DNA Microarray (II)

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http://www.soest.hawaii.edu/marinefungi/OCN403webpage.htm
Overview

• Common genomics tools (Blastn & Blastp)
• DNA microarray
  – History
  – Procedure and principle
DNA Microarray Methodology

1. Research questions
   - Gene expression
   - Community analysis
   - Genotyping

2. Microarray design and probe preparation

3. Microarray fabrication

4. Sample preparation and labeling with fluoresceins

5. Microarray hybridization

6. Microarray scanning

7. Data mining and analysis

8. Modeling and prediction

A. RNA Isolation

B. cDNA Generation

C. Labeling of Probe
   - Reverse Transcriptase
   - Fluorescent Tags

D. Hybridization to Array

E. Imaging
   - Sample A > B
   - Sample B > A
   - Sample A - B

computer analysis
Hybridization of Microarray

Schematic drawing that shows how oligonucleotides molecules are analyzed by using DNA microarrays (More, 2001)
Microarray Types and Applications

Based on immobilized probe

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>cDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesized in situ or printed</td>
<td>Prefabricated and printed</td>
</tr>
<tr>
<td>Short Probes (25-70 Bases)</td>
<td>Long Probes (300-1000)</td>
</tr>
<tr>
<td>Multiple probes/gene</td>
<td>One probe/gene</td>
</tr>
<tr>
<td>Homemade or Commercial</td>
<td>Homemade or Commercial</td>
</tr>
</tbody>
</table>

Applications

- Basic biology, ecology, and physiology (regular array).
- Tumor classification, risk assessment, and prognosis prediction (comparative genomic hybridization)
- Drug development, drug response, and therapy development (Expression analysis (transcriptomics)).
- Environmental biotechnology.
Advantages of Microarrays

• High-throughput and parallel analysis
  – Small surface is uniformly deposited with thousands to hundreds of thousands of array elements or probes

• High sensitivity
  – Small volume probe & small area.
  – High sample concentration & rapid hybridization kinetics
• COMPARE the activity of many genes
  – Different target samples labeled with different fluorescent tags.

• Automation
  – Cost-effective

Drawback:

• Too much data all at once. Can take quite a while to analyze all the results.

• The results may be too complex to interpret

• The results are not always reproducible

• The results are not always quantitative enough

• The technology is still too expensive
Microarray Fabrication

• Two current DNA microarray fabrication techniques
  – in-situ synthesis of oligonucleotides;
  – Deposition of presynthesized oligonucleotides.

• In-situ synthesis of oligonucleotides
  – highly parallel process that synthesizes all probes on a substrate base by base simultaneously using photolithography method.
  – High probe density, almost no space between the spots and spotsizes below 10 µm. (Gene Chips, Affymetrix) \(10^6\) in 1.28cm\(^2\).
  – Relatively short oligonucleotides (25 bases)
  – Drawbacks
    • there need a significant time and cost for designing/fabricating of masks,
    • all process has to be done in clean rooms using expensive facilities,
    • only short DNA oligonucleotides can be synthesized (maximum 25 bases).
In situ synthesis using masks. Extension of DNA oligonucleotides are determined by light that passes through a mask (step 2). The light destroys a photolabile protective group (step 1–2) on the phosphoamidite making selective extension possible (step 3). This process requires 100 masks and thus 100 repetitions of step 1–3 for production of arrays with 25-bases long probes.
• Spotted microarrays (deposition)
  - Depositing presynthesized DNA onto a substrate using printing facility using a relevant surface chemistry.
  - Surface of these substrates needs to be functionalized with a chemical group and modified or unmodified DNA can be deposited on those substrates.
Attachment of nucleic acids to solid surfaces through electrostatic interaction. The microarray substrate contain amine group (NH3+) attached to covalently to the glass surface. At the neutral pH, the amines forms ionic bonds with the negatively charged phosphate backbone. Covalent attachment of the DNA to the surface can also be further achieved by treatment with UV light or heat.

Attachment of nucleic acids to solid surfaces through covalent bonding. The microarray substrate contain aldehyde groups attached to covalently to the glass surface. Primary amino linkers (NH2) on the DNA attach the aldehyde groups to form covalent bonds. Dehydration reaction stabilize this attachment.
– Advantages
  • no restriction of the length of DNA to be printed
  • It can be used to fabricate microarrays containing 3kb long DNA molecules.

