Comparing Fast and Standard Data on Applied Biosystems 7500 and 7500 Fast Real-Time PCR Systems

Introduction

The Applied Biosystems 7500 Fast Real-Time PCR System reduces quantitative PCR run times to approximately 35 minutes, generating results almost 75 precent faster than previous generation instruments and allowing multi-user laboratories to more easily share their real-time PCR platforms.

The ability of the 7500 Fast System to complete real-time PCR experiments in less than 40 minutes results from a combination of a new, high-speed thermal cycling block, TaqMan® Fast Universal PCR Master Mix, and Optical Fast 96-well thermal cycling plates.

Transitioning from standard to fast thermal cycling modes is simple with either Applied Biosystems TaqMan® Fast Universal PCR Master Mix and

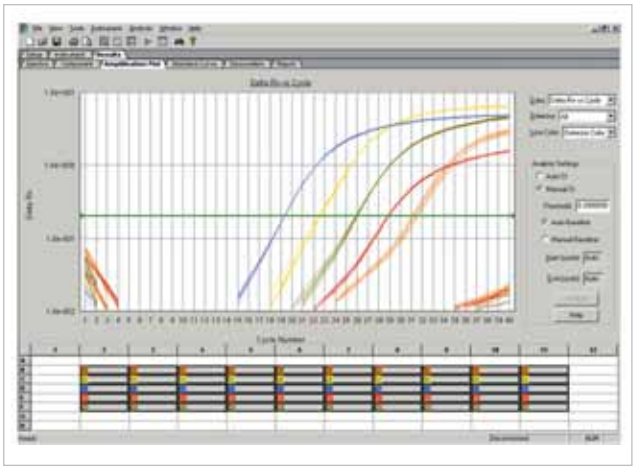


Figure 1. Performance of assays in fast mode on the 7500 Fast System using fast chemistry. Run time: 37 minutes.

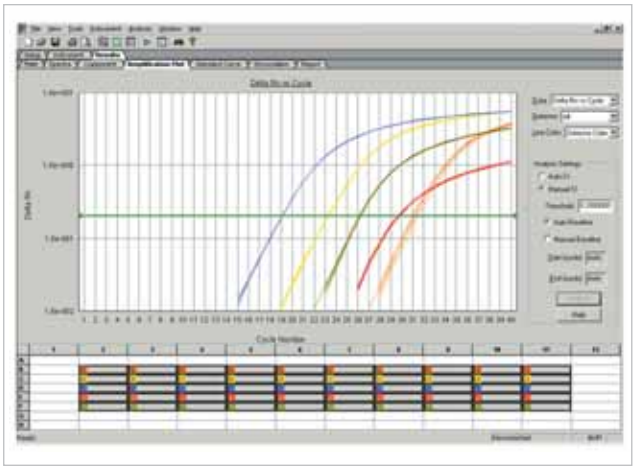


Figure 2. Performance of assays in standard mode on the 7500 System with standard chemistry. Run time: 1 hour and 40 minutes.

Table 1. Fast vs. Standard Mode Data Comparison Results

			Fast Mode: 37-min. run		Standard Mode: 1 hr., 40-min. run		
Gene Symbol	**Assay ID #**	**Target* Expression Level**	**Average C_T**	**Standard Deviation**	**Average C_T**	**Standard Deviation**	**ΔC_T from 7500 Standard Run**
AGER	Hs00153957_m1	Low	31.27	0.20	31.15	0.18	0.12
CCT7	Hs00362446_m1	Medium	22.52	0.08	23.26	0.05	-0.74
GAPDH	Hs99999905_m1	High	19.24	0.02	19.06	0.02	0.18
Gene Symbol	**Assay ID #**	**Template** Sequence Content**	**Average C_T**	**Standard Deviation**	**Average C_T**	**Standard Deviation**	**ΔC_T from 7500 Standard Run**
PGRMC1	Hs00198499_m1	GC-Rich	28.84	0.06	29.67	0.07	-0.83
RAB14	Hs00249440_m1	AT-Rich	25.91	0.08	26.13	0.05	-0.22

*Expression levels are designated as low (C_T > 27), medium (C_T 20–27), and high (C_T < 20).

