MPCL PROTOCOL

Title: QuantStudio 6 Pro Relative Quantification Experiments

SCOPE:

This protocol covers how to utilize QuantStudio 6 Pro Real-Time PCR System to perform relative quantification experiments.

BACKGROUND

The Applied Biosystems QuantStudio 6 Pro Real-Time PCR System uses fluorescent-based polymerase chain reaction (PCR) reagents to provide quantitative research detection of target nucleic acid sequences (targets) using real-time analysis, post-PCR (endpoint) analysis and analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR). The Applied Biosystems QuantStudio 6 Pro Real-Time PCR System features a six-color filter set that supports all Applied Biosystems dyes.

- The QuantStudio 6 Pro supports Comparative C_T experiments using TaqMan or SYBR Green Reagents, Singlex or Multiplex PCR and 1- and 2-Step PCR.

IMPORTANT! SYBR Green reagents cannot be used for multiplex PCR.

- For more information on the principle of Comparative C_T, refer to the Applied Biosystems QuantStudio 6 Pro System Getting Started Guide for Comparative C_T Experiments (Booklet 3, Part 2).

- The QuantStudio 6 Pro Real-Time PCR system consists of

    - QuantStudio 6 Pro Instrument
    - Computer containing QuantStudio 6 Pro Software

- The QuantStudio 6 Pro Real-Time PCR system is available in Centre for Heart Lung Innovation's Core 1 facility and features a sample block module suited for 384 well plates.

RESPONSIBILITIES

- If you have any questions regarding the proper operations of the QuantStudio 6 Pro instrument, please contact the Core 1 technicians (ext. 63535). The Applied Biosystems representatives can be contacted at: 1-800-831-6844.
- Please use the online equipment booking system to sign your name, local and time slot of use prior to using the QuantStudio 6 Pro. After each use, please sign your name under the appropriate lab on the usage log sheet.

SAFETY PRECAUTIONS

- The QuantStudio 6 Pro Real-Time PCR system features moving parts. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.
- For more information refer to the Applied Biosystems QuantStudio 6 Pro Real-Time PCR System User Guide

MATERIALS and/or EQUIPMENT LIST

- 384-well clear optical reaction plates (Applied Biosystems: Part number: 4309849) containing sample, reference sample (calibrator), endogenous control, replicates and negative control and reaction mixture as dictated by your protocol.
- Sealed 384-well plate containing 20uL ddH20 for background validation.

