQuant® Analysis Tool from the Promega web site. The template will not be overwritten if the PowerQuant® Analysis Tool is opened directly without first opening the Excel® software.

2. Depending on the computer's security settings, a "Security Warning Macros have been disabled" message may appear the first time the PowerQuant® Analysis Tool is opened on the computer. If this message appears, select the **Enable Content** button at the top of the screen to enable macros.

3. If the question "Do you want to make this a trusted document?" appears, select **Yes**.

4. The Home worksheet will appear (Figure 98). Select the Admin Settings worksheet at the bottom of the screen.
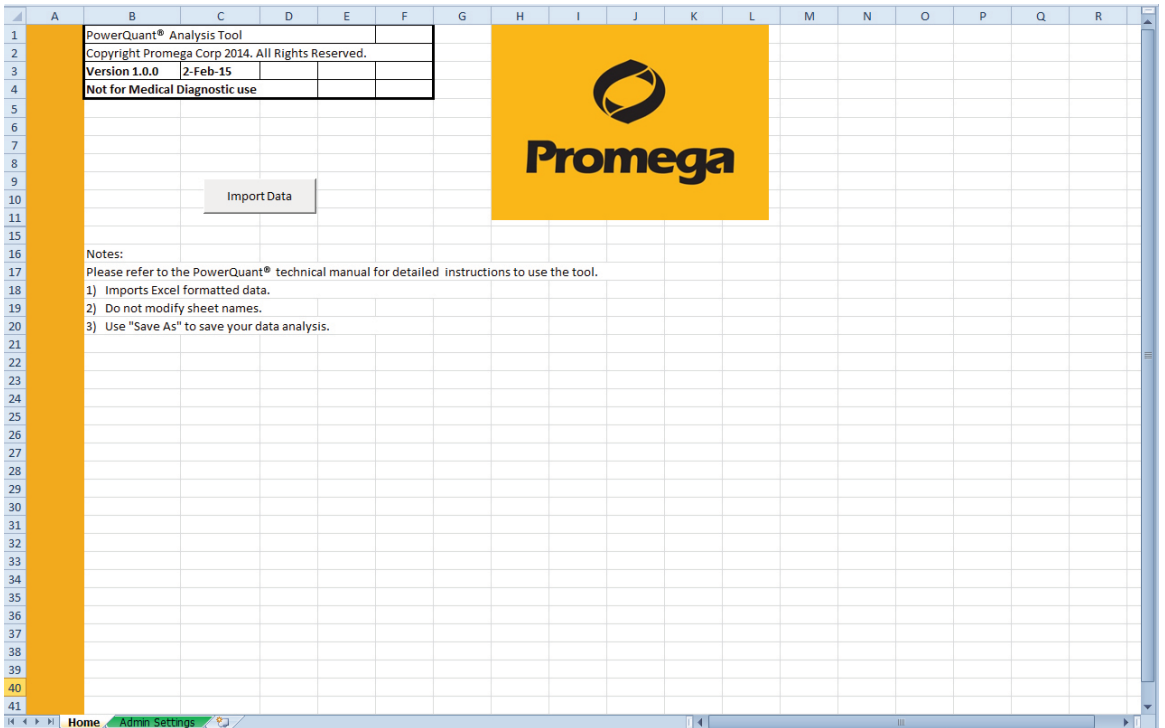
Figure 98. The Home worksheet.

12.D. Using the PowerQuant® Analysis Tool (continued)

5. The Admin Settings worksheet will appear (Figure 99). The default value for each parameter is shown. These values are based on the results of testing during product development. You may need to change these values based on the results of your laboratory's internal validation.

A password is required to change values within the Admin Settings worksheet. To change a parameter, double-click on the cell. You will be prompted to enter the password (Figure 100).

Notes:

1. See Step 7 for a description of each parameter.

2. For instructions to change the password, see Section 12.E.

Figure 99. The Admin Settings worksheet.
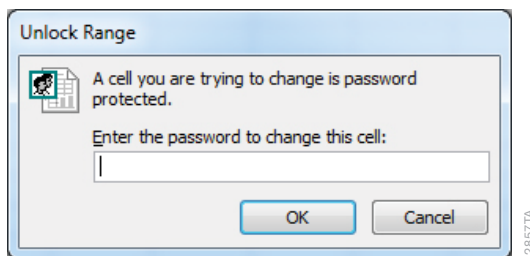
Figure 100. The Admin Settings password window.

6.   Enter the appropriate password, and select **OK**.

      Note: The default password is "Admin".

7.   Type the new value for that cell. To change other parameters in the Admin Settings worksheet, highlight the appropriate cell and type the new value. You can define values for parameters in each of the following sections:

Target Name: In the Target Name column, enter the target name for each of the amplification targets in the Applied Biosystems® 7500 Real-Time PCR System software (see Section 6.A or 7.A). This name must be identical to the name of the amplification target in the Excel® file exported from the Applied Biosystems® 7500 Real-Time PCR System. If the target names in the export file and the Admin Settings worksheet do not match, no data will appear for that amplification target in the PowerQuant® Analysis Tool.

Standard Curve Assessment: Specify the minimum passing R^2 value that is acceptable to your laboratory for the standard curve for each quantification target (autosomal, Y and degradation). The software will flag a standard curve with an R^2 value that falls below the minimum passing value. Specify the upper and lower limits that are acceptable for the slope of the standard curve for each quantification target. The PowerQuant® Analysis Tool will flag any standard curve with slope values outside of the upper and lower limits.

Sample Assessment: In the Possible inhibitor field, specify the minimum IPC shift value at which you may expect to encounter PCR inhibition. The IPC shift is the calculated difference in C_q values for the IPC in an unknown sample and the IPC of the closest DNA concentration in the standard curve.

In the Possible Male/Female Mixture and Possible degraded sample fields, specify the minimum [Auto]/[Y] and [Auto]/[Deg] ratios that are indicative of a potential male/female mixture or degraded DNA sample, respectively.

