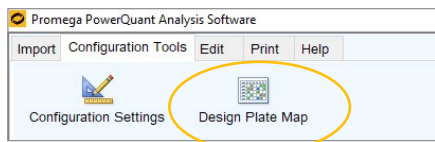


## 1. Introduction

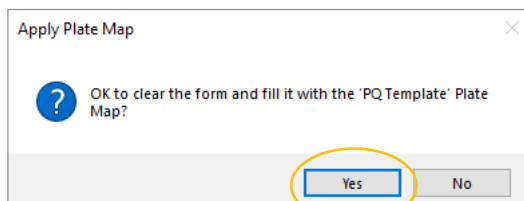
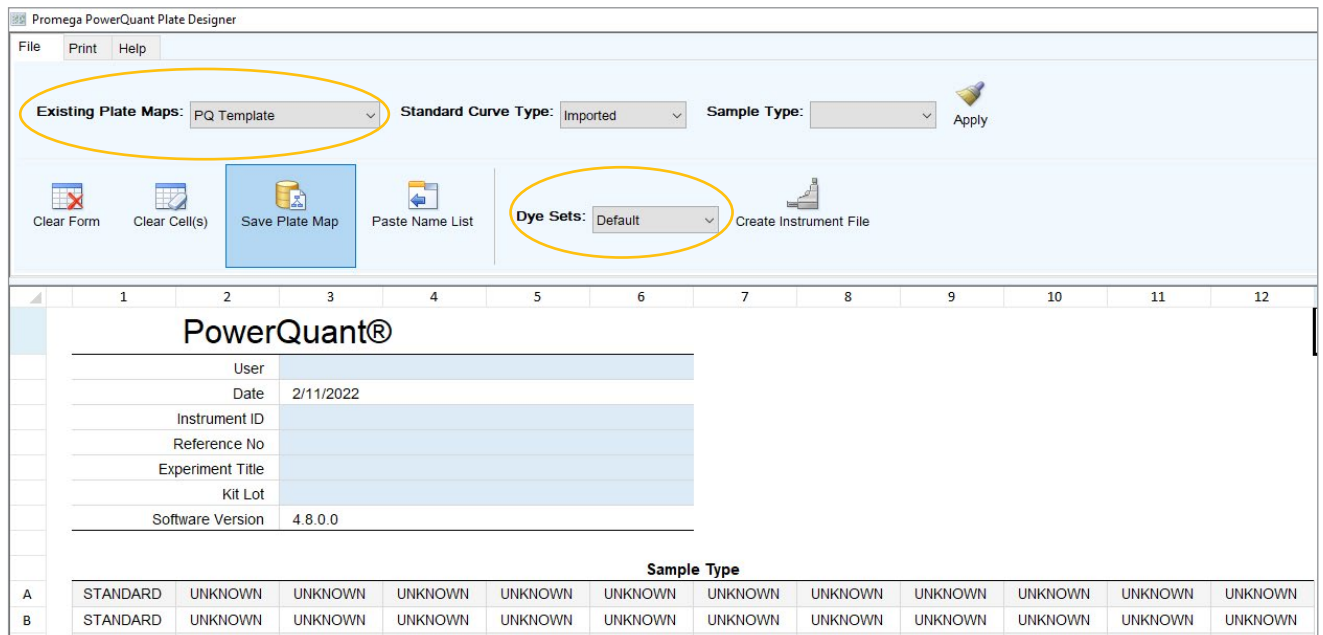
This guide serves as a quick reference for laboratories navigating between the PowerQuant® Analysis Software and running PowerQuant® on the QuantStudio™ 5 Real-Time PCR System. This guide is intended to be used by laboratories who have already performed initial installation and template setups in the PowerQuant® Analysis Software and the QuantStudio™ Design and Analysis Software. Please refer to the PowerQuant® System Technical Manual (TMD047) for detailed setup instructions.

## 2. Create an Import Plate Map Using PowerQuant® Analysis Software

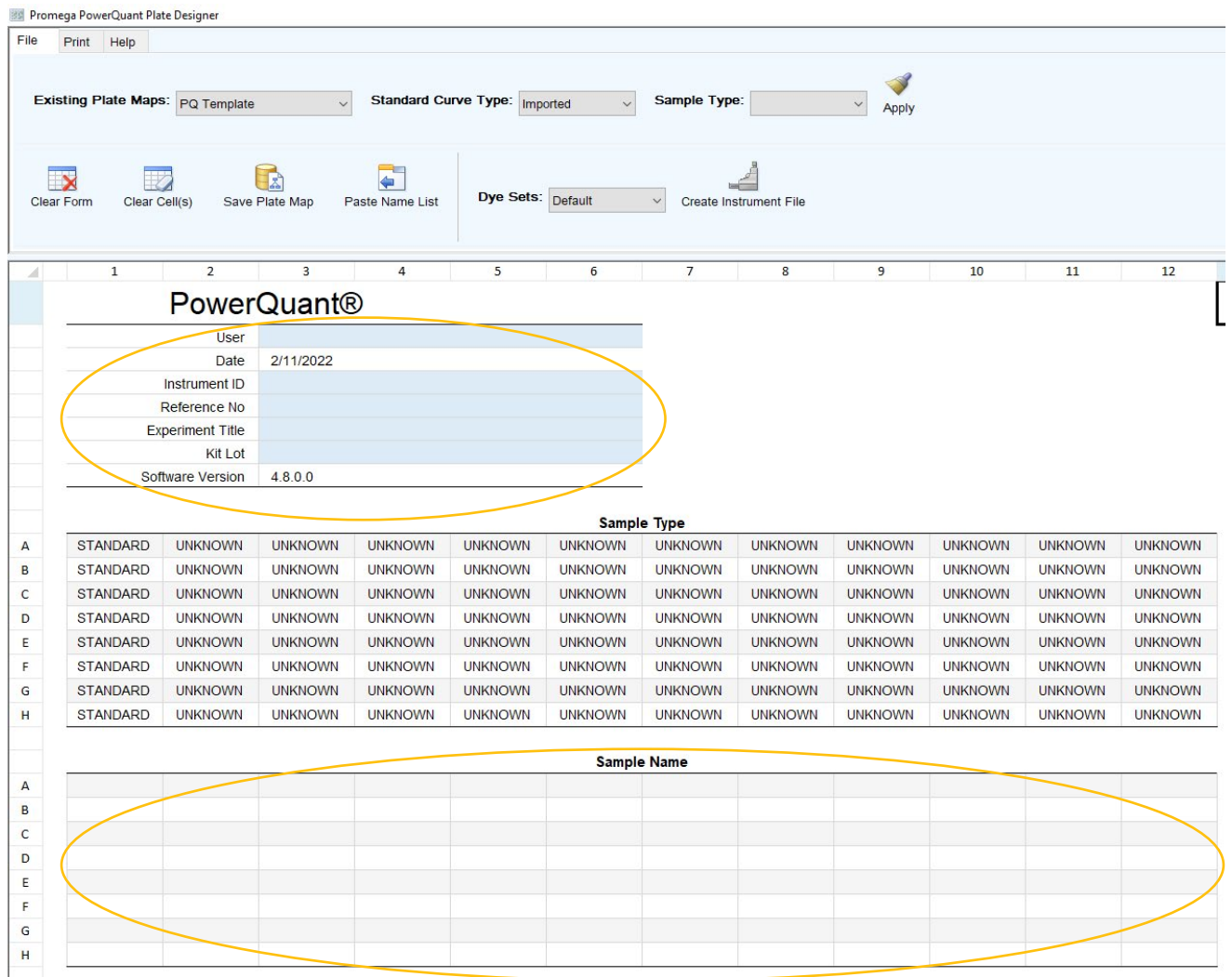
1. Open PowerQuant® Analysis Software to create a plate map for import into the QuantStudio™ 5 Real-Time PCR System.
2. Click on the **Configuration Tools** tab and select **Design Plate Map** icon.



