



Technical Note

Affymetrix Technology Demonstrates Top Performance in Microarray Quality Control Study (MAQC)

Affymetrix Provides Shortest Path to Array-based Clinical Tests and Diagnostics

The results of the MicroArray Quality Control Study (MAQC) demonstrate why Affymetrix is considered the gold standard in microarrays and why scientists have published over 7,000 papers using our products—Affymetrix technology generated the most reproducible, sensitive and robust expression data. Affymetrix was the only participant in the MAQC study that did not exclude data from the results or run replacement arrays. Although the MAQC study was not designed to be a stringent platform comparison trial, the results were clear. The Affymetrix platform delivered:

- **Highest intrasite and inter-site reproducibility**—key for multi-site clinical studies that require the highest quality data from every sample, at every site
- **Highest sensitivity**—key for detecting subtle gene expression changes in real-world samples
- **Highest robustness**—key for minimizing wasted samples and money, through failed arrays
- **Most comprehensive external controls**—key for assessing source of variability, for accurate interpretation of results

Combining the highest quality performance, and the first instrument platform to be approved by the FDA for *in vitro* diagnostics, Affymetrix provides the shortest path to success for microarray-based clinical research.

MAQC Study Details

Two years ago the FDA initiated a study to determine the reproducibility of microarray results generated by different platforms, and to provide standards for researchers who plan to use microarrays in a clinical setting. The results of the MAQC, published in the September 8, 2006 issue of *Nature Biotechnology* (Shi, *et al.*, 2006), showed that by practicing good scientific method in the laboratory, researchers can obtain accurate, reproducible data from microarrays.

The MAQC study put seven microarray platforms and three alternative technologies through more than 1,300 tests at different labs. Gene expression levels were measured from two distinct RNA samples, in four titration pools. The study assessed each platform at three independent test sites and five replicates were assayed at each site.

MAQC Study Results

HIGHEST REPRODUCIBILITY AND ROBUSTNESS
To examine intrasite reproducibility, microarray data from each reference were examined for repeatability of expression signals within tests sites for all genes that were common among platforms. Figure 1 of Shi, *et al.* shows a comparison in terms of the coefficient of variation (CV). Only the Affymetrix platform met a five percent mean CV threshold. The mean CV for the next-ranked platform was nearly 10 percent (Figure 1).

Microarray data from each sample were also examined for repeatability of expression signals across test sites for all genes that were common among platforms.

Only the Affymetrix platform demonstrated a mean CV for intersite variability below 10 percent. Additionally, Affymetrix was the only platform that did not drop data or run replacement arrays across any of the 60 samples processed at three sites (as described in Table S3 of Shi, *et al.*).

Microarray experiments represent a significant investment of time, money, and valuable samples that often cannot be replaced. The MAQC results show that Affymetrix is the best choice for getting the most reliable results the first time.

HIGHEST SENSITIVITY AND ROBUSTNESS

Figure 2 (refer to Supplemental H of Shi, *et al.*) demonstrates that the Affymetrix platform has the highest sensitivity and consistency to detect small gene expression changes across different types of test sites—both manufacturer (blue line) and customer sites (red and gray lines). For example, at 0.8 power, each Affymetrix test site was able to detect 10 - 20 percent more gene expression changes than other platforms. Also, some platforms show much worse performance at customer sites, suggesting that these platforms are more influenced by site variables, especially when the expression changes are small in magnitude (most commonly found in true biological systems).

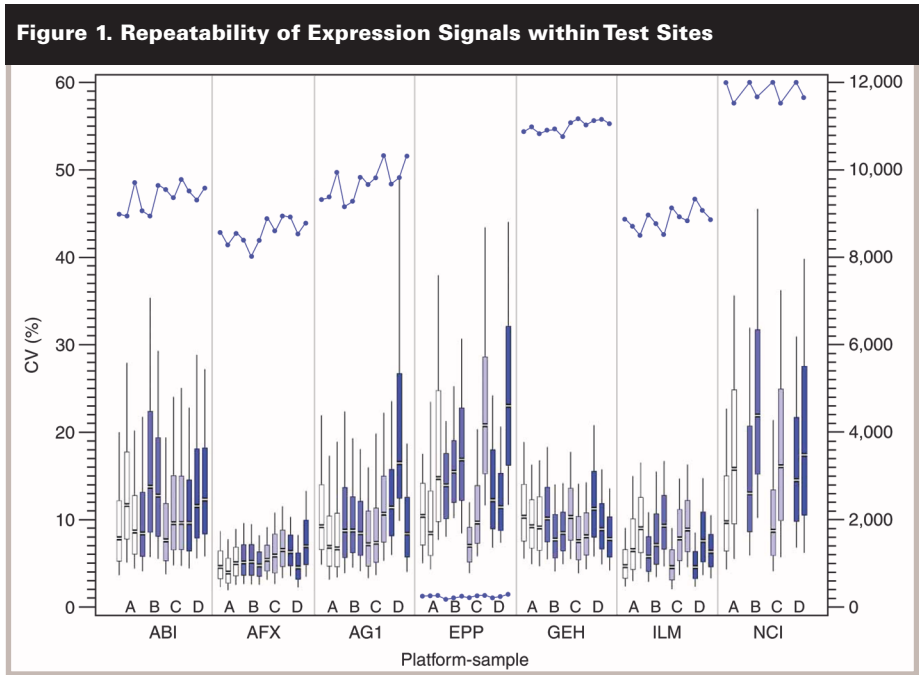
Typical experiments require higher sensitivity than was represented in the MAQC study. The study authors agreed that “expected differences between sample types (e.g., treated versus control animals) are usually much smaller compared to those seen between sample types A and B.”

