

Utilization of DNA Microarrays Increasing

Persistent Doubts about Reproducibility and Compatibility of Data Are Being Overcome

K. John Morrow Jr., Ph.D.

One of the hottest research tools these days within the academic, biotech, and pharma communities is DNA microarrays. The concept morphed from the now-classical Southern blotting procedure into the present highly automated systems for screening hundreds of thousands of gene sequences. Because of their huge scanning capability, DNA microarrays can drive R&D programs that would have been impossible before the advent of this technology.

Southern blotting, now over three decades old, is based on the transfer to nitrocellulose of DNA molecules separated on gels, and their subsequent identification with DNA probes. This was one of the early applications of the highly specific pairing properties of single strands of DNA for their homologous partners. Using radioactively labeled probes, a single gene could be picked out from an entire genome.

The principle was expanded and automated in the development of DNA microarrays, which consist of an arrayed series of thousands of microscopic spots of

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DNA oligonucleotides, each containing tiny amounts of a specific DNA sequence.

The arrays are printed on a platform, usually a glass slide. The probe will hybridize with samples of homologous nucleic acids, as detected by fluorophore-labeled targets whose signal is proportional to the relative abundance of nucleic acid sequences in the target, making it possible to examine the relative activity of thousands of genes at any given time.

Applications

Microarray technology is being applied to the investigation of heart disease, mental illness, infectious diseases, and cancer. It is now possible to classify cancers based on the patterns of gene activity in the tumor cells, suggesting that it will be feasible to design treatment strategies focused on specific malignancies.

According to Justin O'Kelly, a spokes-

person for Roche NimbleGen's 12 plex array offers 12 samples per array and 135 K features on each of 12 subarrays.



person for Affymetrix (www.affymetrix.com), among his company's most notable genotyping products are the Genome-Wide Human SNP Array 6.0 and the new Genotyping Console 3.0.1 software (GTC 3.0.1).

The SNP Array 6.0 is a single microarray that measures more than 1.8 million markers for genetic variation, according to the company. It consists of 906,600 SNPs and 946,000 copy-number probes. The

array enables researchers to perform whole-genome association studies by genotyping more markers from more individuals at a lower cost per sample. These studies increase the probability of discovering genes associated with adverse drug response or complex diseases such as Alzheimer's, diabetes, heart disease, and Parkinson's disease.

Integrating SNP genotyping, copy-num-
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Companies with DNA Microarray Products

Company	Application/Product(s)	Specifications
Affymetrix www.affymetrix.com		
	Genome-Wide Human SNP Array 6.0	1.8 million genetic markers; 906,000 SNPs and 946,000 copy-number variation probes
	Genome-Wide Human SNP Array 5.0	500,000 SNPs and 420,000 extra nonpolymorphic probes
	GeneChip®Chromosome 21/22 2.0R Array	Over 2.3 million probe pairs to interrogate chromosomes 21, 22
	GeneChip ENCODE 2.0R Array	High-resolution 7 base pair tiling design; 18 base pair overlap
	GeneChip Human Promoter 1.0R Array	4.6 million probes tiled through over 25,500 human promoter regions
	GeneChip Human Tiling 2.0R Array Set	45 million oligonucleotide probes to interrogate the human genome on a few arrays
Agilent Technologies www.agilent.com		
	Gene expression	Human genome (whole), mouse development, mouse genome (whole), rat genome (whole), <i>Arabidopsis</i> , barley, bovine, canine, <i>C. elegans</i> , chicken, <i>Drosophila</i> , <i>E. coli</i> , frog, horse, <i>M. grisea</i> 2, maize, mosquito, porcine, rabbit, rhesus monkey, rice, salmon, sheep, tobacco, yeast (VS), zebrafish (V2)
	ChIP-on-chip	Human ENCODE, human promoter, mouse promoter, zebrafish promoter, <i>Arabidopsis</i> whole genome, <i>C. elegans</i> whole genome, <i>Drosophila</i> whole genome, <i>S. pombe</i> whole genome, yeast whole genome
	DNA Methylation CGH	Human and mouse CpG island Human (3), Mouse (3), rat (2), and chicken
	miRNA	Human (2), mouse and rat
Aviva Systems Biology www.avivasysbio.com		
	GLAS-20K-chip H4K Chip	20,000 human promoters 4,000 human promoters
Combimatrix www.combimatrix.com		
	CustomArray™ 12K CustomArray 4x2K	Open architecture on chip design Four independent 2,240-feature microarrays on a single slide
Eppendorf www.eppendorfna.com		
	DualChip® Microarray Kits	16 products including RNAi side effect, inflammation, cancer, hepato, GPCR, apoptosis, general, aging DNA damage and repair, metabolic syndrome and signaling