3. Using the dropdown menus, choose an Existing Plate Map and Dye Set that were previously created for use with PowerQuant® on the QuantStudio™ 5 (see Section 9.H “Designing a Plate Map” in the PowerQuant® System Technical Manual). Select “Yes” in the **Apply Plate Map** dialog box that appears.



- Type in the Instrument ID, Reference No, Experiment Title, and Kit Lot, if desired. Enter sample names in the **Sample Name** plate map. Sample names can be entered manually into the **Sample Map** or copied and pasted (using keyboard shortcuts Ctrl + C and Ctrl + V) in a 96-well format.



**PowerQuant®**

|                  |           |
|------------------|-----------|
| User             |           |
| Date             | 2/11/2022 |
| Instrument ID    |           |
| Reference No     |           |
| Experiment Title |           |
| Kit Lot          |           |
| Software Version | 4.8.0.0   |

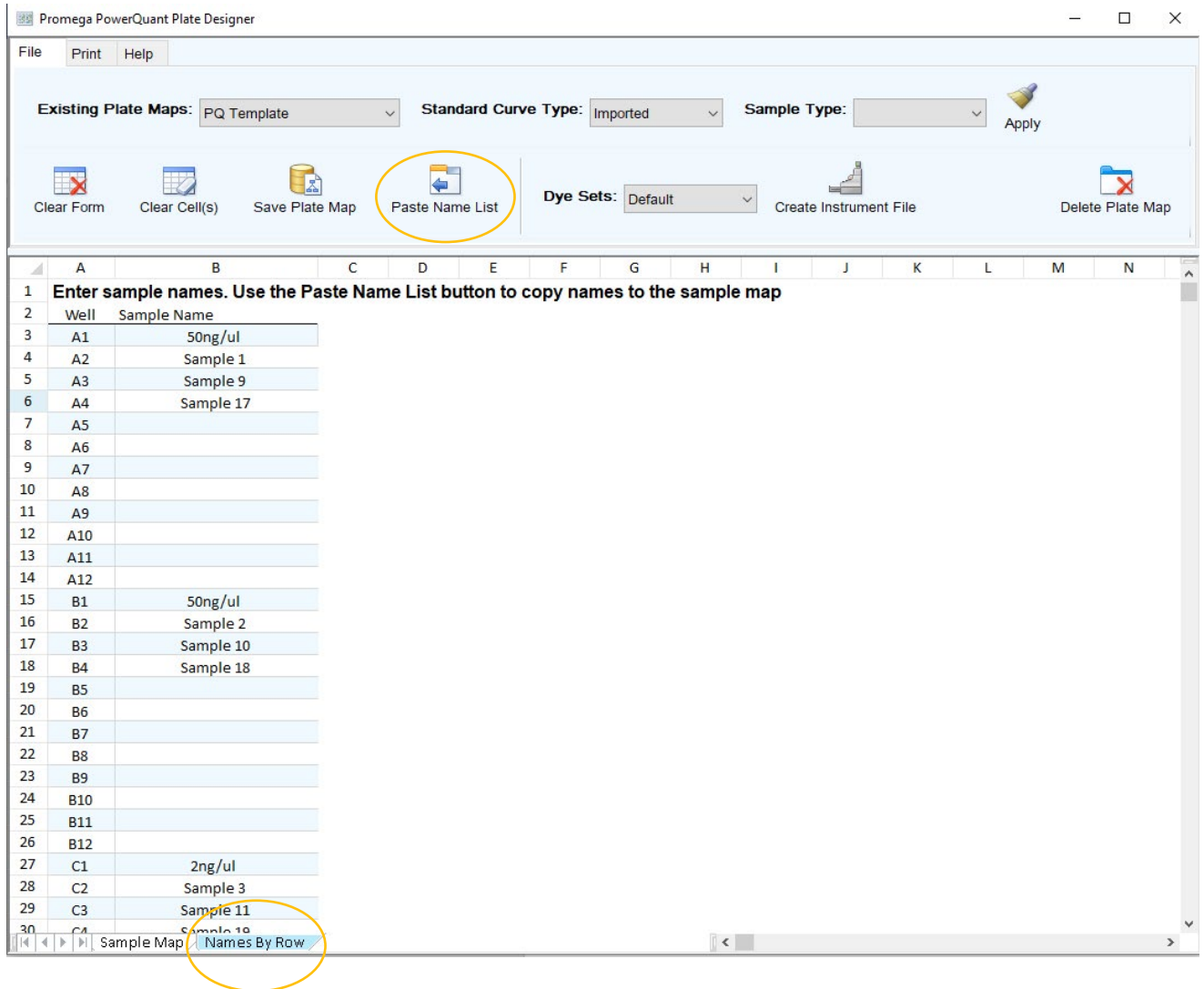
**Sample Type**

|   | 1        | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|---|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| A | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| B | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| C | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| D | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| E | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| F | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| G | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |
| H | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN | UNKNOWN |

**Sample Name**

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

- 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.



Existing Plate Maps: PQ Template Standard Curve Type: Imported Sample Type: Apply

Clear Form Clear Cell(s) Save Plate Map Paste Name List Dye Sets: Default Create Instrument File Delete Plate Map

| 1  | Enter sample names. Use the Paste Name List button to copy names to the sample map |             |
|----|--|-------------|
| 2  | Well   | Sample Name |
| 3  | A1   | 50ng/ul     |
| 4  | A2   | Sample 1    |
| 5  | A3   | Sample 9    |
| 6  | A4   | Sample 17   |
| 7  | A5   |             |
| 8  | A6   |             |
| 9  | A7   |             |
| 10 | A8   |             |
| 11 | A9   |             |
| 12 | A10  |             |
| 13 | A11  |             |
| 14 | A12  |             |
| 15 | B1   | 50ng/ul     |
| 16 | B2   | Sample 2    |
| 17 | B3   | Sample 10   |
| 18 | B4   | Sample 18   |
| 19 | B5   |             |
| 20 | B6   |             |
| 21 | B7   |             |
| 22 | B8   |             |
| 23 | B9   |             |
| 24 | B10  |             |
| 25 | B11  |             |
| 26 | B12  |             |
| 27 | C1   | 2ng/ul      |
| 28 | C2   | Sample 3    |
| 29 | C3   | Sample 11   |
| 30 | C4   | Sample 19   |

