Applied Biosystems® 7900HT Fast Real-Time PCR System: A Comprehensive Comparison of Standard and Fast System Data

Introduction

The Applied Biosystems® 7900HT Fast Real-Time PCR System, the flexible leading-edge real-time PCR instrument, reduces the time-to-result from almost 2 hours to about 35 minutes, a 75% time reduction. For laboratories equipped with the ABI PRISM® 7900HT Sequence Detection System, an affordable service upgrade to the 7900HT Fast Real-Time PCR System is also available, providing the same access to accelerated results without the need to invest in a completely new system.

Applied Biosystems has extensively evaluated the transfer of existing methods from the standard 96-well to the new fast 96-well real-time PCR run. This data sheet compares performance across a range of experiments on the fast and standard blocks. Specifically, the two blocks were evaluated for:

1. Performance robustness (Table 1)
2. Precision (Figure 1)
3. Relative quantitation (RQ) in gene expression (Figure 2 and Table 2)
4. Customer results (Figures 3 and 4)

The data comparisons that follow clearly indicate that both fast and standard modes of the 7900HT Fast Real-Time PCR System achieve highly comparable results with the same level of performance and reproducibility you have come to expect from Applied Biosystems.

Performance Robustness

The Applied Biosystems® 7900HT Fast Real-Time PCR System, whether in fast or standard mode, delivers highly robust results, even in GC-rich and low-expressing templates (Table 1).

Table 1. Robust Results Across Variable Targets.

		Fast Mode		Standard Mode	
Gene	**Expression Level**	**Average C_t**	**Standard Deviation**	**Average C_t**	**Standard Deviation**
AGER	Low	30.54	0.06	30.84	0.05
CC17	Medium	24.62	0.02	23.92	0.02
GAPD	High	17.65	0.01	17.00	0.04
Gene	**Template Sequence Content**	**Average C_t**	**Standard Deviation**	**Average C_t**	**Standard Deviation**
PGRMC1	GC-rich	26.60	0.03	26.89	0.10
RAB14	AT-rich	24.15	0.02	23.89	0.02

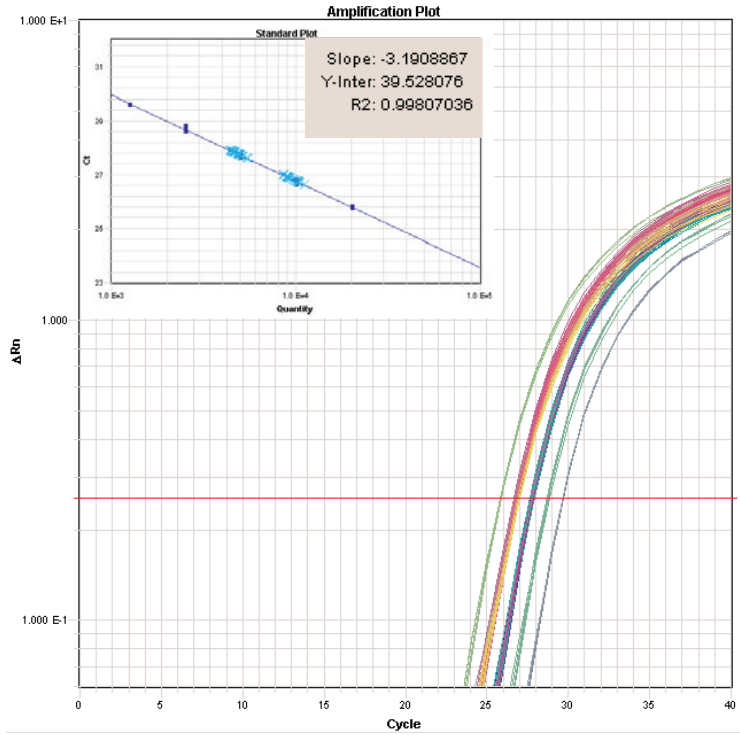
Amplification of variable targets from human Raji cDNA (1 ng/µL) in a 20 µL reaction volume (fast mode) or a 25 µL reaction volume (standard mode). Expression levels are designated as Low (C_t >27), Medium (C_t 20–27), and High (C_t <20). GC-rich is defined as >50% GC content of the amplified template region. AT-rich is defined as >50% AT content of the amplified template region.

Precision

Precise and accurate data are an essential component for success in real-time PCR. Applied Biosystems provides verification test plates for accuracy and precision testing of the 7900HT Fast Real-Time PCR System in customer laboratories. This helpful tool is used to validate proper instrument function. Using these verification test plates, we tested the compatibility of the fast and standard block systems.

