MicroArray Quality Control (MAQC) Project: An Overview of the Published Findings

Introduction

The MicroArray Quality Control (MAQC) project, headed by Leming Shi* at the U.S. Food and Drug Association – National Center for Toxicological Research (FDA-NCTR), was established to generate a set of quality control tools for the research community. MAQC includes validated reference samples, a large collection of multiplatform reference data sets, and analysis tools. The project involved 137 participants representing 51 organizations, including the U.S. FDA, National Institutes of Health, Environmental Protection Agency, U.S. Department of Agriculture, all major whole-genome gene expression platform providers, and several alternative gene expression platform providers. The purpose of this document is to give a brief overview of the MAQC Project and to review the major results of the published findings[1].

Experimental Design

Illumina, Affymetrix, and GE Healthcare were the three platform providers who participated in both pilot phases, which were designed to finalize the experimental design and research materials for the main study. Pilot phase 1 examined four potential RNA sources: Stratagene Universal RNA, Clontech Universal RNA, Ambion brain RNA, and Ambion liver RNA. Although all four tissues proved useful, the Stratagene Universal RNA and Ambion brain RNA samples were chosen based on a series of predetermined criteria, including number of genes detected and magnitude of fold changes between the two tissues. Pilot phase 2 tested various mixture ratios between the two RNAs to help evaluate microarray and algorithm performance. From a series of 11 mixtures, two mixtures were chosen for the final study design: 75% Stratagene Universal RNA/25% Ambion brain RNA and 25% Stratagene Universal RNA/75% Ambion brain RNA.

The main study was performed by five commercial whole-genome gene expression microarray providers: Applied Biosystems, Affymetrix, Agilent, GE Healthcare, and Illumina. A number of other non-commercial, non-whole-genome array and alternative technology providers also contributed. Each platform provider identified three site locations for the main study design. Starting from a common source of aliquoted material, each site processed five labeling replicates for each of the four samples (Stratagene Universal RNA, Ambion brain RNA, mixture 1, and mixture 2) for a total of 60 arrays per platform provider. To ensure objective assessment and to avoid complications arising from splice variation, all analyses in this study tested a common list of 12,091 genes that cross-mapped to all whole-genome platforms by stringent sequence comparison criteria. The following section provides a guide to the tables and figures in the MAQC publication[1].

Visit The MAQC Website

http://www.fda.gov/nctr/science/centers/toxicoinformatics/maqc/index.htm

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Results

Table 11 summarizes the study design, which compared five commercial whole-genome microarray platforms and a home-brew spotted array technology produced by the National Cancer Institute. The remainder of the MAQC publication describes the analysis of this data set and compares the platform reproducibility, self- and cross-platform concordance, and concordance to non-array-based gene expression technologies, including the Applied Biosystems Taqman reverse transcription PCR assay.

Figures 11 and 21 show a comparison of the repeatability of array hybridization platforms within test sites and across sites in terms of the coefficient of variation (CVs) of normalized hybridization signals. The Illumina and Affymetrix platforms are the only two platforms that showed median replicate CVs of < 10% for all samples within all sites (Figure 11) and < 12% for all samples across all sites (Figure 21). These results show the Illumina hybridization platform to be highly reproducible across ovens, scanners, technicians, days, and arrays, all of which differed across the three test sites.

Figure 31 shows a platform comparison for concordance of detection calls. The plot shows the percentage of genes that show consistent presence or absence of calls across all test sites. Of the commercial array platforms, Illumina showed the highest concordance, with > 85% of all calls in agreement. As with the CV of hybridization signal shown in Figures 11 and 21, these results demonstrate that the Illumina platform provides highly reproducible results in different laboratories.

Figure 41 represents the concordance of differential expression calls made by the platforms. For each platform, lists of genes were determined to be differentially expressed if they showed ≥ twofold change in hybridization signal and a significance of $P < 0.001$ between Sample A (100% Human Reference RNA) and Sample B (100% Human Brain Sample). The percent overlap of the lists for each platform was then compared to the others in a matrix of all platforms. All platforms produced similar lists (> 60% for all cross-platform comparisons of all platforms). Of all platforms examined, Illumina provided the most similar results to the Affymetrix platform (80.4% agreement) and this relationship was reciprocal, with Affymetrix being more similar to Illumina than any of the others compared.

Figures 51 and 61 compare the array platforms to each other and to

> "For example, data from Affymetrix test sites, which use multiple short oligonucleotide probes per target with perfect match and mismatch sequences, and Illumina test sites, which use plasma-etched silicon wafers containing beads with long oligonucleotide probes, were remarkably similar in detection and detection consistency, gene list overlap and ratio compression analyses. In other words, the expression patterns generated were reflective of biology regardless of the differences in technology."
>
> Excerpt from "MicroArray Quality Control (MAQC) project shows interplatform reproducibility of gene expression measurements."1

Taqman real-time PCR assays in terms of correlations of log ratios for expression signals between different samples. Log expression ratios for Samples A and B were calculated for each platform and the rank-based correlation (R) of these ratios was calculated for each cross-platform comparison. As with the comparisons of differential expression in Figure 41, this comparison showed the results from the Affymetrix and Illumina platforms to be most similar to each other, with an R value of 0.936. Other commercial platforms gave R values ranging from 0.792 and 0.933 when compared to Illumina and Affymetrix (Figure 5b1 and Table S111). Comparisons to Taqman reverse transcription PCR showed the results from the Illumina and Affymetrix platforms to be the most similar to this orthogonal technology, with R values of .902 and .905, respectively. The other whole-genome platforms gave R values ranging between 0.839 and 0.894 when compared to Taqman. These results show the Illumina platform to produce results highly consistent with other commonly used commercial array platforms as well as gene expression technologies that are not based on microarrays.