Sample Map Names By Row

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

Promega PowerQuant Plate Designer

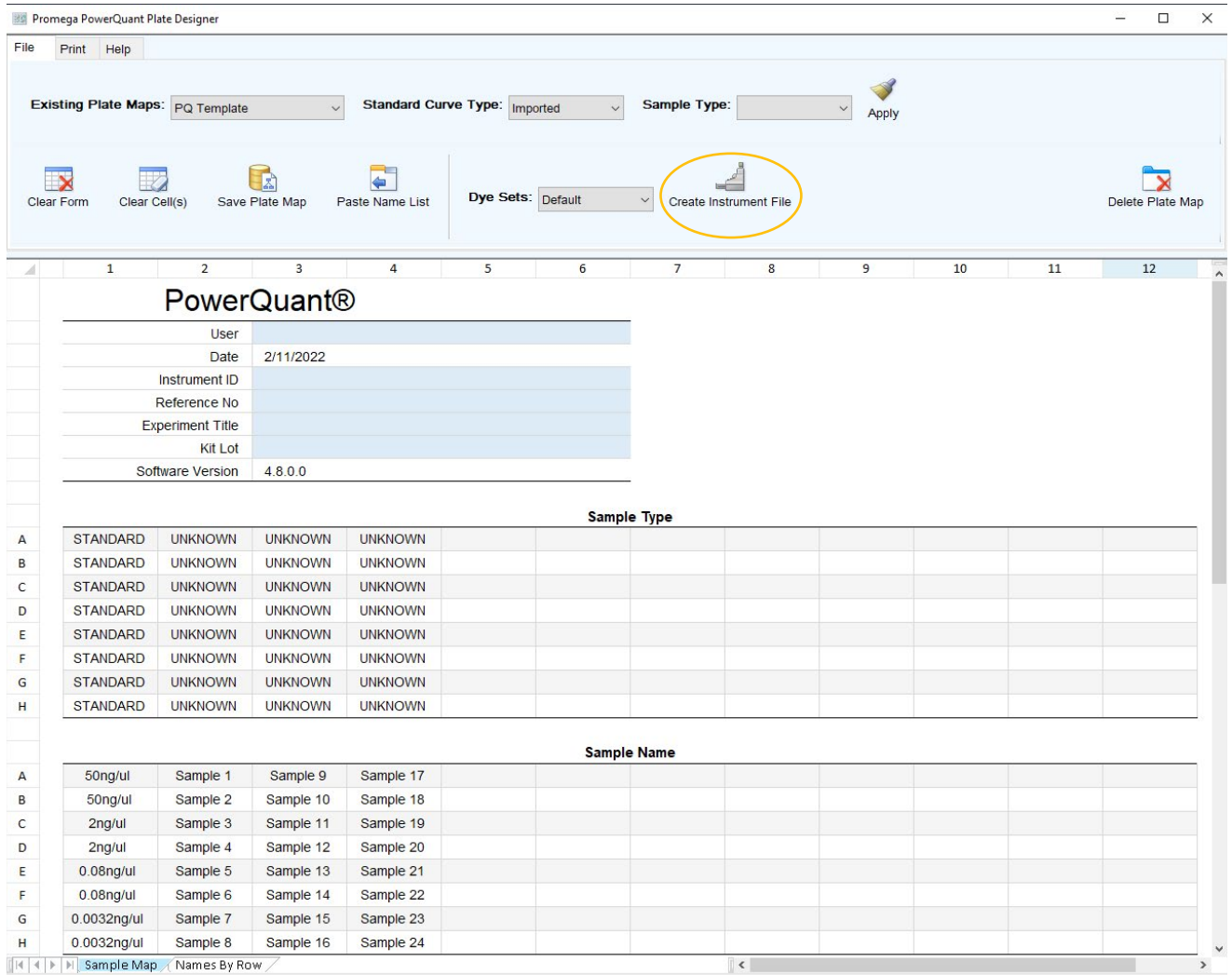
File Print Help

Existing Plate Maps: PQ Template Standard Curve Type: Imported Sample Type: Apply

Clear Form **Clear Cell(s)** Save Plate Map Paste Name List Dye Sets: Default Create Instrument File

|                    | 1                  | 2        | 3         | 4         | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------|--------------------|----------|-----------|-----------|---|---|---|---|---|----|----|----|
| <b>PowerQuant®</b> |                    |          |           |           |   |   |   |   |   |    |    |    |
|                    | User               |          |           |           |   |   |   |   |   |    |    |    |
|                    | Date               |          | 2/11/2022 |           |   |   |   |   |   |    |    |    |
|                    | Instrument ID      |          |           |           |   |   |   |   |   |    |    |    |
|                    | Reference No       |          |           |           |   |   |   |   |   |    |    |    |
|                    | Experiment Title   |          |           |           |   |   |   |   |   |    |    |    |
|                    | Kit Lot            |          |           |           |   |   |   |   |   |    |    |    |
|                    | Software Version   |          | 4.8.0.0   |           |   |   |   |   |   |    |    |    |
|                    | <b>Sample Type</b> |          |           |           |   |   |   |   |   |    |    |    |
| A                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| B                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| C                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| D                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| E                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| F                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| G                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
| H                  | STANDARD           | UNKNOWN  | UNKNOWN   | UNKNOWN   |   |   |   |   |   |    |    |    |
|                    | <b>Sample Name</b> |          |           |           |   |   |   |   |   |    |    |    |
| A                  | 50ng/ul            | Sample 1 | Sample 9  | Sample 17 |   |   |   |   |   |    |    |    |
| B                  | 50ng/ul            | Sample 2 | Sample 10 | Sample 18 |   |   |   |   |   |    |    |    |
| C                  | 2ng/ul             | Sample 3 | Sample 11 | Sample 19 |   |   |   |   |   |    |    |    |
| D                  | 2ng/ul             | Sample 4 | Sample 12 | Sample 20 |   |   |   |   |   |    |    |    |
| E                  | 0.08ng/ul          | Sample 5 | Sample 13 | Sample 21 |   |   |   |   |   |    |    |    |
| F                  | 0.08ng/ul          | Sample 6 | Sample 14 | Sample 22 |   |   |   |   |   |    |    |    |
| G                  | 0.0032ng/ul        | Sample 7 | Sample 15 | Sample 23 |   |   |   |   |   |    |    |    |
| H                  | 0.0032ng/ul        | Sample 8 | Sample 16 | Sample 24 |   |   |   |   |   |    |    |    |

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



The screenshot shows the Promega PowerQuant Plate Designer software interface. The window title is "Promega PowerQuant Plate Designer". The menu bar includes "File", "Print", and "Help". The main toolbar contains several icons: "Clear Form", "Clear Cell(s)", "Save Plate Map", "Paste Name List", "Dye Sets: Default", "Create Instrument File" (circled in yellow), and "Delete Plate Map". The "Existing Plate Maps" dropdown is set to "PQ Template", "Standard Curve Type" is "Imported", and "Sample Type" is empty. The "Apply" button is visible next to the "Sample Type" dropdown.

The main workspace displays a "PowerQuant®" form with the following fields:

|                  |           |
|------------------|-----------|
| User             |           |
| Date             | 2/11/2022 |
| Instrument ID    |           |
| Reference No     |           |
| Experiment Title |           |
| Kit Lot          |           |
| Software Version | 4.8.0.0   |

Below the form are two tables:

**Sample Type**

|   | 1        | 2       | 3       | 4       | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|----------|---------|---------|---------|---|---|---|---|---|----|----|----|
| A | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| B | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| C | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| D | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| E | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| F | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| G | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |
| H | STANDARD | UNKNOWN | UNKNOWN | UNKNOWN |   |   |   |   |   |    |    |    |

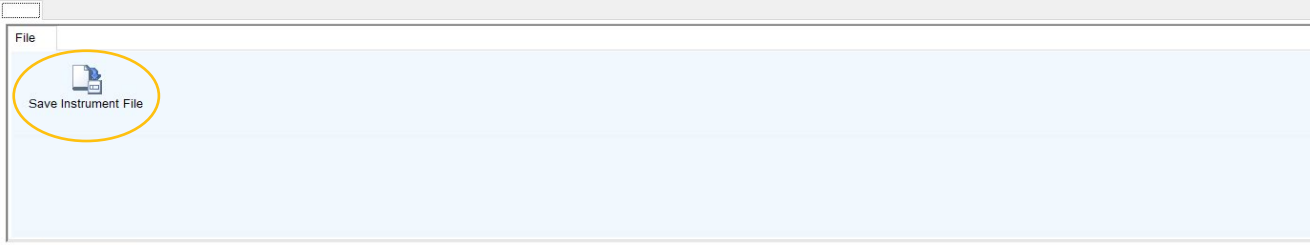
**Sample Name**

|   | 1           | 2        | 3         | 4         | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------------|----------|-----------|-----------|---|---|---|---|---|----|----|----|
| A | 50ng/ul     | Sample 1 | Sample 9  | Sample 17 |   |   |   |   |   |    |    |    |
| B | 50ng/ul     | Sample 2 | Sample 10 | Sample 18 |   |   |   |   |   |    |    |    |
| C | 2ng/ul      | Sample 3 | Sample 11 | Sample 19 |   |   |   |   |   |    |    |    |
| D | 2ng/ul      | Sample 4 | Sample 12 | Sample 20 |   |   |   |   |   |    |    |    |
| E | 0.08ng/ul   | Sample 5 | Sample 13 | Sample 21 |   |   |   |   |   |    |    |    |
| F | 0.08ng/ul   | Sample 6 | Sample 14 | Sample 22 |   |   |   |   |   |    |    |    |
| G | 0.0032ng/ul | Sample 7 | Sample 15 | Sample 23 |   |   |   |   |   |    |    |    |
| H | 0.0032ng/ul | Sample 8 | Sample 16 | Sample 24 |   |   |   |   |   |    |    |    |

The status bar at the bottom shows "Sample Map" and "Names By Row".

