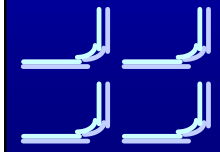
Microarrays in Three Easy Steps

Priti Hegde



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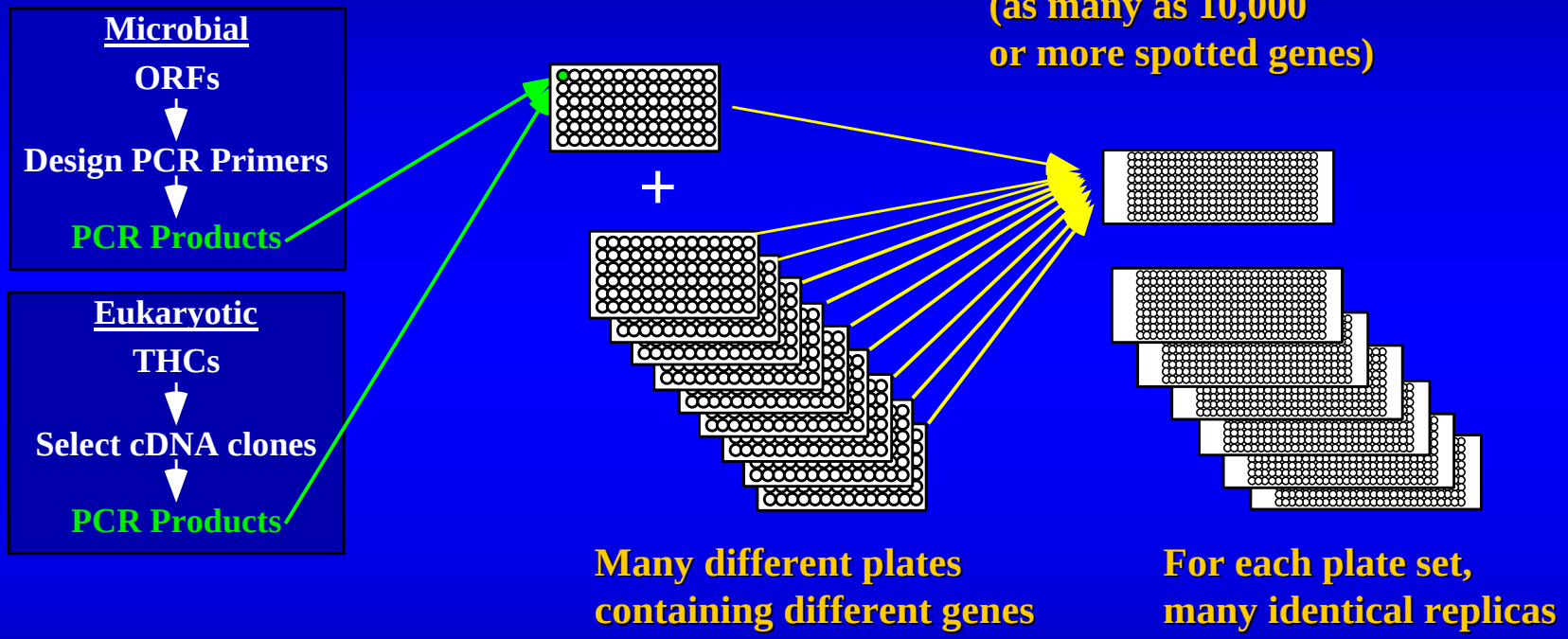
Microarray Analysis Stages

- Array Fabrication
- Probe Preparation and Hybridization
- Data Collection, Normalization, and Analysis

Array Fabrication



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The TIGR Gene Indices <http://www.tigr.org.tdb/tdb/tgi.html>

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Development of the TIGR "30k cDNA Gene Set"

Goal:

Array 30,000 genes and study gene expression in human cancer to develop stage and tissue specific expression fingerprints.

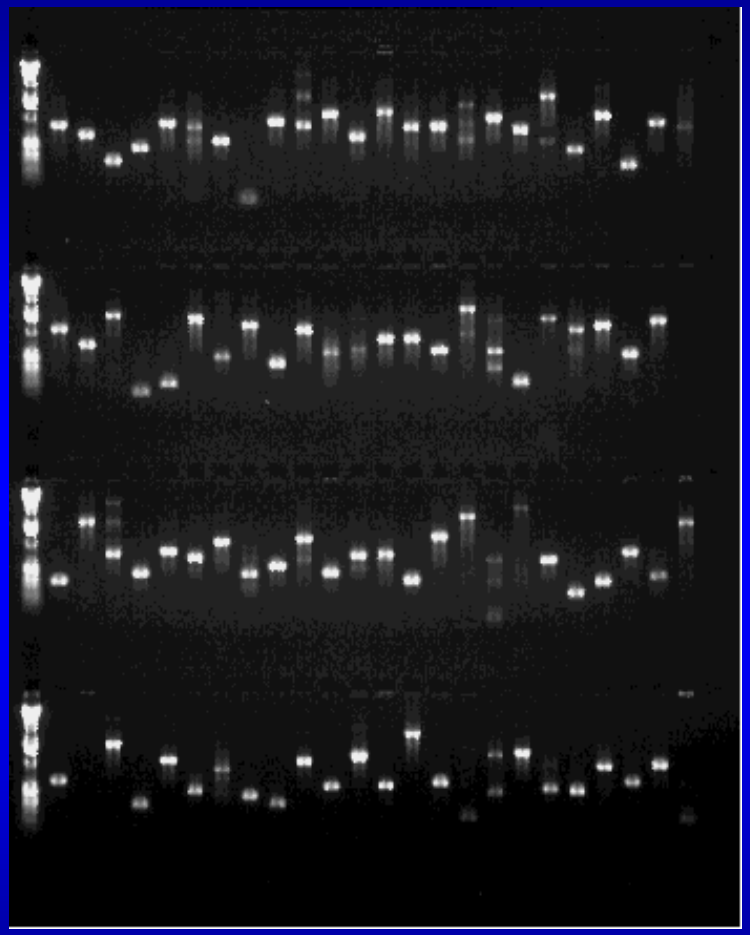
Progress:

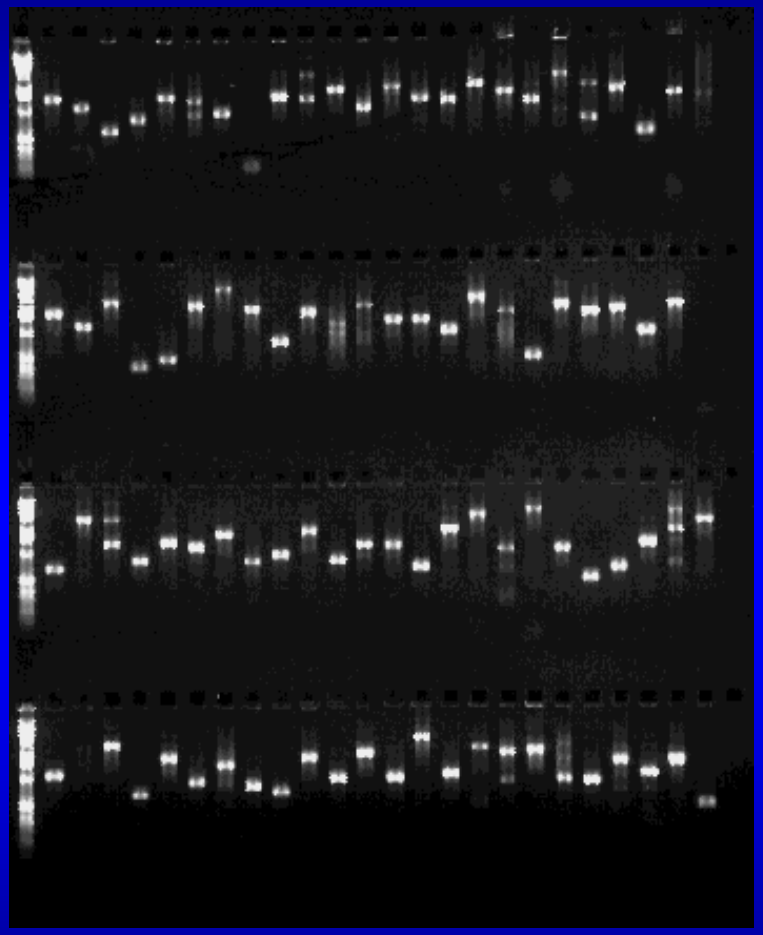
- Nearly 48,000 cDNA clones have been selected using the EST Assemblies (THCs) in the TIGR Human Gene Index*; 40,000 have been amplified by PCR and are ready for use in array studies. Funding has been secured to expand to 60,000 clones.
- Priority has been given to arraying known genes and genes with mapping information.
- Additional clones have been chosen representing genes of unknown function.
- Pilot studies are underway with 7,200 and 19,200 clone arrays.