These values should be determined experimentally as part of internal validation studies. The PowerQuant® Analysis Tool will flag any DNA sample that has values that exceed the values entered for these parameters.

Normalization: In the Max Dilution Volume (µl) field, specify the largest volume of diluent that you can add to a concentrated DNA sample to try to achieve the desired final DNA concentration (e.g., the maximum tube volume or maximum well volume on a 96-well plate).

The PowerQuant® Analysis Tool will flag DNA samples that require additional attention for normalization based on the normalization parameters defined in the Admin Settings worksheet. Choose a character (e.g., !,^,*) to be used as a flag for each of the parameters listed below, and enter that character in the appropriate symbol field.

This flag will indicate that the sample exceeds the indicated specifications.

- The Above Max Dilution Volume Symbol indicates that a volume greater than the specified Max Dilution Volume is required to dilute the specified volume of DNA sample to the desired final DNA concentration.

- The Below Min Volume Symbol indicates that the DNA sample volume is less than the minimum volume to pipet based on the specified dilution volume. This symbol also indicates that the volume of diluent you need to add is less than the Min Pipetting Volume (µl) specified on the Admin sheet.

- The Below DNA Target Mass Symbol indicates that the DNA concentration determined using the autosomal or Y target (for the Autosomal or Y Normalization worksheets, respectively) is too low to achieve the specified target DNA mass (in ng), even when using the maximum volume of DNA sample.

Normalization for Autosomal STR: The PowerQuant® Analysis Tool uses the values defined in this section and the autosomal DNA concentration for a DNA sample to determine the volume of undiluted DNA required to achieve the target mass in an autosomal STR amplification reaction. If a DNA sample is too concentrated, the user-defined values are used to calculate the sample dilution needed to achieve the target mass of DNA. The software will flag any sample that does not reach the target mass of DNA based on the maximum sample volume.

In the Min Pipetting Volume (µl) field, specify the minimum volume of undiluted DNA sample that can be added to the autosomal STR amplification reaction. This field also indicates the minimum volume of diluent that you can use before the Below Min Volume Symbol appears. This volume is used to determine the volume of sample to add to the reaction after dilution if a dilution step is necessary to reach the desired target mass of DNA to be amplified.

In the Max Sample Volume (µl) field, specify the maximum volume of undiluted DNA sample that can be added to the autosomal STR amplification reaction. This volume is used to calculate Diluent to Add to Reaction (µl) for a DNA sample that does not require dilution prior to autosomal STR amplification reaction assembly as well as Filler Diluent to Add to Reaction (µl) for a sample that does require dilution.

In the DNA per reaction (ng) field, specify the desired mass (in ng) of DNA to be amplified.

Normalization for Y-STR: The PowerQuant® Analysis Tool uses the values defined in this section and the male DNA concentration (Y target) for a DNA sample to determine the volume of undiluted DNA required to achieve the target mass in a Y-STR amplification reaction. If a DNA sample is too concentrated, the user-defined values are used to calculate the sample dilution needed to achieve the target mass of DNA. The software will flag any sample that does not reach the target mass of DNA based on the maximum sample volume.

In the Min Pipetting Volume (µl) field, specify the minimum volume of undiluted DNA sample that can be added to the Y-STR amplification reaction. This field also indicates the minimum volume of diluent that you can use before the Below Min Volume Symbol appears. This volume is used to determine the volume of sample to add to the reaction after dilution if a dilution is necessary to reach the desired target mass of DNA to be amplified.

In the Max Sample Volume (µl) field, specify the maximum volume of undiluted DNA sample that can be added to the Y-STR amplification reaction. This volume also is used to calculate Diluent to Add to Reaction (µl) for a DNA sample that does not require dilution prior to Y-STR amplification reaction assembly as well as Filler Diluent to Add to Reaction (µl) for a sample that does require dilution.

In the DNA per reaction (ng) field, specify the desired mass (in ng) of DNA to be amplified.

Note: To save any changes made to the Admin Settings parameters, save a copy of the PowerQuant® Analysis Tool as an Excel® macro-enabled template (.xltm file). This template can be saved as a read only file to prevent others from making changes to the template.

8. Return to the Home worksheet, and select the **Import Data** button.

9. Browse to the .xls file exported from the Applied Biosystems® 7500 Real-Time PCR System software in Section 6.D or 7.D, highlight the file and select **OK**.

    The following new worksheets are created, with tabs for each worksheet at the bottom of the template.

Standards: The Standards worksheet displays the standard curve results for each target (e.g., R^2 value, slope, Y-intercept and efficiency) and indicates whether the R^2 value and slope meet the criteria defined on the Admin Settings worksheet. If the R^2 value is at or above the R^2 threshold set on the Admin Settings worksheet, the PowerQuant® Analysis Tool displays "At or Above". If the R^2 value falls below the R^2 threshold, the PowerQuant® Analysis Tool displays "Below". If the slope is within the range set on the Admin Settings worksheet, the PowerQuant® Analysis Tool displays "Within". If the slope is outside of this range, the PowerQuant® Analysis Tool displays "Outside". The R^2 value and slope values also are displayed. This worksheet includes a table with the observed C_q value for each target at each DNA standard concentration on the standard curve (Figure 101). This worksheet also includes the Threshold set from Admin sheet table. For more information about how the slope and R^2 value can be used to evaluate the standard curve, see Section 10.