8. Select the **Save Instrument File** button, give the file a unique name, and save as a .txt file in the desired location. Close out of the PowerQuant® Analysis Software.

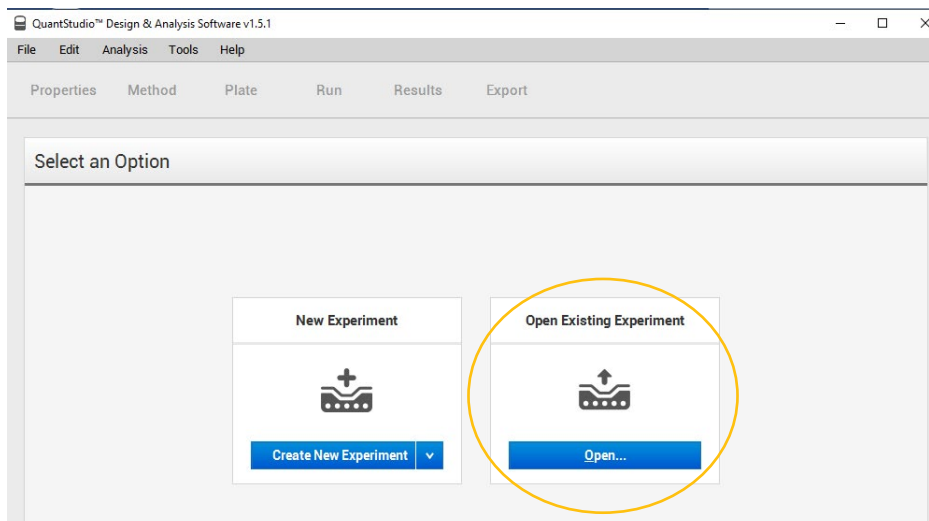
Excel Data Bucket



|    | A                           | B                                       | C                  | D             | E              | F           | G                  | H        | I         | J        | K        | L        |
|----|-----------------------------|---|--------------------|---------------|----------------|-------------|--------------------|----------|-----------|----------|----------|----------|
| 1  | * Block Type =              | 96alum                                  |                    |               |                |             |                    |          |           |          |          |          |
| 2  | * Chemistry =               | TAQMAN                                  |                    |               |                |             |                    |          |           |          |          |          |
| 3  | * Experiment File Name =    | C:\Applied Biosystems\7500\bin\Untitled |                    |               |                |             |                    |          |           |          |          |          |
| 4  | * Experiment Run End Time = | Not Started                             |                    |               |                |             |                    |          |           |          |          |          |
| 5  | * Instrument Type =         | sds7500                                 |                    |               |                |             |                    |          |           |          |          |          |
| 6  | * Passive Reference =       | PQ_CXR                                  |                    |               |                |             |                    |          |           |          |          |          |
| 7  | [Sample Setup]              |   |                    |               |                |             |                    |          |           |          |          |          |
| 8  | Well                        | Sample Name                             | Sample Color       | Biogroup Name | Biogroup Color | Target Name | Target Color       | Task     | Reporter  | Quencher | Quantity | Comments |
| 9  | A1                          | 50ng/ul                                 | "RGB(132,193,241)" |               |                | Autosomal   | "RGB(139,189,249)" | STANDARD | PQ_FAM    | NFQ-MGB  | 50       |          |
| 10 | A1                          | 50ng/ul                                 | "RGB(132,193,241)" |               |                | Degradation | "RGB(204,153,255)" | STANDARD | PQ_Q670   | NFQ-MGB  | 50       |          |
| 11 | A1                          | 50ng/ul                                 | "RGB(132,193,241)" |               |                | IPC         | "RGB(255,0,0)"     | UNKNOWN  | PQ_TMR    | NFQ-MGB  |          |          |
| 12 | A1                          | 50ng/ul                                 | "RGB(132,193,241)" |               |                | Y           | "RGB(0,255,0)"     | STANDARD | PQ_CFG540 | NFQ-MGB  | 50       |          |
| 13 | A2                          | Sample 1                                | "RGB(132,193,241)" |               |                | Autosomal   | "RGB(139,189,249)" | UNKNOWN  | PQ_FAM    | NFQ-MGB  |          |          |
| 14 | A2                          | Sample 1                                | "RGB(132,193,241)" |               |                | Degradation | "RGB(204,153,255)" | UNKNOWN  | PQ_Q670   | NFQ-MGB  |          |          |
| 15 | A2                          | Sample 1                                | "RGB(132,193,241)" |               |                | IPC         | "RGB(255,0,0)"     | UNKNOWN  | PQ_TMR    | NFQ-MGB  |          |          |
| 16 | A2                          | Sample 1                                | "RGB(132,193,241)" |               |                | Y           | "RGB(0,255,0)"     | UNKNOWN  | PQ_CFG540 | NFQ-MGB  |          |          |
| 17 | A3                          | Sample 9                                | "RGB(168,255,222)" |               |                | Autosomal   | "RGB(139,189,249)" | UNKNOWN  | PQ_FAM    | NFQ-MGB  |          |          |

## 3. Run a PowerQuant® Plate on the QuantStudio™ 5

1. Open the QuantStudio™ Design and Analysis Software and select the **Open Existing Experiment** button to open the previously created PowerQuant® run template (see Section 8.B "Creating a Run Template" in the PowerQuant® System Technical Manual).