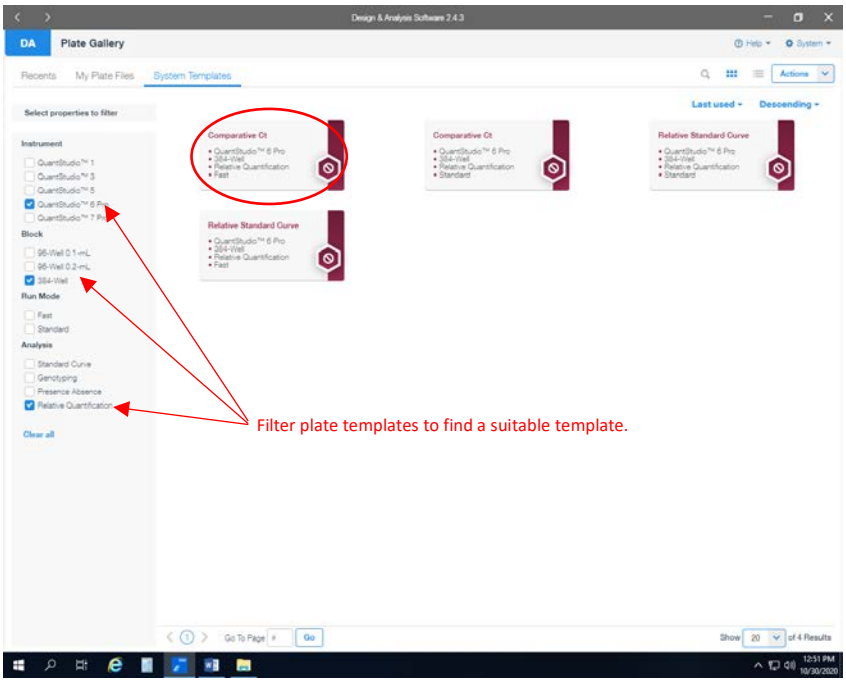
PROCEDURE

1. USING THE DESIGN AND ANALYSIS SOFTWARE TO RUN A PLATE:

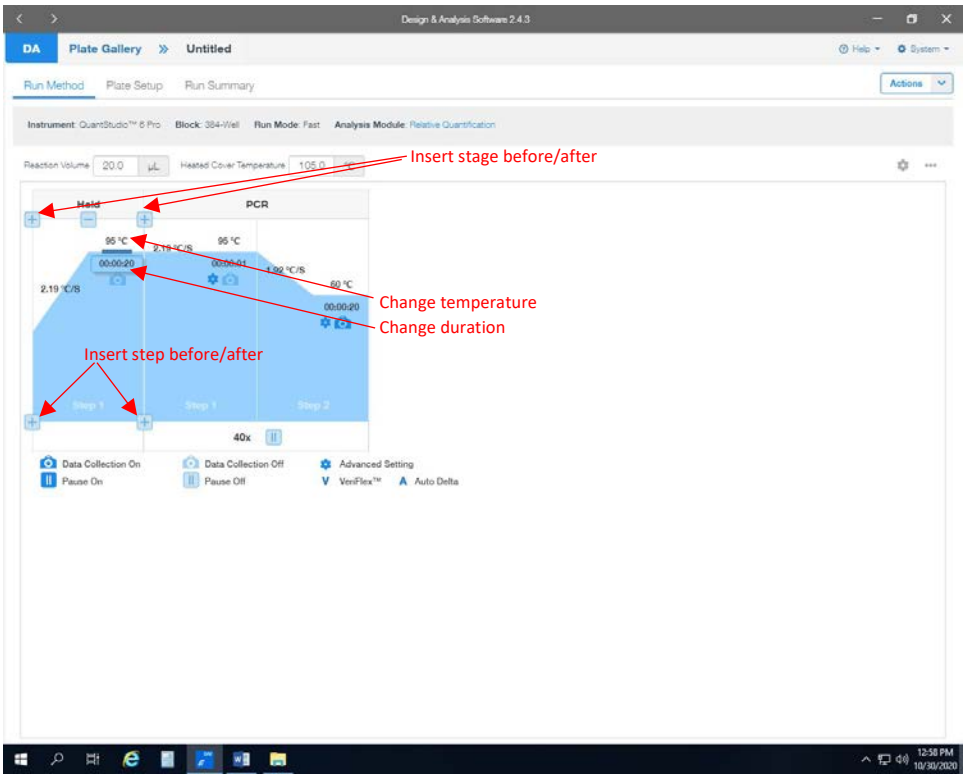
1. Start the QuantStudio Design and Analysis Software from the desktop.
2. Perform a routine background check (See MPCL Procedures for How to run a background plate)
3. Click SET UP PLATE
4. Using the filter list on the left, filter for QuantStudio 6 Pro under instruments, 384 well under block and Relative quantification under analysis. **→See Appendix A for an example**
5. Choose the appropriate template for your experiment setup and mastermix (Fast means the cycling conditions are 1sec at 95C and 20sec at 60C while standard is 15sec and 1min respectively.) **→See Appendix A for an example**
6. In the **Run method** screen adjust the run method according to the instructions for your mastermix. **→See Appendix A for an example**
    a. To change the temperature or time, simply click on the value to be edited.
    b. To add a step click the (+) at the bottom of the stage you want to add to;
    c. To add a stage click the (+) at the top of the stage you want to add before (left top) or after (right top).
        i. To add a melt curve, simply add a stage and click melt curve
    d. Adjust the volume in your wells.
7. In the **Plate Setup** screen; **→See Appendix A for an example**
    a. Highlight the wells you would like to process, add a sample and a target by pressing the (+) on the right panel and assign by clicking the check box next to the sample/target name.
    b. You can enter Biogroup information by clicking the cell underneath biogroup. Once a value is entered, it will appear in the drop down menu.
    c. You can change the Reporter and Quencher by clicking in the cell underneath and scrolling through the drop down menu.
8. Check the Passive Reference is configured correctly according to your mastermix instructions. **→See Appendix A for an example**
    a. You can also import Plate setup, Taqman assay plate, export plate setup or flip the plate setup from the dropdown menu at the right top of plate panel or print the layout.
    b. You can copy and paste sample information directly from Excel as long as the headings are identical.
9. Review your setup in the Run Summary screen and save your plate file. **→See Appendix A for an example**
10. Select the QuantStudio 6 Pro and send your plate to Run Queue **→See Appendix A for an example**
11. On the instrument screen,
    a. Click Set up run, Run Queue and choose your file to run.
    b. You can also select Network drive and find the saved plate file under your own folder.
    c. Choose a data file name, choose the network drive location you would like to save your data file.
    d. Check your Method and Plate and press **Start run**.

Appendix A: Examples using the Design and Analysis software to run a plate

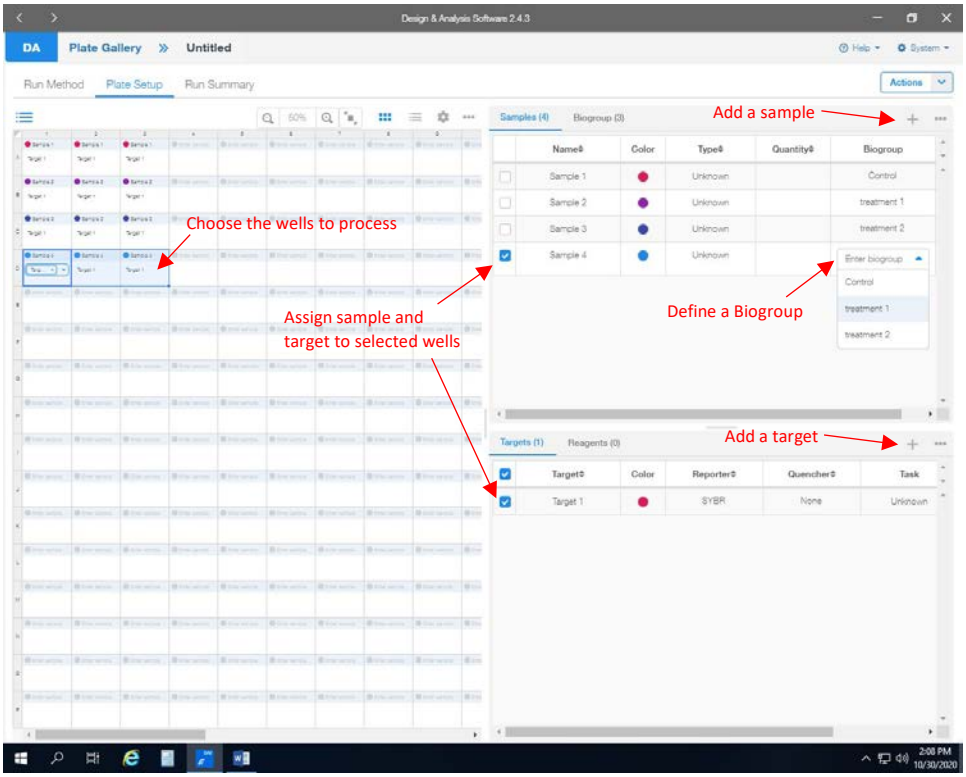
5. Choose the appropriate template for your experiment setup and mastermix



