1. Preparing the DNA Standards

1.1. Label eight microcentrifuge tubes to be used for dilution of standards (i.e. 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 Quantifier 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, vortex and spin briefly in a centrifuge.
1.5. Add the calculated amount of the Std. 1 to the buffer in Std. 2 , vortex and spin briefly in a centrifuge.
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.

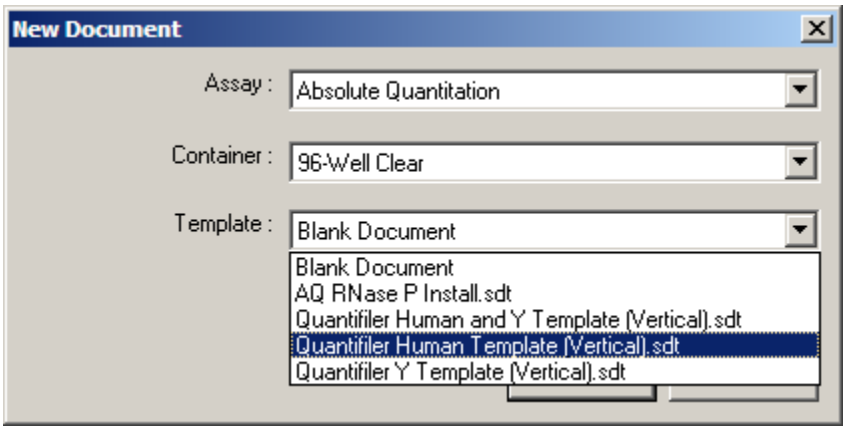
Standard	Concentration (ng/ul)	Example Amounts	Minimum Amounts	Dilution Factor
Std. 1	50.000	50ul [200ng/ul stock] + 150ul TE/glycogen buffer	10ul [200ng/ul stock] + 30ul TE/glycogen buffer	4x
Std. 2	16.700	50ul [Std. 1] + 100ul TE/glycogen buffer	10ul [Std. 1] + 20ul TE/glycogen buffer	3x
Std. 3	5.560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

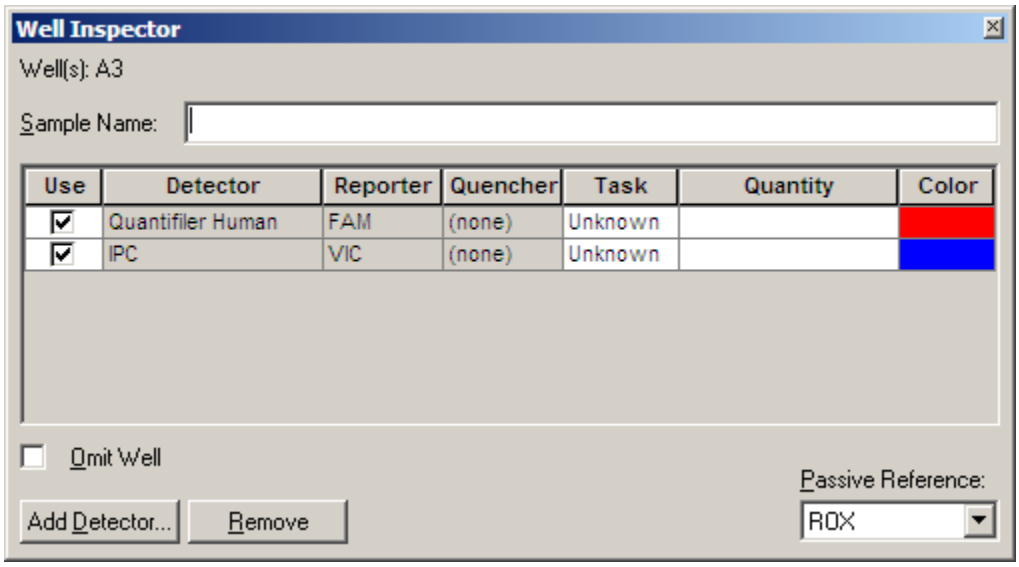
2.1. Calculate the amount of reagents needed for the number of samples you are analyzing by using the ABI Quantifiler Setup Worksheet.
2.2. Save the sample setup by copying and pasting into a notepad file and saving the file on the CODIS Laptop that you will be using.
2.3. Vortex the Quantifiler Human 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 sterile microcentrifuge tube.
2.6. Vortex the mixture for 3-5 seconds and then centrifuge briefly.
2.7. Dispense 23 µl of the reagent mixture into each reaction well.
2.8. Vortex the DNA samples and standards for 3-5 seconds then centrifuge briefly.
2.9. Add 2 µl of the DNA sample into the corresponding reaction well. For the NTC (negative control) add 2 µl of TE buffer.
2.10. Add 2 µl of the DNA standard into the corresponding reaction well. The DNA standards shall be run in duplicate.
2.11. Seal the reaction plate with the optical adhesive cover. Use plastic tool to seal optical cover in place. Tear off white edges.
2.12. Centrifuge the plate at 2000 rpm 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 the "Omit Well" box in the Well Inspector (optional).



