USE AND CALIBRATION OF QUANTSTUDIO 3™ REAL-TIME PCR SYSTEM

1. INTRODUCTION

1.1. This method is based on the Applied Biosystems QuantStudio 3™ Real-Time PCR manuals. The method describes general steps for Quant Studio™ Real-Time PCR System to set up a qPCR run.

2. EQUIPMENT AND SUPPLIES

2.1. Equipment and Supplies –

Equipment/Supply Name	Notes
QuantStudio3™ _1 Real-Time PCR System	S/N 272311740
QuantStudio3™ _2 Real-Time PCR System	S/N 272314393
CBS Optimizer PCR Workstation #1	S/N 007631
CBS Optimizer PCR Workstation #2	S/N 007632
Optical 96 Well PCR Reaction Tray	N/A
Optical Adhesive PCR Reaction Tray Tape	N/A
Optical 96 Well Plate Holder and Stand	N/A
Thermo Megafuge ST4F Plus Centrifuge	S/N 43085295
Fisher Mini Vortexes	N/A
Gloves, Latex or Nitriles	N/A
Tube Racks	N/A
Micropipettes	N/A
Sterile PCR Grade Filter Tips	N/A
Or Equivalent	

3. REAGENTS AND STANDARDS

3.1. Deionized water

3.2. Molecular Biology grade water. Corning Molecular Biology Grade Water tested to USP Sterile Water (Catalog Number: MT46000CV).

4. SETUP AND USE OF QUANTSTUDIO 3™ INSTRUMENT FILES

4.1. Create an Import File:

4.1.1. In \\floridadep\data\LABS\Lab3\Biology\qPCR\Import files, select the corresponding year folder, then test ID folder and open the appropriate marker text file template. “Save as” with the current worksheet number.

4.1.2. If needed, update the standard concentration, SPK serial numbers for the third well spike, and LFB serial numbers for the extraction control (EC) using the “Replace” tool.

4.1.3. Use Edit/Replace from the menu to replace “Sample1” (starting at row A7) with the first sample ID. Repeat with all the samples and refer to the plate layout so that the right sample IDs go into the correct wells.

4.1.4. Check the naming conventions for the DUP samples, as well as for purified (PUR) samples.

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4.1.5. Empty wells do not need to be removed from the import file. The software may not recognize the text file if columns are shifted due to deleted or extra spaces. Empty wells can be cleared later in the software..

4.1.6. Follow spacing, capitalization, and underscores as shown in table 1 below.

Table 1- Naming Conventions

Type	Crude name	PUR name
Standard curve	HF183	PURHF
	PMAHF	PMAHF
	HumM2	PURH2
	BacR	PURBR
	DG3	PURD3
	HoF	PURHO
	CowM2	PURC2
	GULL2	PURG2
	GFD	PURGD
	SalmonDNA	SalmonDNA
Sample	SampleID_	SampleID_PUR
Sample surrogate	SUR_SampleID_	SURPUR_SampleID_PUR
Lab duplicate, target assay	SampleID_DUP_A_	SampleID_DUP_A_PUR
Lab duplicate, surrogate assay	SUR_SampleID_DUP_A_	SURPUR_SampleID_DUP_A_PUR
3RD well spike control	LFB_XXSPK#	LFB_XXSPK#
3rd well spike, samples	SampleID_SPK_XXSPK#	SampleID_SPK_PUR_XXSPK#
3RD well spk, lab duplicates	SampleID_SPK_DUP_A_XXSPK#	SampeID_SPK_DUP_A_PUR_XXSPK#
Target extraction control	LFB_XXLFB#	LFB_XXLFBP#
Surrogate extraction control	SUR_XXLFB#	SURPUR_XXLFB#
Method blank	MBLK	MBLK
Extraction blank	EXBLK	EXBLK
No Template Control	NTC	NTC

corresponds to LIMS serial number assigned in Standard Preparation Tracker.

XX corresponds to the unique 2-letter naming convention of the target assay (HF, BR, D3, HO, C2, H2, GD, G2)

Note: If multiple extraction batches are analyzed on the same plate, add an underscore followed by the worksheet number. Ex.: MBLK_477, EXBLK_477. Remove worksheet numbers before importing the file to QCM; but save original file with designated worksheet numbers.

