Real-Time PCR ABI Prism 7500

Every time you use any of the 7500 instruments, you MUST sign the log book. Use the calendar to reserve a time.
Each instrument has it's own log book and calendar. Use only optical grade tubes, flat top caps or plates, see below for order information.
The 7500 uses different plates and tubes than the 7500 FAST systems.
For the 7500 instrument, place tubes in the black tray labeled INDIVIDUAL TUBES ONLY and make sure they are balanced. Place plate in the black tray labeled PLATES ONLY.

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First time users, please check out the following website before doing your first run. It has 4 documents about setting up your experiment, how real-time pcr works, selecting reagents and a comparison of real-time vs. traditional pcr.
http://www.appliedbiosystems.com/support/apptech/#rt_pcr The PDF documents above (ABI seminars) are also very helpful. These are from a seminar by David Chappell, Field Applications Scientist. To order individual manuals (they are very helpful), see below, Section K for order #'s.

Decisions to be Made
The first decision to be made is whether or not to use SYBR Green or Taqman probes. SYBR Green is cheaper and you don't have to design the Taqman probe. But SYBR Green labels all DNA, while the Taqman probes bind to 1 spot on the template.
Second decision to be made is whether to run Relative or Absolute. All experiments compare something to something else - Absolute compares your samples to a standard curve; Relative compares Endogenous/control to a Target/sample. David recommends starting with an Absolute run to get all your parameters set (concentrations of primer and sample; different run temperatures and times. After that is setup, you should switch to a Relative run. For this type of experiment, you run a Relative Plate and then use Relative Study to compare different plates for difference between controls and samples.

Differences between the old 7500 and the new Fast 7500 systems

• Old 7500 runs are usually 2 hours. The Fast 7500 runs are 30 - 40 minutes if appropriate master mix is used.
• Old 7500 reactions are usually 25 - 50 ul. The Fast 7500 reactions can be as low as 10 ul, saving considerably on expensive reagents.
• Plates and tubes are different for the old 7500 and the Fast 7500 systems (NOT interchangeable).
• You must use the Fast master mix to get the 30 - 40 minute runs on the Fast 7500 systems.
• You can run using the regular (old) master mix on any of the instruments. You just have to select a standard run in the 7500 FAST instrument settings instead of the Fast run (default).

Terms for 7500

1. Relative quantification. Determines the change in expression of a target sample to a control sample (ex. Untreated vs. treated or a time course study with the control time zero). This is commonly used to compare expression levels of wild type vs. mutant or the expression levels of a gene in different tissues. You don't need standard curves or the exact copy # of the template.
2. Absolute quantification. Determines the absolute quantity of a single target within an unknown sample. Using Real time PCR, you monitor the progress of the PCR as it occurs, collecting data through out the PCR rather than at the end of the PCR. If you want a standard curve, you must use absolute.
3. Real time PCR is characterized by the point in time during cycling when amplification of a target is first detected rather than by the amount of target accumulated at the end of the PCR.
4. Reporter dye. The dye attached to the 5' end of a TagMan probe. The dye provides a signal that is an indicator of specific amplification.
5. Detector. A virtual representation of a gene or allele-specific nucleic acid probe reagent used for all analyses. Before you can start a run, you need to apply appropriate detectors for all samples on the plate.
6. Baseline. The initial cycles of PCR in which there is little change in fluorescence signal.
7. Threshold. Used to determine CT values; above the baseline, but within the exponential growth region of the amplification curve.
8. Threshold cycle (CT). The cycle number at which the fluorescence passes the threshold.
9. NTC. No Template Control. A sample that does not have template, used to verify amplification quality.
10. Standard. A sample of known quantity used to construct a standard curve.
11. Passive reference. A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. This is usually ROX and comes with the TaqMan Master mix.
12. Rn. Normalized reporter. The ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye.
13. Delta Rn (ΔRn). The magnitude of the signal generated by the specified set of PCR conditions ($\Delta Rn = Rn - \text{baseline}$).

