



Technical Note

High-Quality Data Obtained with NimbleGen Gene Expression Arrays

Microarray analysis is a powerful tool for quantifying genome-wide changes in gene expression. To facilitate wider application of microarray data in biomedical research and promote the use of microarray analysis in diagnostic and regulatory fields, it is important that data obtained using this technology is accurate and reliable. As such, we evaluated the performance of NimbleGen gene expression arrays using standard RNA samples recently used by the Microarray Quality Control (MAQC) Consortium, a project established to evaluate the reproducibility and quality of data obtained using microarrays from multiple sources. Here we show that high interarray reproducibility was achieved for both the 1-plex and 4-plex expression microarrays, demonstrating the reliability of NimbleGen array data. We also show a concordance between data obtained from NimbleGen array analysis and TaqMan analysis, the current “gold standard” for mRNA level quantitation, indicating the accuracy of NimbleGen array data in assessing gene expression values.

Introduction

Gene expression microarray analysis is widely used to answer important biological questions at a molecular level that is not easily achieved using other detection techniques. As with all innovative technologies, and especially with high-content analysis technology that requires complex sequence design and sophisticated data analysis, the reliability of the data acquired through microarray analysis is closely scrutinized. Additionally, the concordance between data acquired using different microarray platforms is paramount for enabling evaluation and comparison of results from multiple sources.

A seminal study aimed at evaluating the data concordance between gene expression microarrays from multiple sources was performed by the Microarray Quality Control (MAQC) Consortium and published in *Nature Biotechnology* (1). The high level of data reproducibility between the platforms and test sites concluded by this study provides confidence for the continued use of this technology in research as well as future use in clinical and regulatory settings. Furthermore, the public availability of the data and reference RNA samples facilitate the evaluation of new gene expression microarray platforms.

Roche NimbleGen was unable to participate in the MAQC project because the company provided only full array analysis services at the time. At the end of 2006, the company added microarray delivery as an option for our customers. As an extension to the MAQC project, we evaluated our NimbleGen 1-plex and 4-plex gene expression arrays using the same RNA reference samples as those in the MAQC project. The results show that the generation of gene expression values using NimbleGen microarrays compares in concordance with the other platforms reported. Additionally, the accuracy of the NimbleGen data is supported by the high correlation between the NimbleGen data and TaqMan data reported by the MAQC project.

Materials and Methods

The RNA samples used were universal human reference RNA (Stratagene, La Jolla, CA) and human brain reference RNA (Ambion, Austin, TX). The NimbleGen gene expression microarrays used were the *Homo sapiens* 1-plex array (Roche NimbleGen, catalog number: A4542-00-01, 385,000 features/array) and the *Homo sapiens* 4-plex array (Roche NimbleGen, catalog number: A4487001-00-01, 72,000 features/array).

Ten micrograms of the appropriate RNA were processed and labeled per the standard NimbleGen protocol. Briefly, RNA was converted into cDNA using the SuperScript II cDNA Conversion Kit (Invitrogen, Carlsbad, CA). Double-stranded cDNA was random-prime labeled with Cy3-nonamers and hybridized to the microarrays for 16 hours at 42°C. The arrays were washed, dried, and scanned at 5µm resolution using a GenePix 4000B microarray scanner (Molecular Devices, Sunnyvale, CA). Data were extracted from scanned images using NimbleScan software (Roche NimbleGen). Quantile normalization was performed across replicate arrays, and RMA (Robust Multichip Average) analysis was performed to generate gene expression values (2).

Results and Discussion

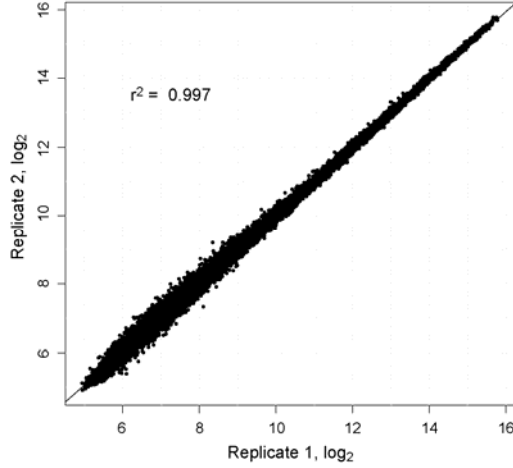
Reproducibility of Expression Signals. A critical aspect of microarray data acquisition and analysis is the signal reproducibility between array replicates. We analyzed signals for universal reference RNA and brain reference RNA from five 1-plex array replicates and five 4-plex array replicates. Representative scatter plots from two NimbleGen 1-plex arrays (Figure 1A) and two NimbleGen 4-plex arrays (Figure 1B) for universal reference RNA show very high interarray reproducibility. Table 1 shows the mean interarray correlation between all 1-plex and 4-plex array replicates for the universal or the brain reference RNA samples.

Sample	Correlation, r^2	
	1-Plex Arrays	4-Plex Arrays
Universal Reference RNA	0.991 ± 0.001	0.990 ± 0.001
Brain Reference RNA	0.990 ± 0.005	0.990 ± 0.003

Table 1. Reproducibility of signals from NimbleGen arrays. Universal reference RNA and brain reference RNA were analyzed using NimbleGen 1-plex and 4-plex arrays. Correlations between signals from each replicate were compared to each other and presented as mean ± SD (n = 5).