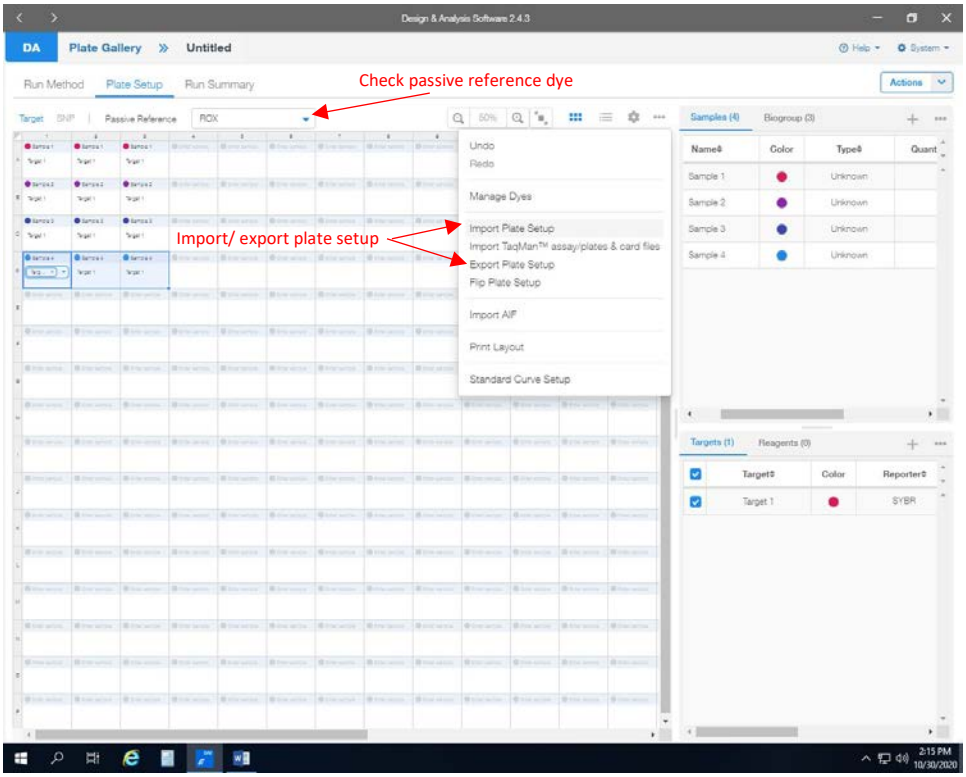
6. Setup run method:



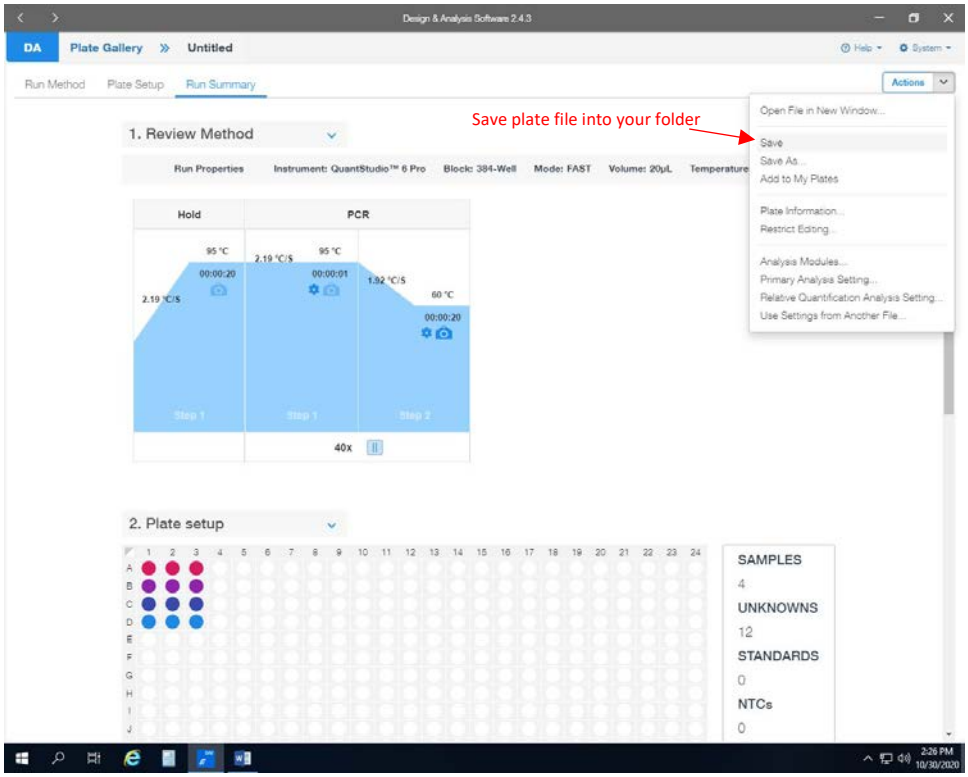
7. Plate Setup:

