User Bulletin

Applied Biosystems 7500 Fast Real-Time PCR System

July 2006

SUBJECT: Using Expert Mode on the Applied Biosystems 7500 Fast Real-Time PCR System with the Applied Biosystems 7500 Fast System SDS Software v1.4

IMPORTANT! Expert Mode is available when selecting Absolute or Relative Quantitation assay types and is not available for Allelic Discrimination or Plus/Minus assays. In addition, Expert Mode is only available when selecting Fast thermal cycling mode on the Applied Biosystems 7500 Fast System and not for other Real-Time PCR instruments or software.

In This User Bulletin

This user bulletin includes the following topics:

Overview

This bulletin provides information on how to use Expert Mode in the Sequence Detection Systems Software version 1.4 (SDS Software v1.4) for the 7500 Fast Real-Time PCR System (7500 Fast System).

Run times may be reduced to 30 minutes, or less, using Expert Mode on the 7500 Fast System by selecting only those filter sets (for a particular set of reporter dyes in an assay) necessary for data collection. This user bulletin describes how to use Expert Mode on the 7500 Fast System and explains some limitations to avoid loss of data.

The information in this bulletin supplements the application-specific *Getting Started Guides* (see "Related Documentation" on page 2) and *Sequence Detection Systems Software version 1.4 Online Help (SDS Online Help)* for the 7500 Fast System. Refer to the *SDS Online Help* for details on how to use the 7500 Fast System.

Note: To access the *SDS Online Help*, select **Help > SDS Online Help** from the SDS Software v1.4 menu bar.

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Related Documentation

For more information about using the 7500 Fast System and SDS Software v1.4, refer to:

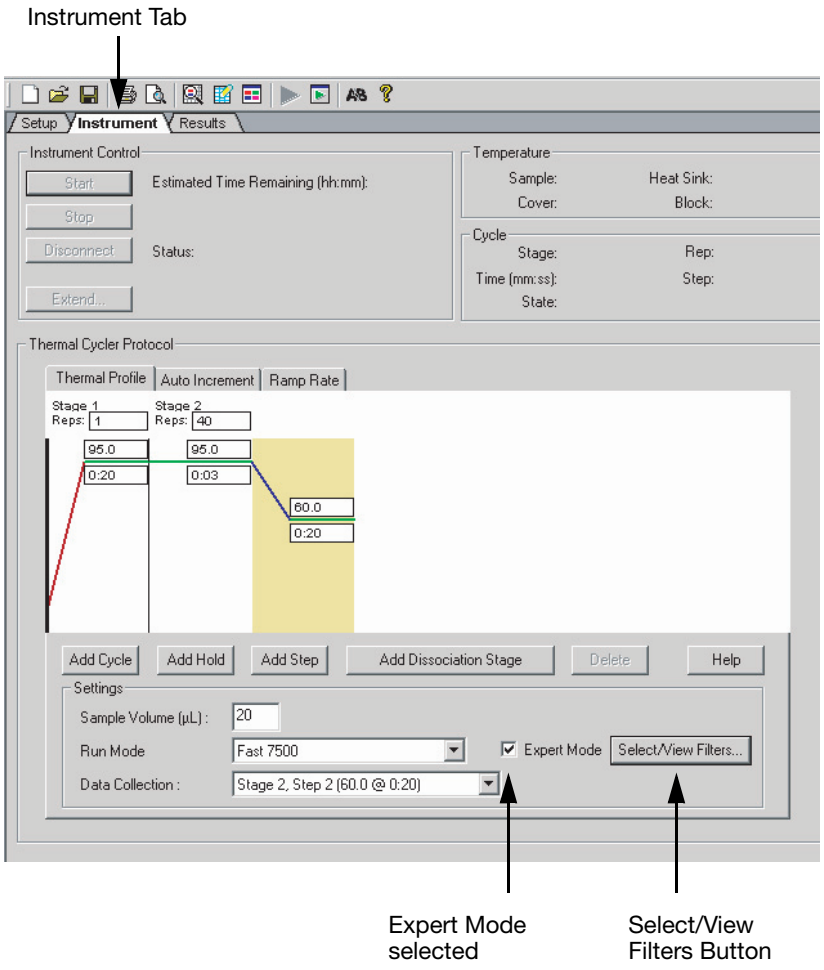
- *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Getting Started Guide* (PN 4347825)
- *Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantitation Getting Started Guide* (PN 4347824)
- *Sequence Detection Systems Software version 1.4 Online Help (SDS Online Help)*

