Concordance of Clariom D microarrays and TaqMan real-time PCR assays

Introduction

Microarrays and real-time PCR provide comprehensive solutions for gene expression profiling, screening, and verification studies. Transcriptome analysis using the Applied Biosystems™ Clariom™ D microarray, combined with Applied Biosystems™ Transcriptome Analysis Console (TAC) Software 4.0, empowers scientists to gain information about RNA expression at the gene level and alternative splicing at the exon level in different biological samples. Data from array technology are routinely verified by Applied Biosystems™ TaqMan® Assays due to their high sensitivity and wide dynamic range. In this technical note, we verified the fold-change results obtained with the Clariom D microarray with results obtained using the Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit followed by real-time PCR using Applied Biosystems™ TaqMan® Gene Expression Assays and TaqMan® Fast Advanced Master Mix. We describe guidelines for this verification and show excellent inter-platform concordance between the microarray and real-time PCR data.

Key observations

- TaqMan qPCR fold-change measurements correlate well with Clariom D microarray measurements
- "Best coverage" TaqMan Gene Expression Assays (with convenient online search tool) can reliably confirm gene-level expression data from Clariom D microarrays
- TaqMan Fast Advanced Master Mix provides a fast and complete solution for performing real-time PCR

Materials and methods

To compare transcript levels, we used a strategy based on the MicroArray Quality Control (MAQC) study [1]. Well-characterized RNA transcripts from Stratagene™ Universal Human Reference RNA (Cat. No. 740000) and Applied Biosystems™ FirstChoice™ Human Brain Reference RNA (Cat. No. AM6050) were used to compare performance of the different platforms. For microarray analysis, we used 50 ng of starting material with the Applied Biosystems™ Clariom™ D Assay (Cat. No. 902922), which includes the whole-transcriptome Clariom D microarray and the Applied Biosystems™ GeneChip™ WT PLUS Reagent Kit (50–500 ng). Based on microarray data, 80 targets spanning a wide range of expression levels were picked for further verification by qPCR. For qPCR, best coverage TaqMan Gene Expression Assays (Cat. No. 4351372) targeting each of the 80 targets were chosen. A total of 100 ng of each reference RNA sample was reverse-transcribed using the SuperScript VILO cDNA Synthesis Kit (Cat. No. 11754). The resulting cDNA was analyzed using individual TaqMan Gene Expression Assays and TaqMan Fast Advanced Master Mix (Cat. No. 4444963). Real-time PCR data were analyzed using the Relative Quantitation (RQ) app within Thermo Fisher Connect. A C_t of 35 was used as a cutoff for real-time PCR data.

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Results

Figure 1 shows a scatter plot that demonstrates the correlation of fold-change data between the Clariom D Assay and TaqMan Assays. The coefficient of determination (r^2) of 0.89 indicates good correlation.

Conclusions

Data presented in this technical note show that best coverage TaqMan Assays can be reliably used to validate and confirm differential expression patterns from microarray data. TaqMan Gene Expression Assays have long been considered the gold standard for studying gene expression. In this technical note, we show concordance of assays on 80 differentially expressed genes in two samples across two platforms. We found a high degree of concordance between the two platforms based on differential expression status (i.e., differentially expressed or not differentially expressed). Although the values of the fold-change measurements were not identical in these comparisons, the low fold changes were confirmed to be low, and high fold changes were confirmed to be high. The results support previous reports that the degree of concordance between microarrays and qPCR increases as fold change increases [2]. Conversely, concordance between microarrays and qPCR dropped for genes with low fold changes (<1.4), due to increased noise [2]. Nevertheless, we found that genes with low fold changes were consistently low, even if the absolute value of the magnitude of change varied. Other features such as transcript length, number of exons, splice variants, and read quality may also have decreased the concordance of individual genes in this study, but these possibilities were not investigated further.

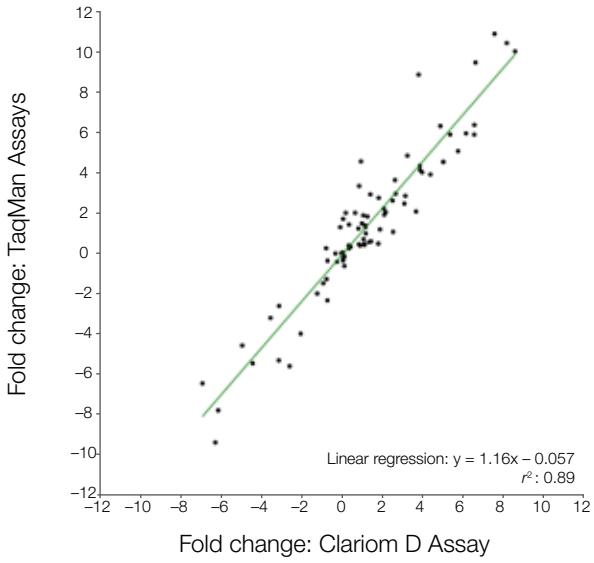

Figure 1. Fold-change correlation between the Clariom D Assay and TaqMan Gene Expression Assays. The scatter plot shows that the data sets are highly correlated.

Ordering information

Product	Cat. No.
Clariom D Assay, human (includes the whole-transcriptome Clariom D microarray and the GeneChip WT PLUS Reagent Kit)	902922
TaqMan Gene Expression Assay, single tube*	4351372

* For additional TaqMan Assay formats, including Applied Biosystems™ TaqMan® Array Cards and OpenArray™ Plates for screening and verification studies, go to **thermofisher.com/taqman**.

References

1. MAQC Consortium (2006) The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol* 24:1151-1161.
2. Morey JS, Ryan JC, Van Dolah FM (2006) Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR. *Biol Proced Online* 8:175-193.

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