The Applied Biosystems 7900HT Micro Fluidic Card System with Assays-on-Demand™ Products for Gene Expression

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Introduction:

Applied Biosystems offers a complete, high-throughput, easy-to-use real-time PCR gene expression quantification system. Featuring the new Applied Biosystems 7900HT Micro Fluidic Card, with new, off-the-shelf Assays-on-Demand Gene Expression Products, and analysis with the new SDS 2.1 software, the ABI PRISM® 7900HT Sequence Detection System allows researchers to move from gene lists to large-scale real-time PCR gene expression profiling results quickly and easily. Several key feature enhancements of the system are shown in Table 1.

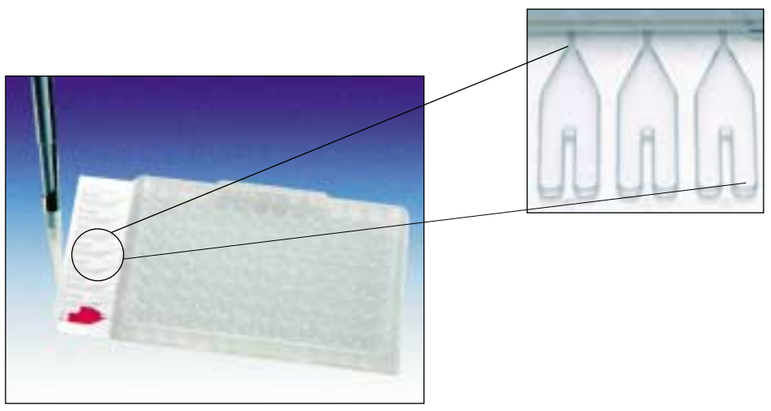

Figure 1. *Loading the 7900HT Micro Fluidic Card*

Micro Fluidic Card

The Applied Biosystems 7900HT Micro Fluidic Card is a new consumable consisting of 384 wells connected by a series of channels. The Micro Fluidic Card is designed for custom assay configuration, and so acts as a low-density custom array. TaqMan® probe based gene expression assays are loaded and dried in the wells of the Micro Fluidic Card during the manufacturing process. At run time, up to eight template cDNA samples/card in TaqMan® Universal PCR Master Mix are added to 8 loading ports, allowing delivery of a single sample-specific reaction mix to 48 wells via a centrifugal filling process. The Micro Fluidic Card technology allows multiple targets to be analysed per sample with very few pipetting steps, streamlining reaction set-up time and eliminating the need for liquid handling robotics. This, combined with only a 2 µL well volume (includes well and channel volume), results in very efficient use of biological samples combined with low reagent consumption.

Assays-on-Demand Products

Customers can choose assays from the Applied Biosystems Assays-on-Demand Gene Expression product list with over 25,000 human and mouse assays now available. Assays-on-Demand Gene Expression Products are gene-specific TaqMan® probe & primer sets designed using Applied Biosystems sophisticated bioinformatics pipeline. Each assay design undergoes extensive *in silico* QC, including BLASTing against both transcript and genome databases to ensure gene specificity. Manufactured assays further undergo analytical QC, ensuring synthesis and formulation integrity.

SDS 2.1 software

High-throughput gene expression quantification is further enabled with the new SDS 2.1 software. Key new gene expression features include a new Relative Quantification Study application and automatic CT determination. The Relative Quantification Study application completely automates analysis when using the DDCT/comparative CT method, and allows 10 individual cards to be combined in a study with a single analysis. The SDS 2.1 Enterprise (database) software upgrade with companion RQ Manager software further streamlines data handling and analysis, and maximizes the throughput capability of the 7900HT system when used with the optional Automation Accessory.

Component	Advantages
7900HT Micro Fluidic Card	Requires no liquid handling robotics
	Efficient use of biological samples and reagents
	Quality results: consistent quantification results for gene sets
Assays-on-Demand Products	Over 25,000 off-the-shelf, gene-specific TaqMan probe & primer sets
	Design by validated bioinformatics pipeline
	Mouse and Human
SDS 2.1 Software	Relative Quantification Study application using the DDCT/comparative CT method
	Automatic CT determination
	Enterprise (database) software upgrade with companion RQ Manager software

Table 1. *Key Enhancements of ABI PRISM® 7900HT Sequence Detection System*

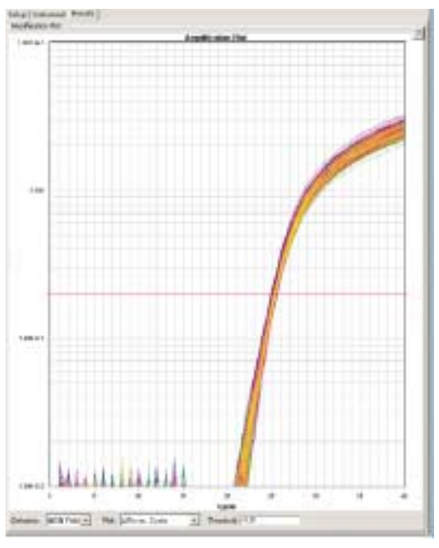

Figure 2. *Uniformity across a single 7900HT Micro Fluidic Card*
7900HT Micro Fluidic Card uniformity results. 384-wells contain the assay (primers and FAM™ dye labelled MGB probe) for transforming growth factor, beta 1 (TGFB1), plus template at identical concentrations.
Ct standard deviation = 0.097.