Selecting Expert Mode

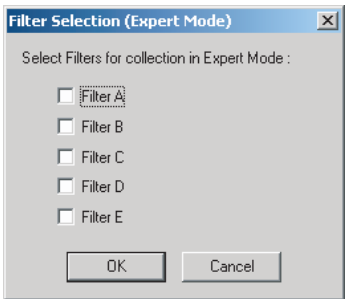
To select Expert Mode in SDS Software v1.4:

1. Set up your plate document as explained in the appropriate Getting Started Guide or *SDS Online Help*.
2. At the Instrument tab, verify that Expert Mode is selected. Click the **Select/View Filters** button.

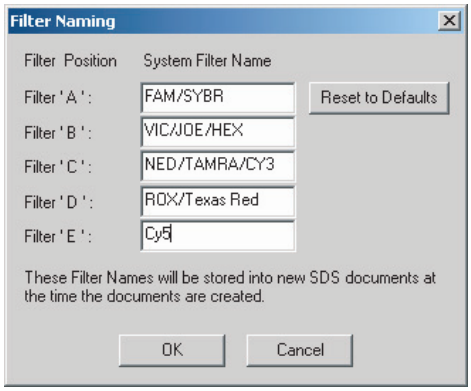
   Note: Expert Mode is only enabled for the Fast 7500 thermal cycling Run Mode. This feature is not available for Standard or 9600 Emulation mode.



1. At the Filter Selection (Expert Mode) dialog box, select the desired filters.



2. (Optional) To rename the filter labels, select **Tools > Filter Configuration** to open the Filter Naming dialog box. You can change Filter names to indicate the reporter dyes calibrated for the instrument.



Selecting Filters in Expert Mode

Using Expert Mode, you can select any single filter or group of filters for data collection. However, when using ROX™ as a passive reference dye, you must use certain combinations of filters (see Table 1). All Applied Biosystems' master mixes contain ROX™ as a passive reference dye to correct for fluorescent fluctuations which may occur during the run (due to bubbles or variations resulting from pipetting error). Using ROX™ as a passive reference dye, in all runs, is strongly recommended.

Table 1 Filter selection options with or without ROX™ dye passive reference

Reporter Dye/Assay Type	Filter Selection *with* ROX™ Dye Passive Reference Normalization	Filter Selection *without* ROX™ Dye Passive Reference Normalization
FAM™ Single Reporter Assay (using non-fluorescent quencher)	A, D	A
FAM™ Single Reporter Assay (using TAMRA quencher)	A, C, D	A, C
SYBR® Green I Dye	A, D	A
VIC® Single Reporter Assay (using non-fluorescent quencher)	A[a], B, D	B

Reporter Dye/Assay Type	Filter Selection *with* ROX™ Dye Passive Reference Normalization	Filter Selection *without* ROX™ Dye Passive Reference Normalization
VIC® Single Reporter Assay (using TAMRA quencher)	A[a], B, C, D	B, C
NED™ Single Reporter Assay (using non-fluorescent quencher)	A[a], C, D	C
Cy3 Single Reporter Assay (using non-fluorescent quencher)	A[a], C, D	C
Cy5 Single Reporter Assay (using non-fluorescent quencher)	A[a], D, E	E
FAM™/VIC® Multiplex Assay (using non-fluorescent quencher for both FAM™/VIC® reporters)	A, B, D	A, B
FAM™/VIC® Multiplex Assay (using TAMRA® quencher for both FAM™/VIC® reporters)	A, B, C, D	A, B, C

a. When using Applied Biosystems Master Mixes, both FAM and ROX™ dye filters are required for correct use of the ROX™ dye passive reference normalizer.

IMPORTANT! Selecting the incorrect filter for a particular assay (i.e., Filter C for a FAM dye single reporter assay) results in loss of data for that experiment, since no data will be collected for the FAM® dye in filter A.

Using Expert Mode

Thermal Cycling Modes on 7500 Fast Systems

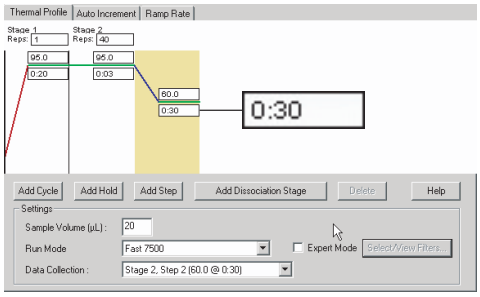
The 7500 Fast System has three thermal cycling run modes equating to three different sample temperature ramp rates. The run mode options are Fast, Standard and 9600 Emulation modes.

