Quantifiler Trio Quantitation Setup using the QIAgility

Background

The Qiagen® QIAgility™ is a liquid handling robot that can perform automated setup of quantitation, amplification, normalization of sample extracts and capillary electrophoresis setup in efforts to reduce human error and allow laboratory personnel to use time elsewhere. The instrument uses carbon impregnated pipette tips and liquid-level sensing for precision pipetting and the QIAgility™ software provides the user with a clean and simple interface for ease of programming. The versatility of the robot permits pipetting from and into a variety of tube and plate conformations, allowing the instrument to work in tandem with other instrumentation in the laboratory.

Summary of Procedure

A Quantifiler® Trio quantitation setup protocol has been created for quantitation setup on the QIAgility™, including preprogrammed wells for master mix, diluent (Quantifiler® THP DNA Dilution Buffer) and Quantifiler® THP DNA Standard standards. Sample names are imported and the software is programmed to designate the sample wells that the instrument will pipette from and reaction wells the instrument will pipette into. In addition, the QIAgility™ has been programmed to perform a serial dilution of Quantifiler® THP DNA Standard standards and prepare master mix. The instrument is utilized in conjugation with the Quantifiler® Trio Quantitation Kit and sample extracts to perform quantitation setup into a 96-well half-skirted PCR reaction plate or 8-well optical strips.

Becoming Familiar with the Worktable

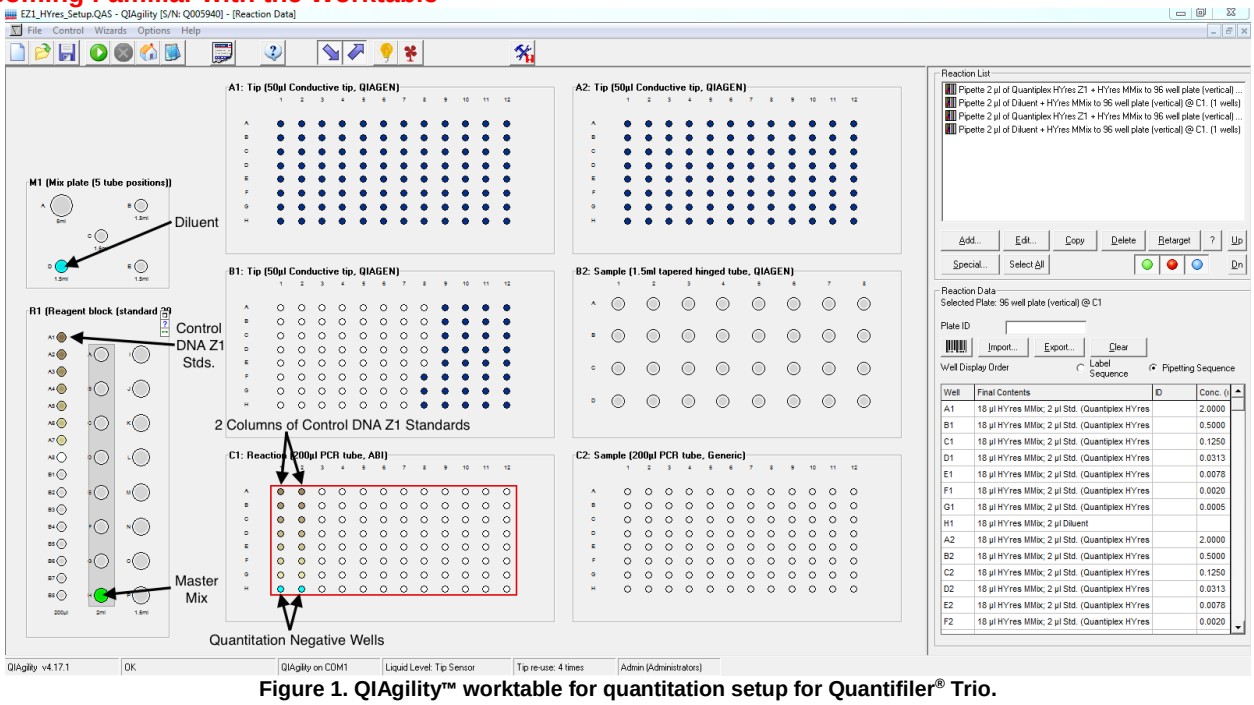

Figure 1. QIAgility™ worktable for quantitation setup for Quantifiler® Trio.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, *Forensic Evidence Handling*

After completion of the run, samples are to be securely stored at 2-8 °C.