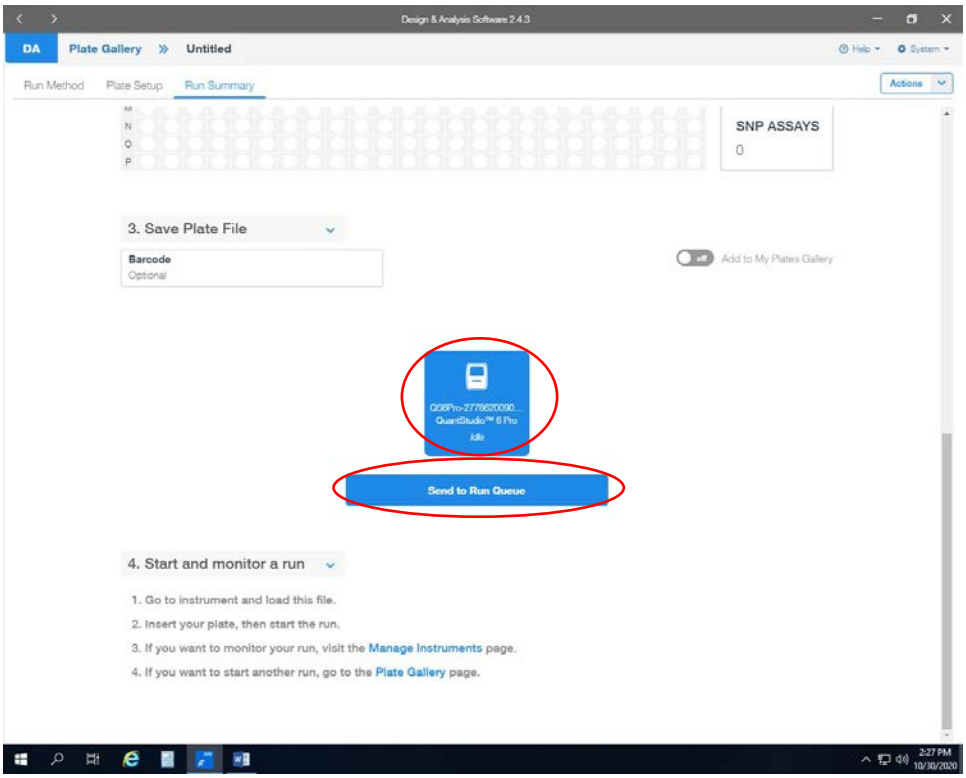


8. Passive reference and plate setup import/export



9. Run summary and plate queue:





2. USING THE QUANTSTUDIO 6 PRO INSTRUMENT INTERFACE TO RUN A PLATE:

1. Bring the Instrument out of sleep more by tapping the touch screen.
2. Tap **Set up Run**
3. Under **Templates**, tap on QS6Pro-384-Well-RQ-Fast.edt or QS6Pro-384-Well-RQ-Fast.edt **→See Appendix B for an example**
4. Determine a Data File Name and the save location (Set the save location to xe3-iot-2019/PRO/Experiments/YourFolder) **→See Appendix B for an example**

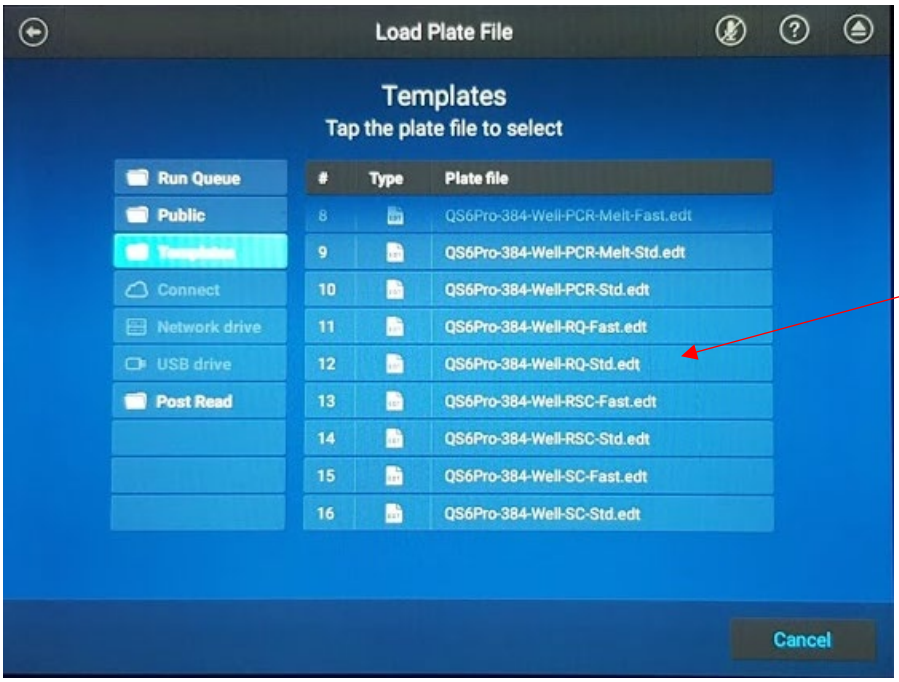
   The credentials for the Network Drive are:
   xe3-iot-2019/PRO
   username**: tmp**
   password**: tmp77**

