Phalanx Biotech Group

Switching to Low Cost DNA Microarrays Makes Research Dollars Go Further

Low cost DNA microarrays that equal or surpass the quality of brand name microarrays are a business imperative for budget-conscious and results-driven research projects.

Scientists who want to stretch their research budgets so that they can do more and understand more in their gene expression work, may find that they can do just that with a new class of microarrays.

Challenges Researchers Face

Research budgets can be challenged by the high cost of materials—including expensive microarrays.

Researchers studying genes and genomes using DNA microarrays must be confident that the tools they are using are of the highest caliber to ensure that accurate data is obtained. The high price of quality materials and supplies—including the DNA microarrays used in gene expression work—can cause the cost of research projects to skyrocket. Because of this, researchers often face budgetary constraints, which can limit the number of experiments that can be performed, thus limiting the results of their work.

Ensuring that DNA Microarrays are High Quality

The DNA microarrays used in gene expression study must adhere to the highest quality standards to ensure that the results obtained from experiments are correct and accurate. To ensure this, the microarray technology used in experimental settings must have the following three attributes:

- Optimization of immobilization conditions
- Consistent reproducibility
- Defined sensitivity and dynamic range

Optimization of Immobilization Conditions to Enhance Microarray Performance

Surface Chemistry

Immobilization conditions must be optimized to ensure consistent reproducibility in DNA microarrays.

DNA microarrays are widely used to compare the gene expression profiles of specific cell types within normal and diseased individuals. Therefore, the quality and reproducibility of DNA microarrays are essential for obtaining meaningful data from such experiments. Factors that influence the quality of inkjet-spotted microarrays fabricated with pre-synthesized probes include, the consistency of microarray surface properties, the density of immobilized probe molecules, and the concentration of salts and other additives in the printing buffer.

A microarray typically combines a solid base and a reactive surface that allows the attachment of probe molecules. Biomolecules, such as DNA fragments, oligonucleotides, peptides, or proteins, are used as probes.

Printing Buffer

In addition to microarray surface properties, key components of the printing buffer and their respective concentrations also play an important role in the immobilization of probes. Buffers containing salts such as SSC are commonly used in printing to improve immobilization efficiency. One major drawback of adding salts to printing buffers is the formation of crystals during the array fabrication process due to water evaporation. Crystallization of the printing buffer significantly reduces the immobilization efficiency and compromises the circularity of spots.

Reproducibility for Accurate Data

Inter-array Data Reproducibility of DNA

High reproducibility is an absolute pre-requisite when using multiple microarrays.

A high degree of reproducibility is an absolute pre-requisite for obtaining meaningful microarray data using multiple microarrays. While performing DNA microarray hybridization experiments, multiple repeats with the same samples are often required in order to acquire data that are statistically significant. Thus, high data reproducibility both within and between arrays is essential during the production of high-density microarrays.

Data Collection of DNA Microarray Information

High data reproducibility also enables the researchers, in some cases, to compare data obtained from different samples when no suitable control is available to normalize multiple sets of data or when the amount of available sample is limited such that only a small number of arrays can be used.

Standardized arrays with proven reproducibility are critical for data comparison.

As the popularity and usage of microarrays as a research tool rapidly advances, construction of databases that encompass a vast amount of microarray information will provide researchers with the opportunity to quickly compare the specific data obtained in one experiment to a variety of different data sets. In order for this comparison to be meaningful, standardized arrays with proven data reproducibility will be required.

Further, from the point of view of practical application, high data reproducibility will also enhance the efficiency and applicability of microarrays as diagnostic tools in medicine. Therefore, optimized immobilization conditions for consistent reproducibility must be established for the microarray technology used in gene expression study.

Sensitivity and Dynamic Range

Signal Detection

Signal detection in experiments involving the use of DNA microarray relies on the hybridization of specific targets in the population to immobilized probes on the slide. Thus, factors that affect the hybridization efficiency will have profound influence on the overall performance and functionality of the array. For example, it is well documented that the surface environment provided by the chemical composition on the slide can dramatically alter the hybridization rate (?) Furthermore, the probe sequence chosen by a particular software program for a certain gene has to have adequate GC content to ensure efficient hybridization. In addition, probe immobilization efficiency directly affects the probe concentration on the array and the rate of hybridization in the reaction. Taken together, these factors determine the sensitivity and dynamic range of a particular microarray platform.