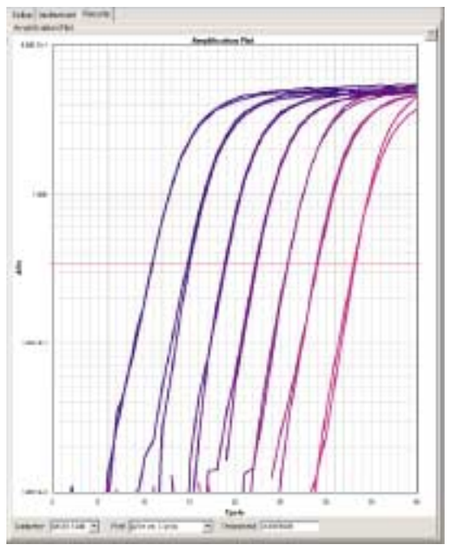

Figure 3. *Dynamic range is over 6 logs.*
10-fold dilution series, demonstrating >6 log dynamic range.

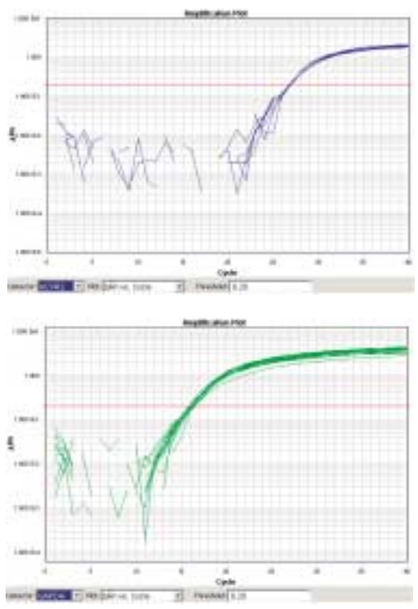

Figure 4. *Reproducibility across 7900HT Micro Fluidic Cards.*
Universal Reference cDNA (2ng/µl) was run on each of four 7900HT Micro Fluidic Cards. Two replicates/gene/sample/card, for eight replicates, are displayed.

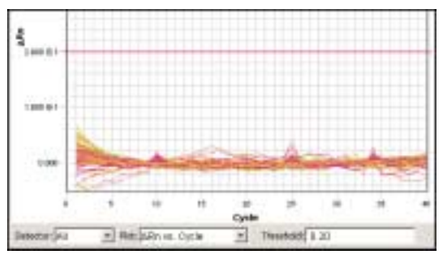

Figure 5. *No Template controls*
Universal Master Mix with no added cDNA template was run on 2 ports of a 7900HT Micro Fluidic Card. 2 replicates/gene x 44 genes are displayed.

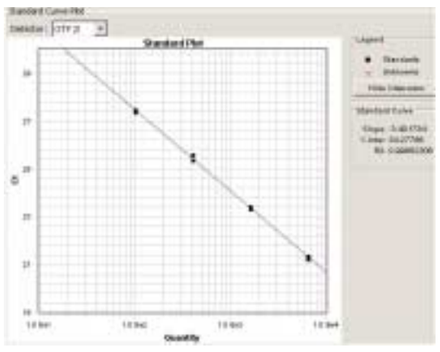

Figure 6. *Standard Curve and PCR Efficiency*
Representative assay, GTFI (Hs00231018_m1).
Slope indicates 97% PCR efficiency.

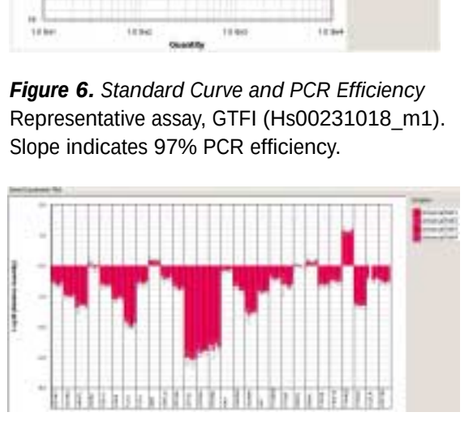

Figure 7. *Relative Quantification of gene expression across 7900HT Micro Fluidic Cards*
Universal Reference cDNA run on four 7900HT Micro Fluidic Cards, analysed using the SDS 2.1 Relative Quantification software "RQ Manager," by the DDCT/comparative CT method. A brain cDNA run on a single card was used as calibrator sample, and GAPDH (separate well) was used as Endogenous Positive Control. All genes with an average Ct <34 cycles in both Brain and Universal Reference samples are shown. Error bars indicate 95% confidence interval.