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 the handle at the 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.

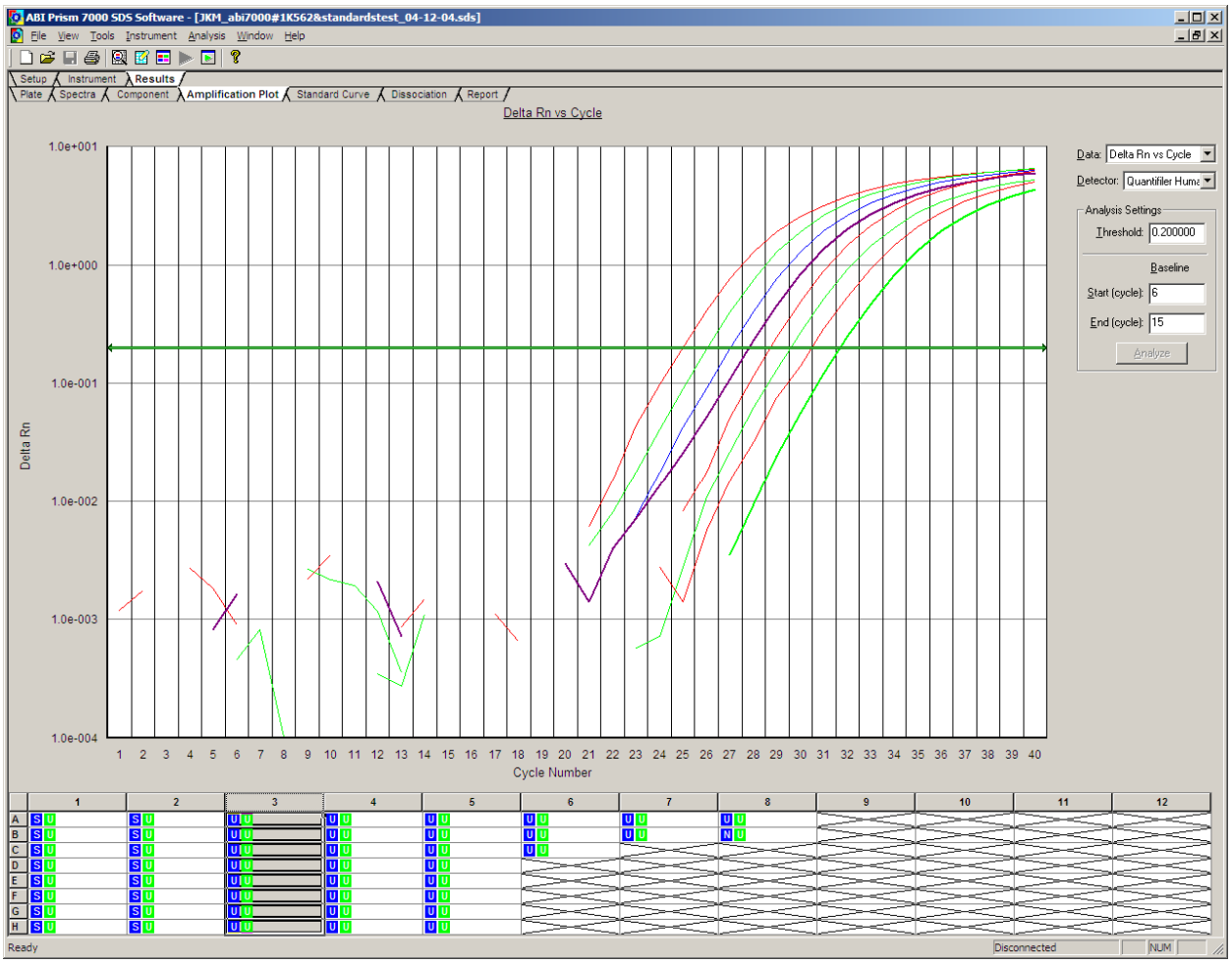
4.4. Close the machine door.

