



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

Important notes

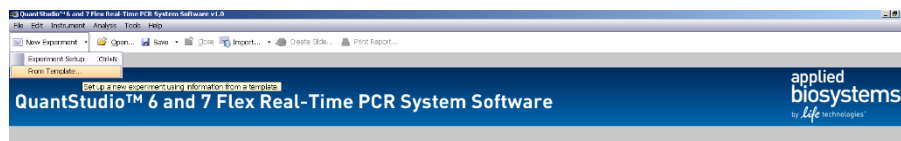
- This document is only a quick start guide, please refer to the official instrument user manual for *in depth* instructions. Contact us if you have questions or need help with troubleshooting.
- Check the compatibility of your mastermix with this instrument. In particular, mastermixes containing « high 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).

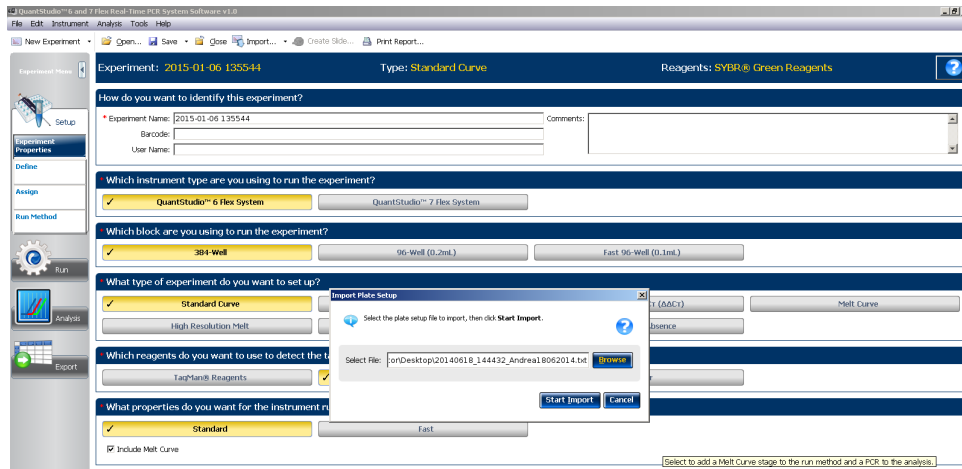
- Booking is done through the PPMS GECF portal. Pricing is available on the GECF web site.

Starting the run

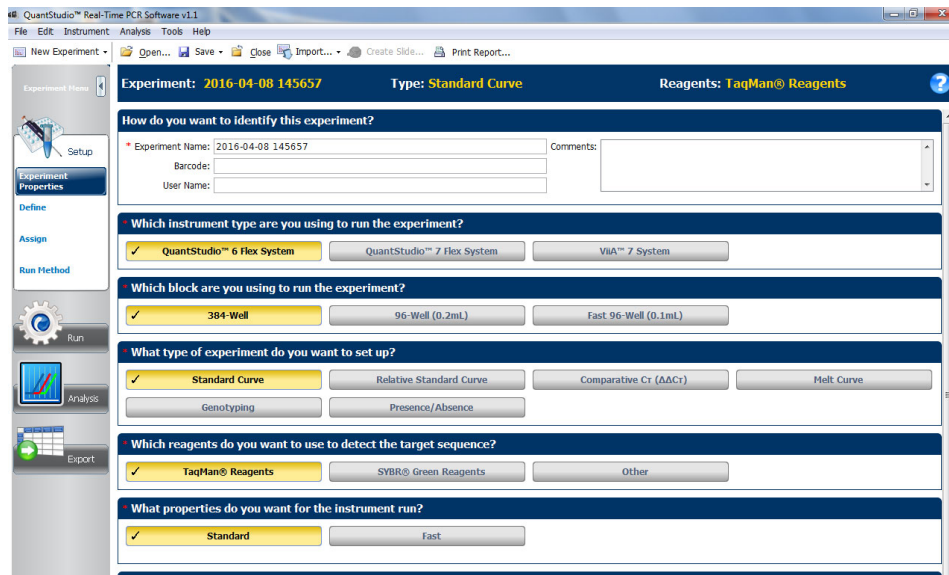
- Wake-up the instrument from the touch screen (or switch it on if needed). If the instrument was already on, make sure no run is ongoing.
- Open the “QuantStudio 6 and 7 Flex Real-Time PCR System software”.
- “New Experiment” button → “From template”.