*Human Gene Index: < http://www.tigr.org/hgi/hgi.html>

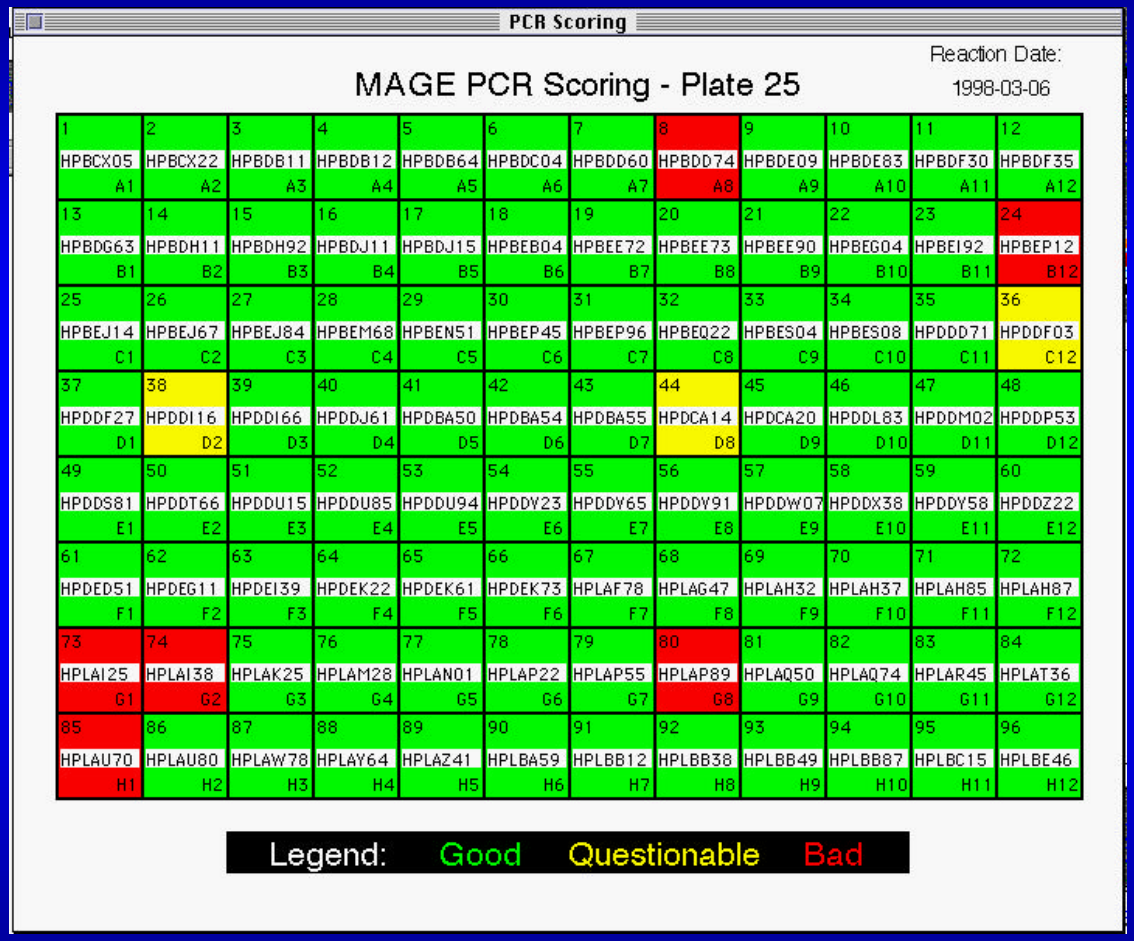
- **PCR Amplify Them**
  - Grow clones overnight
  - Dilute 1:20 (5ml:95ml) in water
  - "Pop" the cells, spin out debris
  - Amplify in 50 μl reaction with Platinum Taq (Life Technologies)
- **Purify the PCR products**
  - 96-well Millipore multiscreen glass filter plate
  - Bind products in high salt (1:5 5.3M Guanidine-HCl/150 mM KAc)
  - Elute in water/TE

PCR Amplification





Microarray PCR Scoring Tool



88% Good
6% Questionable
6% Bad

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The Beast: Microarray Robot from Intelligent Automation

- **Array The Clones**

<http://www.ias.com>

The Beast in Action: #1

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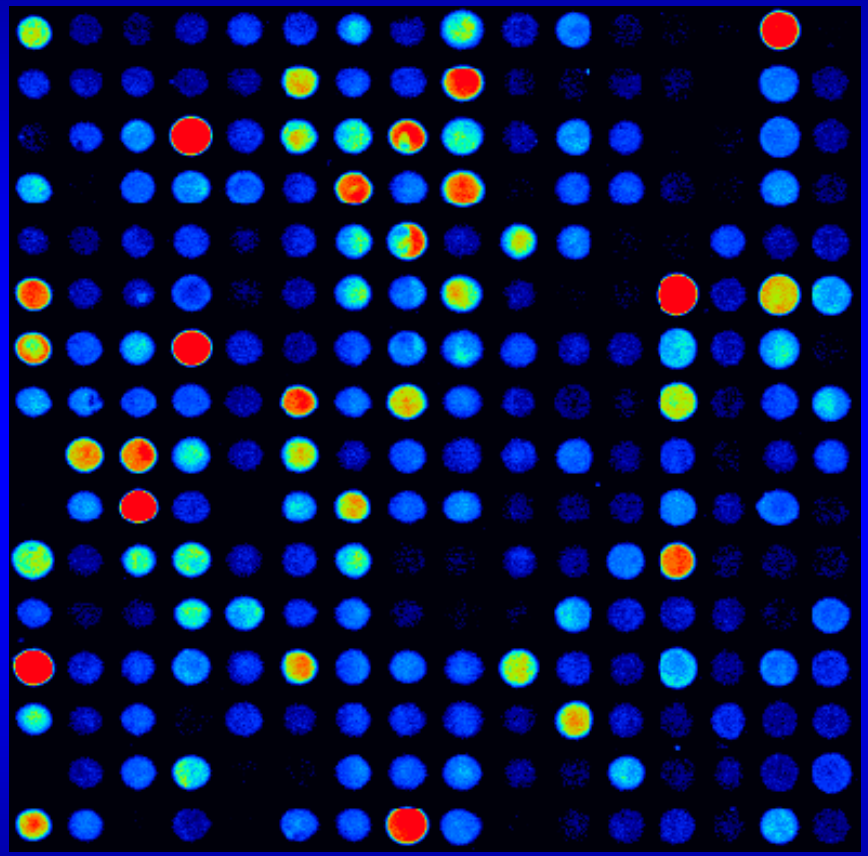
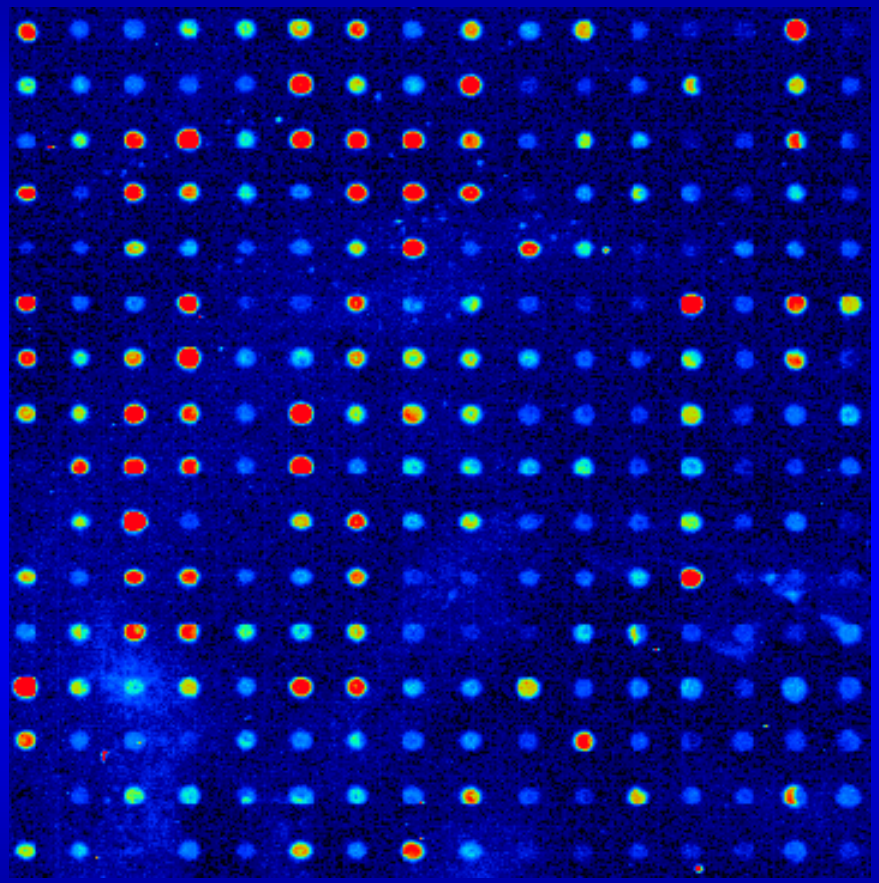
The Beast in Action: #2

- **The Glass is Crucial!**

Corning CMT-GAPS Slide



Another Slide



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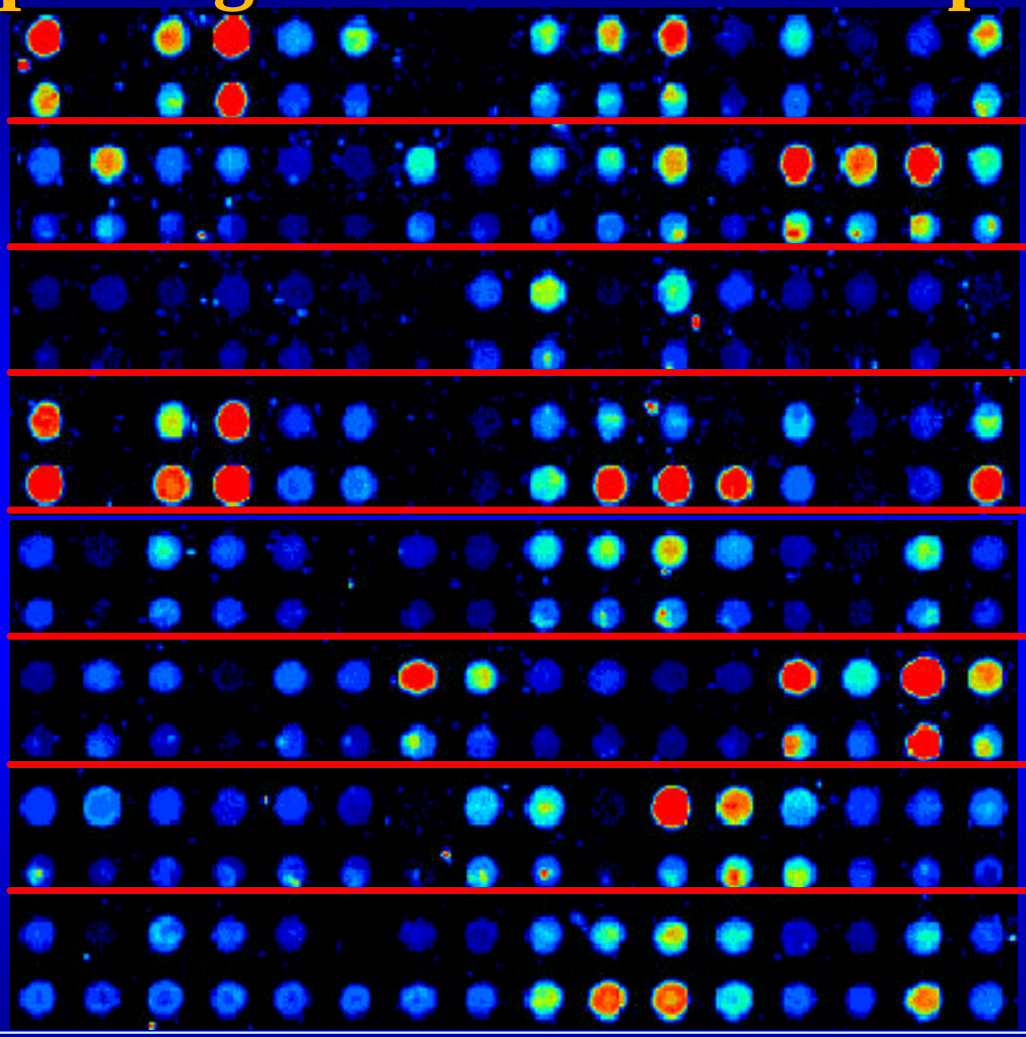
The Effects of Spotting Buffer and PCR Clean-up