12.D. Using the PowerQuant® Analysis Tool (continued)

	A	D	E	F	G	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1	Concentration ng/µl	Auto Cq	Y Cq	Deg Cq	IPC Cq				Auto	Y	Deg							
3	0.0032	35.2404	35.6007	35.5886	20.08													
4	0.0032	35.2003	34.5221	35.5752	20.23			R^2	0.999	0.997	1.000							
5	0.0800	30.5430	30.3509	30.5874	19.96			Slope	-3.43	-3.37	-3.44							
6	0.0800	30.2623	30.4830	30.4435	20.06			Intercept	26.63	26.68	26.89							
7	2.0000	25.2913	25.6011	25.8162	20.03			Amplification Efficiency	95.66%	98.18%	95.40%							
8	2.0000	25.6772	25.6051	25.7601	20.01													
9	50.0000	21.0259	20.9562	21.1166	20.48			R^2 Thresholds	At or Above	At or Above	At or Above							
10	50.0000	20.7226	21.0026	21.1636	20.29			Slope Limits	Within	Within	Within							
11																		
12								Threshold set from Admin sheet										
13								R^2 Thresholds	0.99	0.99	0.99							
14								Slope Upper limit	-3.10	-3.10	-3.10							
15								Slope Lower limit	-3.60	-3.60	-3.60							

Home   Admin Settings   Standards   Standard Curves   Results   Results with Averages   Autosomal Normali

Figure 101. The Standards worksheet.

Standard Curves: The Standard Curves worksheet displays a plot of C_q value versus DNA concentration (ng/µl) for each quantification target and the equation for the line of best fit (Figure 102).
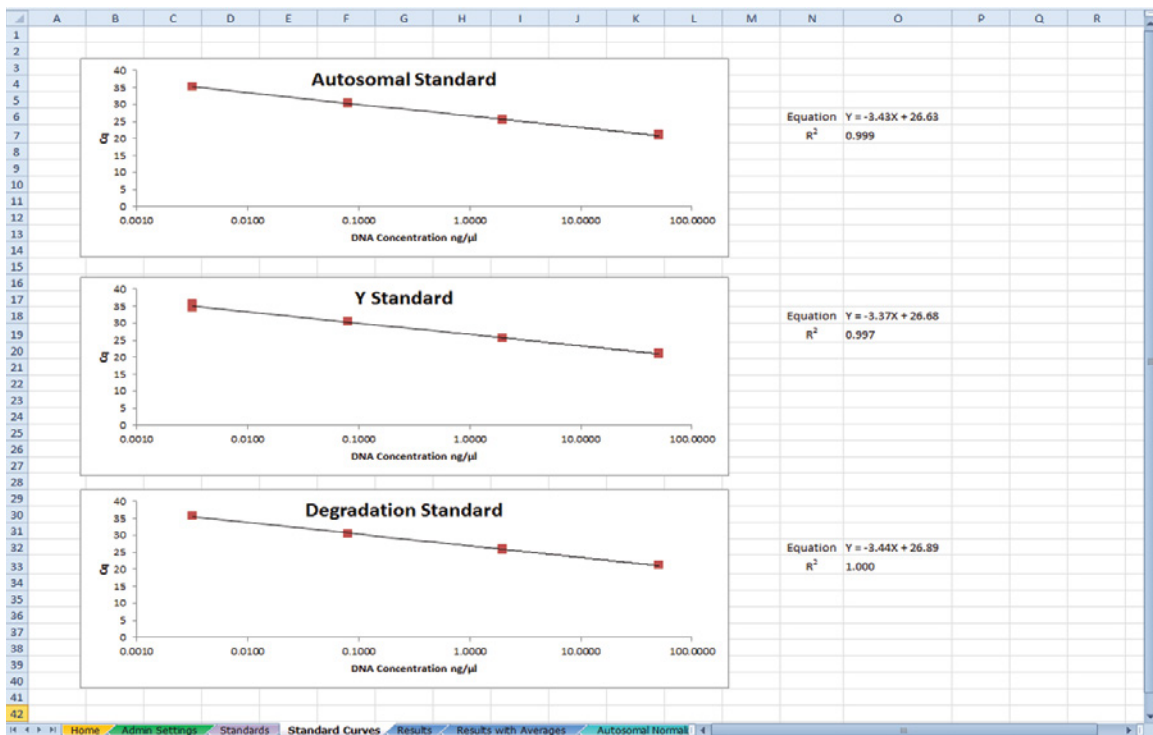
Figure 102. The Standard Curves worksheet.

Results: The Results worksheet (Figure 103) displays the data output sorted by well position. The Instrument Target Name and Sample Assessment settings from the Admin Settings worksheet are displayed at the top of page. The DNA concentration and C_q value for each target are displayed in individual columns. The DNA concentrations and C_q values are imported from the Applied Biosystems® 7500 software.

The IPC C_q value for the DNA standard with the closest DNA concentration is displayed for each sample in the Closest IPC column. The difference in C_q values for the IPC of the closest DNA standard and the IPC of the DNA sample is displayed in the IPC Shift column. The IPC Threshold column displays either "At or Above" or "Below" based on the IPC Shift value for the sample and the IPC Shift value set in the Sample Assessment section of the Admin Settings worksheet (Figure 99).

The ratio of autosomal DNA concentration and male DNA concentration is calculated and displayed in the [Auto]/[Y] column. The [Auto]/[Y] Threshold column displays either "At or Above" or "Below" based on the [Auto]/[Y] ratio for the sample and the [Auto]/[Y] ratio set for the Possible Male/Female Mixture field in the Sample Assessment section of the Admin Settings worksheet.

12.D. Using the PowerQuant® Analysis Tool (continued)

The ratio of autosomal DNA concentration and degradation target DNA concentration is calculated and displayed in the [Auto]/[D] column. If no value is displayed in the [Deg] column for a sample, then the [Auto]/[D] column will display "Undetermined". The [Auto]/[D] Threshold column displays either "At or Above" or "Below" based on the [Auto]/[Deg] ratio for the sample and the [Auto]/[Deg] ratio set for the Possible degraded sample field in the Sample Assessment section of the Admin Settings worksheet. The Results worksheet is not locked and is sortable by column.

