

SDS Compendium

7700 - Ver 3.0

This is an e-mail distributed document in Acrobat (.pdf) format with information on the ABI PRISM® 7700 Sequence Detection Systems from Applied Biosystems in Australia and New Zealand. You are welcome to pass it on to others who may be interested. If you received it indirectly and would like to receive updates, e-mail me at "maselam@appliedbiosystems.com" with "SDS Compendium subscribe" as the subject or body text. If you wish to unsubscribe send me a similar message with "SDS Compendium unsubscribe".

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Introduction

This compendium provides summarised procedures for setting up, operating and analysing data generated from the ABI PRISM® 7700 Sequence Detector. It is intended to get you up and running quickly as well as answer some commonly asked questions. Please refer to your ABI PRISM® 7700 Sequence Detection System Users Manual for more detailed information.



Materials and Methods

You should be amplifying your DNA template with your specific primers and TaqMan fluorescent probe using the TaqMan Universal PCR Master Mix (Part No. 4304437). This is supplied in 2 × concentration and contains heat-activated AmpliTaq Gold DNA Polymerase, AmpErase UNG for carryover prevention, dNTPs with dUTP, Passive Reference dye, and optimised buffer components.

Working Reagents

TaqMan Universal PCR Master Mix	2 × stock
Forward primer	9 μM (see (b) below)
Reverse primer	9 μM (see (b) below)
TaqMan probe	2.5 μM (see (a) below)
Water	ddH ₂ O or MQ
DNA sample	2-20 ng/μl (gDNA, cDNA) or 10 ⁶ -10 ⁷ targets/μl (clones)

Equipment

1. Optical tubes (N801-0933), caps (N801-0935) or Adhesive covers (4313663)	5. Setup racks
2. Eppendorf tubes	6. Pens
3. Pipetmans: P20 and P200	7. Gloves
4. Pipette tips: filter-tipped 10–200 μl	8. Benchtop microcentrifuge

Precautions

Use accurate pipetting technique

Wear gloves when handling all reagents and tubes to avoid contamination

Filtered tips should always be used to avoid aerosol contamination

If possible, use screw cap (O-ring) sealed tubes for all primers, probes and stock nucleic acid solutions to guard against possible cross contamination when opening tubes

Discard all used tips, tubes and gloves into the pathological waste

Keep tubes on ice

Setup Protocols

(a) Probe dilution

1. Your TaqMan probe is usually supplied as 60 μl of a 100 μM solution (confirm this on your Probe specification sheet) and must be diluted to a 2.5 μM working reagent.
2. Pipette 30 μl into each of 2 screwcap tubes.
3. Add 1.17 ml of ddH₂O to each of these tubes.
4. Close cap, mix by inversion 2–3 times and spin briefly.
5. Pipette 120 μl aliquots of your working probe reagent into 20 \times 1.5 ml tubes.
6. Store these probe aliquots at -70°C .

(b) Primer dilution

1. Your primers are supplied desiccated and must be diluted to 9 μM for use as working reagent. (The primers final concentration will be 900nM after pipeting out your Mother Mix volumes)
2. Resuspend each primer separately in 100 μl of ddH₂O. For example, if you had 10,000 pMol of primer it will now be at a concentration of 100 μM . Calculate the concentration of your solutions.
3. Using the following example calculation, you will need to add X μl of your resuspended primer in a total of 150 μl of ddH₂O:

$$X \mu\text{l} = \frac{\text{final vol } (\mu\text{l}) \times \text{final primer stock } (\mu\text{M})}{\text{primer yield (pMol) / resuspend vol } (\mu\text{l})^*} = \frac{150 \mu\text{l} \times 9 \mu\text{M}}{(10,000 \text{ pMol} / 100 \mu\text{l})^*} = 13.5 \mu\text{l}$$

*Substitute your concentration following resuspension in 100 μl of ddH₂O (from 2. above)

4. Now mix X μl of your primer with (150 – X) μl of ddH₂O.
5. Repeat this process for your second primer.
6. You will now have one 150 μl aliquot for each of your primers for immediate use. The remaining concentrated stock primer solutions can be left for you to take away with you and store at -70°C .

(c) Sample Dilution Series Preparation

1. Label 5 Eppendorf tubes with your name and number them 1 to 5.
2. Pipette 45 μl of ddH₂O into each tube and stopper each tube (to prevent contamination by aerosols from more concentrated solution).
3. Pipette 5 μl of your stock DNA template into tube #1. This is your first ten-fold dilution.
4. Mix carefully and spin solution down for 5 seconds in a benchtop microcentrifuge.
5. Pipette 5 μl from tube #1 into tube #2.
6. Mix and spin tube #2. This is your second ten-fold dilution.
7. Continue this process until tubes #3 and #4 have had DNA added. Leave tube #5 free of DNA (add water only) as this will be used for your No Template Controls (NTCs).
8. Leave these samples on ice until needed.

(d) PCR Setup

1. Assemble reagents in the rack provided. Thaw and mix any frozen reagents.
2. The 7700 plate will be set up complete with 8 rows of 12 tubes.
3. You will prepare duplicate tubes with the No Template Control (NTC) and each of your 5 dilutions (total of X tubes). Prepare a PCR Mother Mix (MM) as follows:

Reaction Component	x MM (µl)	13 x MM (µl)	Final Sample Vol. (µl)	Final Conc.
TaqMan Univ. PCR Master Mix (2x)		325	25	1x
Forward primer (9 µM)		65	5	900 nM*
Reverse primer (9 µM)		65	5	900 nM*
TaqMan probe (2.5 µM) Fam		65	5	250 nM*
DNA sample		–	5	–
Water		65	5	–
Total		585	50	–

* These are Applied Biosystems recommended Universal Concentrations but you should optimise both the Primers and the Probe concentrations as outlined in the Master Mix Protocols

4. Prepare the mother mix for X x 50 µl samples as indicated in the previous table.
5. Pipette 45 µl of this mother mix to the bottom of each of X PCR tubes in the 7700-plate row or column that you have been assigned.
6. Pipette 5 µl of water (No Template Controls) or DNA serial dilution sample as appropriate. Prepare the NTC tubes first, followed by your dilution series in order of decreasing dilution (Dil.).

Row # _____ Column # _____

1	2	3	4	5	6	7	8	9	10	11	12
Stock DNA	Stock DNA	Dil. #1	Dil. #1	Dil. #2	Dil. #2	Dil. #3	Dil. #3	Dil. #4	Dil. #4	NTC #5	NTC #5

7. Seal tubes with the strip caps or a plate with an Adhesive Cover. The reactions are now ready for thermal cycling. **Optical Adhesive Covers can only be used with plates.**
8. Put the 7700 plate into the 7700 Sequence Detector. Close the lid and click the run button.
9. The universal thermal cycling protocol is as follows:

Step	Temperature (°C)	Time	
1. Carryover decontamination via UNG	50	2 min	
2. AmpliTaq Gold pre-activation	95	10 min	
3. Melting step	95	15 sec	x 40 cycles
4. Anneal/Extend step (combined)	60	1 min	

Setup & Analysis of a TaqMan[®] Run

Running the 7700 SDS

1. The 7700 SDS must be switched on before the computer at least **30 min** before run, but we recommend leaving the instrument on at all times.
2. You must **Re-Start the Mac** computer before each run.
3. Double click on the **Sequence Detector 1.7 icon**.
4. **Open new plate** (under “file”) if new plate hasn’t opened automatically.
5. **Select Reporter Dye** (Default is **FAM**, so this step isn’t necessary if using **FAM**). If you are multiplexing (also using **VIC**) a second Dye layer must be set up.
6. **Label plate** – i.e. NTC, Standards, Unknowns
 - To do this, select cells and then select label found under “**Sample Type**”.
 - Also label cells with a “**Sample Name**”.
 - Type in “**Quantity**” (necessary for standards).
 - Can also specify if samples are “**Replicates**” (see page C-6 of User Manual).
7. Go to “**Thermal Cycling Conditions**”.
 - Specify Extension conditions.
 - Specify Reaction volume.
 - Go to “**Show Data Collection**” – collect all Data points.
 - Must always have 50⁰C for 2 min (Activation of UNG) and 95⁰C for 10 min (Activation of AmpliTaq Gold and deactivation of UNG).
8. Add a “**Comment**” as to what the run is about.
9. “**Save Plate**” (“**Save As**” found under “File”).
10. Click on “**Show Analysis**” to view the Analysis screen.
11. If using an **Adhesive Cover** go to “**Instrument**”, “**Diagnostics**”, “**Advanced Options**” and select the checkbox – “**Set 7700 Exposure Time for Plates**” and change the time from 25 to **10 (Very Important)**.
12. Place sample tubes or plate in 7700 SDS.
13. Press “**Run**” (when cover temp reaches 105⁰C, the 7700 will beep & you will hear the shutters open).
14. “**Time Remaining**” of run is displayed on the screen.

