# Using the PowerQuant® System on the Applied Biosystems® QuantStudio™ 5 Real-Time PCR System

*Promega Corporation*


**Materials Required:**

- PowerQuant® System, 200 reactions (Cat.# PQ5002) or 800 reactions (Cat.# PQ5008)
- PowerQuant® Calibration Kit (Cat.# DS1221)
- Sterile, aerosol-resistant pipette tips
- Tubes (5ml or larger) for diluting the PowerQuant® Calibration Standards

**Instrument Requirements:**

- Applied Biosystems® QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific)

## Introduction

This application note provides instructions for using the Promega PowerQuant® System (Cat.# PQ5002 and PQ5008) on the Applied Biosystems® QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific). The analysis is performed using the QuantStudio™ Design and Analysis Software (ver. 1.4.1 or higher). Refer to the current version of the *PowerQuant® System Technical Manual* #TMD047, Promega Corporation, for general considerations regarding the PowerQuant® System chemistry, components, storage conditions, user-supplied materials and interpretation of data.

## Calibrating the QuantStudio™ 5 Real-Time PCR Instrument

We recommend performing the Regions of Interest (ROI)/uniformity calibration and background calibration as described in Chapter 5 of the *QuantStudio™ 5 Real-Time PCR Instrument User Guide for Human Identification* #MAN0017162, Thermo Fisher Scientific, before performing the PowerQuant® dye calibration.

You will use the PowerQuant® Calibration Kit (Cat.# DS1221) to calibrate the QuantStudio™ 5 Real-Time PCR instrument for the five PowerQuant® dyes (FAM, CAL Fluor® Gold 540, TMR, Quasar® 670 and CXR).

### Setting up the Calibration Plate

Follow the instructions in the *Calibration Plate Setup* section of the *PowerQuant® System Technical Manual* #TMD047.

### Performing a PowerQuant® Dye Calibration

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. Choose ‘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. Choose the corresponding dye you wish to calibrate in the Custom Dye menu. Enter '60°C' for the calibration temperature.
8. Press 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 Figure 1.
3. Choose '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 choose '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* #MAN0017162, Thermo Fisher Scientific.

## Setting up the QuantStudio™ Design and Analysis Desktop Software

Download the current version of the QuantStudio™ Design and Analysis Software before using the PowerQuant® System on the QuantStudio™ 5 Real-Time PCR System for the first time.

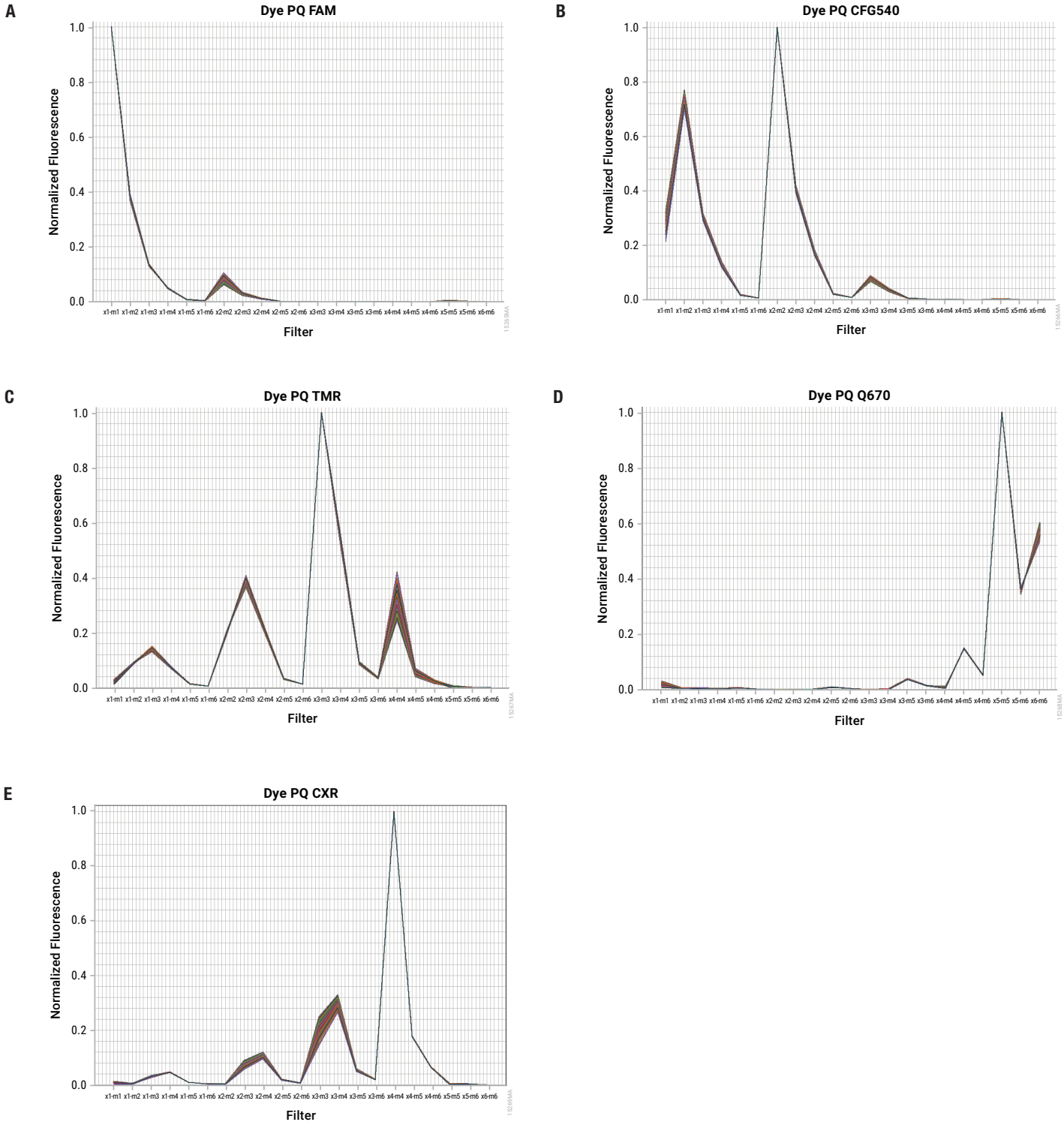
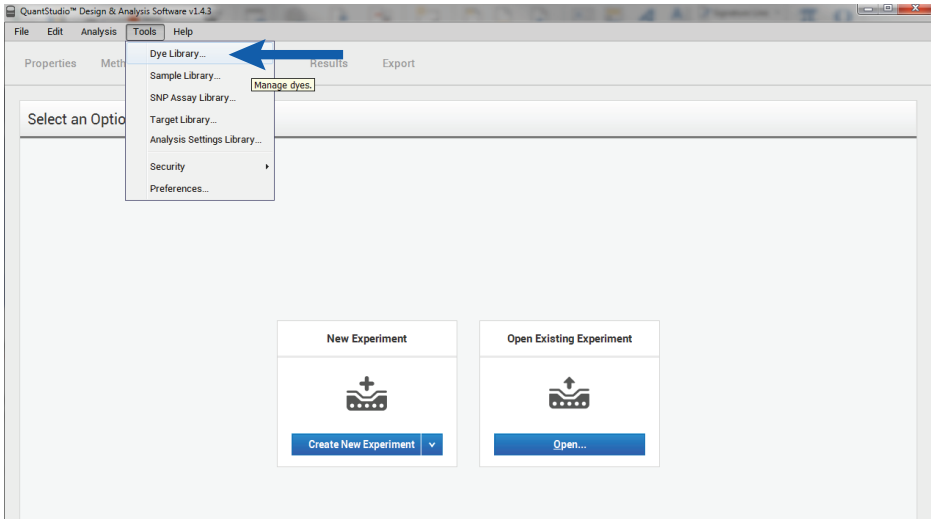



**Figure 1. Example dye calibration spectra with peak filter for the strongest signal denoted in parentheses.** Panel A: PQ_FAM (x1-m1). Panel B: PQ_CFG540 (x2-m2). Panel C: PQ_TMR (x3-m3). Panel D: PQ_Q670 (x5-m5). Panel E: PQ_CXR (x4-m4).

