1. Preparing the DNA Standards

1.1. Label eight microcentrifuge tubes: Std. 1, Std. 2, Std. 3, etc.
1.2. Add required amount of TE/glycogen buffer to each tube (See table below)
1.3. Thaw the Quantifiler Human DNA Standard and vortex for 3-5 seconds.
1.4. Add the calculated amount of Quantifiler Human DNA Standard to the tube for Std. 1 and vortex.
1.5. Add the calculated amount of the Std. 1 to the buffer in Std. 2 tube and vortex.
1.6. Keep repeating this until all standards are made.

NOTE: Be very careful when making the DNA standards. Precise pipetting is of utmost importance. Any poor pipetting will skew your results.

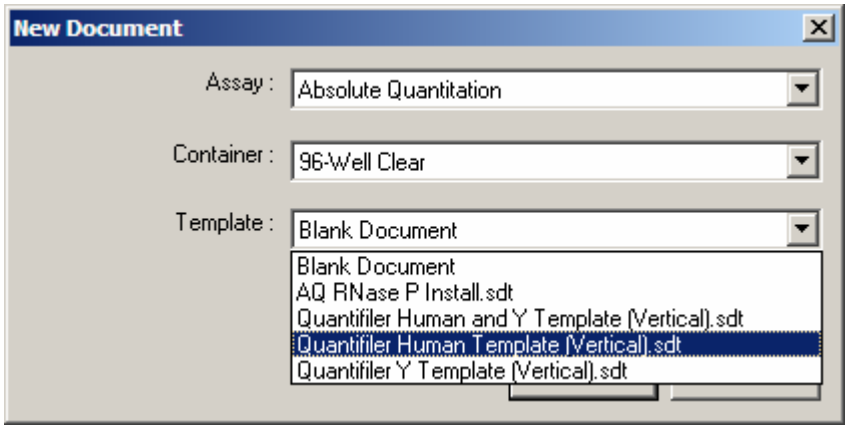
Standard	Concentration (ng/ul)	Example Amounts	Minimum Amounts	Dilution Factor
Std. 1	50.000	50ul [200ng/ul stock] + 150ul TE/glycogen buffer	10ul [200ng/ul stock] + 30ul TE/glycogen buffer	4x
Std. 2	16.700	50ul [Std. 1] + 100ul TE/glycogen buffer	10ul [Std. 1] + 20ul TE/glycogen buffer	3x
Std. 3	5.560	50ul [Std. 2] + 100ul TE/glycogen buffer	10ul [Std. 2] + 20ul TE/glycogen buffer	3x
Std. 4	1.850	50ul [Std. 3] + 100ul TE/glycogen buffer	10ul [Std. 3] + 20ul TE/glycogen buffer	3x
Std. 5	0.620	50ul [Std. 4] + 100ul TE/glycogen buffer	10ul [Std. 4] + 20ul TE/glycogen buffer	3x
Std. 6	0.210	50ul [Std. 5] + 100ul TE/glycogen buffer	10ul [Std. 5] + 20ul TE/glycogen buffer	3x
Std. 7	0.068	50ul [Std. 6] + 100ul TE/glycogen buffer	10ul [Std. 6] + 20ul TE/glycogen buffer	3x
Std. 8	0.023	50ul [Std. 7] + 100ul TE/glycogen buffer	10ul [Std. 7] + 20ul TE/glycogen buffer	3x

2. Setting up Quantifiler Reactions

2.1. Calculate the amount of reagents needed for the number of samples you are analyzing by using the ABI Quantifiler Setup Worksheet.
2.2. Save the sample setup by copying and pasting into a notepad file and saving the file on the CODIS Laptop that you will be using.
2.3. Vortex the Quantifiler Human Primer Mix for 3-5 seconds and then centrifuge briefly before opening tube.
2.4. Swirl Quantifiler PCR Reaction Mix gently and mix by pipetting up and down a few times before using.
2.5. Pipette the proper amounts of the reagents into a polypropylene tube.
2.6. Vortex the mixture for 3-5 seconds and then centrifuge briefly.
2.7. Dispense 23ul of the reagent mixture into each reaction well.
2.8. Vortex your DNA samples for 3-5 seconds, centrifuge briefly, then add 2ul of the DNA sample into the corresponding reaction well. (For negative control add 2ul of TE buffer)
2.9. Seal the reaction plate with the optical adhesive cover.
2.10. Centrifuge the plate at 2000rpm for 2 minutes and check for bubbles at the bottom of each well.