4.1.7 Save the text file in the current year and target assay folder.

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4.2. Create an Experiment in QuantStudio™ Design & Analysis software v1.5.1

4.2.1. Under **Properties**, **Experiment Properties**, add the corresponding Experiment Name (XX-X-###-YYMMDD). Where XX corresponds to test ID (HF, BR, G2, GD, D3, H2, HO, or C2); X refers to the DNA extraction method (C for crude and P for purified); ### corresponds to the worksheet number and YYMMDD specifies the date of the run). "TBD" can be used in place of a run date if the file is made in advance of the run date.

4.2.2. Type the analyst initials under **User name**.

4.2.3. Make sure the experiment selections are correct (the defaults are 96-Well 0.1-mL Block, Standard Curve, TaqMan® Reagents, and Standard).

4.2.4. Select **File/Import Plate Setup** from the menu, choose the corresponding text file from the Import files folder and click **Apply**.

4.2.5. Select the **Method** tab to ensure all running conditions are listed per the SOPs. Standard running conditions will apply if a new template is used. The plate **Volume** is set to 20 ul and **Cover** temperature is 105.0 deg C. Enzyme activation occurs during Step 2 of the "Hold" stage. Denaturation occurs during "Stage 1" of the PCR stage. Annealing/Extension occurs during Step 2 of the PCR stage.

4.2.6. Click the **Plate** tab and under **Assign Targets and Samples**, click **Advanced Setup**. Make sure the samples and controls are in the correct wells. Delete the information from empty wells by un-clicking the assigned target and sample ID or by highlighting the wells, right click, and hit clear.

4.2.7. Select the appropriate task for each well: S for standard curve, U for sample, SPK, SUR, MBLK, EXBLK, and N for NTC

4.2.8. Select appropriate reporter dye. All current assays use FAM. A quencher does not need to be selected.

4.2.9. Enter the concentrations of target standard and SKETA curves.

4.2.10. Confirm correct naming conventions in all wells.

4.2.11. Save the QuantStudio experiment template file with the experiment name (See Section 4.1.1.) in corresponding year folder within \\floridadep\data\LABS\Lab3\Biology\qPCR\Import files\QuantStudio_Template.

4.2.12. The file is now ready for the experiment run.

4.3. Running the Instrument File in QuantStudio™ Design & Analysis Software v1.5.1

4.3.1. Load the EDT file saved in folder from step 4.2.12.

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4.3.2. On the instrument touch screen, press the arrow in top right corner to open the sample tray. Position the plate so that well A1is located in the top left corner of the sample block. Press the arrow again to close the drawer.

4.3.2. Click the **Run** tab in the software. Then, click the blue "Start Run" button in the upper right-hand corner of the screen.

4.3.4. Click on the instrument serial number that appears below the button. Accept or edit the name for the EDS file.

4.3.5. Save to corresponding year folder in \\floridadep\data\LABS\Lab3\Biology\qPCR\Instrument Files.

Note**:** Do not leave the instrument or computer until you verify that the run has started. Run progress can be viewed from the touch screen of the instrument or from the software on the computer.

4.3.6. Once the run has completed, the file will automatically save in the network folder selected in 4.3.5. If any edits are made to the file, such as adjusting the threshold, select the blue "Analyze" button in upper right corner of **Results** tab. Save the file again.

4.3.7. Click done on the instrument touch screen and select the arrow button to remove the reaction plate. Discard the reaction plate in biohazardous waste.

4.4. Running the Instrument File from the Instrument Touch Screen

4.4.1. If there is a computer-instrument connection or software issue, use the touch screen to operate the instrument.

4.4.2. Transfer the import template to a USB drive. Then, connect the USB to the port located on the front panel of the instrument.

4.4.3. On the touch screen, select **Load Experiment**, then **USB**. Select the import template file.

4.4.2. A message will appear as a reminder to load the plate. Select yes or OK, then press the arrow in the top right corner of the instrument to load the plate. Position the plate so that well A1 is in the top left corner of the sample block. Press the arrow to close the sample drawer.

4.4.3. Select **Start Run**. Wait to ensure the run has started.

4.5. qPCR Run Analysis and Exporting File

4.5.1. Review QC and sample amplification per applicable target assay SOPs. Select the **Export** tab.

4.5.2. The file name is automatically filled in and corresponds to the **Name** field entered in the **Properties** tab.

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4.5.3. **File type** is defaulted to "QuantStudio". Select "*.txt" file in the second drop- down list. Select **Browse** to choose the network file location. Export to \\floridadep\data\LABS\Lab3\Biology\qPCR\Export Files. Select the folder for the current year and save to the corresponding target assay folder.