– Drawback
  • the printing resolution \((10^4)\) of DNA spots and the fabricated probe density are much lower than photolithography based method.

– can be produced either by contact printing or noncontact printing.
  • Microarray fabrication using contact printing
    – based on high definition pins that upon contact with the microarray substrate deposits a small amount of probe solution. The pins are attached to a robotic arm that moves the pins between the different probe solutions, the glass slides where the microarray is created and a washing station.

ArrayIt® sets the standard for microarray manufacturing with ChipMaker, Stealth and 946 microarray pins and printheads.

Glass slide
Stealth 3 Pins and Corresponding Microarrays. (Top panel) Shown is an SMP3 Pin with a regular uptake channel (top left), an SMP3B Pin with a "bubble" uptake channel (top center), and an SMP3XB Pin with an "extended bubble" channel (top right). The SMP3 pin produces 200 spots of 110 µm diameter, the SMP3B pin produces 510 spots of 120 µm diameter, and the SMP3XB pin produces 780 spots of 130 µm diameter.
**SMP15XB Pin Printing.** A 500 fmole/µl solution of Cy3-labeled oligonucleotide in 1X Micro Spotting Solution (MSS) was printed onto a SuperAldehyde Substrate (SMA) at 16°C and 45% relative humidity using a PixSys 5500 robot (Cartesian). Spot spacing was 750 µm. The printed microarray was scanned at 80% PMT and 90% laser power on a ScanArray Express (PerkinElmer) and the .tif data were coded to a rainbow palette. The space bar equals 750 µm. SMP15XB pins load 1.25 µl of sample and print 88 spots of 590 µm diameter under these conditions, and are excellent for diagnostic applications.
• Microarray fabrication using noncontact printing
  – similar in terms of robotics but instead of pins, small dispensing systems are mounted on the robotic arm. The dispensing system can be based on inkjet, bubblejet or piezo actuation technology and can usually dispense in the range of \(100 \text{ pl}\) to \(2 \mu\text{l}\). Non-contact printing can have slightly higher spot density.
  – Sample taken from the source plate, and a droplet of sample is ejected from the print head onto the surface of a substrate.
Both contacting and noncontact ink-jet printing allow the spotting of virtually any biological molecule of interest, including cDNA, genomic DNA, antibodies, and small molecules.

In contrast to contact printing, ink-jet printing avoid direct surface contact, introducing surface-contact feature anomalies, and resulting in consistent spot uniformity and traceability.

Can not manufacture as dense as those prepared by photolithography or spotting approaches.
TABLE 2. Genes targeted for multiplex PCR and microarray hybridization