**GC-rich is defined as > 50% GC content of the amplified template region. AT-rich is defined as > 50% AT content of the amplified template region.

Results of standard-mode amplification of human Raji cDNA (reaction: 1 ng/µL, 25 µL) using the 7500 Real-Time PCR System and TaqMan® Universal PCR Master Mix with five TaqMan® Gene Expression Assays, and fast-mode amplification of human Raji cDNA (reaction: 1 ng/µL, 25 µL) using the 7500 Fast Real-Time PCR System and TaqMan® Fast Universal PCR Master Mix with five TaqMan Gene Expression Assays.

TaqMan® Gene Expression Assays, or assays designed with Primer Express® software and run under universal thermal cycling conditions. The transfer requires no re-optimization, and assay performance is comparable in both modes.

Applied Biosystems scientists recently compared data from the standard Applied Biosystems 7500 Real-Time PCR System and from the 7500 Fast Real-Time PCR System to verify performance. Data from these experiments show that both systems and chemistries provide superior performance, accurate, reproducible results, a high level of sensitivity, outstanding precision, and a broad dynamic range. Specifically, the two platforms and chemistries were evaluated for performance (precision and C_T value), gene expression measurements using the relative quantitation (RQ) or $\Delta\Delta C_T$ method, and test-site results.

Performance

As can be seen in Table 1 and in Figures 1 and 2, the Applied Biosystems 7500 Fast Real-Time PCR System, with its approximtely 35-minute run times, delivers data with C_T values and precision comparable to that generated on the standard 7500 platform with its 1 hour and 40-minute run time. This fact indicates that the performance of the 7500 Fast System and fast chemistry is highly robust, even with GC- and AT-rich or low-expressing templates.

Precision and Validation Testing

Precision is an essential component of successful real-time PCR. Applied Biosystems provides customer laboratories with verification test plates for accuracy and precision testing of the 7500 Fast System. Verification test plates, which also validate proper instrument function, were used in these comparison studies to test both the 7500 Fast System and 7500 System. The data showed almost no variation in replicate reproducibility (Figure 3). The expected two-fold difference in sample quantity is represented by a single-cycle (C_T) separation for the two unknown populations of 5,000 and 10,000 copies.

Measuring Gene Expression using the RQ (Comparative or ΔΔCT) Method

Relative quantitation (RQ) studies determine gene expression changes in a target sample, relative to a calibration sample. When using the comparative or $\Delta\Delta C_T$ method, an endogenous control normalizes the amount of cDNA that is added to the reaction.

In the experiment detailed in Table 2 (Experimental Plate Layout view shown in Figure 4), gene expression measurements were made for cDNA derived from total RNA that was isolated from three human tissues (brain, liver, kidney) and from a universal reference RNA pool. The endogenous control was 18S rRNA, and universal reference RNA was used as the calibrator (i.e., the sample against which gene expression changes are measured).

Applied Biosystems SDS (sequence detection system) RQ Study software calculates fold changes directly and provides an easy-to-understand visual display of the measured expression