QuantStudio™ Design & Analysis Software v1.5.1

File Edit Analysis Tools Help

Properties Method Plate Run Results Export


Select an Option

**New Experiment**



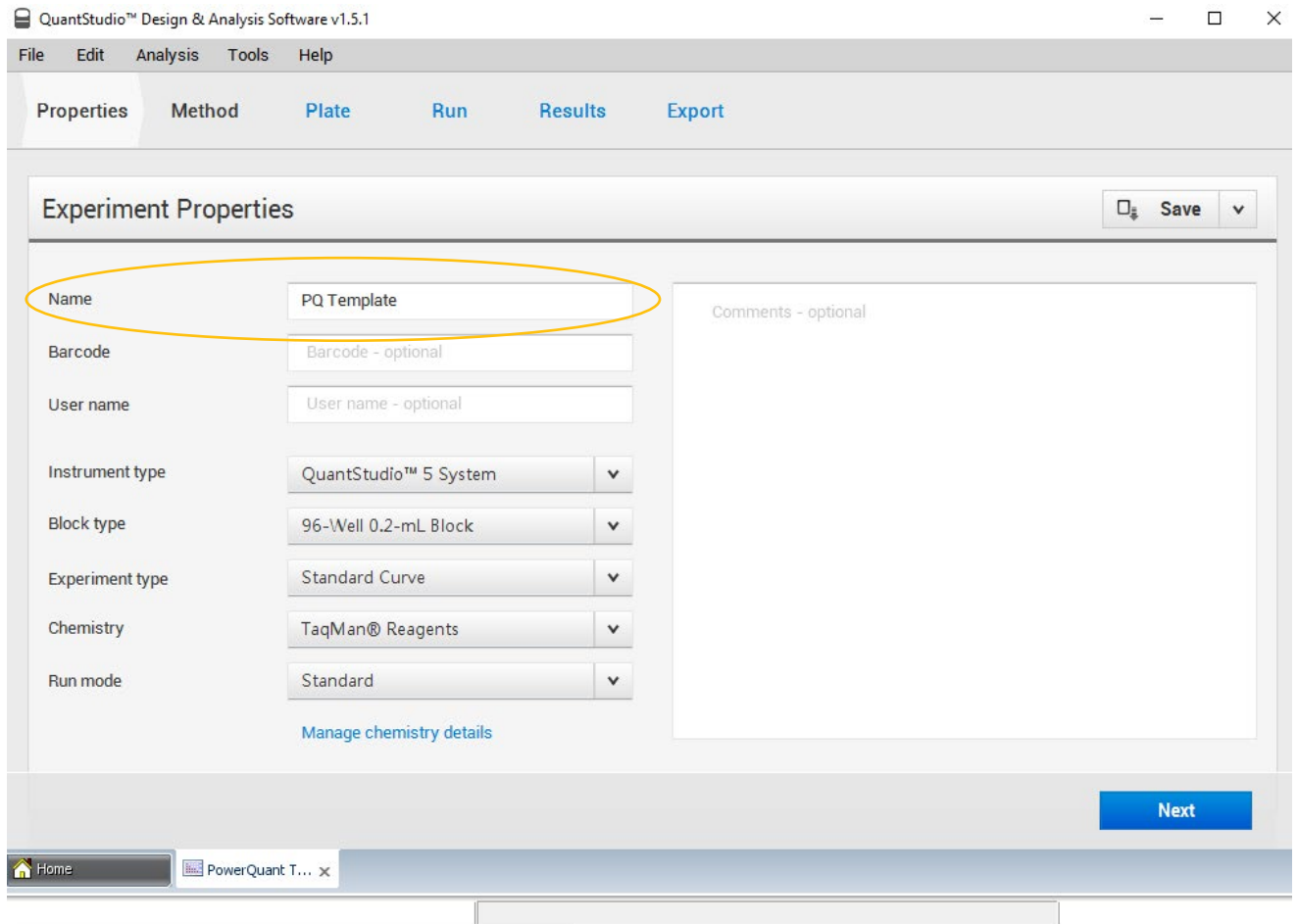
Create New Experiment ▾

**Open Existing Experiment**

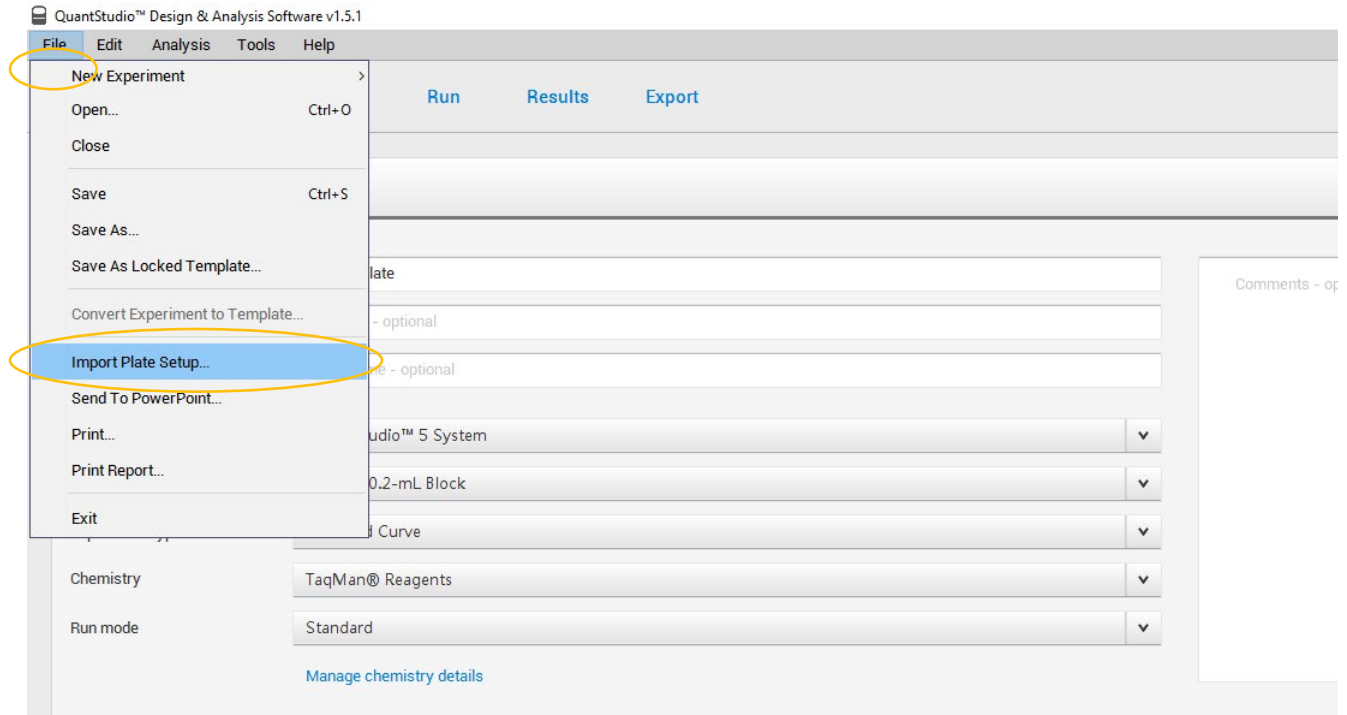


Open...

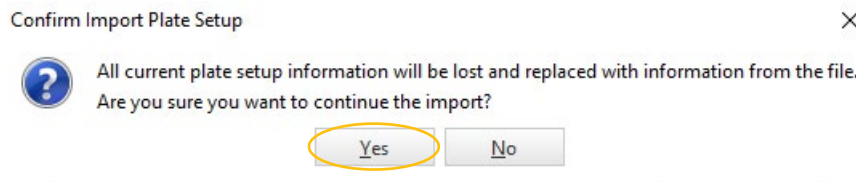
2. Rename the experiment with a unique name.



3. Select "File > Import Plate Setup" and browse to find the .txt file created with the PowerQuant<sup>®</sup> Analysis Software. Select **Apply** button.