Affymetrix expression arrays are the gold standard because it is recognized that finding a signature with the highest predictive value requires the ability to see even very low levels of expression. To bring that signature successfully to the clinic, researchers must be able to reproduce the results of their initial experiment through validation, product development, and regulatory review.

MOST COMPREHENSIVE EXTERNAL CONTROLS
The MAQC study concluded that external controls should be used to evaluate sample quality throughout the experimental process. Only Affymetrix provides both poly-A and pre-labeled controls at a range of concentrations, helping to ensure quality control for the most reliable clinical results. While the MAQC study was a structured and controlled experiment, scientists need external controls to account for the wide diversity found in real-world clinical samples.

Conclusions

The MAQC results show that Affymetrix arrays generated the most repeatable, reproducible, sensitive, and robust expression data within and across laboratory sites; no data were excluded or replaced.



Reproducibility is critical for developing tests and diagnostics, but only Affymetrix provides all the tools necessary to successfully take your research to the clinic.

Affymetrix has the first and only microarray instrument to be cleared by the FDA for *in vitro* diagnostics (IVD) use, providing a standardized platform

for nucleic acid diagnostics. Affymetrix partners are developing signature-based assays for a wide range of malignancies, including leukemia, lymphoma, breast, lung, colon, and prostate cancers. Affymetrix will soon be the only microarray manufacturer to offer a CLIA-approved laboratory for development of clinical assays and processing of samples.

Figure 2. Apparent Power Analysis. Only comparisons between sample A (Universal RNA from Stratagene) and sample B (brain total RNA from Ambion) are shown (for more information, refer to Supplemental H of Shi, *et al.*, 2006). The percentage of genes detected as differentially expressed is plotted on the y-axis and the calculated power of detection on the x-axis. The sensitivity of the platform can be determined by comparing the percentage of genes represented on the y-axis at any given power on the x-axis—more genes on the y-axis at a given power equals higher sensitivity.

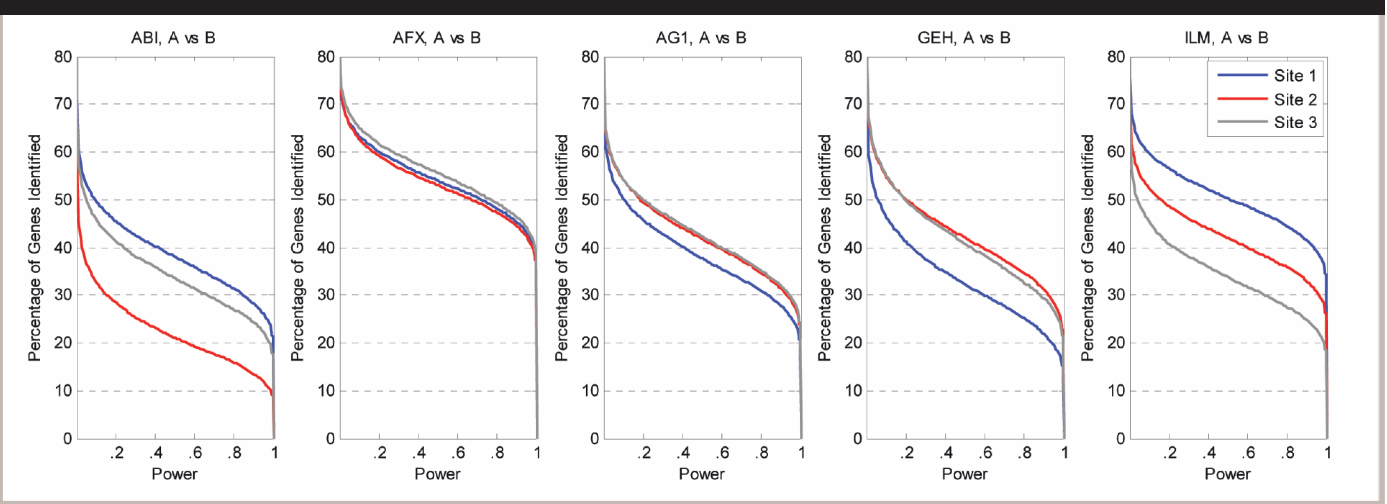


Table 1. Summary of Microarray Processing Controls. This table is based on information published in Figure 1 of Tong, *et al.*, 2006.