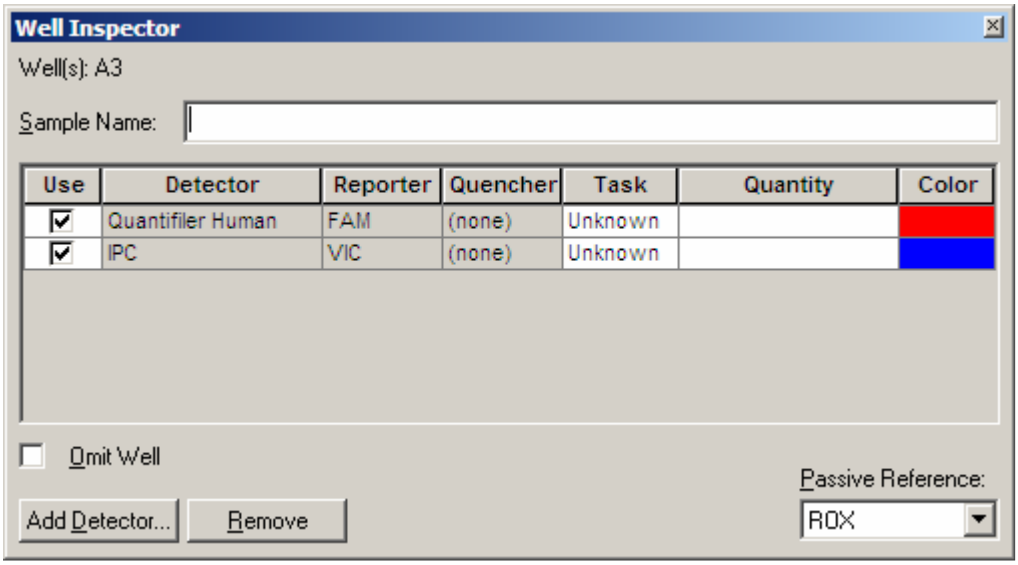
3. Setting up a Plate Document

3.1. Turn on the ABI 7000
3.2. Open up ABI PRISM 7000 SDS Software
3.3. Click File and choose New
3.3.1. A box will appear in the middle of the screen that looks like this:



3.3.2. Select the appropriate template for your reactions.
3.3.3. Click on File in the menu bar and click on Import Sample Setup
3.3.4. Choose the file you saved on that computer from step 2.2

3.3.5. Click on View in the menu bar and click on Well Inspector. Highlight all the wells that you are not using and click Omit Well box in the Well Inspector



3.3.6. After completing the plate setup, click on the Instrument tab and check the settings. The settings should look like this:

	Stage 1	Stage 2	
# of cycles (Reps)	1	40	
Temp. (Celsius)	95	95	60
Time	10 minutes	15 seconds	1 minute

3.3.7. Also make sure that the sample volume is set to 25ul and that the box next to “9600 Emulation” is checked

3.3.8. Click File on the menu bar and click Save as to save and name your document (this must be done before the machine will start collecting Data

4. Operating the ABI PRISM 7000

4.1. Lift handle at bottom of the door and push the door all the way to the back until it locks into place.

4.2. Place compression pad over the optical adhesive cover with the brown side facing up and the holes in the compression pad corresponding to the wells in the reaction plate.

