

DNA Microarrays

Introduction

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Bio 210

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Admin

- Syllabus & class info web site posted
- Note: Link changed slightly
- <http://www.soe.ucsc.edu/~lowe/courses/MicroarrayW03/>

Reading Assigned

- “Comprehensive Identification of Cell Cycle-related Genes of the Yeast *Saccharomyces cerevisiae* by Microarray Hybridization”
Spellman, Sherlock, Zhang, Iyer, Anders, Eisen, Brown, Botstein, & Futcher *Molecular Biology of the Cell*, December 1998
- Review: Quackenbush, “Computational Analysis of Microarray Data”, *Nature Reviews Genetics* **2**: 418-27.

Terms

- Oligonucleotide = oligo = N' mer
 - A short piece of single-stranded DNA, usually less than 100 nucleotides (40 nt oligo = 40' mer)
 - Can be synthesized chemically (easily) without biological systems or enzymes
- PCR product – a piece of DNA, usually over 100 nucleotides, produced by an enzymatic reaction (polymerase chain reaction -- for more background, see chapter 15 of Current Protocols in Mol Bio)

Terms

- Probe – DNA immobilized on solid substrate (i.e. array surface)
 - Known sequence / gene
 - aka “spot”
- Target – Complex DNA or RNA mixture, in solution
 - Unknown composition

Several types of DNA arrays

- Spotted DNA arrays
 - Developed by Pat Brown's lab at Stanford
 - PCR products of full-length genes (>100nt)
- Affymetrix "GeneChips"
 - Photolithography technology from computer industry allows building many 25-mers
- Ink-jet / Bubble jet microarrays
 - a) Deposition of cDNAs / oligos pre-made
 - highly uniform spots
 - b) In situ synthesis – building 25-60mer oligos directly on array; very flexible, good for prototyping; expensive!

Basis: The Southern Blot

Basic DNA detection technique that has been used for over 30 years, known as Southern blots:

1. A “known” strand of DNA is deposited on a solid support (i.e. nitocellulose paper)
2. An “unknown” mixed bag of DNA is labelled (radioactive or flourescent)
3. “Unknown” DNA solution allowed to mix with known DNA (attached to nitro paper), then excess solution washed off
4. If a copy of “known” DNA occurs in “unknown” sample, it will adhere by base-pairing (hybridization), and labeled DNA will be detected on photographic film

Massive Increase in Measurements

- Most commonly, 5-50 samples can be tested in each traditional Southern experiment
- Affymetrix chips have >350,000 oligos per chip (multiple oligos per gene)
- Microarray “spotters” are high-precision robots with metal pins that dip into DNA solution & tap down on glass slide (pins work like a fountain pen)
 - Currently, ~48,000 different DNA spots can fit on one glass microscope slide

Pros/Cons of Different Technologies

Spotted Arrays

- relative cheap to make (~\$10 slide)
- flexible - spot anything you want
- Cheap so can repeat experiments many times
- highly variable spot deposition
- usually have to make your own
- Accuracy at extremes in range may be less

