QuantStudio 6/7 qPCR instrument (Applied Biosystems) quick start guide

Important notes

− This document is only a quick start guide, please refer to the official instrument user manual for *in depth* instructions. Contact us if you have questions or need help with troubleshooting.

− Check the compatibility of your mastermix with this instrument. In particular, the passive reference concentration is important. Mastermixes containing « high rox » concentrations should not be used with the QS6/7, rather choose the "low rox" version.

Note: Many mastermixes contain a passive reference (most often ROX), which is a dye not involved in the qPCR reaction itself but used to normalize for putative volume differences between wells. If you don't know if your mastermix includes ROX or its concentration, please check your mastermix user guide, or open a previous run, check the "Multicomponent plot" under "analysis" tab: A high ROX signal will typically appears around 1mio fluorescence units, while a "low rox" signal will show up around 100'000 units. If your mastermix does not contain rox at all, the signal will be flat at 0 unit. If the concentration of rox in your mastermix is too high, you can still save the analysis by unchecking the "rox passive dye" option during data analysis (see later).

- Booking is done through the BBS GECF portal. Pricing is available on GECF web site.

Starting the run

− Wake-up the instrument from the touch screen (or switch on if needed). If the instrument was already on, make sure no run is still ongoing.

− Open the "QuantStudio 6 and 7 Flex Real-Time PCR System software".

− "New Experiment" button → "From template".



− Then, either use the relevant GECF template amongst the ones called "DontForgetToLoadPlate_QS6/7_384w...edt". The templates are well suited for plates assembled with our Hamilton robot:
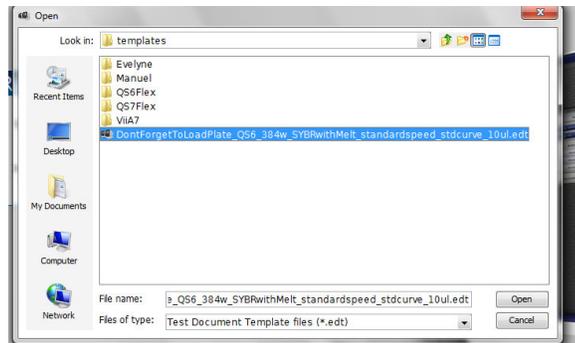
… Or open the "QS6/7 Flex" folder and choose the relevant generic template for your experimental design. *In these templates, the volume in the well is 20 ul by default, therefore you'll have to change it to 10 ul later on.*

− Click on "import" (at the top), select the txt file created by the Hamilton robot, click on "start import" and answer "yes":
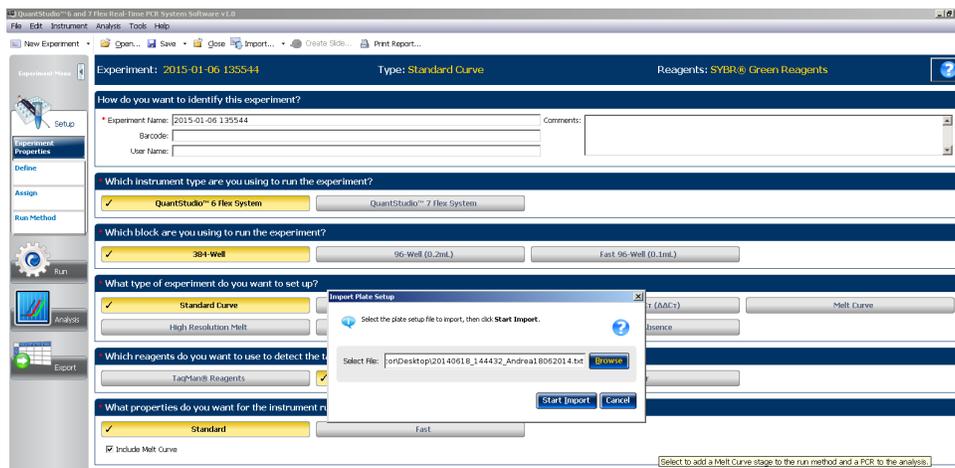