4.5. Click the start button (within the instrument section) on the right-hand side of the screen. It takes approximately 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.



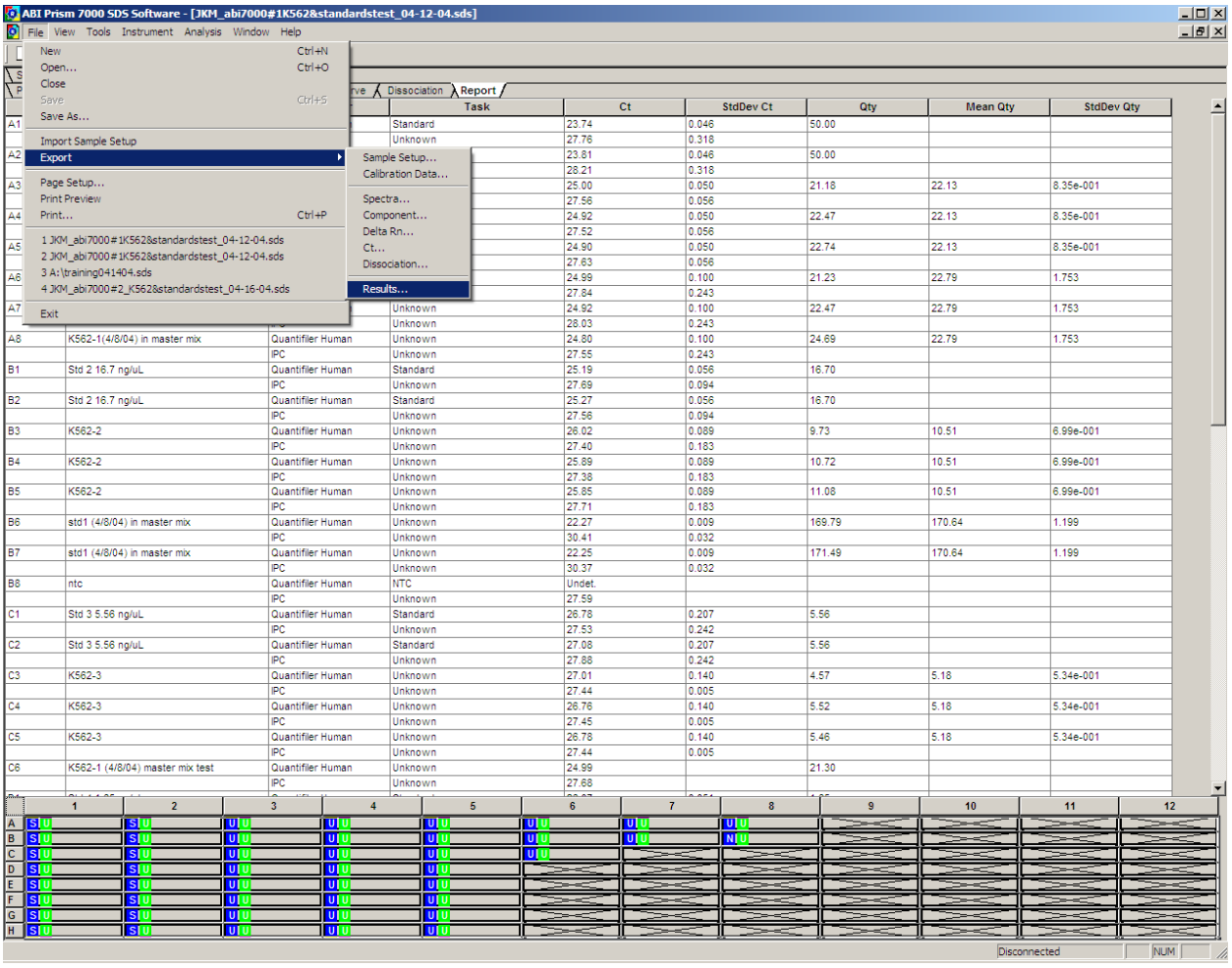
5.3. Click on the Amplification Plot tab and check all of your data to make sure there is no unacceptable activity in the negative control and that the internal positive control (IPC) reaction worked in each well.

