Validating Microarray Results Using the QuantiGene Reagent System

Introduction

The QuantiGene Reagent System has been used to quantitate gene expression in a variety of life science applications including RNAi knockdown, predictive toxicology and microarray validation. Here we demonstrate that the QuantiGene assay is an ideal tool for microarray validation. The model for study was the inflammatory response by U937 cells to phorbol myristate ester (PMA) followed by lippopolysaccarhide (LPS) treatment.

To elucidate which genes might be involved in the regulation of the inflammatory response, a microarray was fabricated by spotting 1044 different gene-specific 70-mer oligonucleotides on amine-coated slides. These 70-mer oligonucleotides were selected to represent regulatory genes from four gene classes and include 534 kinases, 260 transcription factors, 192 phosphatases and 58 translation factors.

Experiments comparing gene expression of U937 cells treated with PMA/LPS with control U937 cells revealed changes of expression in 20% of the genes. Nine genes were reexamined using the QuantiGene assay and six of the nine genes, which had commercially available TaqMan probes, were reexamined using the TaqMan assay. The results from all three assays were then compared and the data are presented below. The data indicate that the QuantiGene and microarray assay results are highly correlated.

The QuantiGene Technology

QuantiGene products use branched DNA (bDNA) technology which relies on cooperative hybridization between target RNA and a gene-specific probe set. Hybridization is followed by signal amplification with a luminescent readout. The assay requires no RNA purification or target amplification, is performed in standard 96-well plates and requires standard molecular biology equipment and techniques.

An overview of the technology is shown in Figure 1. Detailed descriptions of the QuantiGene technology, and the probe sets used in the experiments described in this Application Note, have been previously published, and are available at www.panomics.com.

Materials and Methods

Microarray Assay

1044 unique gene oligos were obtained from Operon Human 2.0 Array ready panel. Each of the 1044 oligos was printed on Corning Ultra-GAPS slides with 18-fold redundancy. Printing was done using a novel and proprietary FiberPrint technology for highly parallel fiber optic-based oligonucleotide deposition of 10,000 elements simultaneously. The housekeeping gene GAPDH was included as a control. Poly A+ RNA from control (vehicle uninduced) and treated (PMA/LPS induced) U937 cells were labeled with both Cy3 and Cy5-labeled dCTPs using CyScribe cDNA labeling protocol (Amersham Biosciences) and used in a two-color hybridization. Data were normalized to expression of GAPDH.

Figure 1. Overview of the QuantiGene Technology.
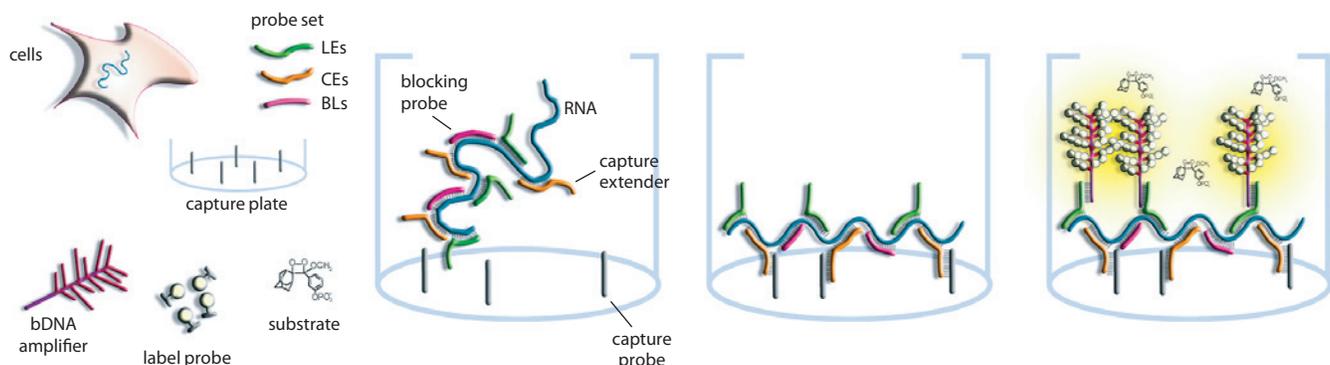
QuantiGene Assay