	Well	Sample Name	Task	[Auto]	[Deg]	[Y]	Auto Cq	IPC Cq	Y Cq	Deg Cq	Closest IPC	IPC Shift	IPC Threshold	[Auto]/[Y]	[Auto]/[Y] Threshold	[Auto]/[D]	[Auto]/[D] Threshold
6	A1	PQ_50ng/ul	STANDARD	50.0000	50.0000	50.0000	21.03	20.48	20.96	21.12	20.39	0.09	Below	1.00	Below	1.00	Below
7	A2																
8	A3																
9	A4	Unknown 1	UNKNOWN	0.1385	0.1254	0.1190	29.58	20.08	29.79	29.99	20.01	0.07	Below	1.16	Below	1.10	Below
10	A5	Unknown 1	UNKNOWN	0.1464	0.1275	0.1216	29.49	19.77	29.76	29.96	20.01	-0.24	Below	1.20	Below	1.15	Below
11	A6	Unknown 8	UNKNOWN	223.7019	170.3264	0.3227	18.57	19.98	28.33	19.22	20.39	-0.41	Below	693.16	At or Above	1.31	Below
12	A7	Unknown 12	UNKNOWN	0.1773	0.1498	0.1693	29.21	19.91	29.27	29.72	20.01	-0.11	Below	1.05	Below	1.18	Below
13	A8	Unknown 14	UNKNOWN	0.0018	0.0016	0.0021	36.04	19.98	35.68	36.54	20.16	-0.17	Below	0.86	Below	1.16	Below
14	A9	Unknown 15	UNKNOWN	0.1911	0.1691	0.1504	29.10	19.99	29.45	29.54	20.01	-0.02	Below	1.27	Below	1.13	Below
15	A10	Unknown 17	UNKNOWN	0.0031	0.0023	0.0009	35.25	20.00	36.87	35.94	20.16	-0.15	Below	3.29	At or Above	1.33	Below
16	A11	Unknown 18	UNKNOWN	1.2771	1.2691	1.4329	26.27	20.03	26.15	26.53	20.02	0.01	Below	0.89	Below	1.01	Below
17	A12																
18	B1	PQ_50ng/ul	STANDARD	50.0000	50.0000	50.0000	20.72	20.29	21.00	21.16	20.39	-0.09	Below	1.00	Below	1.00	Below
19	B2																
20	B3																
21	B4	Unknown 2	UNKNOWN	0.1653	0.1487	0.1284	29.31	19.91	29.68	29.73	20.01	-0.10	Below	1.29	Below	1.11	Below
22	B5	Unknown 2	UNKNOWN	0.1391	0.1450	0.1286	29.57	19.77	29.67	29.77	20.01	-0.25	Below	1.08	Below	0.96	Below
23	B6	Unknown 8	UNKNOWN	231.4745	188.7329	0.3468	18.52	19.88	28.22	19.07	20.39	-0.50	Below	667.51	At or Above	1.23	Below
24	B7	Unknown 12	UNKNOWN	0.1985	0.1607	0.1772	29.04	19.65	29.21	29.62	20.01	-0.36	Below	1.12	Below	1.24	Below
25	B8	Unknown 14	UNKNOWN	0.0031	0.0029	0.0015	35.23	19.62	36.13	35.61	20.16	-0.54	Below	2.01	At or Above	1.07	Below
26	B9	Unknown 15	UNKNOWN	0.2277	0.1768	0.1601	28.83	19.94	29.35	29.47	20.01	-0.07	Below	1.42	Below	1.29	Below
27	B10	Unknown 17	UNKNOWN	0.0032	0.0023	0.0022	35.19	20.04	35.64	35.97	20.16	-0.12	Below	1.48	Below	1.40	Below
28	B11	Unknown 18	UNKNOWN	1.3474	1.3139	1.4945	26.19	19.67	26.09	26.48	20.02	-0.35	Below	0.90	Below	1.03	Below
29	B12																
30	C1	PQ_2ng/ul	STANDARD	2.0000	2.0000	2.0000	25.29	20.03	25.60	25.82	20.02	0.01	Below	1.00	Below	1.00	Below
31	C2																
32	C3																
33	C4	Unknown 3	UNKNOWN	0.1696	0.1470	0.1146	29.27	19.85	29.84	29.75	20.01	-0.16	Below	1.48	Below	1.15	Below
34	C5	Unknown 3	UNKNOWN	0.1809	0.1418	0.1036	29.18	19.94	29.99	29.80	20.01	-0.07	Below	1.75	Below	1.28	Below
35	C6	Unknown 9	UNKNOWN	202.9561	162.4162	0.1148	18.71	19.57	29.84	19.29	20.39	-0.82	Below	1767.63	At or Above	1.25	Below
36	C7	Unknown 12	UNKNOWN	0.2014	0.1443	0.1659	29.02	19.87	29.30	29.78	20.01	-0.15	Below	1.21	Below	1.40	Below
37	C8	Unknown 14	UNKNOWN	0.0018	0.0019	0.0026	36.08	19.55	35.40	36.27	20.16	-0.60	Below	0.69	Below	0.94	Below
38	C9	Unknown 15	UNKNOWN	0.2056	0.2107	0.1713	28.99	19.85	29.26	29.21	20.01	-0.16	Below	1.20	Below	0.98	Below
39	C10	Unknown 17	UNKNOWN	0.0020	0.0020	0.0025	35.90	19.71	35.44	36.18	20.16	-0.45	Below	0.80	Below	1.00	Below
40	C11	Unknown 18	UNKNOWN	1.2646	1.4456	1.5006	26.28	20.04	26.08	26.34	20.02	0.02	Below	0.84	Below	0.87	Below

Settings header: IPC Shift Threshold 0.35 | M/F mixture Threshold 2 | Deg Threshold 2 | Autosomal | Degradation | IPC | Y

Worksheet tabs: Home | Admin Settings | Standards | Standard Curves | Results | Results with Averages | Autosomal Normal

Figure 103. The Results worksheet.

