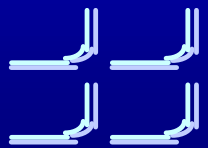


Microarrays in Three Easy Steps

Priti Hegde

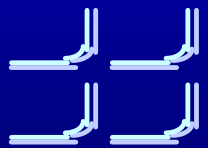


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Microarray Analysis Stages

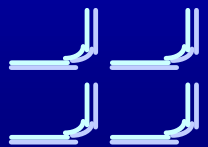
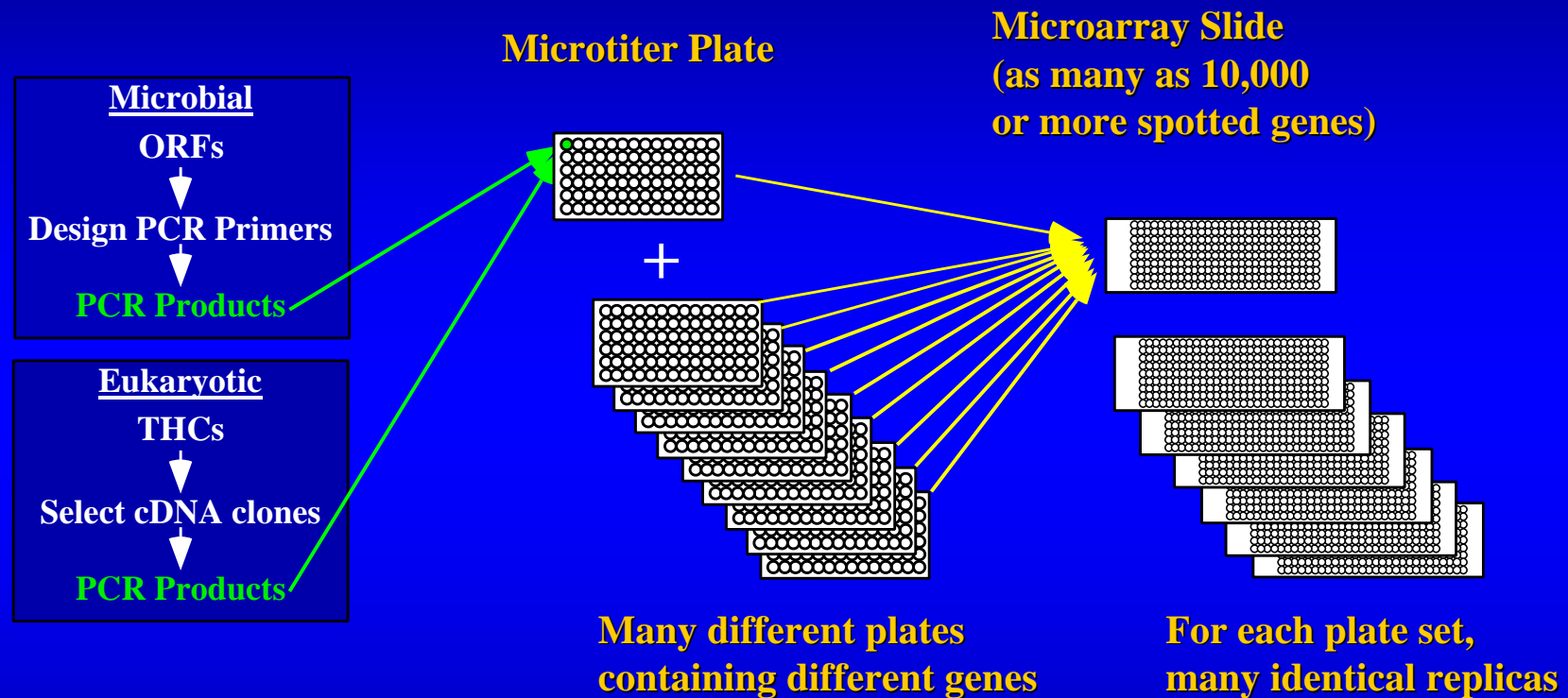
- **Array Fabrication**
- **Probe Preparation and Hybridization**
- **Data Collection, Normalization, and Analysis**



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Array Fabrication



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

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TIGR Gene Indices

Integrating data from international EST sequencing and gene research projects, the Gene Indices are an analysis of the transcribed sequences represented in the world's public EST data.