7700 Data Analysis

1. When the run has finished, **Save your Data immediately.**
2. Analyse Data (under “**Analysis**”). To View Data you must always be in the Analysis screen.
3. Select wells to view & go “**Analysis**” & select either “**Amplification Plot**”/“**Standard Curve**” etc.
4. Curves can be viewed in either log or linear form. Double click on the y-axis to change this view.
5. It is also possible to re-label samples as standards after a run is complete:
 - Go to “**Set-up Screen**”.
 - Re-label samples as standards.
 - Give each sample a quantity value— very Important!
 - Go back to “**Analysis**” screen and **re-analyse** data.
 - Now it is possible to quantify unknown samples compared to the nominated standards.
6. **Make sure for complete and correct analysis of data: Use the OBT Principle!**
 - **O:** Exclude **Outlying** wells (wells that have failed) by going to “**Edit**” then “**Clear**”. Outliers are best viewed on a linear amplification plot. It is also easy to detect outliers in the Ct vs. well position view of the data. Exclude any samples that produce atypical plots or that “creep”, i.e. that do not exhibit bona-fide amplification (defined as having 3 points in the log phase). This may include NTCs that creep up over the threshold at 40 cycles.
 - **B:** Set **Baseline**— Default range is 3 to 15. Set it to cover as many cycles as possible, typically 1-2 cycles *before* the reporter dye signal begins to increase. If amplification occurs after cycle 15 we do not recommend changing the baseline. The 13 data points within that range are sufficient for the baseline. It should only be changed when amplification occurs before cycle 15.
 - **T:** Set **Threshold** Bar to the halfway point in the linear part of the amplification plot. Thresholds are best set using the semi-log scale (this makes the linear phase of the PCR clear). A threshold for a particular assay can be set to the same value from run-to-run (but this must be checked).
 - If Multiplexing (**Fam** + **Vic**) go to “**Instrument**”, “**Diagnostics**”, “**Advanced Options**” and select the checkbox— “**Use Spectral Compensation for Real Time**” and re-analyse.
7. Once you are happy with the Data go to “**Window**”, “**Experimental Report**” and print out results.
8. This Report contains sample C_T values, sample quantity and standard deviations.
9. To see any abnormalities of the run (if it was aborted or no standards were labeled, for example) go to “**Event Log**” under “**Window**”.

Trouble-shooting, Tips and Important User Maintenance

1. Back up Data— Results should be regularly saved to a ZIP disk to avoid an eventual crash.
 - Go to the SDS Runs Folder (This entire **SDS Runs Folder** should be **deleted monthly**).
 - Files without Fluorescent icon can be copied onto ZIP and then deleted from this folder.
 - Files with fluorescent icons are the responsibility of users and should be backed up.
2. 7700 thermal cycler block must be kept clean – use 70% EtOH and cotton buds to clean.
3. Do not use any Organic Solvents or Radioactive samples in the 7700 SDS.
4. **Mac Preferences** (All under **Apple, Control Panels – These must be set correctly**)
 - **Energy Saver** – set to Never
 - **General Controls** – Shut Down Warning “Not Ticked” and Documents set to “Folder that is set by the application”.
 - **Memory** – Virtual Memory must be “OFF”.
5. **Rebuild the Mac desktop weekly** by holding down **Apple & Option** Keys & Select **Re-Start**. Hold down both keys until this message appears; “**Do You Want To Rebuild the Desktop?**” – Click **OK**.
6. To take a “**Picture**” – Hold down the **SHIFT + APPLE + 4** keys. The cursor becomes a crosshair. You can then lasso any part of the screen. When you release the mouse button you will hear a camera click. A picture has been saved to the hard disk. Find Picture 1 on hard drive. This can be used for data presentation or attached to an email for support (to ABOZSupport@appliedbiosystems.com).
7. Occasionally you will **NOT be able to Analyse** a run because the link between the raw data file and the run file have been lost. You must do the following:
 - After quitting out of the SDS 1.7 software, double-click on your **HD**, go to **System Folder, Preferences, SDS** and you will find a file labeled **SDS preferences** – drag this to the trash and empty it (Be careful NOT to trash the Spectra Components folder).
 - Open up the SDS 1.7 software and select **File, New Plate** making sure you select the correct preferences for your specific application (i.e. the run that just finished).
 - **VERY IMPORTANT** – You must re-setup a fresh plate **exactly** as you had labeled your previous plate and that includes dual dye layers if you were using **Fam** and **Vic**.
 - Then go **File, Import, LabView Format Raw Data** – you will be asked to assign the raw data file from the run that failed to analyse properly.
 - You must navigate to the **SDS Runs f folder** where the raw data files are located. The date and time are stamped on these so it is very easy to get the correct one. A merging data icon will tell you that all is successful.
 - You can then analyse as normal but please save this plate immediately.

Trouble-shooting, Tips and Important User Maintenance cont.

8. Norton's Disk Doctor and Speed Disc should be run at least **monthly** via the **Norton's Utilities CD**.

- Insert **Norton Utilities** CD into the CD drive and do one of the following:
 - Hold down the C key while starting up or
 - Use the Startup Disk Control Panel to select the CD-ROM as the startup drive
(Different startup will depend on MAC OS and Version of **Norton Utilities**)
- Go to "Special", "Restart"
- Double-click on the Norton Utilities CD
- Double-click on the Norton Utilities Icon
- *Never Install **Norton Utilities** on your Hard-drive as it interferes with our software.*
- Click Norton Disk Doctor in the Norton Utilities menu
- In the Disk Doctor window select your hard-drive and then click Examine
- If you wish to save the report do so now or click cancel
- Norton Disk Doctor will begin to test the selected drive
- If any errors or recommendations are reported – select Fix
- Click Done once finished checking your Hard-drive
- If there were any fixes to be done you must again select your hard-drive and then click Examine until you get a "No Problems Found" reported.
- Go to "Utilities", "Main Menu or Norton Utilities" and click Speed Disk
- Click Ok
- Choose your hard-drive to be optimised and click Optimize
- When finished go "File", "Quit"
- Select "Special", "Restart" if you used the C key to startup Norton Utilities or
- Go to "Apple", "Startup Disk" and select your hard-drive, then select "Special", "Restart"

Pre-Developed TaqMan® Assay Reagents for MULTIPLEXING

Choosing Endogenous Controls for Normalizing Gene Expression

Using the comparative C_T method, endogenous controls can normalize the expression levels of target genes by correcting differences in the amount of cDNA that is loaded into PCR wells.

Three steps are important in the endogenous control selection process:

1. **Verify that the endogenous control is consistently expressed in the sample set to be tested.**

Because the endogenous control normalizes differences in the amount of cDNA that is loaded into PCR reaction wells, endogenous control expression must be uniform across all samples in the study. Endogenous controls can be tested for uniform expression by comparing the gene expression of several samples. Samples should be representative of the target's gene expression and should span the target's expected range of expression. Specifically, it is important to demonstrate that while the target's expression levels may range widely, expression of the endogenous control remains constant. When conducting this test, it is crucial to load identical amounts of cDNA for each test sample. To ensure this, measure cDNA concentrations spectrophotometrically. Gene expression of endogenous controls should vary only slightly. The TaqMan® Human Endogenous Control Plate (P/N 4309920) can evaluate 11 housekeeping genes for their potential as endogenous controls. These 11 [endogenous controls](#) are also available as individual reagents.