4.3. Place the reaction plate into the plate holder with well A1 in the top left corner.

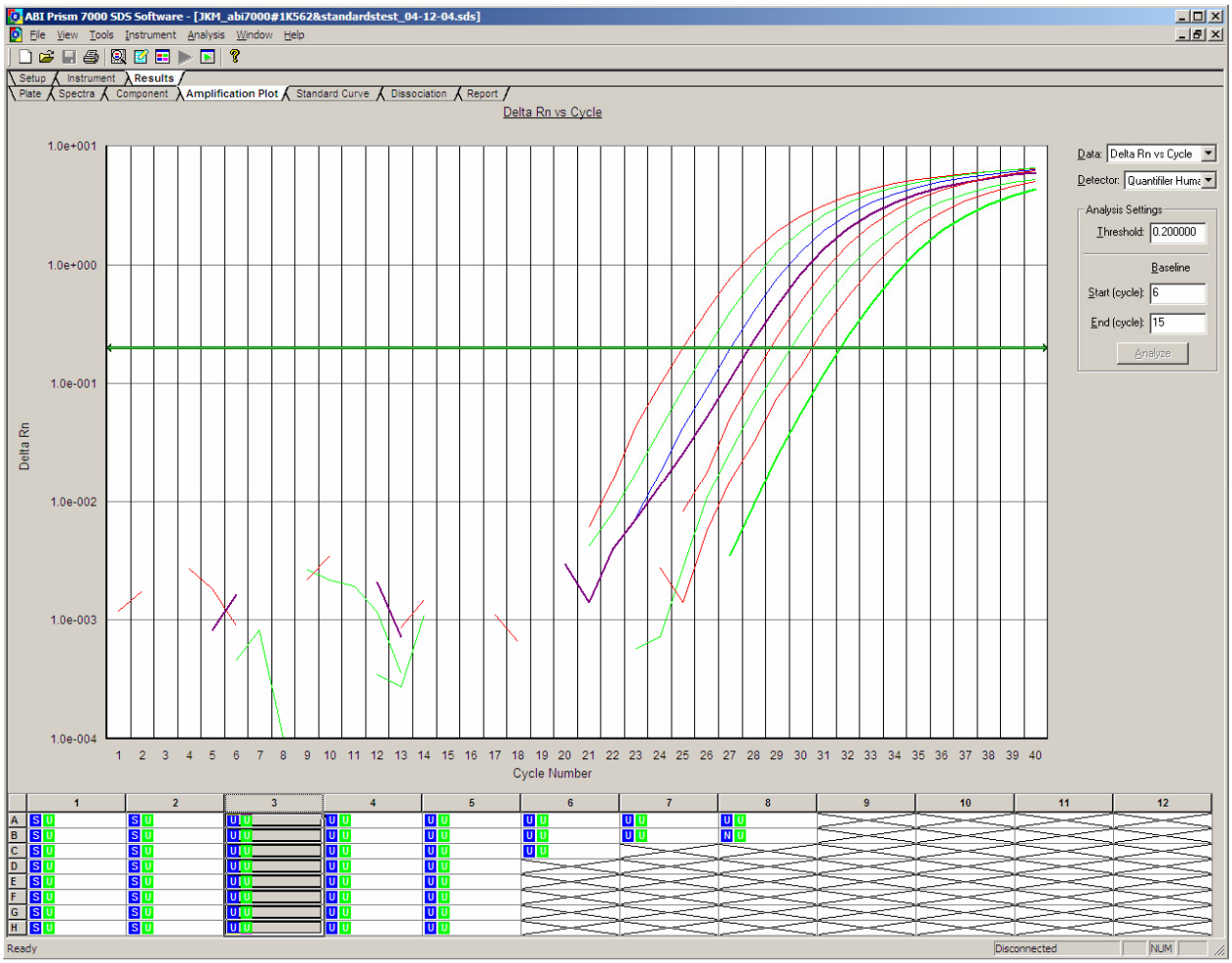
4.4. Close the machine

4.5. Click the start button (within the instrument section) on the right-hand side of the screen. It takes one hour and forty-six minutes for the PCR to run and give the results.

5. Analyzing Data in SDS Software

5.1. When the ABI 7000 run is finished, click on the Analyze button (green arrow in the tool bar).

5.2. Click the Results tab and highlight all the wells to see your results. This will show your quantity results in a plate format.



