AB **Applied Biosystems**

Analysis of Gene Expression in Normal and Tumor Liver Tissue

A Comparison of the Applied Biosystems Expression Array System with a Leading Competitive Platform

Introduction

The Applied Biosystems Expression Array System is equipped with the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer that houses electro-optical systems for fast, sensitive detection of gene expression. In combination with optimized chemiluminescent chemistry kits and microarrays that survey whole genomes, this system is designed to measure the very low gene expression levels that platforms equipped only with fluorescence detection often miss.

In this study we designed a series of experiments to compare the Applied Biosystems Expression Array System with a leading competitive platform.

The sensitivity of each platform was determined by comparing the number of genes that are detectable and the fold changes in differential gene expression as measured between normal and tumor tissue samples.

| System Specifications	Applied Biosystems Expression Array System	Competitive Microarray
Oligo probe length	60-mer	25-mer
Manufacturing method	Spotting with contact technology	*In situ* photolithography
Dye or detection chemistry	Chemiluminescence with alkaline phosphatase enzyme	Fluorescence with phycoerythrin dye
Genes studied	10,687 cross-mapped common genes between platforms	
Input RNA labeling	1 µg/replicate (Applied Biosystems Chemiluminescent RT-IVT Kit)	4 µg/replicate (recommended RT-IVT Kit)
Data analysis	• Gene detection sensitivity • Differential gene expression (normal vs. tumor)	

Results
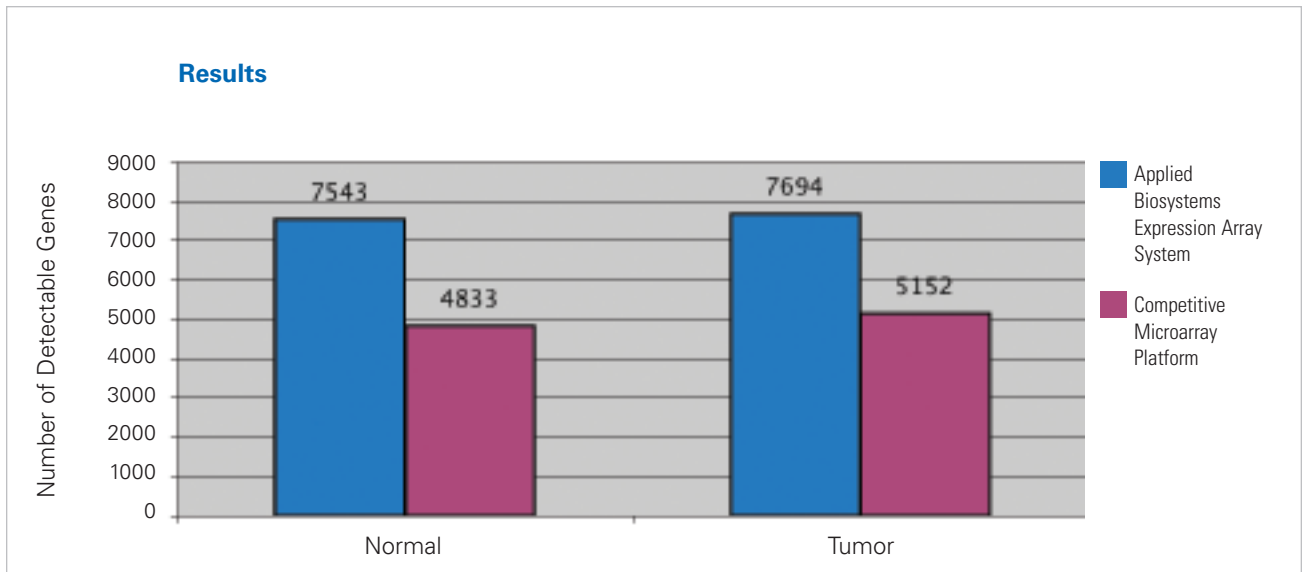
Figure 1. Number of detectable genes in both normal and tumor liver tissues. Detection thresholds used for each platform are as follows: Applied Biosystems platform, S/N > 3; leading competitive platform, Present/Absent (p < 0.05).