- Spotting "Ink" and Clean-up matter



50% DMSO
3xSSC

50% DMSO
3xSSC

50% DMSO
3xSSC

Ethanol Precipitation/DMSO
Glass Filter Cleanup/DMSO

50% DMSO
3xSSC

50% DMSO
3xSSC

50% DMSO
3xSSC

Ethanol Precipitation/DMSO
Glass Filter Cleanup/DMSO

When Temperature and Humidity Go Bad

- **Laboratory Conditions Matter**

72°F (22.2°C)
40-45% Relative Humidity

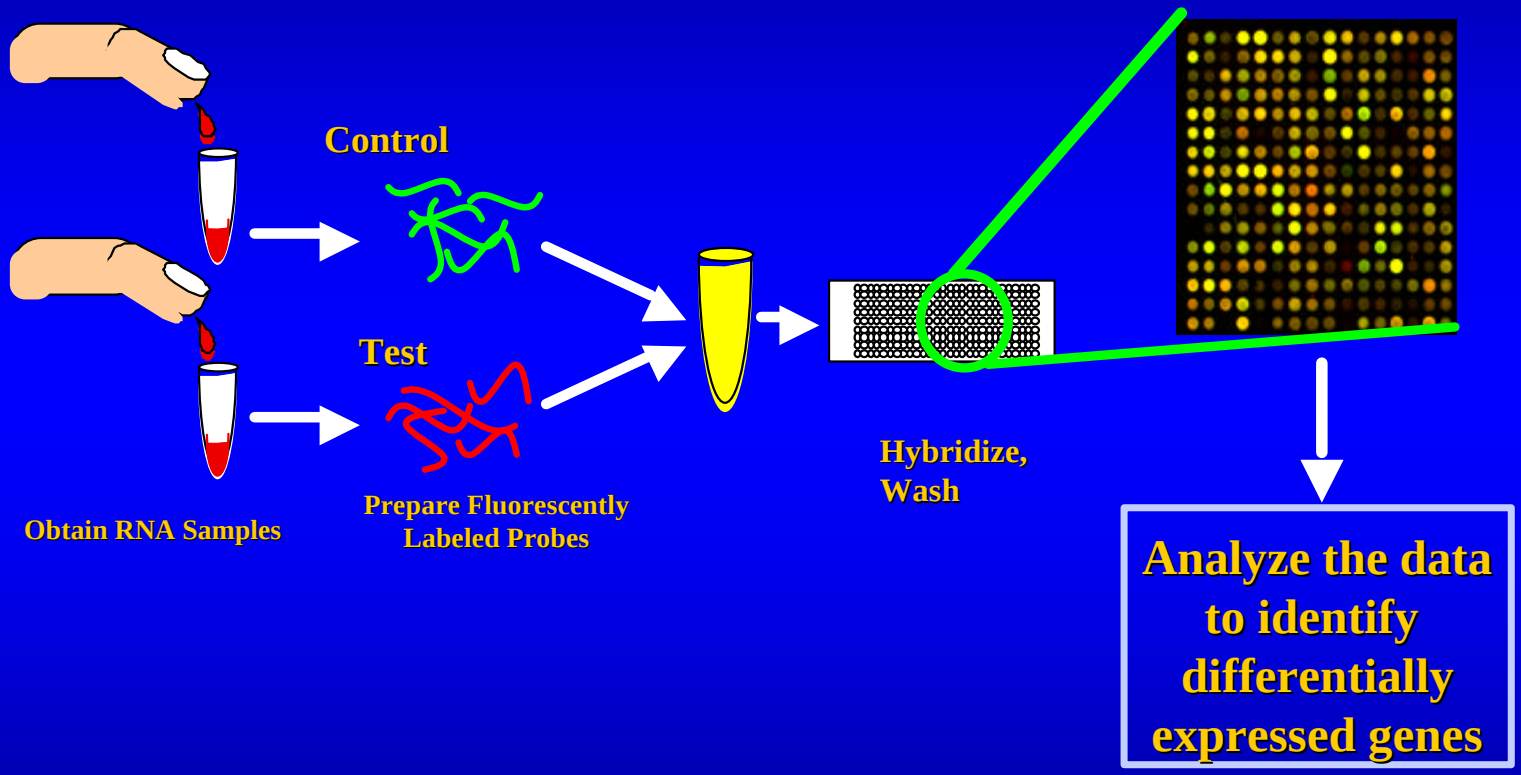
62°F (16.7°C)
40-45% Relative Humidity

80°F (26.7°C)
80-85% Relative Humidity

62°F (16.7°C)
40-45% Relative Humidity

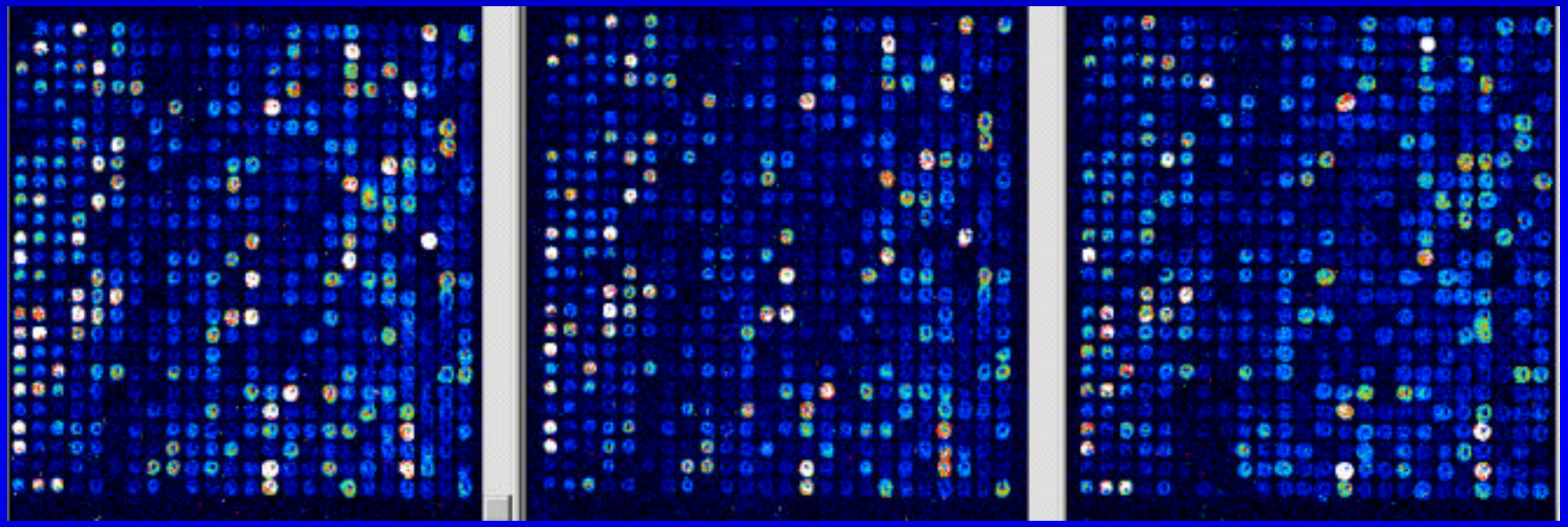
- **Arraying Conditions**
  - **72°F (22.2°C), 40-45% Relative Humidity**
  - **50% dimethyl-sulfoxide (DMSO), 20mM Tris HCl, 50mM KCl, pH 6.5**
    (Thanks to Robin Cline, Erik Snesrud, Karen Ketchum)
  - **Corning CMT-GAPS silane coated slides**
  - **UV Cross-link at 90 mJ, bake at 80 ° C for 2 hours**