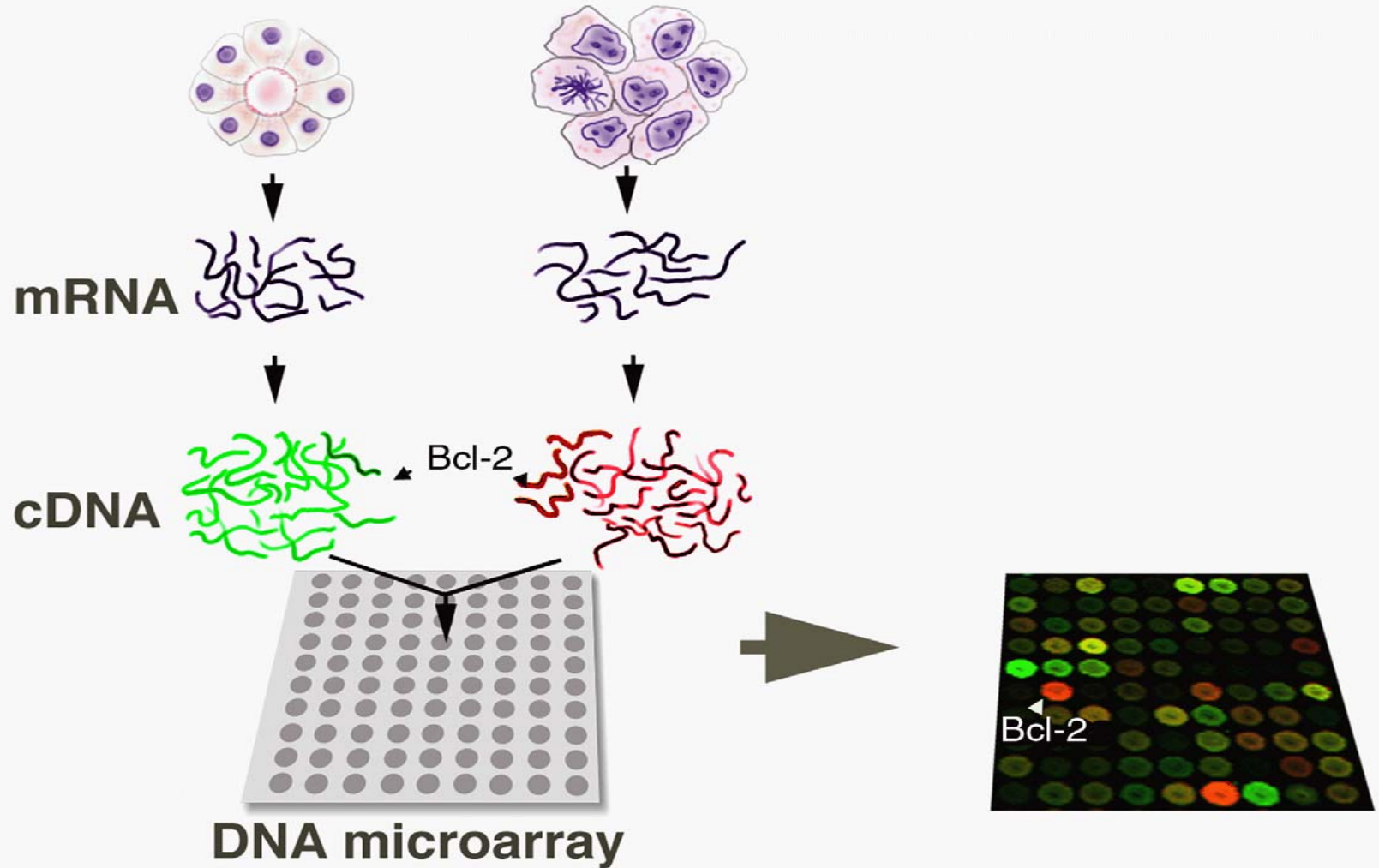
Affy Gene Chips

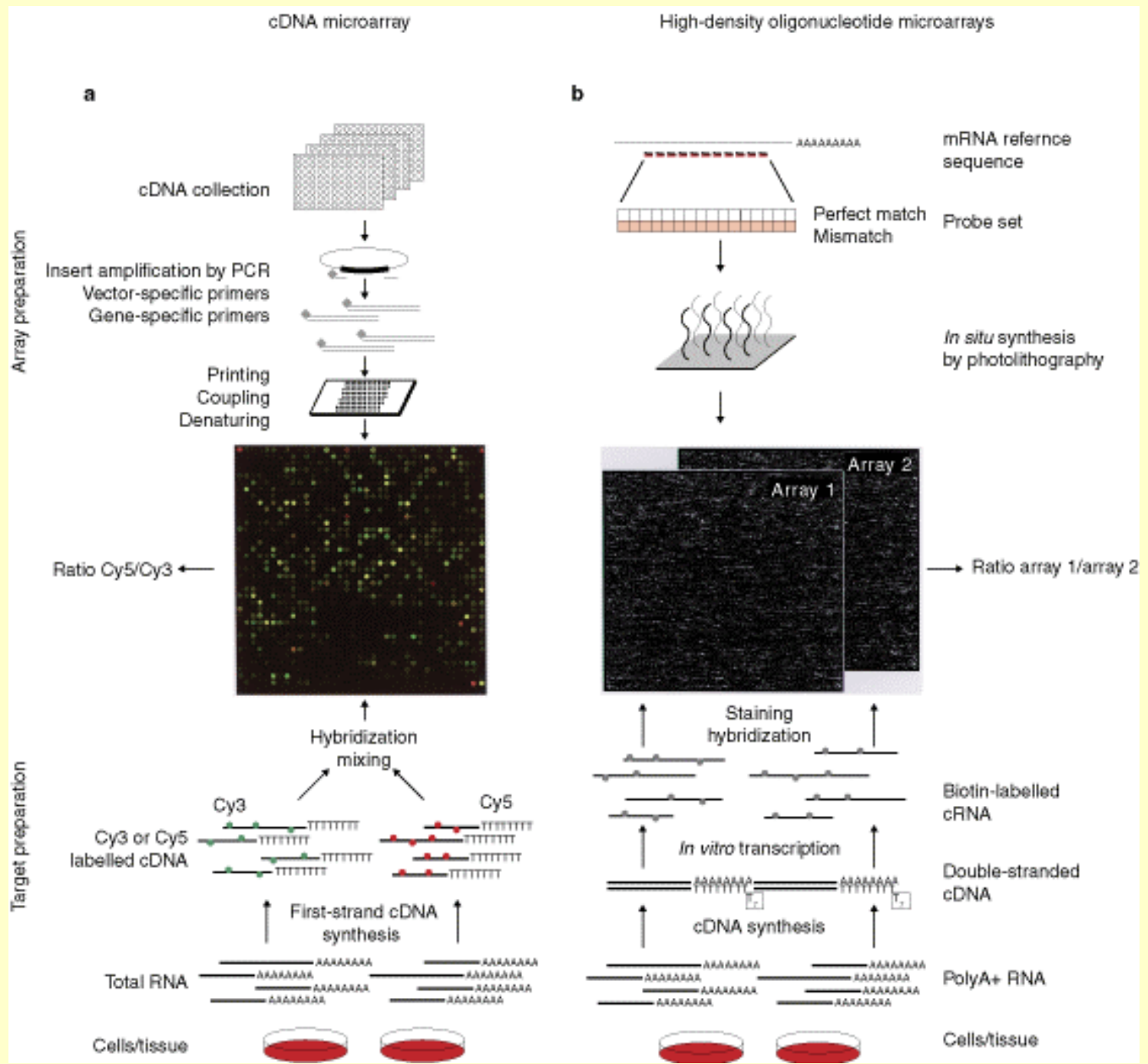
- expensive (\$500 or more)
- limited types avail, no chance of specialized chips
- fewer repeated experiments usually
- more uniform DNA features
- Can buy off the shelf
- Dynamic range may be slightly better

Types of Array Exp

- mRNA transcription analysis
 - Single experiment (control v. experimental)
 - Time course (multiple samples in same exp)
- Genomic DNA -- similarity of genomes
 - Genetic Footprinting
 - Species cross hybridization (existence of a specific pathway in a related species)
 - SNP's (single nucleotide polymorphisms)

