

## Introducing our new Agilent DNA Microarray Platform “...the platform that’s open to anything...”

IMG M Laboratories is glad to inform you about the availability of our **new Agilent DNA Microarray Platform**. The system successfully passed our internal cross-platform validation and is now ready to go. (for details on our validation, please see below)

The Agilent DNA Microarray Platform is characterized by superior sensitivity, high reproducibility as well as excellent flexibility and cost effectiveness.

By switching to this powerful microarray platform, we were able to further increase the value of our well accepted whole genome gene expression service. We are now able to offer our customers new applications e.g. gene expression analysis of a wide range of species, oligo aCGH or microRNA analysis. Independent of your application, our service brings together high data quality, sound bioinformatics and detailed and understandable reports.

For customers with new projects, we recommend to process samples with our new high performance microarray platform. However, for customers wishing to extend existing data sets, our AB1700 Chemiluminescent Microarray Analyzer will still be available until the end of 2008.

### **Validation of the Agilent DNA Microarray Platform in comparison to the AB1700 System**

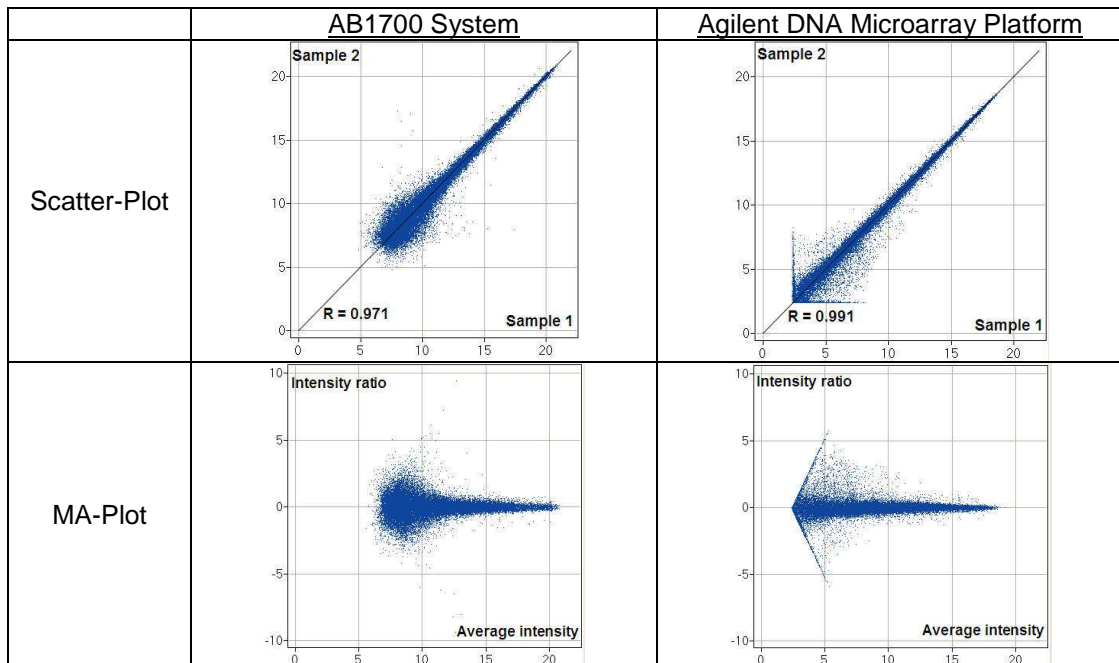
#### **Brief outline of the validation experiment**

For the cross-platform validation experiment, the following materials, protocols and analysis parameters were used:

- Total RNA of two different neuroblastoma cell lines (different in MDR status)
- Three biological replicates per cell line (> 500ng each)
- Input amount, labeling and hybridization according to manufacturer’s recommendations
  - AB1700: single round RT-IVT labeling
  - Agilent: One-Color labeling
- Biostatistics according to manufacturer’s recommendations
  - AB1700: R/Bioconductor package ABarray
  - Agilent: GeneSpring GX 9
- Analysis was performed based on the 12091 genes published in the MAQC common gene set
  - Nature Biotechnology 24 (9), 1151 – 1161, 2006

