Quantifiler Duo DNA Quantification Work Instructions

Making the dilution series of the Human genomic DNA standard.

A dilution series is made (as needed) using the Quantifiler Duo DNA Standard (200 ng/µl) and the Quantifiler Duo DNA Dilution Buffer supplied with the kit. The standard dilutions used for amplification/quantitation are 50 ng/µl, 16.67 ng/µl, 5.56 ng/µl, 1.85 ng/µl, 0.62 ng/µl, 0.21 ng/µl, 0.068 ng/µl, and 0.023 ng/µl.

1. Remove the 200 ng/µl Quantifiler Duo DNA Standard from the +4°C refrigerator, vortex for 30-60 seconds, and then **very** briefly pulse-spin down the contents of the tube.

2. The 50 ng/µl standard is made by diluting the 200 ng/µl stock with Quantifiler Duo DNA Dilution Buffer. Mix **50** µl of the stock with **150** µl of DNA Dilution Buffer. For more accurate and precise results, use the same P-100 pipettor to aliquot the 50 µl and 100 µl volumes (i.e. 3 aliquots of 50 µl for the 150 ul volume). Vortex the mixture for 10-15 seconds, then briefly spin down.

3. Then mix **50** µl of the 50 ng/µl standard with **100** µl of DNA Dilution Buffer to make the 16.67 ng/µl standard (again using the same P-100 pipettor for dispensing both volumes). Vortex 10-15 seconds, then spin down.

4. Repeat step 3 for each subsequent dilution of the series substituting for the 50 ng/µl standard with the current dilution standard to make the subsequent dilution standard (i.e. use the 16.67 ng/µl to make the 5.56 ng/µl, the 5.56 ng/µl to make the 1.85 ng/µl, etc.).

5. The standard dilution series is stored at 2-8°C. The 200 ng/µl stock is stored with the kit at 2-8°C. The standard dilutions were stable for at least 2 months during the in-house validation. The expiration date on the standard dilutions will be 1 month from the preparation date. The slope, Y-intercept, and R^2 values of the standard curve of each run can be monitored for signs of degradation. A decrease in slope and/or increase in Y-intercept ct outside of acceptable ranges (see page 5 of this work instruction) could indicate degradation of the standard. Remake standard dilution, if appropriate. Document all QC testing using DNA QR-248.

Setting up the amplification reactions for the Quantifiler kits.

1. Use the **Quantifiler Duo DNA Quantitation Worksheet (QR-15a** (a copy is located on the U:\ Drive, instructions on how to use the optional macro elements of the worksheet are included with the worksheet) to locate the placement of samples in the 96-well block of the 7500 instrument and the number of reactions and solution volumes that will be needed. Print out and fill in the sheet as necessary.

2. Any sample less than 250 ng/5 µl (50 ng/µl) does not need to be diluted before amplification with the Quantification kits. If a sample is suspected of being greater than 50 ng/µl, a dilution of the sample can be used for quantitation (include dilution factor in sample name or note on worksheet). Samples greater than approximately 10 ng/µl can inhibit the Internal Positive Control (IPC) amplification somewhat.

3. In the PCR machine room, turn on the computer for the 7500 real-time instrument. Type in "administrator" for the username and "password" for the password at the logon prompt and wait till the computer has completely booted up before pressing the switch to turn on the 7500 instrument. At this point, you can perform steps 1 through 6 of the next section of the instructions (**Creating a plate document and running your samples**). Performing these steps now will allow you to start the run immediately after loading your prepared samples onto the 7500 instrument.

4. Remove the Reaction and Primer Mixes from the kit box stored in the 4°C refrigerator (amp room). Mix the **Primer Mix** (only) by vortexing the tube for 5-10 seconds, then briefly spin down. Mix the **Reaction Mix** by **swirling** for 5-10 seconds (**do not vortex**). Make a master mix of Reaction Mix (12.5 ul/reaction) and Primer Mix (10.5 ul/reaction). It may be necessary to make N+2 or N+3 reactions total (N=number of reactions). In addition to your samples, you will need 8 reactions for the standard dilution series and 1 reaction for a No Template/Negative Control (NTC/NEG). Vortex the master mix for 5 seconds and then spin down.