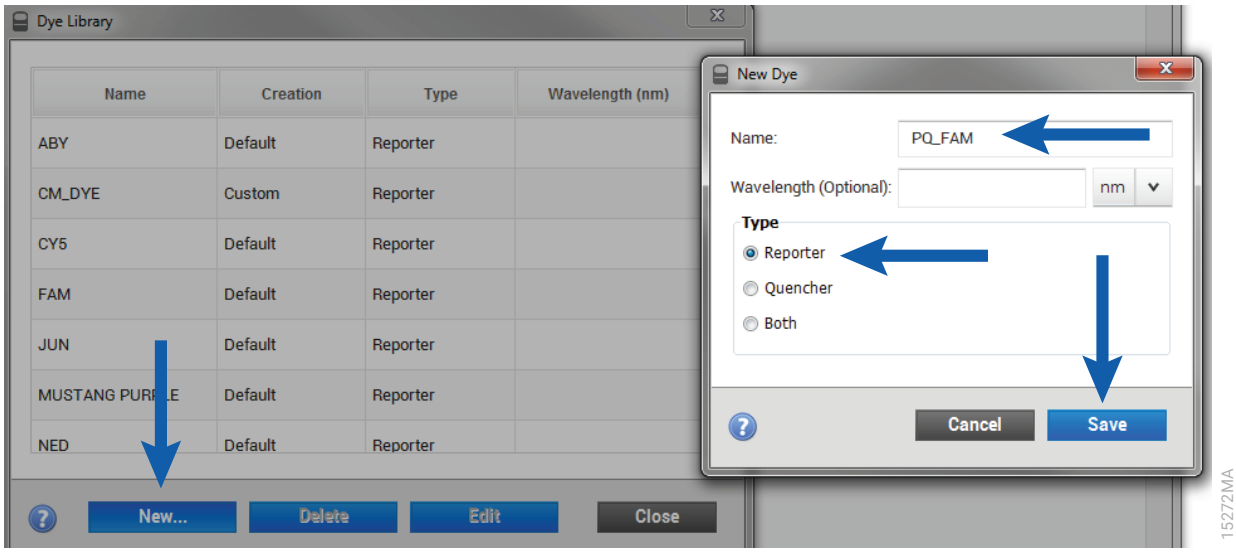
### Adding the PowerQuant® Dyes

1. Select 'Tools > Dye Library' from the menu at the top of the home screen.



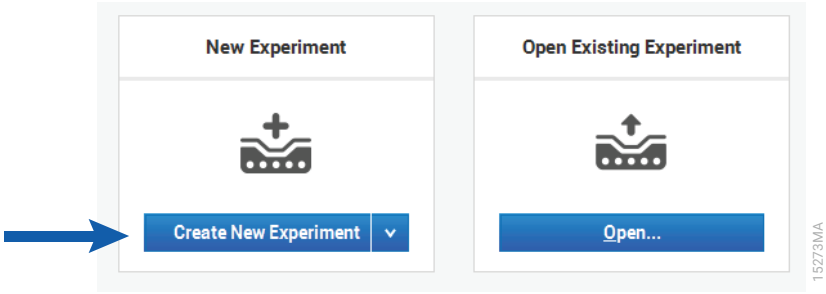

2. Add the five PowerQuant® custom dye names by selecting the **New** button.
3. Enter and save the following dye names: 'PQ_FAM', 'PQ_CFG540', 'PQ_TMR', 'PQ_Q670' and 'PQ_CXR'.
4. Confirm that 'Reporter' is selected as the Type for each dye.

   **Note:** The dye names must match those entered in the 'Custom Dye' area when the dye calibrations were performed in the *Performing a PowerQuant® Dye Calibration* section.



### Creating a Run Template

1. Open the QuantStudio™ Design and Analysis Software and select 'Create New Experiment' from the home screen.




2. Enter the following information into the 'Experiment Properties' section:

   Instrument type: QuantStudio™ 5 System

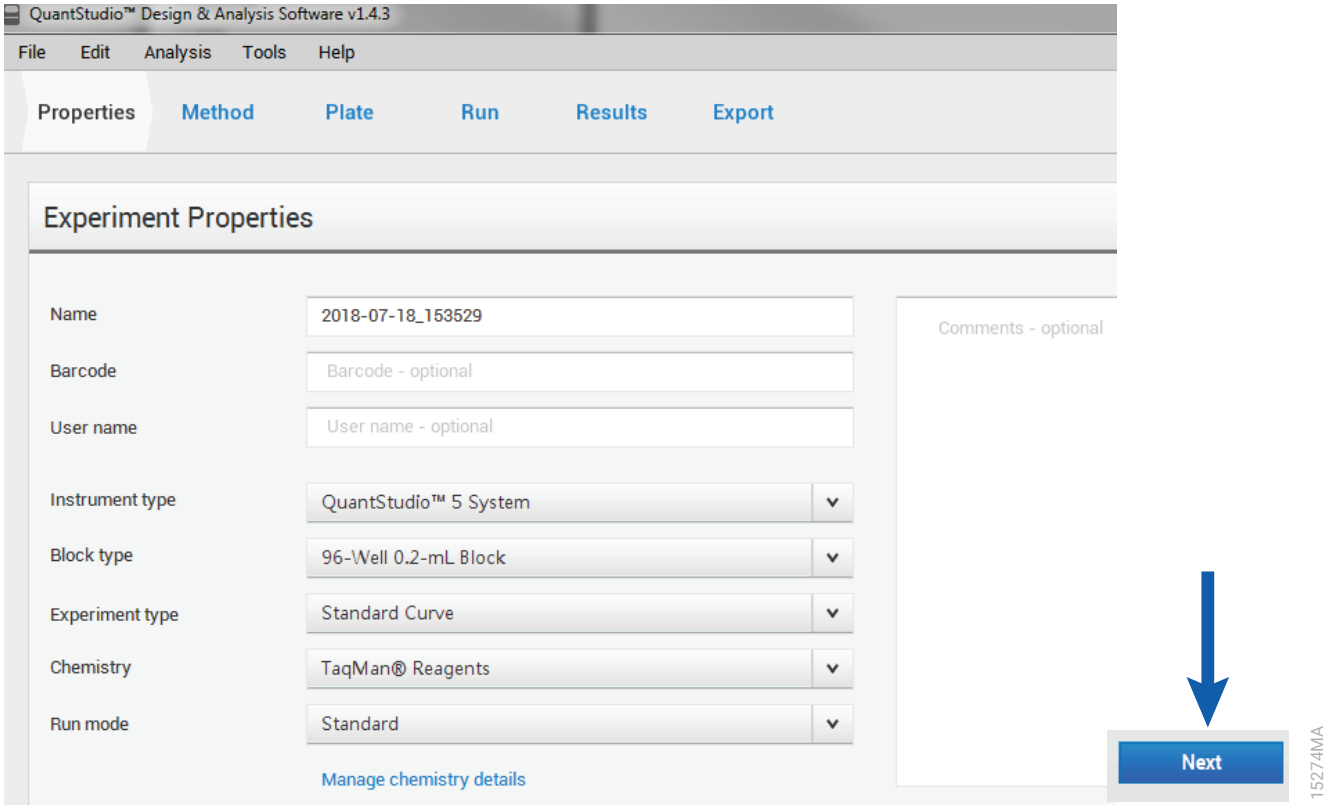
   Block type: 96-Well 0.2ml Block

   Experiment type: Standard Curve

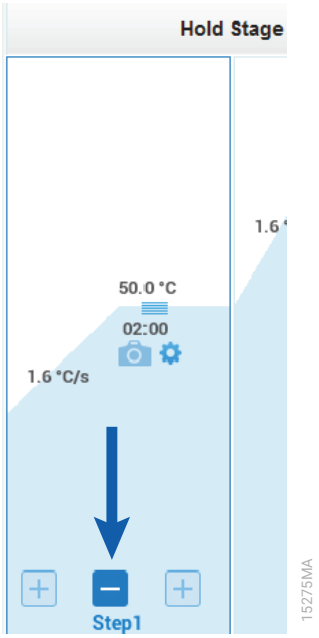
   Chemistry: TaqMan® Reagents

   Run mode: Standard

   Click the **Next** button.