2. **Ensure that the target(s) and endogenous control have identical PCR efficiency.**

The endogenous control and target assays must have identical PCR efficiency. TaqMan® PDARs (both controls and targets) are carefully designed for optimal performance in gene expression quantification. It is not necessary, therefore, to test PCR efficiency when using TaqMan® PDAR controls for normalizing TaqMan® PDAR targets. However, when using TaqMan® PDAR controls to normalize targets that are not TaqMan® PDARs, ensure that PCR amplification efficiencies are identical. This can be done by serially diluting a cDNA sample and demonstrating that the C_T difference between the target and endogenous control remains constant. A constant C_T difference across a range of at least 3 logs (1000-fold) of initial template concentration will verify identical PCR efficiency.

3. **[Necessary for multiplexing only]: Ensure that the gene expression level of the endogenous control is greater than that of the target.**

An additional step is required for researchers who wish to multiplex an endogenous control and target on the ABI PRISM® 7700 Sequence Detection System. PCR amplification of the more highly expressed gene must be primer limited. This guarantees that the less abundantly expressed gene will have adequate resources during PCR amplification to achieve the maximum efficiency required for accurate quantification results.

All TaqMan® PDAR controls are primer limited and contain probes labeled with the VIC™ reporter dye. This allows multiplexing of TaqMan® PDAR controls with TaqMan® PDAR targets, provided that the control gene is more abundantly expressed than the target gene. To test that the control is more abundantly expressed than the target, use test samples that span the expected range of target expression. It is important to run separate control and target reactions for each sample. All control C_T s should be lower than target C_T s. If this is not the case, two options are available: (1) Repeat Step 3, using another endogenous control, or (2) Conduct the study by running the control and the target in separate wells.

FAQs

These are the top 16 Frequently Asked Questions from our Website

Description	Solution
1. What can be used to dilute my TaqMan® probe?	The probe can be diluted in 10 mM Tris-HCl, pH 8.0 (at room temperature); 1 mM EDTA or ddH ₂ O.
2. Whenever I turn on the 7700, I get a Firmware Incompatibility Error – How do I stop this?	Applied Biosystems recommends leaving your 7700 Sequence Detection System on at all times.
3. What are VIC labeled probes?	Applied Biosystems designed the VIC dye as a replacement for the TaqMan® JOE dye currently in use with ABI PRISM Sequence Detection Systems. The VIC dye features improvements in fluorescent signal strength, spectral resolution, and production cost. VIC and JOE both have emission peaks at 554 nm. However, the fluorescent signal from VIC dye is nearly four times stronger than JOE. The increased signal strength of VIC results in greater sensitivity and, therefore, lower CTs at lower amplicon concentrations. VIC's emission spectrum consists of a narrow peak with half bandwidth 15 percent thinner than that of JOE. Consequently, the VIC spectrum overlaps less with surrounding dyes and introduces less error into the system. Eventually, all current kits that use Joe labeled probes will be replaced with VIC labeled probes. The TaqMan Cytokine Gene Expression Plate 1 (P/N 4304671) was the first product released with VIC labeled probes. VIC labeled TaqMan probes are currently available in 40 nmole (P/N 450025), 0.2 mole (P/N 450024), and 1 mole (P/N 450003) scale DNA synthesis. For additional information on VIC dye labeled TaqMan probes refer to User Bulletin #5: Multiplex PCR with TaqMan VIC Probes (P/N 4306236).
4. If I want to do multiplex PCR, how do I tell the software that there are two probes with different labels?	Use the "Dye Layer" button from the set-up screen. E.g.: use FAM dye layer for your target and VIC dye layer for the control target. From the Sample Type Set-up menu, you need to designate acronyms with the dye of choice. You cannot use the same acronym twice e.g.: if use "UNK" with FAM then for VIC you need to designate "UNK-V".
5. What's the best way to ensure good communication between the Mac and 7700?	A. Always re-start both the Mac and the 7700 before a new run. B. Trash "SDS Preferences" once a week or whenever there is a lockup. C. Re-build Mac desktop once a week D. Run Norton's Utilities once a month
6. Which dye label should I use for my target and control?	Always choose FAM for your target of interest as all of our Controls and PDAR's are labeled with VIC.
7. Now that my probes have arrived, what's the first optimisation experiment I should perform?	Providing you are using our Universal Master Mix and Universal Cycling conditions their will only be two (2) optimisation experiments required. A Primer concentration matrix (50, 300, 900 nM of each primer) and a Probe concentration titration (50, 100, 200, 250 nM) Choose the concentrations, which give the smallest Ct value and largest delta Rn.
8. Can I use 25ul reactions instead of 50ul?	The instrument performance specification is based on 50ul reactions but 25ul is possible. Smaller volumes are not recommended due to difficulties in optical detection of very small volumes. Remember to change the reaction volume in the cycler setup so that the 9600 cycler knows what the thermal load is.
9. Do I really need to use 8 of each control for my Allelic Discrimination Assay?	YES: if the automated allele calling routine in the software is to function correctly 8 replicates of each type (NTCs, Allele#1 and Allele#2) are required. This gives a 99.7% confidence level. Manual allele calling with less replicates is possible but confidence is reduced and that is why it is not recommended.

10. In the TaqMan® Rodent GAPDH Control Reagents (P/N 4304104) kit, what rodent is the Control RNA isolated from?	The control RNA is total RNA isolated from mouse.
11. What is Baseline?	The Baseline default is cycles 3 to 15. It is used to calculate the “background” of un-reacted probe. It is very important to shorten the Baseline when the samples become positive before cycle 15 or the plot will be artificially “skewed” in a negative direction.
12. Can the ABI Prism 7700 Sequence Detection System Computer be hooked up to a Network using AppleShare?	Yes, but during data collection the AppleTalk should be turned off. If the network communicates with the Mac during data collection the run will crash.
13. How do I use Primer Express for designing primer and probe sets for TaqMan?	Primer and probe sets for TaqMan can be designed by using an easy to follow tutorial for Primer Express, which is located at the following URL: http://www.appliedbiosystems.com/techsupp/tutorials/taqman/
14. What extensions are required to run the ABI Prism 7700 Sequence Detection System?	No extensions are required to run the SDS software. The SDS software will work with all the extensions turned off.
15. Can Macintosh computers that are connected to 7700s or 7200s, running MAC O/S 7.6.1, be upgraded to Mac O/S 8.0?	Below is a compatibility list of Macintosh Computers and Operating Systems tested with the 7700s and 7200s: PowerMac 7200 and Power Mac 4400 are compatible with Mac OS 7.6.1 and Mac OS 8.0; PowerMac G3 is compatible with Mac OS 8.0 only. Note: Mac OS 8.1 is not supported and should not be used on the Mac to run the 7200 and 7700 Sequence Detection Systems. A bug in Mac OS 8.1 can render the 7200s and 4400s inoperable.

16. What are the Molar Extinction Coefficients for the TaqMan® dyes, when using a spectrophotometer to quantitate TaqMan probe concentrations at 260 nm, as well as the Excitation and Emission wavelengths?

Dye	Excitation Wavelength (nm)	Emission Wavelength (nm)	Extinction Coefficient
FAM	488	518	20,958
VIC	488	552	30,100
TET	488	538	16,255
JOE	488	554	12,000
TAMRA	488	582	31,986
SYBR	488	520	N/A
ROX	488	610	N/A

Primer Express 1.5 User Guide

Note - The following procedures are valid only for **version 1.5** of the *Primer Express* software. Primer Express is optimised for use with Applied Biosystems assays, consumables, probes, instrumentation, reagents, and thermal cycling parameters. Melting temperatures may differ substantially if another T_m calculator is used to design the TaqMan probes or TaqMan MGB probes.

TaqMan Probe Design Guidelines

IMPORTANT - When designing probes, it is important to consider probes from both strands.

For designing TaqMan probes, follow the guidelines below in order of priority:

1. Avoid probes with a guanine residue at the 5' end of the probe. A guanine residue adjacent to the reporter dye will quench the reporter fluorescence, even after cleavage.
2. Select probes with a Primer Express software-estimated T_m of 68–70°C.
3. Make TaqMan probes as short as possible without being longer than 30 nucleotides.
4. Avoid runs of an identical nucleotide. This is especially true for guanine, where runs of four or more should be avoided.