4. Select "Yes" to the message that appears. The software will display an Import Successful box. Select "OK".





5. Select the **Method** tab to ensure that the cycling parameters are correct as shown below.

QuantStudio™ Design & Analysis Software v1.5.1

File Edit Analysis Tools Help

Properties **Method** Plate Run Results Export

### Experiment Method

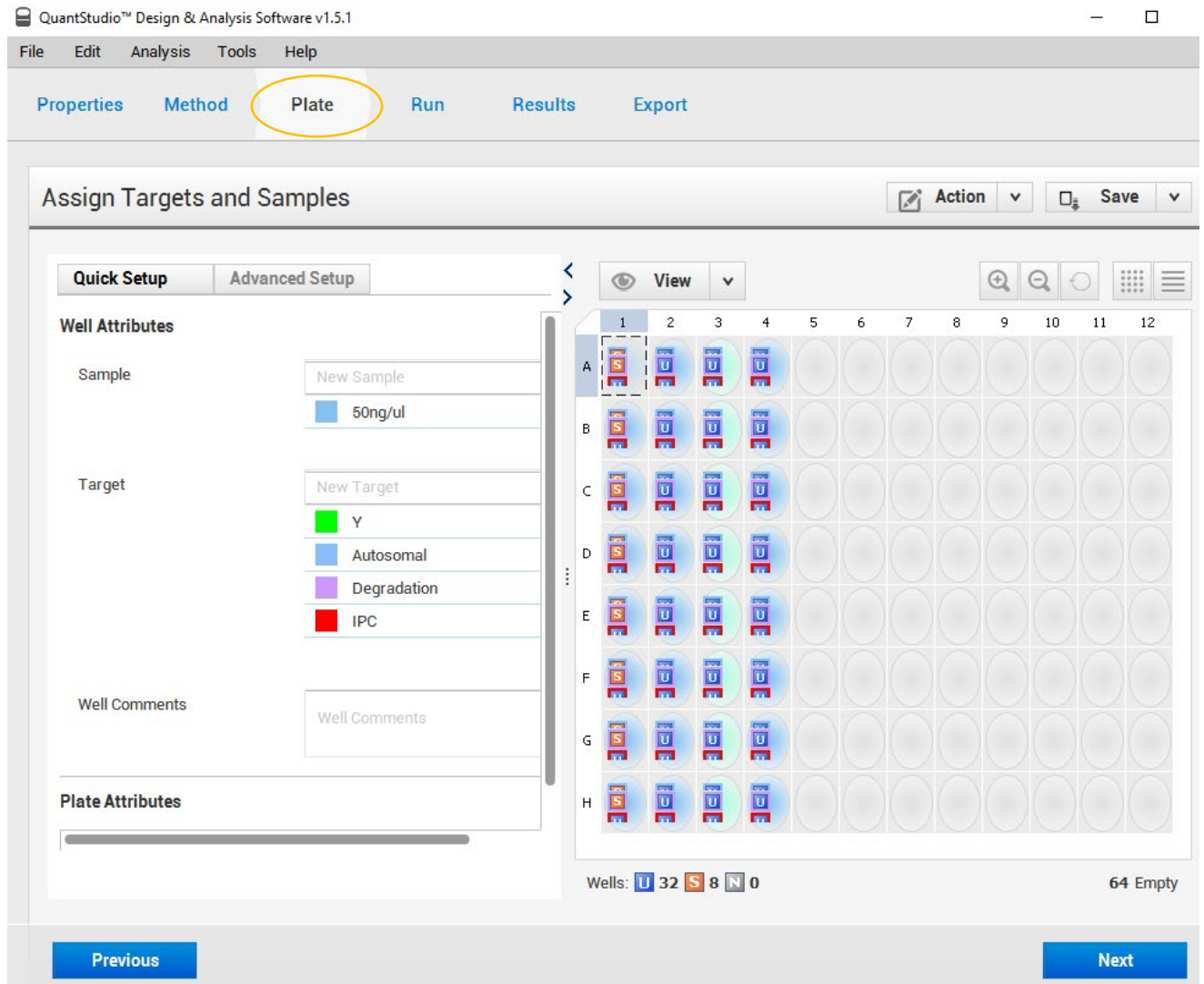
Volume: 20 µL    Cover: 105.0 °C

| Stage      | Step  | Temperature | Ramp Rate | Time  | Actions                 |
|------------|-------|-------------|-----------|-------|-------------------------|
| Hold Stage | Step1 | 98.0 °C     | 2.44 °C/s | 02:00 | Camera, Settings        |
|            | Step1 | 98.0 °C     | 2.44 °C/s | 00:15 | Camera, Settings, Pause |
| PCR Stage  | Step1 | 98.0 °C     | 2.44 °C/s | 00:15 | Camera, Settings, Pause |
|            | Step2 | 62.0 °C     | 2.44 °C/s | 00:35 | Camera, Settings, Pause |

39 x

Legends: Data Collection On    Data Collection Off    Pause On    Pause Off    Advanced Settings    Verif

6. Select the **Plate** tab to ensure that the plate map matches the expected sample layout.



QuantStudio™ Design & Analysis Software v1.5.1

File Edit Analysis Tools Help

Properties Method **Plate** Run Results Export

Assign Targets and Samples

Quick Setup Advanced Setup

Well Attributes

Sample: New Sample, 50ng/ul

Target: New Target, Y, Autosomal, Degradation, IPC

Well Comments: Well Comments

Plate Attributes

View

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | S | U | U | U |   |   |   |   |   |    |    |    |
| B | S | U | U | U |   |   |   |   |   |    |    |    |
| C | S | U | U | U |   |   |   |   |   |    |    |    |
| D | S | U | U | U |   |   |   |   |   |    |    |    |
| E | S | U | U | U |   |   |   |   |   |    |    |    |
| F | S | U | U | U |   |   |   |   |   |    |    |    |
| G | S | U | U | U |   |   |   |   |   |    |    |    |
| H | S | U | U | U |   |   |   |   |   |    |    |    |