3. Perform the following actions on the 'Experiment Method' tab:
   a. Enter '20 µl' in the 'Volume' box.
   b. Delete the first Hold Stage by hovering over the Step 1 stage box and selecting the **[–]** button.

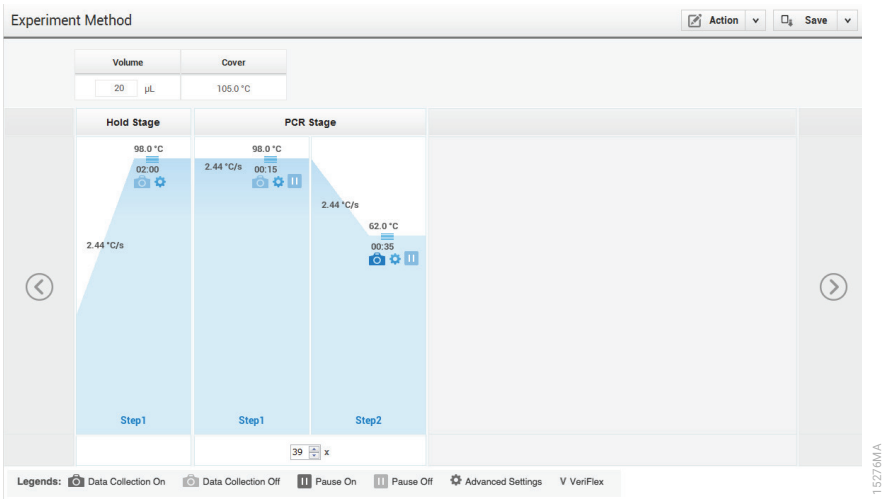



   c. Change the remaining Step 1 of the Hold Stage to '98°C for 2 minutes'.
   d. Change Step 1 of the PCR Stage to '98°C for 15 seconds'.
   e. Change Step 2 of the PCR Stage to '62°C for 35 seconds'.

      Confirm that the 'Data Collection On' icon is active for Step 2 of the PCR Stage.

      **Note:** You can view the icon legends at the bottom of the screen.
   f. Enter '39' for the number of cycles in the box below the PCR Stage.
   g. Change the ramp rates for all three steps to '2.44°C/s'.
   h. Click the **Next** button.

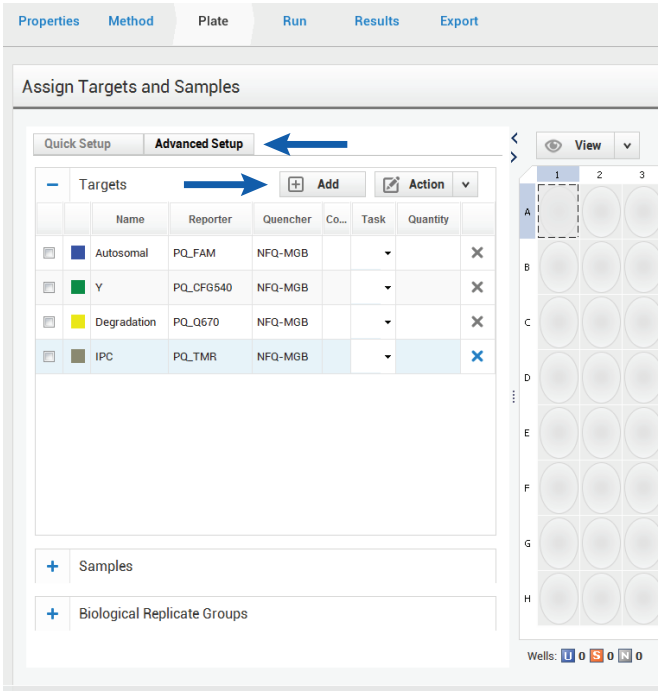
An example of the completed PowerQuant® System run method is shown below:



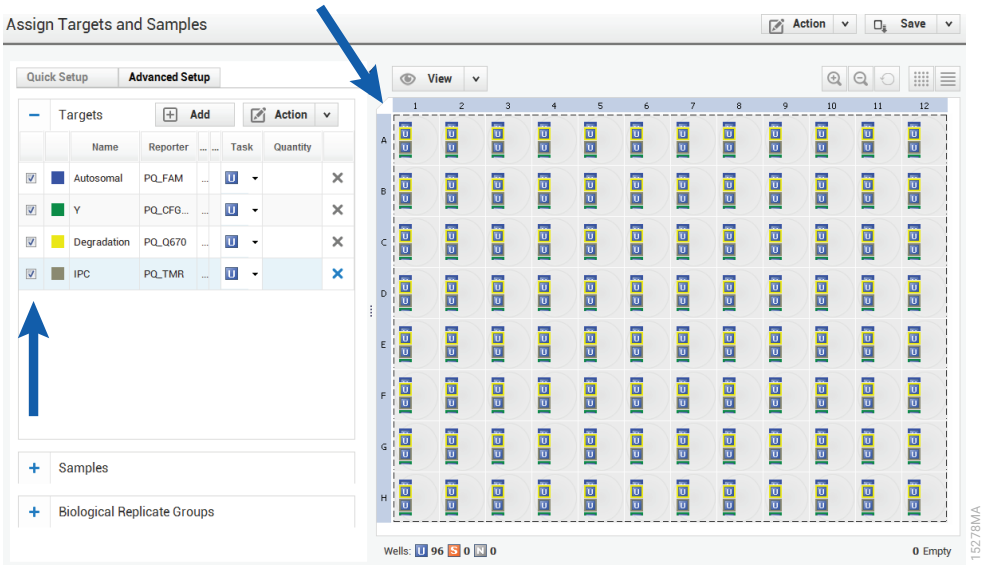
4. Enter the PowerQuant® targets in the 'Targets' section of the 'Advanced Setup' tab in the 'Assign Targets and Samples' area. Click **Add** three additional times and then enter the following target-specific information:

| 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 Tool to recognize these targets.

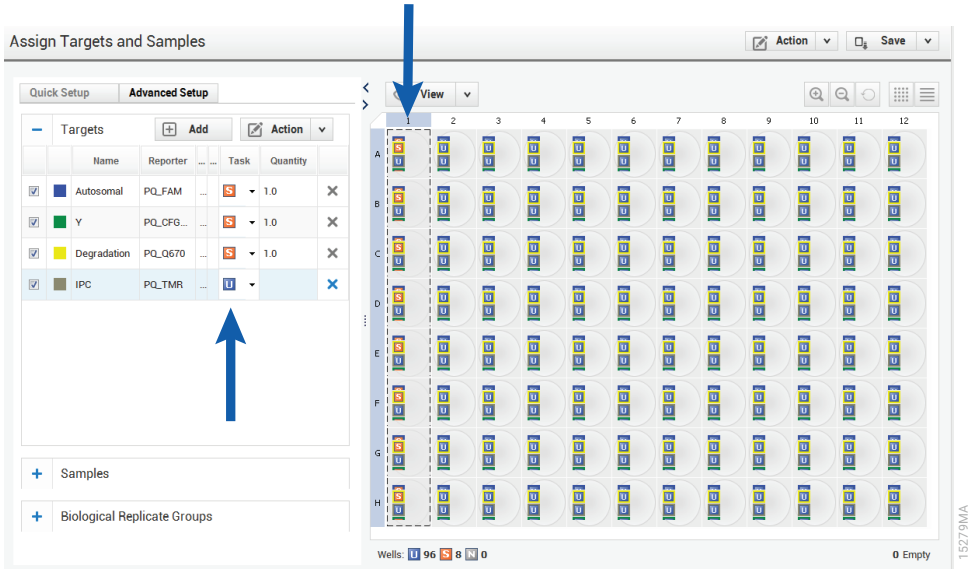



5. Highlight all wells in the plate map. Assign all four targets to all wells by clicking the box next to each target name.