- Human Gene Index
- Mouse Gene Index
- Rat Gene Index
- Drosophila Gene Index
- BLAST Search
- FAQ
- Rice Gene Index
- Arabidopsis Gene Index
- Zebrafish Gene Index
- Fernando Gene Index
- Maize Gene Index
- Yeast Gene Index
- Cattle Gene Index
- M. truncatula Gene Index
- Wheat Gene Index
- C. elegans Gene Index
- T. brucei Gene Index
- T. cruzi Gene Index
- Leishmania Gene Index
- S. mansoni Gene Index

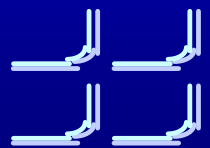
TOGA
The TIGR Orthologous Gene Alignment (TOGA) database provides links between candidate orthologous identifiers using the Tentative Consensus (TC) sequences that comprise the TIGR Gene Indices.

The TIGR Gene Index Project is supported in part by funding from the US Department of Energy, Grant #DE-FG02-96ER6252, and the US National Science Foundation, Grant #IBN-955370. Additional fees are provided by the US National Science Foundation, through grants #IBN-9812292 and #IBN-975584.

Send mail to TIGR Search | Site Map

TIGR Databases | What's New | About TIGR | TIGR Faculty | TIGR Gene Indices | Conferences, Education and Training | TIGR Software | Career Opportunities | Related Links

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Development of the TIGR “30k cDNA Gene Set”

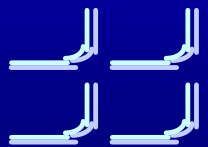
Goal:

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>>



TIGR

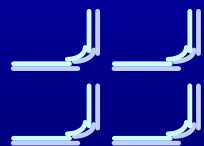
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- **PCR Amplify Them**

- Grow clones overnight
- Dilute 1:20 (5ml:95ml) in water
- “Pop” the cells, spin out debris
- Amplify in 50 ml reaction with Platinum Taq (Life Technologies)

- **Purify the PCR products**

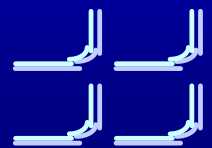
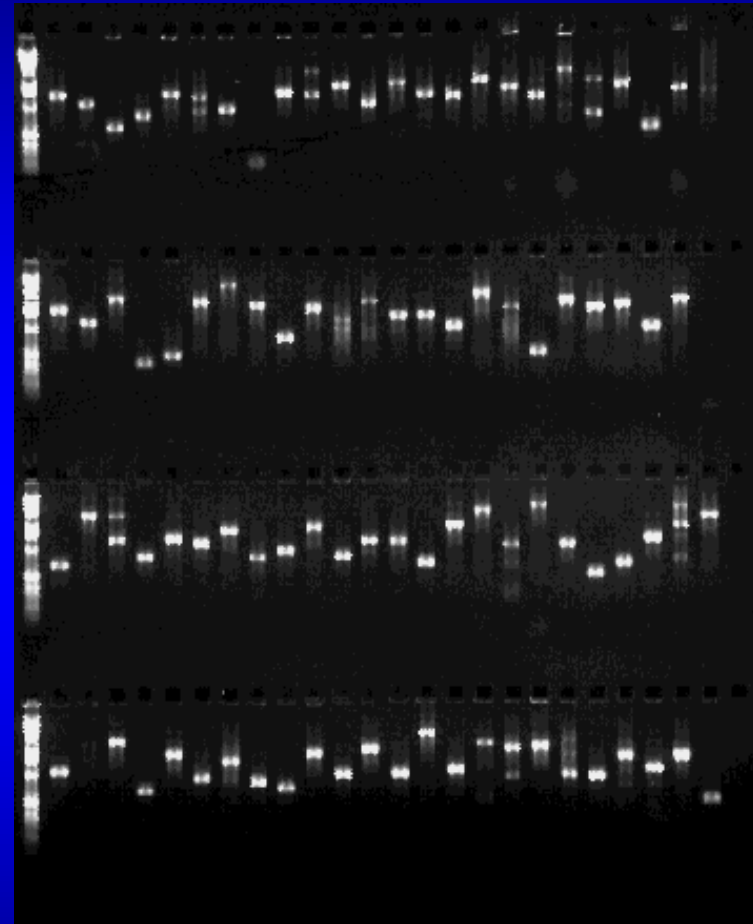
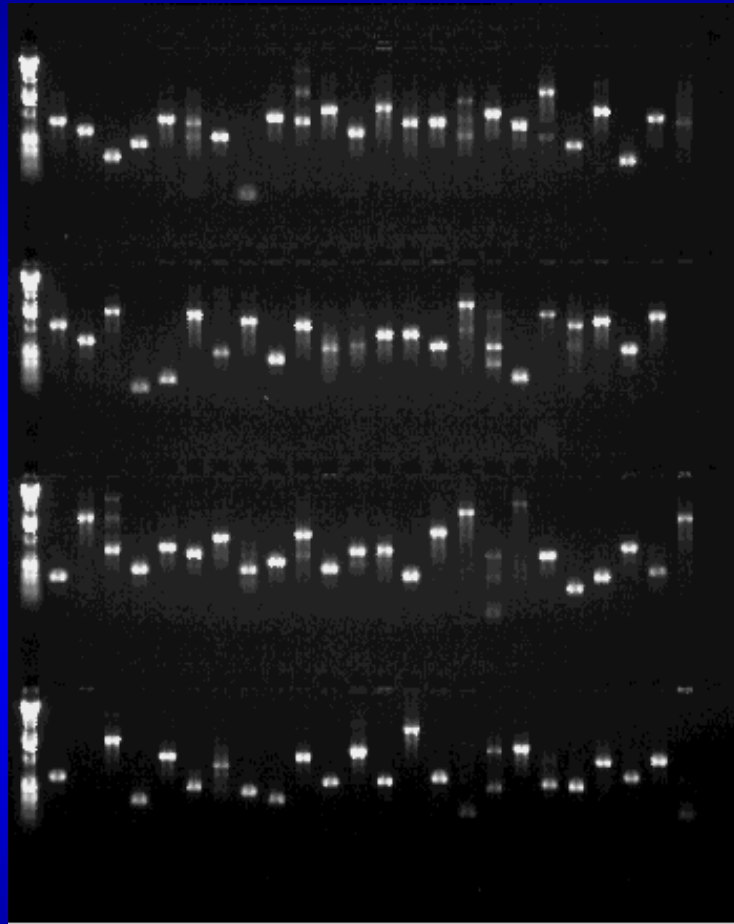
- 96-well Millipore multiscreen glass filter plate
- Bind products in high salt (1:5 5.3M Guanidine-HCl/150 mM KAc)
- Elute in water/TE