Microarray Overview II



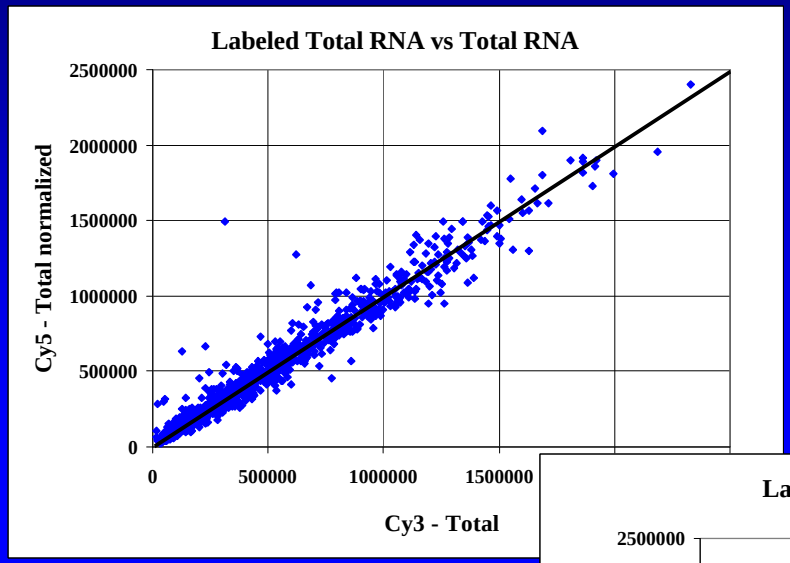
Comparison of Labeling Protocols

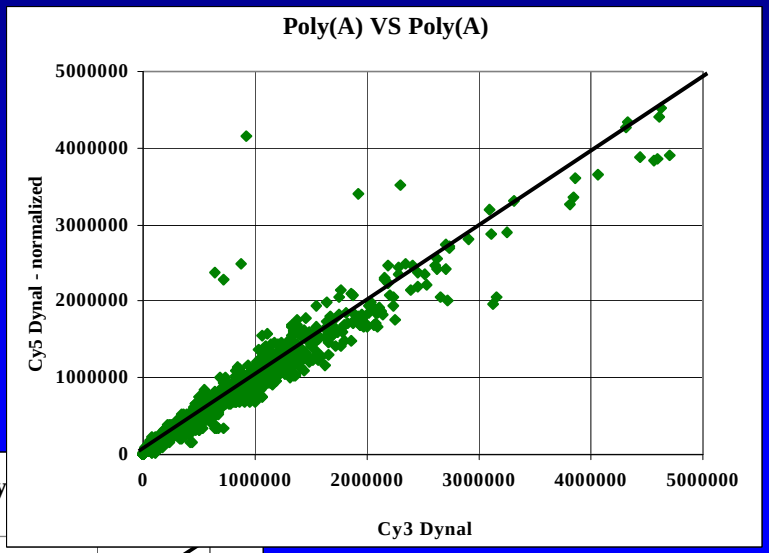
- **Preparing Labeled Probe**

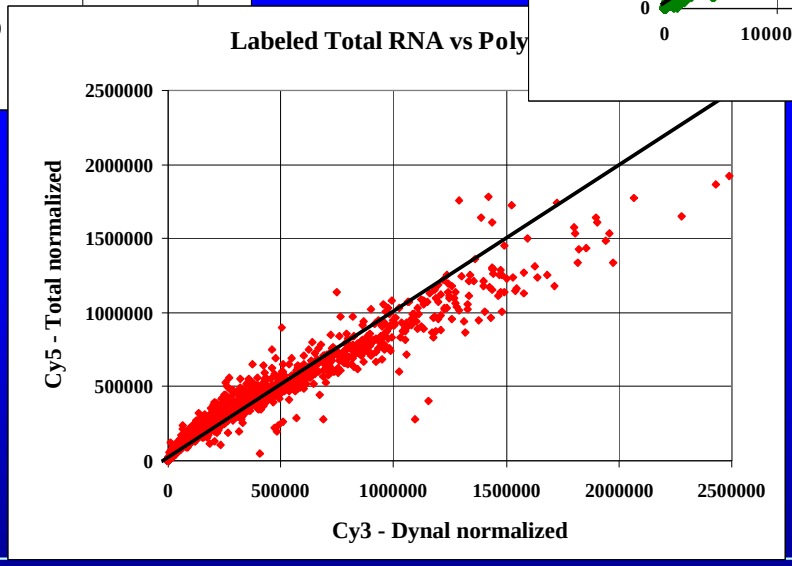


20µg total RNA **4µg total RNA** **~1.5µg Poly(A) RNA (Seradyne Beads)**

Total and Poly(A) RNA give equivalent results







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- **Labeling Protocols**
  - **Oligo(dT) primed labeling using SuperScript II (Life Technologies) and 4-8 mg total RNA**
  - **Clean Probes using Pharmacia GFX Columns**
- **Hybridization**
  - **Prehybridize slides with 5´SSC, 0.1% SDS and 1% BSA to block free amine groups**
  - **Combine probes with 10´SSC, 0.2% SDS, 50% formamide and hybridize at 42°C O/N**

Confocal Laser Scanner: ScanArray 3000

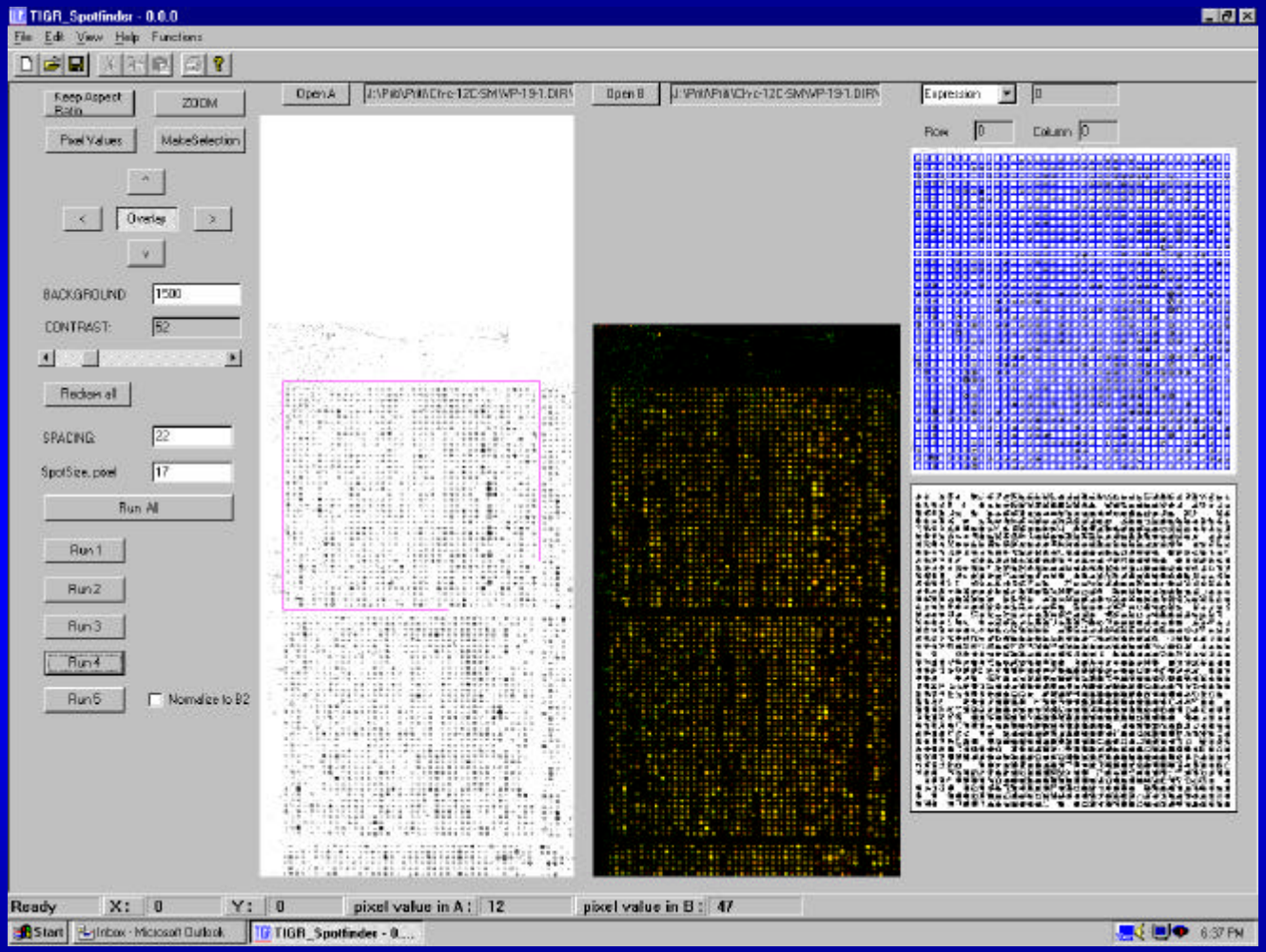
- **Data Collection, Normalization, and Analysis**

General Scanning <http://www.genscan.com>

Image Processing Issues

- Spot Finding
- Background Subtraction
- Reproducibility
- Measure - median *vs.* mean (integrated intensity)
- Quality measures

TIGR Spotfinder



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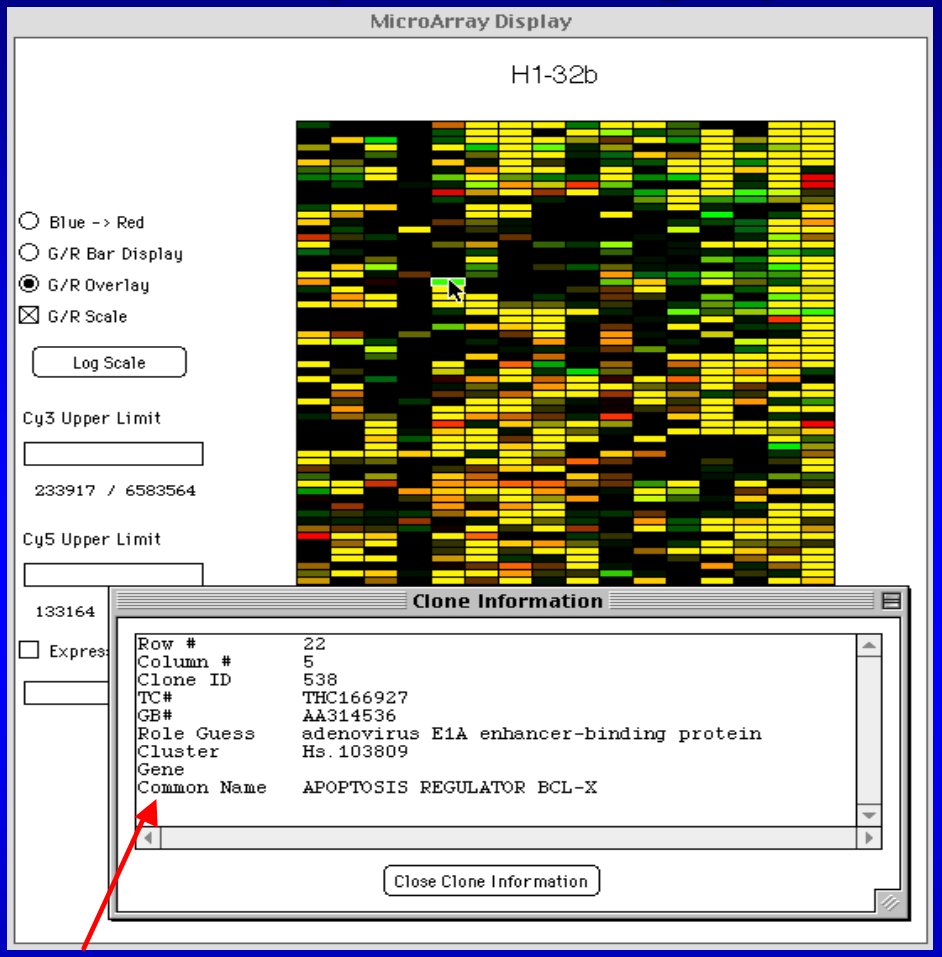
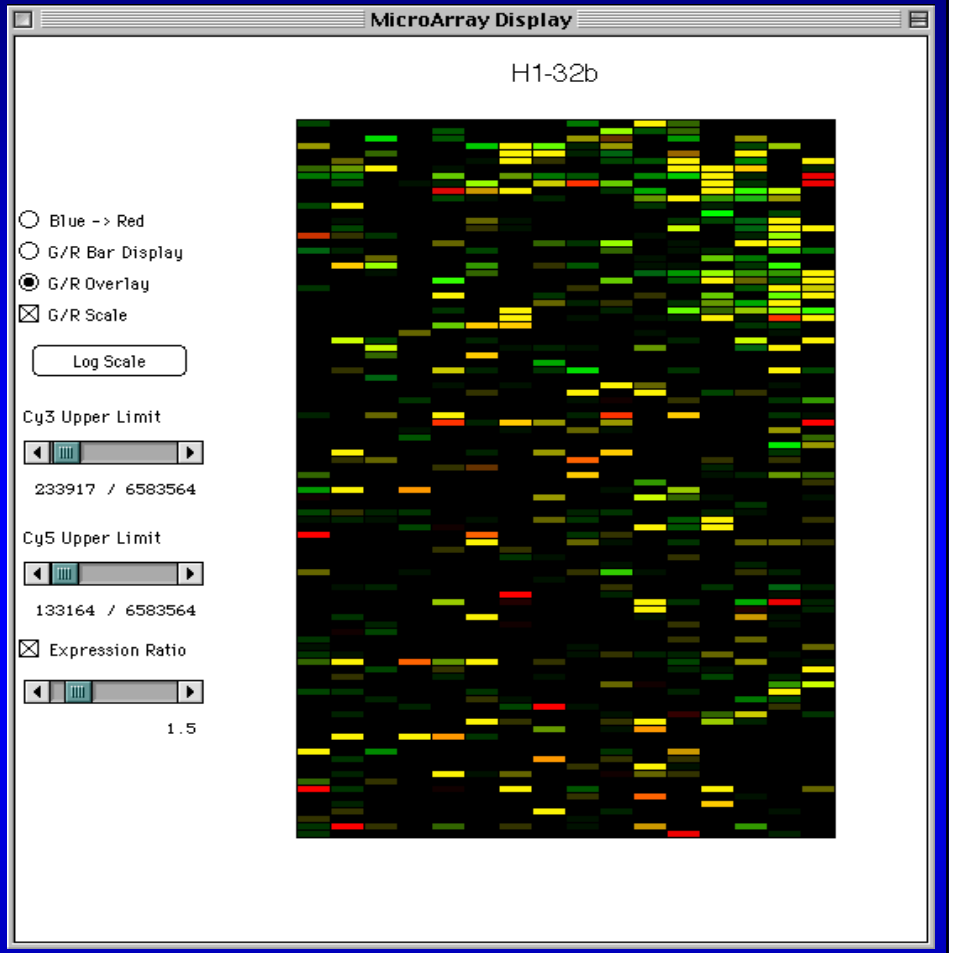
Data Analysis Issues