Conventionally, sensitivity indicates the lowest amount of targets present in the population that can be reliably detected by the probes on the array. The dynamic range defines the boundaries of signal intensity that can accurately reflect differences in amounts of targets present in a sample. Since the molecular amount of any target within an *in vivo* derived sample can not be determined quantitatively in a straightforward way, externally spiked-in targets are generally used in these experiments.

The Solution: Low Cost Microarrays

OneArray™ DNA Microarray Fits the Bill

The OneArray DNA Microarray offers researchers a low cost option without sacrificing quality.

Built on next-generation platform technology, the OneArray™ DNA Microarray is the low cost solution that enables researchers to complete more tests with the same dollar amount using microarrays that are equal to or better than the brand name microarrays used in the past.

OneArray provides a low cost DNA microarray solution for researchers with quality that is equal to or better than high priced name brand microarrays. Thus, OneArray allows researchers to do more and understand more in their gene study work.

Optimized immobilization conditions and consistent reproducibility—two critical factors in the microarray technology used in gene expression study—demonstrate how switching to low cost microarrays can increase research results.

OneArray is Optimized for Enhanced Performance

Surface Chemistry

OneArray is a glass microarray substrate coated with a co-polymer of two functionally diverse components. One compound provides highly active functional moieties to covalently bind with the amine groups of probe molecules while the other serves as a structural support and provides the hydrophobicity crucial for high-density immobilization of probes. By altering the ratio of the hydrophobic material and the functional group bearing compound in the co-polymer, the level of hydrophobicity can be adjusted.

The hydrophobic property of the co-polymer determines the contact angle of the probe droplet on the surface of the microarray substrate, which directly affects the size and morphology of the spots delivered. Figure 1 shows the side view of a OneArray microarray slide specifically formulated to maintain the circularity and high contact angle of droplets by confining them in a hydrophobic environment. Figure 1 provides a three-dimensional reconstruction of a portion of the OneArray surface (surface area: 250 x 300 µm).

The homogeneity of the surface coating is illustrated by a color gradient that represents the height of the co-polymer layer. The homogeneity of the co-polymer layer is excellent after coating to the glass substrate.

Each OneArray slide is specifically designed to maintain the integrity of the spots delivered in the probe droplet.

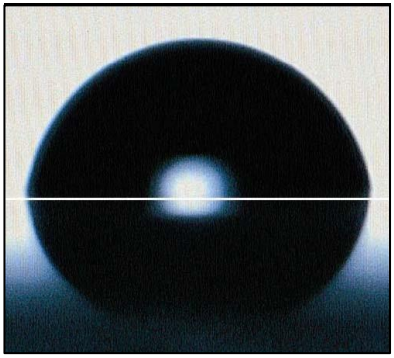

Figure 1. Side view of a OneArray slide spotted with a probe droplet.
The slide surface is delineated by the white line.

Printing Buffer

OneArray uses anti-evaporation to prevent spots from crystallizing.

To overcome the crystallization problem, an anti-evaporation formulation was developed that can effectively prevent the formation of the salt crystals and maintain appropriate spot morphology during the immobilization process. Figure 2 shows the comparison of the OneArray anti-evaporation printing buffer with conventional printing solutions.

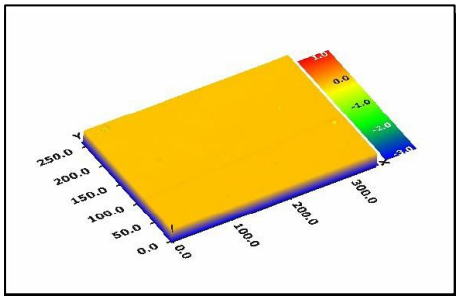

Figure 2. Three-dimensional reconstruction of a 250 x 300 µm portion of the OneArray surface coating.