Company	Application/Product(s)	Specifications
Illumina www.illumina.com		
	Infinium DNA Analysis Bead Chips Human1M-Duo	Whole-genome genotyping, CNV, LOH: Dual-sample array with more than 1M SNPs per sample
	Human660W-Quad	Whole-genome genotyping, CNV, LOH: Four-sample array with coverage of both common SNPs and CNV regions
	iSelect Custom Genotyping Panels	6K-2,000K genotyping panels: Twelve-sample product that targets any SNP for any species.
	iSelect Standard	HumanCVD, BovineSNP50, Genotyping Panels CanineSNP20, EquineSNP50, HumanMethylation27.
	HumanCytoSNP-12	Whole-genome genotyping; cyogenetic screening; CNV; LOH
Miltenyi Biotec www.miltenyibiotec.com		
	PIQOR Series	Immunology, toxicology, skin, cell death, oncology and skin patho microarrays
Roche NimbleGen www.roche.com		
	12 x 135	12 samples per array; 135 K features on each of 12 subarrays
	385 K	1 sample per array; 385 K features on the array
	4 x 72 K	4 samples per array; 72 K features on each of 4 subarrays
	2.1 M	1 sample per array; 2.1 million features on the array
	3 x 720 K	3 samples per array; 720 K features on each of 3 subarrays
Phalanx Biotech Group www.phalanxbiotech.com		
	Human OneArray™	30,968 human genome probes; 1,082 experiment control probes
	Mouse OneArray	31,802 oligonucleotides: 29,922 mouse genome probes, 1,880 experimental control probes
SABiosciences www.sabiosciences.com		
	Oligo GEArray	Available in two formats: HybTube and HybPlate
Takara Bio www.takara-bio.com		
	IntelliGene® Series	For eukaryotes, the microarrays are spotted with cDNA regions including 3'-UTR without Alu sequence, and the ones for prokaryotes are spotted with full-length or 3'region of ORFs.

DNA Microarrays

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ber polymorphism (CNP) genotyping, and rare copy number variation identification in one data analysis application, the SNP 6.0 Array and the GTC 3.0.1 enable researchers to bridge CNP genotyping and classical SNP genotyping analysis in one genome-wide association study and/or high-resolution cytogenetic analysis, the company reports.

Focused DNA Microarrays

“Oligo GEArrays are an effective alternative to genome-wide microarrays,” says Jeffrey Hung, Ph.D., director of marketing at SABiosciences (www.sabiosciences.com). He explains that the standard genome-wide array may result in the production of so much data that interpretation becomes a daunting task, requiring elaborate software and complex analytical procedures.

and hematology-immunology markers. According to Dr. Hung, the system constitutes a useful research tool for investigators studying gene-expression profiles related to disease states. Discovery of overexpressed genes provides possible drug targets and new ways of looking at pathological states.

SABiosciences also offers a complement to the microarrays in the form of a methodology for amplifying picogram amounts of RNA. The TrueLabeling-picoAMP kit takes advantage of a two-round in vitro transcription procedure to amplify and label the cRNA for use in determining gene-expression profiles.

The kit lends itself especially to situations in which the target RNA is present in small quantities, such as specifically staining cells present in a fixed tissue sample or cells isolated through the use of fluorescence-activated cell sorting, Dr. Hung says. The kit

and other complex diseases.

For researchers interested in targeted analysis of chromosomal regions, genes, or copy-number variants, Roche NimbleGen offers customized arrays for any eukaryotic organism. Custom CGH array designs consisting of either uniform or mixed-density probe spacing can be created for all available array formats (12 x 135 K, 2.1 M, 385 K, 4 x 72 K) and may include whole genomes, single chromosomal regions, or multiple loci of interest.