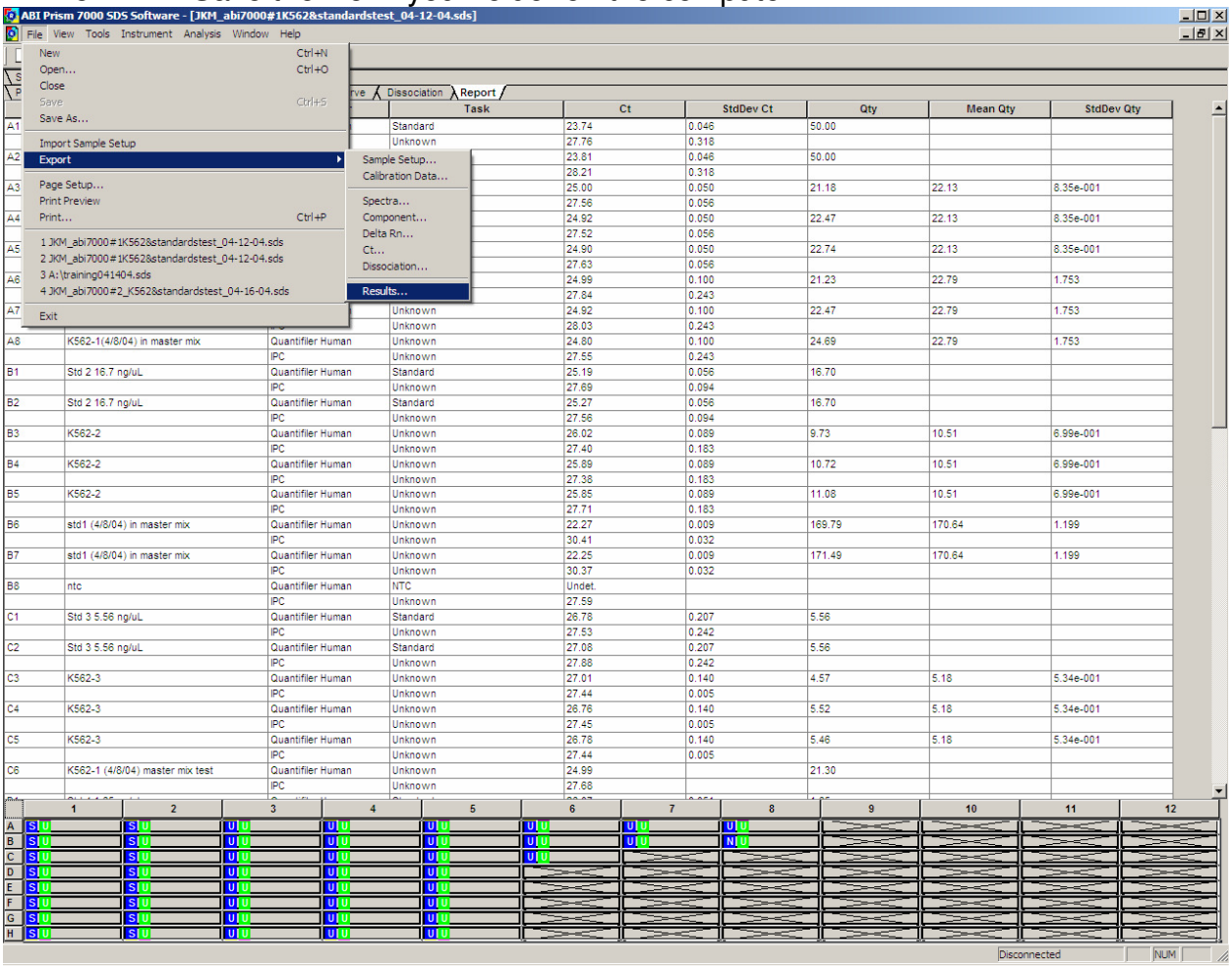
5.3. Click on the Amplification Plot tab and check all of your data to make sure there is no contamination in your negative control and that the internal positive control (IPC) reaction worked in each well.

5.4. Click on the Standard Curve tab and check the values of your slope and R2. The slope should be between -2.9 and -3.3, while the R2 value should be greater that 0.98. The intercept just indicates what the Ct value of a 1ng/ul sample would be for that run.