- Then open the template folder and:
 - Either choose the relevant generic template for your experimental design. *The wells volume is 20 ul by default, therefore you'll have to change it to 10 ul later on if you have 384-w plates.*
 - Or use the relevant GECF template amongst the ones called “DontForgetToLoadPlate_XXX.edt”, which are pre-edited by the GECF for our main applications. These templates are notably well suited for plates assembled with our Hamilton robot.
- Then, the following step depends on whether you have prepared the plate with our Hamilton robot or not:
 - If you did use the robot: Click on “import” (at the top), select the txt file created by the robot → “start import” → “yes”:

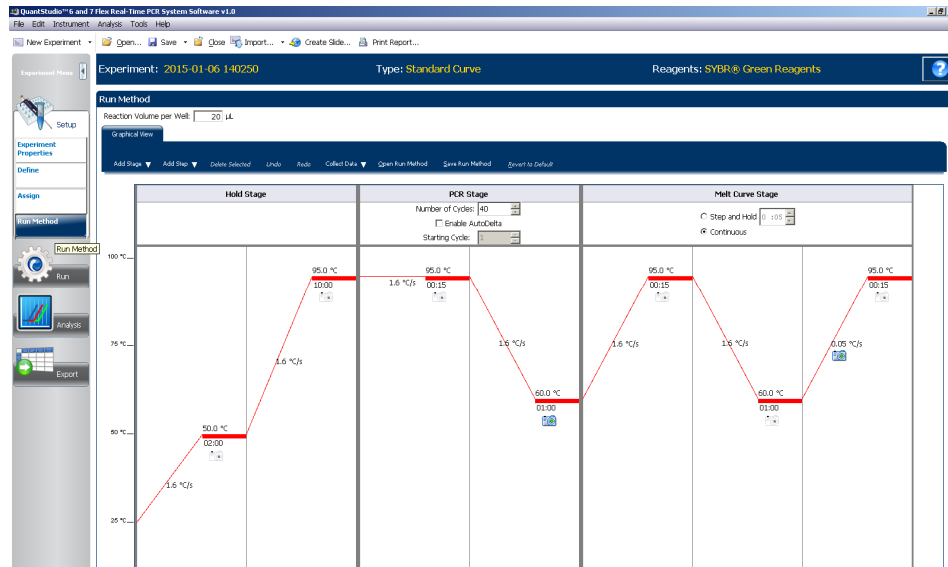


- If you did not use the robot, create genes (targets) and samples in the Define tab. Then, in the Assign tab, assign them to wells, and define their “tasks” (N for negative controls, U for samples, S for standards). *Avoid non-standard characters in samples and detectors names.*
- If needed, in the “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):

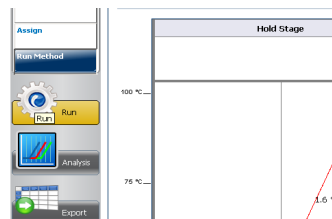


- In the “setup” tab, “define” sub-tab, adjust the passive reference at the bottom (by default ROX is selected).
- Press on the “Run method” button on the left of the screen. For 384-w plates, indicate “10 ul” as “Reaction Volume per Well”. Choose the cycling parameters (refer to your mastermix user guide). The melting curve (=

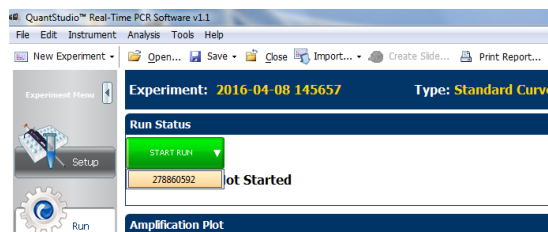
dissociation curve) was added automatically from the template (only relevant for SYBR green).



- Open the tray (button in lower right corner of touchscreen) 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 of the “LectureMiseq” shared GECF network drive, and click again on the “start run” button if the run hasn’t started yet (the button becomes red when the run has begun):