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PCR Amplification



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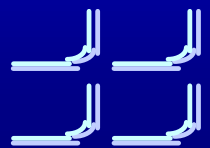
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Microarray PCR Scoring Tool

PCR Scoring											
MAGE PCR Scoring - Plate 25											
Reaction Date: 1998-03-06											
1	2	3	4	5	6	7	8	9	10	11	12
HPBCX05	HPBCX22	HPBDB11	HPBDB12	HPBDB64	HPBDC04	HPBDD60	HPBDD74	HPBDE09	HPBDE83	HPBDF30	HPBDF35
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
13	14	15	16	17	18	19	20	21	22	23	24
HPBDG63	HPBDH11	HPBDH92	HPBDJ11	HPBDJ15	HPBEB04	HPBEE72	HPBEE73	HPBEE90	HPBEG04	HPBEI92	HPBEP12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
25	26	27	28	29	30	31	32	33	34	35	36
HPBEJ14	HPBEJ67	HPBEJ84	HPBEM68	HPBEN51	HPBEP45	HPBEP96	HPBEQ22	HPBES04	HPBES08	HPDDD71	HPDDF03
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
37	38	39	40	41	42	43	44	45	46	47	48
HPDDF27	HPDDI16	HPDDI66	HPDDJ61	HPDDBA50	HPDDBA54	HPDDBA55	HPDCA14	HPDCA20	HPDDL83	HPDDM02	HPDDP53
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
49	50	51	52	53	54	55	56	57	58	59	60
HPDDS81	HPDDT66	HPDDU15	HPDDU85	HPDDU94	HPDDV23	HPDDV65	HPDDV91	HPDDW07	HPDDX38	HPDDY58	HPDDZ22
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
61	62	63	64	65	66	67	68	69	70	71	72
HPDED51	HPDEI11	HPDEI39	HPDEK22	HPDEK61	HPDEK73	HPLAF78	HPLAG47	HPLAH32	HPLAH37	HPLAH85	HPLAH87
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
73	74	75	76	77	78	79	80	81	82	83	84
HPLAI25	HPLAI38	HPLAK25	HPLAM28	HPLAN01	HPLAP22	HPLAP55	HPLAP89	HPLAQ50	HPLAQ74	HPLAR45	HPLAT36
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
85	86	87	88	89	90	91	92	93	94	95	96
HPLAU70	HPLAU80	HPLAW78	HPLAY64	HPLAZ41	HPLBA59	HPLBB12	HPLBB38	HPLBB49	HPLBB87	HPLBC15	HPLBE46
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Legend: Good Questionable Bad

88% Good
6% Questionable
6% Bad



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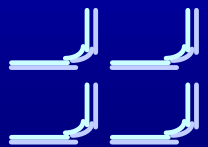
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The Beast: Microarray Robot from Intelligent Automation

- **Array The Clones**



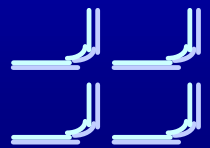
<<http://www.ias.com>>



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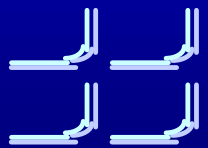
The Beast in Action: #1