TaqMan Primer Design Guidelines

After selecting probes for the assay, choose primers based on the guidelines below, again in order of priority.

Consequently, amplicons are usually 75–150 bp. By limiting the parameters for amplicon design (such as amplicon size), it is possible to run all reactions with a single reaction buffer (such as TaqMan® Universal PCR Master Mix (P/N 4304437) and a single thermal cycling protocol.

1. Avoid runs of an identical nucleotide. This is especially true for guanine, where runs of four or more should be avoided.
2. Set a Primer Express software-estimated T_m for the primers of 58–60°C.
3. Keep the guanine + cytosine content within 20–80%.
4. Make sure the last five nucleotides at the 3' end contain no more than two guanine + cytosine residues.
5. Place the forward and reverse primers as close as possible to the probe without overlapping it.

Please see the Applied Biosystems Amplicon Design Sheet 1.1 for more details

Importing a Sequence and Marking Regions of Interest for Probe and Primer Design

The steps involved in importing a DNA sequence into the Primer Express software and subsequent design of appropriate primers and probe are as follows:

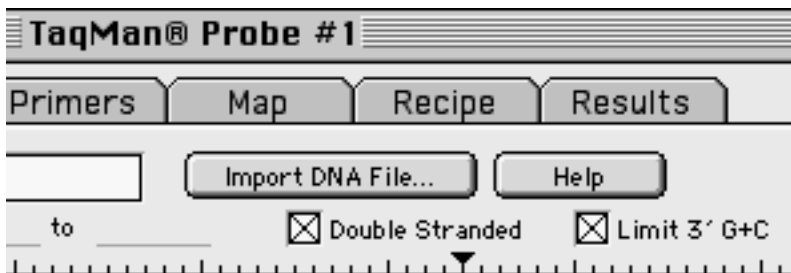
1. Launch the Primer Express software.

2.

To select a probe from...	Then...
a DNA file	a. From the File menu, scroll to New, and select TaqMan Probe & Primer Design. A TaqMan Probe document appears. b. Click Import DNA File. c. Locate and select a DNA file in the browser. d. Click Open (Try to limit the sequence too less than 1000 bp). The software loads the sequence and displays it in the Sequence tab.
An existing primer/probe document (located in the Document Archive)	a. From the File menu, select Open. The Document Archive box appears. b. Double-click the document to load, or select the sequence and click Open. The software loads the sequence and displays it in the Sequence tab.
a text document or GenBank sequence	a. Select the sequence from the text document or the navigator window. b. From the Edit menu, select Copy. c. From the File menu, scroll to the New submenu, and select TaqMan Probe & Primer Design. A TaqMan Probe document appears. d. From the Edit menu, select Paste. The software pastes the nucleotide sequence into the Sequence tab. Note - Edit the GenBank sequence before saving it as a Primer Express document.

3. In the **"Sequence"** view (see diagram below), the following checkboxes should be checked prior to primer selection:

- Double Stranded
- Limit 3' G+C (You will have to de-select if the PEX software comes up with No Primer Pairs Found or manually lock in your own)



The sense and antisense sequences appear on the Sequence tab when the **"Double Stranded"** checkbox is selected.

4. Label any Intron/Exon boundaries within the sequence using the Junction tool:

a. From the Tools palette, click the Junction Tool.



b. Select the Intron/Exon sequence (i.e. the 2 bp spanning the Intron/Exon boundary).

TCNANACGAC	NCACTANTAG	GGCGAATTCG	AGCTCGGTAC	CCGGGGATCC	50
TCTAGAGTCA	GACCTGCAGG	CATGAAGCTT	GAGTATTCTA	TAGTGTCCACC	100
TAAATAGCTT	GGCGTAATCA	TGGTCATAGC	TGTTTCCTGT	GTGAAATTGT	150
TATCCGCTCA	CAATTCCACA	CAACATACGA	GCCGGAAGCA	TAAAGTGTA	200
AGCCTGGGGT	GCCTAATGAG	TGAGCTAACT	CACATTAATT	GCGTTGCGCT	250
CACTGCCCCG	TTCCAGTCG	GGAAACCTGT	CGTGCCAGCT	GCATTAATGA	300
ATCGGCCAAC	GCGCGGGGAG	AGGCGGTTTG	CGTATTGGGC	GCTCTTCCGC	350
TTCCCTCGCT	ACTTGACTCG	CTGCGCTCGG	TCGTTCCGGCT	GCGGCGAACG	400
GTTATCAGCT	CACTCCAAAG	GCGGTAAATA	CGGTTTATCC	ACCAGNATCA	450
AGGGGGATAA	CCNCAGNAAA	GAACATGTGA	GCNAAAAGGN	CAAGCAAAAAG	500
GCCCAGG					507

5. Exclude any sequence where you **DO NOT** want primers/probe designed by using the Exclude tool:

a. From the Tools palette, click the Exclude tool.



b. Select the excluded sequence, i.e. where you **DO NOT** want primers to be designed.

TCNANACGAC	NCACTANTAG	GGCGAATTCG	AGCTCGGTAC	CCGGGGATCC	50
TCTAGAGTCA	GACCTGCAGG	CATGAAGCTT	GAGTATTCTA	TAGTGTCCACC	100
TAAATAGCTT	GGCGTAATCA	TGGTCATAGC	TGTTTCCTGT	GTGAAATTGT	150
TATCCGCTCA	CAATTCCACA	CAACATACGA	GCCGGAAGCA	TAAAGTGTA	200
AGCCTGGGGT	GCCTAATGAG	TGAGCTAACT	CACATTAATT	GCGTTGCGCT	250
CACTGCCCCG	TTCCAGTCG	GGAAACCTGT	CGTGCCAGCT	GCATTAATGA	300
ATCGGCCAAC	GCGCGGGGAG	AGGCGGTTTG	CGTATTGGGC	GCTCTTCCGC	350
TTCCCTCGCT	ACTTGACTCG	CTGCGCTCGG	TCGTTCCGGCT	GCGGCGAACG	400
GTTATCAGCT	CACTCCAAAG	GCGGTAAATA	CGGTTTATCC	ACCAGNATCA	450
AGGGGGATAA	CCNCAGNAAA	GAACATGTGA	GCNAAAAGGN	CAAGCAAAAAG	500
GCCCAGG					507

Designing the TaqMan Probe

Steps involved in the design of a TaqMan probe are as follows:

6. Select the “Params” Tab and select the “Factory Defaults” button:

Note – Very Important to do! This resets the default parameters each time you are designing an assay. It is very important if the PEX software is on a network as the values may be different from their defaults.

TaqMan® Probe #1

Sequence **Params** Rxn Cond Primers Map Recipe Results

Primer Tm Requirements
 Min Tm Max Tm Optimal Tm
 Maximal Tm difference

Primer GC Content Requirements
 Min %GC Max %GC 3' GC clamp residues

Primer Length Requirements
 Min length Max length Optimal length

Amplicon Requirements
 Min Tm Max Tm
 Min length Max length

TaqMan® Probe Criteria
 TaqMan® Probe Tm must be greater than PCR Primer
 TaqMan® Probe should not begin with G

7. Click the **Sequence** Tab, followed by “Options” and then “Find Primers / Probes Now”.

The PEX software will aim to design a Probe over the Intron/Exon boundary that you designated.

```

TATCCGCTCA CAATCCACA CAACATACGA GCCGGAAGCA TAAAGTGTA 200
ATAGGCGAGT GTTAAGGTGT GTTGATGCT CGGCCTTCGT ATTTCACATT

AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT 250
TCGGACCCCA CGGATTACTC ACTCGATTGA GTGTAATTAA CGCAACGCGA

CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA 300
GTGACGGGCG AAAGGTCAGC CCTTGGACA GCACGGTCGA CGTAATTACT

ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC 350
TAGCCGGTTG CGCGCCCTC TCCGCCAAC GCATAACCCG CGAGAAGGCG

TTCCTCGCTC ACTTGACTCG CTGCGCTCGG TCGTTCCGGCT GCGGCGAACG 400
AAGGAGCGAG TGAAGTGAAG GACGCGAGCC AGCAAGCCGA CGCCGCTTGC

GTTATCAGCT CACTCCAAAG GCGGTAATA CGGTTTATCC ACCAGNATCA 450

```

A total of 47 primer pairs found. To examine primer pairs, click the 'Primers' tab.