   a. Tap the checkbox "Export run results with…" to disable auto export.
5. Tap on the **Method** tab to set up run method according to the instructions for your mastermix.
   a. To change the temperature or time, simply tap on the value to be edited.
   b. To add a step first tap advanced, then tap add/remove step, then tap the (+) where you want to add your step.
   c. To add a stage first tap advanced, then tap add/remove stage tap the then tap the (+) where you want to add your stage.
   d. To add a melt curve, tap (+) to add stage and select melt curve.
   e. Adjust the volume in your wells. **→See Appendix B for an example**
6. Tap on the **Plate** tab to configure your plate.
   a. Tap on Actions to import sample setup, edit reference dye or import AIF file. **→See Appendix B for an example**
   b. **Optional:** Tap and drag the wells to assign samples and targets. **→See Appendix B for an example**

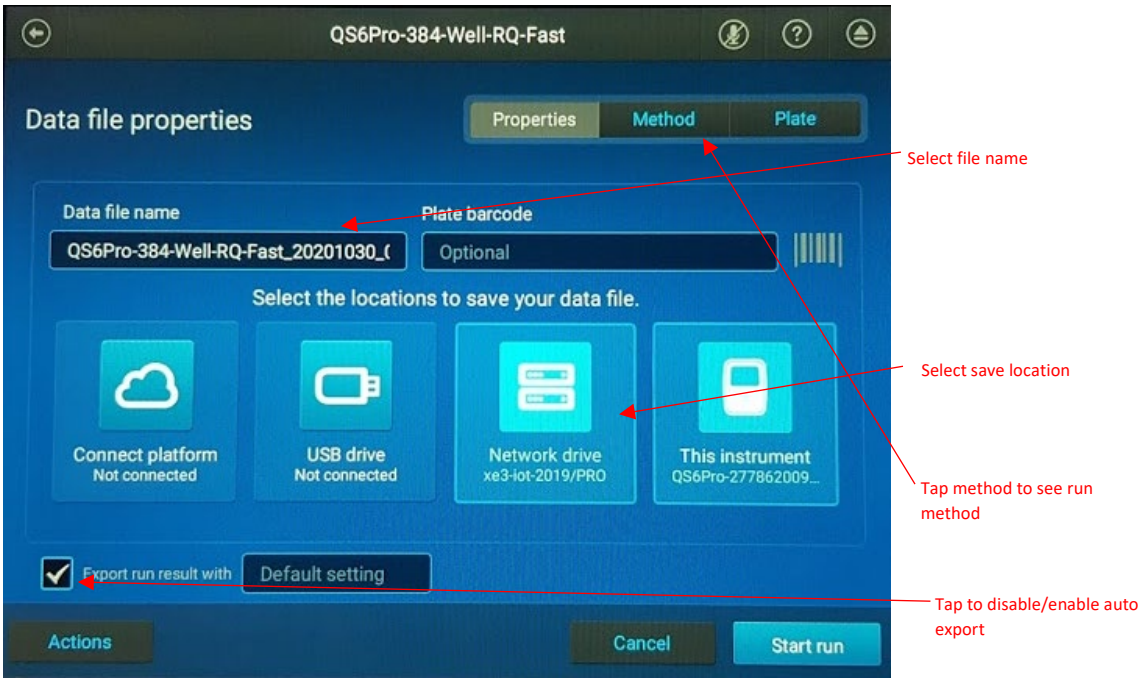
      (Tap the right panel to get to the ample/target menu)
7. Tap start run.

Appendix B: Examples using the QuantStudio 6 Pro Interface to run a plate

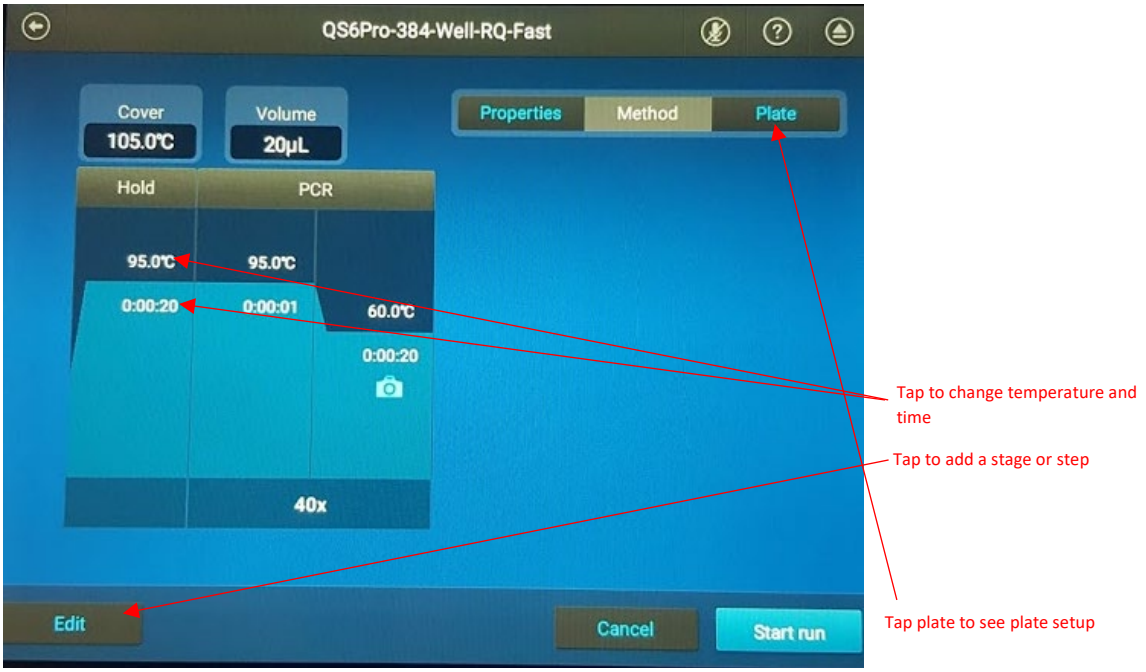
3. Choose a template:



4. Set file name and save location:

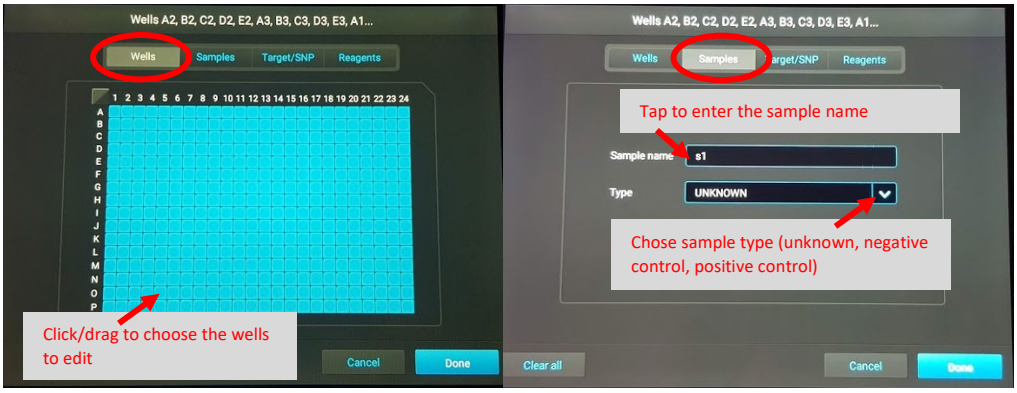


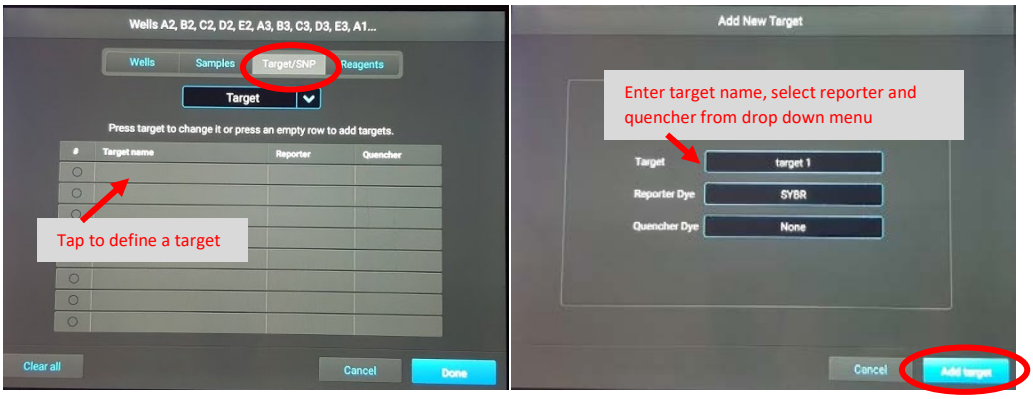
5. Set up run method:



6. a. Plate set up:

6. b. **Optional:** Defining and assigning samples and targets:





3. DATA ANALYSIS USING DESIGN AND ANALYSIS SOFTWARE:

1. When the run is complete your data will be saved in the specified location. You can now log into the OES network to carry your data to your HLI Network folder.
2. Open the .eds file with the Design and Analysis Software.
    a. You can download the free software at: www.thermofisher.com/qpcrsoftware
3. In the **Plate Setup** screen; **→See Appendix A –7. for an example**
    a. Highlight the wells you would like to process, add a sample and a target by pressing the (+) on the right panel and assign by clicking the check box next to the sample/target name.
    b. You can enter Biogroup information by clicking the cell underneath biogroup. Once a value is entered, it will appear in the drop down menu.
    c. You can change the Reporter and Quencher by clicking in the cell underneath and scrolling through the drop down menu.
4. Check the **Passive Reference** is configured correctly according to your mastermix instructions. **→See Appendix A – 8. for an example**
    a. You can also import Plate setup, Taqman assay plate, export plate setup or flip the plate setup from the dropdown menu at the right top of plate panel or print the layout.
    b. You can copy and paste sample information directly from Excel as long as the headings are identical.
5. In the **Quality Check** screen:
    a. On the left panel click to filter by sample type, name or target to view your data.
    b. Hovering over a well or curve will highlight it in the other panels and clicking on it will filter the results. You can also click and drag.
    c. On the plate outline you can color by different attributes like sample, target, Cq, Cq confidence, Amplification score and Amplification status.
    d. On the Well table, click on the checkbox to omit samples that fail the QC.
    e. Click **analyze** to re-run the analysis.