- Presentation
- Multiple Views
- Normalization
- Identification of Differentially Expressed Genes
- Multiple Experiments

Microarray Data Display Software



Software displays array data with links to database information about the underlying genes



Differentially expressed clones can be selectively displayed

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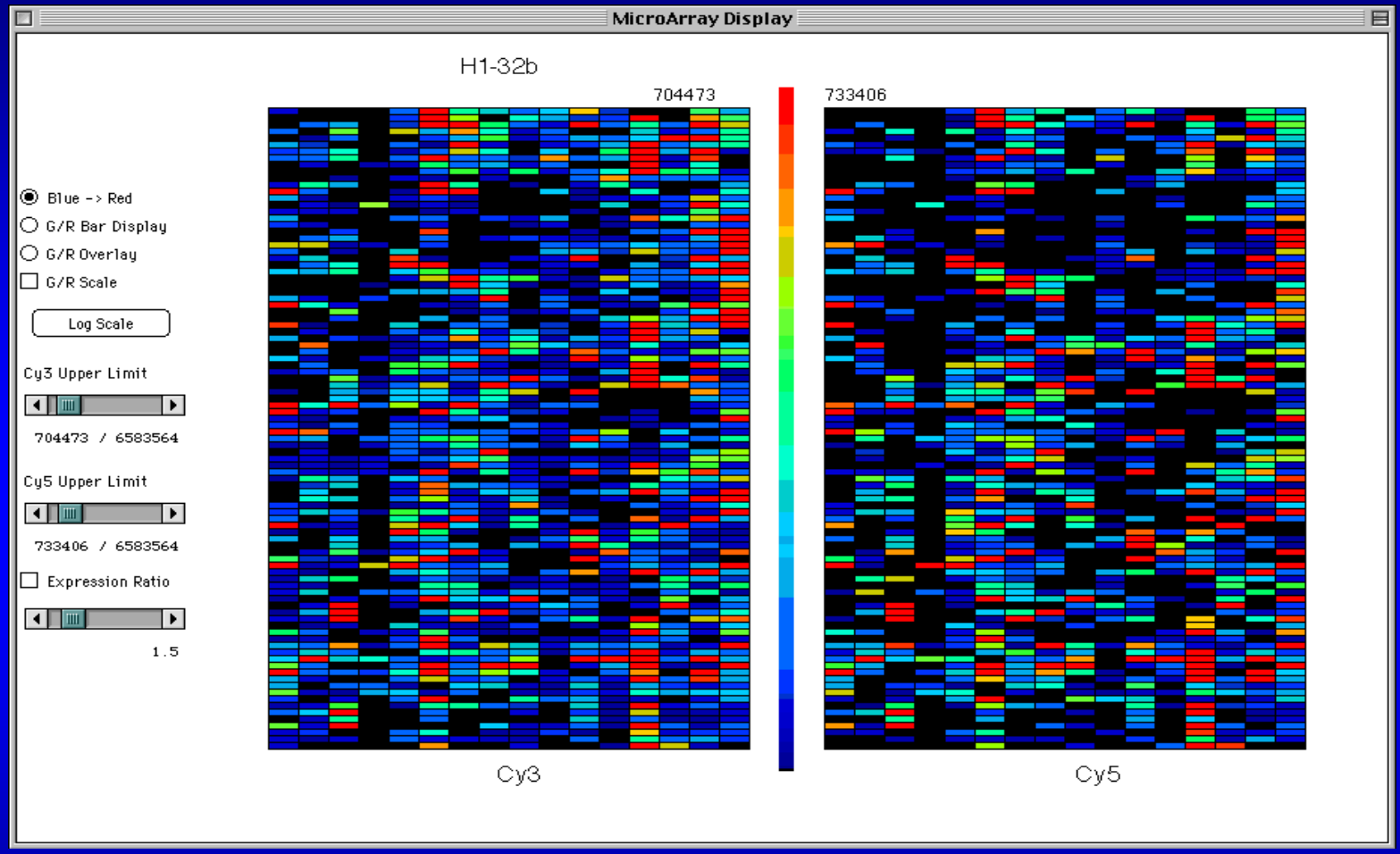
Microarray Data Display Software



Pseudo-false color display allows assessment of hybridization signal strength

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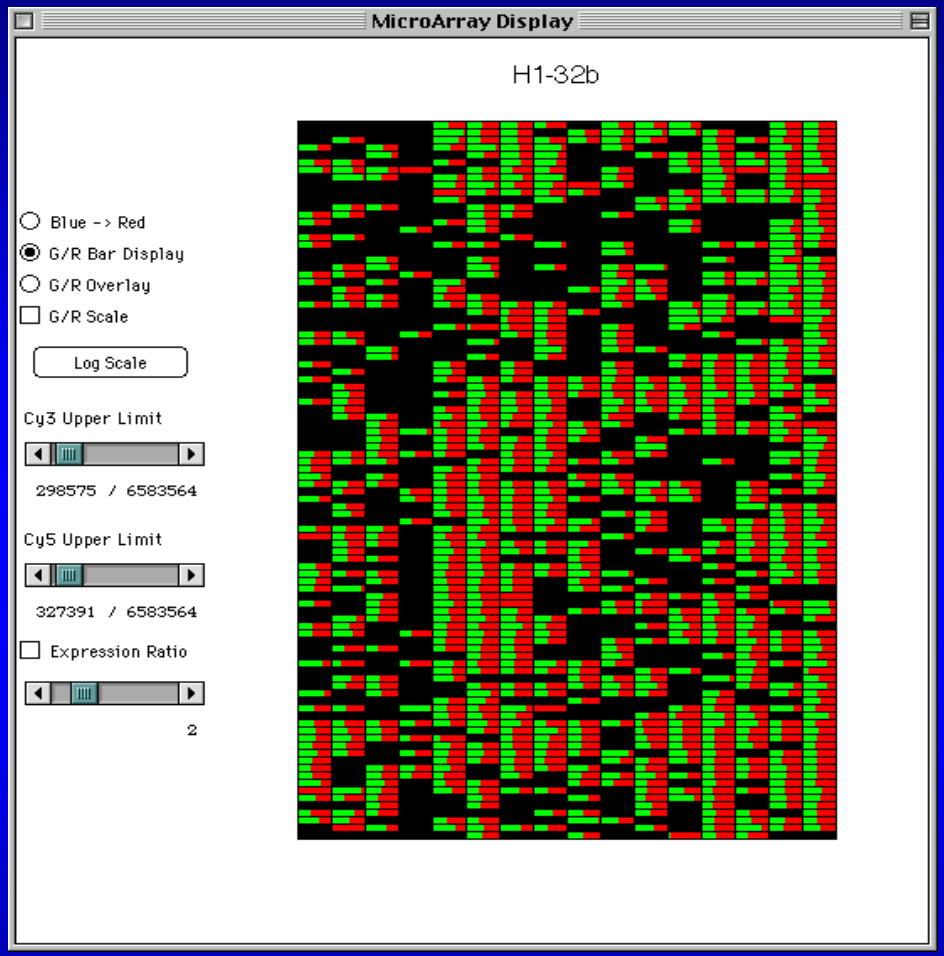
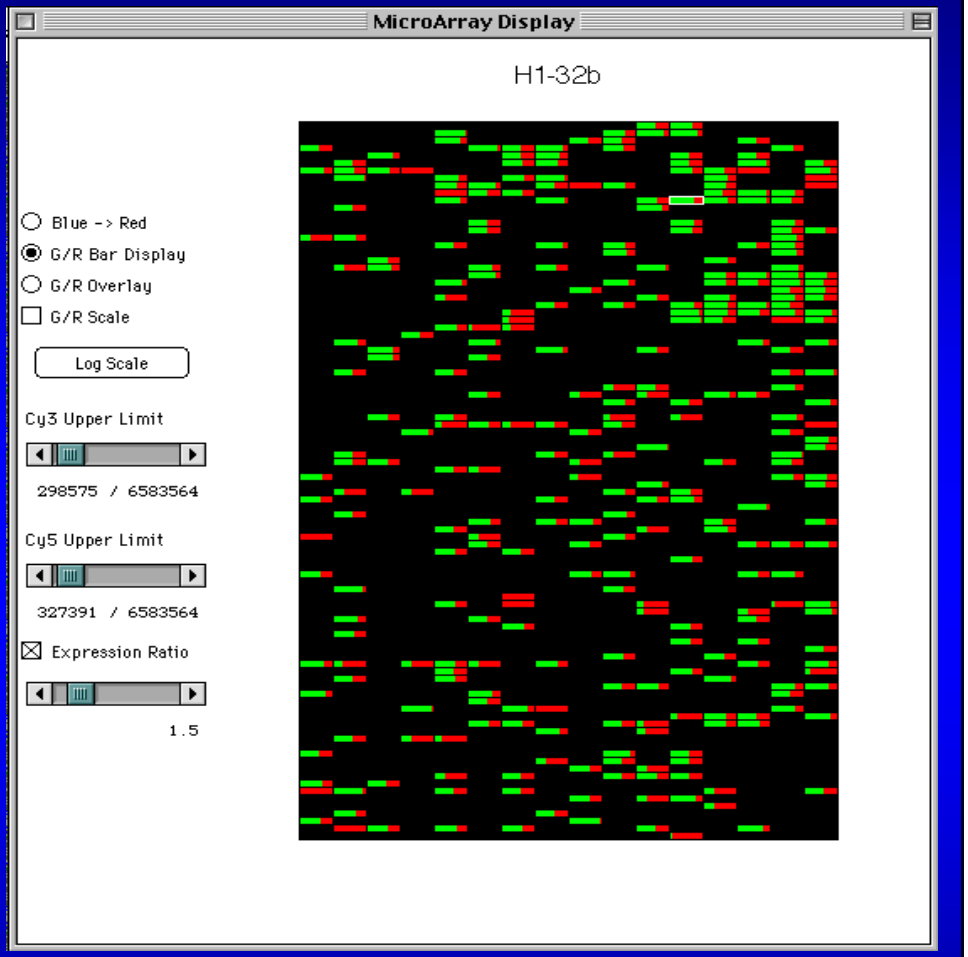
Microarray Data Display Software



