Microarray - Part 2

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BII
What is a DNA microarray?

An array that contains large number of DNA molecules spotted on modified glass slides, nylon membranes or silicon wafers.

Old: 1 gene, 1 experiment
New: 1000 genes, 1 experiment!

What’s similar between microarray and a radio?
Array types

- Complementary DNA RNA -> DNA
- Oligonucleotide based (~60 mer)
cDNA Arrays

- Extract mRNA (from cell line or tissue)
- Generate a labeled sample (target)
- Hybridize in parallel to DNA sequences
- Detection
- Analysis
- Data management
Oligo arrays

- 20-25 mer oligos synthesized in situ
- Technology: photolithography, ink-jet
- 95% efficiency at each step
- Alternative: Print presynthesized oligos on glass
Microarray variants

Antibody M.
Application: Protein Regulation >> Degradation, Phosphorylation, Dephosphorylation

Antigen M.
Application: SLE, Rheumatoid Arthritis, MS

BAC M.
Application: High resolution, High throughput Genomic Profiling

Bead M.
Application: High surface to volume ratio
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Microarray variants

Proteome arrays
Protein-protein interaction chips
RNA chips
SNP chips
Suspension arrays
Synthetic DNA arrays
Tiling arrays
Tissue microarrays
Tox chips
Transcript microarrays
Universal microarrays
Cy3 / Cy5

Normalised Cy Dye emission

Fluorescence emission

Wavelength (nm)
An overview

From experiment to analysis

**GREEN** represents **Control** DNA

**RED** represents **Sample** DNA

**YELLOW** represents an equal combination of **Control & Sample** DNA

**BLACK** represents areas where neither the **Control nor Sample DNA** hybridized to the target DNA.
The Making of Microarrays
Another view

1. Many bits of single-stranded DNA corresponding to specific genes are arrayed on a small glass or plastic slide on a grid.

2. Messenger RNA (mRNA) is extracted from the sample, converted to complementary DNA (cDNA) to make it easier to work with, and a fluorescent label is added. (mRNA is the molecule produced when a gene is turned on.)

3. The fluorescently tagged cDNA sample is applied to the microarray. If the sample contains a sequence that matches the single-stranded DNA on the chip, those complementary strands will bind. The resulting fluorescent signal corresponds to the quantity of that gene in the sample.

4. The array is scanned to measure the amount of fluorescent label, and a picture is generated showing how much each gene on the chip was expressed in the sample, relative to the other genes that were studied.
Microarray hardware

- Slides
- Spotter
- Photolithography
- Pin heads
- Scanner
- Computer analysis
- Affymetrix instrument system
Image analysis

Array Target Segmentation

Target detection

Background intensity extraction

Target intensity Extraction
Do-it-yourself

- Building arrays in-house
  
  **The MGuide. Version 2.0**
  
  Patrick Brown Lab (Stanford)

Probe deposition

**Pins**: Slotted, capillary tubes, piezoelectric ink jets, solid pins, pin-and-loop system.

Carrying capacity: 0.1-0.6 ul
Deposit/element: 0.3-1 nL

Total number of spots: 400 / loading

Confoundling factor: Evaporation
Parallel loading: 32 pins
Printing time: 1-2 spots/s/pin

**Gene Machine**: 32 pins, 34,000 element microarray 100 slides, 17 hours
Microarray plates

Best result

High background fluorescence

Irregular spot morphology

Comet tails

Low signal

Streaks
Yeast on microarray
Chip statistics

cDNA per spot: 2-10 ng of cDNA
Spot diameter: 150 µm - 600 µm
Distance between spots: 0.6 - 2 mm
Array Shelf life: 9 months
Optimum storage: 2 - 8°C
Total time scale: ~ 3 days
  Sample preparation: ~ 2 days
  Hybridization: ~ O.N. (16 hours)
  Washing and staining: ~ 1 hour
  Scan: ~ 10 minutes
FAQs

Q How reliable are microarray results?
A :( Error rate 30% -50%

Q Can microarrays be re-used?
A Nylon ?

Q How much RNA is needed to process the sample?
A 10 µg total RNA (X2)

Q How many sample we must run to generate useable data?
A As many as possible
FAQs

Q. Should I prehybridize the slides?
A. No.

Q. What is the volume of probe required when using the chamber?
A. ~ 1.8 ml / hybridization

Q. What types of fluorescent dyes can be used?
A. Cy3, Cy5, Alexa, Bodipy, FITC, Texas Red.

Q. Can fluorescent microscopes be used for detection?
A. Yes, but…?
Q. How are signals normalized?

Q. When comparing differences in expression levels between two arrays, what value is considered significant?

Q. What results can I expect if I use RNA from a different species than the one for which the blot was made?

A: Unpredictable!
An ideal strategy

- 3 sets of replicate experiments
- 2 sets of slides for each expt.
- Duplicate scanning for each slide

Objective: to identify & remove noise
Future Advances

- Further automation in tracking sample from start to finish
- Pin and printer design - to improve spot uniformity, spot density, printing speed
- Improvement in microfluidics
- Upgraded multi-channel scanners
Question time

The following section will discuss possible scenarios that arise during experiments with microarrays.
Problem 1

A researcher is scanning a cDNA microarray and obtains an image with the following characteristics: a few spots are very bright but many spots are not visible. A colleague suggests that increasing the PMT intensity to visualize missing spots. Describe what happens if the PMT gain is increased. Is it true that many spots currently not visible might become visible? Should this be done?
Problem 2

You collect mRNA from cells from a 30-year old patient and from an unaffected 30 year old person. You label the patients’ mRNA with red fluorescence tags and the normal mRNAs with green fluorescence tags. You mix the two labeled mRNAs and hybridize them on a microarray. You find a significant similarity between patients’ gene expression profile and the profile of the unaffected 30-year old person. However, a small subset of genes that cluster in this analysis also cluster when the patients’ mRNA is compared to an unaffected 80-year old person's mRNA. What conclusion can you derive from such an experiment?
Problem 3

While studying the developmentally regulated pathway in C. elegans, a graduate student obtained following results at different time intervals. Which gene(s) do you think are developmentally regulated? Why?
Problem 4

Devise an experiment that can help infer the function of an unknown gene
Problem 5

Assuming that this microarray plate shows results from an experiment at a single time point, what would be your most likely conclusion? Can you identify co-regulated genes from this picture?
Suggested Reading

• Cell 2000: 102, 9-15
• Nature Reviews 2001: 2, 441-443
• Nature Cell Biol 2001: 3, E175-178
• Nature Reviews 2002: 3, 579-82
• TIG 2002: 18(8), 395-398