Setting up reaction. For run using SYBR Green.

1. For more information about this, please see manual. You can check out the manual to look at. Contact Debby – (404) 413-5363. See below for order information.
2. Always include NTCs.
3. Use Dissociation curves and NTC to optimize primer concentrations (start with 50 nM for forward and reverse primer; no labeled probe for SYBR Green).
   a. Primer concentrations selected should provide a low CT and high ΔRn when run against the target template, but should not produce nonspecific product formation with NTCs.
   b. If the dissociation curve data shows that the melting temperature of the product generated in the absence of template is lower than the melting temperature of the specific product generated with template, then you probably have a primer-dimer formation. Using lower primer concentrations may provide more optimal results.
4. Tubes and caps must be from ABI. See below for order information.
5. Set up reactions.
6. Press lids on VERY FIRMLY.
7. Turn 7500 on (right most button, circle with a line). Press dark gray depression to open door (2nd most right button).
8. Place tubes in black tray. Tubes need to be balanced in the tray so that when the heated lid comes into contact with the tubes it's not slanted in thermal cycler. To close, press the dark gray depression and push the door closed. The door won't close if you press anywhere else.

Setting up Rxn. for run using Labeled probe (single reporter + quencher)

1. For more information about this, please see manual. You can check out the manual to look at. Contact Debby – (404) 413-5363. See below for order information.
2. Tubes and caps must be from ABI. See below for order information.
3. Set up reactions.
4. Press lids on VERY FIRMLY.
5. Turn 7500 on (right most button, circle with a line). Press dark gray depression to open door (2nd most right button).
6. For old 7500 instrument. Place tubes in black tray labeled INDIVIDUAL TUBES ONLY. Tubes need to be balanced in the tray so that when the heated lid comes into contact with the tubes it's not slanted in thermal cycler. Place plate in black tray labeled PLATES ONLY. To close, press the dark gray depression and push the door closed. The door won't close if you press anywhere else. For new 7500 Fast instruments, there is only 1 tray for both tubes and plates.

Quantitate samples with Quanti-iT PicoGreen kit

1. When running allelic discrimination/SNP experiments, it is all about the clusters. When the experiment is run, you want to know which cluster your sample falls in. So, tight clusters are very important. If the DNA concentration is not accurate, the clusters could lose resolution. Using a standard lab spectrophotometer may not be accurate enough.
2. A fluorometric method is recommended to get accurate DNA concentrations. This can be done in the Real-Time instrument. This is the second company who has recommended using fluorescence for more accurate quantification, so I don't think it's a sales pitch.
3. ABI also suggested using 10 ng DNA if possible.
4. Real-Time protocol. (guide.pdf files/PicoGreen [DNA].pdf)

Run

1. Log on.
2. Open 7500 System SDS software on desktop.
3. Old 7500: File/New. 7500 Fast: window opens, select Create New document.
4. Numerous drop down lists.

a. Assay. Select relative plate or absolute. Be careful selecting type of assay – data stored in one is not accessible to the other. You must select absolute if you want a standard curve. Do relative plate not relative study, the study is for analyzing multiple plates.

b. Container. Default – 96-well clear (only 1 choice with 7500).

c. Template. Blank. If you have created a template for your expt., you can select it. See manual for instructions for setting up a template.

d. Operator. You can put your name if you want. Default is the log in name.

e. Comments. Put any information you would like for the specific run if you want.

f. Default plate name. Name your plate so you can find it later – ex. Use the date and maybe your name, just some way of identifying your run afterwards. Not really needed, since you will be saving the file in your folder before starting the run.

5. Next.
6. Select detectors to add to the plate document. These are the detectors that your probes are labeled with. Selecting the detectors means that they will appear when you open the Well Inspector. If you forget one – don't worry, there is a button on the well inspector to add detectors. If you are doing a relative plate, you must have a separate detector for each gene you are using in the run; the detector also must be setup with a different color for each gene. This is so that when you setup your relative study, you will be able to see the gene expression data.

a. Highlight the detectors you used.

b. See below for instructions about adding new detectors to your list.

c. Click Add.

d. Finish, not Next. It's easier to label each well using the full screen view, which you get with "finish".