Methods

The purpose of the study was to test assay performance and relative quantification using the complete Applied Biosystems 7900HT Micro Fluidic Card system. In this study, four Micro Fluidic Cards with Assays-on-Demand reagents were run as Relative Quantification cards using SDS 2.1 software in Enterprise mode, using the 7900HT System's automation accessory.

Each card was formatted for 4 samples run against 44 Assays-on-Demand Gene Expression Products, in duplicate. cDNA samples were made from commercially available total RNA (Stratagene), using the Applied Biosystems "High Capacity cDNA archiving kit" for reverse transcription. Table 2 shows the cDNA samples loaded across the four cards, with the sample names receiving sequential numbers to facilitate analysis of replicates.

File name	Ports/cDNA samples (final conc)
Card1.sds	1/2: Universal Ref1 (2 ng/ul)
	3/4: Brain1 (2 ng/ul)
	5/6: Liver1 (2 ng/ul)
	7/8: Kidney1 (2 ng/ul)
Card2.sds	1/2: Universal Ref2 (2 ng/ul)
	3/4: Brain2 (2 ng/ul)
	5/6: Liver2 (2 ng/ul)
	7/8: Kidney2 (2 ng/ul)
Card3.sds	1/2: Universal Ref3 (2 ng/ul)
	3/4: Universal Ref 1:4 dil. (0.5 ng/ul)
	5/6: Universal Ref 1:16 dil. (0.125 ng/ul)
	7/8: Universal Ref 1:64 dil. (31 pg/ul)
Card4.sds	1/2: Universal Ref4 (2 ng/ul)
	3/4: Raji (0.4 ng/ul)
	5/6: Thymus (2 ng/ul)
	7/8: No Template Control

Table 2. *cDNA samples on Micro Fluidic Cards*

Results

Ease-of-use and Throughput

Sample specific reaction mixes are prepared and loaded into the fill ports (Figure 1) of the Applied Biosystems Micro Fluidic Card using a standard pipette. The Micro Fluidic Card is then centrifuged to load the sample specific reaction mixes into the individual wells of the Micro Fluidic Card, and sealed, before loading into the 7900HT system. Throughput of 10 – 12 card/day (over 4000 wells/day) can be easily achieved using the automation accessory for the 7900HT. Preparation of 10 Micro Fluidic Cards can be done in 1 – 2 hours by a trained researcher, with no liquid-handling robotics.

Assay performance on the Micro Fluidic Card is excellent

- Uniformity across a single Micro Fluidic Card was tested (Figure 2). A typical 384 identical-well card with TGFB (FAM-MGB) and a control cDNA template gave a Ct standard deviation of 0.097. This easily enables discrimination of 2-fold changes in gene expression with >99% confidence. (SD of <1.67 required for 2x discrimination).
- Dynamic range is typical of other Applied Biosystems real-time PCR platforms. A 10-fold dilution series (Figure 3) shows linear response, and good PCR efficiency, over at least 6 logs.

Performance of the Assays-on-Demand gene expression reagents was very reliable.

- Reproducibility across cards was excellent. Amplification Plot data from replicate cards was indistinguishable (Figure 4).
- No detectable amplification was seen in No Template Controls (Figure 5).
- An Absolute Quantification card run with a dilution series of cDNA was analysed (Figure 6). PCR efficiency calculation from the slope of the Ct/Quant curve (Efficiency = $2\text{-}2^{(-1/slope)}$) gives assay-specific PCR efficiencies typically above 90%. A representative assay, GTFI (Hs00231018_m1) is shown; slope indicates 97% PCR efficiency.

Relative Quantification using SDS 2.1 Software

- Relative Quantification of gene expression across cards was compared in order to demonstrate card-to-card reproducibility (Figure 7). A single input cDNA (Universal Reference) was run on 4 cards, and the results were analysed using the SDS 2.1 Relative Quantification software "RQ Manager," by the DDCT/comparative CT method. Excellent reproducibility of RQ values was seen, with the 95% confidence intervals for the 4 cards on all well-expressed genes overlapping.

Summary

Applied Biosystems has released 3 new enhancements to its ABI PRISM® 7900HT Sequence Detection System, now a complete, high-throughput, easy-to-use real-time PCR gene expression quantification system.

Product developments:
- 7900HT Micro Fluidic Card
- Off-the-shelf Assays-on-Demand Gene Expression Products
- SDS 2.1 software

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