Erik Bjeldanes
Product Manager, Catalog Microarrays
March 12, 2003

Practical Applications of Mouse Development Oligo Microarrays

11:00 a.m. EST
Telephone Number: 888-840-7694
International Number: 703-871-3887
Chairperson: Rita Willis

Agilent Technologies
Erik Bjeldanes
Product Manager, Catalog Microarrays
March 12, 2003

Practical Applications of Mouse Development Oligo Microarrays

11:00 a.m. EST
Telephone Number: 888-840-7694
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Starts in Five Minutes
Erik Bjeldanes  
Product Manager, Catalog Microarrays  
March 12, 2003

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Starts in One Minute
Erik Bjeldanes is the Catalog Microarray Product Manager for Agilent’s Bio-Research Solutions unit in Palo Alto, CA. His primary focus is to bring the highest quality 60mer oligo microarray products to the market faster than any other provider on the market.

Mr. Erik Bjeldanes
Product Manager, Catalog Microarrays
Agenda

• **SurePrint* in situ Manufacturing Platform**
• Mouse Development Microarray Description
• Microarray Performance
• Biological Comparison Study
Agilent Custom Microarrays

- Standard Phosphoramidite Chemistry
- *in situ* Synthesis
- Software Driven

*in situ* synthesized oligonucleotide arrays offer maximum flexibility. Microarray formats supporting up to 22.5K features are available.

Custom In-situ Microarrays

2X11K feature array

22.5K feature array
60-mer Oligo Platform Performance

Sensitivity 8X Over 25mers

Average LLD
60mers: 0.004pM
25mers: 0.032pM
60-mer Oligo Platform Performance

Accuracy of Ratio Measurements

Avg vs Expected Log Ratios

Within 1.4X of Expected Ratio
60-mer Oligo Platform Performance

Linear Dynamic Range

Concentration of synthetic target vs gBGSubSignal. Replicates Averaged

5pM = ~1000 copies/cell

Average LLD = .004pM = ~0.6 copies/cell

Dynamic Range of Over 3 Orders of Magnitude
Linear to 0.004pM (~0.6 Copies per Cell)
60-mer Oligo Platform Performance
Ratio Reproducibility Across Microarrays

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
<th>Non-Weighted</th>
<th>Weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible</td>
<td>0.995</td>
<td>0.997</td>
</tr>
<tr>
<td>Selected</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Common Signature</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td>All (no fails/controls)</td>
<td>0.995</td>
<td>0.997</td>
</tr>
<tr>
<td>P&lt;=0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-Value <= 0.01
99.9 % Correlation
A New Standard…

**Human 1A Oligo Microarray Kit**
Biologically Validated Probes Designed to Over 17,000 Full-Length Genes, All of Which Have Been Mapped and Assembled to the Human Genome.

**Mouse Development Oligo Microarray Kit**
Representing Over 22,000 Genes from the Mouse Stem Cell and Development Clone Libraries of Dr. Minoru Ko of the National Institute on Aging.

**Yeast Oligo Microarray Kit**
Designed to 6256 Known Open Reading Frames in the Yeast Genome.
Agenda

• SurePrint *in situ* Manufacturing Platform
• Mouse Development Microarray Description
• Microarray Performance
• Biological Comparison Study
The Mouse Development 60-mer Microarray

Developed in collaboration with the laboratory of Dr. Minoru Ko of the National Institute on Aging - NIA / NIH
Mouse Development Microarray Design

- Sequences derived from the NIA Mouse Developmental/Stem-Cell 15K and 7.4K clone sets
- 23,324 sequences used in probe design
- Collection of 22,927 unique gene clusters + 397 GenBank
- Unique 60 mer probes were developed for 21,939 sequences
- 20,986 sequences from 3’ end and 556 from 5’ end.
- Final commercial product is comprised of the top 20,371 probes from initial design
Content Information

• Coverage:
  • 6,711 match RefSeq
  • 5,760 match other known genes
  • 1,458 similar to other known genes
  • 8,009 unknowns

• Annotated gene content and transcript sequence information can be found at the NIA Mouse cDNA project website

• Clone reagents are available throughout the research community (American Type Culture Collection)

• Information is located via the Internet at:
  http://lgsun.grc.nia.nih.gov/
Annotation Information

- **Systematic Name:** H3107C11-3
- **Probe Name:** A_65_P18312
- **Gene Name:** Efemp2
- **GenBank ID:** NM_021474.1
- **Unigene ID:** Mm.41781
- **Gene Description:** Mus musculus epidermal growth factor-containing fibulin-like extracellular matrix protein 2 (Efemp2), mRNA
Annotation Information

- Systematic Name: H3107C11-3
- Universal Identifier for the sequence used in the design of the 60mer probe sequence used on the microarray. Use as link to the NIA website for additional information on that sequence:
  - http://lgsun.grc.nia.nih.gov/
Mouse Development Annotation

• Gene Symbol: Efemp2

• This is the Official Gene Symbol as designated by the HUGO Gene Nomenclature Committee (HGNC).