7. The plate document is created with tabs setup and plate selected. You cannot save a plate unless you name the samples either by using the Well Inspector or typing directly into the well.
8. Specify the detectors and tasks for each well.

a. Open Well Inspector. Either double click on a well; View/well inspector; CTRL 1; or use the well inspector button (square with a magnifying glass).

b. Click on a well or drag the cursor over a # of wells.

c. Check box to select a detector for the selected wells (this list comes from the detectors added in Step #6. You can select more than 1 detector if applicable. 2 colors will appear in the well.

d. Click under the Task column to assign the detector Task. The available tasks depend on whether you are doing absolute or relative.

i. Absolute. Unknown is the default; contains target sequences you are quantifying. Standard for standard curve (You have to type in the quantity. You can't use scientific notation; it does it when you are finished. Don't use units either, it's relative. Ex. One well is .1 and the next is .01 and the next .001. NTC for No Template Control is the negative control that contains PCR reagents, but no template.

ii. Relative. Target is the default; wells that contain PCR reagents for the amplification of target sequences. ENDO for Endogenous Control; wells that contain reagents for the amplification of the endogenous control sequence. Make sure that when you selected the detectors, that you have a separate detector for each gene you are using in the run; the detector also must be setup with a different color for each gene. This is so that when you setup your relative study, you will be able to see the gene expression data.

e. Standard. If you are doing a standard, select STD in Task column. You have to type in the quantity. You can't use scientific notation (it does it when you are finished).

Don't use units either, it's relative. Ex. One well is .1 and the next is .01 and the next .001.

f. You can name the sample. You can highlight a group of wells and type in a name under Sample Name and it will label each one. If you want to put units and more details for your Std, you can do it here. You can also click on a well and type the name without going into Well Inspector. For a relative plate, you must use the same name for samples within a gene set. This is so that when you setup your relative study, you will be able to see the gene expression data.

9. If you forgot a detector: Add detector, select the appropriate detector. Add to Plate Doc. Button/Down.

10. Close Well Inspector when all wells are named.

11. Make sure sample names are correct and detectors are correct. You can change by going back into the Well Inspector.

12. Select the Instrument tab. Change any parameters as needed by highlighting and typing changes in.

a. To delete a step, double click on the step or click and drag across the step. Delete.

b. Add a stage by moving the black thick bar to the right of the step to add after (click the mouse on the line to move it). Select Add cycle (repeats stage steps to right of black bar) or step (adds 1 step to a stage).

c. Change sample volume if needed.

d. Run Mode. Old 7500 - 2 choices; Standard 7500 (default) and 9600 Emulation (slower ramp rate). 7500 Fast - 2 choices Fast 7500 (default) and Standard 7500 (runs like old 7500).

e. Add Dissociation Stage if using SYBR Green.

f. The default Data Collection is at end of run . You can pick another stage to collect data, but in the 7500 program you can only pick 1.

13. File/Save as/D: Drive. Find folder for your lab/name file (usually name + date)/Save. If you forget this step, you will get an Error message prompting you to Save the file when you select Start.

14. Load plate/tubes into instrument. A1 is top left hand corner. Make sure black tabs on plate are above opening. Make sure plate and tube caps are very flat. Tubes should be balanced. If you only have a few tubes, place some blank ones in appropriate positions so that the heated lid will sit flat on your tubes. Make sure your samples are in the same place in the rack as in your plate setup.

15. Start. Don't walk away until you see the #'s in the temp. settings and other places. The heated lid sometimes gets stuck on the tube lids and the run will stop. If you wait until the #'s show up, you have passed the problem spot. During run, do not close software. You can do other things – this isn't as sensitive as the 7700 computer. After about 6 cycles, you can start looking at Results. What you see, though is delayed by a couple of cycles.