    →See Appendix C for an example

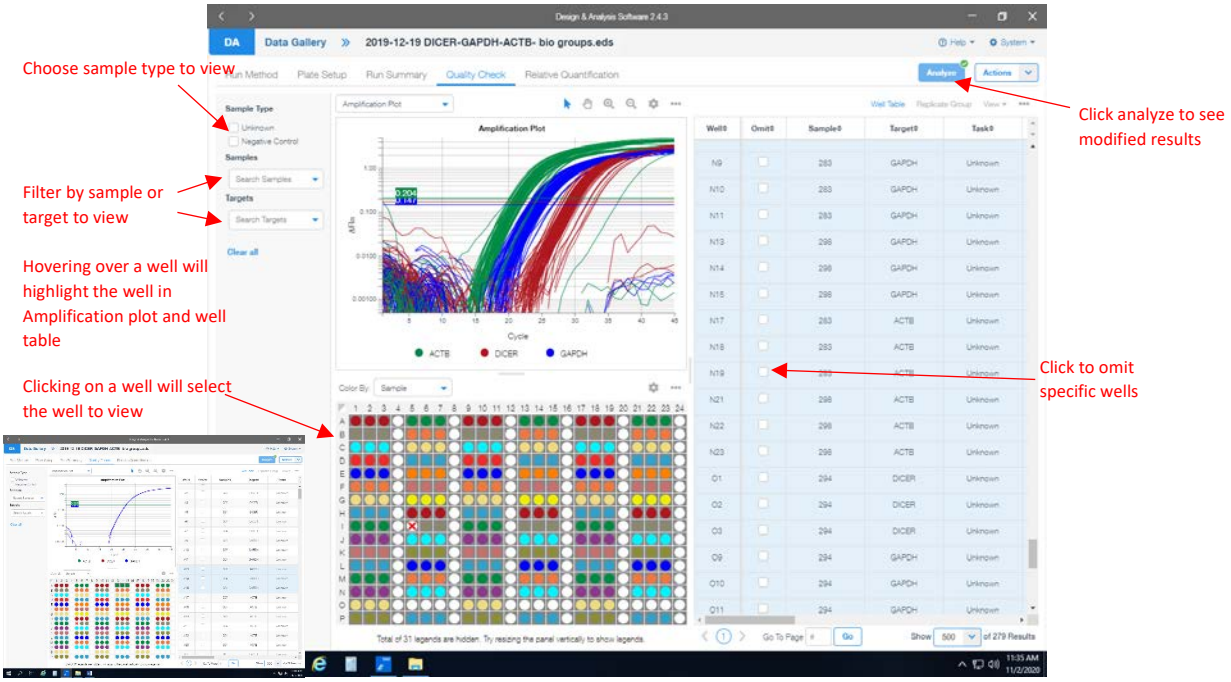
6. In the **Relative Quantification** screen:
    a. View your results (RQ) by sample or Biological group.
    b. On the plate outline you can color by different attributes like sample, Cq, Bio group.
    c. Click on the (…) on the RQ graph panel or Plate outline panel to save an image.
    d. Click on the (…) on the results table panel to export results only as a .csv file.
    e. Click **Actions -> Export** to export all the parameters like QC, Cq, Results into a .csv, .xls or another format.
    f. Click Actions -> Generate report to generate a .pdf report with all of the run parameters.

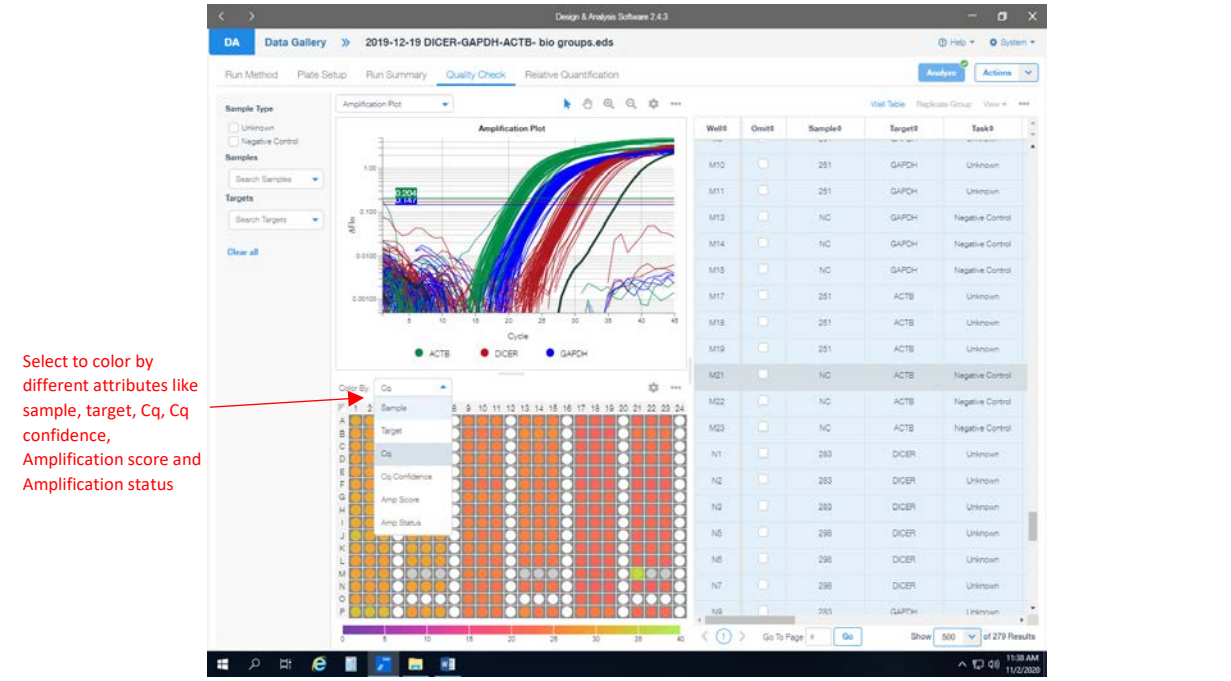
    →See Appendix C for an example

***NOTE: If you chose PCR and not RQ as a template, you will need to set the analysis mode by clicking **Actions -> Analysis modules -> Relative Quantification**

