Detection of Immune Response Genes Using the Applied Biosystems® ViiA™ 7 Real-Time PCR System

Researchers at the Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Austria. From left to right: Martin Svoboda (PhD student), Ursula Smole (PhD student), Thomas Halama (Senior Field Application Specialist, Life Technologies), Diana Mechtcheriakova (Associate Professor), and Paolo Geroldi (Field Service Engineer, Life Technologies).

Features	ViiA™ 7 Real-Time PCR System
Block Configurations	96-well, Fast 96-well, 384-well (runs Fast or standard), TaqMan® Array Micro Fluidic Cards
Run Time	30 minutes expected (Fast 96-well); 35 minutes (384-well)
Resolution	Down to 1.5-fold changes for singleplex reaction
Excitation Source	OptiFlex™ System with halogen lamp
Detection Channels	Decoupled—6 emission, 6 excitation
21 CFR p11 Compliance Module	Optional software module
Remote Monitoring	Available to monitor up to 4 instruments in real time and the status of up to 15 instruments
Data Export Format	User configurable: *.xls, * .xlsx, *.txt, and 7900 formats, as well as the new MIQE-compliant RDML format

Introduction

Associate Professor Diana Mechtcheriakova and her research team from the Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Austria focus on the identification and characterization of novel pathways and targets contributing to pathophysiological conditions including inflammation, aberrant immunity, and cancer. Their main goal is the establishment and validation of disease-relevant, multi-gene signatures as part of a strategy towards genomic medicine. In a previous study, the group used the Applied Biosystems® 7900HT Fast Real-Time PCR System with the Applied Biosystems® TaqMan® Array Gene Signature Card (Human Immune Panel) for immune profiling of dendritic cells generated from peripheral blood monocytes of allergic and healthy samples [manuscript in preparation]. Here, our collaborators evaluate the reproducibility of those results using the same RNA samples, reagents, and consumables on the new Applied Biosystems® ViiA™ 7 Real-Time PCR System.

Materials and Methods

Immature dendritic cells (DCs) generated from peripheral blood monocytes of allergic and healthy samples were cultured *in vitro* in the presence of GM-CSF and IL-4. Next, immature DCs were treated with recombinant allergens (20 µg/mL), maturation cocktail [TNFα (25 ng/mL), IL-1β (10 ng/mL), and GM-CSF (500 U/mL)], or both, in kinetics for 0.5 hr, 1 hr, 2.5 hr, 4 hr, and 24 hr, or left untreated. Cells were harvested, and total RNA was isolated using the Absolutely RNA RT-PCR Miniprep Kit (Stratagene). cDNA was prepared from 1 µg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

To analyze the gene expression pattern, Applied Biosystems® TaqMan® Array Gene Signature Cards (Human Immune Panel) were used in conjunction with the Applied Biosystems® ViiA™ 7 Real-Time PCR System according to the manufacturer's protocols. Samples were run in duplicate. The data obtained were compared to data generated from a previous study in which the same set of samples had been tested using the human immune panel arrays on the Applied Biosystems® 7900HT Fast Real-Time PCR System.

Results

Figure 1 shows raw C_t values generated by the 7900HT Fast Real-Time PCR System and the new ViiA™ 7 Real-Time PCR System. There is a high correlation of results between the two systems at the C_t level, as shown by the R^2 values. These results further validate the performance of the TaqMan® Array Micro Fluidic Cards as a dependable workflow on both the 7900HT Fast Real-Time PCR System and the ViiA™ 7 Real-Time PCR System.

Comparison of ΔC_t values using GAPDH as an endogenous control gene is shown in Figure 2. Again, results between the systems show a high degree of concordance.

Finally, relative quantities were compared by calculating 2^(-$\Delta\Delta C_t$) for all targets using GAPDH as the endogenous control and calibrating against the untreated cells. Examples of regulated genes are shown in Figure 3.

Conclusions

The ViiA™ 7 Real-Time PCR System produced results that were fully concordant with those obtained on the 7900HT Real-Time PCR System, demonstrating the continued robustness of Applied Biosystems real-time PCR systems and TaqMan® Array Micro Fluidic Card technology. Assistant Professor Diana Mechtcheriakova states, "The experiments performed on the ViiA™ 7 Real-Time PCR System using the TaqMan® Array Micro Fluidic Card format with blood-derived primary cells from different samples confirmed the scientific hypothesis and are in 100% agreement with the results obtained on the Applied Biosystems® 7900HT Real-Time PCR System using the TaqMan® Array Card format."

Acknowledgement

The authors greatly appreciate the opportunity to collaborate with Professor Dr. Erika Jensen-Jarolim and Assistant Professor Dr. Diana Mechtcheriakova and researchers Ursula Smole and Martin Svoboda from the Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Austria.