Fast thermal cycling mode attains sample ramp rates of +/- 3.5 °C/sec (up and down ramp). Standard mode attains +/-1.6 °C/sec. The 9600 Emulation mode matches the sample ramp rate achieved for the ABI PRISM® 7700 Sequence Detection System at up ramp of 0.8 °C/sec and down ramp of 1.6 °C/sec, ensuring assays that were designed for older generation platforms achieve comparable performance on newer systems.

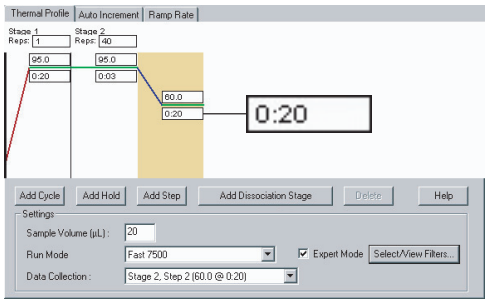
Extension Times for Fast Thermal Cycling Protocols

Fast thermal cycling run mode, in combination with TaqMan® Fast Universal PCR Master Mix (2✕) No AmpErase UNG and Optical Fast Thermal Cycling plates, allows a reduction in thermal cycling protocols to approximately 40 minutes.

The default Fast thermal cycling protocol uses a denaturation time of 3 seconds at 95 °C and an extension time of 30 seconds at 60 °C. The requirement for a 30 second extension time results from the time required to collect data through all 5 filters plus the time needed for the master mix enzyme to complete the polymerization process.



Fast Thermal Cycling Protocol Time (Expert Mode Not Selected)



Expert Mode Thermal Cycling Time (Expert Mode Selected)

When Expert Mode is selected, the default extension time changes to 20 seconds. This is the minimum recommended extension time (when using TaqMan® Fast Universal PCR Master Mix (2✕) No AmpErase® UNG) needed for the polymerization process to complete. If you are selecting fewer than 2 filters for data collection, you may optionally choose to select extension times lower than 20 seconds. However, note that extension times of less than 20 seconds have not been validated using TaqMan® Fast Universal PCR Master Mix (2✕) No AmpErase UNG and may impact the quality of results. Validating your assay performance, when using extension times of less than 20 seconds, is therefore strongly recommended.

At the start of each run, the SDS Software verifies the extension time, as set by the user, against the number of filters selected. If the minimum time needed to collect data through the selected number of filters exceeds the extension time, the SDS Software will generate a warning message that the run cannot be started. You must then enter a longer extension time to allow for the data collection process to complete and the run to be started. Refer to the table below for the minimum required extension times in relation to the number of filters selected.

Table 2 Minimum extension times for filter selections in Expert Mode

Number of Filters Selected	Minimum Extension Time/Seconds[a]
Default	20[b]
1	10[c]
2	14
3	19
4	23
5	25

a. Note that the minimum times listed may vary slightly by 1 - 2 seconds for different combinations of filters and from instrument to instrument.

b. The default extension time is set at 20 seconds for all Expert Mode filter selections. This selection can be modified by the user, however, to avoid observable data quality issues (related to extension times) it is strongly recommended that the default time be used.

c. In Expert Mode, these times are the absolute minimum extension times needed for the number of filters selected.

Dissociation Stages and Expert Mode

Dissociation stages are typically added to real time PCR runs when using SYBR® Green I dye as the reporter dye. If you use Expert Mode and add a dissociation stage to the thermal cycling protocol, dissociation data is only collected for those filters selected using Expert Mode. The number of data points collected during dissociation varies depending on the number of filters selected. As the number of filters selected decreases, the number of data points collected during the dissociation stage increases.

Applied Biosystems does not currently provide a SYBR® Green I Dye master mix which can be used for runs in Fast thermal cycling mode. It is strongly recommended that Standard or 9600 emulation modes be selected for all runs using Applied Biosystems SYBR® Green I Dye containing master mixes.