Results with Averages: The Results with Averages worksheet (Figure 104) contains output results sorted alphabetically by sample name, with averages calculated for replicate samples (i.e., samples with the same sample name). Data for the standards are not displayed. The Instrument Target Name and Sample Assessment settings from the Admin Settings worksheet are displayed at the top of page. The DNA concentration for each target is displayed in an individual column.

The IPC C_q value for the sample is displayed. The difference in C_q values for the IPC of the closest DNA standard and the IPC of the DNA sample is displayed in the IPC Shift column. The IPC Threshold column displays either "At or Above" or "Below" based on the IPC Shift value for the sample and the IPC Shift value set in the Sample Assessment section of the Admin Settings worksheet.

The ratio of autosomal DNA concentration and male DNA concentration is calculated and displayed in the [Auto]/[Y] column. The [Auto]/[Y] Threshold column displays either "At or Above" or "Below" based on the [Auto]/[Y] ratio for the sample and the [Auto]/[Y] ratio set for the Possible Male/Female Mixture field in the Sample Assessment section of the Admin Settings worksheet.

Well	Sample Name	[Auto]	[Deg]	[Y]	IPC Cq	IPC Shift	IPC Threshold	[Auto]/[Y]	[Auto]/[Y] Threshold	[Auto]/[D]	[Auto]/[D] Threshold
A4	Unknown 1	0.1385	0.1254	0.1190	20.08	0.07	Below	1.16	Below	1.10	Below
A5	Unknown 1	0.1464	0.1275	0.1216	19.77	-0.24	Below	1.20	Below	1.15	Below
Average	Unknown 1	0.1424	0.1265	0.1203	19.93	-0.09	Below	1.18	Below	1.13	Below
E6	Unknown 10	205.9642	163.1381	0.0250	19.64	-0.75	Below	8227.81	At or Above	1.26	Below
F6	Unknown 10	218.2691	168.3754	0.0267	19.60	-0.79	Below	8187.92	At or Above	1.30	Below
Average	Unknown 10	212.1166	165.7567	0.0258	19.62	-0.77	Below	8207.23	At or Above	1.28	Below
G6	Unknown 11	134.4298	109.0514	0.0027	19.71	-0.68	Below	49795.54	At or Above	1.23	Below
H6	Unknown 11	136.8869	112.9007	0.0024	19.92	-0.47	Below	57316.07	At or Above	1.21	Below
Average	Unknown 11	135.6583	110.9761	0.0025	19.81	-0.57	Below	53325.70	At or Above	1.22	Below
A7	Unknown 12	0.1773	0.1498	0.1693	19.91	-0.11	Below	1.05	Below	1.18	Below
B7	Unknown 12	0.1985	0.1607	0.1772	19.65	-0.36	Below	1.12	Below	1.24	Below
C7	Unknown 12	0.2014	0.1443	0.1659	19.87	-0.15	Below	1.21	Below	1.40	Below
D7	Unknown 12	0.1857	0.1697	0.1655	19.78	-0.23	Below	1.12	Below	1.09	Below
Average	Unknown 12	0.1907	0.1561	0.1694	19.80	-0.21	Below	1.13	Below	1.22	Below
E7	Unknown 13	0.0188	0.0196	0.0173	19.86	-0.30	Below	1.09	Below	0.96	Below
F7	Unknown 13	0.0188	0.0199	0.0189	19.70	-0.46	Below	1.00	Below	0.95	Below
G7	Unknown 13	0.0214	0.0155	0.0200	19.87	-0.29	Below	1.07	Below	1.38	Below
H7	Unknown 13	0.0173	0.0158	0.0183	20.02	-0.14	Below	0.95	Below	1.09	Below
Average	Unknown 13	0.0191	0.0177	0.0186	19.86	-0.29	Below	1.03	Below	1.08	Below
A8	Unknown 14	0.0018	0.0016	0.0021	19.98	-0.17	Below	0.86	Below	1.16	Below
B8	Unknown 14	0.0031	0.0029	0.0015	19.62	-0.54	Below	2.01	At or Above	1.07	Below
C8	Unknown 14	0.0018	0.0019	0.0026	19.55	-0.60	Below	0.69	Below	0.94	Below
D8	Unknown 14	0.0020	0.0015	0.0013	20.01	-0.15	Below	1.45	Below	1.34	Below
Average	Unknown 14	0.0022	0.0019	0.0019	19.79	-0.37	Below	1.14	Below	1.11	Below
A9	Unknown 15	0.1911	0.1691	0.1504	19.99	-0.02	Below	1.27	Below	1.13	Below

Figure 104. The Results with Averages worksheet.

The ratio of autosomal DNA concentration and degradation target DNA concentration is calculated and displayed in the [Auto]/[D] column. If no value is displayed in the [Deg] column for a sample, then the [Auto]/[D] column will display "Undetermined". The [Auto]/[D] Threshold column displays either "At or Above" or "Below" based on the [Auto]/[Deg] ratio for the sample and the [Auto]/[Deg] ratio set for the Possible degraded sample field in the Sample Assessment section of the Admin Settings worksheet. The Results with Averages worksheet is locked and is not sortable.

All values for individual wells displayed on the Results with Averages worksheet are identical to those on the Results worksheet.

Autosomal Normalization: The Autosomal Normalization worksheet (Figure 105) contains the output normalization parameters for each DNA sample based on the DNA concentration determined using the autosomal target for that sample and the autosomal normalization parameters set in the Admin Settings worksheet. If multiple DNA quantification results are available for a sample, these values are displayed for individual replicates and for the average autosomal quantification result for each set of replicates. The Normalization for Autosomal STR and Normalization settings from the Admin Settings worksheet are displayed at the top of page.

The Autosomal Normalization worksheet displays the sample name and well position of the DNA sample in the quantification plate. The Sample Concentration (ng/µl) column displays the DNA concentration of the sample based on the quantification results from the autosomal target.