In this example, the PEX software has come up with a list of 47 assays. These are temporary and the maximum (default) is 200. You now need to interrogate all primers and the probe selections individually until a final list of 1 assay is displayed meeting all guidelines.

8. Click the **Primers** Tab and scroll across to the **TaqMan Probe** column:

	Start	Length	Tm	%GC	Probe	Start	Length	Tm	Reve
CA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	312	20	59	
CA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	20	59	
CA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	312	20	59	
CA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	312	19	58	
CA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	19	58	
CA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	312	19	58	
CCA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	20	59	
CCA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	312	20	59	
CCA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	312	20	59	
CCA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	19	58	
CCA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	312	19	58	
CCA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	312	19	58	
CA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	313	16	60	
CA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	313	16	60	
CA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	313	16	60	
CCA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	313	16	60	
CCA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	313	16	60	
CCA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	313	16	60	
TT	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	312	20	59	
TT	265	23	69	61	CAGTCGGGAAACCTGTCGTGCCA	312	20	59	
TT	265	24	70	63	CAGTCGGGAAACCTGTCGTGCCAG	312	20	59	
TT	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	20	59	
TT	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	312	20	59	
CA	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	314	15	59	
CA	269	22	68	64	CGGGAAACCTGTCGTGCCAGCT	314	15	59	
CA	268	23	70	61	TCGGGAAACCTGTCGTGCCAGCT	314	15	59	
TT	268	22	69	64	TCGGGAAACCTGTCGTGCCAGC	312	19	58	
TT	265	24	70	63	CAGTCGGGAAACCTGTCGTGCCAG	312	19	58	
TT	265	23	69	61	CAGTCGGGAAACCTGTCGTGCCA	312	19	58	

A total of 47 primer pairs found. Click any entry in the list to select it. Details in Primer Data window.

Select the Probe from the list that satisfies all requirements from Page 12 and our SDS Amplicon Design Sheet 1.1. **Note** - The Probe can be Top or Bottom strand depending on the **“More C’s than G’s guideline”**.

Note – The Probe/Primer List is ranked according to a Penalty Score (see Appendix B of the PEX User Manual) from Top to Bottom (i.e. The lower the score the better). Penalty scores are calculated during amplicon assembly, and are used to dynamically select the best 200 amplicons from among all possible amplicons. Penalty Score is displayed on the Primers page in the column at the far right (you must scroll to the right to view the penalty score).

9. Click on the **Sequence** Tab again and select the **Probe** Tool:

a. From the Tools palette, click the Probe Tool



b. Then select the sequence designated inside the **Green box**. If successful, the Probe sequence will turn **green** and lowercase.

TCNANACGAC NCACTANTAG GGCGAATTCG AGCTCGGTAC CCGGGGATCC	50
AGNTNTGCTG NGTGATNATC CCGCTTAAGC TCGAGCCATG GGCCCCTAGG	
TCTAGAGTCA GACCTGCAGG CATGAAGCTT GAGTATTCTA TAGTGTCCAC	100
AGATCTCAGT CTGGAGCTCC GTA CTTCGAA CTCATAAGAT ATCACAGTGG	
TAAATAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT	150
ATTTATCGAA CCGCATTAGT ACCAGTATCG ACAAAGGACA CACTTTAACA	
TATCCGCTCA CAATTCACACA CAACATACGA GCCGGAAGCA TAAAGTGTAA	200
ATAGGCGAGT GTTAAGGTGT GTTGTATGCT CGGCCTTCGT ATTTACACATT	
AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT	250
TCGGACCCCA CGGATTACTC ACTCGATTGA GTGTAATTAA CGCAACGCGA	
CACTGCCCGC TTTCCAG tcg ggaaacctgt cgtgccagc T GCATTAATGA	300
GTGACGGGCG AAAGGTC agc cctttggaca gcacggtcg A CGTAATTACT	
ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC	350
TAGCCGGTTG CGCGCCCCTC TCCGCCAAAC GCATAACCCG CGAGAAGGCG	
TTCCTCGCTC ACTTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAACG	400
AAGGAGCGAG TGA ACTGAGC GACGCGAGCC AGCAAGCCGA CGCCGCTTGC	
GTTATCAGCT CACTCCAAAG GCGGTAATA CGGTTTATCC ACCAGNATCA	450

A total of 47 primer pairs found. To examine primer pairs, click the 'Primers' tab.

- Again select "Options", then "Find Primers / Probes Now" from the drop-down menu.
- The PEX software will now design forward and reverse primers that are most compatible with your chosen probe sequence.

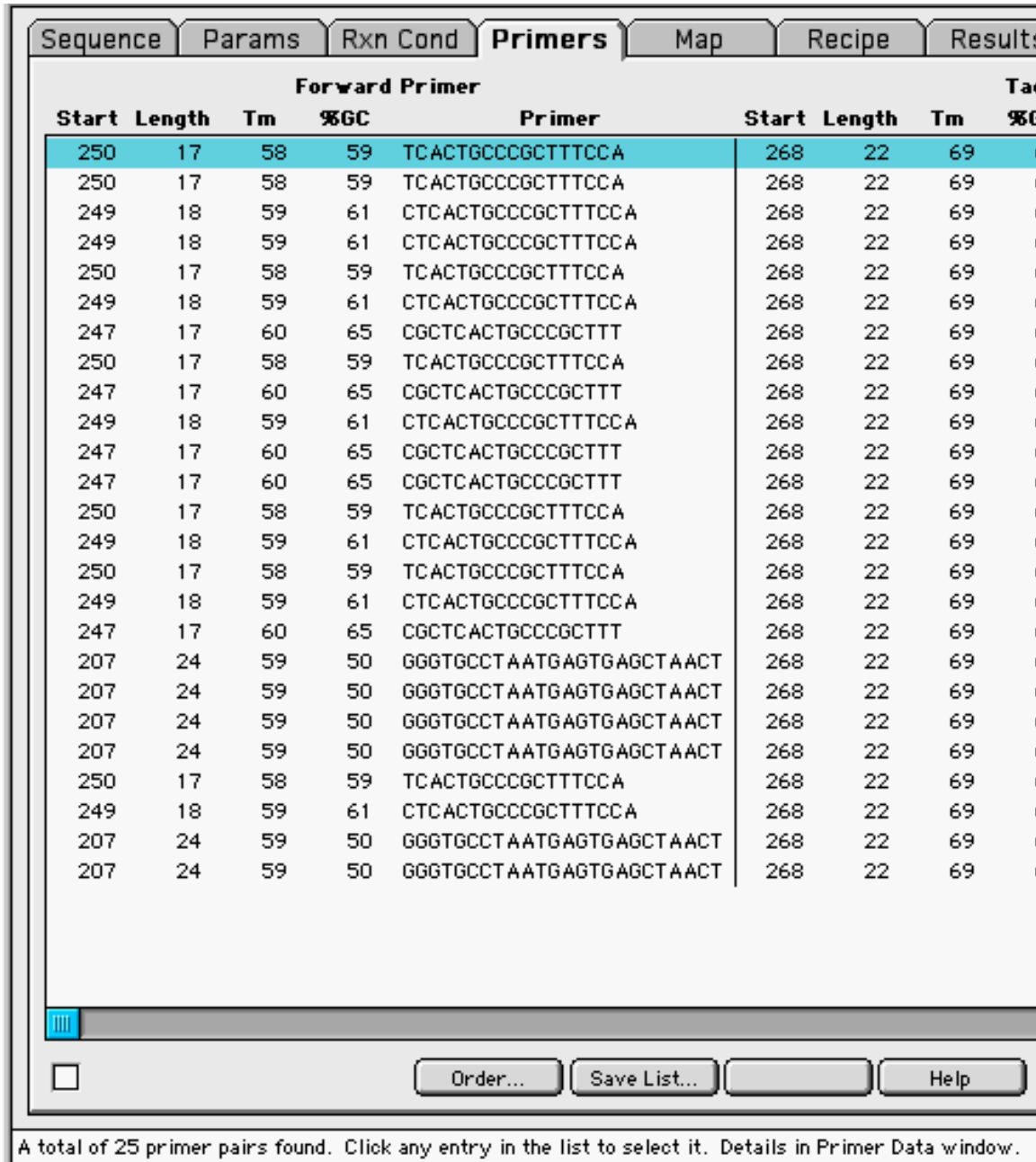
TCNANACGAC NCACTANTAG GGCGAATTCG AGCTCGGTAC CCGGGGATCC	50
AGNTNTGCTG NGTGATNATC CCGCTTAAGC TCGAGCCATG GGCCCCTAGG	
TCTAGAGTCA GACCTGCAGG CATGAAGCTT GAGTATTCTA TAGTGTCCAC	100
AGATCTCAGT CTGGAGCTCC GTA CTTCGAA CTCATAAGAT ATCACAGTGG	
TAAATAGCTT GGCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT	150
ATTTATCGAA CCGCATTAGT ACCAGTATCG ACAAAGGACA CACTTTAACA	
TATCCGCTCA CAATTCACACA CAACATACGA GCCGGAAGCA TAAAGTGTAA	200
ATAGGCGAGT GTTAAGGTGT GTTGTATGCT CGGCCTTCGT ATTTACACATT	
AGCCTGGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT	250
TCGGACCCCA CGGATTACTC ACTCGATTGA GTGTAATTAA CGCAACGCGA	
CACTGCCCGC TTTCCAG tcg ggaaacctgt cgtgccagc T GCATTAATGA	300
GTGACGGGCG AAAGGTC agc cctttggaca gcacggtcg A CGTAATTACT	
ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC	350
TAGCCGGTTG CGCGCCCCTC TCCGCCAAAC GCATAACCCG CGAGAAGGCG	
TTCCTCGCTC ACTTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAACG	400
AAGGAGCGAG TGA ACTGAGC GACGCGAGCC AGCAAGCCGA CGCCGCTTGC	
GTTATCAGCT CACTCCAAAG GCGGTAATA CGGTTTATCC ACCAGNATCA	450

