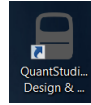
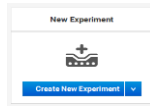


## Gene Expression- SYBR QuantStudio™ Experimental Set-Up

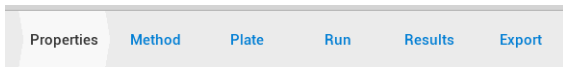
1. Launch the QuantStudio™ Design and Analysis Software.



2. Click: Create New Experiment.



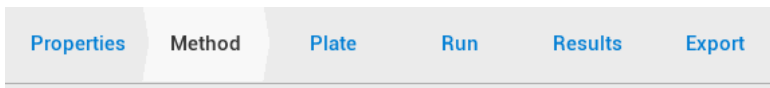
3. Under Properties: Complete the following fields:



- 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 Comparative Ct.
- e. Select SYBR Green.
- f. Select the appropriate Run Mode based on the Master Mix you will be utilizing: Standard or Fast

A screenshot of the "Experiment Properties" form. The fields are: 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 (Comparative Ct (ΔΔCt)), Chemistry (SYBR® Green Reagents), and Run mode (Standard). There is a "Manage chemistry details" link at the bottom.

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



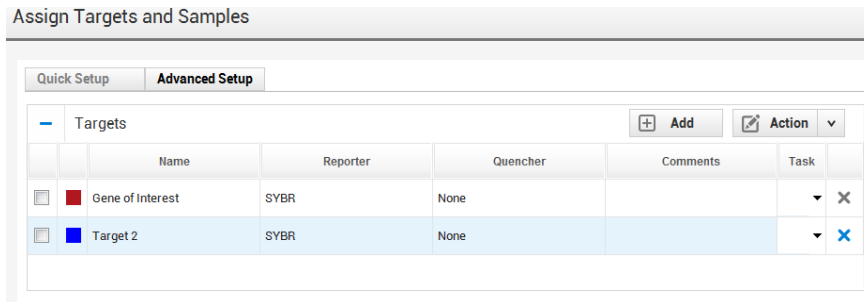
5. Under Plate: Complete the follow items:



a. Click Advanced Setup

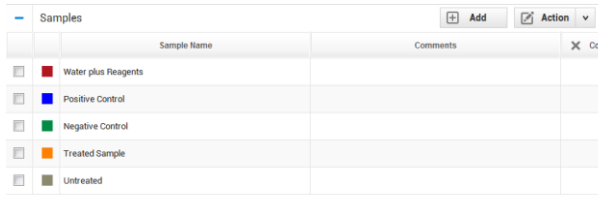
i. Define Targets:

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.

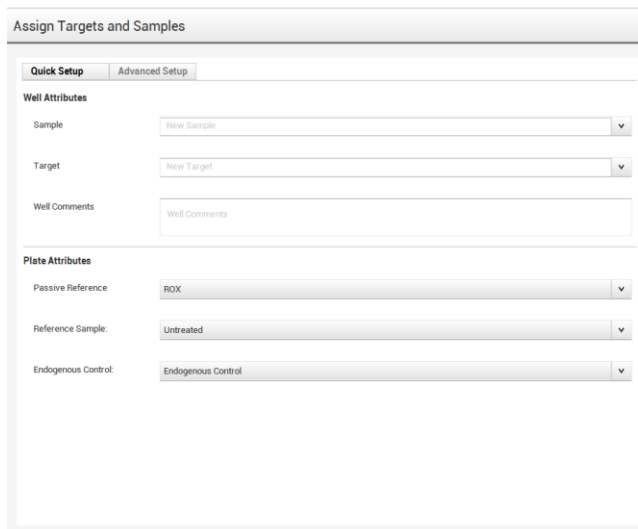


ii. Define Samples:

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 additional Samples to your plate.



b. In the Quick Setup:



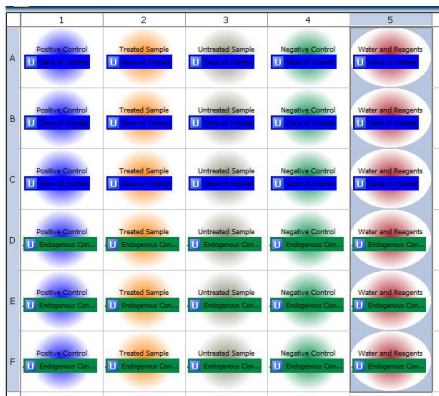
- i. Define your Passive Reference: If you are utilizing an Applied Biosystems™ Master Mix please leave the Passive Reference as “ROX.” Please direct all other inquires to the vendor of the Master Mix.
- ii. Select the appropriate Reference Sample and Endogenous Control for your experiment.

c. Click on Advanced Setup.

d. 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.



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 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.