*Avoid non-standard characters in samples and detectors names.*

− If needed, in the "setup" tab on the left, "experiment properties" sub-tab:
  • choose the relevant dye chemistry (TaqMan or SYBR).
  • choose "Standard" or "Fast" settings (avoid "Fast" unless you have an assay and mastermix carefully optimized for "Fast" settings).
  • We recommend keeping the default "standard curve" setting for "experiment type" (this can be changed after the run if needed, more in the analysis section of this guide):
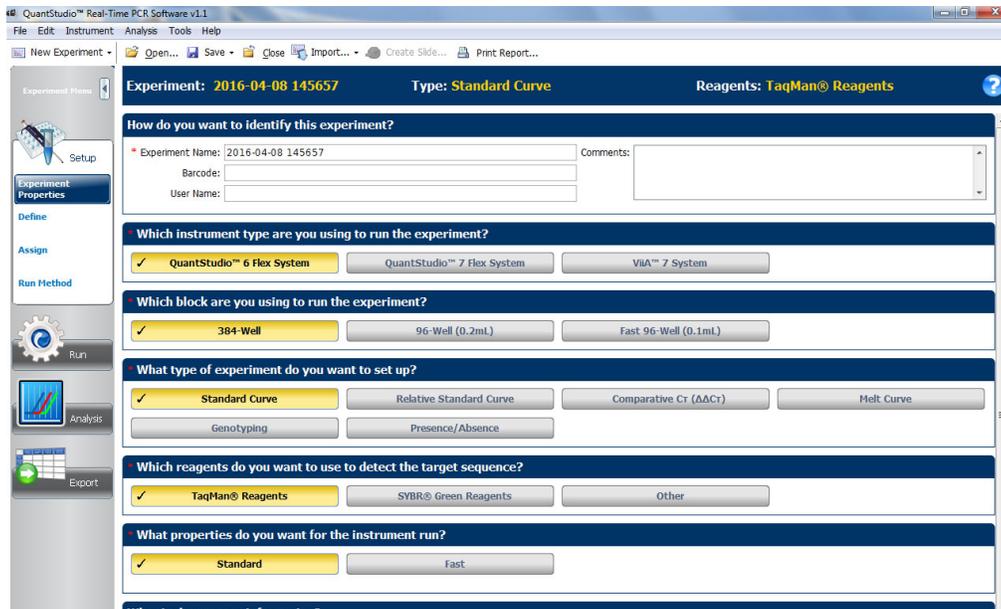
- In the "setup" tab, "define" sub-tab, adjust the passive reference on the bottom (by default ROX is selected).

- Press on the "Run method" button on the left of the screen. If needed, indicate "10 ul" as "Reaction Volume per Well". Choose the cycling parameters (refer to your mastermix user guide). The melting curve (= dissociation curve) was added automatically from the template (only relevant for SYBR green).
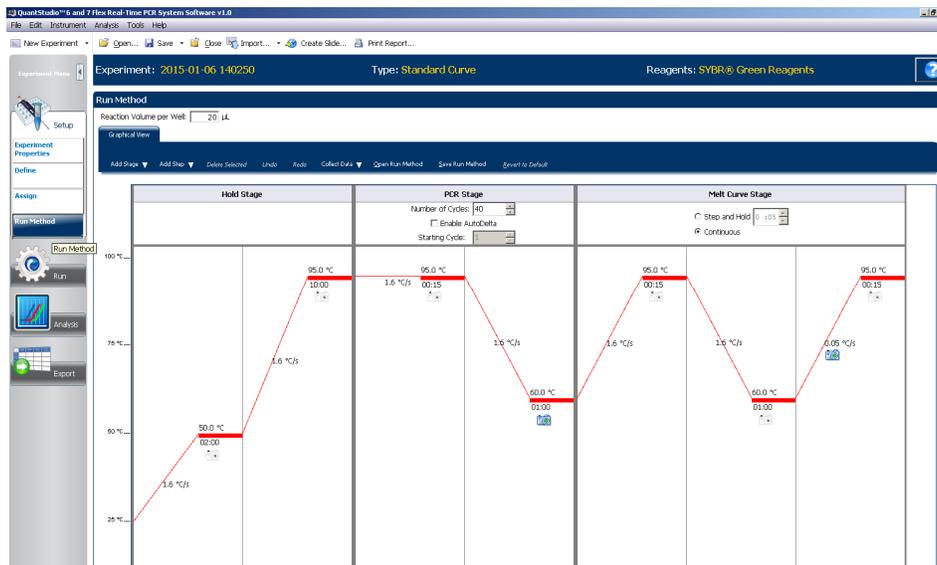
- Open the tray and insert your plate into the instrument. If the tray comes out empty, make sure a plate is not stuck inside.