According to Burkhard Ziebolz, Ph.D., head of global communications at the Roche Applied Science Group (www.roche-applied-science.com), the high-density arrays of long oligo nucleotide probes provide greater information content and higher data quality revealing the full diversity of genomic and epigenomic variation. The company has developed a technology referred to as Maskless Array Synthesis, which uses digital light processing and rapid, high-yield photochemistry to synthesize long oligonucleotide, high-density DNA microarrays with extreme flexibility.

The system is a benchtop, solid-state, high-density DNA array fabrication instrument composed of a maskless light projector, a reaction chamber, a personal computer, and a DNA synthesizer.

Hot New Applications

Mirus Bio (www.mirusbio.com) offers an extensive line of products for the investigation of microRNAs (miRNAs). These are small, noncoding RNAs of 19 to 24 nucleotides involved in regulation of messenger RNA.

MicroRNAs are members of a class of small regulatory RNAs including small interfering RNAs (siRNAs) that control the expression of downstream gene targets, including transcription factors, oncogenes, and tumor suppressor genes. Because of their association with cancer development, diagnosis, and prognosis, it is believed that their signatures can be used to detect and classify cancers and predict the severity of disease.

Technological advances by Mirus and other companies in expression profiling and in the ability to collect minute quantities of tissues allow a growing number of global transcriptional studies. Microarray technology is now well established for miRNA studies.

According to Shannon Bruse, Ph.D., director of scientific operations at Mirus, “MicroRNA expression-profiling analysis has become a prominent application of microarray technology, measuring the presence and relative amount of specific miRNAs.” In order to quantify the fluorescent signal from the hybridization step, the Mirus Bio’s Label IT platform covalently attaches label to nucleic acids. “Samples labeled directly with the Label IT reagents do not require enzymatic replication, while at the same time they yield sensitive hybridizations,” Dr. Bruse adds.

Taking the RAP

“Rapid pathogen testing is an unmet

clinical need that PCR combined with microarrays can fill,” says Christof Henne, Ph.D., key account manager for diagnostics at Eppendorf Diagnostics (www.eppendorf-biochip.com). To address this void, the company has developed the RAP™ (Real-Time Array PCR) that it believes combines the advantages of qPCR and those of microarrays.

According to Dr. Henne, RAP technology brings together the sensitivity and reproducibility of PCR with the rapid multiplexing capability of microarrays.

A critical feature in the construction of the Eppendorf platform was the genetic engineering of the active site of the polymerase enzyme so it would function at high salt concentrations. This allows stable surface binding under conditions that would ordinarily be nonpermissible. Microarrays do not lend themselves to quantification, and they have a low dynamic range, but these drawbacks can be overcome when they are combined with effective PCR.

Dr. Henne mentions that the Eppendorf platform can be coupled to commercial systems for PCR-based DNA testing. “This is a niche market that is rapidly expanding with an increasing number of assays,” he notes. One example of an integrated commercial system for PCR-based DNA testing is designed by Cepheid (www.cepheid.com), combining sample preparation, DNA amplification, and detection. “The system was originally developed for anthrax testing, but the company is now expanding its diagnostic offerings,” Dr. Henne comments.