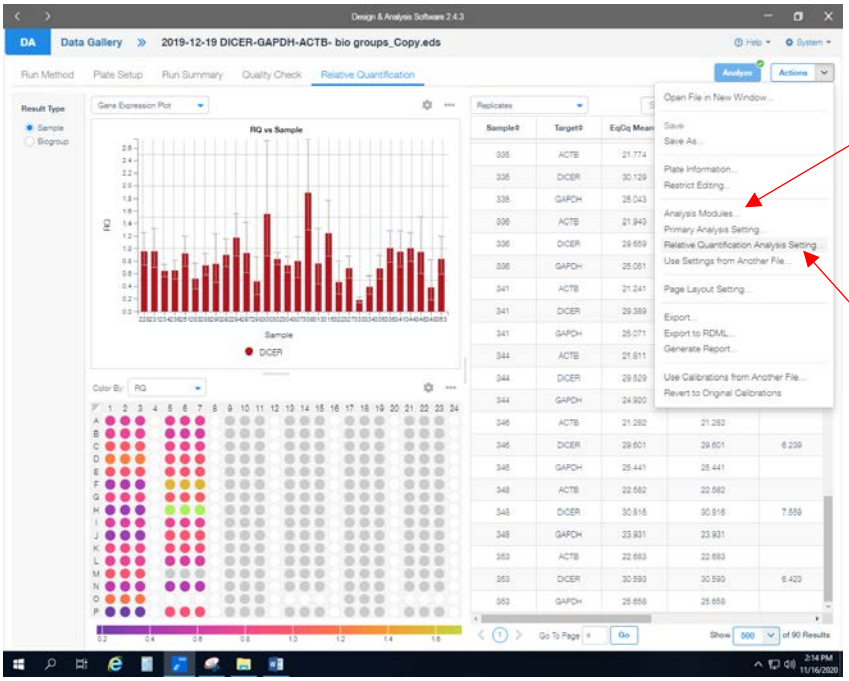
Appendix C: Examples for data analysis

5. Quality Check tab





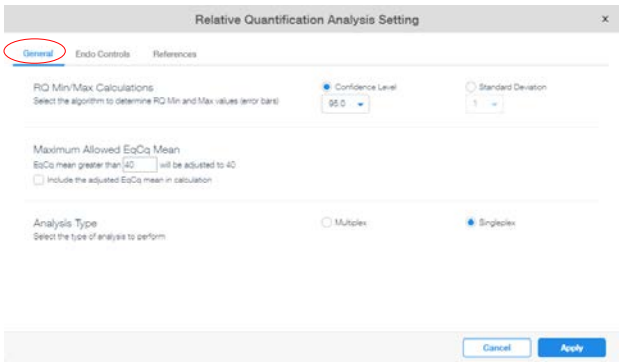
6. Relative Quantification tab



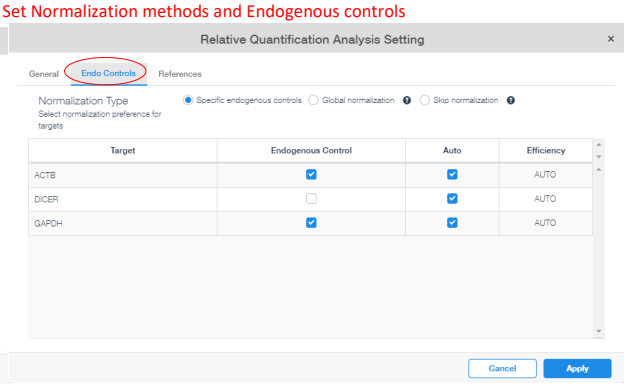
Set the analysis mode – Relative quantification

Set the Primary analysis settings and Relative quantification Analysis settings (Housekeeper, multiplex vs singleplex etc.

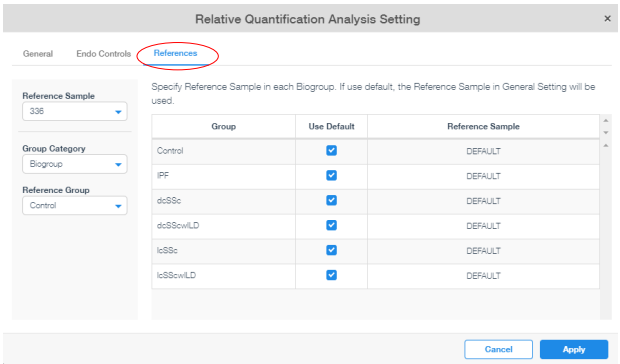
Set confidence level and analysis type



Set Normalization methods and Endogenous controls



Set reference sample/group



6. Relative Quantification tab - continued