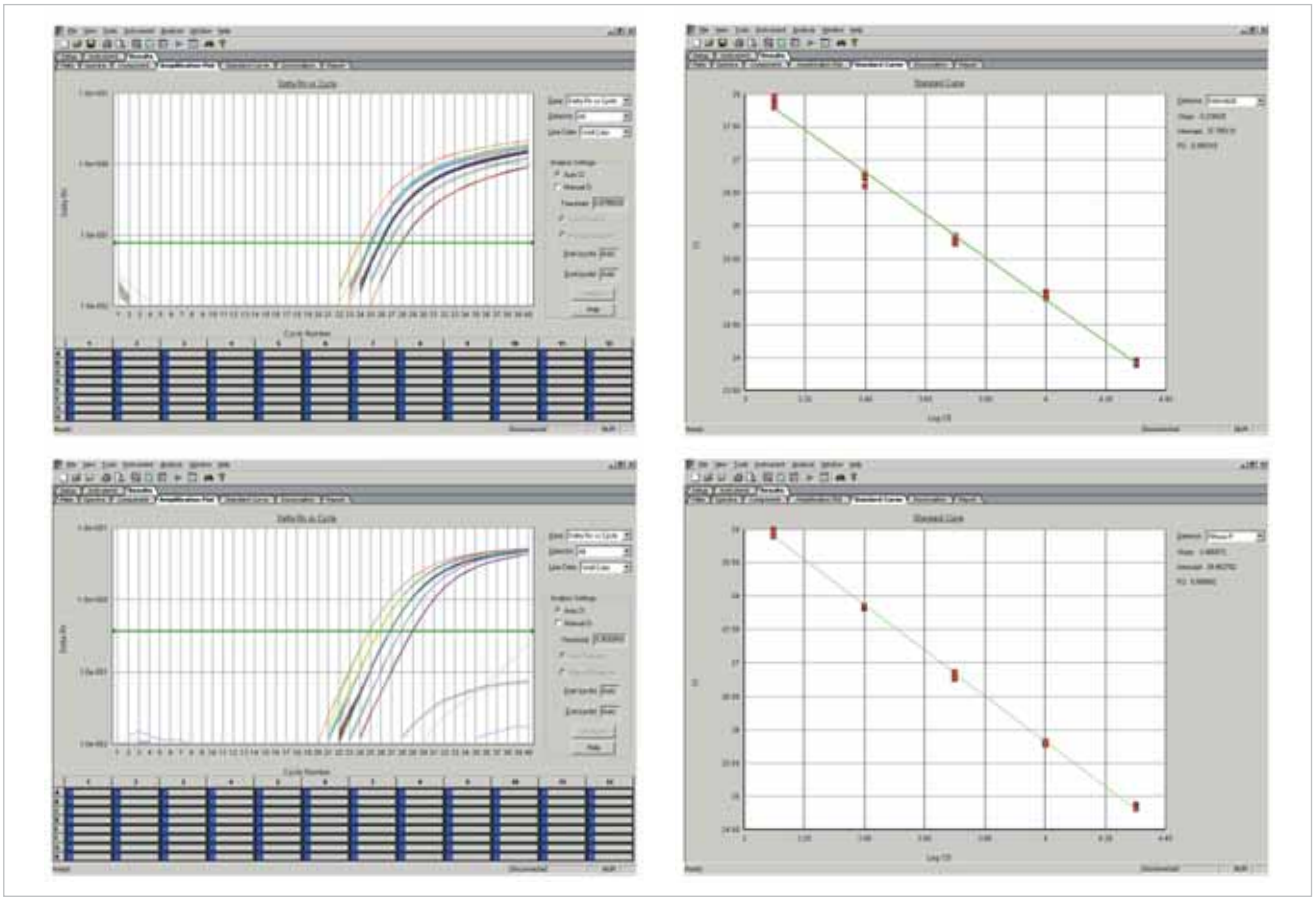


Figure 3. Amplification plots and standard curves showing C_T values plotted against the log of copy number of the RNase P gene from human genomic DNA. Samples were run in replicates of 36, using the fluorogenic 5' nuclease assay. Both systems can distinguish, with a confidence level of 99.7 percent, between two samples containing 5,000 and 10,000 template copies. The R^2 values for both the fast and standard thermal cycling modes were > 0.99, and the standard deviations of C_T were < 0.075 for all samples. Top: 7500 Fast System using fast mode and fast chemistry. Run time: 37 minutes. Bottom: 7500 System using standard mode and standard chemistry. Run time: 1 hour and 40 minutes.