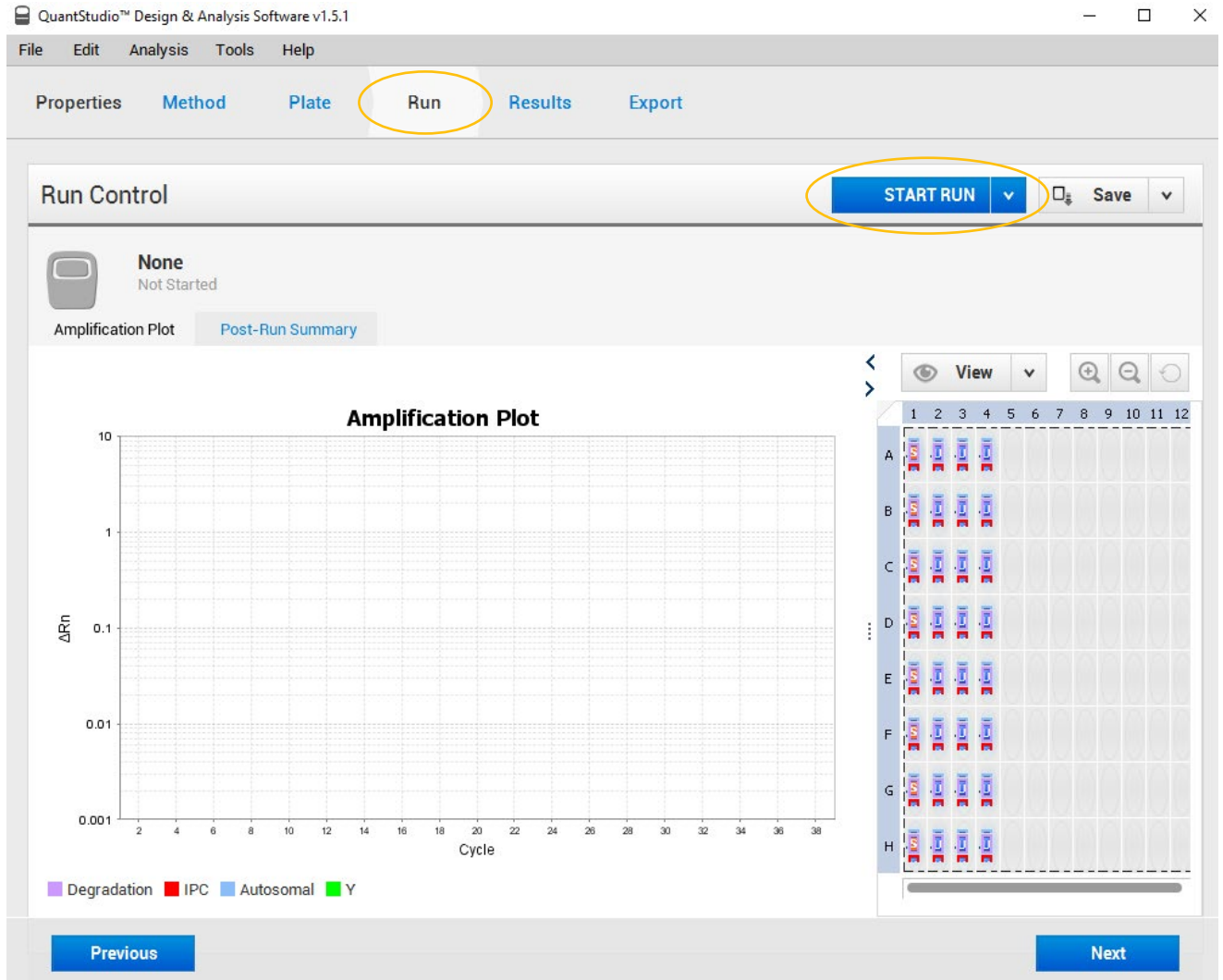
Wells: U 32 S 8 N 0 64 Empty

Previous Next

7. Select "Analysis > Analysis Settings". On the **CT Settings** tab, ensure the laboratory validated thresholds are entered for each target. The **Automatic Baseline** box should be selected.
8. Select "File > Save As" and save file as a "Test Document Template (.edt)" file with a unique name.
9. Ensure the prepared plate has been loaded into the QuantStudio™ 5 instrument.

10. Select the **Run** tab. Click on the arrow next to the “Start Run” button to select the instrument to be used for the run. Click “Start Run”. The software will prompt you to save your experiment as an .eds file. Run time is approximately 1 hour.

*Note:* If the QuantStudio<sup>™</sup> 5 is being operated as a standalone instrument without a laptop or desktop, the previously created .edt template file must be imported via the touch screen.



QuantStudio<sup>™</sup> Design & Analysis Software v1.5.1

File Edit Analysis Tools Help

Properties Method Plate **Run** Results Export

Run Control **START RUN** Save

None  
Not Started

Amplification Plot Post-Run Summary

**Amplification Plot**

ΔRn

Cycle

Degradation IPC Autosomal Y

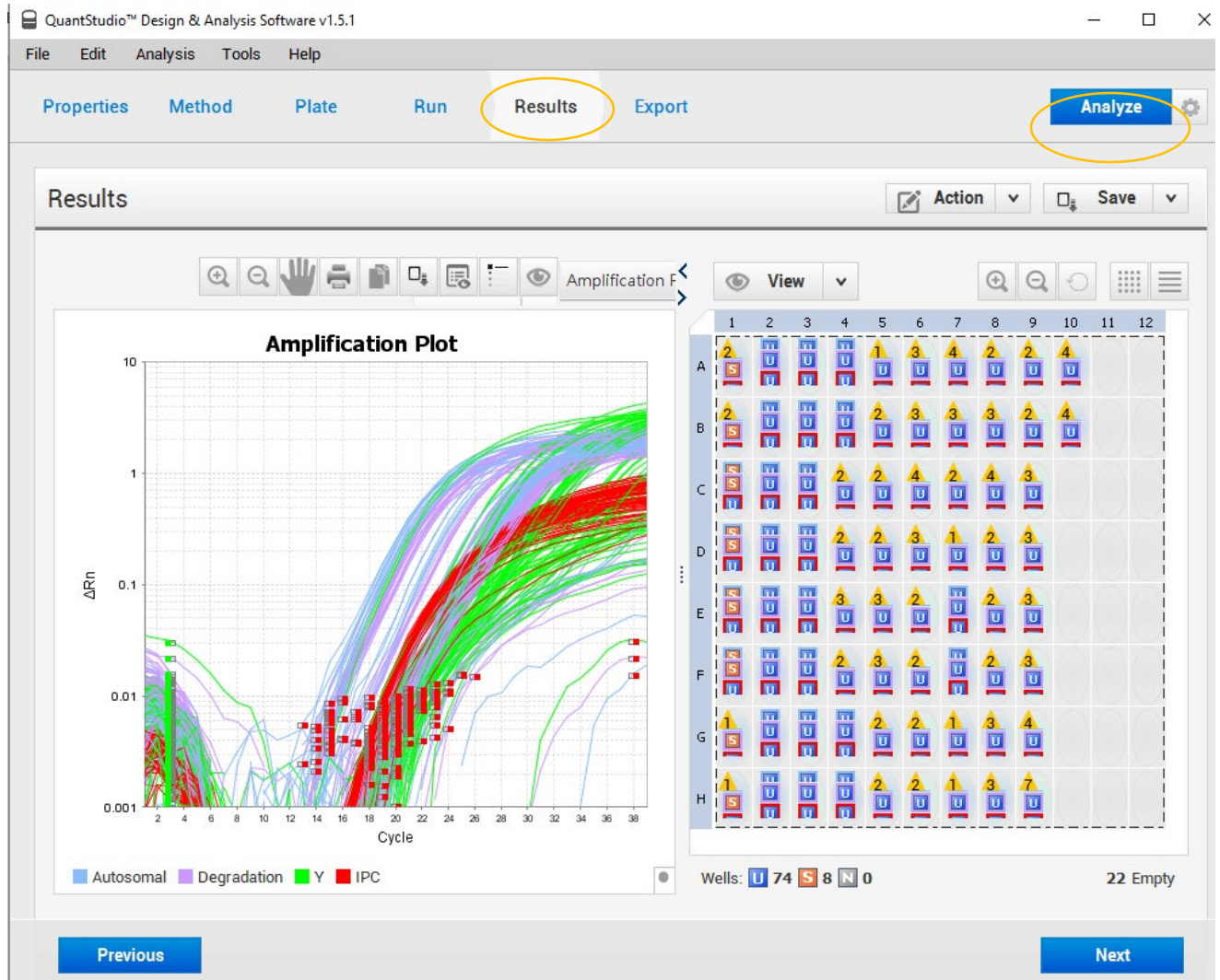
View

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H

Previous Next

11. Once the run is complete, select the **Results** tab and click “Analyze”.



The screenshot shows the QuantStudio Design & Analysis Software v1.5.1 interface. The **Results** tab is selected and circled in yellow. The **Analyze** button is also circled in yellow. The main window displays an **Amplification Plot** on the left and a **View** panel on the right. The plot shows  $\Delta Rn$  on the y-axis (log scale from 0.001 to 10) versus **Cycle** on the x-axis (from 2 to 38). The plot contains multiple colored lines representing different samples: Autosomal (blue), Degradation (purple), Y (green), and IPC (red). The View panel shows a grid of 12 columns and 8 rows (A-H) with various icons and numbers. Below the grid, the wells are listed as U 74, S 8, N 0, and 22 Empty. The interface includes a menu bar (File, Edit, Analysis, Tools, Help) and a toolbar with icons for zooming, panning, and printing.

12. Evaluate the standard curves in the **Results** tab. Highlight all wells with standard samples. Select "Standard Curve" from the drop-down menu located above the **Amplification Plot** section.
13. The standard curve for each target may be viewed by choosing the appropriate target from the "All Target" drop-down menu. The standard curve parameters are located below the standard curve plot.