The next two columns apply to samples that do not require dilution prior to STR amplification reaction assembly. These columns will be blank for samples that require dilution prior to autosomal STR amplification reaction assembly.

• The Sample to Add to Reaction (µl) column displays the volume of undiluted DNA to be added directly to the autosomal STR amplification reaction to achieve the desired target DNA mass (in ng).

• The Diluent to Add to Reaction (µl) column displays the difference in volume between the Max Sample Volume (µl) and the Sample to Add to Reaction (µl) volume. This value represents the volume of diluent to add to the reaction.

The next four columns apply to samples that require dilution prior to autosomal STR amplification reaction assembly. These columns will be blank for samples that do not require dilution prior to reaction assembly. Using the volumes indicated in this section, dilute the high-concentration DNA samples in a separate tube or plate so that the desired target DNA mass (in ng) can be added to the autosomal STR amplification reaction.

• The name of the column to the right of the Diluent to Add to Reaction (µl) column is based on the value entered as the Min Pipetting Volume (µl) on the Admin Settings worksheet. For example, if the Min Pipetting Volume (µl) value was set to 1.0, the name of the column is Diluent to Add per 1.0 µl Sample (µl). This column displays the volume of diluent to add to the minimum volume (e.g., 1.0µl) of DNA sample when diluting these DNA samples.

• The Max/Min Dilution Volume Warning column displays the appropriate character entered on the Admin Settings worksheet if the diluent volume required to dilute the specified volume of DNA sample to the desired final DNA concentration exceeds the specified Max Dilution Volume or if the diluent volume is less than the Min Pipetting Volume.

• The Diluted Sample to Add to Reaction (µl) column displays the volume of diluted DNA to add to the autosomal STR amplification reaction. This value is the Min Pipetting Volume (µl) set on the Admin Settings worksheet.

• The Filler Diluent to Add to Reaction (µl) column displays the difference in volume between the Max Sample Volume (µl) and the Diluted Sample to Add to Reaction (µl) volume. This value represents the volume of diluent to add to the reaction.

The DNA template (ng) column displays the target mass of DNA for the autosomal STR amplification reaction based on the volumes and dilution steps specified in the Autosomal Normalization worksheet for that sample.

The Below DNA Target Mass Warning column displays the character entered on the Admin Settings worksheet to indicate that the DNA concentration determined using the autosomal target is too low to meet the specified target mass of DNA (in ng), even when using the maximum volume of DNA sample.

	PowerQuant Autosomal Normalization Utility			DNA Target Mass (ng)	Min Pipetting Volume (µl)	Max Sample Volume (µl)	Max Dilution Warning (µl)					
				0.5	1	15	200					
	LEGEND:	Above Max Dilution Warning		Below Min Volume Warning		Below DNA Target Mass Warning						
		^		*		!						
Well	Sample Name	Sample Concentration (ng/µl)	Sample to Add to Reaction (µl)	Diluent to Add to Reaction (µl)	Diluent to Add per 1 µl Sample (µl)	Max / Min Dilution Volume Warning	Diluted Sample to Add to Reaction (µl)	Filler Diluent to Add to Reaction (µl)	DNA template (ng)	Below DNA Target Mass Warning
---	---	---	---	---	---	---	---	---	---	---
A4	Unknown 1	0.1385	3.6	11.4					0.500	
A5	Unknown 1	0.1464	3.4	11.6					0.500	
Average	Unknown 1	0.1424	3.5	11.5					0.500	
E6	Unknown 10	205.9642			410.9	^	1.0	14.0	0.500	
F6	Unknown 10	218.2691			435.5	^	1.0	14.0	0.500	
Average	Unknown 10	212.1166			423.2	^	1.0	14.0	0.500	
G6	Unknown 11	134.4298			267.9	^	1.0	14.0	0.500	
H6	Unknown 11	136.8869			272.8	^	1.0	14.0	0.500	
Average	Unknown 11	135.6583			270.3	^	1.0	14.0	0.500	
A7	Unknown 12	0.1773	2.8	12.2					0.500	
B7	Unknown 12	0.1985	2.5	12.5					0.500	
C7	Unknown 12	0.2014	2.5	12.5					0.500	
D7	Unknown 12	0.1857	2.7	12.3					0.500	
Average	Unknown 12	0.1907	2.6	12.4					0.500	
E7	Unknown 13	0.0188	15.0	0.0					0.282	!
F7	Unknown 13	0.0188	15.0	0.0					0.282	!
G7	Unknown 13	0.0214	15.0	0.0					0.321	!
H7	Unknown 13	0.0173	15.0	0.0					0.259	!
Average	Unknown 13	0.0191	15.0	0.0					0.286	!
A8	Unknown 14	0.0018	15.0	0.0					0.027	!
B8	Unknown 14	0.0031	15.0	0.0					0.047	!
C8	Unknown 14	0.0018	15.0	0.0					0.026	!
D8	Unknown 14	0.0020	15.0	0.0					0.029	!
Average	Unknown 14	0.0022	15.0	0.0					0.032	!
A9	Unknown 15	0.1911	2.6	12.4					0.500	
B9	Unknown 15	0.2277	2.2	12.8					0.500	
C9	Unknown 15	0.2056	2.4	12.6					0.500	
D9	Unknown 15	0.1929	2.6	12.4					0.500	
Average	Unknown 15	0.2043	2.4	12.6					0.500	
E9	Unknown 16	0.0272	15.0	0.0					0.408	!

Sheet tabs: Standards / Standard Curves / Results / Results with Averages / Autosomal Normalization / Y Normalization

Figure 105. The Autosomal Normalization worksheet.

Y Normalization: The Y Normalization worksheet (Figure 106) contains the output normalization parameters for each DNA sample based on the male DNA concentration determined using the Y target for that sample and the Y normalization parameters set in the Admin Settings worksheet. If multiple quantification results are available for a sample, these values are displayed for individual replicates and for the average male quantification result for each set of replicates. The Normalization for Y-STR and Normalization settings from the Admin Settings worksheet are displayed at the top of page.