5. Place the 96-well Support Base (SUP-Base, **for 8-strip tubes**) or Splash Free 96-well Base (SF-Base, **for optical plates**) in the hood for use as a tube/plate rack. Remove the appropriate number of optical 8-strip tubes **OR** 96-well optical plate from their sealed plastic bag packaging and place them in the appropriate SUP- or SF-Base. If using 8-strip tubes, remove a packet of optical 8-strip caps from their sealed plastic bag packaging and place them in the hood, **careful not to place them on a surface that has been exposed to bleach or other contaminants that could be transferred to the 7500 instrument block during amplification**.

Suggestion: Using the 1.5 ml tube rack in the amp hood, prop up the SF-Base at an angle towards you so you can see better into the tubes or wells of the plate for pipetting. Make sure the

SF-Base is supported on both sides by the tube rack so there is no contact between the bottoms of the strip tubes or plate with the tube rack.

6. Pipet 23 µl of the master mix into each tube or well being used.

7. Pipet 2 µl of each standard or sample into the appropriate tube or well. For the NTC/NEG, use 2 ul of DNA Dilution Buffer, Milli-Q purified water or the Reagent Blank.

8. If using strip tubes, label each cap strip on the **underside** of the **tab at one end of the strip** (**doing it this way prevents transfer of marker ink to the heated lid or block of the 7500 instrument**). Then, place a cap strip on each tube strip by gently pressing on the cap at each end of the strip. Use the ABI tool designed for pressing caps onto strip tubes to get a good seal. Avoid using gloved fingers to press the caps onto the tubes as transfer of any chemical or liquid could obscure the optically clear caps. Transfer the plate or strips, **leaving behind the base in the amp hood**, to a base in the 7500 instrument room. **IMPORTANT! The Bases in the amp room should not enter the other room to prevent contamination of the pre-amp room with post-amp product**.

If you using an optical plate, remove a piece of optically clear tape from the box, peel off the backing from the sticky side, and place the tape (sticky side down) onto the top of the plate covering all of the wells. Use the ABI tool designed to seal the tape to the plate by pressing down and dragging it across the top of the tape from side to side and top to bottom, etc. Remove the excess tape from both sides.

9. Using other SF-Bases and strip tubes or a 96-well plate found on top of the 7500 instrument in the PCR machine room, balance your current SF-Base with tube strips or plate in the 96-well plate spinner and spin down your samples for at least 1 minute to remove bubbles produced during pipetting and to bring each sample to the bottom of the tube or well.

10. Open the 7500 instrument tray by pressing on the right side of the tray. Make sure the appropriate tray holder is in the instrument tray, there is one for optical strip tubes and one for 96-well plates. Each tray holder is labeled as to which it is designed for. 96-well plates fit into the tray with well A1 in the upper left. Place tubes in the tray holder in a **symmetrical pattern** to prevent the heated lid (which can tilt on a cam system) from tilting to one side or the other across the tube strips. Make note of the tube strip and sample locations so you can fill out the plate record properly.

<u>Creating a plate document and running your samples.</u>

1. Double click on the SDS software icon on the desktop to open the software.

2. Open the file **Duo Template.sdt** (or Quantifiler Template.sdt) from the **D:/SDS 1.2/Documents/Template** folder. From the **Setup/Plate** tab view open the **Well Inspector ('Ctrl' and '1' or double-click on any well)**. Referring to your filled out**QR-15a** worksheet, use the **Well Inspector** to select all the wells where you will be running samples and check off on all 3 detectors. Every sample well should have the **Duo Human**, **Duo Male**, and **IPC** detector checked.
3. Using the Well Inspector, select the column of wells containing the DNA Standards and change the task for both the Duo Human and Duo male detector to standard. Using the Well Inspector, select the sample well containing the **50** ng/µl standard and type in **50** for the Quantity entry for **both** the Duo Human and Duo Male. Repeat this process for the sample wells containing the **16.67**, **5.56**, **1.85**, **0.62**, **0.21**, **0.068**, and **0.023** ng/µl standards with the appropriate quantity entered for each (**both Duo Human and Duo Male**). Leave the sample well containing the No Template/Negative Control as an unknown. Using the Well Inspector, sample names can be added to each well.