The same source of Poly A+ RNA used in the microarray assay was used in the QuantiGene assay. Based on the microarray results, nine genes were selected to be reassayed. The nine genes selected represented all four gene classes, showed a varied level of expression ranging from 3 standard deviations (SD) above background to near signal saturation and spanned a broad range in fold induction (i.e., the ratio of induced signal over uninduced signal). Probe sets were designed for each gene and each sample was run in triplicate. The assay was run according to the QuantiGene Reagent System User Manual. To test the accuracy and robustness of the assay, three 2-fold dilutions of mRNA samples (i.e., 2, 4, 8 ng of Poly A+ RNA) were run for each gene. The fold inductions shown represent the average of the triplicate samples. The expression of GAPDH was used to normalize the data.

TaqMan Assay

Out of the nine genes selected, only six of the TaqMan probes (Applied Biosystems Inc.) were commercially available for the correlation study. The cDNA was generated using the CyScribe kit. Each gene sample was run in triplicate with the equivalent cDNA generated from 4 ng of Poly A+ RNA. GAPDH expression was used to normalize the data.

The workflow of the experiment is displayed in Figure 2 and shows the QuantiGene assay proceeding directly from cell lysates and the other two assays requiring the RNA from the lysates to be processed before running.
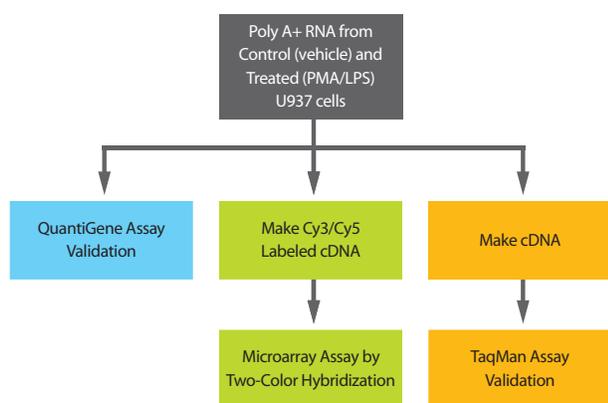

Figure 2. Experiment workflow of microarray validation experiment.
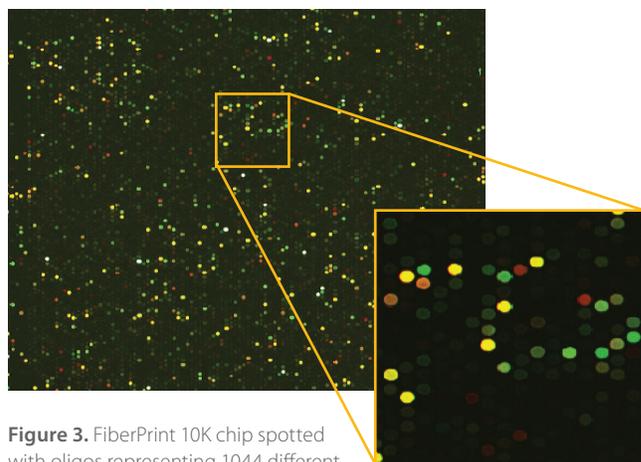

Figure 3. FiberPrint 10K chip spotted with oligos representing 1044 different genes with 18-fold redundancy. Cy3 (green) is induced, Cy5 (red) is uninduced.

Results

Microarray Assay Data

An image of the FiberPrint microarray experiment is shown in Figure 3. The inset shows an enlarged view of spotted oligos of the two-color hybridization with high resolution of spot morphology.