16. If you get an error message and have to fix the heated lid, the Start button is inactive. To fix the stuck lid, see below, Troubleshooting. You have to close the program and reopen it. You haven't lost anything since you saved your plate.

Analysis

After run is complete, click green arrow or go to Analysis and choose Analyze. If you make any changes in well information or delete a well, you have to reanalyze. If you change the baseline or threshold settings, you have to reanalyze.

Absolute Quantification

1. Terms

a. Baseline. The initial cycles of PCR in which there is little change in fluorescence signal.

b. Threshold. Used to determine CT values; above the baseline, but within the exponential growth region of the amplification curve.

c. Threshold cycle (CT). The cycle number at which the fluorescence passes the threshold.

d. Passive reference. A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. This is usually ROX and comes with the TaqMan Master mix.

e. Rn. Normalized reporter. The ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye.

f. Delta Rn (ΔRn). The magnitude of the signal generated by the specified set of PCR conditions (ΔRn = Rn – baseline).

2. Set Analysis Settings. Analysis/Analysis Settings. Select All in the Detector box. Select Auto Ct (the software will automatically set the baseline and threshold. Select Use system Calibrator. OK and Reanalyze.

3. Review Baseline and Threshold values. Even though you have the software set to do this automatically, there are many reasons that these values won't be accurate. View all wells and all detectors.

a. Baseline. Make sure that amplification curve starts after the baseline setting. If you have to change the setting: go to Analysis settings and select Manual baseline. Enter values in the Start (cycle) and End (cycle) windows. Make sure that the amplification curve growth begins at a cycle after the End Cycle value. Reanalyze.

b. Threshold. Make sure that the threshold is set in the geometric phase of the amplification curve. If the threshold is too low or too high, the standard deviation # is too high. If you have to change the setting: go to Analysis settings and select Manual CT. Drag the threshold setting bar until the threshold is above the background, below the linear or plateaued regions of the curve and within the geometric phase of the curve. The software adjusts the threshold value and displays it in the Threshold field after the analysis. Be sure to adjust the threshold for each detector. Reanalyze.

4. Result tab. To see anything under the Results tab, you have to select the wells to show. Either drag the mouse over the wells to select or select all 96 by clicking on the gray button between the A and the 1 (left top of the plate).

5. Results tabs:

a. Plate tab. Displays the results data of each well including the sample name and detector and the Rn value.

b. Spectra tab. Displays the fluorescence spectra of selected wells. The cycles slider near the bottom allows you to see spectra for each cycle by dragging it with the pointer. The cycle # text box shows the current position of the slider.

c. Component tab. Displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

d. Amplification plot tab. 3 plots to choose from to view amplification.

i. Rn vs. Cycle (linear) view. Displays normalized reporter dye fluorescence (Rn) as a funtion of cycle. You can use this plot to identify and examine irregular amplification.

ii. ΔRn vs. Cycle (Log) view. Displays dye fluorescence (ΔRn) as a function of cycle number. You can use this plot to identify and examine irregular amplification and also to manually set the threshold and baseline values.

iii. CT vs. Well Position view. Displays threshold cycle (CT) as a function of well position. You can use this plot to locate outliers and consider omitting a sample.

e. Standard Curve. The SDS software will calculate the amount of unknown samples using the standard curve. If nothing there – are the wells selected? Did you label the plate correctly (well labeled Standard and a quantity entered)? If not labeled correctly, you can go back and add them in, but you have to reanalyze and reselect the wells.

f. Dissociation. For SYBR Green only. Displays the melting curve (TM). See SYBR Green analysis below.

g. Report. Displays data for selected wells in an Excel format. This isn't actually Excel. You need to Export the file to Excel to go back to your lab. See below for how to do this. The columns show well, sample name, detector, task, Ct, StdDev Ct, Qty, Mean Qty, and StdDev Qty. The values in the Qty column are calculated by extrapolation from the Standard curve.

h. Omitting Samples. Identify Outliers using the Ct vs. Well position under the Amplification tab. Once identified, select View/Well Inspector, select the omit check box for the appropriate well. Analyze.

