Gene Expression Analysis with Pathway-Centric DNA Microarrays

SuperArray Bioscience Corporation
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Manager, Customer Education
Topics to be Covered

- Introduction to DNA Microarrays
  - What are they? How are they used?
  - Different platforms
  - Low-density arrays applications & advantages
- Important Array Performance Parameters
  - Reproducibility, Sensitivity, Reliability
- Examples
  - Similar results across different array platforms
DNA Microarrays

- Used to profile changes in gene expression between experimental conditions
  - Cultured cell line with or without treatment
  - WT versus mutant or KO animals; Normal versus patient
- Elements complementary to RNA
  - Gene-Specific Oligonucleotides or Fragments of cDNA
- Printed on solid support (chip, membrane, etc.)
- Various densities
  - High (genome-wide) or Low (Pathway-Centric™)
# Oligo Versus cDNA Microarrays

<table>
<thead>
<tr>
<th></th>
<th>Oligonucleotides</th>
<th>cDNA Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length of Element</strong></td>
<td>25 to 80 nucleotides</td>
<td>250 to 600 base pairs</td>
</tr>
<tr>
<td><strong>Sequence Source</strong></td>
<td>Entire gene sequence</td>
<td>Entire gene sequence</td>
</tr>
<tr>
<td></td>
<td>But usually 3’ biased</td>
<td></td>
</tr>
<tr>
<td><strong>Design Philosophy</strong></td>
<td>Genome-Wide Pathway-Centric™</td>
<td>Genome-Wide Pathway-Centric™</td>
</tr>
<tr>
<td><strong>Performance Parameters</strong></td>
<td>Equivalent</td>
<td>Equivalent</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Easier and less expensive to produce</td>
<td>Allows for cross-species hybridization</td>
</tr>
</tbody>
</table>
Pathway-Centric™ Design

Knowledge-Based Design +

Genomic Technology =

Application-Specific Tools ⇒

Perform Hypothesis-Driven Research &

Answer Questions in Systematic Manner
Applications for Low-Density Microarrays
Pathway-Centric™ Array Approach

Application:
- Genome-Wide Survey
- Pathway Survey
- Gene Family Survey
- Study (Verification) of Individual Genes

Methodology:
- High-Density Microarrays
- Low Density, Focused Pathway or Application-Specific Microarrays
- Pathway-Centric™ GEArray®
- Other Molecular Biology Techniques (Real-Time or Conventional RT-PCR)
Advantages to Pathway-Centric™ Microarray Design

- Focuses your research on the genes you care about
- Only hundred genes instead of thousands
- Less data to examine
- Not nearly as overwhelming to interpret
- Learn More in Less Time
- Verifies high-density array data before RT-PCR
- Easy to use & inexpensive experimental platform
How to Perform Microarray Analyses:

Control CELLS \[\rightarrow\] Treated Cells or Tissue \[\rightarrow\] Isolate RNA \[\rightarrow\] Synthesize Labeled Probe or Target \[\rightarrow\] Microarray Hybridization \[\rightarrow\] Detection of Signal & Data Analysis

Experimental CELLS
Properties of Microarray Platform Required for Successful Results

- Identical for both low and high density arrays
- **Reproducibility**
  - Good correlation between replicates
  - Low coefficient of variation
- **Sensitivity**
  - Wide dynamic range
  - Low limit of detection
- **Reliability**
  - Good cross-platform validation with RT-PCR
  - Similar profiles regardless of initial microarray conditions
Low-Density Microarray Reproducibility

XpressRef™ Human Universal Reference RNA (GA-004) (3 x 3 µg) → AmpoLabeling-LPR™ Probe Synthesis (L-03) → GEArray® Human NFκB Signaling Pathway Microarray (n=3)

Coefficient of Variation = Standard Deviation / Mean

Array to Array %CV

Less than 10% Coefficient of Variation Between Microarrays
Low-Density Microarray Reproducibility

High Correlation Observed Between Duplicate Microarrays

$R^2 = 0.9935$

GEArray® Human Trial Microarray (OHS-000)
Sensitivity and Dynamic Range

Spike with different amounts of \textit{in vitro} transcribed copies of four different mouse genes: Acox1, Bcl2l, Fbp1, Ucp2

AmpoLabeling-LPR & GEArray® Mouse Insulin Signaling Pathway Microarray (MM-030)

\begin{align*}
\text{Signal Intensity (fold over background)} & \\
0 & 5 & 10 & 15 & 20 & 25 & 30 & 35 \\
\text{Millions of copies of RNA added} & 0 & 50 & 100 & 150 & 200 & 250 & 300 \\
\end{align*}

\begin{align*}
\text{3-fold over background} & \\
\sim 30 \times 10^6 \text{ copies} & \\
\sim 3 \times 10^6 \text{ cells} & \\
= & \\
\sim 10 \text{ copies/cell} & \\
\sim 30 \text{ million copies} &
\end{align*}
In vitro transcribed firefly luciferase mRNA was TrueLabeling-AMP™ Linear RNA Amplification (GA-010) and hybridized to GEArray® Human Trial Microarray (OHS-00)