The color gradient to the right maps the vertical dimensions (unit: µm) of the co-polymer layer.

OneArray Anti-evaporation Solution

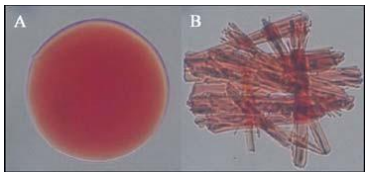

Figure 3. Pictures showing spots (60-mer oligonucleotides) printed with proprietary anti-evaporation printing buffer (3A) and conventional SSC buffer (3B).

The pictures above were taken four hours (at room temperature) after spotting. Spot printed with proprietary anti-evaporation spotting solution maintained its original morphology, while the spot printed with SSC buffer crystallized due to loss of its water content.

Figure 3, above, shows how using OneArray's anti-evaporation formula, the probe droplet in panel A is able to maintain its original morphology after being on the microarray surface for four hours. While in panel B, the probe droplet was spotted using conventional formula was completely crystallized after the same period of time. OneArray's anti-evaporation printing buffer is able to maintain the water content of deposited probe droplets for longer periods of time, which allows the probes to be immobilized under optimal conditions.

Probe Concentration

Probes on the OneArray slides are immobilized under optimal conditions.

Under the same immobilization conditions, the density of immobilized probe molecules on the microarray surface generally increases as the concentration of probes in the printing buffer increases. Because hybridization efficiency depends largely on the amount of available probes for target hybridization, performance occurs in probes with significant concentration variation. In order to assess the effects of probe concentration on immobilization efficiency, a Cy3-labeled 60-mer oligonucleotide probe was deposited on a OneArray slide at three different concentrations: 1 µM, 5 µM, 20 µM. Unbound probes were washed away after immobilization and the slide was scanned using a commercially available scanner[1] at the resolution of 5 µM, PMT 500 volts, and 33% laser power. Figure 4 shows the fluorescent image of the spots; Table 1 lists the averaged signal intensities of different probe concentrations. Fluorescent intensities of the immobilized spots were measured to evaluate the amount of immobilized probe molecules on the slide surface.

Table 1. Table showing pixel intensity data extracted from the fluorescent image in Figure 4.

	1 :M	5 :M	20 :M
Mean	4852	15129	14792
Median	4784	15124	14769
Median/mean	0.99	0.99	0.99
SD	740	1152	1120
CV	15.3%	7.6%	7.4%

The mean, medium, SD, and CV were calculated using the entire population of pixel intensities from the replicates.

1 GenePix® -400B scanner and GenePix® 4.1 software, Molecular Devices.

Immobilization of OneArray Spots

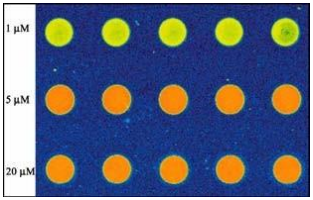

Figure 4. Fluorescent image of Cy3-labeled 60-mer oligos immobilized on a OneArray at three different concentrations.

Hybridization Performance

Applying optimal conditions for immobilization results in the demonstrated homogeneous hybridization performance of OneArray.

To further evaluate the hybridization efficiency of OneArray immobilized under optimal conditions, three 60-mer oligo probes (A, B, and C in Figure 5, below) derived from three different genes were deposited on a OneArray slide in replicates of five and processed under optimized immobilization conditions before undergoing hybridization. Total RNA was isolated from HepG2 cells using a commercially available kit.[1,2] Hybridization targets were prepared using a commercially available scanner[3] at the resolution of 5m, µm, PMT 500 volts, and 33% laser power.

Probe A, B, and C in the OneArray corresponded to genes that have very different expression levels in HepG2 cells (low, medium, and high expression levels). Signal intensity data of the three probes are summarized in Table 2, below. Signal intensity was averaged across the five replicates. Results show that the ratios of median over mean for Probe A, B. and C are very close to one and the CV values are 15.5%, 22.3%, and 30.8% respectively. The homogeneous hybridization performance is the result of evaluating and applying the optimal conditions for immobilization.