Warnings and Precautions

Risk of personal injury is possible when the instrument lid is raised and the robotic arm is moving. Hands and arms should remain away from the arm when the lid is raised.

The instrument lid should remain closed and tip ejector must be covered when performing UV irradiation in the instrument. An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp and under no circumstances should this interlock be disabled.

DO NOT use bleach, solvents or reagents containing acids, alkalis (e.g. ELIMINase®), or abrasives to clean the QIAgility™.

Reagents and Materials

General Materials
Gloves
Lab Coat
Permanent Marker
Kimwipes
Vortex
Microcentrifuge
Protective Eyewear
Tube rack

QIAgility™ Specific Materials
Qiagen® QIAgility™
50 µl liquid-level sensing pipette tips
1.5 mL round-bottom screw-cap tubes (Diluent)
2.0 mL flat-bottomed screw-cap tubes (Master Mix)
Tip disposal box

Quantitation Specific Materials and Reagents
Applied Biosystems® **Quantifiler® Trio** Quantitation Kit:
- Quantifiler® THP PCR Reaction Mix
- Quantifiler® Trio Primer Mix
- Quantifiler® THP DNA Standard
- Quantifiler® THP DNA Dilution Buffer

10% bleach (or other decontaminate solution)
TE^{-4}
MicroAmp® Optical 96-Well Reaction Plate or MicroAmp® Optical 8-Tube Strip
MicroAmp® Optical 8-Cap Strips (for use with plates or 8-tube strips)
MicroAmp® Optical Adhesive Seal (for use with plates)
7500 Real-Time PCR System with SDS Software

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Quantifiler® Trio Quantitation Worksheet using the QIAgility™** and an appropriate **Sample Orientation Worksheet** is required for documentation. All information must be completed.

Positive Control

If a positive control was initiated during extraction, it should be added to the quantification plate.

Quantifiler® THP DNA Standard is the sample with known human and male DNA concentrations used to make the standard curve dilution series for quantification of unknown samples. The standard dilution series is set up on the reaction plate only once.

An internal positive control (IC) is included in the reaction mix. It is added to every sample at the same concentration and therefore has an expected C_t. The IC monitors for successful amplification and possibly for the presence of PCR inhibitors.

Negative Control

All extraction negative controls must be added to the quantification plate.

A quantification negative control will be setup to monitor for contamination in the Quantifiler® Trio reagents. This negative control consists of all reagents used in the procedure but contains no DNA sample. Quantifiler® THP DNA Dilution Buffer is added in place of a DNA sample.

Procedure

A. Opening the Quantifiler® Trio Protocol and Importing Sample Data

1. Turn on the instrument, followed by opening the software, in that order.
2. Select the “Recent” tab and double-click on “Quantifiler Trio TEMPLATE RUN” protocol.
    a. If the protocol is not visible in the recent tab, double-click on the “last used layout” in the “New” tab. Once the worktable opens, select “File” → Open and choose the “Quantifiler Trio TEMPLATE RUN” protocol.
3. Left-click on the sample block at B2.
4. Once the right-hand panel opens for the sample block, select the “Import” button.
5. In the “Import Well Data” window (figure 2), be sure that under column separator, “Comma” is selected.
6. Click on the small grey box next to “Import File” and browse for your *.CSV file.
7. Under “Import Options” verify that only “Load ID from column” and “Sample name from column” are checked. Set both as “from column 2.”