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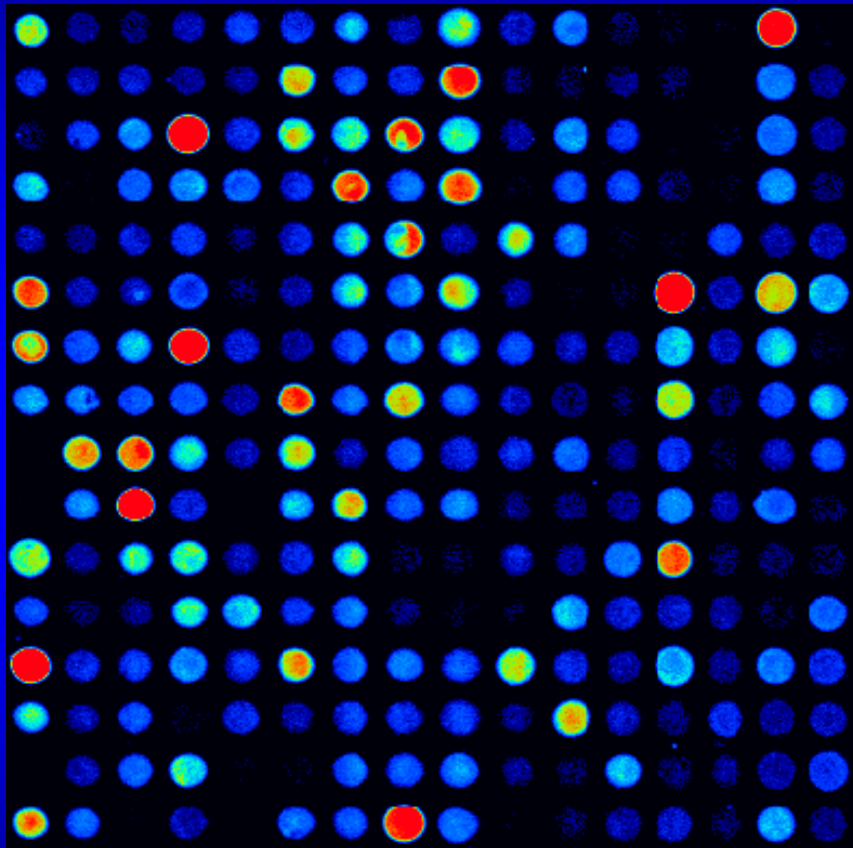
The Beast in Action: #2



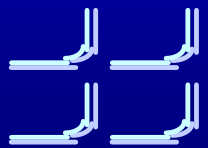
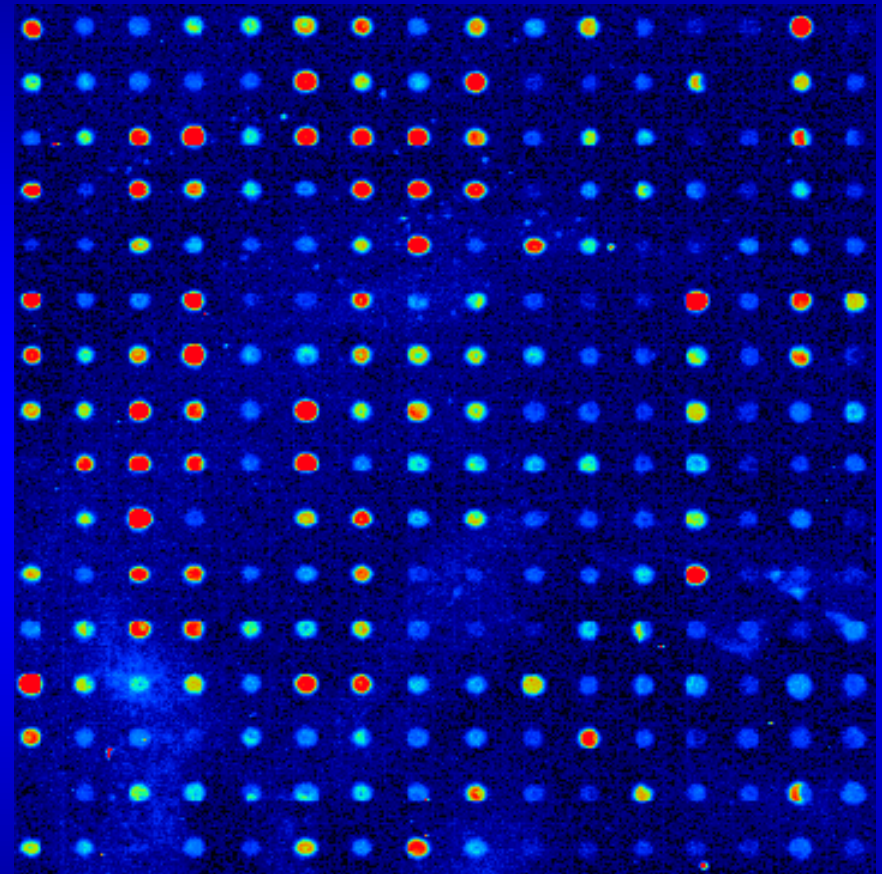
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- **The Glass is Crucial!**
Corning CMT-GAPS Slide