Table 2. Table showing pixel intensity data extracted from the fluorescent image in Figure 5.

	Probe A	Probe B	Probe C
Mean	39,220	12,040	4732
Median	38,534	11,780	4607
Median/mean	0.98	0.97	0.97
SD	608	2686	1462
CV	15.5%	22.3%	30.8%

The mean, medium, SD, and CV were calculated using the entire population of pixel intensities from the replicates.

OneArray consistently demonstrates homogeneous hybridization performance.

1 RNeasy Midi Kit, Qiagen

2 3DNA Array 350 labeling kit, Genisphers

3 GenePix-400B scanner and GenePix 4.1 software, Molecular Devices.

Homogeneous Hybridization Performance of OneArray

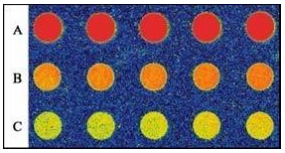

Figure 5. Fluorescent image of oligonucleotide probes (A, B, C, and D), derived from four differentially expressed genes.

The 60-mer oligonucleotide probes were spotted in replicates of five and hybridized with cDNA targets prepared from HepG2 total RNA.

Reproducibility of OneArray

OneArray's Inter-array Data Reproducibility

As outlined previously, it is critical that DNA microarrays used in gene expression research perform with a highly consistent reproducibility both within and between arrays in order to produce data results that are relevant and correct. OneArray fulfills this requirement as demonstrated in the experiment described below.

Materials and Methods

In this example, OneArray shows outstanding reproducibility.

Total RNA from the HepG2 cell lines was isolated and amplified according to the protocol of a commercially available RNA amplification kit (1). Two µg of amplified RNA (aRNA) was direct-labeled with Cy5-CTP or Cy3-CTP while performing *in vitro* transcription, and hybridized under standard conditions[2] to five 6K test arrays on which 6053 (60-mer) oligonucleotide probes—each probe corresponding to an individual known human gene—were printed in duplicates. The fluorescent signal for each spot was obtained using a commercially available scanner .[3] The z-score normalization procedure and the subsequent statistical calculation were performed using in-house software programs that employed a single channel data processing method written in R language.

Results and Discussion

Detection of Signal Intensity

In the same experiment, OneArray also shows a high degree of sensitivity.

Since five arrays were used in the experiment, and each sequence was printed in duplicate, the maximum number of spots (features) that were available for analysis for each gene was 10. We first determined that the number of features that showed detectable signal for each gene (see Table 3), and found that approximately 76% of them (4586/6053) had at least five of the ten spots showing detectable signal. Among them, 64% (2938/4593) had all ten features available for quantitative analysis.

Thus, a majority of the genes printed on the arrays consistently showed enough detectable signals on multiple arrays, a critical requirement for any accurate assessment of data reproducibility. This finding also is testimonial to the high degree of sensitivity of the OneArray DNA microarrays developed by Phalanx Biotech.

Table 3. Number of detectable genes.

Genes with Signal	Number of Genes
Total number of genes including non-expressed genes	6053
>50% showing positive signals from 10 replicate spots	4586
100% of genes showing positive signals from all 10 replicate spots on five arrays	2938

Total: Total number of genes on each array, including non-expressed genes.

>50%: Number of genes showing 50% positive signals from 10 replicate spots on five arrays.

100%: Number of genes showing 100% positive signals from all 10 replicate spots on five arrays.

1 RNeasy Midi Kit, Qiagen

2 3DNA Array 350 labeling kit, Genisphers

3 GenePix-400B scanner and GenePix 4.1 software, Molecular Devices.

Comparison of Signal Intensity Across Arrays

In order to achieve maximal comparison of signal intensity across arrays, the 2938 genes for which all 10 features are available for quantitative studies were chosen for further analysis. The range of signal intensity among these 2938 genes is more than two orders of magnitude and the distribution of intensity is wide. Thus, they should form a reasonably representative group for statistical analysis.

Results conclude that OneArray shows a statistically significant high degree of reproducibility.