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Locus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GenBank accession no. (reference)</th>
<th>Name&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sequence</th>
<th>Annealing temp (°C)</th>
<th>Product (bp)</th>
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</thead>
<tbody>
<tr>
<td><em>Aeromonas salmonicida</em></td>
<td>vapA</td>
<td>M64655 (13)</td>
<td>F-A.sal-1</td>
<td>ATTAGCCCGGAACGACACAC</td>
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<td>177</td>
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<td></td>
<td></td>
<td>R-A.sal-2</td>
<td>GTCGTTGAAATTGCCCTC</td>
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<td></td>
<td></td>
<td></td>
<td>P-A.sal-vapA</td>
<td>AACTAAGCAGCCGGTGACTGAG</td>
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<td></td>
<td>X64214 (18)</td>
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<td>R-A.sal-4</td>
<td>TTATCGAGGAGCACAAACAT</td>
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<tr>
<td></td>
<td></td>
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<td>P-A.sal-plas</td>
<td>TCGACACAAATTCAATTACACC</td>
<td>65</td>
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<td><em>Listonella anguillarum</em></td>
<td>rpoN</td>
<td>U36585 (33)</td>
<td>F-V.ang-1</td>
<td>CCAAGGAAGAGATCCCAAGAGG</td>
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<td>125</td>
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<td>R-V.ang-2</td>
<td>ACACCTCAAGCACTGGCTCT</td>
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<td>P-V.ang-rpoN</td>
<td>CGCTGATGTTCACTAGCATCAATG</td>
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<td><em>fatA</em></td>
<td>Z12000 (39)</td>
<td></td>
<td>F-V.ang-3</td>
<td>GTCCGCAAAGATGGAATGAAT</td>
<td>60</td>
<td>137</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>R-V.ang-4</td>
<td>ACTGCTGCCACITTTGTTG</td>
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<td></td>
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<td>P-V.ang-fatA</td>
<td>AGTTCAAGCAAACCTCCACAAAT</td>
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<tr>
<td><em>Photobacterium damselae</em></td>
<td>ureC</td>
<td>U40071</td>
<td>F-P.dam-1</td>
<td>CACAGGGGCTCTGGAATATG</td>
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<td>subsp. <em>damselae</em></td>
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<td>R-P.dam-2</td>
<td>GCTCCAGCTCAATTTCGTC</td>
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<td></td>
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<td></td>
<td>P-P.dam-ureC</td>
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<tr>
<td><em>dly</em></td>
<td>L.16584 (26)</td>
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<td>R-P.dam-4</td>
<td>CGTCCGATGGAAATGCCTTG</td>
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<td></td>
<td></td>
<td></td>
<td>P-P.dam-dly</td>
<td>GTCAATATGGAAGCAATTTG</td>
<td>65</td>
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<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>gyrB</td>
<td>AF007287 (42)</td>
<td>F-V.par-1</td>
<td>GCTAAGCAGGGGTCCGTAATCG</td>
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<td>145</td>
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<td></td>
<td></td>
<td>R-V.par-2</td>
<td>GACCGATACACAGCCGCAAGT</td>
<td>60</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P-V.par-gyrB</td>
<td>CCGAAGAAGTTGCAAGCTTTAC</td>
<td>65</td>
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<td><em>toxR</em></td>
<td>L.11929 (27)</td>
<td></td>
<td>F-V.par-3</td>
<td>CTGGAATCCCCAGCGTTATT</td>
<td>60</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R-V.par-4</td>
<td>TGATTGCGGGTGATTTAC</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-V.par-toxR</td>
<td>ATTCGATCTCCGCAGATGCTG</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>cya</td>
<td>MD4670 (44)</td>
<td>F.V.vul-1</td>
<td>TTTCAATCCGAGGCGTTAG</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R-V.vul-2</td>
<td>ATCAATACCAGCCGACTGC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P-V.vul-cya</td>
<td>CCAAAGGCTGAGATCTTATACCC</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

* Genetic locus targeted by the described PCR primers and probes: *cya*, cytolysine; *gyrB*, gyrase B; *ureC*, urease C; *dly*, phospholipase D; A.plas., *A. salmonicida* plasmid.
FIG. 1. Positive control hybridizations. (Upper left panel) Specificity for *L. anguillarum* with the multiplex PCR and microarray hybridization. Genotypes for R-82 (O1), ATCC 43305 (O1), and ATCC 43306 (O2) match the respective genotypes for *rpoN* and *fatA*. (Upper right panel and lower left panel) Hybridization with probes complementary to *V. vulnificus* (cyr), *V. parahaemolyticus* (*gyrB* and *toxR*), *P. damselae* (*ureC* and *dly*), and *A. salmonicida* (*vapA* and *A.plas.*) in four different PCRs. (Lower right panel) Positions of oligonucleotide probes and the biotin control on the microarray.
A. 20% (398 of 2065) of the ORFs did not appear to be significantly expressed under either growth condition.

B. Of the remaining 1,667 ORFs, the expression of 125 of them (8%) differed by more than fivefold between the two cultures, and 82 of the 125 (65%) appear to be part of operons, indicating extensive coordinate regulation.

FIG. 1. Fluorescence intensities of DNA microarrays. (A) cDNA vs cDNA derived from two independent cultures of cells grown with peptides as the carbon source. (B) cDNA versus cDNA derived from two independent cultures of cells grown with peptides or maltose as the carbon source. In panel A, the upper and lower diagonal line pairs indicate twofold and fivefold changes in the signal intensities, respectively, while only the lines indicating fivefold changes are given in panel B. See text for details.
Overview

- DNA microarray hybridization
- DNA Fabrication
  - In situ synthesis using masks
    - Procedure
    - Advantage and drawback
  - Spotted microarray
    - Contact printing
      - Advantage and drawback
    - Noncontact ink jet printing
      - Advantage and drawback.

- Application and analysis
  - Fish pathogens
  - Whole-genome wide microarray of archaea