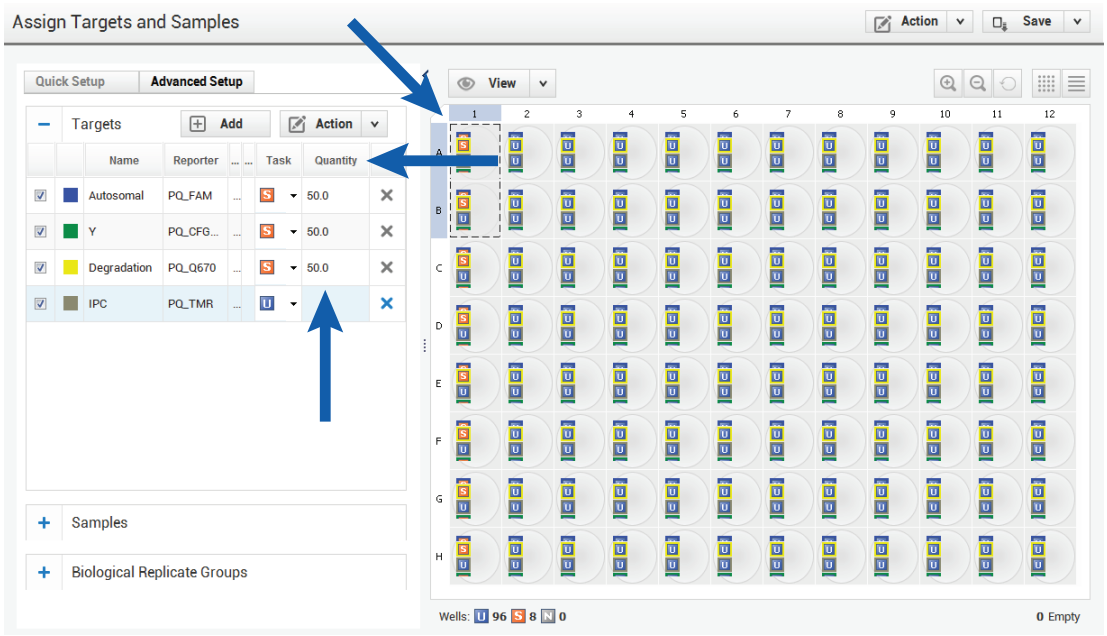
6. Highlight the wells containing DNA standards. Assign the Task to 'S' for the Autosomal, Y and Degradation targets.

   **Note:** The Task for the IPC target should remain 'U'.

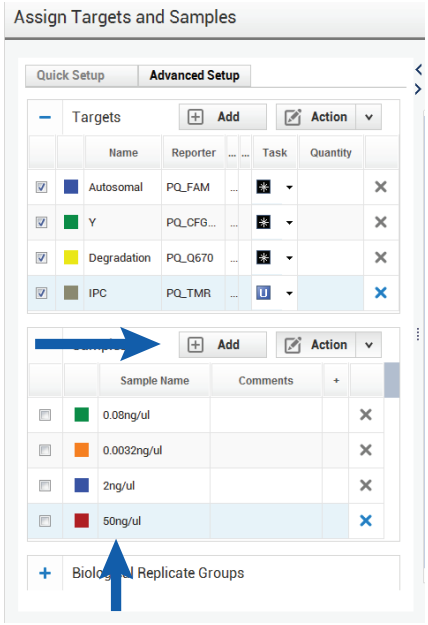



7. Enter the concentration of each DNA Standard in the 'Quantity' field without a unit of measure.

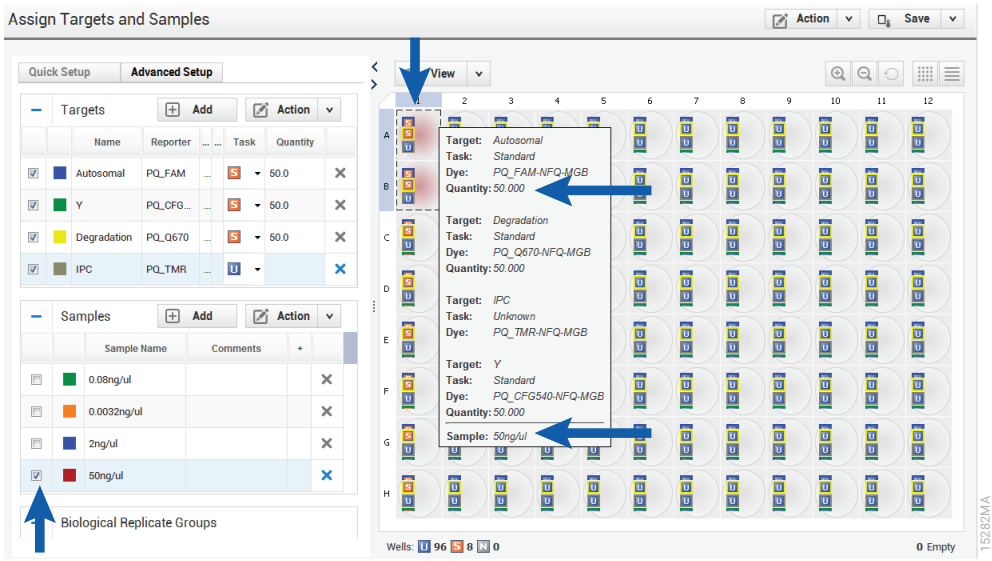
   **Example:** Enter '50' for 50ng/µl, '2' for 2ng/µl, '0.08' for 0.08ng/µl and '0.0032' for 0.0032ng/µl. Highlight wells with DNA standards of the same concentration simultaneously, then enter the value. Repeat for each DNA standard concentration.



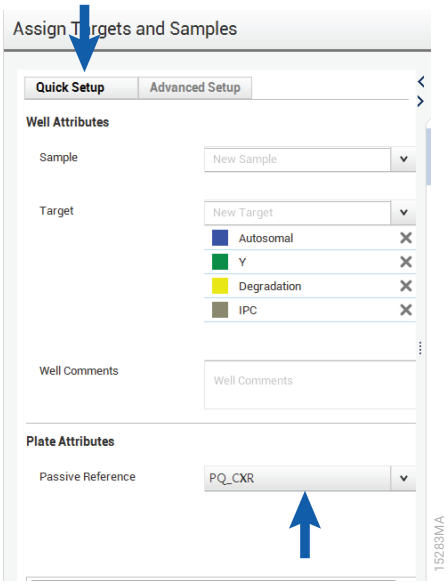
8. Enter the names of the DNA standards in the ‘Samples’ section of the ‘Advanced Setup’ tab. Click **Add** three additional times and then type the name of each standard once.



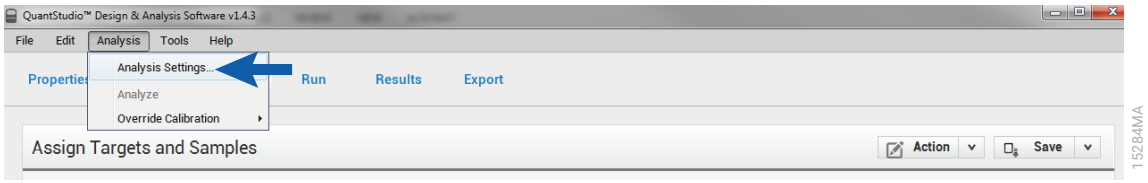

9. Highlight all wells with DNA standards of the same name. Assign the DNA standard name to the selected wells by clicking the box adjacent to the corresponding DNA standard name. Repeat for each set of DNA standards.