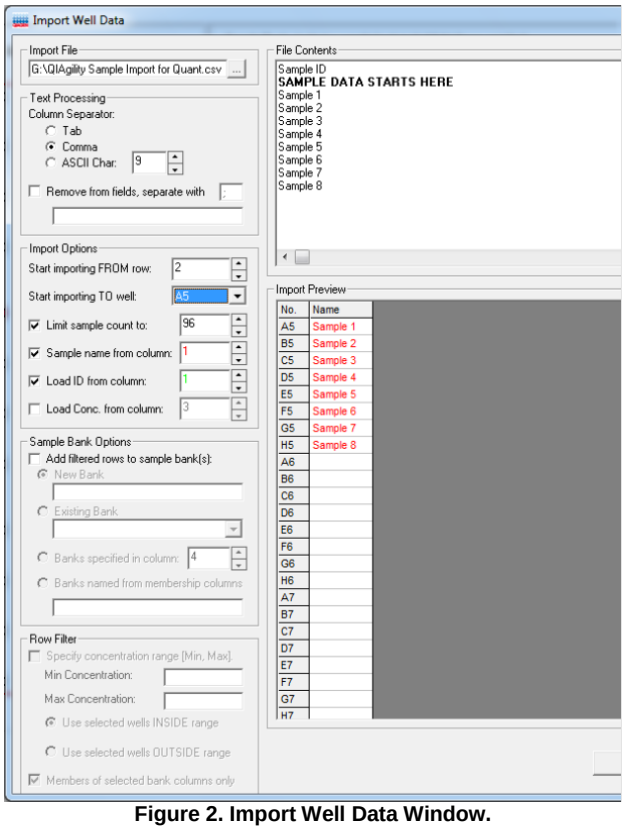

Figure 2. Import Well Data Window.

8. Select “Import” and then “Finish,” and Sample Names and ID’s should be present in the right-hand pane.

B. Adding Sample Wells to a Sample Bank

1. Left-click on the well(s) of interest in the sample block.
2. Right-click and select “Add selected wells to sample bank…”
3. Create a new sample bank for selected well(s) or choose from existing banks.
4. Select “Add selection and close.”
    a. To verify, the added well(s) should now be colored.

C. Remove Sample Wells from Sample Bank

1. To remove wells from sample bank, left-click well(s) of interest followed by right-clicking well(s) and selecting “Remove selected samples from sample bank…”
2. Verify that the correct existing sample bank is highlighted and choose “Delete selection and close.”
    a. To verify, the added well(s) should no longer be colored.

D. Adding Reaction Well to Reaction Plate

***Note that reaction wells A1 through F1 are already added to the reaction list as the serially diluted Quantifiler® THP DNA Standard standards and negative control. These wells can be moved (Procedure G) and duplicated (Procedure F).**

1. Left-click the Reaction Plate at block C1 on the worktable.
2. Select “Add…” on the right-hand reaction window, which will open up a reaction configuration window.
3. Select the desired sample bank you wish to add in the drop down menu.
4. Check the box next to Master Mix, “Mix A,” to include it in the final reaction (You should see the final volume read 18 µl).
5. Set the sample volume to 2µl and verify that the final volume reads 20 µl.
    a. If the final volume is not at 20 µl, check that everything is being added to the reaction well and proper volumes have been designated.

6. Select "OK" and verify that the well has gone from grey to colored. The color should correspond with the color of the sample well being taken from.
    a. In the reaction window on the right, final contents will be displayed for each reaction well under "Reaction Data."
7. Repeat from step one until all reaction wells have been established.

E. Skipping a Well

1. Left-click the Reaction Plate and select the "Add…" button in the right-hand window that appears.
2. Leave everything blank and verify that the sample drop-down window says "No samples (empty wells)."
    a. Verify that the "Mix A" is unchecked and the "Reagent" is set to zero.
3. To add more than one blank well in sequence, change the "number of wells," under "target wells."

F. Rearrange Well Order

1. Left-click on the Reaction Plate.
2. In the right-hand "Reaction List" that appears, select the reaction you wish to move.
3. Use the "Up" and "Down" buttons to move the reaction.
    a. Note that when reactions are moved in the reaction list, their destination well is also moved.

G. Duplicate Well

1. Left-click on Reaction Plate and select the reaction under the "Reaction List" you wish to duplicate.
2. Select the "Copy…" button under the lists of reactions.
3. Choose "Copy in place?" to copy the selected reaction under the current reaction or "Copy to end of list?" to copy the selected reaction to the bottom of the reaction list.