5.5. Click on the Report tab.

5.6. Click the File button, go to Export, and click Results.

5.7. Save the file in your folder on the computer.



6. Analyzing data in Microsoft Excel

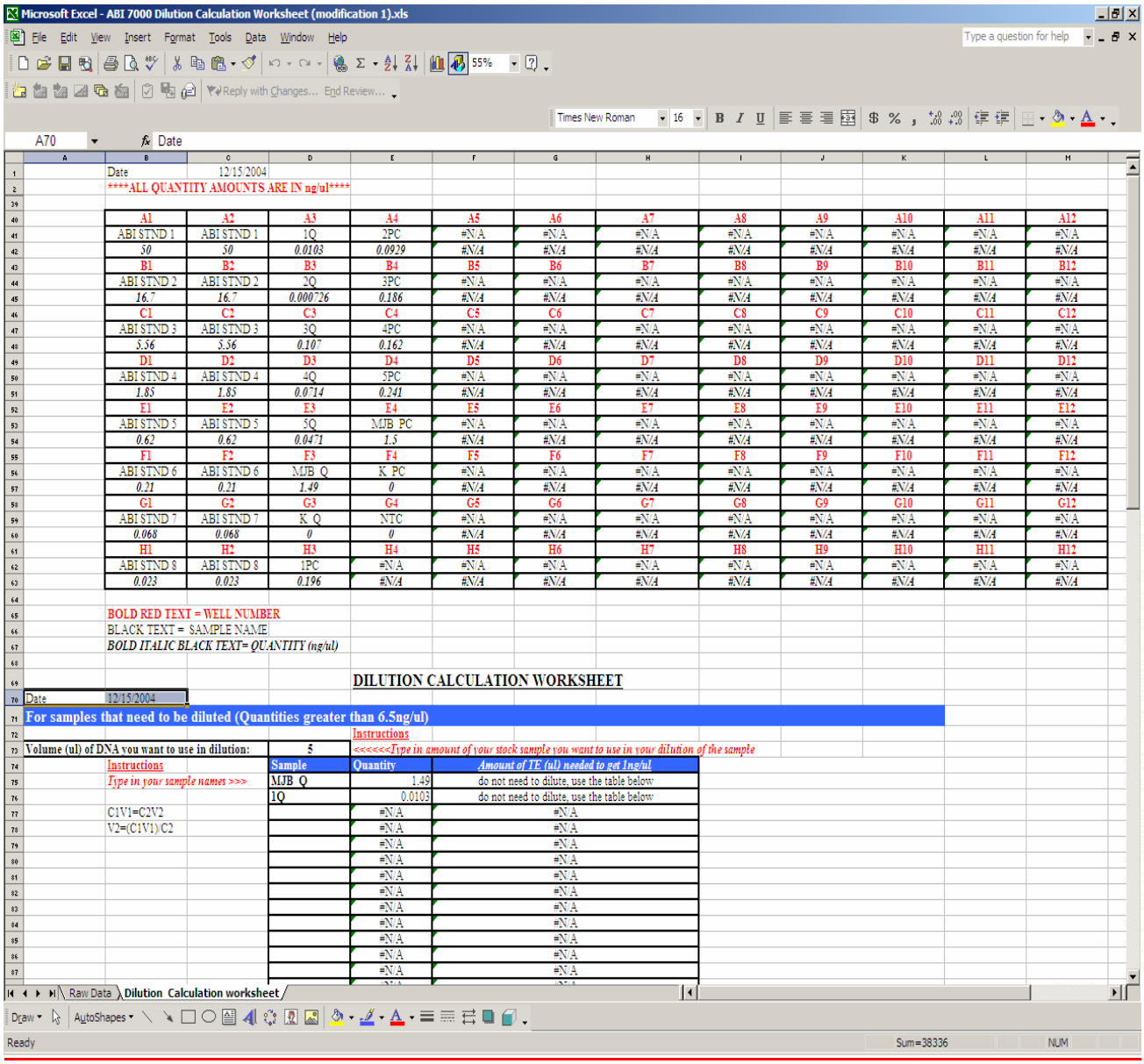
6.1. Open your results document.

6.2. Open the ABI 7000 Dilution Calculation Worksheet

6.3. Select all the data on your results document by pressing Ctrl + A.

6.4. Click on cell A1 of the Results section of the ABI 7000 Dilution Calculation Worksheet and paste your results

6.5. Click on the Dilution Calculation Worksheet tab and you will see your results in plate format.



6.6. Type in the amount of your sample you want to use for the dilution of your sample to get 1ng/ul, then type in your sample names in the spreadsheet according to whether you will need to dilute that sample or not. (There needs to be approximately 1ng of DNA in the Identifiler reaction, therefore samples that

are~6.5ng/ul and lower do not need to be diluted.) The spreadsheet will automatically calculate how much TE you need to add to you dilution or how much of your DNA you need to add to your Identifiler reaction.

6.7. Print out your results on the calculation worksheet and use it to prepare your samples for Identifiler amplification reactions

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Revision History		
Effective Date	Revision Number	Reason
Revision History		
December 23, 2004	00	Original Document

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	