Correlation between NimbleGen Array and TaqMan Data. To assess the accuracy of NimbleGen array data, the brain to universal \log_2 ratio for 961 genes was compared to the TaqMan brain to universal \log_2 ratio reported by the MAQC Consortium. The correlation is shown as scatter plots in Figure 2 and is also represented by the correlation coefficient (r). The high correlations of 0.91 for 1-plex arrays and 0.92 for 4-plex arrays are comparable to the best platforms tested in the MAQC project (Table 2), indicating a high relative accuracy of the gene expression values obtained using NimbleGen gene expression arrays.

1A. NimbleGen 1-plex arrays



1B. NimbleGen 4-plex arrays

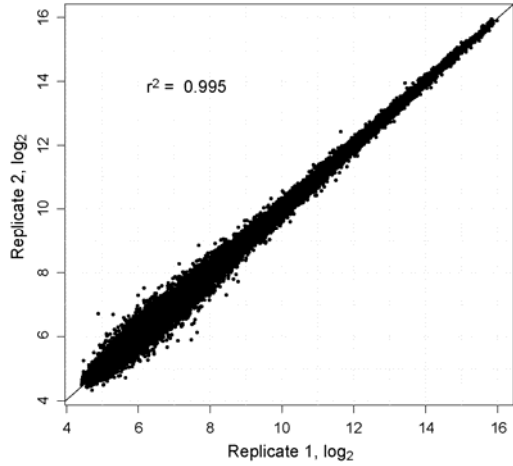
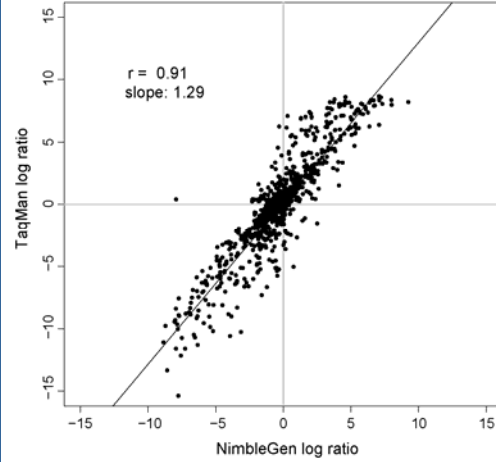


Figure 1. Reproducibility of signals from NimbleGen arrays. Universal reference RNA was analyzed using NimbleGen 1-plex (1A) and 4-plex (1B) arrays. Each point represents the signal for one gene, in \log_2 .

2A. NimbleGen 1-plex arrays



2B. NimbleGen 4-plex arrays

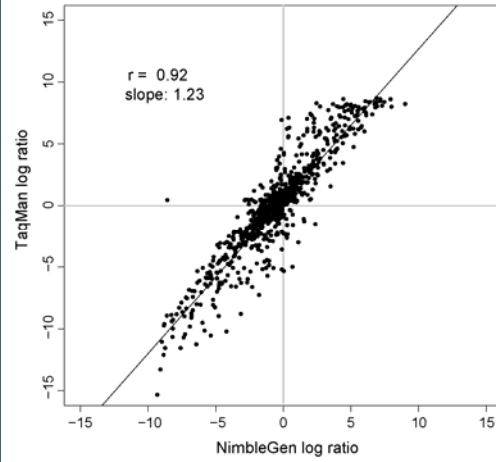


Figure 2. Correlation between NimbleGen microarray and TaqMan data. The \log_2 ratio of brain RNA to universal RNA signals from 1-plex (2A) and 4-plex (2B) microarrays were compared to TaqMan signals obtained from the MAQC report (1). Each point represents one of the 961 genes for which TaqMan values are available.

Source	r
NimbleGen 4-plex arrays	0.92
NimbleGen 1-plex arrays	0.91
Affymetrix	0.92*
Applied Biosystems	0.85*
Agilent	0.91*
Illumina	0.91*

Table 2. Accuracy of signals from commercial microarrays. \log_2 ratios of brain RNA to universal RNA signals from microarrays from the indicated sources were compared to the same ratio from TaqMan analysis.

*Mean for three test sites, as provided by the MAQC Consortium (1)

Conclusion

The reliability of microarray analysis as a high-throughput gene expression detection technology is critical in the acceptance of the technology for wider research use, as well as for expansion into clinical and regulatory arenas.

Confidence that data obtained using microarrays from different providers are comparable facilitates application and translation of the information into further research. Towards achieving this goal, Roche NimbleGen analyzed the performance of our human whole-genome gene expression microarrays for signal reproducibility and relative accuracy and compared them to data from other microarray sources reported by the MAQC Consortium (1). Both NimbleGen 1-plex and 4-plex human arrays generate highly reproducible gene expression values, allowing comparison of data from multiple arrays and experiments. Additionally, the NimbleGen expression values correlate strongly with TaqMan expression values, the current “gold standard” for gene expression quantitation, indicating the accuracy of data obtained using this technology.

In addition to highly reproducible and accurate expression values, Roche NimbleGen’s unique combination of high-density arrays, long oligos, and flexible design capabilities together provide superior results for advanced gene expression analysis of any sequenced and annotated genome. NimbleGen gene expression microarrays are available as catalog designs for a large number of eukaryotic and prokaryotic genomes, or you can order a custom array for a nominal design fee. NimbleGen microarrays can be delivered for processing in your facility, or samples can be sent to Roche NimbleGen’s full-service laboratory for analysis.

References

1. MAQC Consortium, The MicroArray Quality Control (MAQC) project shows inter- and intra-platform reproducibility of gene expression measurements. *Nat. Biotechnol.* (2006) 24:1151-1161.
2. Irizarry R. et al., Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* (2003) 4:249-264.

For More Information

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