The signal intensity for each of the 2945 genes was first ranked within each array. As the most stringent test, the quantitative mean and standard deviation of the log2 values of signal intensity for all ten spots of each gene were first calculated without any normalization. The coefficient of variation (CV) was determined for each case and the global statistical analysis is shown in Table 3. For comparison, the z-score normalization method, which used the 40%-70% signal intensity rankings to normalize any systematic difference within and between arrays, was also performed.

After normalization, the mean plus the standard deviation of the log2 values of signal intensity for all 10 features and the coefficient of variation (CV) in each case was determined and analyzed as above (Table 4). In either case, the mean CV and the standard deviation of CV for the signal intensity across arrays of these 2938 genes suggests a statistically significant high degree of reproducibility across arrays.

Table 4. Signal intensity statistics across five arrays.

Genes with Signal	CV_mean	CV_SD
2938 (genes) set—without normalization	2.6%	1.6%
2938 (genes) set—with normalization	2.3%	1.5%

Correlation Between Any Two Arrays

Strong positive correlation between arrays indicates the high degree of reproducibility of OneArray.

To further demonstrate the high quality of OneArray, the relationship between any two arrays was examined by calculating the correlation coefficient (r) of the log2 values of signal intensity in a pair-wise fashion for all possible combinations using all 2938 genes. The average log2 intensity for each gene on an individual slide was determined and plotted against the corresponding one on another slide and the correlation coefficient determined. A representative graph is shown in Figure 6, and the results are summarized in Table 5, below. In all cases, strong positive correlation is evident indication a high degree of reproducibility.

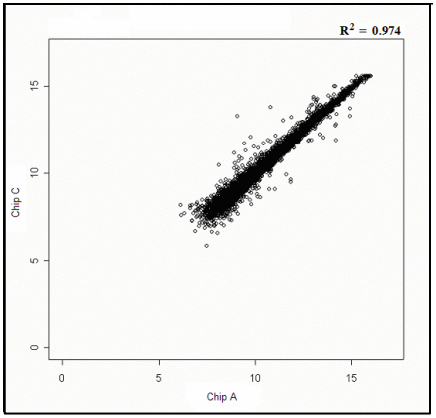

Figure 6. Scatter plot showing pair-wise correlation of two 6K Test Arrays developed by Phalanx Biotech Group.

Table 5. Pair-wise intensity correlation.

	A	B	C	D	E
A	1.0	0.97	0.974	0.962	0.968
B	0.971	1.0	0.971	0.956	0.964
C	0.974	0.971	1.0	0.964	0.972
D	0.962	0.956	0.964	1.0	0.972
E	0.968	0.964	0.963	0.972	1.0

One Array Demonstrates Superior Sensitivity and Dynamic Range

Experimental results support the high sensitivity and dynamic range of OneArray.

It is well known that the level of mRNA expression of different genes can vary greatly within any given cell type. The ability of a particular type of DNA microarray in detecting global gene expression is reflected by its sensitivity while the dynamic range determines the output intensity boundary for which changes in gene expression can be quantified. Undoubtedly, in order to obtain a significant amount of useful data in any experiment involving DNA microarray, the sensitivity and dynamic range of the array being used have to be sufficient.

Control Probe Sequences

Unique control probe sequences and corresponding spike-in target mRNAs are commercially available. They can be used as indicators of the general printing qualities of microarrays. In addition, 60mer DNA sequences used as probes on the OneArray are chosen by the IMPORT program. Using the same criteria, we have selected six probe sequences (each one from a specific gene) from *Arabidopsis thaliana* that do not cross-hybridize with human sequences and printed the corresponding oligonucleotides on OneArray as controls. Collectively, they are termed ETQC (External Quality Controls). These sequences were chosen using the similar criteria as the human probes and the oligonucleotides were processed under the same conditions as the human probes.

Thus, the performance of these ETQC probes can serve as indicators for the overall quality of the set of human probes printed on OneArray. In this report, we first printed the corresponding probe sequences to the commercially available controls and the six ETQC probes, then used varying amounts of amplified aRNA as well as labeled anti-sense oligonucleotides in the case of ETQCs complementary to the probes in order to determine the sensitivity and the dynamic range of OneArray.