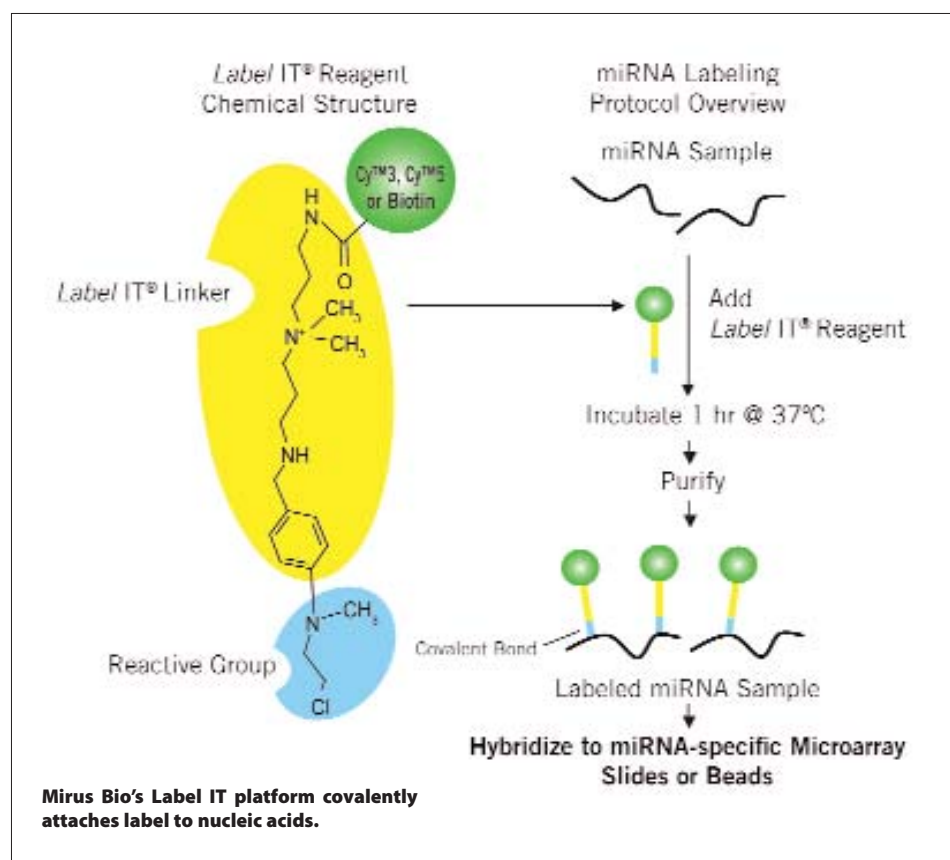
“For a point-of-care test for infectious disease diagnosis to be acceptable, it must have robustness and it must have built-in controls that inform the user of his mistakes, with clear positive and negative controls,” he continues. “The more automated the platform is, the better for the patient, so the only remaining variable is the sample quality.”

Personalized medicine is a rapidly developing area to which the RAP technology lends itself, Dr. Henne notes. Voluminous information is generated per patient, and it will be necessary to describe the patient in much more individualized terms, taking into account genetic subtypes that describe sensitivity to cancer and other disease processes.

“The combination of multiplexing ability with rapid testing will be essential in building of personalized medicine platforms,” he concludes.

An Array of Possibilities

Since array technology came on the scene in the early 1990s, it has experienced a number of advances that have greatly expanded its range of possibilities, and it is widely used today for gene-profiling studies that may produce drug targets or potential biomarkers. Yet doubts still exist concerning the reproducibility and compatibility of array data generated in different laboratories and on different platforms. Until these technical and experimental issues can be resolved, the technology will not reach its full potential as a tool for drug discovery.



GEArrays are focused array panels that allow investigators to narrow in on a select group of genes representing a particular biological pathway or disease state. The arrays are constructed with a nylon membrane that is spotted with gene-specific 60 mer oligos for up to 440 specific genes. The arrays operate with as little as 10 pg of RNA, he adds, and are detected with a chemiluminescence method.

The oligo GEArrays are evaluated for specificity, sequence complexity, secondary structure, melting temperature, GC content, and distance to the 3' end of the transcript. The company favors nylon membranes as a support structure as opposed to glass or other impermeable materials, reporting that the nylon provides larger probe immobilization and an increased hybridization and detection area.

The available pathway options cover a wide range of cogent scientific targets, including atherosclerosis, cancer biomarkers, signal transduction pathway markers,

amplifies and labels cRNA for hybridization to microarrays from picogram quantities of RNA, employing as few as 20 cells. It also contains the enzymes and buffer components needed to complete the synthesis of labeled cRNA in a 48-hour period.

“The technology provides an opportunity to find candidate genes for biomarker discovery rapidly and efficiently,” Dr. Hung concludes.

Tracking Genetic Diseases

Roche NimbleGen (www.nimblegen.com) recently made available a comparative genomic hybridization (CGH) microarray system for analysis of DNA copy-number variation. The 12 x 135 K format permits simultaneous analysis of 12 independent sample pairs on a single microarray slide, each with 135,000 oligonucleotide probes. This new array format is a research tool designed for rapid and cost-effective analysis of DNA copy-number variation associated with human genetic disorders, cancer,