	Affymetrix	Agilent	Applied Biosystems	GE Healthcare	Illumina
Target Labeling Controls	4 controls with varying concentrations	10 controls with varying concentrations	6 controls at a single fixed concentration	None	None
Array Processing Controls	4 controls with varying concentrations	None	3 controls at a single fixed concentration	6 controls with varying concentrations	None

Additionally, only Affymetrix offers:

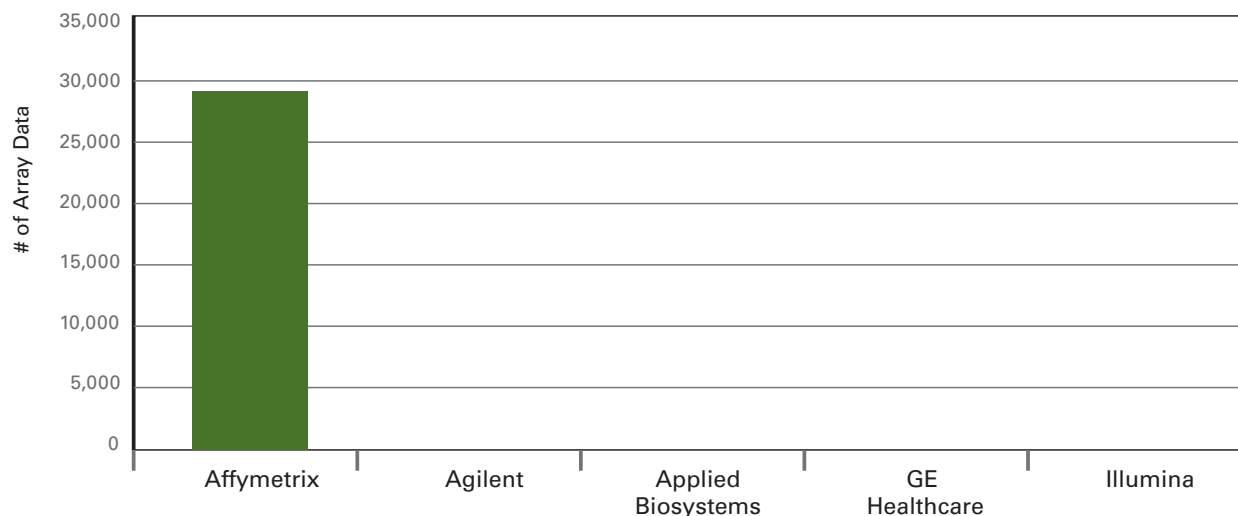
- Arrays, reagents, and process controls to quickly obtain data from as little as 1 µg of total RNA for standard assay or 10 ng for amplified procedure
- Multiple choices for downstream statistical and pathway analysis for users to quickly turn array results into novel findings
- Most comprehensive annotation accessible freely on the NetAffx™ Analysis Center web site to identify biological mechanisms and quickly design downstream studies
- 1,000 times more array data deposited in Gene Expression Omnibus (GEO) compared with all other microarray platforms, for a wealth of comparative data to rapidly dissect pathways and targets (Figure 3).

To learn more about the Affymetrix Translational Medicine Program for enabling affordable, large clinical studies, or to find out more about the Affymetrix Clinical Labs, call 1-888-DNA-Chip or your local sales representative. To learn more about setting up a comprehensive comparison study, please refer to the Affymetrix Technical Note entitled “GeneChip® Expression Platform: Comparison, Evolution, and Performance.”

REFERENCES

1. Shi, *et al.* The MicroArray Quality Control (MAQC) project shows interplatform reproducibility of gene expression measurements. *Nature Biotechnology* 24(9):1151-1161 (2006).
2. Tong, *et al.* Evaluation of external RNA controls for the assessment of microarray performance. *Nature Biotechnology* 24(9):1132-1139 (2006).
3. Shippy, *et al.* Using RNA sample titrations to assess microarray platform performance and normalization techniques. *Nature Biotechnology* 24(9):1123-1131 (2006).

Figure 3. Number of Array Data Accessible Through Gene Expression Omnibus (GEO)



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