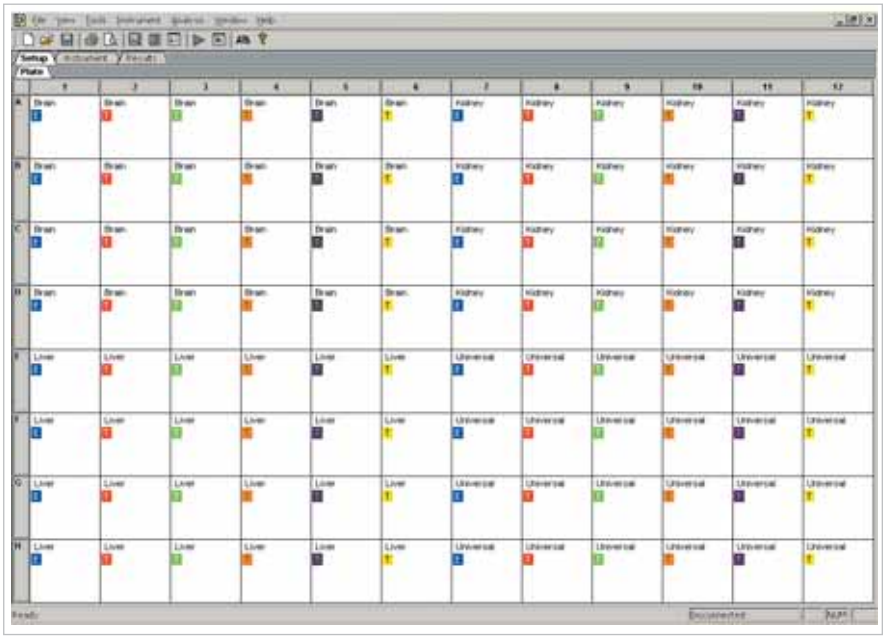


Figure 4. Plate set-up view (gene expression comparison).

changes. If replicate samples are used, RQ Min/Max error bars provide a visual estimate of the error inherent in the measurement. Up to 10 plates of data can be combined into a single study. The results for gene expression levels are consistent between fast and standard modes on the 7500 System (Figures 5 and 6).

Test-Site Data

Several external test-sites participated in the development of the 7500 Fast System and the 7500 Fast Upgrade Kit. Test-site conditions are listed in Table 3, and results can be found in Table 4 and Figure 7.

Table 2. Experimental Conditions for Gene Expression Comparison

Instrument	7500 Fast System		7500 System	
cDNA Template	Human brain, liver, kidney, and universal reference RNA pool (1 µL of 50 ng/µL)		Human brain, liver, kidney, and universal reference RNA pool (1 µL of 50 ng/µL)	
Assays	**Name**	**Assay ID #**	**Name**	**Assay ID #**
	EGR3	Hs00231780_m1	EGR3	Hs00231780_m1
	MAOB	Hs00168533_m1	MAOB	Hs00168533_m1
	OGDH	Hs00159768_m1	OGDH	Hs00159768_m1
	OSGEP	Hs00215099_m1	OGSEP	Hs00215099_m1
	SERPING1	Hs00163781_m1	SERPING1	Hs00163781_m1
Endogenous control	18S rRNA		18S rRNA	
Replicates	Quadruplicates for all targets and endogenous controls		Quadruplicates for all targets and endogenous controls	
Samples	Brain, liver, kidney, and universal reference RNA		Brain, liver, kidney, and universal reference RNA	
Calibrator	Universal Reference RNA		Universal Reference RNA	
Reagent	TaqMan® Fast Universal PCR Master Mix (2X)		TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)	
Reaction volume	20 µL		50 µL	
Plate	Optical 96-well fast thermal cycling plate with barcode (code 128)		ABI PRISM® 96-well optical reaction plate with barcode (code 128)	
Thermal cycling conditions	**Fast Mode** Hold: 20 sec./95°C 40 cycles: 3 sec./95°C 30 sec./60°C		**Standard Mode** Hold: 10 min./95°C 40 cycles: 15 sec./95°C 60 sec./60°C	
Run times	37 minutes		1 hour, 40 minutes	