5.4. Click on the Standard Curve tab and check the values of your slope and R^2. The slope should be between -2.9 and -3.3, while the R^2 value should be greater that 0.98. If the value falls outside of this range, then one point of the slope may be dropped to account for pipetting variations. If a point is dropped, then both the original and adjusted slopes must be printed and placed in the case notes. The intercept indicates what the Ct value of a 1ng/µl sample would be for that run.

5.5. Click on the "Report" tab.

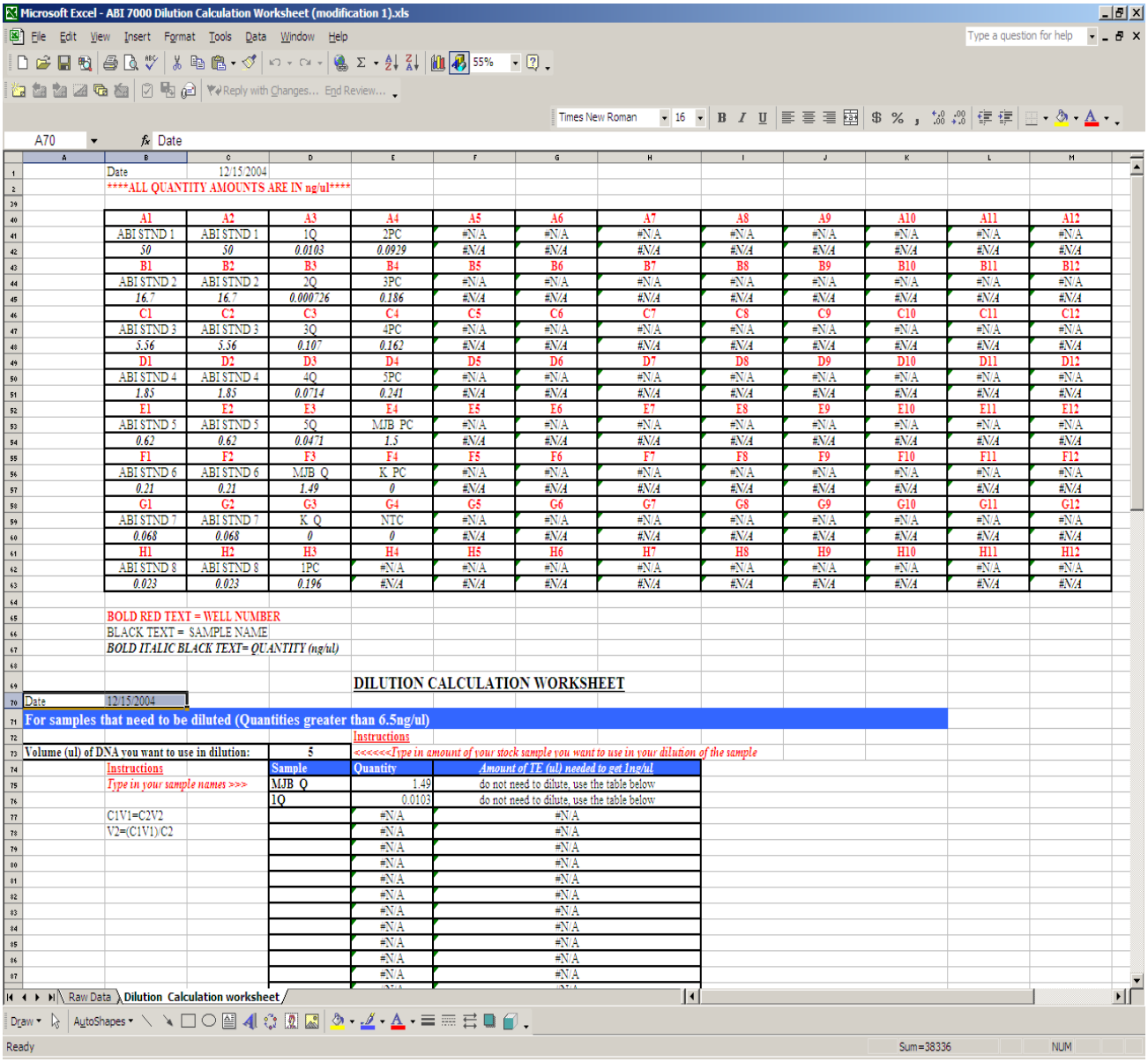
5.6. Click the "File" button, go to "Export", and click "Results".