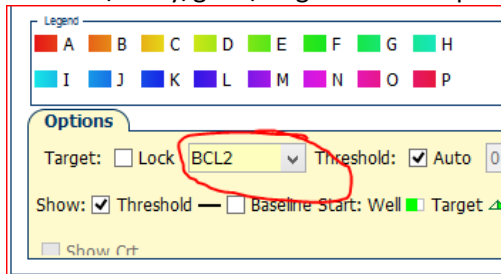


After the run

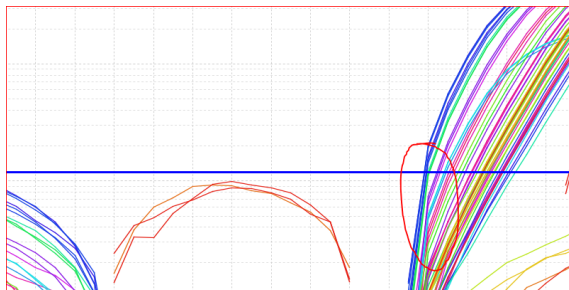
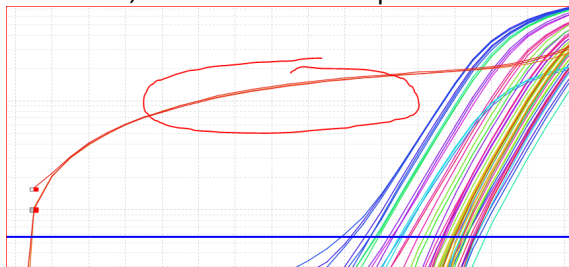
Analyzing the run

- 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).

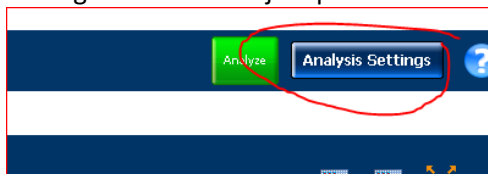
- In the toolbar, choose “analysis” -> “analyze” to automatically set the threshold and background and calculate the Ct’s of your samples.
- Inspect the amplification plot for putative issues, by selecting the samples in the table, and the detector/assay/gene/target in the drop-down list below the amplification 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.

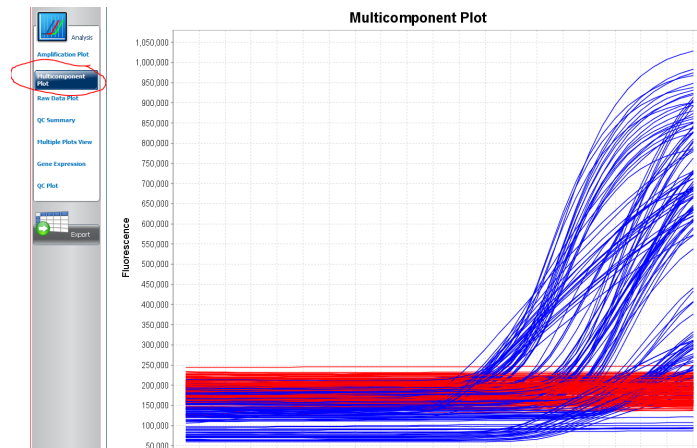


Melting curve analysis

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 QS6/7 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, even though with limited functionalities as compared to more advanced software (see below). For that, you need to first convert the run file into a relative quantification file in the setup tab (choose comparative Ct). Note that after conversion, any exported txt file is much less broadly recognized by third party macros and software, therefore we recommend exporting a standard version of the Ct's data before the conversion (see the “exporting data” section above).
- 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.

Advanced Software options

Even though data analysis can be done directly in the QuantStudio software, which can be sufficient in certain

cases, we can recommend other options for more advanced analysis:

- **Thermo ExpressionSuite Software** <https://www.thermofisher.com/ch/en/home/technical-resources/software-downloads/expressionsuite-software.html>
- **Thermo cloud apps** <https://www.thermofisher.com/ch/en/home/digital-science/thermo-fisher-connect/all-analysis-modules.html>
- **Genex** The licence works through a USB dongle that we can lend you. <https://www.biomcc.com/genex-software.html>
- **Clarida** Previously known as qBase+. Paid but a free tier is available. <https://clarida.bio/>

Diverse

- On the QS7, we have additional thermal blocks for 96w plates and TLDA (Thermo arrays, e.g. for miRNAs). Only semi-skirted 96w plates can be used (e.g. Thermo N8010560 or 4306737, or 4-titude 4ti-0760). Contact us if interested.

Versions log

- v1.04-v1.08: many small changes, including analyses guidelines, and high/low rox recommendations. Brief descriptions of melt curve analysis, and absolute/relative quantification analyses. Do not perform an analysis while a run is ongoing. 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.
- v1.11: Mentioned how to open the tray door. Clarified the procedure when not using the Hamilton robot.
- v1.12 (09.06.2026): Mentioned where to save the run data (*LectureMiseq* shared GECF network drive).
- v1.13 (19.06.2026): Compatible 96w plates indicated.
- v1.14 (24.09.2026): List of data analysis solutions. Clarified better the workflows when using or not our robot.