10. Switch to the 'Quick Setup' tab. Select 'PQ_CXR' as the 'Passive Reference'.




11. Select 'Analysis > Analysis Settings' from the menu at the top of the screen.



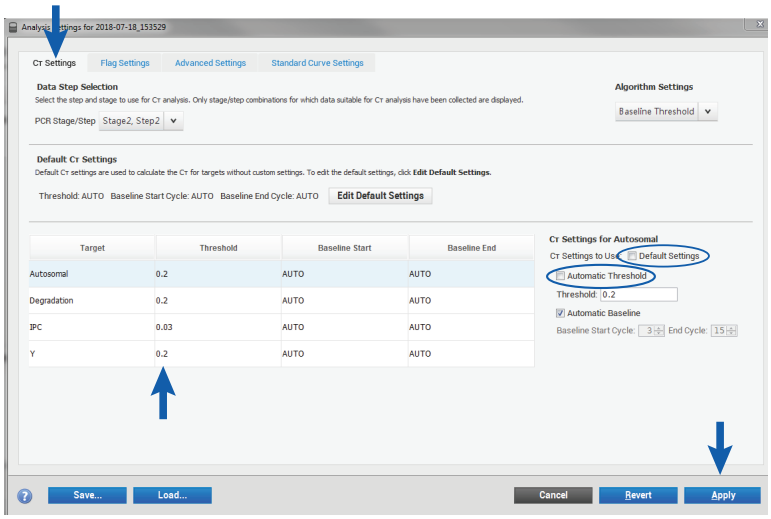

12. Navigate to the '$C_T$ Settings' tab. Highlight a target, then uncheck the 'Default Settings' and 'Automatic Threshold' boxes for each target.

    **Note:** Leave the 'Automatic Baseline' box checked.

    Enter the following threshold values for each target:

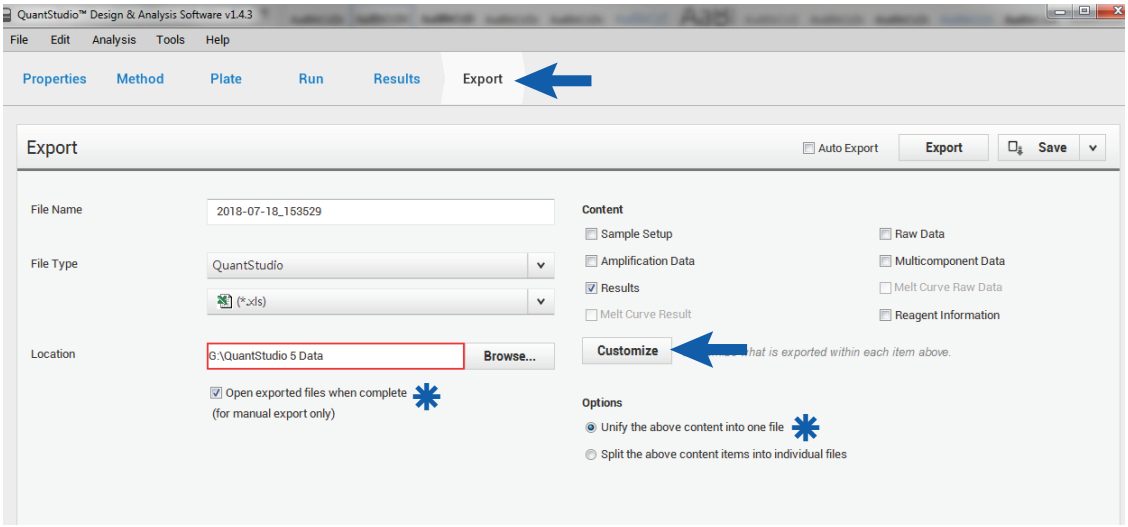
    | | |
    |---|---|
    | Autosomal: | 0.2 |
    | Degradation: | 0.2 |
    | IPC: | 0.03 |
    | Y: | 0.2 |

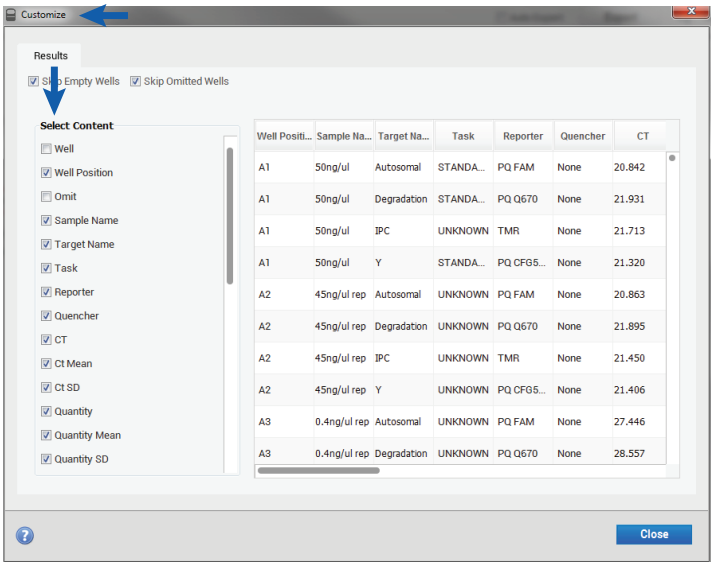
    Click the **Apply** button.



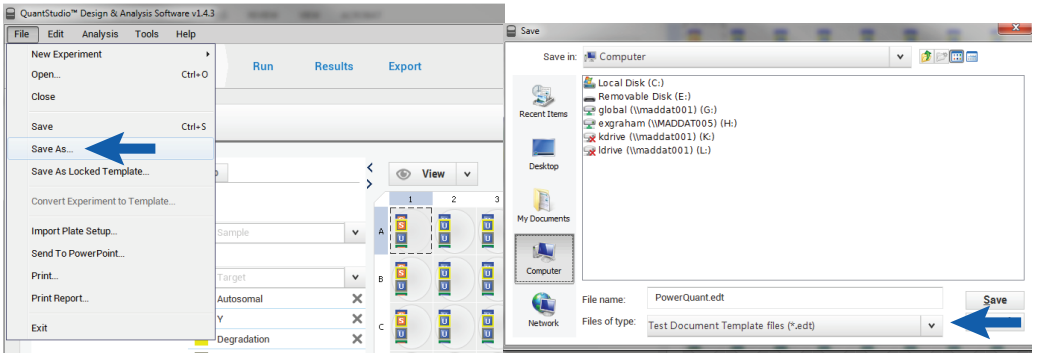
13. Navigate to the ‘Export’ tab. Review the following parameters and adjust them as needed:
    - Set the File Type to ‘QuantStudio’ and ‘.xls’.
    - The ‘Open exported files when complete’ box should be checked.
    - The ‘Results’ box should be the only box checked under the Content section. Deselect the ‘Sample Setup’ and ‘Amplification Data’ boxes.
    - ‘Unify the above content into one file’ should be selected under the ‘Options’ section.
    - Click the **Customize** button and confirm that the following items are deselected:

      Well, Omit, Y-Intercept, $R^2$, Slope, Efficiency, Amp Status, $C_q$ Conf, Rn(last cycle) and Delta Rn(last cycle).
    - Leave the boxes for ‘Skip Empty Wells’ and ‘Skip Omitted Wells’ at the top of the Customize screen checked if your software version displays them.



14. Select 'File > Save As' from the file menu at the top of the screen. Choose a location to save the template file as a Test Document Template (.edt). The template file can now be used to create future PowerQuant® experiment documents on the QuantStudio™ 5 System.