• Can be used with:

  1. LocusLink

  • http://www.ncbi.nlm.nih.gov/LocusLink/

• Links to:

  RefSeq    OMIM    GenBank - Protein
  Unigene   Homology Maps  Genbank - Nucleotide
  Gene Ontology  Chromosomal Locus
Mouse Development Annotation

• Gene Description: Mus musculus epidermal growth factor-containing fibulin-like extracellular matrix protein 2 (Efemp2), mRNA

• Iterative BLAST-based algorithm
  • oligo probe sequences vs. NCBI RefSeq and non-redundant (nr) databases to identify perfect matches to the sense strand of mRNA entries then...
  • parent clone sequence used to design the oligo against the same databases for a match to an mRNA entry of at least 90% identity with 80% overlap.
  • No matches: “unknown”.

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Mouse Development Annotation

• GenBank ID: NM_021474.1
• Unigene ID: Mm.41781

• Best representation of design sequence
• Is actual design sequence accession

Content comparisons can be performed on the Resourcer page of TIGR’s website: (Use NIA_All as Query Set)

http://pga.tigr.org/tigr-scripts/magic/r1.pl
Mouse Development Annotation

- Feature ID: A_65_P18312

- Universal Agilent ID for exact 60-mer sequence on Microarray
- Qualified customers can download 60-mer probe sequences for all genes via secure connection on Agilent’s microarray website:
  - www.agilent.com/chem/DNA
  - Click: “Catalog Oligo & cDNA Microarrays”
  - Click: “Request Sequence Information”
- Use the sequence for BLAST analysis, confirmation of microarray results (RT-PCR, Northern), cloning, mapping, designing primers, building your database, as biological markers, etc.
Break Number 1

For questions, at break please dial 1 on your phone, or type onto the chat screen at any time during the presentation.
Agenda

• SurePrint *in situ* Manufacturing Platform
• Mouse Development Microarray Description
• Microarray Performance
• Biological Comparison Study
Sample Labeling Techniques

• Use Either Amplification and Direct Labeling protocols

• Direct Labeling
  • Uses a recommended 10ug of total RNA or 200ng of poly A+ RNA per experiment
  • Generates labeled sample in hours
  • Introduces more system noise

• Linear Amplification
  • Uses a recommended 5ug of total RNA or 200ng of poly A+ RNA; enough for several experiments
  • Generates labeled sample in several hours
  • Introduces less system noise

• Not intended to be interchangeable within experiments.
Sample Labeling Techniques

Human 1A Oligo microarray hybridized with Self vs. Self targets (HeLa-Cy3 vs HeLa-Cy5), 1 microarray

aRNA Targets

St. Dev. = 0.064

Green Processed Signal

Red Processed Signal

Up-regulated
Unchanged
Down-regulated (P < 0.001)

St. Dev. = 0.127

Green Processed Signal

Red Processed Signal

Up-regulated
Unchanged
Down-regulated (P < 0.001)

Agilent Technologies
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Sample Labeling Techniques

Human 1A Oligo microarrays hybridized with Self vs. Self targets (HeLa-Cy3 vs. HeLa-Cy5), 8 microarrays

aRNA Targets

Median = 0.013

St Dev Of Log Ratio

Avg Of Processed Signal

Median = 0.029

St Dev Of Log Ratio

Avg Of Processed Signal

Agilent Technologies
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Sample Labeling Techniques

Human 1A Oligo microarray hybridized with Self vs. Self targets (Clontech Universal Reference-Cy3 vs Placenta-Cy5), 1 microarray

aRNA Targets

cDNA Targets

- Up-regulated
- Unchanged
- Down-regulated
(P < 0.001)

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Dial 1-816-650-0621 for e-Seminar Audio
Agenda

• SurePrint *in situ* Manufacturing Platform
• Mouse Development Microarray Description
• Microarray Performance
• **Biological Comparison Study**
E12.5 Embryo v. Placenta

- First differentiation event = TE v. ICM
- Placenta v. embryo are downstream results
- Placenta-specific genes identified
Biological Comparison