- Click on the "run" button on the left of the screen:
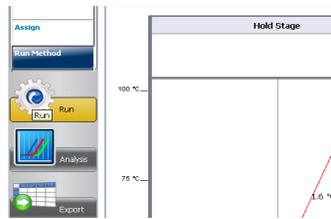
- Click on "start run", selecting the instrument number that appears. Save your file in your own folder, and click again on the "start run" button if the run hasn't started yet (the button becomes red when the run has begun):
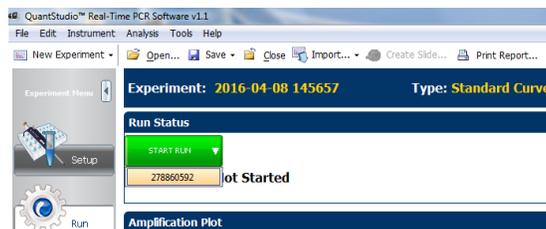

## After the run

Analyzing the run

- Make sure you don't perform the analysis while another run is ongoing (for instance the run of the next user). *If needed, we can provide you with the installation file for the QuantStudio software (PC only).*

- In the toolbar, choose "analysis" -> "analyze" to automatically set the threshold and background and calculate the Ct's of your samples.

- Inspect the amplification plot for putative issues, by selecting the samples in the table, and the detector/assay/gene/target in the drop-down list below the amplification plot:
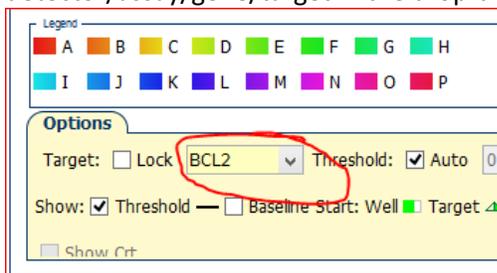
- You should check for each gene that the threshold and baseline were correctly assessed: 1) Check that the automatic threshold is in the exponential part of the amplification curve (appearing linear due to log scale); 2) Check that the baseline is correctly set by checking for absence of aberrant behavior of the beginning of the curves, such as the ones depicted below:
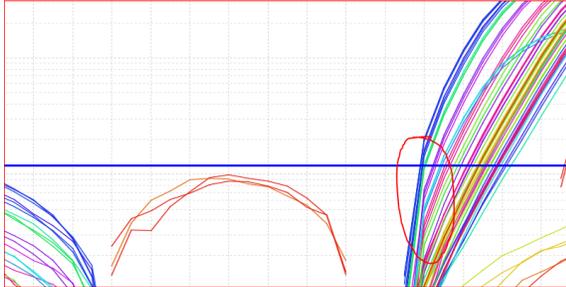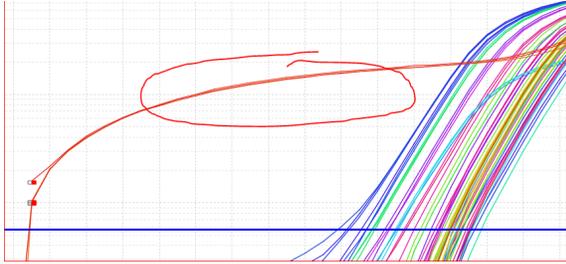
− In case the automatically-set threshold and baseline need to be manually adjusted, press on the "analysis settings" button to adjust parameters manually.
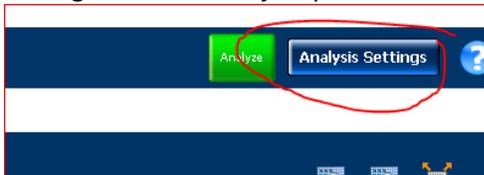