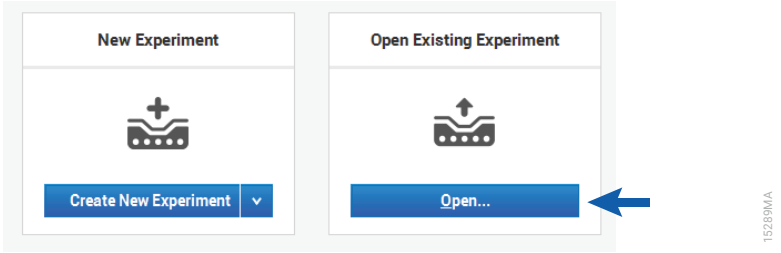



## Reaction Plate and Run Setup

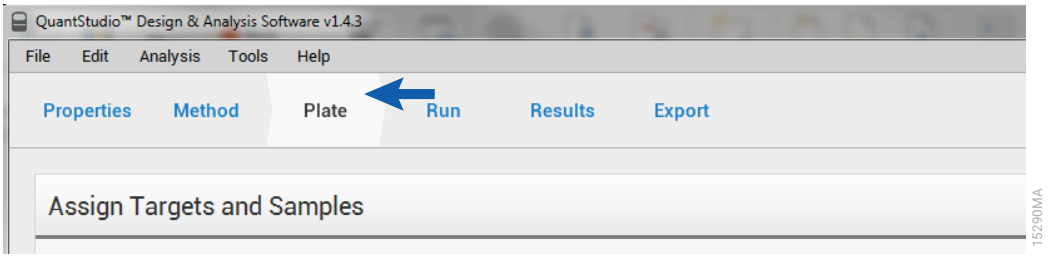
Follow the instructions in the *Reaction Plate Setup* section of the *PowerQuant® System Technical Manual* #TMD047.

### Starting a Run

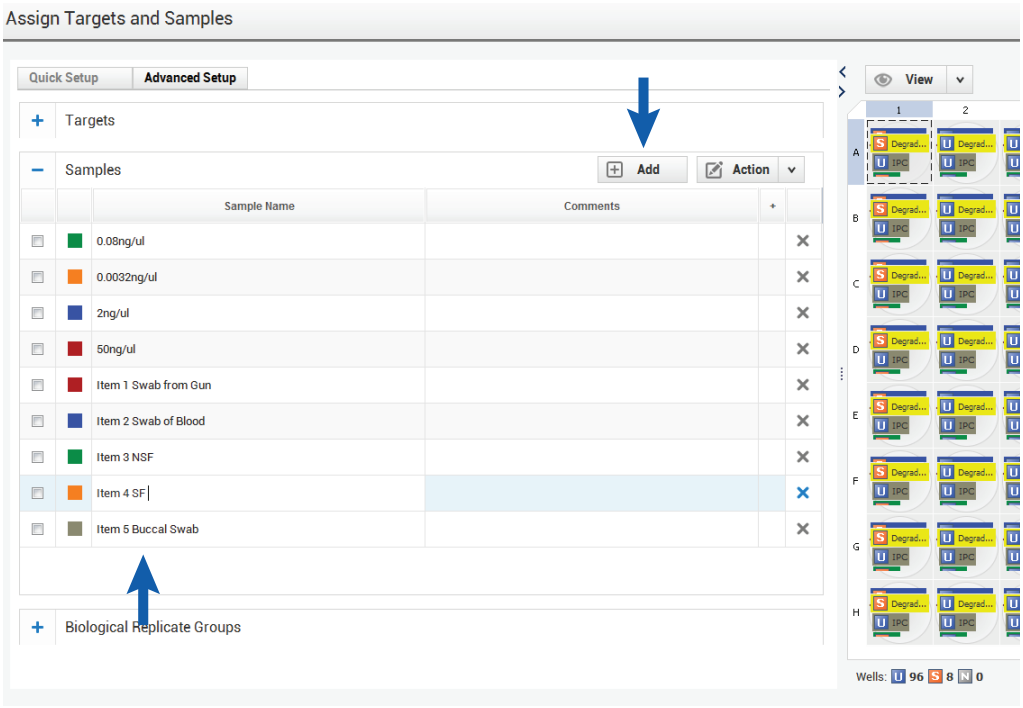
1. Open the QuantStudio™ Design and Analysis Software and select 'Open Existing Experiment' from the home screen.




2. Open a previously created PowerQuant® .edt template file and navigate to the 'Plate' tab.



3. Enter the names of the DNA samples in the 'Samples' section of the 'Advanced Setup' tab by clicking **Add** until the appropriate number of sample name lines are present. Type the name of each sample only once.

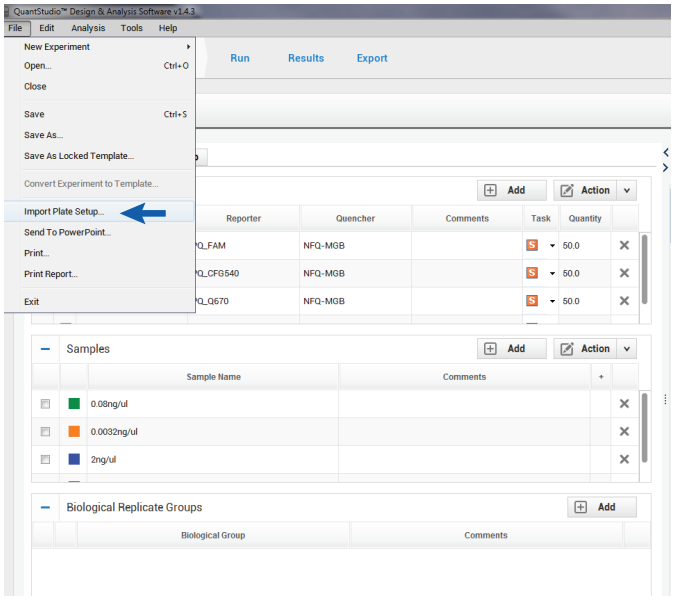


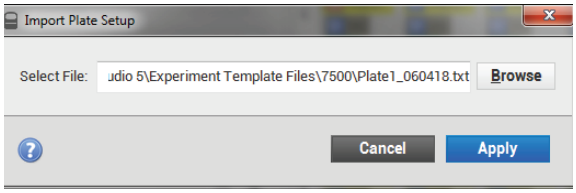

4. Highlight the corresponding well(s) on the plate map and assign the appropriate sample name to the selected well(s) by clicking the box adjacent to the matching sample name. Repeat until all sample names are assigned to a well in the plate map.

   **Note:** If a sample name is inadvertently assigned to the wrong well, reselect that well and uncheck the box next to the wrong sample name. Locate and assign the correct sample name.

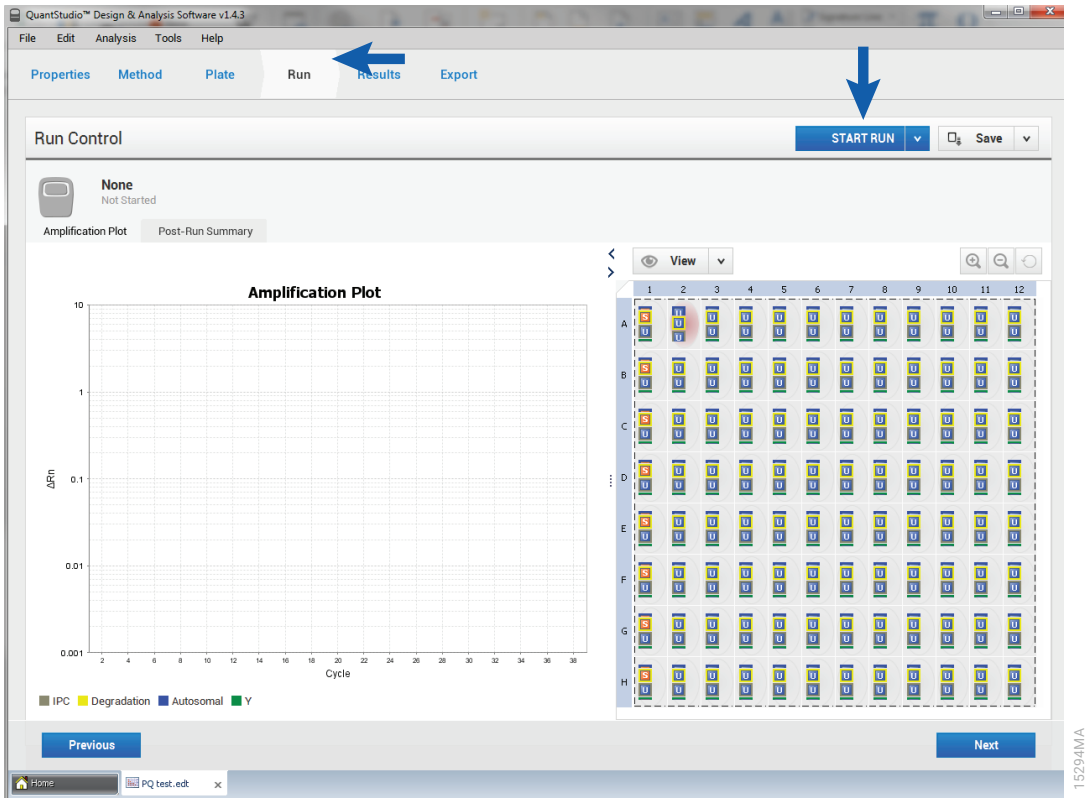
5. Alternatively, you can use a plate setup import file by selecting 'File > Import Plate Setup'. Browse to the location where your plate .txt file is stored. Select the file and click the **Apply** button. Select 'Yes' and continue with the import when prompted. The software will display an 'Import Successful' box. Click **OK** to move forward with starting the run.