Probe Sensitivity

Graphs provide visual evidence that OneArray consistently achieves high results.

Figures 1-3 showed the intensity plots for either the probes from the two commercially available sources or from the ETQCs when varying amounts of amplified aRNA targets were used for hybridization. In all three cases, the lowest signal intensity that could be reliably detected was around 0.05pM. Similar result was obtained when labeled anti-sense oligonucleotides complementary to ETQCs were used in the experiment (Figure 4). Approximately, the concentration of 0.05pM is equivalent to *x* transcript per *y* cells and such sensitivity should be able to detect a great majority of expressed genes under normal conditions.

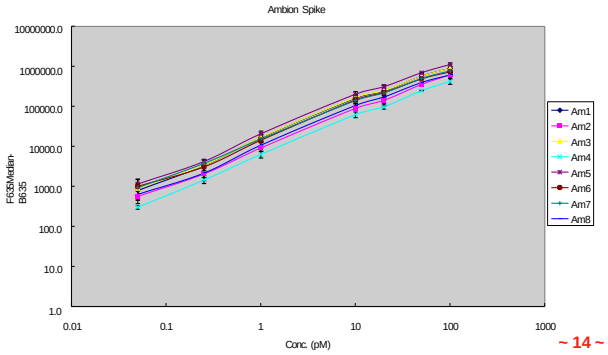

Figure 7. Hybridization of Spiked-in aRNAs to Ambion Control Probes.

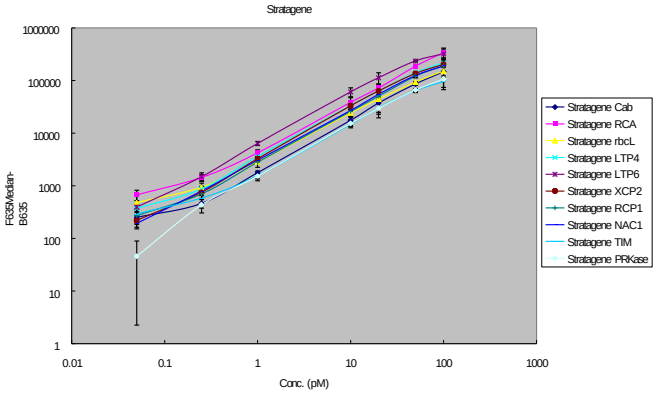

Figure 8. Hybridization of Spiked-in aRNAs to Stratagene Contol Probes.

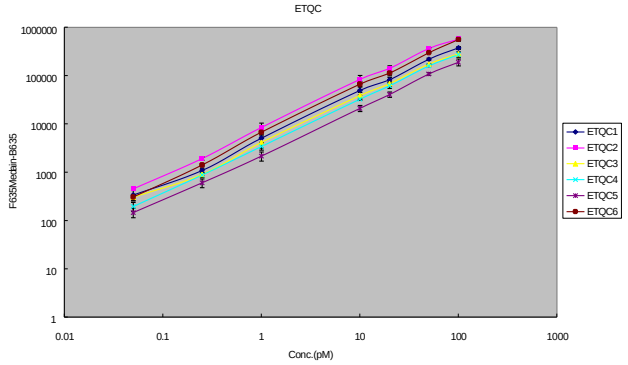

Figure 9. Hybridization of Spiked-in aRNAs to proprietary ETQC probes.

Conclusion

Inexpensive microarrays that are comparable in quality to better-known, commercial brands give researchers in the field of gene expression study a solution to budgetary restrictions on their work—and the result is increased productivity without increased costs.

OneArray provides researchers with a low cost, high quality DNA Microarray for increased productivity.

Using the low cost OneArray DNA Microarray, which equals or surpasses the quality of brand name microarrays, are a business imperative for budget-conscious and results-driven gene expression study projects.

For More Information About OneArray . . .

For more information about OneArray DNA Microarray products and services, contact Phalanx Biotech Group at:

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