H. Verifying Enough Tips are Present and Adding Tips to the Worktable

1. Left-click on any tip designated plates.
2. A right-hand "tip info" window will appear and provide you with information regarding how many tips will be needed, how many are marked available and if enough are present.
3. If more tips are needed, add 50 µl tips to an empty tip rack.
    a. Highlight wells in the software that correspond to the wells that contain a tip in the tip rack.
    b. Right-click highlighted wells and select "set selected tips to 'Available'"
        i. These wells will now be dark blue in color.

I. Adding Master Mix, Quantifiler® THP DNA Standard and Quantifiler® THP DNA Dilution Buffer to the Worktable

1. In accordance with the quantitation worksheet, be sure you have enough volume for each of the reagents being used.
2. Vortex the Quantifiler® THP PCR Reaction Mix, vortex and centrifuge the Quantifiler® Trio Primer Mix tubes and add both to the designated location on the worktable (be sure to have at least the amount calculated above). Loosen the caps of each.
3. Add an empty 5 mL screw-cap tube to the designated "Master Mix" well on the worktable. This is the tube the Master Mix will be generated by the QIAgility™.
4. Briefly vortex and centrifuge the tube containing Quantifiler® THP DNA Standard. Load 25 µl of the stock standard and 25 µL of Quantifiler® THP DNA Dilution Buffer into the first well of an 8-well strip tube.
5. Load 40 µL Quantifiler® THP DNA Dilution Buffer into the sixth well of the 8-well strip tube. Cap the first and sixth well with an 8-well strip cap and place the 8-well strip tube on the Reagent Block at wells A1 to A8.
6. Vortex and centrifuge samples. Loosen caps and add sample to sample block B2 of the worktable in accordance with your imported data.
    a. If samples are in a 96-well format, vortex and centrifuge the plate. Remove coverings on plate and place the sample plate in sample block C2.
7. Add a new 96-well half-skirted PCR reaction plate or 8-well optical strip tube(s) to reaction block C1 on the worktable.
8. Remove tube caps from the Quantifiler® THP PCR Reaction Mix, Quantifiler® Trio Primer Mix, and Quantifiler® THP DNA Dilution Buffer and wrap them in a KimWipe®. Remove tube caps from the "master

mix" and standards, and toss them in the trash. Label sample tube caps if it has not been done. Remove sample tube caps and place them to the left side of the QIAgility™, wrapped in a KimWipe®.

J. Starting a Run

1. Check to see if the tip disposal box is emptied. If it has not been, empty used tips into a non-biohazard sharps container.
2. Double-check that the software and worktable are setup properly. Be sure that all tubes are in correct locations on the worktable, in accordance with the software.
3. Select the green play button, save your protocol and complete the checklist given. Select "OK" when finished and the run will begin.

K. After the Run

1. Save the post-run report, if desired.
2. Discard master mix and diluent tubes.
3. Cap Quantifiler® THP DNA Standard standards, label them and place them in a small manila envelope. The manila envelope should be labeled with the type of DNA standards ("QIAgility prepared Trio standards"), initials of preparer, preparation date, expiration date (one week from preparation date), lot number of kit and kit expiration date.
4. Recap sample tubes and store at 2-8 °C.
5. Use an optical seal or optical caps to conceal reaction wells of the 96-well plate or optical strip tube(s).
    a. The reaction plate is now ready to be run on the Real-Time 7500 System.
6. Empty tip disposal box into a labeled non-biohazard sharps waste bin.
7. Perform regular maintenance on the QIAgility™.

Comments on Storage

Store samples extracts at 2-8 °C after completion of the run.

Technical Assistance

For technical assistance and more information regarding the QIAgility™, visit the Technical Support Center at www.qiagen.com/goto/TechSupportCenter or contact the QIAGEN Technical Service Department at 1-800-362-7737.

Reference

1. Qiagen®. QIAgility® User Manual. June 2013.
2. Applied Biosystems®. Quantifiler® Trio DNA Quantification Kit. July 2014.