**Comparative analysis of data generated at IMG Laboratories**

- Reproducibility**

**Figure 1:** Correlation of replicate samples (normalized log<sub>2</sub> data; all represented genes)


**Conclusion:** The AB1700 System showed a very high correlation for two biological replicate samples ( $R = 0.971$ ). With an  $R$  value of 0.991, the Agilent DNA Microarray Platform performed even better.

- Sensitivity**

**Table 1:** Total number of genes significantly detected above background (present calls)

	AB1700 System (32878 probes)		Agilent DNA Microarray Platform (41000 probes)	
	Cell line 1	Cell line 2	Cell line 1	Cell line 2
Mean present calls	18030 (54.8%)	18717 (56.9%)	27572 (67.2%)	27734 (67.6%)
Standard deviation	544 (1.7%)	764 (2.3%)	198 (0.5%)	255 (0.6%)

**Table 2:** Total number of genes significantly up- or down-regulated by a p-value of  $\leq 0.01$  (Welch's t-test) and a factor of  $\geq 2$ 

	AB1700 System		Agilent DNA Microarray Platform	
	up-regulation	down-regulation	up-regulation	down-regulation
Absolute number	443	519	1555	1641
Out of whole genome	1.3%	1.6%	3.8%	4.0%

**Conclusion:** Compared to the AB1700 System, the sensitivity of signal detection is highly increased with the Agilent DNA Microarray Platform. This is documented by an increase of present calls (from 55% to 67%) and a decrease in the related standard deviations (from ~ 2% down to 0.55%). This finding is further substantiated by the Scatter-plots and MA-plots and explains the significantly better detection of low expressed genes with the Agilent System (see Figure 1 and Table 1).

Sensitivity in detecting de-regulation is increased with the Agilent DNA Microarray Platform. About three times more de-regulated genes were detected compared to the AB1700 System (see Table 2).

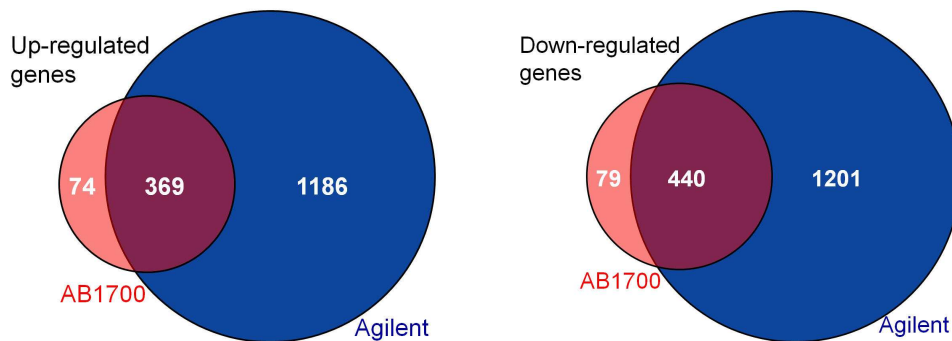
The data presented here confirm Agilent's claim that their DNA Microarray Platform has a linear dynamic range of > 5 orders of magnitude (see Figure 1).

- **Comparability**

**Table 3:** Analysis of significantly de-regulated genes by identifying the overlap between both platforms

	Specifically detected with AB1700	Specifically detected with Agilent	Detected with both platforms
up-regulated	74	1186	369
down-regulated	79	1201	440

**Figure 2:** Graphical representation of the overlap of de-regulated genes between both platforms



**Conclusion:** More than 83% of the de-regulated genes identified with the AB1700 System were also significantly detected with the Agilent DNA Microarray Platform.