Another Slide

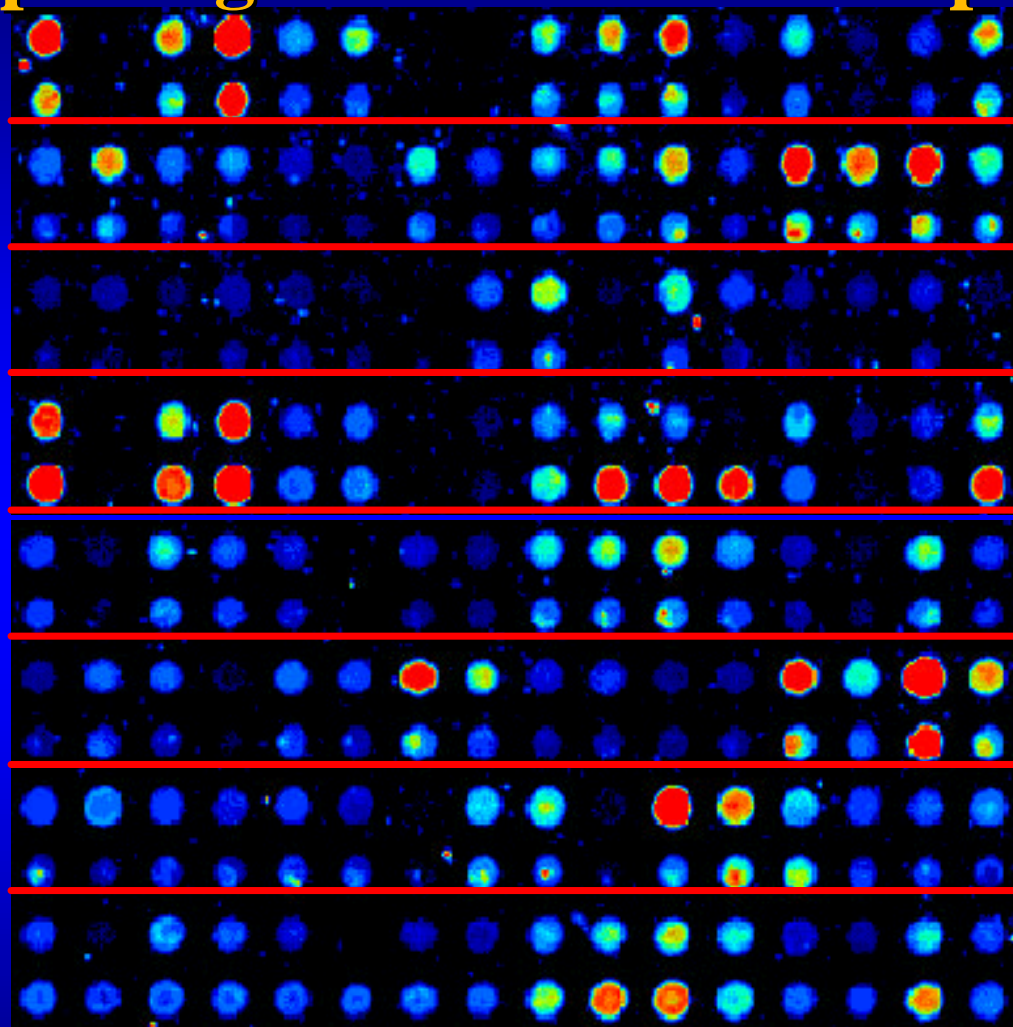


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The Effects of Spotting Buffer and PCR Clean-up

- Spotting 'Ink' and Clean-up matter



50% DMSO
3xSSC

50% DMSO
3xSSC

50% DMSO
3xSSC

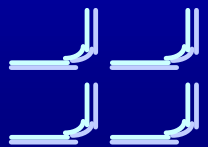
Ethanol Precipitation/DMSO
Glass Filter Cleanup/DMSO