- Improved array?
  - more genes
  - more tissues
  - better data quality?
- Comparison of outcome
  - are the same genes identified?
  - what are the differences?
  - new biology?
Platform Specifications

15K cDNA array
- nylon support
- cDNA probes
- 1-100 µg total RNA
- radioactive direct labeling
- normalized, single channel data
- student’s t-test
- $p < 0.05$

22K 60-mer oligo array
- glass support
- 60-mer oligo probes
- 6 µg total RNA/channel
- fluorescent amplification labeling
- two-channel log ratio data
- high/low end correction
- error models
- $p < 0.05$
Data Images
Experimental Design

(pooled by litter)

self v. self controls

differential (experimental)

mean log ratios

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Self v. Self Controls

Legend:
- + Unchanged (20,901/20,901)
- + Signature (790/790)
- + Downregulated (601/601)
- + Upregulated (189/189)
- Selection (0/0)
Self v. Self Controls

Legend
- Unchanged (20,601/20,601)
- Signature (68/68)
- Downregulated (66/66)
- Upregulated (2/2)
- Selection (0/0)

P <= 0.05

Log 10(Intensity)

Log 10(Data on a Linear Scale)
Experimental Design

Self v. self controls
(pooled by litter)

Differential (experimental)

Mean log ratios
# Reliable Measurement Rates

<table>
<thead>
<tr>
<th></th>
<th>15K cDNA</th>
<th>22K oligo</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>720/15,264</td>
<td>8,096/21,393</td>
</tr>
<tr>
<td>(4.7%)</td>
<td>(38%)</td>
<td></td>
</tr>
<tr>
<td>&gt;10 fold</td>
<td>114 (16%)</td>
<td>127 (1.6%)</td>
</tr>
<tr>
<td>5-10 fold</td>
<td>119 (17%)</td>
<td>366 (4.5%)</td>
</tr>
<tr>
<td>2-5 fold</td>
<td>300 (42%)</td>
<td>2,626 (32%)</td>
</tr>
<tr>
<td>1.2-2 fold</td>
<td>188 (26%)</td>
<td>5,064 (62%)</td>
</tr>
</tbody>
</table>
cDNA v. Oligos

10% anti-correlative features
Data Set Validation by QPCR

\[
\begin{align*}
\text{Log (intensity)} & \\
-1.5 & \quad -1 & \quad -0.5 & \quad 0 & \quad 0.5 & \quad 1 & \quad 1.5 & \quad 2 & \quad 2.5 & \quad 3 & \quad 3.5 & \quad 4 & \quad 4.5 & \quad 5
\end{align*}
\]

\[
\begin{align*}
\text{Log (ratio)} & \\
-1.5 & \quad -1 & \quad -0.5 & \quad 0 & \quad 0.5 & \quad 1 & \quad 1.5 & \quad 2 & \quad 2.5 & \quad 3 & \quad 3.5 & \quad 4 & \quad 4.5 & \quad 5
\end{align*}
\]
Data Set Validation by QPCR

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Data Set Validation by QPCR

Log (ratio)

Log (intensity)
cDNA v. QPCR

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Microarray Validation – Northernns