A total of 25 primer pairs found. To examine primer pairs, click the 'Primers' tab.

Note – There are now only 25 primer pairs compared to the previous 47.

12. Click the **Primers** Tab again and scroll across to the **Forward Primer** column:

Select the Forward Primer sequence from the list that satisfies all requirements from Page 1 and our SDS Amplicon Design Sheet 1.1.



Forward Primer								
Start	Length	Tm	%GC	Primer	Start	Length	Tm	%GC
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
247	17	60	65	CGCTCACTGCCCCGCTTT	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
247	17	60	65	CGCTCACTGCCCCGCTTT	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
247	17	60	65	CGCTCACTGCCCCGCTTT	268	22	69	
247	17	60	65	CGCTCACTGCCCCGCTTT	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
247	17	60	65	CGCTCACTGCCCCGCTTT	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	
250	17	58	59	TCCTGCCCCGCTTTCCA	268	22	69	
249	18	59	61	CTCACTGCCCCGCTTTCCA	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	
207	24	59	50	GGGTGCCTAATGAGTGAGCTAACT	268	22	69	

A total of 25 primer pairs found. Click any entry in the list to select it. Details in Primer Data window.

13. Click on the **Sequence** Tab again and select the **Forward Primer** Tool:

a. From the **Tools** palette, click the **Forward Primer** Tool



b. Then select the sequence designated inside the blue/red box. Once the mouse button is released, the primer sequence will be underlined with a solid blue arrow.

TCNANACGAC	NCACTANTAG	GGCGAATTCG	AGCTCGGTAC	ECGGGGATCC	50
AGNTNTGCTG	NGTGATNATC	CCGCTTAAGC	TCGAGCCATG	GGCCCCTAGG	
TCTAGAGTCA	GACCTGCAGG	CATGAAGCTT	GAGTATTCTA	TAGTGTCCACC	100
AGATCTCAGT	CTGGACCTCC	GTA <u>CTTCGAA</u>	CTCATAAGAT	ATCACAGTGG	
TAAATAGCTT	GGCGTAATCA	TGGTCATAGC	TGTTTCCTGT	GTGAAATTGT	150
ATTTATCGAA	CCGCATTAGT	ACCAGTATCG	ACAAAGGACA	CACTTTAACA	
TATCCGCTCA	CAATTECACA	CAACATACGA	GCCGGAAGCA	TAAAGTGTA	200
ATAGGCGAGT	GTTAAGGTGT	GTTGTATGCT	CGGCCTTCGT	ATTCACATT	
AGCCTGGGGT	GCCTAATGAG	TGAGCTAACT	CACATTAATT	GCGTTGCGCT	250
TCGGACCCCA	CGGATTACTC	ACTCGATTGA	GTGTAATTAA	CGCAACGCGA	
<u>CACTGCCCGC</u>	<u>TTTCCAG</u>	<u>tcg ggaaacctgt</u>	<u>cgtgccagc</u>	T GCATTAATGA	300
<u>GTGACGGGCG</u>	<u>AAAGGTC</u>	<u>agc cctttggaca</u>	<u>gcacggtcg</u>	A <u>CGTAATTA</u> CT	
A TCGGCCAAC	GCGCGGGGAG	AGGCGGTTT	CGTATTGGGC	GCTCTCCGC	350
<u>TAGCCGGTTG</u>	<u>CGCGCCCC</u> TC	TCCGCCAAC	GCATAACCCG	CGAGAAGGCG	
TTCTCGCTC	ACTTGACTCG	CTGCGCTCGG	TCGTTCCGGCT	GCGGCGAACG	400
AAGGAGCGAG	TGAACTGAGC	GACGCGAGCC	AGCAAGCCGA	CGCCGCTTGC	
GTTATCAGCT	CACTCCAAAG	GCGGTAATA	CGGTTTATCC	ACCAGNATCA	450

A total of 25 primer pairs found. To examine primer pairs, click the 'Primers' tab.

14. Again select “Options” “Find Primers / Probes Now”

15. The PEX software will design reverse primers that are most compatible with the selected probe and forward primer sequences:

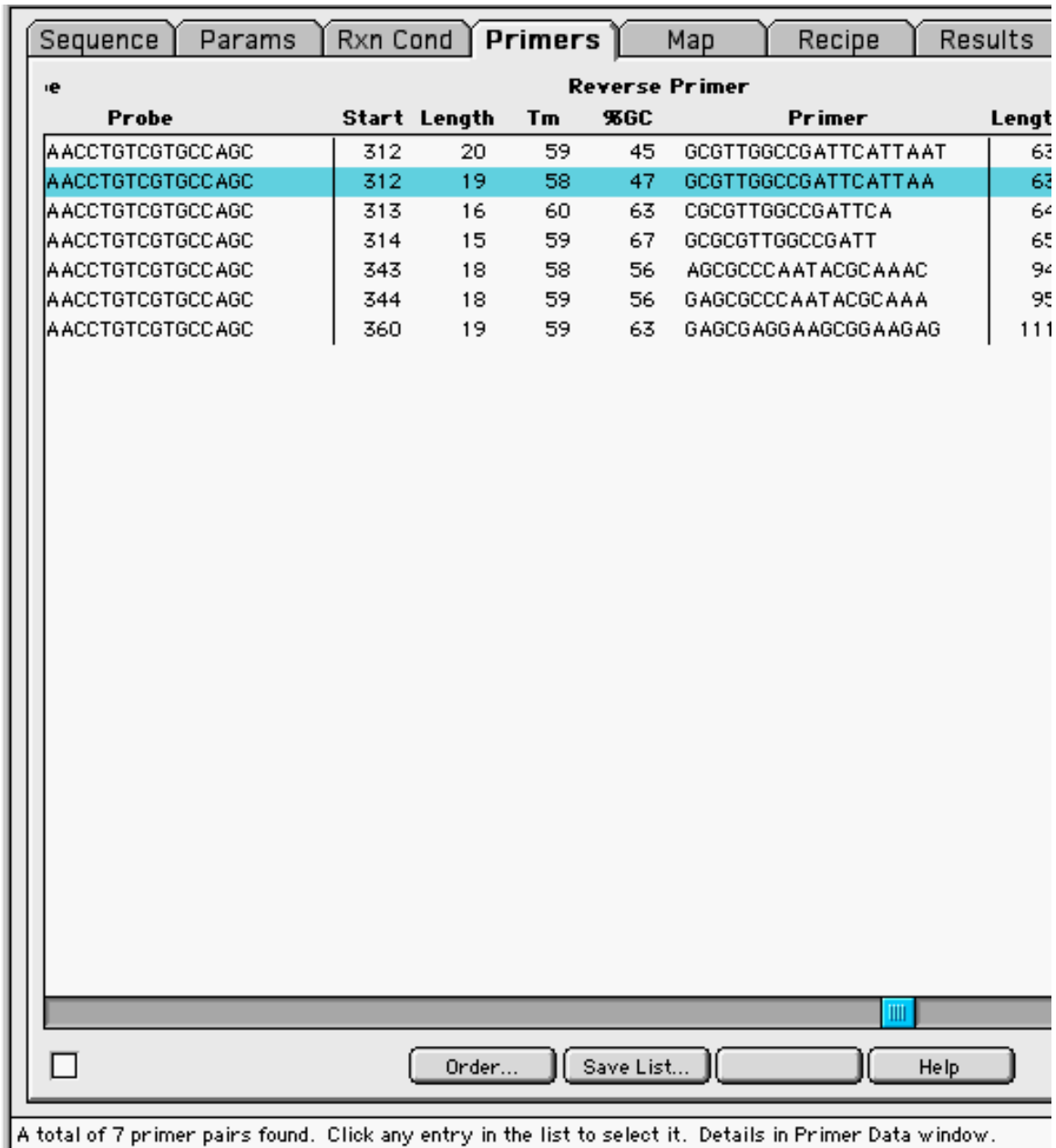
Note – There are now only 7 primer pairs compared to the previous 25.