50% DMSO
3xSSC

50% DMSO
3xSSC

50% DMSO
3xSSC

Ethanol Precipitation/DMSO
Glass Filter Cleanup/DMSO

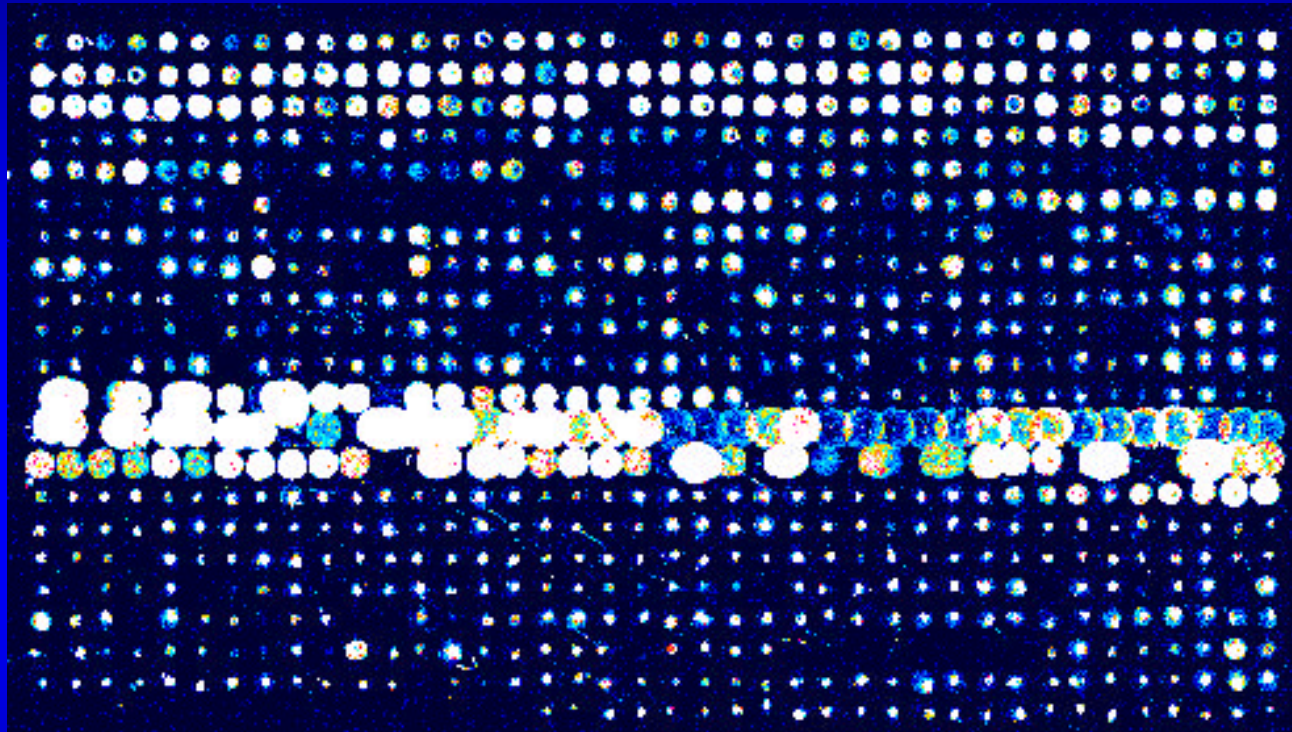


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When Temperature and Humidity Go Bad

- **Laboratory Conditions Matter**



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



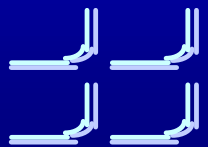
**62°F (16.7°C)
40-45% Relative Humidity**