- **TSA-1**
  - H3027D05
  - chip: 4.9:1
  - blot: 2.9:1

- **HSP-25**
  - H3082D09
  - chip: 6.7:1
  - blot: 30:1

- **Hmgi/Y**
  - H3029B11
  - chip: 8.6:1
  - blot: 3.4:1

- **unknown**
  - H3090F05
  - chip: 6.2:1
  - blot: 4.5:1

- **IMP-3**
  - H3035G11
  - chip: 12.6:1
  - blot: 5.3:1

- **ank**
  - H3042C06
  - chip: 3.3:1
  - blot: 1.17:1

- **AP-27**
  - H3097E03
  - chip: 1:6.4
  - blot: 1:2.3

- **G-protein?**
  - H3020G09
  - chip: 1:6.6
  - blot: 1:13

- **lynB tk**
  - H3001B08
  - chip: 1:6.4
  - blot: 1:2.3

- **Myosin 1b**
  - H3001A08
  - chip: 1:12.8
  - blot: ~1:100

---

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## Placenta-Specific Genes

<table>
<thead>
<tr>
<th><strong>endocrine-related proteins</strong></th>
<th><strong>ratio:cDNA</strong></th>
<th><strong>oligos</strong></th>
<th><strong>QPCR</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>secretin</td>
<td>H3001A02</td>
<td>&gt;100</td>
<td>41</td>
</tr>
<tr>
<td>placental lactogen II (PL-II)</td>
<td>H3046B04</td>
<td>&gt;100</td>
<td>7.9</td>
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<tr>
<td>calcyclin</td>
<td>H3087H09</td>
<td>35</td>
<td>51</td>
</tr>
<tr>
<td>prolactin-like protein-A precursor</td>
<td>H3007C10</td>
<td>8.7</td>
<td>19</td>
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<tr>
<td>prolactin-like protein M (Prlpm)</td>
<td>H3018B06</td>
<td>16</td>
<td>56</td>
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<tr>
<td>proliferin-related protein (PRP)</td>
<td>H3021D07</td>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>similar to NVLp.2</td>
<td>H3076C01</td>
<td>13</td>
<td>1.3</td>
</tr>
<tr>
<td>MDGI</td>
<td>H3104D07</td>
<td>47</td>
<td>4.1</td>
</tr>
<tr>
<td>similar to human HEM45</td>
<td>H3108A01</td>
<td>27</td>
<td>1.1</td>
</tr>
</tbody>
</table>
### Placenta-Specific Genes

<table>
<thead>
<tr>
<th>epithelial proteins</th>
<th>ratio: cDNA</th>
<th>oligos</th>
<th>QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>EndoA cytokeratin</td>
<td>H3031C01</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>EndoB cytokeratin</td>
<td>H3104F03</td>
<td>20</td>
<td>39</td>
</tr>
<tr>
<td>band 3-related protein</td>
<td>H3137D09</td>
<td>26</td>
<td>2.7</td>
</tr>
<tr>
<td>similar to TIG2</td>
<td>H3137E09</td>
<td>83</td>
<td>20</td>
</tr>
<tr>
<td>Bteb2</td>
<td>H3102C04</td>
<td>2.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>oxidative- and stress-related proteins</th>
<th>ratio: cDNA</th>
<th>oligos</th>
<th>QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP25</td>
<td>H3082D09</td>
<td>18</td>
<td>16</td>
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<tr>
<td>carbonic anhydrase IV</td>
<td>H3095H12</td>
<td>&gt;100</td>
<td>25</td>
</tr>
<tr>
<td>Gpx3</td>
<td>H3125A09</td>
<td>41</td>
<td>3.8</td>
</tr>
</tbody>
</table>
# Placenta-Specific Genes

<table>
<thead>
<tr>
<th></th>
<th>ratio: cDNA</th>
<th>oligos</th>
<th>QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>homeobox / transcriptional proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eHAND</td>
<td>H3020C07</td>
<td>4.9</td>
<td>2.8</td>
</tr>
<tr>
<td>pem</td>
<td>H3027G05</td>
<td>33</td>
<td>63</td>
</tr>
<tr>
<td>PSX1</td>
<td>H3120B02</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>CREG</td>
<td>H3037D10</td>
<td>50</td>
<td>34</td>
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<tr>
<td>msg1</td>
<td>H3076B01</td>
<td>16</td>
<td>4.7</td>
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<tr>
<td>transcriptional repressor SIN3B</td>
<td>H3129D02</td>
<td>18</td>
<td>9.3</td>
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<tr>
<td>similar to FRAG1</td>
<td>H3036E12</td>
<td>7.7</td>
<td>0.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>misc. proteins</strong></th>
<th>ratio: cDNA</th>
<th>oligos</th>
<th>QPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS-binding protein</td>
<td>H3086G08</td>
<td>15</td>
<td>3.3</td>
</tr>
<tr>
<td>protein kinase inhibitor p58</td>
<td>H3094A04</td>
<td>10</td>
<td>7.0</td>
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<tr>
<td>pyruvate carboxylase</td>
<td>H3137A09</td>
<td>26</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Slides Courtesy of Dr. Mark Carter/ NIA*
Placenta-Specific Genes

- Rex-1
- desmoplakins, desmocollins
- keratins
- annexins
- proliferin- and prolactin-related proteins
- CEBP
- Clim1
- Gata2, Gata3
- osteoclastogenes inhib. factor
- alpha synuclein
- minopontin
- g-protein signalling receptors, activators
- N-myc downstream regulated 1
- Ring1 interactor
- immune receptors, antigens
- unknown (at least 60)
## Acknowledgements

### Agilent Technologies
- Condie Carmack
- Pius Brzoska
- S. Stuart Hwang
- Scott Vacha

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- Toshio Hamatani
- Tetsuya Tanaka
- Yong Qian
- Dawood Dudekula
Wrap-up E-Seminar Questions

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