TCNANACGAC	NCACTANTAG	GGCGAATTCG	AGCTCGGTAC	ECGGGGATCC	50
AGNTNTGCTG	NGTGATNATC	CCGCTTAAGC	TCGAGCCATG	GGCCCCTAGG	
TCTAGAGTCA	GACCTGCAGG	CATGAAGCTT	GAGTATTCTA	TAGTGTCCACC	100
AGATCTCAGT	CTGGACCTCC	GTA <u>CTTCGAA</u>	CTCATAAGAT	ATCACAGTGG	
TAAATAGCTT	GGCGTAATCA	TGGTCATAGC	TGTTTCCTGT	GTGAAATTGT	150
ATTTATCGAA	CCGCATTAGT	ACCAGTATCG	ACAAAGGACA	CACTTTAACA	
TATCCGCTCA	CAATTECACA	CAACATACGA	GCCGGAAGCA	TAAAGTGTA	200
ATAGGCGAGT	GTTAAGGTGT	GTTGTATGCT	CGGCCTTCGT	ATTCACATT	
AGCCTGGGGT	GCCTAATGAG	TGAGCTAACT	CACATTAATT	GCGTTGCGCT	250
TCGGACCCCA	CGGATTACTC	ACTCGATTGA	GTGTAATTAA	CGCAACGCGA	
<u>CACTGCCCGC</u>	<u>TTTCCAG</u>	<u>tcg ggaaacctgt</u>	<u>cgtgccagc</u>	T GCATTAATGA	300
<u>GTGACGGGCG</u>	<u>AAAGGTC</u>	<u>agc cctttggaca</u>	<u>gcacggtcg</u>	A <u>CGTAATTA</u> CT	
A TCGGCCAAC	GCGCGGGGAG	AGGCGGTTT	CGTATTGGGC	GCTCTCCGC	350
<u>TAGCCGGTTG</u>	<u>CGCGCCCC</u> TC	TCCGCCAAC	GCATAACCCG	CGAGAAGGCG	
TTCTCGCTC	ACTTGACTCG	CTGCGCTCGG	TCGTTCCGGCT	GCGGCGAACG	400
AAGGAGCGAG	TGAACTGAGC	GACGCGAGCC	AGCAAGCCGA	CGCCGCTTGC	
GTTATCAGCT	CACTCCAAAG	GCGGTAATA	CGGTTTATCC	ACCAGNATCA	450

A total of 7 primer pairs found. To examine primer pairs, click the 'Primers' tab.

16. Click the **Primers** Tab again and scroll across to the **Reverse Primer** column:

- a. Select the Reverse Primer sequence from the list that satisfies all requirements from Page 1 and the SDS Amplicon Design Sheet 1.1.
- b. If possible, select the Reverse Primer that matches the Forward Primer in Tm – in this case, 58°C.



Reverse Primer						
Probe	Start	Length	Tm	%GC	Primer	Length
AACCTGTCGTGCCAGC	312	20	59	45	GCGTTGGCCGATTCATTAAT	63
AACCTGTCGTGCCAGC	312	19	58	47	GCGTTGGCCGATTCATTA	63
AACCTGTCGTGCCAGC	313	16	60	63	CGCGTTGGCCGATTCA	64
AACCTGTCGTGCCAGC	314	15	59	67	GCGCGTTGGCCGATT	65
AACCTGTCGTGCCAGC	343	18	58	56	AGCGCCCAATACGCAAAC	94
AACCTGTCGTGCCAGC	344	18	59	56	GAGCGCCCAATACGAAA	95
AACCTGTCGTGCCAGC	360	19	59	63	GAGCGAGGAAGCGGAAGAG	111

A total of 7 primer pairs found. Click any entry in the list to select it. Details in Primer Data window.

17. Click on the **Sequence** Tab again and select the **Reverse Primer** Tool:

- a. From the **Tools** palette, click the **Reverse Primer** Tool.



- b. Select the sequence designated inside the blue/red box. If successful the primer sequence will be underlined with a solid blue arrow.

A total of 7 primer pairs found. To examine primer pairs, click the 'Primers' tab.

18. It is VERY IMPORTANT to select “Options”, “Find Primers / Probe Now” for a final analysis. The PEX software will analyse the interaction of all 3 oligonucleotide sequences to determine if the combination conforms to the recommended TaqMan guidelines.

A successful design is designated by the message " A Total of 1 Primer Pairs Found." (See text at the base of the Sequence view, below).

A total of 1 primer pairs found. To examine primer pairs, click the 'Primers' tab.

19. You must see at the bottom of the document – A Total of 1 primer pairs found (not 2 or more).
20. This is a 100% Guaranteed Assay from Applied Biosystems providing:
 - a. The target sequence information is correct.
 - b. *Applied Biosystems* instruments, software, reagents and consumables are used to run the assay (the guarantee does not extend to other manufacturers products).
21. There are two ways to save this final assay:
 - a. “**File**” then “**Save As**” is an internal save within the Archive File or
 - b. “**File**” then “**Export**” is an external save to the computer’s hard-drive. You can send your assay to Applied Biosystems - ABOZSupport@appliedbiosystems.com for checking and confirmation by one of our Field Application Specialists.
22. As a final check, all details on the SDS Amplicon Design Sheet 1.1 should be completed. In addition, Applied Biosystems recommends that you do a BLAST search on your primers/probe to confer specificity.

Primer Express Tips if the above instructions fail to design any primers

1. Deselect the Intron/Exon boundaries with the **Eraser** Tool if the PEX software fails to come up with any Primer pairs and use the **Line** Tool instead. You will then have to manually choose your own primers in conjunction with the **Exclude** Tool to get the software to design some Primers in the area that you want.
2. Deselect the **Limit 3’ G+C** checkbox if the PEX software fails to come up with any Primer pairs and carefully choose a Primer from the **Primers** Tab that satisfies the “**No More the 2 G/C’s in the last five bases**” guideline. If none choose the least number of G/C’s.
3. If you are still having problems, you will manually have to come up with Primers yourself. Use the **Primer Test Document** to come up with a Primer that meets the correct Tm range (i.e. 58-60⁰C). Copy the sequence and select “**Edit**”, then “**Find Sequence**”, paste in the sequence and select OK. The sequence will be underlined in the **Sequence Page** so you can then lock it in with the respective **Forward or Reverse Primer Tool** button to see if it passes. You may have to do this several times until you come up with an acceptable primer.
4. If you are still having problems use new **TaqMan MGB Probe and Primer Design** document. As the probes are much shorter you should be able to come up with a list in the Primers Page. The rest of the design is as standard.
5. When designing Primers for a **SYBR Green** assay, do exactly as outlined in pages 12-22 above, i.e. lock in the probe and primers but don’t get the probe synthesised. This way if you need it later the probe is ready to be synthesised.
6. **Two final issues:**
 - A** – Use the Primer Express software for also designing standard PCR, RT-PCR and when designing Sequencing Primers.
 - B** – No matter what document you are using, remember to click on the **Factory Defaults** button (**Params Tab**) before you start as well as locking in all relevant primers. You must get down to a **final list of 1 Primer Pairs found** to be confident in the primers selected by the software.