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



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

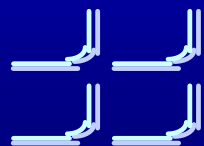


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- **Arraying Conditions**

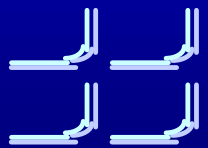
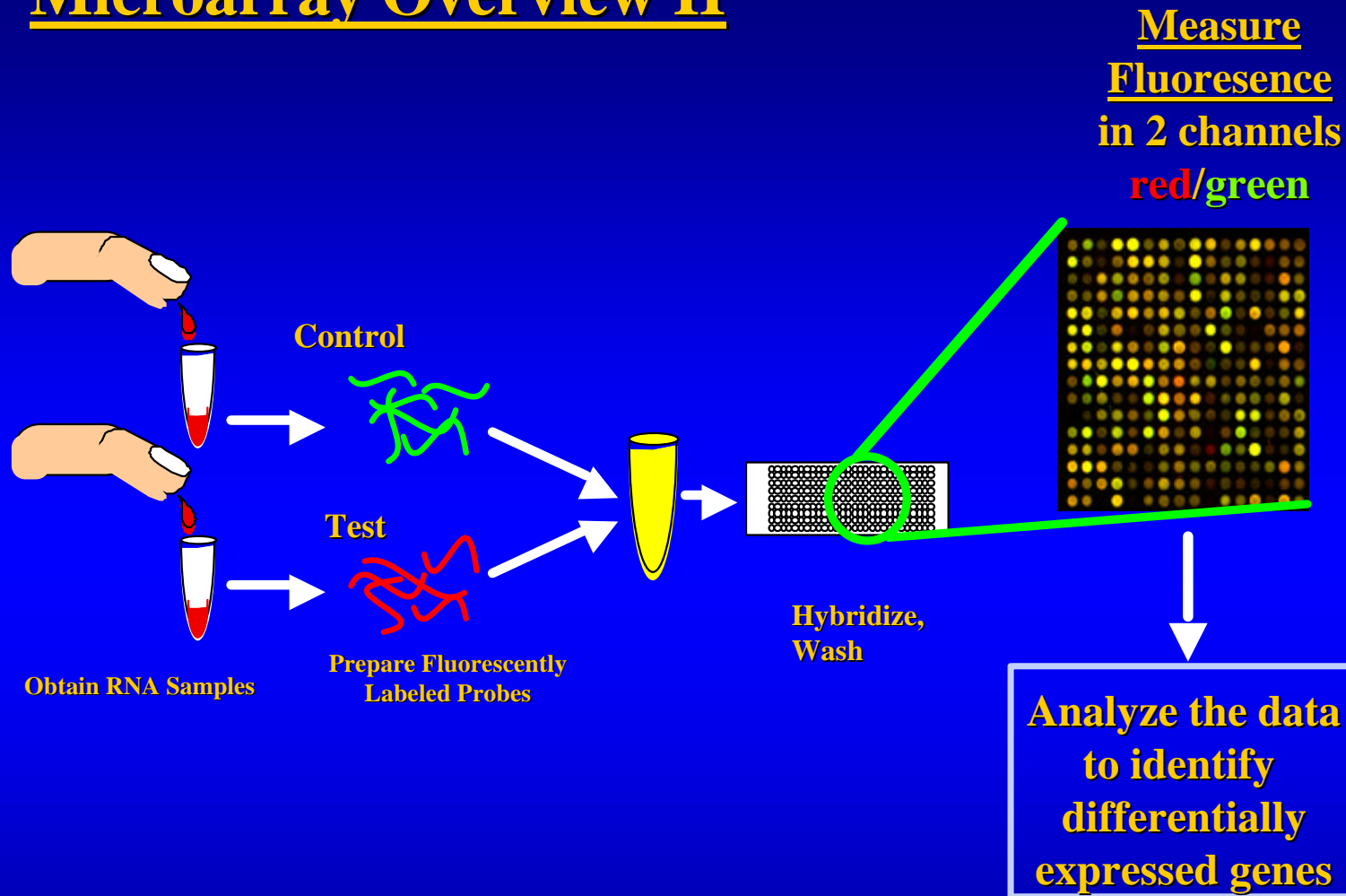
- **72°F (22.2°C), 40-45% Relative Humidity**
- **50% dimethyl-sulfoxide (DMSO), 20mM Tris HCl, 50mM KCl, pH 6.5**
(Thanks to Robin Cline, Erik Snestrud, Karen Ketchum)
- **Corning CMT-GAPS silane coated slides**
- **UV Cross-link at 90 mJ, bake at 80 ° C for 2 hours**