However, about three times more de-regulated genes could be detected with the Agilent DNA Microarray Platform.

After reviewing the results from our cross-platform validation experiment, we are confident that we now have the perfect microarray platform in place to process your samples and generate the highest quality data for your whole genome gene expression project. We are convinced that this superior performance will also extend to all other applications available on the **Agilent DNA Microarray Platform**.

The Agilent DNA Microarray Platform offers a broad set of applications designed to meet your specific research needs e.g. basic research, identification of new drug targets or discovery of biomarkers.

**“Select your application. Select your genome. Open up to possibilities.”**

- **Gene Expression**

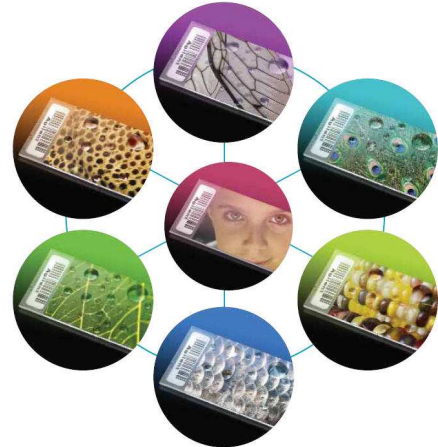
Explore gene transcription on a genome-wide basis across a variety of model systems. Besides Human, Mouse and Rat Whole Genome arrays you can choose from a great inventory of catalog array designs or compose your own custom arrays.

- **microRNA**

Profile microRNA's (miRNA's) from Human, Mouse or Rat and explore the role they play in gene regulation. Agilent provides frequent array design updates (currently Sanger miRBase, v. 10.1) that reflect new discoveries in this emerging field. Due to the broad usability of the Agilent System, IMGM can offer to analyze miRNA arrays from different vendors (e.g. Invitrogen, Ambion).

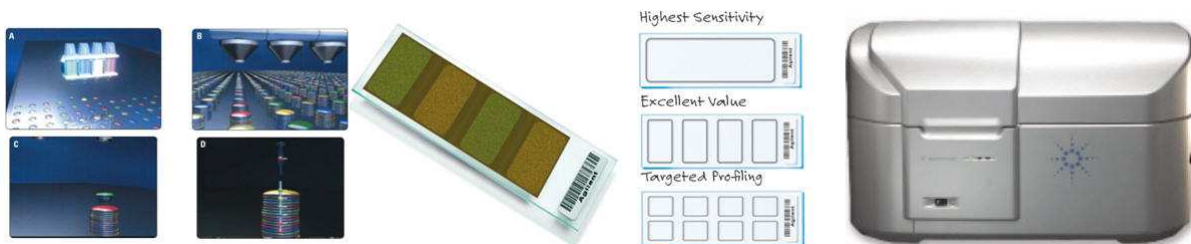
- **Comparative Genomic Hybridization (oligo aCGH)**

Conduct high-resolution, genome-wide profiling of DNA copy number changes associated with cancer and other genetic diseases. The average probe distance across the genome is 6.4 kb.



Agilent's 60-mer oligonucleotide probes are synthesized in situ, resulting in highly reproducible features, superior signal-to-noise ratio and more than 5 orders of magnitude of linear signal intensity.

Agilent's SurePrint inkjet technology prints oligonucleotides on the fly, enabling total control over array content (inventoried catalog arrays or custom design) with no compromise in cost, data quality or scalability (1-pack, 4-pack or 8-pack format).



In summer 2008, Agilent will introduce the **1 Million Feature Arrays** in conjunction with a 2µm Scanner Upgrade. This exciting development will bring the following **key benefits**:

- Increased resolution and comprehensive coverage
- Improved experimentation workflow with fewer slides
- Profile multiple samples per slide, reducing total price per experiment
- Greatly reduced price per data point

The IMGM team is now ready to use our **new Agilent DNA Microarray Platform** and we are looking forward to conducting your project.