Of 10,687 common genes that are cross-mapped between Applied Biosystems Expression Array System and the leading competitive platform, Applied Biosystems platform (blue) detected 2,710 more genes in normal tissue and 2,542 more genes in tumor tissue than the competitive platform (purple).
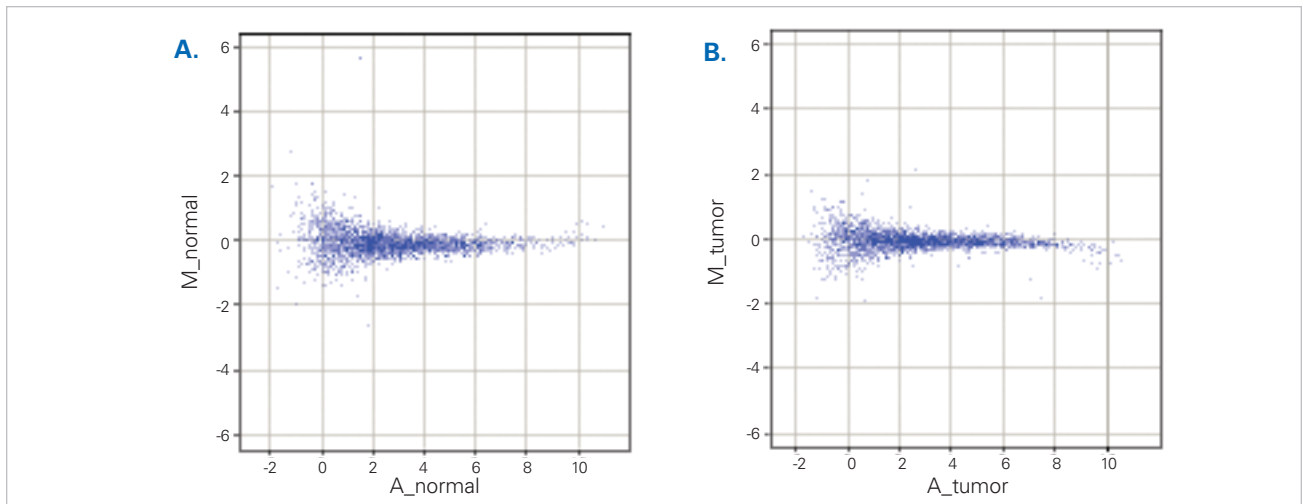
Figure 2. The reproducibility of the Applied Biosystems Expression Array System was also evaluated using both normal and tumor samples. This figure shows a high degree of correlation in both normal vs. normal and tumor vs. tumor samples. The technical replicates for normal **(A)** and tumor **(B)** samples were plotted as follows:

X axis: $A = 0.5 \times \log_2 (\text{Signal\_rep1} \times \text{Signal\_rep2})$; Y axis: $M = \log_2 (\text{Signal\_rep1}/\text{Signal\_rep2})$.

Only genes detectable in both replicates are shown in the figures, corresponding to chemiluminescent signal/noise ratios that are greater than 3.
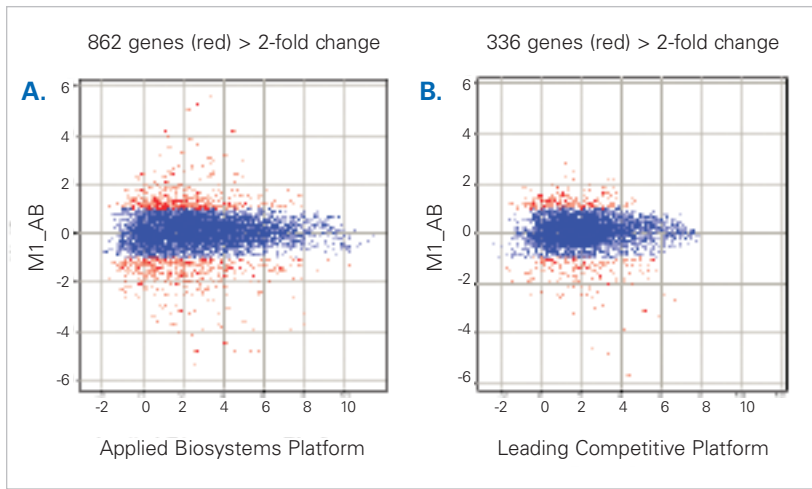
862 genes (red) > 2-fold change

336 genes (red) > 2-fold change

337 genes (red) > 2-fold change

Figure 3. Y axis: M = log$_2$ (Signal_tumor/Signal_normal); X axis: A = 0.5*log$_2$ (Signal_tumor*Signal_normal).