Relative Red/Green areas represent relative expression levels



Setting expression ratios show only over/under expressed genes

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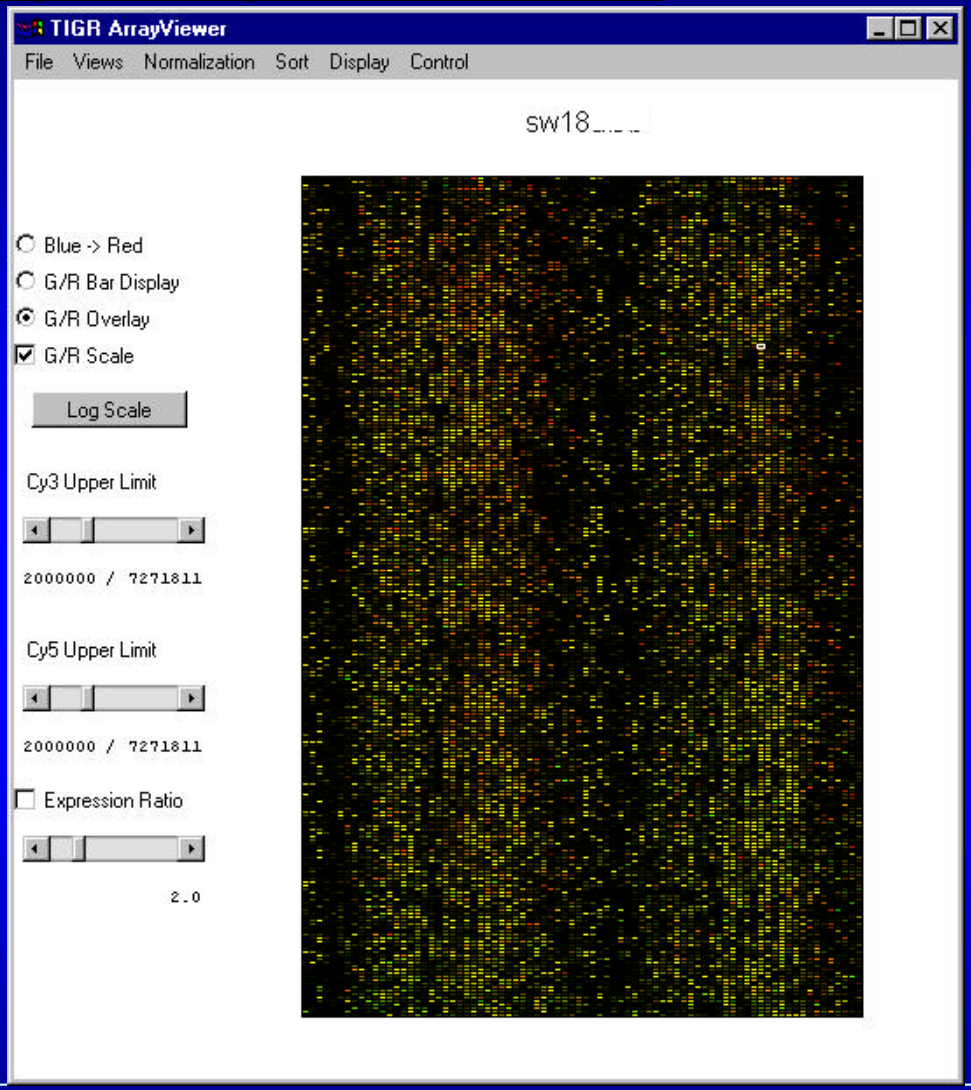
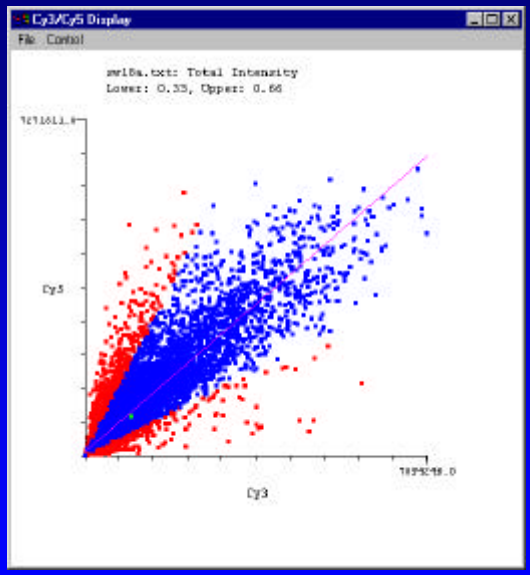
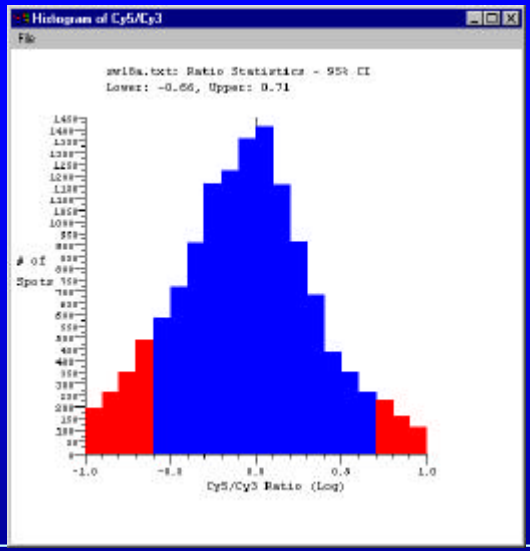
Data Display/Analysis Software







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Normalization Approaches

- Total Intensity
- Linear Regression
- Ratio statistics described by Chen *et al.*,

  J. Biomed. Optics (1997) 2(4) 364-374

Any of these using:

- Entire Data Set
- User-defined Data Set/Controls

Normalization Approaches (II)

Entire Data Set

- Probe Quantification less important
- No assumption on which genes constitute “housekeeping” set
- Uses all the data
- No independent confirmation

User-defined Data Set/Controls

- Requires definition of “housekeeping” set

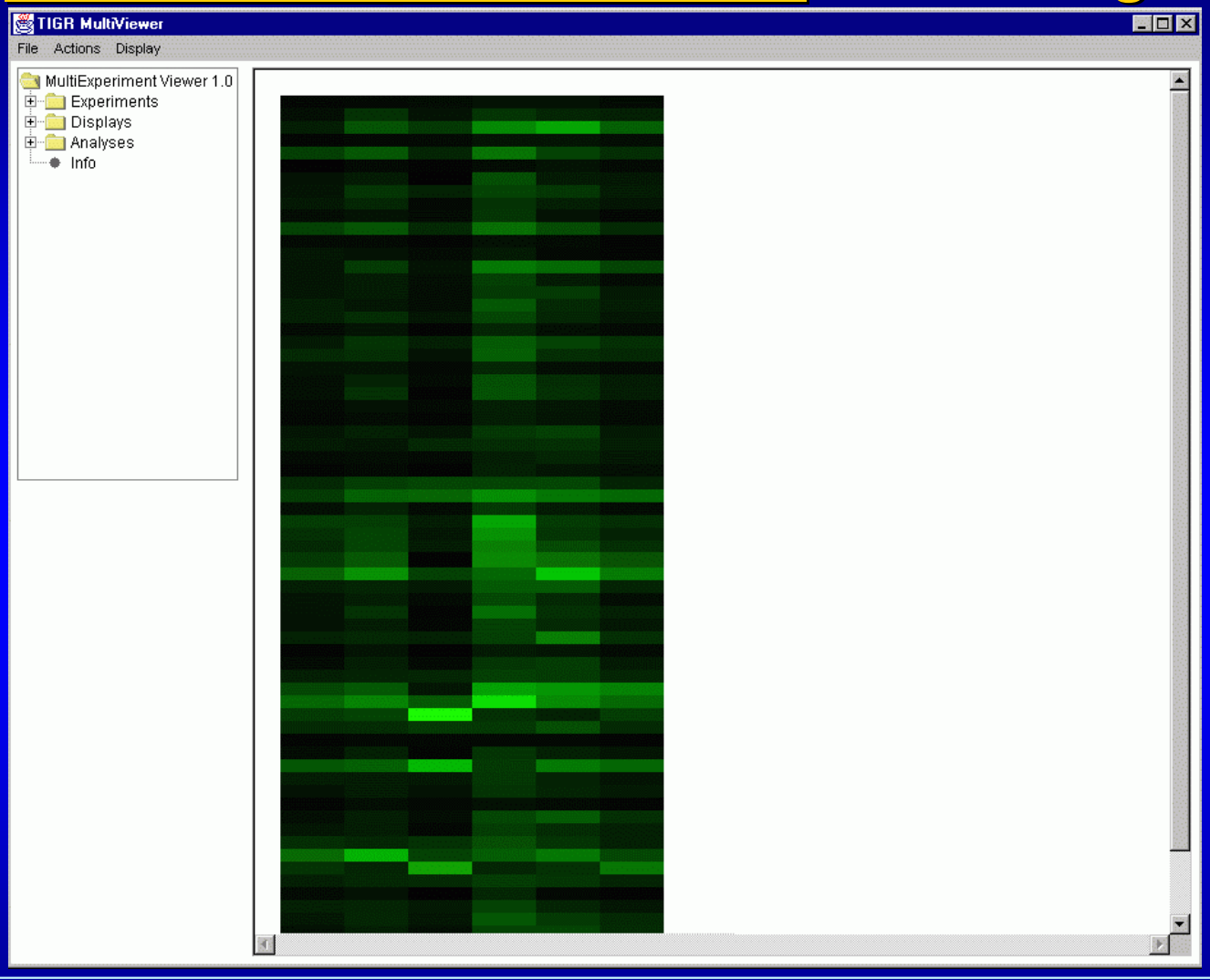
  or good added controls
- Requires good RNA quantitation
- Ignores much data

Normalization Approaches (III)

Solution(?)