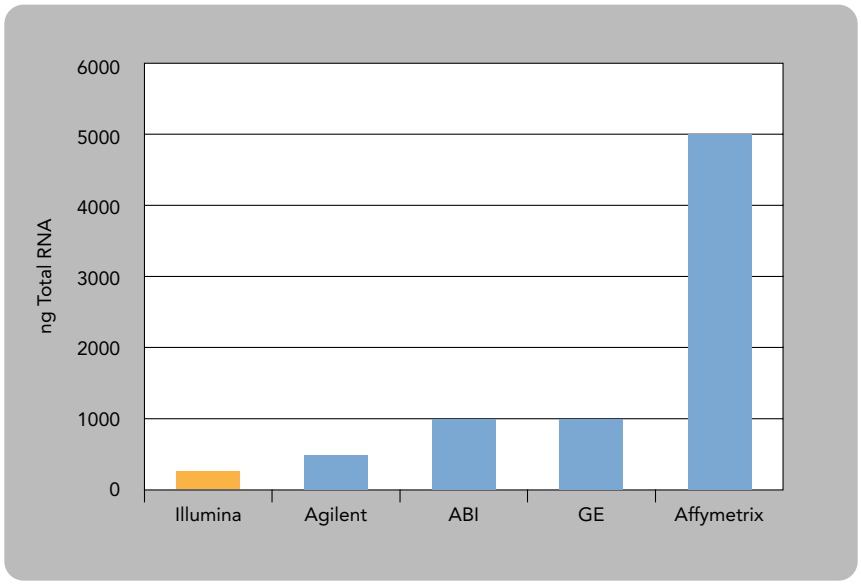
Conclusions

The outcome of the MAQC project was a successful comparison of multiple whole-genome gene expression profiles across various commercial platforms at an unprecedented scale. This scale, combined with sequence-based probe mapping and a large number of alternative technology gene expression measurements (such as TaqMan assays), sets this study apart from previous cross-platform comparison studies. Despite the use of very different technologies, a relatively high level of interplatform concordance was observed, especially in the results produced by Illumina BeadChips and Affymetrix GeneChips. Given such high levels of concordance, choosing a gene expression platform should primarily be driven by such factors as technical performance, cost, usability, input requirements] (Figure A), and content quality. The Illumina BeadChips, which were shown to consistently be among the very best performers across the various technical measurements, are also substantially less expensive (Figure B) than all other commercial whole-genome arrays used in this study. With array and reagent costs less than half that of the other commercial arrays, the use of Illumina BeadChips allows for experimental designs to be expanded, yielding more powerful and far-reaching results with the same research budget.

Reference

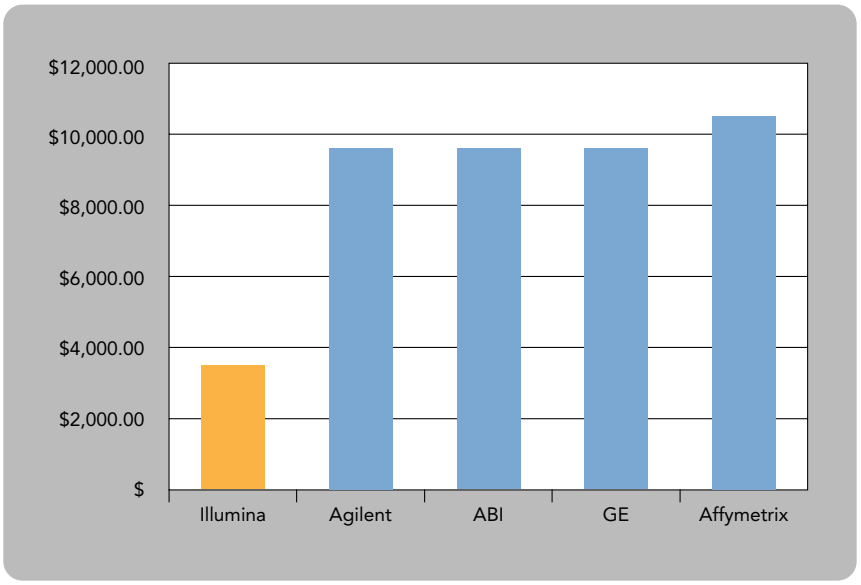
1. MAQC Consortium. (2006). The MicroArray Quality Control (MAQC) project shows interplatform reproducibility of gene expression measurements. Nature Biotechnology 24(9), 1151-1161.

Figure A: Input RNA Required



The bar graph above shows the RNA required for input into the platform specified on the X-axis.

Figure B: Materials Cost



The approximate cost for materials to run 20 samples (i.e., per site cost) is shown above with the platform specified on the X-axis.

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