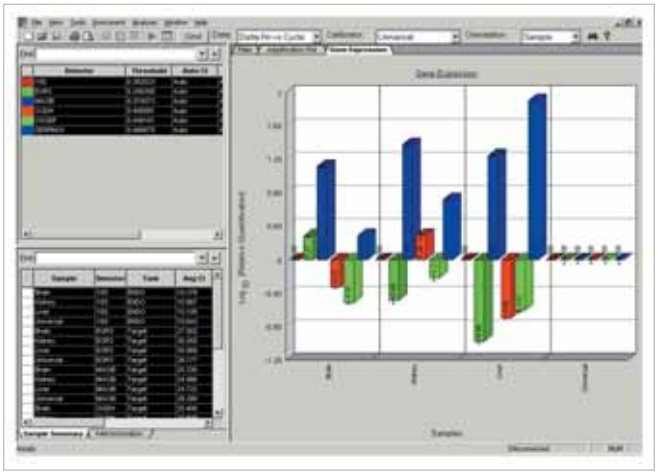


Figure 5. Display of $\log_{10}$ fold change in gene expression for five genes from four samples using the 7500 Fast System in fast mode. Run time: 37 minutes.

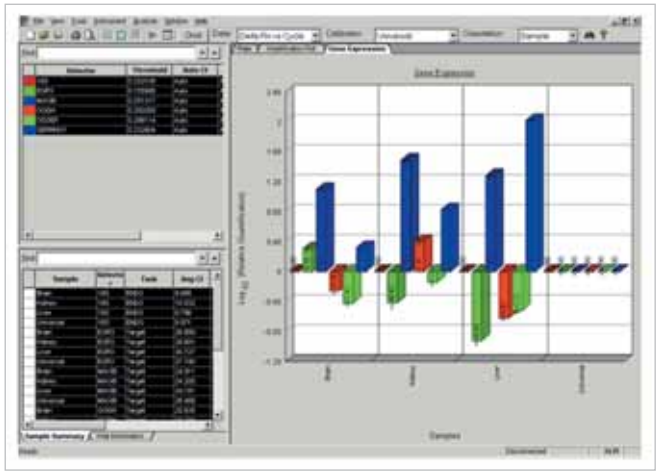
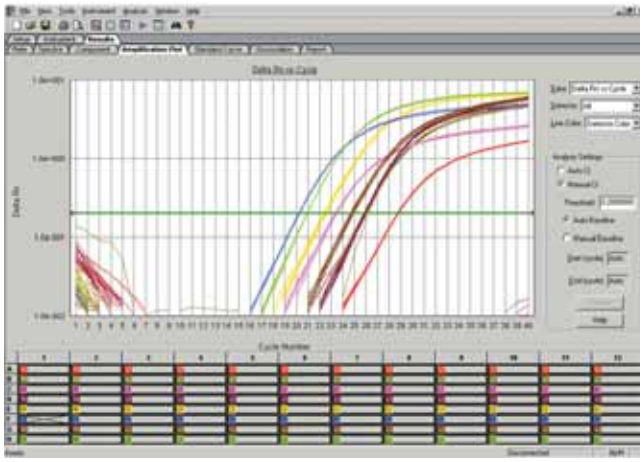


Figure 6. Display of $\log_{10}$ fold change in gene expression (five genes) from four samples using the 7500 System in standard mode. Run time: 1 hour, 40 minutes.