6. To adjust the x- or y- axes, click on the axes you want to change and the Graph Settings dialog is shown.

Relative Quantification

1. Terms

a. Baseline. The initial cycles of PCR in which there is little change in fluorescence signal.

b. Threshold. Used to determine CT values; above the baseline, but within the exponential growth region of the amplification curve.

c. Threshold cycle (CT). The cycle number at which the fluorescence passes the threshold.

d. NTC. No Template Control. A sample that does not have template, used to verify amplification quality.

e. Standard. A sample of known quantity used to construct a standard curve.

f. Passive reference. A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. This is usually ROX and comes with the TaqMan Master mix.

g. Rn. Normalized reporter. The ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye.

h. Delta Rn (ΔRn). The magnitude of the signal generated by the specified set of PCR conditions ($\Delta Rn = Rn - baseline$).

2. Result tab. To see anything under the Results tab, you have to select the wells to show. Either drag the mouse over the wells to select or select all 96 by clicking on the gray button between the A and the 1 (left top of the plate).

3. Results tabs:

a. Plate tab. Displays the results data of each well including the sample name and detector and the Rn value.

b. Spectra tab. Displays the fluorescence spectra of selected wells. The cycles slider near the bottom allows you to see spectra for each cycle by dragging it with the pointer. The cycle # text box shows the current position of the slider.

c. Component tab. Displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

d. Amplification Plot. Displays a plot of Rn as a function of cycle number for the selected detector and well(s).

4. Any changes such as omitting wells, or run information must be reanalyzed.

5. Analysis of a RQ plate must be done in a RQ study. This also allows you to compare more than 1 RQ plate. Make any changes to samples, detectors or detector tasks before going into the study – don't forget to reanalyze after any changes.

RQ Study

1. All RQ plates added to a study MUST have the same thermal cycling parameters, same steps, volume and cycles.
2. File/New
3. Assay drop down list – select Relative Quantification (ddCt) Study.
4. All the rest as usual. Next.
5. Add RQ plates to the study. Select Add plates. Select the plates you want (up to 10) and Open. The plates selected are displayed. Finish.
6. RQ main view has 3 panels:

a. RQ Detector grid. Allows you to select detectors to associate with the loaded study. For each detector, color, detector name, Threshold value, Auto Ct and Baseline are displayed. (Threshold (0.200000), Auto Ct (manual) and Baseline (6, 15) are set for default values.)

b. RQ Sample grid. Displays the samples associated with the selected detector(s). The sample grid displays numerical results of RQ calculations and has 2 subtabs: Sample Summary and Well Information.

c. RQ Results. Contains the 3 results-based tabs: Plate (default), Amplification Plot, and Gene Expression.

7. Set Analysis Settings. Analysis/Analysis Settings.

a. Select All in the Detector box.

b. Select Auto Ct (the software will automatically set the baseline and threshold.

c. Select Use system Calibrator. If your experiment uses only a single plate, there must be at least 2 different samples that have different names and have their own endogenous controls. You can go back to a saved RQ plate and change sample names if necessary.

d. Select the Endogenous Control Detector.

e. Select the Control Type if the study contains both multiplex and nonmultiplex reactions.

f. Select the RQ Min.Max Confidence level. The software uses these values to calculate error bars for expression levels.

g. Select Remove Outliers which will allow the software to automatically identify and filter outliers. Or you could do this manually.

h. Select OK and Reanalyze.

i. This information is now displayed in the RQ Detector grid. Threshold values are automatically calculated, The AutoCt and Baselines are now set to Auto.