Analysis of the microarray data revealed that approximately 20% of the genes from all four classes of genes were regulated by treatment with PMA/LPS. A table identifying those genes that were subsequently evaluated by the QuantiGene and TaqMan

GAPD	Glucose dehydrogenase	NM_002046
ATF3	Activating transcription factor 3	NM_004024
CDKN1A	Cyclin-dependent kinase inhibitor	NM_000389
EEF2	Eukaryotic translation elongation factor 2	NM_001961
EIF5A	Eukaryotic translation initiation factor 5A	NM_001970
PLAUR	Plasminogen activator, urokinase receptor	NM_002659
PPP4C	Protein phosphatase 4, catalystic subunit	NM_002720
PKM2	Pyruvate kinase, muscle	NM_002654
P381P	Transcription factor, p38 interacting protein	NM_017569
TPT1	Tumor protein, translationally-controlled 1	NM_003295

Table 1: Nine genes which demonstrated a broad range of fold induction were chosen from the microarray results for follow-up evaluation by QuantiGene and TaqMan assays.

Table 2. Microarray assay data of nine genes chosen for follow-up analyses.

Gene	Cy5/Cy3 Dye Order (induced/uninduced)			Cy3/Cy5 Dye Order (induced/uninduced)			Average Fold Induction (normalized)
	Average Signal Cy3 Uniduced	Average Signal Cy5 Induced	Fold Induction (normalized)	Average Signal Cy5 Uninduced	Average Signal, Cy3 Induced	Fold Induction (normalized)	
GAPD	2013	2335.1	1	1886.7	3149.9	1	**1**
ATF3	16.5	118.3	12.46	15.4	266.5	10.25	**11.36**
CDKN1A	11.7	1153.6	90.73	16.6	2552.1	132.65	**111.70**
EEF2	1555.9	805.7	0.63	1447.7	1558.9	0.61	**0.62**
EIF5A	823.4	272.2	0.47	616.3	708.6	0.4	**0.43**
PLAUR	37.8	2513.8	71.93	43.9	5320.0	67.0	**69.47**
PPP4C	488.0	197.0	0.76	299.3	391.7	0.53	**0.64**
PKM2	1299.5	2056.3	2.85	997.3	5080.9	1.91	**2.38**
P381P	967.1	803.5	1.24	806.3	699.3	0.95	**1.09**
TPT1	2561.5	6238.4	3.69	2008.9	13864.2	2.85	**3.27**

assays is shown below.

Data from the microarray experiment are shown in Table 2. For consistency, microarray experiments were performed with dye swapping. The average CV for the microarray assay was 12.3% and the CV ranged from 5% to 23.8% for the uninduced samples and 5.7% to 27.9% for the induced samples.

Values for the 4 ng mRNA and the average of all three dilutions are shown in Table 3. The average CV for the QuantiGene data was 3.2% and the CV ranged from 0.57% to 19.3% for uninduced samples and 0.15% to 12.69%.

Microarray Validation

Using the QuantiGene Assay

The same nine genes shown in Table 1 were reexamined using the QuantiGene assay. To confirm the accuracy and robustness of the assay, three two-fold dilutions of mRNA samples (at 2, 4, and 8 ng of Poly A+ RNA) were examined for each gene.

Microarray Validation by the TaqMan Assay

Six of the nine genes examined in the microarray and QuantiGene assays were also evaluated using the TaqMan assay. The data are shown in Table 4. The average CV for the data was 11.6% and the CV ranged from 0.6% to 16.1% for uninduced samples and from 2.5% to 37.2% for induced samples.

Table 3. QuantiGene assay data from nine genes.