   **Note:** The plate setup import file must be in .txt format. Please contact your Promega representative for further information on preparing a plate setup import file.



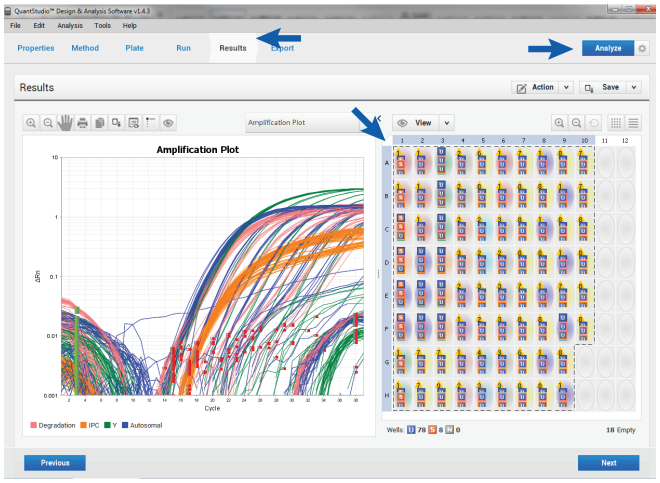
6. Highlight all unused wells on the plate map and deselect all targets by unchecking the boxes next to the target names.
7. Load the appropriate PowerQuant® chemistry plate onto the instrument. You can open and close the tray door by touching the **Eject** icon on the home screen.
8. Navigate to the 'Run' tab. Click the **Start Run** button. The software will prompt you to save your experiment as an .eds file. Run time is approximately one hour.



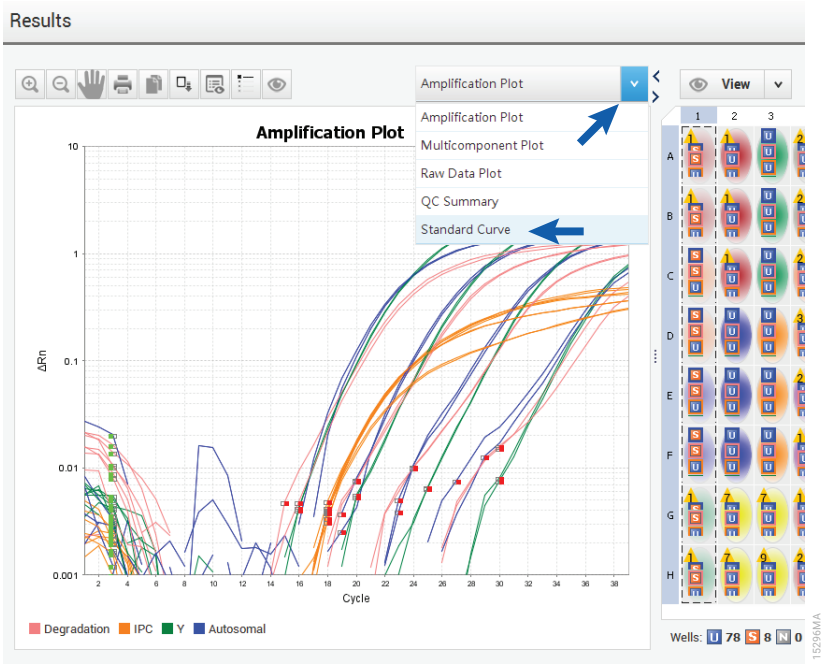

## Evaluating and Exporting Data

### Evaluating Standard Curves Using the QuantStudio™ Design and Analysis Software

1. Navigate to the 'Results' tab. Highlight all the wells you want to analyze on the plate map. Click the **Analyze** button.

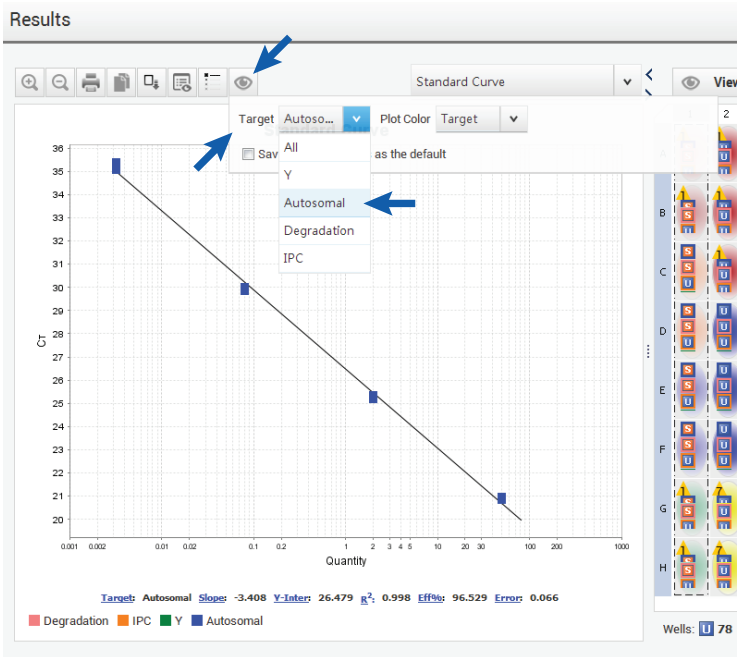


2. Display the standard curves by selecting ‘Standard Curve’ from the drop-down menu located above the ‘Amplification Plot’ box.




3. Display the standard curve for each target by selecting the **Eye** icon. Show the standard curve for each target by choosing the appropriate target from the ‘Target’ drop-down menu. Information regarding the standard curve values is located below the standard curve graph.