8. Review Baseline and Threshold values. Even though you have the software set to do this automatically, there are many reasons that these values won't be accurate. View all wells and all detectors.

a. Baseline. Make sure that amplification curve starts after the baseline setting. If you have to change the setting: go to Amplification Plot tab, select Delta Rn vs. Cycle in the Data drop-down list. In the RQ Detector grid, select a detector (you can only select 1 detector at a time). Analysis settings and select Manual baseline. Enter values in the Start (cycle) and End (cycle) windows. Make sure that the amplification curve growth begins at a cycle after the End Cycle value. Reanalyze.

b. Threshold. Make sure that the threshold is set in the geometric phase of the amplification curve. If the threshold is too low or too high, the standard deviation # is too high. If you have to change the setting: go to Amplification Plot tab, select Delta Rn vs. Cycle in the Data drop-down list. In the RQ Detector grid, select a detector (you can only select 1 detector at a time). Under Analysis settings and select Manual CT. Drag the threshold setting bar until the threshold is above the background, below the linear or plateaued regions of the curve and within the geometric phase of the curve. The software adjusts the threshold value and displays it in the Threshold field after the analysis. Be sure to adjust the threshold for each detector. Reanalyze.

9. Results. Select detector to include in Results Graphs. Select by clicking on a detector in RQ Detector Grid or CTR-click to select more than 1 detector. The corresponding samples appear in the RQ Sample Grid. Results display depends on which tab is selected in the RQ Results Panel.

a. Plate -

b. Amplification Plot – Select 3 choices from Data drop down menu.

i. Rn vs. Cycle (linear) view. Displays normalized reporter dye fluorescence (Rn) as a function of cycle. You can use this plot to identify and examine irregular amplification.

ii. ΔRn vs. Cycle (Log) view. Displays dye fluorescence (ΔRn) as a function of cycle number. You can use this plot to identify and examine irregular amplification and also to manually set the threshold and baseline values.

iii. CT vs. Well Position view. Displays threshold cycle (CT) as a function of well position. You can use this plot to locate outliers and consider omitting a sample.

c. Gene Expression Plot. Shows the expression level or fold-difference of the target sample relative to the calibrator. Gene Expression Plot Orientation – Detector: Detectors are plotted on the x-axis and each bar shows the detector value of a single sample. Gene Expression Polo Orientation – Sample: Samples are plotted on the x-axis and each bar shows the set of sample values of a single detector.

10. Gene Expression Plot.

a. Select the gene of interest in the RQ Detector Grid. After changing the Analysis settings, you should now see a bar graph representation of gene expression for the gene you selected.

b. If not, make sure that when you selected the detectors, that you have a separate detector for each gene you are using in the run; the detector also must be setup with a different color for each gene. Also, you must use the same name for samples within a gene set. If you forgot to do both of these, you won't be able to see anything. You can still go back to your relative plate and add the appropriate detectors, detector color and sample name. Just don't forget to analyze.

11. Error bars for Gene Expression Plots. Error bars are displayed as long as there is a group of 2 or more replicates. The error bars display the calculated maximum (RQMax) and minimum (RQMin) expression levels that represent standard error of the mean expression level (RQ value). Collectively, the upper and lower limits define the region of expression within which the true expression level value is likely to occur. The error bars are based on the RQ Min/Max Confidence Level in the Analysis Settings dialog box.

12. **DON'T FORGET!!!** If you change analysis settings, baseline, threshold values, endogenous controls, detectors, sample name, control type or the RQ Min/Max parameters, you must reanalyze the data.

To open an old run

1. My Computer/D:/SDS Data/name of expt.

Creating a plate template

1. Template using save as function.

a. Create and label sample plate as usual. File/Save as. Browse to your folder or create a folder for your templates. Name template. Change file type to template.

b. Using saved template. Open software. File/New. Select assay type as usual. Browse to appropriate template at plate selection. If you don't see your template, be sure that file type shows ALL Files. The sample plate will open with all the well labels and thermal cycling parameters, but no detectors. Make any changes you need to.

