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

4.2. 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.

4.3. 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.

4.4. Check the naming conventions for the DUP samples, as well as for purified (PUR) samples. Refer to Table 6.1 Naming Conventions in SOP PCR-5.1.

4.5. Save the text file.

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4.6. **In QuantStudio™ Design & Analysis software v1.5.1**, click "Create New Experiment".

4.6.1. Under **Properties**, **Experiment Properties**, add the corresponding Experiment Name (XX-X-###-YYMMDD). Where XX corresponds to test ID (HF, BR, G2, GD, D3, H2, 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).

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

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

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

4.6.5. 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 corresponds to Step 2 of the Hold stage. Denature corresponds to Stage 1 of the PCR stage. Anneal/Extend correspond to Step 2 of the PCR stage.

4.6.6. 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.

4.6.7. Save the file with the experiment name (See Section 4.6.1.) in \\floridadep\data\LABS\Lab3\Biology\qPCR\Import files.

4.6.8. The file is now ready for the experiment run. Load the plate into the instrument. To start the experiment, click the blue "Start Run" button in the upper right-hand corner of the screen. Click on the box that appears below the button and accept or edit the name for the EDS file, then save to\\floridadep\data\LABS\Lab3\Biology\qPCR\Import files\QuantStudio_Template.

4.6.8.1. Run progress can be viewed from the touch screen of the instrument. At the beginning of a run do not leave the instrument or computer until you verify that the run has started.

4.6.9. When the run is complete, save the file on the thermocycler PC so that the run information is saved correctly to the server. Remove the reaction plate and discard in hazardous waste.

5. qPCR RUN ANALYSIS AND EXPORTING FILE

5.1. In the instrument file, review the standard curve for both assays. Efficiency should be between 85–110% and R^2 should be ≥0.99 for the standard curves. Omit

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individual wells as needed and hit "Analyze" again. If the efficiency is below 70%, the R^2 is below 0.97, or the standard curves do not amplify properly the entire plate may need to be re-run. If more than 1 NTC well amplifies or if the extraction blank (EB) or method blank (MB) is above the method detection limit (MDL), the entire plate may need to be re-run. Discuss with supervisor or senior analyst. Compare the 3rd well spikes and SKETA assay controls with the samples to check for inhibition.

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

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

6.2. 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.

6.2.1. 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.

6.2.2. 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.

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

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

6.2.5. Vortex the plate for 5 seconds.

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

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

6.2.8. 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.

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

6.2.10. 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.

6.2.11. 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.

6.2.12. 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.

6.2.13. 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.

6.2.14. 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

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

6.3. 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.

7. QUALITY CONTROL

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

8. 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 Manuals and Software\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.

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