Generating 7700 SDS Amplification Plots in a Spreadsheet Program

The goal of this tutorial is to demonstrate the procedure through which analyzed data generated within a 7700 data file can be exported & regenerated in Microsoft® Excel in order to generate an amplification plot.

The following tutorial should be used as a guideline only. For further information on how to use Microsoft® Excel and Chart Wizard, please refer to the Microsoft® Excel user manual. Applied Biosystems does not support Microsoft® Excel or any other Microsoft® Software.

Step 1: Manipulations within the 7700 software:

1. Open the SDS data file to be analyzed.
2. Analyze the data and set the baseline & threshold according to Applied Biosystems guidelines. To obtain information on baseline & threshold setting, please refer to pages 23-32 in the TaqMan Cytokine Gene Expression Plate I Protocol, available on-line at: <http://www.pebiiodocs.com/pebiiodocs/04306744.pdf>
3. From the **File** menu, select "**Export**" and "**Clipped data**". You may either export all of the data or data from selected wells (click the appropriate button).
4. The default file name will be "**data.clipped**". Provide a name and file location for this data. It is suggested that you keep the suffix .clipped.

Step 2: Open a new Workbook in Microsoft Excel:

1. Select "**File**", "**Open**". Open the .clipped file.
2. Create a chart by either selecting "**Chart**" from the "**Insert**" tab, or by clicking the Chart Wizard button. Select **XY (Scatter)** as the chart type, and select the Chart sub-type "**Scatter with Data Points Collected by Smoothed Lines**". Click "**Next**".
3. Select the "**Series in: Columns**" button. The Data Range will default to the entire chart. Click "**Next**".
4. Begin to format the plot by adding a title to the chart and defining the axes. Click "**Next**".
5. **Save** the plot either as a separate file or as an object in the current file. Click "**Finish**".
6. This is the rough plot as seen after clicking "Finish". The plot can now be formatted and adjusted.
7. **Double-click on the X-axis** to re-scale and **Select** Check Boxes: Minimum: 0, Minor Unit: 0.4 and Value (Y) Axis Crossed at: 0. Patterns, font, and number notation can all be adjusted. Click "**OK**".
8. Double-click on the Y-axis to re-format. The formatting options are similar as to those for the X-axis.
9. Since Y-axis scaling is based on fluorescence values, it is important to note that this scale will be assay dependent. Click "**OK**".
10. To convert the plot to the Logarithmic view, check the "**Logarithmic**" box while formatting the Y-axis. Scales may need to be re-adjusted at this point. Click "**OK**". Your plot should be finished. Please go to: www.appliedbiosystems.com/techsupp/tutorials/7700amp/ for the above tutorial.

Generating 7700 SDS Standard Curve Plots in a Spreadsheet Program

The goal of this tutorial is to demonstrate the procedure through which analyzed data generated within a 7700 data file can be exported and regenerated in Microsoft Excel. This tutorial demonstrates how to generate a Standard Curve.

The following tutorial should be used as a guideline only. For further information on how to use Microsoft® Excel and Chart Wizard, please refer to the Microsoft® Excel user manual. Applied Biosystems does not support Microsoft® Excel or any other Microsoft® Software.

Step 1: Manipulations within the 7700 software:

1. Open the SDS data file to be analyzed.
2. Analyze the data and set the baseline & threshold according to Applied Biosystems guidelines. To obtain information on baseline & threshold setting, please refer to pages 23-32 in the TaqMan Cytokine Gene Expression Plate I Protocol, available on-line at: <http://www.pebiiodocs.com/pebiiodocs/04306744.pdf>
3. From the **File** menu, select **Export** and **Results**. You may either export all of the data or data from selected wells (click the appropriate button).
4. The default file name will be **data.results**. Provide a name and file location for this data. It is suggested that you keep the suffix **.results**.

Step 2: Manipulations within Microsoft Excel:

1. Open a new Workbook in Microsoft Excel.
2. Select **File, Open** and open the **.results file**.
3. Copy and Paste the Quantities and Ct values from this data into a new Worksheet.
4. Create a column between the Quantities column and the Ct column.
5. The Quantities need to be converted to their log values. To do this, click on the **fx icon** on the Toolbar. Click on **Math and Trig**, and select **Log**.
6. Convert the quantities into their log values in the appropriate column. The log values will be displayed on the x-axis and the Ct values on the y-axis.
7. Go to the **Chart Wizard icon** on the tool bar and click to open.
8. For the chart type, select **XY (scatter)** and select the chart sub-type **Scatter. Compares Pairs of Values**. Click **Next**.
9. Chart Wizard now asks for a Data Range:
10. To select a data range, place your cursor at the top corner of the data points to be used. Click and drag to highlight the data points. Include the data for both axes.
11. The data points will be plotted in the Chart Wizard. Click **Next**.
12. Click on the **Titles** tab and name the chart title and axes. Click **Next**.
13. Select a location for the chart. The chart can be created in a new sheet or within the current spreadsheet. E.g. the chart will be created in the open spreadsheet. Click **Finish**.
14. To finish formatting, place your cursor on a point in the graph & click once to highlight the data points.
15. From the Chart tab on the menu bar, select **Add Trendline**.
16. Under the Type tab, highlight **Linear**.
17. Under the Option tab, select the Display equation on chart and Display R-squared value on chart boxes. Click **OK**.
18. The chart should now display the equation, which may be moved by clicking on the equation and dragging it to a new location.
19. To format the X-axis, point your cursor to the X-axis and click.
20. Font size, scales and patterns can also be changed in this view.
21. To Format the Y-axis point the cursor on the Y-axis and click.
22. Your Graph should now be finalised. The format can be changed via the menu bar in Microsoft® Excel. Please go to: www.appliedbiosystems.com/techsupp/tutorials/7700std/ for the above tutorial.

ABI PRISM 7700 SDS User Maintenance Schedule

To keep your ABI PRISM 7700 Sequence Detection System in peak operating condition, check the following items at the interval recommended. Note observations in a logbook for future reference.

Daily Before Running

- *Restart* the Macintosh Computer *before* beginning SDS Software ver. 1.7
- Check that the optical caps are seated correctly in the tubes or plates
- Check that you have set up your plate correctly & each well is labeled correctly in the software
- If using an Adhesive Cover go to “Instrument; Diagnostics; Advanced Options” and select the checkbox; “Set 7700 Exposure Time for Plates”. Then change the time from 25 to 10 (*Very Important*)

After Each Run

- Use the “Save As” function immediately to Save your data
- Use “**OBT**” principles to correctly analyze data (i.e. Remove **O**utliers; set **B**aseline; set **T**hreshold)
- Remove the tubes or plate from the 7700 and discard accordingly

Weekly

- Rebuild Desktop on Macintosh by holding down the Apple and Option keys during startup
- Open the “System Folder; Preferences folder; SDS Folder” and you will find a file labeled “SDS preferences” — drag it to the trash and empty it

Monthly

- Wipe Perspex lid clean
- Update SAM virus definitions file
- Run Norton Disk Doctor and Speed Disk if available

Yearly

During the Annual Planned Maintenance visit, your Applied Biosystems Service Engineer will perform those items listed in the monthly checks, and in addition:

- Check laser alignment and verify that the Thermocycler is working correctly
- Check CCD signal to noise ratio
- Clean interference filters and laser mirrors where necessary
- Verify the Optics system
- Check that safety interlocks operate, and that all safety labels are visible
- Remove dust from computer (inside and outside), clean screen and keyboard
- Run Verify Instrument test
- Perform all required upgrades

To Contact Customer Support:

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