Data Quality When Using Expert Mode

Data quality, when using Expert Mode (with a 20 second extension time) is generally comparable to data obtained using Fast Mode (with a 30 second extension time). Note that when using Expert Mode, ΔRn values will decrease since less time is allowed for the extension phase of the reaction. This results in an overall decrease in fluorescent signal. It is strongly recommended that you keep the default extension time (of 20 seconds in Expert Mode) as the minimum extension time used for any assay. Extension times *below* 15 seconds are known to significantly impact the C_T and precision of data sets.

Two experiments were performed to compare the C_T values and precision of data generated using Expert Mode and Fast Mode:

- A TaqMan® RNase P Instrument Verification Plate assay was run quantitating a genomic DNA target against a standard curve.
- A selection of TaqMan® Gene Expression assays were run using cDNA as a template.

Comparing TaqMan® RNase P Data Between Expert Mode and Fast Mode

The following section lists experimental conditions for running a TaqMan® RNase P Instrument Verification Plate.

Expert Mode

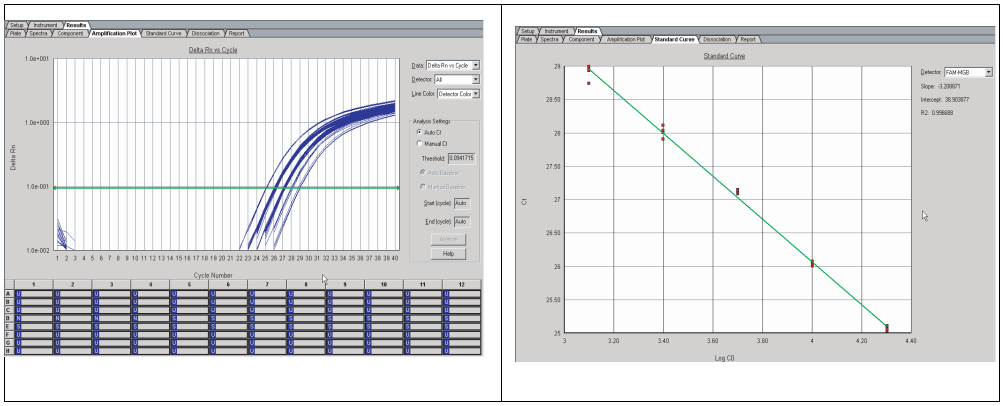


Figure 1 Expert Mode Run. Fast RNase P Instrument Verification plate run on 7500 Fast Real-Time PCR System with default thermal cycling parameters for Expert Mode (95 °C for 20 seconds, 40 cycles at 95 °C for 3 seconds, 60 °C for 20 seconds) using filters A and D (FAM™ and ROX™ dye) were used. Instrument run time was 30 minutes.

Fast Mode

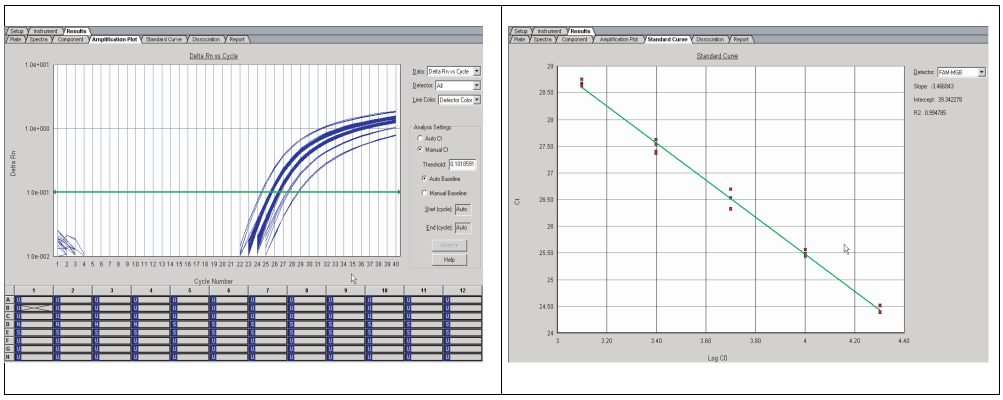


Figure 2 Fast Mode Run. Fast RNase P Instrument Verification plate run on 7500 Fast Real-Time PCR System with default thermal cycling parameters for Fast Mode (95 °C for 20 seconds, 40 cycles at 95 °C for 3 seconds, 60 °C for 30 seconds). Data was collected through all filters. Instrument run time was 40 minutes.

The table below compares the results of Expert Mode and Fast Mode and shows that the precision of data for the RNase P assay is comparable between Expert Mode and Fast Mode.