Genes detected in both normal and tumor samples are shown in the M-A plots above. Differentially expressed genes (> 2-fold changes between tumor and normal samples) are shown in red. Blue indicates genes that are changed < 2-fold. **A.** Of the 10,687 cross-mapped common genes analyzed on the Expression Array System, 6,893 genes are present in both the normal and tumor samples and 862 genes have > 2-fold changes. **B.** Of the 10,687 common genes analyzed on the competitive platform, 4,464 genes are present in both normal and tumor samples, and 336 genes have > 2-fold changes.
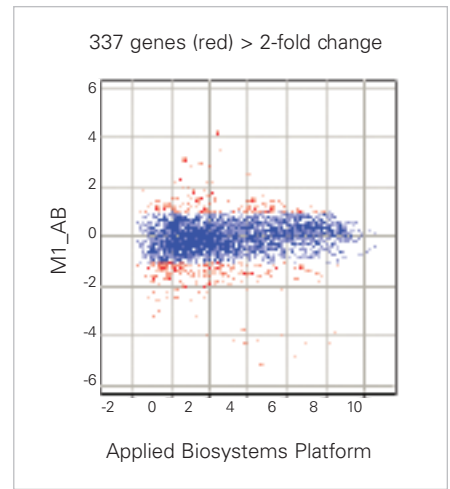
Figure 4. Cross-mapping studies between the Applied Biosystems Human Genome Survey Microarray and the competitive microarray revealed that of the 27,868 genes targeted by the Applied Biosystems microarray, 7,142 genes are not represented on the competitive microarray. Of these unique genes, 3,643 and 3,700 were detected in normal and tumor samples, respectively. The concordance between these tissues (i.e. genes in common) was 3,172 genes of which 337 exhibited > 2 fold changes (red).
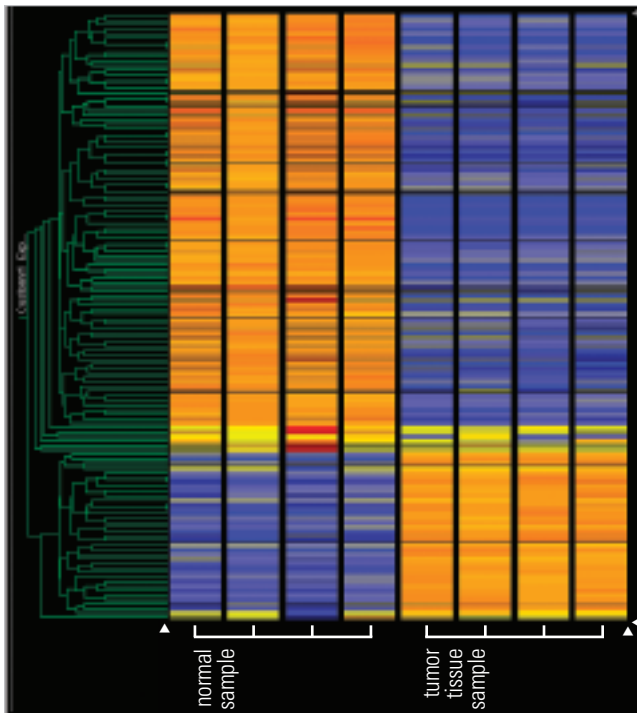

Figure 5. Two-dimensional hierarchical clustering analysis was performed on the Applied Biosystems Expression Array System data set using GeneSpring™ Software. The cluster diagram above shows 1,569 expressed genes (of the 27,868 genes represented on the Applied Biosystems microarray) that were determined to have more than 2-fold differential expression between tissues. In this analysis the genes were identified from an ANOVA analysis of 4 tumor and 4 normal samples with a predicted false discovery rate of < 5% (Benjamini Hochlag multiple testing). Red and blue represent high and low gene expression levels respectively, and yellow represents no, or unchanged expression levels.

Of the 1,569 differentially expressed genes identified by the Applied Biosystems Expression Array System, 1,292 genes (82%) were identified solely by the Expression Array System, and 277 genes (18%) were identified by both platforms. Within these 1,292 genes, 1,102 genes (70%) were identified from the cross-mapped, common genes and 190 genes (12%) were Applied Biosystems unique genes.

Conclusions

- From the 10,687 cross-mapped genes in common, the Applied Biosystems Expression Array System detects 2,710 more genes in normal tissue, and 2,542 more genes in tumor tissue than the leading competitive platform

- The Applied Biosystems Human Genome Survey Microarray contains > 7,142 more genes than the competitors microarray and, therefore, is able to provide more comprehensive gene coverage. Among these additional genes, 3,643 and 3,700 genes were detectable in normal and tumor tissues respectively

- Because the Applied Biosystems Expression Array System consumed less input total RNA than the competitive system, we infer superior detection sensitivity.

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