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



6. Analyzing data in Microsoft Excel

6.1. Open the exported 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.



Microsoft Excel - ABI 7000 Dilution Calculation Worksheet (modification 1).xls

Date 12/15/2004

****ALL QUANTITY AMOUNTS ARE IN ng/ul****

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
ABI STND 1	ABI STND 1	1Q	2PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
50	*50*	*0.0103*	*0.0939*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
ABI STND 2	ABI STND 2	2Q	3PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
16.7	*16.7*	*0.000726*	*0.186*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
ABI STND 3	ABI STND 3	3Q	4PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
5.56	*5.56*	*0.107*	*0.162*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
ABI STND 4	ABI STND 4	4Q	5PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
1.85	*1.85*	*0.0714*	*0.241*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
ABI STND 5	ABI STND 5	5Q	MJB_PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
0.62	*0.62*	*0.0471*	*1.5*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
ABI STND 6	ABI STND 6	MJB_Q	K_PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
0.21	*0.21*	*1.49*	*0*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
ABI STND 7	ABI STND 7	K_Q	NTC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
0.068	*0.068*	*0*	*0*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
ABI STND 8	ABI STND 8	1PC	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A
0.023	*0.023*	*0.196*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*	*#N/A*

BOLD RED TEXT = WELL NUMBER

BLACK TEXT = SAMPLE NAME

BOLD ITALIC BLACK TEXT= QUANTITY (ng/ul)

DILUTION CALCULATION WORKSHEET

Date 12/15/2004

For samples that need to be diluted (Quantities greater than 6.5ng/ul)

Instructions

Volume (ul) of DNA you want to use in dilution: 5 <<<<<<Type in amount of your stock sample you want to use in your dilution of the sample

Instructions	Sample	Quantity	*Amount of TE (ul) needed to get 1ng/ul*
Type in your sample names >>>	MJB_Q	1.49	do not need to dilute, use the table below
	1Q	0.0103	do not need to dilute, use the table below
C1V1=C2V2		#N/A	#N/A
V2=(C1V1)/C2		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A
		#N/A	#N/A

Raw Data | Dilution Calculation worksheet

Ready Sum=38336 NUM

6.6. Type in the volume of the sample you want to use to dilute the sample to 2ng/µl, 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 ~10 ng/µl 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
December 23, 2005	01	Minor wording changes for clarification; Section 2, added information about running standards in duplicate; Section 6, changed cut-off from 6.5 ng/µl to 10 ng/µl.
January 30, 2007	02	Minor wording changes for clarification; Section 5, allow analyst to drop point on standard curve.

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