2. Template using Excel.

a. Create and label sample plate as usual. File/Export/Sample Setup.

b. Open Excel. Browse to the file you exported. You can actually make changes in this format as long as you don't change the well #'s. These files are .cvs. Don't change - this is what the 7500 software needs.

c. Using template. Open software. File/New. Select assay type as usual. Finish. The sample plate will open as a blank plate.

d. File/Import/Sample setup. Browse to your saved Excel file. The sample plate will now have all the labels and detectors and thermal cycling parameters from the template. Make any changes you need.

To open data in Excel

1. Once run is done and analyzed, File/Export/Results

2. Export File Dialog box opens with the file name that you saved run under in the File name. Select Save. Do not move file anywhere else and don't change name.
3. My computer/D/SDS 1.2/Export Files. List has the file you just saved – there should be 2, one is the SDS document and the other should have an Excel icon next to the appropriate file name. Select and data will open in Excel. Do not take SDS document back to your lab – you won't be able to open it.
4. To take back to your lab: Save as/Save. This will save your file as a .csv document. You can open this in Excel, but will have to resave to convert to a true .xsl file. You can also change the Save as type. Pull down menu has 2 Excel choices. I used the first one – Excel 5.0/95 Workbook. This seemed to open as an .xls file and worked fine with later versions of Excel.

Supplies Use ABI optical grade caps and tubes ONLY: Old 7500 Instrument
1. MicroAmp tubes no caps (2000 ea.)– ABI # - N801-0933
2. ABI Prism Optical tubes, 8 tubes/strip (125 strips) – ABI # - 4316567
3. MicroAmp Optical caps (flat top; 8 caps/strip; 300 strips) – ABI #: 4323032
4. MicroAmp Optical 96-well Reaction plate w/o barcode (10 plates) - ABI # - N801-0560; this is a 96 well plate - no caps
5. ABI Prism Optical adhesive covers – ABI# - 4311971
6. Optical support base (10 ea.)– ABI part # - 4312063

7500 Fast Instrument
1. MicroAmp tubes with flat caps, .1 ml (1000 ea.)– ABI part # - 4358297
2. MicroAmp Fast 8-tube strip, .1 ml, (125 strips) – ABI # - 4358293
3. MicroAmp Optical 8-cap strip(flat top; 300 strips) – ABI #: 4323032
4. MicroAmp Optical 96-well Reaction plate w/o barcode, 0.1ml (10 plates) - ABI # - 4346907; this is a 96 well plate - no caps
5. ABI Prism Optical adhesive covers – ABI# - 4311971
6. Optical support base (10 ea.)– ABI part # - 4312063

Kits and Probes
1. There are many kits for Taqman PCR Core Reagents, Universal PCR Master Mix (both for old 7500 and for 7500 Fast) and SYBR Green. Contact Debby for Product list.
2. Single Reporter Probe + Quencher - following are companies besides ABI to order probes.
   a. Brinton Lab: BioSource 5'FAM + 3'TAMRA; 22566 pmole
   b. Attanasio Lab: Lakshmi thinks it's Sigma Genosys; can be ordered with a Fisher discount
   c. Zellars: Sigma Genosys; www.fisheroligos.com Contact (Fisher rep) for more info.

User Guides: Catalog Order #'s
1. Chemistry Guide: #4348358
2. Absolute Quantification: #4347825
3. Relative Quantification: 4347824
4. Allelic Discrimination Assays: 4347822

Troubleshooting
1. If error message comes up just after you have started run (before temp. #'s come up: Heated lid stuck on tube lids
   a. open drawer if you can and remove your tubes. REALLY push down on lids.
   b. Pop open drawer. On the right side of the machine near the front is a small hole. To pop it, use one of the tools in the bottom drawer near the bench by the door. The tools are in a small red box. Put 1 of the tools into the hole and push – don't be gentle. The door will pop open.
   c. Pull the heated lid forward.
   d. Close the door

e. Close the program. This is an important. The Start button won't be active if you don't close and reopen your file.