The Y Normalization worksheet displays the sample name and well position of the DNA sample in the quantification plate. The Sample Concentration (ng/µl) column displays the DNA concentration of the sample based on the quantification results from the Y target.

The next two columns apply to samples that do not require dilution prior to STR amplification reaction assembly. These columns will be blank for samples that require dilution prior to Y-STR amplification reaction assembly.

- The Sample to Add to Reaction (µl) column displays the volume of undiluted DNA to be added directly to the Y-STR amplification reaction to achieve the desired target DNA mass (in ng).

- The Diluent to Add to Reaction (µl) column displays the difference in volume between the Max Sample Volume (µl) and the Sample to Add to Reaction (µl) volume. This value represents the volume of diluent to add to the Y-STR reaction.

The next four columns apply to samples that require dilution prior to Y-STR amplification reaction assembly. These columns will be blank for samples that do not require dilution prior to reaction assembly. Using the volumes indicated in this section, dilute the high-concentration DNA samples in a separate tube or plate so that the desired target DNA mass (in ng) can be added to the Y-STR amplification reaction.

- The name of the column to the right of the Diluent to Add to Reaction (µl) column is based on the value entered as the Min Pipetting Volume (µl) on the Admin Settings worksheet. For example, if the Min Pipetting Volume (µl) value was set to 1.0, the name of the column is Diluent to Add per 1.0 µl Sample (µl). This column displays the volume of diluent to add to the minimum volume (e.g., 1.0µl) of DNA sample when diluting these DNA samples.

- The Max/Min Dilution Volume Warning column displays the appropriate character entered on the Admin Settings worksheet if the diluent volume required to dilute the specified volume of DNA sample to the desired final DNA concentration exceeds the specified Max Dilution Volume or if the diluent volume is less than the Min Pipetting Volume.

- The Diluted Sample to Add to Reaction (µl) column displays the volume of diluted DNA to add to the Y-STR amplification reaction. This value is the Min Pipetting Volume (µl) set on the Admin Settings worksheet.

- The Filler Diluent to Add to Reaction (µl) column displays the difference in volume between the Max Sample Volume (µl) and the Diluted Sample to Add to Reaction (µl) volume. This value represents the volume of diluent to add to the reaction.

The DNA template (ng) column displays the target mass of DNA for the Y-STR amplification reaction based on the volumes and dilution steps specified in the Y Normalization worksheet for that sample.

The Below DNA Target Mass Warning column displays the character entered on the Admin Settings worksheet to indicate that the DNA concentration determined using the Y target is too low to meet the specified target mass of DNA (in ng), even when using the maximum DNA sample volume.

Figure 106. The Y Normalization worksheet.

Raw Data: The Raw Data worksheet contains the imported raw data spreadsheet (image not shown).

1. Save the file by selecting **Save As** from the File menu. Save the file as an .xlsx file to a known location on your computer.

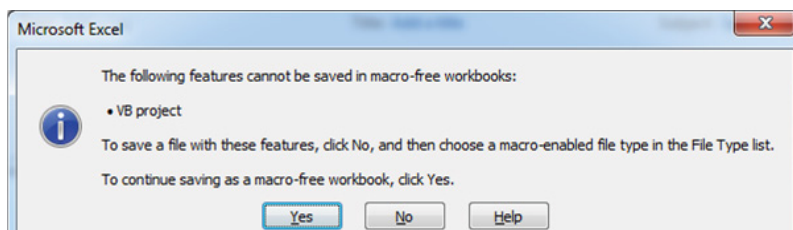2. The window shown in Figure 107 will appear. Select **Yes**.

Figure 107. Saving the file as a macro-free workbook.

12.E. Interpretation of PowerQuant® Data Using the PowerQuant® Analysis Tool

In addition to the concentrations of autosomal and male DNA used to normalize DNA samples for STR amplification, the PowerQuant® System yields information that can be used to evaluate amplification performance, detect PCR inhibitors in the amplification reaction, detect the presence of male and female DNA mixtures and evaluate the degree of DNA degradation.

Note: [Auto]/[Y] and [Auto]/[D] ratios may not be reliable in samples with low DNA concentrations (e.g., less than 1pg/µl) due to stochastic effects.

Both the 7500 Applied Biosystems® Real-Time PCR System Software (see Section 6.C or 7.C) and the PowerQuant® Analysis Tool (see Section 12.D) perform a linear regression to the standard dilution series data and calculate the equation for the line of best fit (the standard curve). The equation is in the form of $y = mx + b$, where x = log concentration and $y = C_q$. The R^2 value is a measure of the fit of the data points to the regressed line. The slope (m in the equation) is an indication of the PCR efficiency. A slope of −3.3 indicates 100% PCR efficiency (i.e., the number of copies of amplification product is doubled at each cycle). The Y-intercept (b in the equation) is defined as the y value (C_q) when x (log concentration) equals 0. Therefore, b corresponds to the C_q value for a sample with a concentration of 1ng/µl [$\log_{10}(1) = 1$].

In general, the standard curve for each target (autosomal, Y and degradation) has an average slope (m) in the range of −3.1 to −3.6 and an R^2 value >0.990. See Section 11 for more information about troubleshooting standard curves that fall out of these ranges. We recommend tracking and evaluating Y-intercept values for any significant changes from run to run.

Internal PCR Control

Amplification performance for the IPC can be used to evaluate overall performance of a PowerQuant® amplification reaction or detect PCR inhibitors in the DNA sample. IPC data analyzed using the PowerQuant® Analysis Tool can be interpreted as follows:

- If a sample yields no detectable amplification for the autosomal, Y and degradation targets but has an IPC amplification curve that crossed the amplification threshold without an IPC shift greater than that specified on the Admin Settings worksheet, then insufficient DNA template was added to the PowerQuant® amplification reaction.