mRNA Expression Profiling





Yeast Genome Expression Array

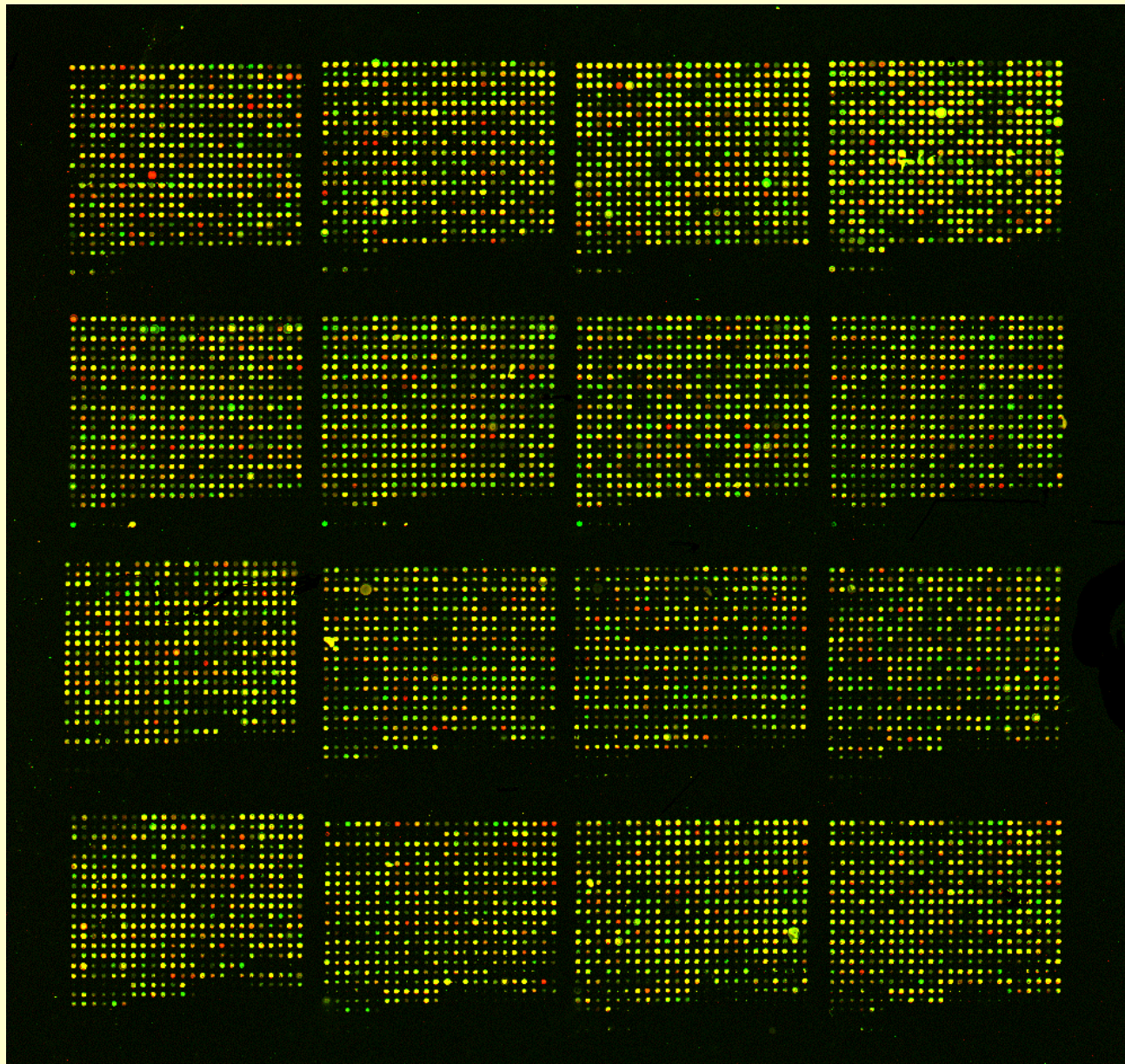
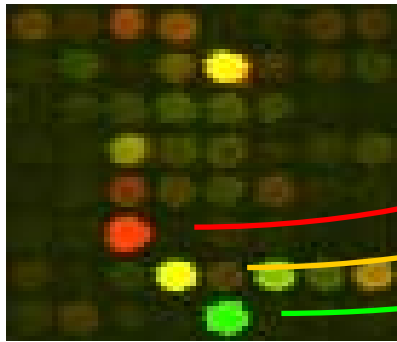


Image Analysis & Data Visualization



Cy3	Cy5	$\frac{\text{Cy5}}{\text{Cy3}}$	$\log_2 \left(\frac{\text{Cy5}}{\text{Cy3}} \right)$	
200	10000	50.00	5.64	Red
4800	4800	1.00	0.00	Black
9000	300	0.03	-4.91	Green