4.5.4 Unselect the option to open the file upon export.

4.5.5. Click **Export** in the upper right corner.

5. MAINTENANCE OF QUANTSTUDIO 3™ REAL-TIME PCR SYSTEM

5.1. Performance maintenance for this instrument is completed bi-annually by a field service engineer. Email ServiceProducts@Lifetech.com to schedule a visit.

5.2. A background calibration plate can be analyzed at any time as a tool for troubleshooting excessive noise, inconsistent, or unusual amplification. It can be performed independently of the other calibration plates.

Note: Do not mark on any of the plates with pens and ensure the plates are prepared on a clean, dry surface.

5.2.1. A background plate comes with the instrument calibration package. The plates should be used within 6 months after opening, and each plate can be used up to 3 times if stored in the original packaging at -15 to -25 °C.

5.2.2. If a background plate is not available, one can be prepared by aliquoting 10-20 µL of deionized water to all 96 reaction wells of a plate. Do not add any master mix. Seal the plate with optical adhesive film. Continue to step 6.2.5.

5.2.3. Allow the background plate to warm to room temperature (at least 5 min).

5.2.4. Remove the plate from its packaging, but do not remove the optical film.

5.2.5. Vortex the plate for 5 seconds.

5.2.6. Centrifuge the plate for 2 min at 750-1,000 g.

5.2.7. Confirm the liquid in each well is at the bottom and free of bubbles. If it is not, centrifuge the plate again.

5.2.8. Load the plate on the instrument so that well A1 is in the top-left corner. Alternatively, the plate can be rotated 180 deg., but make note of the direction of the plate in case troubleshooting is necessary.

5.2.9. In the instrument home screen, select **Settings →Maintenance and Service → Calibrations → Custom → Background**.

5.2.10. Follow the instructions on the home screen to start the calibration. When the screen displays **Calibration Complete**, touch **View Results** to check the calibration status.

5.2.11. View calibration images. If the calibration failed, proceed to troubleshooting in 6.2.13. A passing calibration confirms that the

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instrument block is free of contamination. The instrument light source, heating block, or reagents should be further investigated if unusual or unexpected results continue. After ruling out reagents, contact Thermo Fisher instrument technical support for further instructions. Visit https://www.thermofisher.com/us/en/home/support.html for current contact numbers and information.

5.2.12. If the calibration failed, ensure the correct plate was used for the calibration performed; ensure it was properly thawed, centrifuged, and sealed. Ensure the plate is not damaged and there are no visible signs of contamination such as ink marks, dust, or artifacts. Signals that exceed the limit of normal fluorescence may indicate contaminants on the calibration plate or sample block.

5.2.13. Note the wells that failed calibration. Remove the plate from the instrument, rotate 180 deg., then perform the calibration steps again beginning in section 6.2.9.

5.2.14. If the positions of the failed wells are identical to the first run, the block is contaminated. Proceed to decontaminate the block per the instrument manual. \\floridadep\data\DEAR\Labs\Lab5\Biology\MolecularBiology\Equipment Manuals and Software\Quant Studio3

5.2.15. If the positions of failed wells are reversed, the plate is contaminated. Discard the plate and prepare a new one for calibration.

5.3. Monitor the instrument drawer surrounding the sample block for dust or artifacts. Use a lint free wipe dampened with only DI water to remove dust. Dry completely with another lint free wipe.

6. QUALITY CONTROL

7.1. Document all instrument maintenance and troubleshooting in the Molecular Biology quality control laboratory notebook located in PCR Lab A.

7. REFERENCES

8.1. DEP Laboratory Quality Manual

8.2. Health and Safety Plan

8.3. Applied Biosystems QuantStudio™ 3 Real-Time PCR Systems Installation, Use, and Maintenance manual \\floridadep\data\DEAR\Labs\Lab5\Biology\MolecularBiology\Equipment\Quant Studio3

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Appendix of Significant Changes

01/07/2025	Removed information about StepOne Thermocycler. Added procedure to perform background calibration and troubleshooting of Quant Studio3. Added new Quant Studio instrument and centrifuge. Added file link to instrument manual.
12/18/2025	Updated instructions for creating and saving Quantstudio 3 files in section 4. Added additional steps to create an instrument import file. Added instructions to run a plate without a computer connection. Created a table for naming conventions.

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