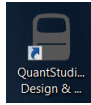
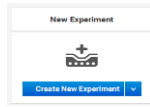


Standard Curve- SYBR QuantStudio™ Experimental Set-Up

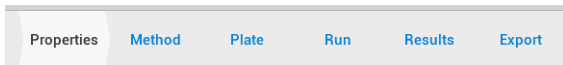
1. Launch the QS Design and Analysis Software.



2. Click: Create New Experiment.



3. Under Properties: Complete the following fields:



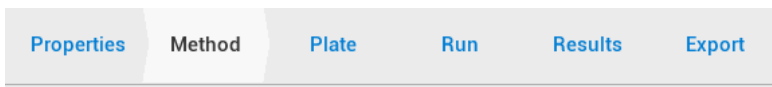
- a. Name: Delete the date and timestamp to create your own nomenclature.
- b. Select the Instrument that you are utilizing: QS3 or QS5
- c. Select the block type that you are utilizing: 384 well, 96-well (0.2mL), or Fast 96-well (0.1mL)
- d. Select Standard Curve.
- e. Select SYBR Green.
- f. Select the appropriate Run Mode based on the Master Mix you will be utilizing: Standard or Fast

The image shows the 'Experiment Properties' form. It has a title bar 'Experiment Properties' and a list of fields with dropdown menus:

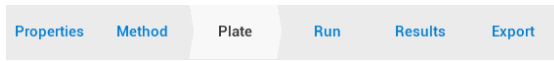
- Name: 2017-03-13_125808
- Barcode: Barcode - optional
- User name: User name - optional
- Instrument type: QuantStudio™ 3 System
- Block type: 96-Well 0.1-mL Block
- Experiment type: Standard Curve
- Chemistry: SYBR® Green Reagents
- Run mode: Standard

At the bottom, there is a blue link that says 'Manage chemistry details'.

4. Under Method: Make the appropriate modifications to ensure the cycling conditions and reaction volumes are correct for your SYBR primers. For additional guidance please select Help → QuantStudio™ Design and Analysis Software Help.



5. Under Plate: Complete the follow items:



a. In the Quick Setup:

Assign Targets and Samples

Quick Setup | Advanced Setup

Well Attributes

Sample:

Target:

Well Comments:

Plate Attributes

Passive Reference:

Reference Sample:

Endogenous Control:

- i. Define your Passive Reference: If you are utilizing an Applied Biosystems™ Master Mix please leave the Passive Reference as “ROX.” Please direct all other inquires to the vendor of the Master Mix.

b. Click Advanced Setup

i. Define Targets:

1. Highlight Target 1 and replace the text with the name of your primer pair.
2. Click “New” to add additional primer pairs to your plate.
3. The Reporter will read SYBR and the Quencher will read None. These are automatic defaults. No changes are required.

Quick Setup | **Advanced Setup**

Targets + Add Action ▾

	Name	Reporter	Quencher	Comments	Task	Quantity
<input type="checkbox"/>	Target 1	SYBR	None		▾	✕

ii. Define Samples:

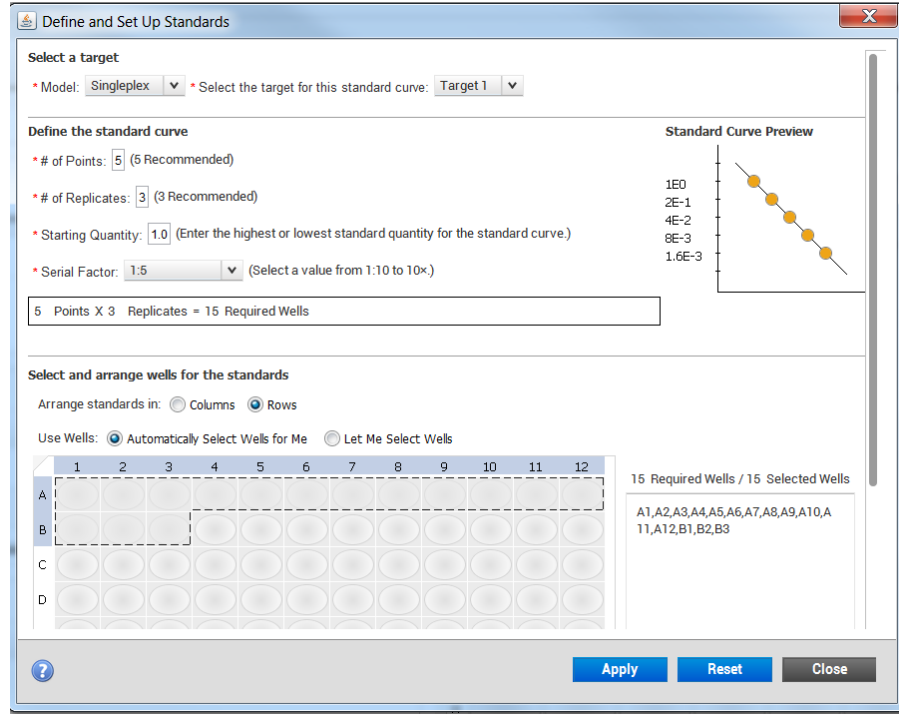
1. Highlight Sample 1 and replace the text with the name of your sample
2. If you have more than one sample click “New” to add new Samples to your plate.

Quick Setup | **Advanced Setup**

Samples + Add Action ▾

	Sample Name	Comments
<input type="checkbox"/>	Water plus Reagents	
<input type="checkbox"/>	Positive Control	
<input type="checkbox"/>	Negative Control	
<input type="checkbox"/>	Treated Sample	
<input type="checkbox"/>	Untreated	

- c. In the Plate Layout right click on any cell and select Define and Set Up Standards. A Pop-Up Window will occur. Define the Standard Curve based on the recommendations below or using a custom method.
 - i. Number of Points: 5
 - ii. Number of Replicates: 3
 - iii. Starting Quantity: Enter the starting mass or the number of copies for your control sample.
 - iv. Serial Factor: 1:10



- d. Click Apply and you will see the standard curve in the Plate Layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target
B	Sample Target	Sample Target	Sample Target									
C												
D												

- e. In the Plate Layout click on a cell and drag to highlight multiple cells. With the cells highlighted click the box to the left of the Target name to assign a target to a specific well.

	1	2	3	4	5
A	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target
B	Sample Target	Sample Target	Sample Target		
C	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target
D	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target
E	Sample Target	Sample Target	Sample Target	Sample Target	Sample Target
F					
G					

- f. In the Plate Layout click on a cell and drag to highlight multiple cells. With the cells highlighted click the box to the left of the appropriate Sample to assign a sample to a specific well.



****Important Note: It is imperative that you set the plate up in the software the same way that you set the plate up in the laboratory.**

6. Save your file.

7. Under Run: Click on Start Run and then click on the instrument Serial Number dropdown.