Table 3 Results summary for RNase P instrument verification plate

TaqMan® RNase P Instrument Verification Plate	Expert Mode	Fast Mode
5K Unknown Standard Deviation	0.061	0.079
10K Unknown Standard Deviation	0.058	0.090
R^2 Value for Standard Curve	0.997	0.995

Comparing Data Quality Between Expert Mode and Fast Mode for TaqMan® Gene Expression Assays

The following section lists experimental conditions for running eight TaqMan® Gene Expression Assays in each Mode. The eight assays are listed below.

Table 4 List of assays used

Gene Symbol	Assay ID
PGRMC1	Hs00198499_m1
RAB14	Hs00249440_m1
SRM	Hs00162307_m1
RAB12	Hs00407832_m1
CCT7	Hs00362446_m1
Beta-2M	4333766F
TGF-beta	4327054F
RNase P	-

The following experimental conditions were used for both Expert and Fast modes.

Table 5 Experimental conditions

Condition	Expert Mode	Fast Mode
Replicates	10 Replicates plus 2 NTCs (No Template Controls) for each assay	10 Replicates plus 2 NTCs (No Template Controls) for each assay
Reagents	TaqMan®Fast Universal PCR Master Mix (2×), No AmpErase UNG	TaqMan®Fast Universal PCR Master Mix (2×), No AmpErase UNG
Reaction Volume	20 µL	20 µL

Data Collection	Filters A & D	Filters A, B, C, D, E
Plate	Optical 96-well Fast Thermal Cycling Plate with Barcode (code 128)	Optical 96-well Fast Thermal Cycling Plate with Barcode (code 128)
Thermal Cycling Parameters	Hold: 20 sec/95 °C 40 Cycles: 3 sec/95 °C then 20 sec/60 °C	Hold: 20 sec/95 °C 40 Cycles: 3 sec/95 °C then 30 sec/60 °C

CAUTION CHEMICAL HAZARD. TaqMan® Fast Universal PCR Master Mix (2✕), No AmpErase UNG may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and glove.

The results for both runs are listed in the table below and the comparable amplifications plot appear in "Amplification plots" on page 10.

Table 6 Experimental results

	Expert Mode		Fast Mode	
Gene	Average C_T	Standard Deviation	Average C_T	Standard Deviation
PGRMC1	28.14	0.031	27.95	0.068
RAB14	25.75	0.048	25.36	0.031
SRM	23.11	0.038	23.00	0.061
RAB12	25.40	0.047	25.28	0.043
CCT7	23.05	0.114	22.64	0.108
Beta-2M	21.00	0.040	20.95	0.043
TGF-beta	24.73	0.075	24.66	0.088
RNase P	18.78	0.063	18.67	0.083

Table 7 Amplification plots

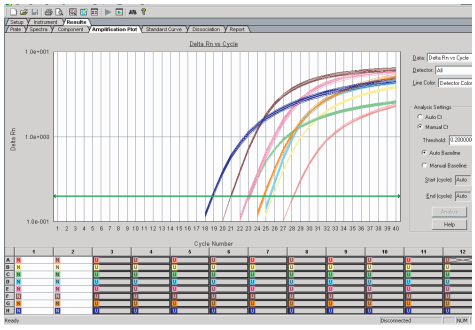


Figure 3 Expert Mode Run. Eight TaqMan® Gene Expression assays run on 7500 Fast System using filters A and D for FAM and ROX dyes with 20 second extension time (see Table 5 on page 8 for experimental conditions)

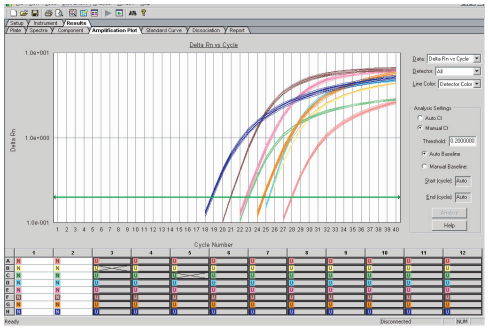


Figure 4 Fast Mode Run. The same eight ® Gene Expression assays run on 7500 Fast System using with data collected through all filters with 30 second extension time (see Table 5 on page 8 for experimental conditions)

Summary

Expert Mode, on the 7500 Fast System provides the capability to reduce run times to 30 minutes while maintaining data quality comparable to that obtained using Fast thermal cycling mode.

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