Gene	Average RLU Signal Uninduced 4 ng mRNA	Average Signal Induced 4 ng mRNA	Fold Induction (Normalized) (Average of 2,4 and 8 ng mRNA)	Microarray Average Fold Induction (Normalized)
GAPD	217.4	275.2	1	1
ATF3	1.02	13.2	19.83	11.36
CDKN1A	1.85	124.3	96.13	111.70
EEF2	96.5	71.64	0.59	0.62
EIF5A	89.64	26.32	0.23	0.43
PLAUR	3.81	246.8	59.25	69.47
PPP4C	17.15	39.9	2.18	0.64
PKM2	72.0	364.8	4.02	2.38
P38IP	2.5	2.4	1.01	1.09
TPT1	103.4	453.7	3.73	3.27

Table 4. TaqMan assay data from six genes.

Gene	Threshold Cycle (Ct) Uninduced	Threshold Cycle (Ct) Induced	Normalized Fold Induction	Microarray Average Normalized Fold Induction
GAPD	17.9	17.54	1	1
ATF3	29.63	22.19	135.45	11.36
CDKN1A	27.87	18.23	623.11	111.70
EEF2	18.46	18.95	0.56	0.62
PLAUR	25.10	18.31	86.22	69.47
PPP4C	21.84	20.89	1.51	0.64
P381P	25.04	25.08	0.76	1.09

Correlation Analysis of QuantiGene with Microarray and TaqMan Assays

The data from the microarray, QuantiGene and TaqMan assays were normalized to GAPD, and the fold of induction (induced/uninduced) was calculated for each gene. The data are shown in Figure 4. The fold induction for microarray was the average of the dye swapping experiment. The data for the QuantiGene assay represents the average ($\pm$ 1 SD) of the three sample concentrations.

The data indicate that a strong correlation exists between the three assays across the tested dynamic range, with the strongest correlation between the microarray and QuantiGene assays. Statistical analysis using a matched pair analysis, shown in Table 5, indicates a correlation of 0.9952 between microarray and QuantiGene assay data, 0.8676 between microarray and TaqMan assay data and 0.8846 between TaqMan and Quantigene assay data.
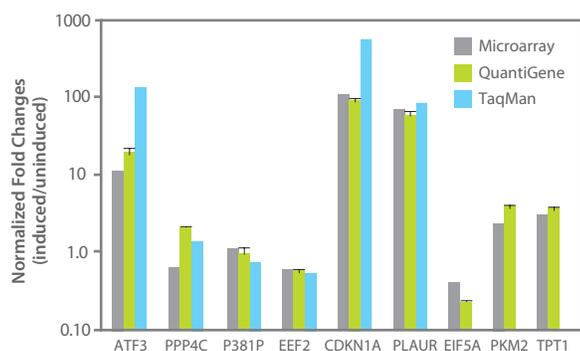

Figure 4. Comparison of normalized fold induction of mRNA quantitated by three methods.

Assay Type	Microarray	QuantiGene	TaqMan
Microarray	1	0.9952	0.8676
QuantiGene	—	1	0.8846
TaqMan	—	—	1

Table 5. Results of the mathed pair analysis.

Discussion

The QuantiGene assay produces results that are highly correlated to other methods of RNA quantitation and does so without the need to purify RNA or perform PCR.

The correlation between QuantiGene assay and the microarray assay was 0.9952, between the microarray and TaqMan assays was 0.8676 and between the TaqMan and QuantiGene assays was 0.8846, indicating that the QuantiGene assay more closely correlates than the TaqMan assay to the microarray data.

The QuantiGene assay is a convenient method that can be used to validate or follow-up studies for microarray analysis. It is one of the only commercially available methods that is capable of directly and accurately quantitating mRNA without purifying or amplifying RNA.

Ordering Information

Product	Size	Catalog No.
QuantiGene Explore Kit	2 – 96-well plate (192 assays)	QG0001
QuantiGene Discover Kit	10 – 96-well plate (960 assays)	QG0002
QuantiGene Discover XL Kit*	50 – 96-well plate (4,800 assays)	QG0003
QuantiGene Screen Kit**	50 – 96-well plate (4,800 assays)	QG0004

* Configured to allow processing of 1 or more plates/run

** Configured for high throughput applications requiring the processing of 10 plates/run

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