- If a sample has an IPC Shift value greater than the value specified in the Admin Settings worksheet or if the C_q value for the IPC is undetermined (regardless of detectable amplification for the autosomal, Y and degradation quantification targets), then this indicates that a PCR inhibitor may be present in the amplification reaction. Repeat the DNA purification if necessary.

- If a sample has an IPC Shift value greater than the value specified in the Admin Settings worksheet and the [Auto]/[D] ratio is less than the ratio specified on the Admin Settings worksheet, then the sample likely contains a PCR inhibitor but the DNA is not likely degraded. Repeat the DNA purification if necessary.

- If a sample has an IPC Shift value greater than the value specified in the Admin Settings worksheet and the [Auto]/[D] ratio is greater than the ratio specified on the Admin Settings worksheet, then the sample likely contains a PCR inhibitor and the DNA is possibly degraded. Repeat the DNA purification if necessary.

  Note: The IPC is the longest target in the PowerQuant® System and therefore is more susceptible to inhibition relative to the shorter autosomal and Y targets. The degradation target is long, and amplification performance also may be affected by inhibitors.

[Auto]/[Y] Ratio

The [Auto]/[Y] ratio can be used to evaluate whether a sample includes a male/female DNA mixture. The results from the [Auto]/[Y] calculations performed by the PowerQuant® Analysis Tool may be interpreted as follows:

- If a sample has no value for the [Auto]/[Y] ratio, then no male DNA was detected.

- If a sample has an [Auto]/[Y] ratio less than the value specified in the Admin Settings worksheet, then the sample may contain male DNA only or low levels of female DNA.

- If a sample has an [Auto]/[Y] ratio greater than the value specified in the Admin Settings worksheet, then the sample contains a possible mixture of male and female DNA.

[Auto]/[D] Ratio

The [Auto]/[D] ratio can be used to evaluate whether a sample was degraded. The results from the [Auto]/[D] calculations performed by the PowerQuant® Analysis Tool may be interpreted as follows:

- If a sample has an [Auto]/[D] ratio less than the value specified in the Admin Settings worksheet, then the DNA in the sample is not likely degraded, regardless of the value in the IPC Shift column.

- If a sample has an [Auto]/[D] ratio greater than the value specified in the Admin Settings worksheet and the value for the IPC Shift is less than the value specified in the Admin Settings worksheet, then the DNA in the sample is likely degraded but the sample does not contain PCR inhibitors.

- If a sample has an [Auto]/[D] ratio and IPC Shift value greater than the values specified in the Admin Settings worksheet then this sample likely contains PCR inhibitors and may or may not contain degraded DNA.

- If a sample has an [Auto]/[D] ratio of "Undetermined" and the IPC Shift value is less than the value specified in the Admin Settings worksheet, then the DNA in the sample is likely severely degraded but the sample does not contain PCR inhibitors.

- If a sample has an [Auto]/[D] ratio of "Undetermined" and the IPC Shift value is greater than the value specified in the Admin Settings worksheet, then the sample likely contains PCR inhibitors and the DNA may be degraded.

12.F. Changing the Password in the PowerQuant® Analysis Tool

Settings on the Admin Settings worksheet of the PowerQuant® Analysis Tool are protected. The default password is "Admin". Instructions to change the password are provided below.

1. Navigate to the Admin Settings worksheet in the PowerQuant® Analysis Tool.

2. Unprotect the Admin Settings worksheet.
   a. Select **Review** in the toolbar.
   b. Select **Unprotect Sheet** in the toolbar.
   c. The 'Unprotect Sheet' dialog box appears.
   d. Enter "Admin Admin" for the password.

3. Select **Review** from the toolbar.

4. Select **Allow Users to Edit Ranges**.
   a. In the 'Allow Users to Edit Ranges' window that appears, ensure "AdminCells" is highlighted, and select **Modify**.
   b. Select **Password**.
   c. Enter the desired password.

5. Select **OK** for all dialog boxes until you are returned to the Admin Settings worksheet.

6. Reprotect the Admin Settings worksheet.
   a. Select **Review** in the toolbar.
   b. Select **Protect Sheet** in the toolbar.
   c. The 'Protect Sheet' dialog box appears. Ensure that the "Protect worksheet and contents of locked cells" box is checked. Under "Allow all users of this worksheet to:" ensure that the boxes for "Select locked cells" and "Select unlocked cells" are checked.
   d. Select **OK**.

12.G. Related Products

STR Systems

Product	Size	Cat.#
PowerPlex® Fusion 6C System	50 (or 100 direct-amp) reactions	DC2705
	200 (or 400 direct-amp) reactions	DC2720
	800 (1,600 direct-amp) reactions	DC2780
PowerPlex® Fusion System	200 reactions	DC2402
	800 reactions	DC2408
PowerPlex® Y23 System	50 reactions	DC2305
	200 reactions	DC2320
PowerPlex® 21 System	200 reactions	DC8902
	4 × 200 reactions	DC8942
PowerPlex® 18D System	200 reactions	DC1802
	800 reactions	DC1808
PowerPlex® ESX 16 Fast System	100 reactions	DC1611
	400 reactions	DC1610
PowerPlex® ESX 17 Fast System	100 reactions	DC1711
	400 reactions	DC1710
PowerPlex® ESI 16 Fast System	100 reactions	DC1621
	400 reactions	DC1620
PowerPlex® ESI 17 Fast System	100 reactions	DC1721
	400 reactions	DC1720
PowerPlex® 16 HS System	100 reactions	DC2101
	400 reactions	DC2100
PowerPlex® CS7 System	100 reactions	DC6613

Not for Medical Diagnostic Use.

12.H. Summary of Changes

The following changes were made to the 8/22 revision of this document.

1.   Corrected System Requirements in Section 9.B.

2.   Revised Installation information in Section 11.B.

3.   Updated cover image and document font.

4.   Made general formatting updates.

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