- **Experiment dependent**
- **Use a combination of techniques**

TIGR MultiExpriment Viewer: Data Mining



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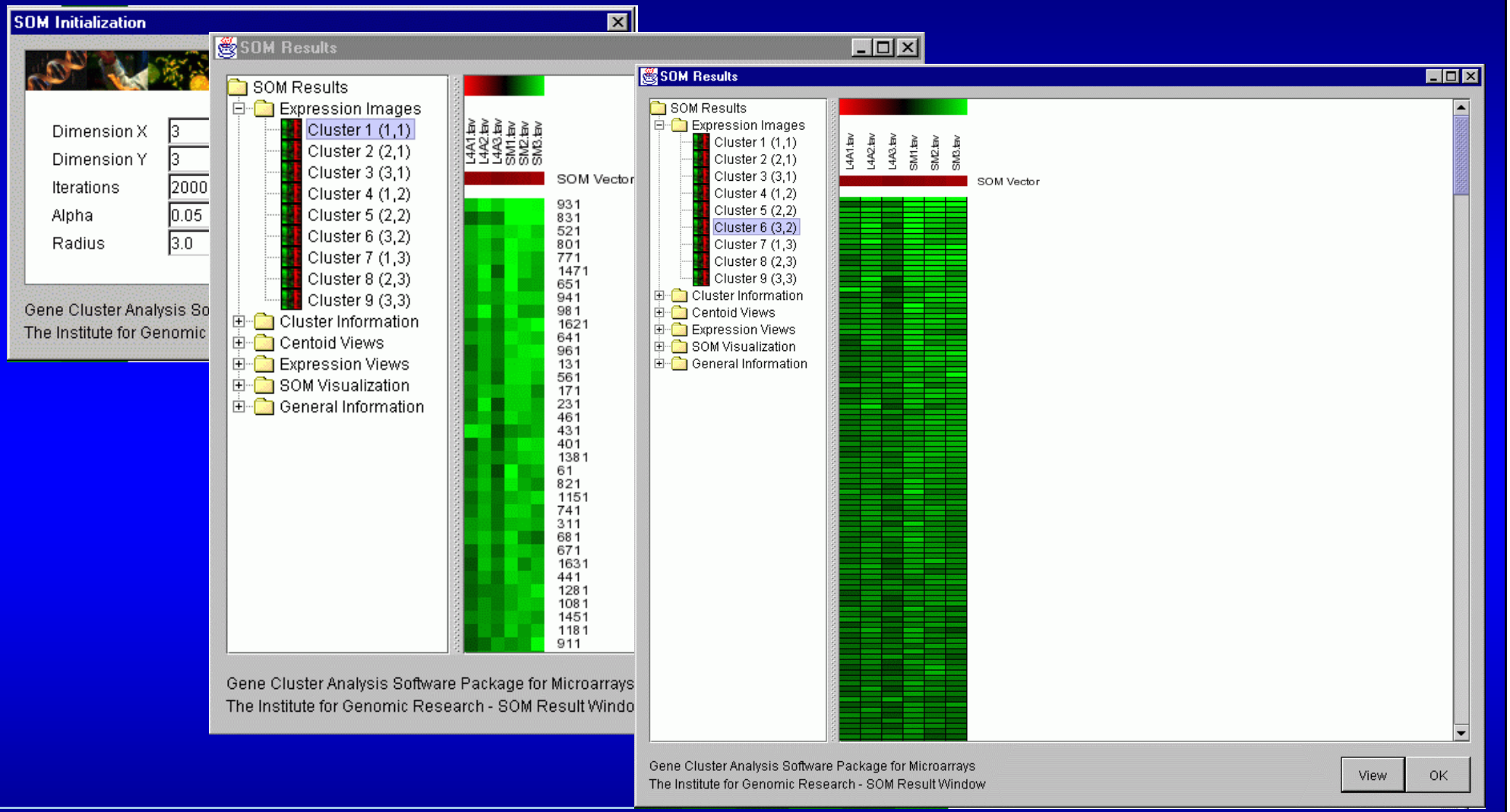
TIGR MultiExpriment Viewer: Self Organizing Maps



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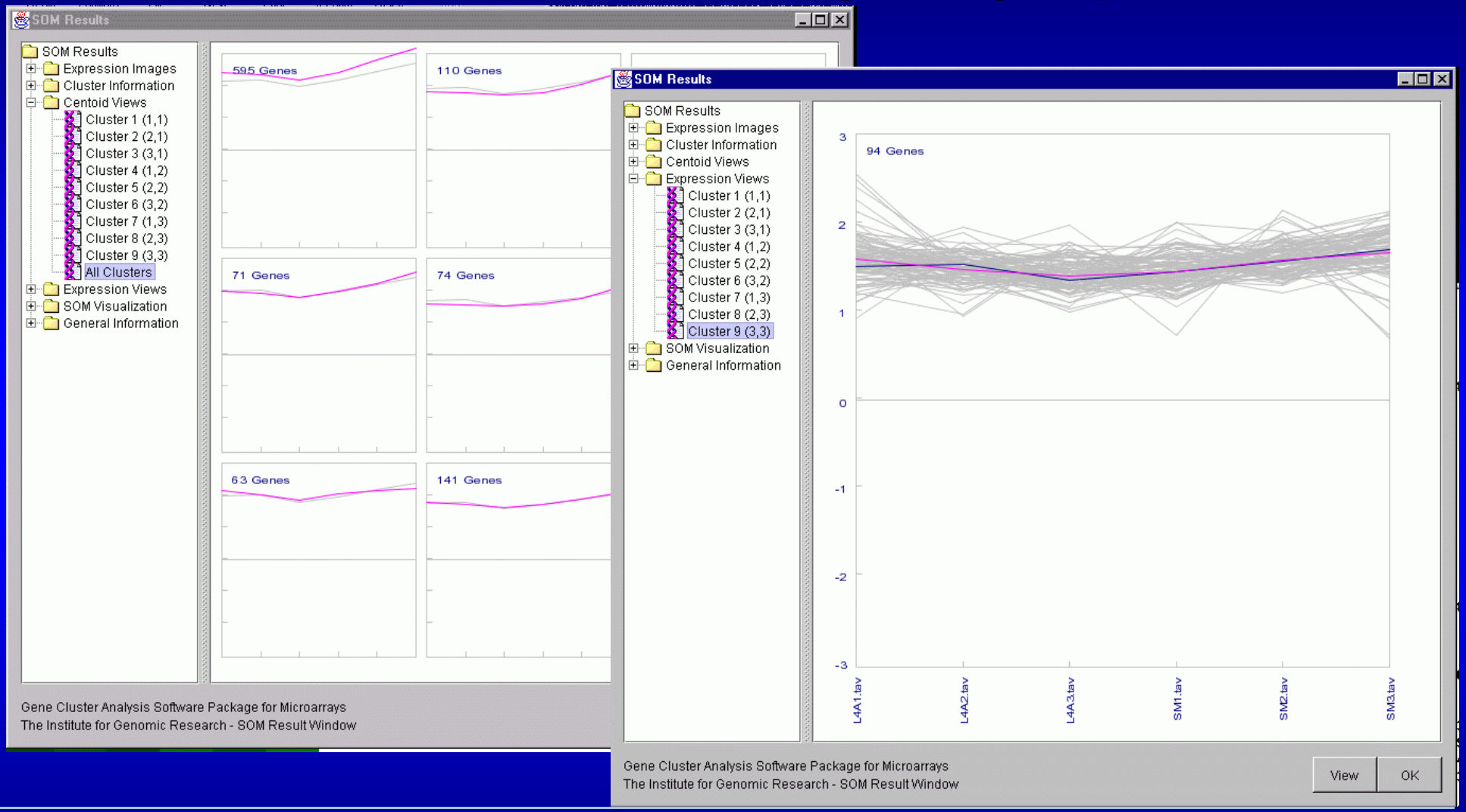
TIGR MultiExpriment Viewer: Self Organizing Map Views