QuantStudio<sup>™</sup> Design & Analysis Software v1.5.1

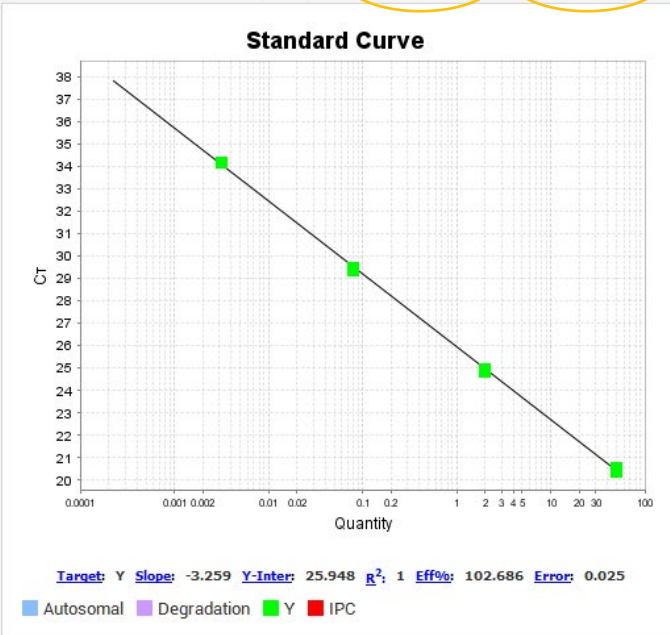
File Edit Analysis Tools Help

Properties Method Plate Run **Results** Export Analyze

### Results

Action Save

Y Standard Curve View



Standard Curve

Target: Y Slope: -3.259 Y-Inter: 25.948  $R^2$ : 1 Eff%: 102.686 Error: 0.025

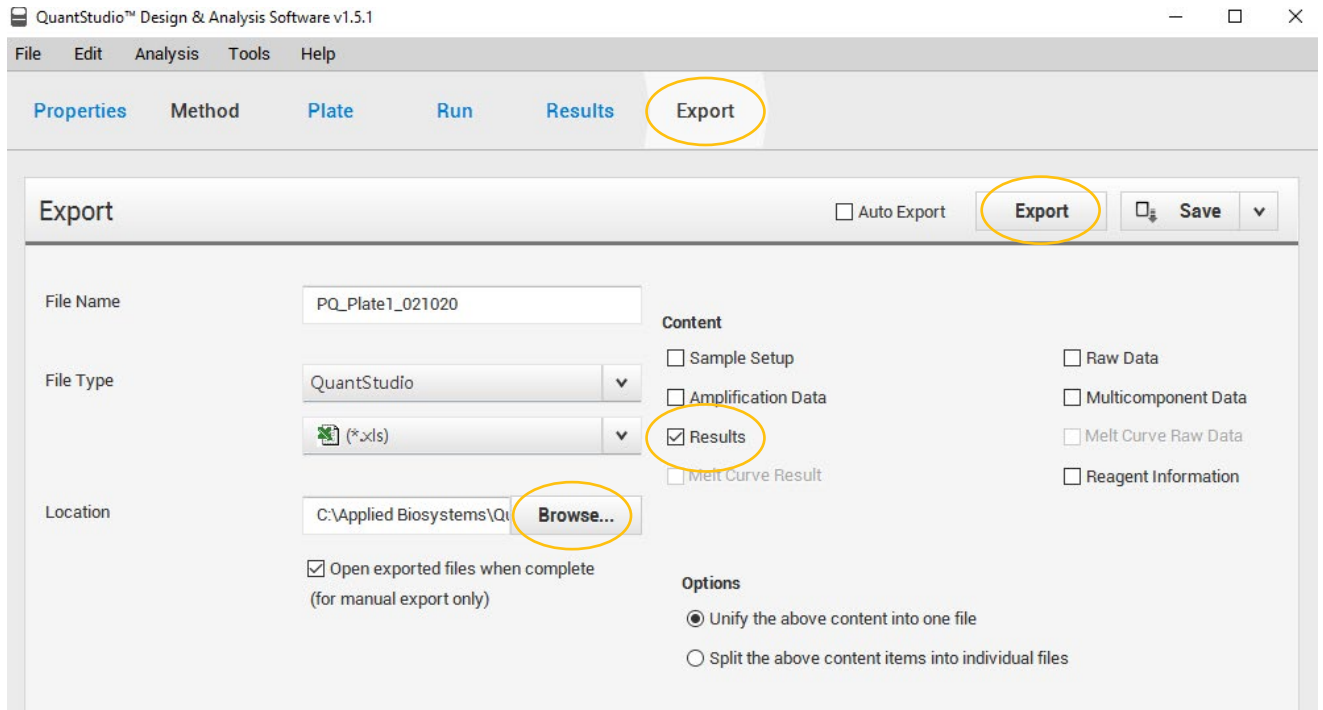
Autosomal Degradation Y IPC

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A | 2 | U | U | U | 1 | 3 | 4 | 2 | 2 | 4  |    |    |
| B | 2 | U | U | U | 2 | 3 | 3 | 3 | 2 | 4  |    |    |
| C | S | U | U | 2 | 2 | 4 | 2 | 4 | 3 |    |    |    |
| D | S | U | U | 2 | 2 | 3 | 1 | 2 | 3 |    |    |    |
| E | S | U | U | 3 | 3 | 2 | U | 2 | 3 |    |    |    |
| F | S | U | U | 2 | 3 | 2 | U | 2 | 3 |    |    |    |
| G | 1 | S | U | U | 2 | 2 | 1 | 3 | 4 |    |    |    |
| H | 1 | S | U | U | 2 | 2 | 1 | 3 | 7 |    |    |    |

Wells: U 74 S 8 N 0 22 Empty

Previous Next

- Once the results are reviewed, export the results by selecting the **Export** tab. Confirm the “Results” box is checked and choose a file location by selecting “Browse”. Select “Export”.

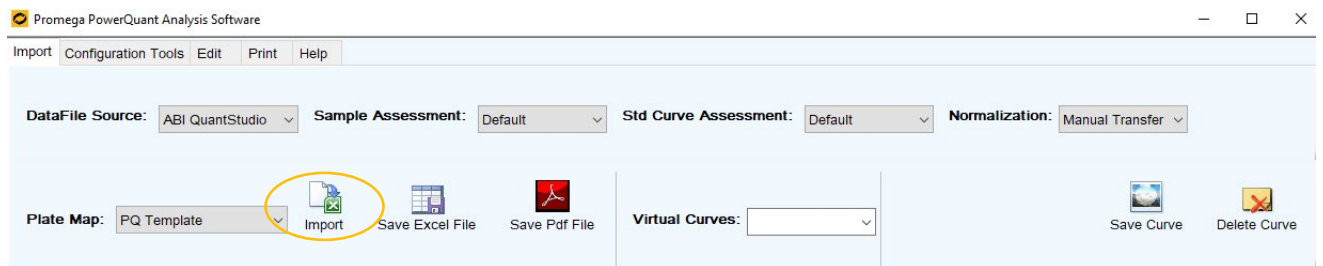


## 4. View Results Using PowerQuant® Analysis Software

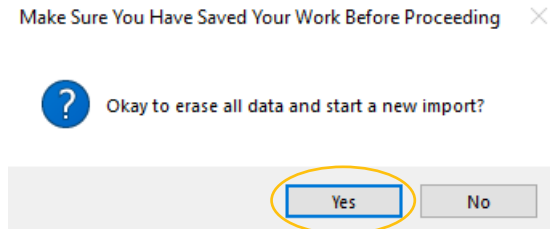
- Open the PowerQuant® Analysis Software and use the drop-down menus to make the following selections: Data File source; Sample Assessment, Standard Curve Assessment, Normalization, Plate Map, and Virtual Curves (for use with virtual standard curves only).

*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.

- Select the **Import** icon.



3. A dialog box labeled **Make Sure You Have Saved Your Work Before Proceeding** will appear. Select "Yes".



4. Select the .xls file exported from the QuantStudio™ Design and Analysis Software.
5. Once results are imported into the PowerQuant® Analysis Software, optional filter and sort functions are provided in the **Table Results** and accessed by selecting the arrow button in the column header.
6. Save analyzed results under the **Import** Tab by selecting either the **Save Excel File** icon or the **Save PDF File** icon. Results are not stored in the software when the program is closed.