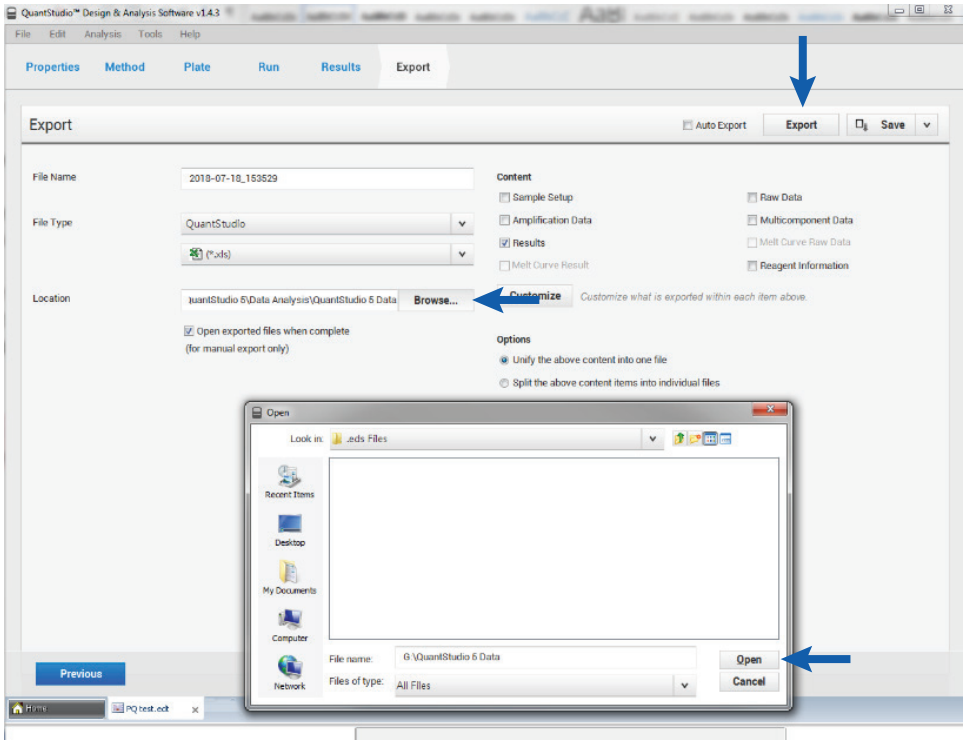
   **Note:** Refer to the *Interpretation of PowerQuant® Data* section of the *PowerQuant® System Technical Manual* #TMD047 for information about how the slope and $R^2$ values can be used to evaluate the standard curve.



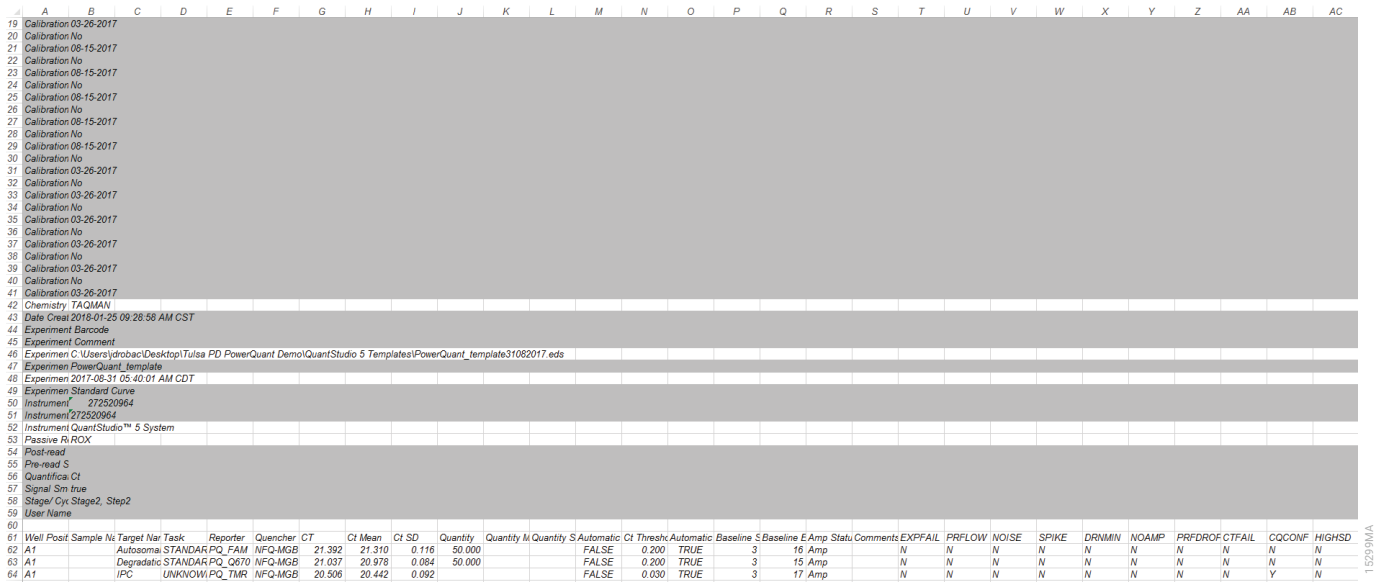

4. Save the analyzed run as an .eds file.

## Exporting Analyzed Data from the QuantStudio™ Design and Analysis Software

1. Confirm that all wells containing data for export are highlighted in the plate map. Navigate to the 'Export' tab.
2. Specify an appropriate export file name. Use the **Browse** button to choose a file location and click the **Export** button.




3. Delete rows 2–41, 43–45, 47, 49–51 and 54–59 from the exported .xls file. Save the changes made to the .xls file.



| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | AA | AB | AC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 20 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 21 | Calibration 08-15-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 22 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 23 | Calibration 08-15-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 24 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 25 | Calibration 08-15-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 26 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 27 | Calibration 08-15-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 28 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 29 | Calibration 08-15-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 30 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 31 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 32 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 33 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 34 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 35 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 36 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 37 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 38 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 39 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 40 | Calibration No | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 41 | Calibration 03-26-2017 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 42 | Chemistry TAQMAN | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 43 | Date Creat 2018-01-25 09:28:56 AM CST | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 44 | Experiment Barcode | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 45 | Experiment Comment | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 46 | Experimen C:\Users\jdrobac\Desktop\Tulsa PD PowerQuant Demo\QuantStudio 5 Templates\PowerQuant_template31082017.eds | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 47 | Experimen PowerQuant_template | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 48 | Experimen 2017-08-31 05:40:01 AM CDT | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 49 | Experimen Standard Curve | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 50 | Instrument 272520964 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 51 | Instrument 272520964 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 52 | Instrument QuantStudio™ 5 System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 53 | Passive Ri ROX | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 54 | Post-read | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 55 | Pre-read S | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 56 | Quantificat Ct | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 57 | Signal Sm true | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 58 | Stage/ Cyc Stage2, Step2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 59 | User Name | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 60 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 61 | Well Posit | Sample Na | Target Nar | Task | Reporter | Quencher | CT | Ct Mean | Ct SD | Quantity | Quantity M | Quantity S | Automatic | Ct Thresh | Automatic | Baseline S | Baseline E | Amp Statu | Comments | EXPFAIL | PRFLOW | NOISE | SPIKE | DRNMIN | NOAMP | PRFDROF | CTFAIL | CQCONF | HIGHSD |
| 62 | A1 | | Autosomal | STANDAR | PQ_FAM | NFQ-MGB | 21.392 | 21.310 | 0.116 | 50.000 | | | FALSE | 0.200 | TRUE | 3 | 16 | Amp | | N | N | N | N | N | N | N | N | N | N |
| 63 | A1 | | Degradatic | STANDAR | PQ_Q670 | NFQ-MGB | 21.037 | 20.978 | 0.084 | 50.000 | | | FALSE | 0.200 | TRUE | 3 | 15 | Amp | | N | N | N | N | N | N | N | N | N | N |
| 64 | A1 | | IPC | UNKNOWN | PQ_TMR | NFQ-MGB | 20.506 | 20.442 | 0.092 | | | | FALSE | 0.030 | TRUE | 3 | 17 | Amp | | N | N | N | N | N | N | N | Y | N | |

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## Using the PowerQuant® Analysis Tool

Refer to the *Using the PowerQuant® Analysis Tool* section of the *PowerQuant® System Technical Manual* #TMD047 for further information. The tool is used to evaluate and calculate quantitation data exported from the QuantStudio™ 5 Real-Time PCR System. You can import the modified exported .xls file into the PowerQuant® Analysis Tool following the instructions in the *PowerQuant® System Technical Manual* #TMD047.

**Note:** An updated PowerQuant® Analysis Tool will be available in the near future to assist with processing of the QuantStudio™ 5 export file. Please contact your Promega representative for further information.



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