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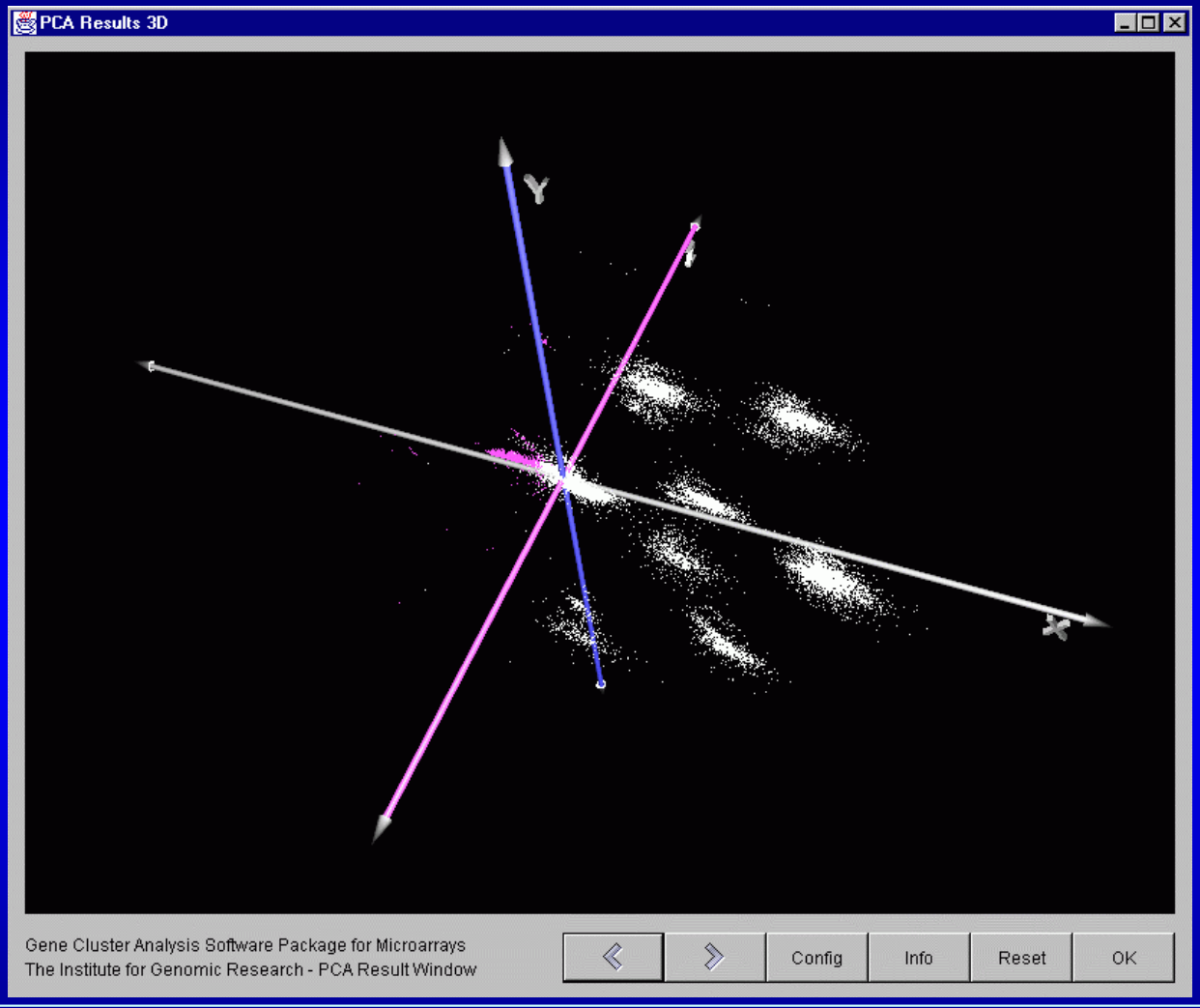
TIGR MultiExpriment Viewer: Principal Component Analysis



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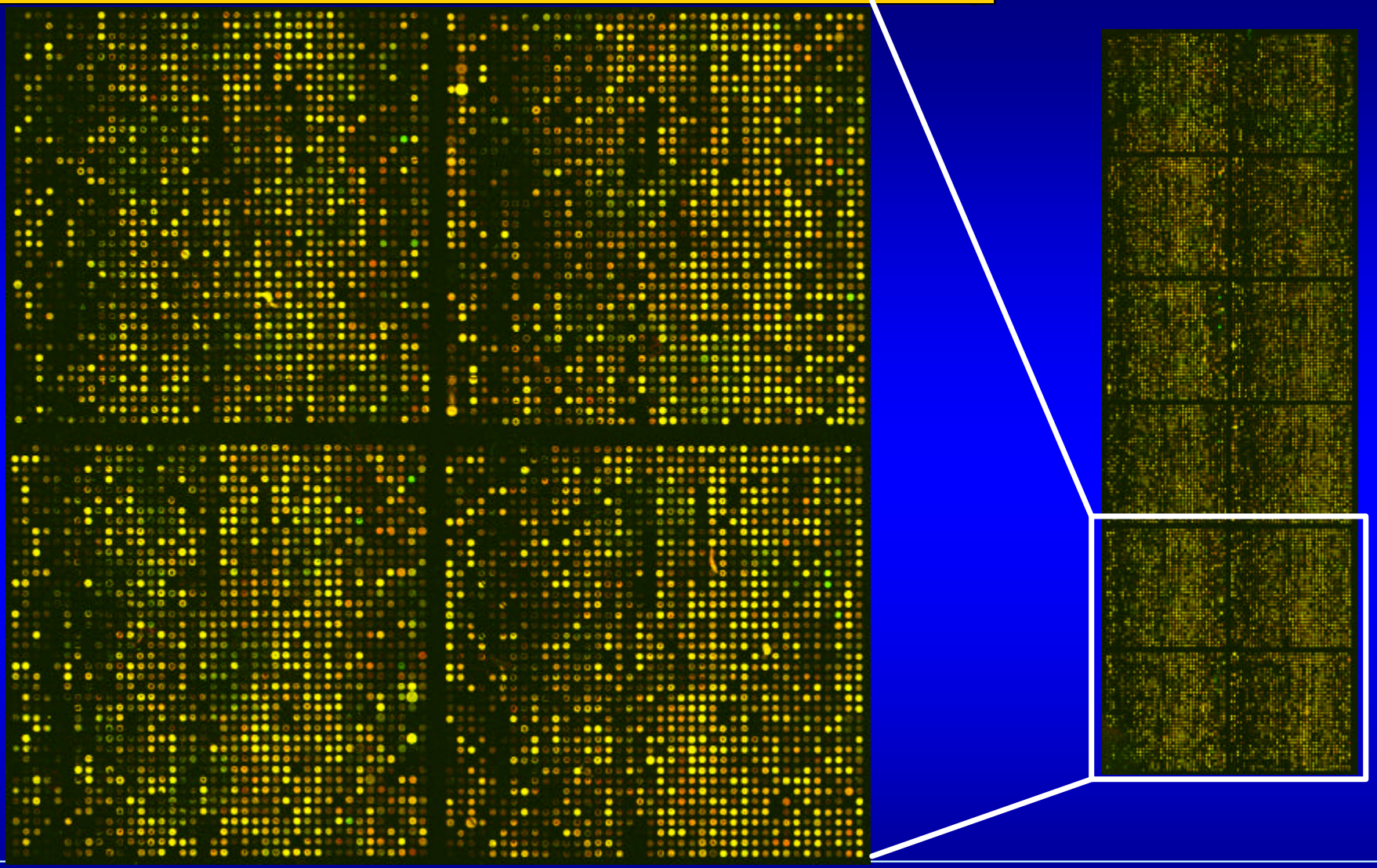
Hybridization to a 19,200 Element Human Array



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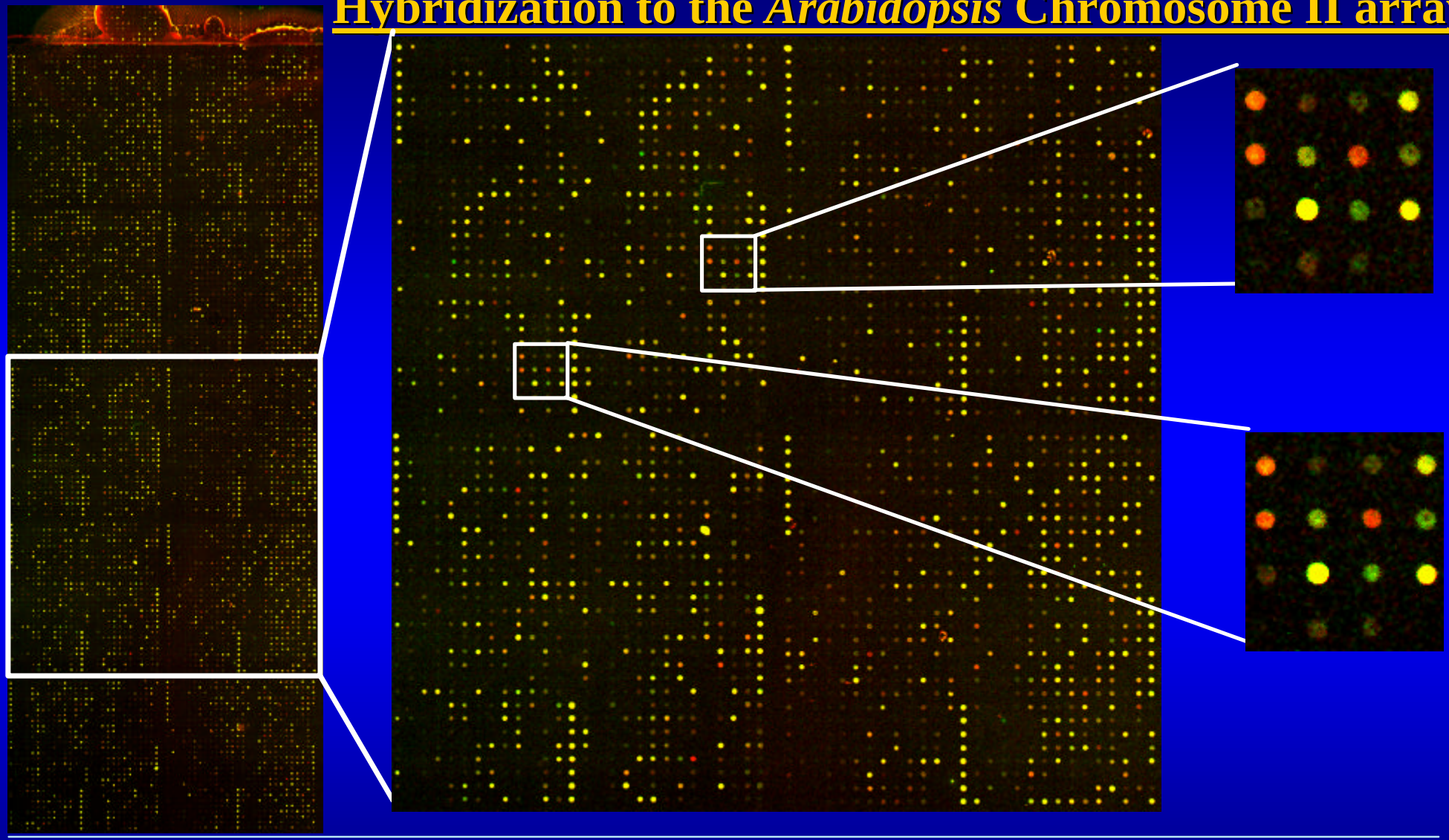
Hybridization to the *Arabidopsis* Chromosome II array



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TIGR Human Expression Team

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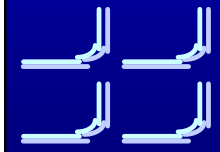
Funding provided by the National Cancer Institute: (R01 CA77049-01; PI: J. Quackenbush)

CMT-GAPS Slides supplied by Corning



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