An Introduction to DNA microarrays
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What is a DNA Microarray?

- Genes or gene fragments attached to a substrate (glass)
- Tens of thousands of spots/genes = entire genome in 1 experiment
- A Revolution in Biology

Hybridized slide
Two dyes
Image analyzed
mRNA Analysis Methods

- **Northern Blot** (Single Gene analysis)
- **Microarray Technology** (Genome Wide Experiment)
NORTHERN BLOTS

- mRNAs separated on gel according to size
- mRNAs transferred to a membrane and hybridized with small number (1-5) of radioactively labeled DNA probes.
  - Probe corresponds to gene of interest
  - Target RNA is spatially fixed and the labeled probe is in solution
  - Low throughput
Number of Genes in Different Organisms

- Human ~ 30,000 genes
- Mouse ~ 30,000 genes
- Yeast ~ 6200 genes
- E. coli ~ 4200 genes
Northern Blots

Immobilized mRNA population hybridized with labeled probe representing one gene

DNA Microarrays

Immobilized probes hybridized with labeled mRNA population representing all expressed genes

We could just 35,000 Northern to monitor expression of all genes!!!
Need to achieve two things:

(i) Immobilize thousands of probes specific for individual genes

(ii) Label mRNA populations

Designing Oligo Probes

70 mer oligo specific to gene of interest
Number of Genes in Different Organisms

- Human ~ 30,000 genes
- Mouse ~ 30,000 genes
- Yeast ~ 6200 genes
- E. coli ~ 4200 genes
- Phage T4 ~ 200 genes
- Influenza ~ 12 genes

Introduction
Overview of fabrication of spotted microarrays

- Liquid Handling
- Resuspension of oligos
- Robotic Printing
- Spotted microarrays
Need to achieve two things:

(i) Immobilize thousands of probes specific for individual genes

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Introduction

cy3 and cy5: Commonly used dyes

Differential dye incorporation
cy5 less well than cy3
Light sensitivity: cy5 more easily degraded
Protect your reactions from light!!
## Introduction

Spotted microarray target preparation

### Direct labeling

<table>
<thead>
<tr>
<th>Target preparation</th>
<th>Spotted Microarray</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFP</td>
<td>EGFP KD</td>
</tr>
<tr>
<td>cy3 Reverse transcription Flourescent dyes</td>
<td>cy5</td>
</tr>
<tr>
<td>cDNA</td>
<td>cDNA</td>
</tr>
<tr>
<td>Combined in equal amounts</td>
<td></td>
</tr>
<tr>
<td>Co-hybridized to array</td>
<td></td>
</tr>
</tbody>
</table>

**EGFP KD**

Combined in equal amounts

- Yellow: $cy3 = cy5$
- Red: $cy5 > cy3$
- Green: $cy3 > cy5$
cDNA “Two Color Chips”

This is the kind of thing you will see in YOUR microarray experiments
What’s happening at each spot?

cDNA Chip vs. Northern Blot

A

hours: 0 2 5 6 7
DMC1
SPS1
hours: 0 2 5 6 7 9 11
DIT1
SPS100

B

hours: 0.5 2 5 7 9 11
DMC1
SPS1
DIT1
SPS100

fold repressed  fold induced
>20 10x 3x 3x 10x >20
Introduction

Two Popular Microarraying Platforms

Spotted microarrays
- cDNA: PCR products (500-1,000bp)
- synthesized oligos (70 mer)
- >10,000 probes

Commercially available Oligo microarray
- Affymetrix “Gene Chip”
- 500,000 probes
- 25 mer (represents a fragment of a gene)

So..what gene probes are represented on the array you will use??

EGFP, p53, EXO1, AAG, ATM, and ATR....

But we added in a bunch more!!
Human DNA Repair Genes
Richard D. Wood, Michael Mitchell, John Sgouros, Tomas Lindahl

Cellular DNA is subjected to continual attack, both by reactive species inside cells and by environmental agents. Toxic and mutagenic consequences are minimized by distinct pathways of repair, and 130 known human DNA repair genes are described here. Notable features presently include four enzymes that can remove uracil from DNA, seven recombination genes related to RAD51, and many recently discovered DNA polymerases that bypass damage, but only one system to remove the main DNA lesions induced by ultraviolet light. More human DNA repair genes will be found by comparison with model organisms and as common folds in three-dimensional protein structures are determined. Modulation of DNA repair should lead to clinical applications including improvement of radiotherapy and treatment with anticancer drugs and an advanced understanding of the cellular aging process.
RNA quality control
Pre-labeling quality control:

Determine RNA Quality
Agilent Bioanalyzer: 50-500 ng
No more formaldehyde gels!!

Gel Image (in silico)
Sharp, Clear Bands

Electropherogram (28S/18S Ratio~2)

Microarray Measurements
Image Analysis: Spotted arrays