7500 Fast System Data (Fast Chemistry)



7500 System Data (Standard Chemistry)

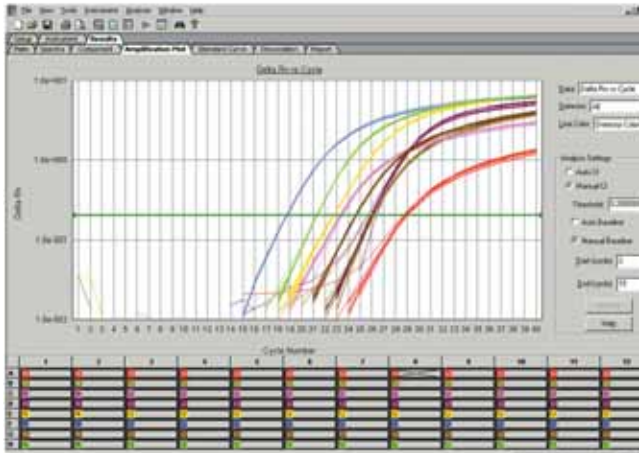


Figure 7. Comparison of TaqMan® Gene Expression Assays and TaqMan® Pre-Developed Assay Reagents (8 technical replicates) on the 7500 Fast System. **Left:** Assay set run using fast mode (run time: 37 minutes). **Right:** The same assay set run using standard mode (run time: 1 hour, 20 minutes). Note that the C_T for the GAPDH assay set (blue) is earlier in fast than in standard mode. Note also that the run time (standard mode) on the 7500 Fast System is shorter than the run time for the same mode on the standard 7500 System. This is expected and is the result of differences between the block designs.

Table 3. Experimental Conditions for Test-Site Comparison

Instrument	7500 Fast System	7500 System
Thermal cycling conditions	**Fast Mode** Hold: 20 sec./95°C 40 cycles: 3 sec./95°C 30 sec./60°C	**Standard Mode** Hold: 10 min./95°C 40 cycles: 15 sec./95°C 60 sec./60°C
Reagent	TaqMan® Fast Universal PCR Master Mix (2X)	TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)
Reaction volume	20 µL	20 µL
Replicates	10 replicates with 2 NTCs	10 replicates with 2 NTCs
Plate	Optical 96-well fast thermal cycling plate with barcode (code 128)	ABI PRISM® 96-well optical reaction plate with barcode (code 128)
Run times	37 minutes	1 hour, 20 minutes

Table 4. Results

Gene Symbol	Assay ID#	Average C_T Fast	Standard Deviation (Fast)	Average C_T Standard	Standard Deviation (Standard)	ΔC_T from 7500 Standard Run
PGRMC1	Hs00198499_m1	28.66	0.041	28.86	0.142	-0.20
RAB14	Hs00249440_m1	25.88	0.114	26.06	0.103	-0.18
SRM	Hs00162307_m1	23.33	0.066	23.30	0.065	0.03
RAB21	Hs00407832_m1	25.87	0.079	25.93	0.191	-0.06
CCT7	Hs00362446_m1	22.41	0.087	22.68	0.05	-0.28
GAPDH	Hs99999905_m1	20.19	0.032	18.60	0.038	1.52
TGF-Beta	4327054F	24.75	0.136	24.57	0.05	0.18
B2M	4333766T	24.44	0.027	24.30	0.033	0.15

Conclusion

The experiments described demonstrate that TaqMan® Gene Expression Assays and TaqMan® Pre-Developed Assay Reagents show comparable performance for C_T value and precision, when run either on the 7500 Fast System using fast chemistry or on the standard 7500 System using standard chemistry. No re-optimization of the assay or primer/probe concentrations was required for transfer to the fast chemistry, and the run times were less than 40 minutes.

Acknowledgements

We would like to express our gratitude to Dr. John Flanagan of the Boston University School of Medicine, Department of Endocrinology, for allowing us to use the department dataset outlined in Figure 7 and Tables 3 and 4.

TaqMan® Genomic Assays

You can search for TaqMan® Genomic Assays by gene symbol, public accession numbers, molecular function, or biological process at **myscience.appliedbiosystems.com**. You'll also find useful data and valuable links to relevant references.

Ordering Information

Description	Part Number
Applied Biosystems 7500 Fast Real-Time PCR System with Notebook Computer	4351106
Applied Biosystems 7500 Fast Real-Time PCR System with Tower Computer	4351107
7500 Fast System Upgrade Kit	4362143
TaqMan® Fast Universal PCR Master Mix (2X), no UNG	4352042
Optical Fast 96-well Microplate with Barcode (code 128)	4346906

Printed in the USA, 06/2005, LD Publication 117MI08-01

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