Different probe amounts

Luciferase cRNA (pM)

Raw Intensity

Noise Level

R² = 0.9969

LOD ~ 10 fM target
Range 0.01 to 10 pM
Detection of Low Abundance Messages: RT-PCR Verification

- Conventional
- AmpoLabeling-LPR™
- RT-PCR

- ACVR1,2
- COL3A1
- INHA
- JUN, MADH2
- TGFBR2
- ACTB

Human Universal Reference Total RNA
GEArray® Human TGFβ/BMP Signaling Pathway Microarray (HS-023)
Accurate Representation of Gene Expression Profiles

AmpoLabeling-LPR

RT-PCR

Mouse Liver or Thymus RNA

GEArray® Mouse Insulin Signaling Pathway Microarray (MM-030)
Accurate Representation of Gene Expression Profiles

Conventional

AmpoLabeling-LPR

RT-PCR

Mouse Liver or Thymus RNA

GEArray® Mouse Insulin Signaling Pathway Microarray (MM-030)
Comparing Relative Gene Expression Profiles Obtained by RT-PCR and the Conventional Method

\[ y = 0.7476x + 0.038 \quad R^2 = 0.186 \]
Comparing Relative Gene Expression Profiles Obtained by RT-PCR and AmpoLabeling-LPR

\[ y = 0.4192x + 0.0449 \quad \text{R}^2 = 0.6456 \]
Cross-Platform Validation Using Real-Time PCR

RNA from MCF or MDA-231 breast cancer cell lines → TrueLabeling-AMP™ Linear RNA Amplification (GA-010) → GEArray® Human Cancer Microarray (OHS-802)

First Strand cDNA Synthesis Kit (Cat # C-01) → RT² Real-Time™ Gene Expression Assay Kits For 84 genes from microarray

Calculate MDA-231 : MCF Ratios of Gene Expression
Cross-Platform Validation Using Real-Time PCR

Plot MDA-231 : MCF Ratios of Gene Expression

Using 1.3-fold threshold in gene expression, > 70 percent (60 / 84) genes changed in the same direction or did not change.
Accurate Representation of Gene Expression Profiles

Two different amounts of XpressRef™ Human Universal Reference RNA (GA-004) and TrueLabeling-AMP™ Linear RNA Amplification (GA-010) were used to generate GEArray® Human Cancer Microarray (OHS-802) profiles. The graph shows a high correlation ($R^2 = 0.976$) observed between profiles generated from two different input RNA amounts.

- 500 ng total RNA
- 3 µg total RNA

High Correlation Observed Between Profiles Generated from Two Different Input RNA Amounts
### Performance Summary

<table>
<thead>
<tr>
<th></th>
<th>Low Density</th>
<th>High Density</th>
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</thead>
<tbody>
<tr>
<td><strong>Sensitivity:</strong> Mass Ratio</td>
<td>1:300,000 (10 copies/cell OR 0.01 pM)</td>
<td>1:200,000-500,000</td>
</tr>
<tr>
<td><strong>Linear Dynamic Range</strong></td>
<td>2-3 logs</td>
<td>2-3 logs</td>
</tr>
<tr>
<td><strong>Input total RNA</strong></td>
<td>1-5 µg total RNA 100 ng with LPR</td>
<td>10-20 µg total RNA 100 ng total RNA with IVT</td>
</tr>
<tr>
<td><strong>Array-to-Array Reproducibility</strong></td>
<td>5-10% average CV</td>
<td>10-20% average CV</td>
</tr>
<tr>
<td><strong>Reliability</strong></td>
<td>Different amounts of RNA</td>
<td>Different amounts of RNA</td>
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<tr>
<td><strong>Cross-Platform Validation</strong></td>
<td>Conventional and Real-Time RT-PCR</td>
<td>Real-Time RT-PCR</td>
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Microarray Summary & Conclusions

- Profile changes in gene expression
- Performance Parameters:
  - Reproducibility, Sensitivity, Reliability
- Equivalent Performance Between:
  - Oligonucleotide or cDNA Based Microarrays
  - Genome-Wide or Pathway-Centric™
- Similar results across different array platforms
GEArray™ Focused DNA Microarrays:
Pathway-Specific Microarrays for Immunology, Cancer, Signal Transduction and More

GEArray® Gene Expression Array

Pathway and Application Specific Gene Expression Profiling
The GEArray® from SuperArray Bioscience: A gene expression profiling tool for every research laboratory