Melting curve analysis

If you used SYBR green, review the melting curves ("melt curve plot") for absence of multiple peaks. Typical peaks will appear between approximately 77° and 88°. Secondary peaks lower than that may represent primer dimers, and higher may represent genomic DNA contamination (amplicon containing an intron).

Detecting issues

− The software automatically detects issues with the amplification and label the affected samples with a yellow triangle in the table. In addition a summary of all the detected issues is found in the "QC summary" tab.

− For troubleshooting purpose, the "multicomponent plot" view can be useful since it shows raw signal for all

relevant channels (FAM/SYBR…, rox). You can select it here:
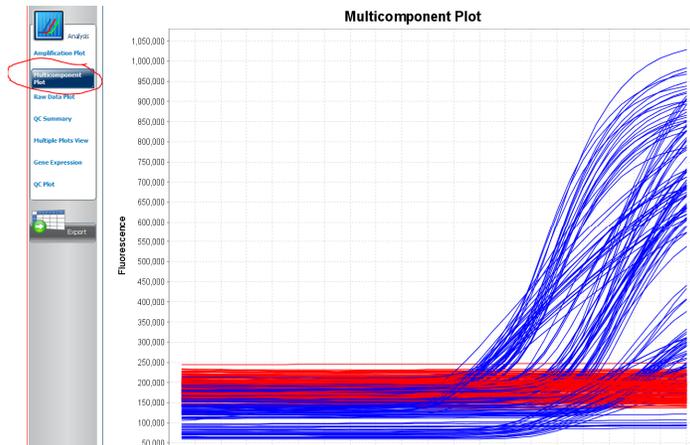
Exporting data

Data can be exported in excel format with the QS6/7 formatting, or with the formatting of other older instruments (options on top of the export window).

Data analysis

Absolute quantification analysis

If your experiment includes a standard curve for absolute quantification of samples, it will be automatically calculated by the software and will appear in the Standard curve tab. Make sure your samples are within the range of the standard curve, and that the curve linear regression fits well enough the standard curve points.

Relative quantification analysis (delta delta Ct)

- The QS6/7 software can automatically calculate the delta delta Ct for relative quantification of gene expression. Most people use dedicated macros and software, but if you want to perform this analysis in the QS6/7 software, first convert the file into a relative quantification file in the setup tab (choose comparative Ct). After conversion, the exported txt file is much less broadly recognized by third party macros and software, therefore we recommend exporting a standard version of the Cts data before the conversion (see the "exporting data" section below).

- Accurately using the delta delta Ct method requires that all your primer couples have a similar efficiency. A serial dilution of a sample or plasmid should be performed to calculate these efficiencies.

- Contact us in case you need some help for the delta delta Ct analysis. We for instance provide access to the commercial Genex software, which allows selecting several housekeeping genes for normalization.

Diverse

- On the QS7, we have additional thermal blocks for 96w plates and TLDA (Thermo arrays, e.g. for miRNAs). Contact us if interested.

Versions log

– v1.04: initial release.

– v1.05: many small changes as compared to initial draft, including analyses guidelines, and high/low rox recommendations.

– v1.06: many minor changes. Addition of brief descriptions of melt curve analysis, and absolute/relative quantification analyses.

– v1.07: Indicated not to perform an analysis while a run is ongoing.

– v1.08: indicated to check no plate is stuck in the instrument when the plate holder goes out empty. Clarified how to tweak analysis settings for baseline issues.

– v1.09: Clarified to avoid non-standard characters in samples/detectors names. Clarified how to book and where to find prices.

– V1.10: Added QS7 mention. Mentioned 96w and TLDA blocks.