We acknowledge Thomas Halama, Senior Field Application Specialist, Molecular Biology, Life Technologies, for his generous contributions to this study.

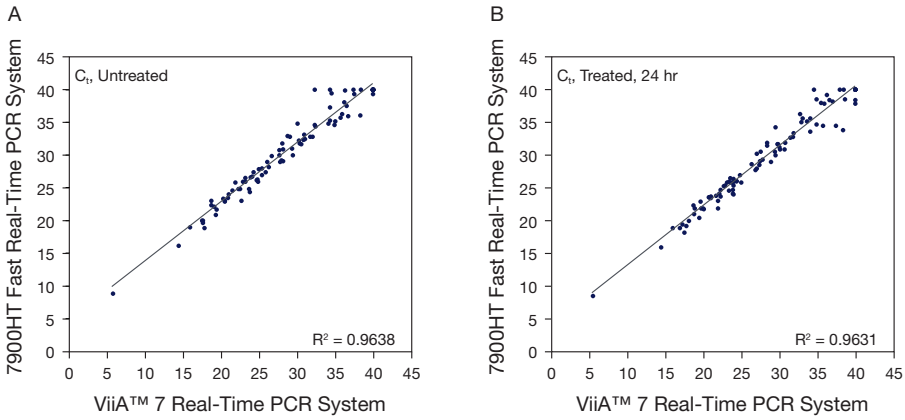

Figure 1. High Correlation of Results Between the ViiA™ 7 Real-Time PCR System and the 7900HT Fast Real-Time PCR System. Panel A, untreated cells; Panel B, cells treated with a maturation cocktail for 24 hr.

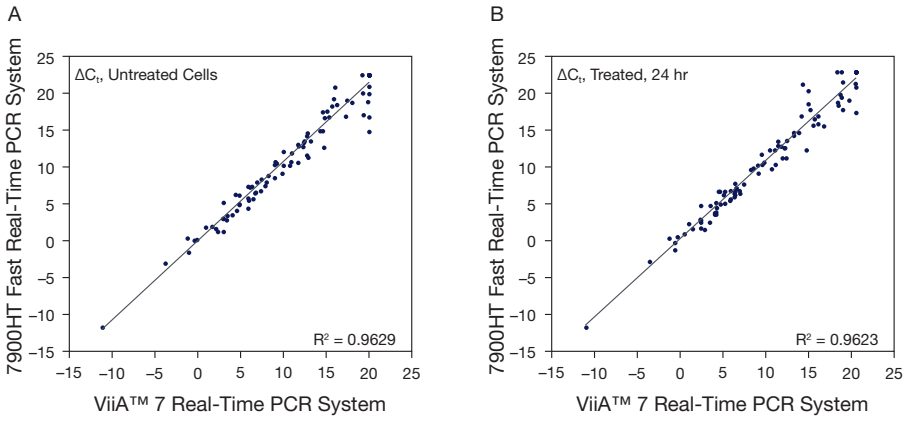

Figure 2. Concordant Results Obtained Using the ViiA™ 7 Real-Time PCR System and the 7900HT Fast Real-Time PCR System. ΔC_t values were generated using GAPDH as the endogenous control. Results are shown for both untreated cells (Panel A) and cells treated with a maturation cocktail for 24 hr (Panel B).

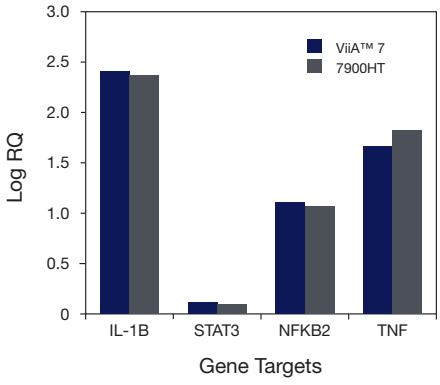

Figure 3. Comparison of Relative Quantities (RQ values). Representative genes that exhibited differential expression.

Dr. Diana Mechtcheriakova, Associate Professor

"The ViiA™ 7 Real-Time PCR System from Life Technologies with 96-well, 384-well, and 384-/micro fluidic card blocks will encourage better experimental practice, allowing fast, reliable, and definite interpretation of data, and as a result, cutting edge science. My scientific philosophy, which I am sure is shared by the majority of scientists, is based on the idea that the most rewarding research is the one which not only delights the curiosity of the researcher, but is beneficial to the humankind. The ViiA™ 7 Real-Time PCR System provides excellent potentialities to fulfill one of the major goals of my research group—establishment and validation of multi-gene signatures as quantitative models for prediction of biological outcomes under diseased conditions, and thereby provides straightforward opportunities for future steps toward personalized medicine."

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