- Focused, Pathway-Specific array design
  - Densities ranging from 96 to 440 genes per array
  - Gene-specific 60-mer 3’-biased oligos or cDNA fragments
  - Minimization of cross-hybridization across array
- No special equipment needed - Accessible to most laboratories
  - Only standard molecular biology reagents and equipment
  - Easier than a Northern blot analysis
- Nylon Membranes (3.8 x 4.8 cm), non-contact printing
- Sensitive, reproducible & reliable gene expression profiling
# Oligo Versus cDNA GEArray®

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<td>Pathway-Centric™</td>
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<td><strong>Performance Parameters</strong></td>
<td>Equivalent sensitivity</td>
<td>Equivalent sensitivity</td>
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<tr>
<td></td>
<td>Equivalent reproducibility</td>
<td>Equivalent reproducibility</td>
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<td><strong>Advantages</strong></td>
<td>More robust performance</td>
<td>Most comprehensive coverage of pathways</td>
</tr>
<tr>
<td></td>
<td>More cost-effective</td>
<td></td>
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Pathway-Centric™ Design

Comprehensive Gene Groupings Determined by:
Literature Study  Database Search
Expert Review  User Feedback

Knowledge-Based Design + Genomic Technology = Application-Specific Tools

Applications Represented:
Immunology  Apoptosis & Cell Cycle
Cancer  Signal Transduction
Disease  Cell & Developmental Biology
Neuroscience  ECM & Adhesion Molecules

Customizable
Example of a Pathway-Centric™ Microarray Design

GEArray® Human Cell Cycle Microarray (HS-001)

G1 phase:
CDK2, 4, 6; CDC6, 7, 37, 34
Cyclin D1, D2, D3, E1, E2
Skp1, 2; Nedd8, Cullin 1, 2, 3, 4A
RB1, p107RB, p130RB2
CDK inhibitors:
(p15, p16, p18, p19, p21, p27 p57)
E2F-1, 2, 3, 4, 5, 6 and DP1, 2
Cks1p9, CKS2

M phase:
CDK1
Cdc16, 20(p55Cdc) and Rbx 1
PRC1, MAD2L1, MAD2L2, MPP2

G2 phase:
CyclinF, B1, B2

S phase:
Cyclin A, A1, C, G2, G1, H
CDK7 and CDK8
Cdc25a, CDC6, CDC45
MCM2, 3, 4(Cdc21); MCM5(Cdc46)
MCM6(Mis5), MCM7(Cdc47)
Ki67, PCNA

DNA damage checkpoint:
p53 and ATM pathways
P53, GADD45, TIMP3, RAD17, E6-AP, Mdm2
Mre11A, Mre11B, RAD50, RAD51
Ubiquitin C, UBE1, SUMO-1 & Nedd8
ATM, rpa, chk1, chk2, c-Abi, p73, Hus1, BRCA1
Bax, Bcl2, Apaf-1
Signal Transduction PathwayFinder™

- **JAK-STAT**
  - A2M
  - IRF1
  - IL2
  - TNFSF6
  - CD5
- **Stress**
  - BAX
  - CDNK1A
  - GADD45A
  - MDM2
  - PIG3
  - MYC
  - ATF2
  - TP53
  - HSF1
  - HSPB1
  - HSPCA
- **NFκB**
  - ICAM1
  - NOS2
  - PECAM1
- **CREB**
  - BMP2
  - HNF3B
  - WNT1
- **NFAT**
  - EGR1
  - CYP19
  - FOS
- **p53**
  - Hedgehog

GEArray™ Focused DNA Microarrays:
Pathway-Specific Microarrays for Immunology, Cancer, Signal Transduction and More
Examples of GEArray® Products

- IMMUNOLOGY
  - Inflammatory Cytokines & Receptors
- CANCER
  - Angiogenesis, Tumor Metastasis, Breast & Prostate Cancer
- SIGNAL TRANSDUCTION
  - MAP Kinase, Insulin, TGFβ/BMP
- CELL & DEVELOPMENTAL BIOLOGY
  - Endothelial Cell Biology, Stem Cell, Osteogenesis
- DISEASE
  - Asthma, Cardiovascular Disease, Cancer
- OTHERS
  - Extracellular Matrix & Adhesion Molecules
  - Drug Metabolism
  - Neuroscience I: Ion Channels & Transporters
GEArray® Support Products

- **RNA Isolation**
  - ArrayGrade™ mRNA Purification Kit (GA-002)
  - XpressRef™ Universal Reference RNA
    - From human, mouse or rat (GA-004, -005 or -006)

- **Probe or Target Synthesis**
  - AmpoLabeling-LPR™ Probe Synthesis Kit (L-03)
    - cDNA probe synthesis for cDNA GEArrays
  - TrueLabeling-AMP™ Linear RNA Amplification Kit (GA-010)
    - cRNA target synthesis for Oligo GEArrays

- **Detection**
  - Chemiluminescent Detection Kit (D-01)

- **Microarray Services & Custom Microarrays**

- **Excellent Customer Service & Technical Support**
Gene Expression Analysis with Pathway-Centric DNA Microarrays

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