4. Alternatively, the definitions of the standards, sample names and detectors can be edited on the plate after the run is complete, **as long as 1 detector was checked off for one well anywhere on the plate before the run was started**.

5. Once finished with entering the appropriate detectors, tasks, quantities, and names, go to the File menu at the top of the window and select **Save As**. **Change** the location to save the file from the **D:/SDS 1.2/Documents/Template** folder to **D:/SDS 1.2/Documents** folder by selecting the **Up One Level** icon. **Change** the name of the file from **Quantifiler or Duo Template** to the Batch name (i.e. XXX010101). Click **Save**.

6. The file name is now different than the one originally opened in this session, so to link this new file to the 7500 instrument, **close** the file (without closing the software) and then **reopen** it.

7. Click on the **Instrument** tab and click the **Start** button at the upper left. The run will take approximately 1 hour and 40 minutes to complete.

8. When the run is done a dialog box prompt will appear saying the run has completed successfully (provided there were no errors during the run). If the run was successful, click the **OK** button on the dialog box prompt.

9. Insert a thumb drive into the USB port on the right side of the laptop computer. Using "My Computer" navigate to the **D:/SDS 1.2/Documents** folder and select your file and **copy and paste** it to your thumb drive. Select the green arrow at the bottom of the main window to

remove your thumb drive safely. Alternatively, your file can be saved on the U drive. Close the SDS software, turn off the 7500 instrument, and shut down the laptop computer.

Analyzing the run data.

1. Plug the thumb drive containing your run file into the computer where you will be doing the analysis. **Cut and paste** the file from the thumb drive to an appropriate folder on your hard drive/desktop. Open the SDS software on the computer and then open your file.

2. If you did not do so before starting the run, enter sample names, tasks, detectors and/or quantities. To do this, click on the **Results** tab then the **Plate** tab. Double click on any well to open the **Well Inspector** and modify wells as needed.

3. Select the **Results** tab then select the **Amplification Plot** tab. Make sure the analysis settings are **Manual Ct**, **Threshold** set to **0.2000**, and **AutoBaseline**. Click on the larger green arrow icon shortcut at the top of the window to analyze the data at the current settings. The horizontal threshold line should change from red to green.

4. Select the **Standard Curve** tab. Make sure to check the standard curve for both **Duo Human** and **Duo Male**. Check to make sure the slope is close to the optimal **-3.32** and the **R^2** value is > **0.98** (the Duo Male curve slope is typically more negative than the Duo Human curve slope). Note the intercept values for both curves. Print each curve separately, so the slope, Y-intercept, and R^2 values appear on the printed page. Based on internal validation and run data, the Y-intercept Ct values for the Duo Human and Duo Male, standard curves should be 29.3 ± 1.4 and 30.1 ± 1.2, respectively. Based on internal validation and run data, the standard curve slopes for the Duo Human and Duo Male should be -3.48 ± 0.41and -3.52 ± 0.56, respectively. If the Y-intercept or slope values fall outside these ranges, consult with the Technical Leader for approval. Individual standards may be removed from the standard curve to attain improved values for the slope, Y-intercept, and R^2. To remove a standard from the curve, open the **Well Inspector** from the **Plate** tab (under the **Results** tab) and change the task for that standard to "**unknown**".

5. Click on the **Report** tab. Then go to **Tools** in the menu header and select **Report Settings**. Select **Portrait** for **Report Orientation**. Select **Show gray/white rows** and change the **# of White/Gray rows** each to **3**. Uncheck all **Data Columns** except **Well Number**, **Sample Name**, **Detector**, **Task**, **Ct**, and **Quantity**. Uncheck all **Graphs to Print** and **Additional Data to Print**. Click **OK**.

6. Select the wells containing your standards and question samples. Go to **File** in the menu header and select **Print Preview**. Check and **Print**. **Save** the file before closing.

7. Write down any comments you feel necessary regarding your quantitation analysis on the **Quantifiler Duo DNA Quantitation Worksheet (QR-15a)** (e.g., the removal of a sample from the standard curve).