The data showed extremely tight replicate reproducibility in both standard and fast modes (Figure 1). The expected two-fold difference in sample quantity is represented by a single-cycle C_t separation.

Fast Mode



Standard Mode

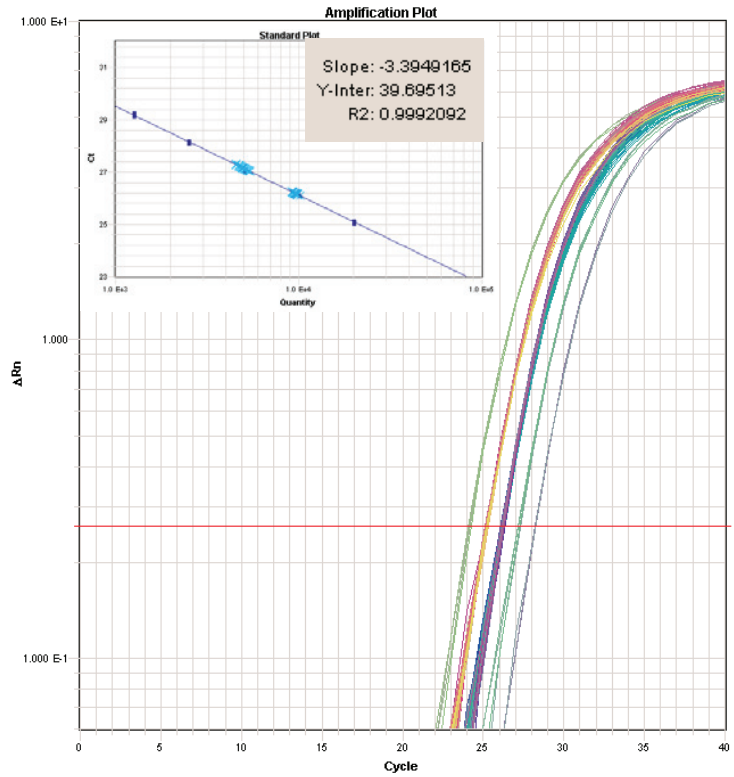


Figure 1. Standard Curves Showing C_t Plotted Against the Log of the Quantity of the RNase P Gene From Human Genomic DNA. Samples were run in replicates of 36, using the fluorogenic 5' nuclease assay. Both systems can distinguish, with a confidence level of 99.7%, between two samples containing 5,000 and 10,000 template copies. The R^2 values for both modes were >0.99, and the standard deviations for the C_t were <0.046 for all samples.

Relative Quantitation (RQ) in Gene Expression Experiments

Relative quantitation (RQ) studies are used to determine gene expression changes in a target sample relative to a calibrator sample. When using the comparative C_t method, an endogenous control is used to normalize the amount of cDNA added to the reaction. In the following experiment (Table 2), 18S rRNA was used as the endogenous control. The calibrator sample was 1X Raji cDNA, and the target samples were the same template at 10X and 100X concentrations. The results for gene expression levels are consistent in both fast and standard modes (Figure 2).

Customer Results

The following results were provided to Applied Biosystems by early-placement customers equipped with the 7900HT Fast Real-Time PCR System. As the following data indicate, customer results independently demonstrate equivalent performance among fast and standard blocks.

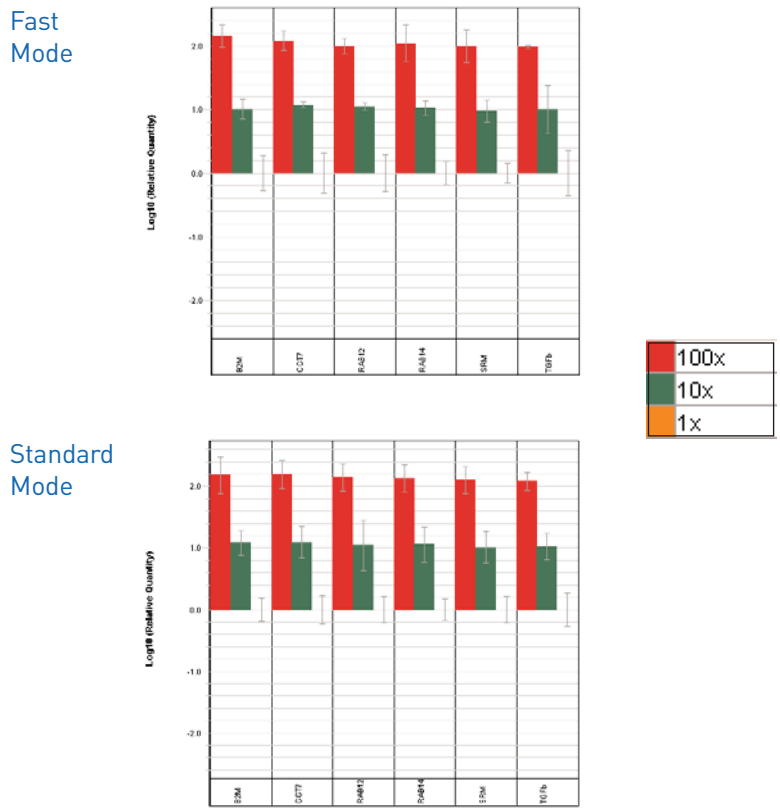


Figure 2. RQ Data From 3 Sample Sizes Are Almost Identical for 6 Genes Studied in This Experiment. Experimental conditions are listed in Table 2. Once again, results from both the fast and the standard modes were highly comparable.

Table 2. Experimental Conditions for the RQ Study.

	Fast 96-Well Block	Standard 96-Well Block
Template	Human Raji cDNA (1 ng/µL)	Human Raji cDNA (1 ng/µL)
Assays	4 TaqMan® Gene Expression Assays; 2 TaqMan® Endogenous Controls	4 TaqMan® Gene Expression Assays; 2 TaqMan® Endogenous Controls
Endogenous Control	18S rRNA	18S rRNA
Samples	1X, 10X, 100X	1X, 10X, 100X
Calibrator	1X sample	1X sample
Reagent	TaqMan® Fast Universal PCR Master Mix (2X)	TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X)
Reaction Volume	20 µL	25 µL
Plate	MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	MicroAmp® 96-Well Reaction Plate with Barcode, 0.2 mL
Thermal Cycling Conditions	Fast mode Hold: 20 sec/95°C 40 cycles: 1 sec/95°C, 20 sec/60°C	9600 Emulation mode Hold: 10 min/95°C 40 cycles: 15 sec/95°C, 60 sec/60°C
Run Time	35 min	1 hr 50 min

Customer 1:
The study utilized TaqMan® Gene Expression Assays to amplify four genes in five replicates. Data confirm that the fast and standard results are highly comparable (Figure 3).

Customer 2:
In this study, the customer laboratory designed their primers and TaqMan® probes with Primer Express® software. Samples were amplified in four replicates. Data verified highly comparable fast and standard results (Figure 4).

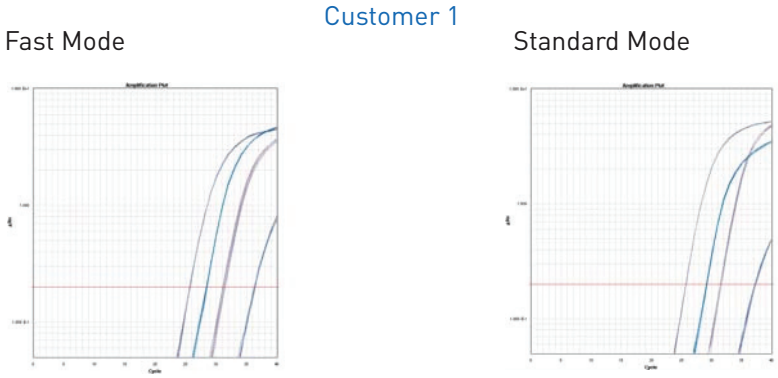


Figure 3. Customer 1 Results. In the experiment conducted at a customer 1 laboratory, equivalent amplification results were obtained for both fast and standard modes, using TaqMan® Gene Expression Assays selected by the laboratory. Each sample was run in five replicates.

Figure 4. Customer 2 Results. In the experiment conducted at a customer 2 laboratory, equivalent amplification results were obtained for both fast and standard modes, using custom-designed primers and probes selected by the laboratory. Each sample was run in four replicates.

Conclusion

All experiments conducted to evaluate fast and standard 96-well blocks found no significant differences in performance. In fact, compared to the standard block, the fast block obtained equivalent data in about one-third less time. Transferring assays from the standard block to the new fast block was easy because the assays were developed in accordance with the Rapid Assay Development Guidelines.

Design changes in TaqMan® probes and/or TaqMan® assays were not necessary to achieve optimal results. Furthermore, method transfer to fast mode was accomplished without the need to alter primer or probe concentrations. All assays were successfully transferred directly from standard to fast mode.

Acknowledgments

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Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

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