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Microarray Overview II

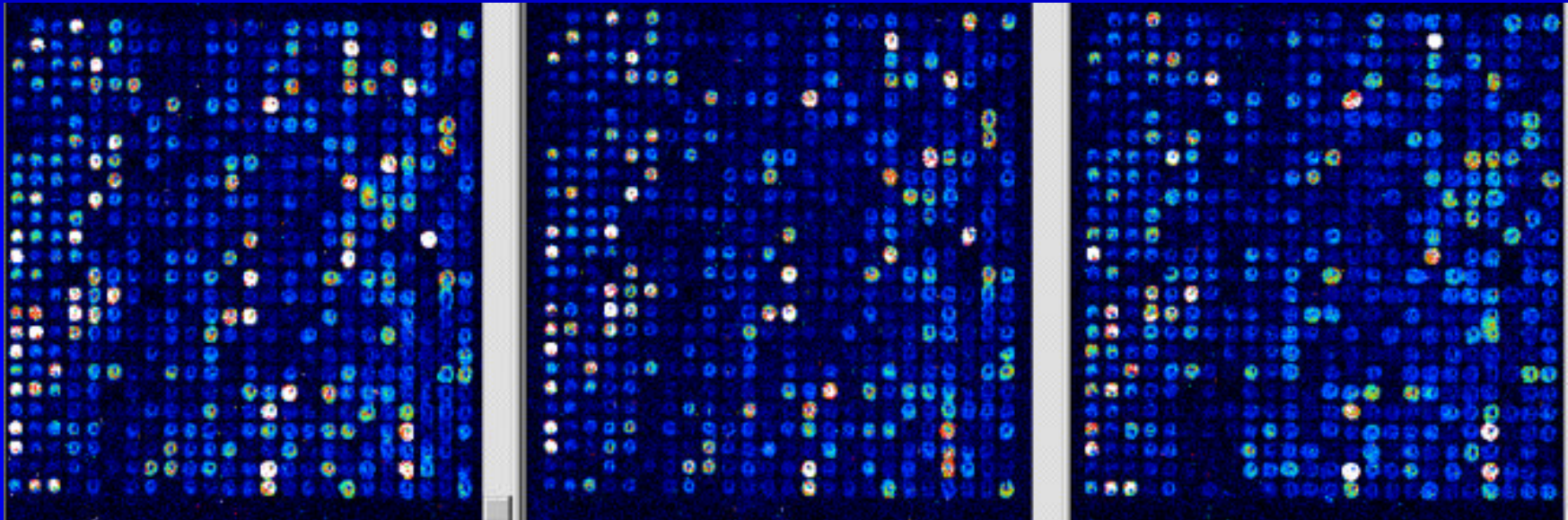


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Comparison of Labeling Protocols

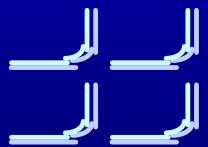
- **Preparing Labeled Probe**



20µg total RNA

4µg total RNA

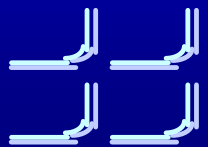
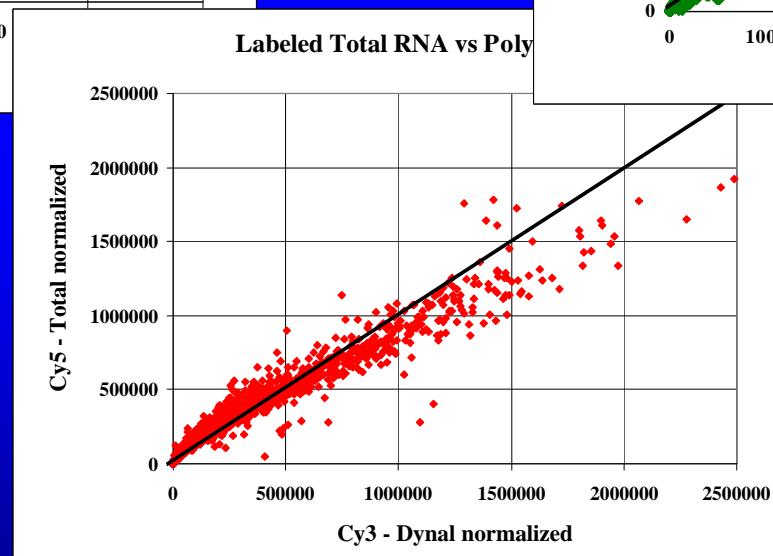
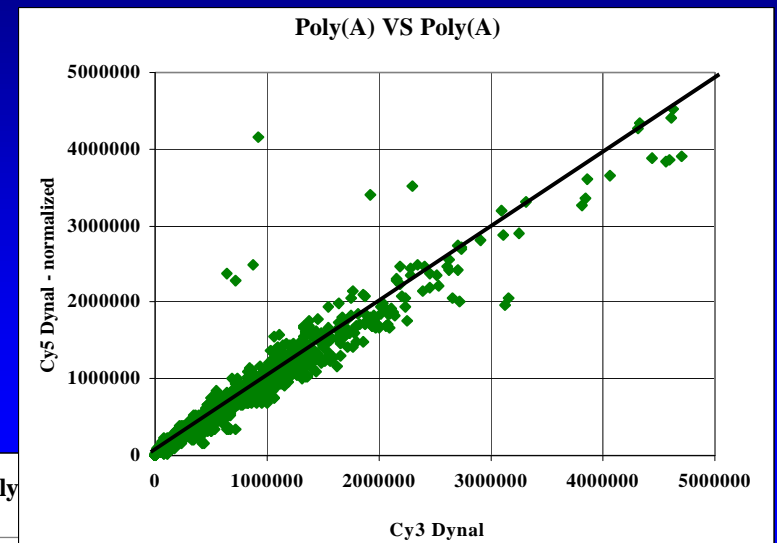
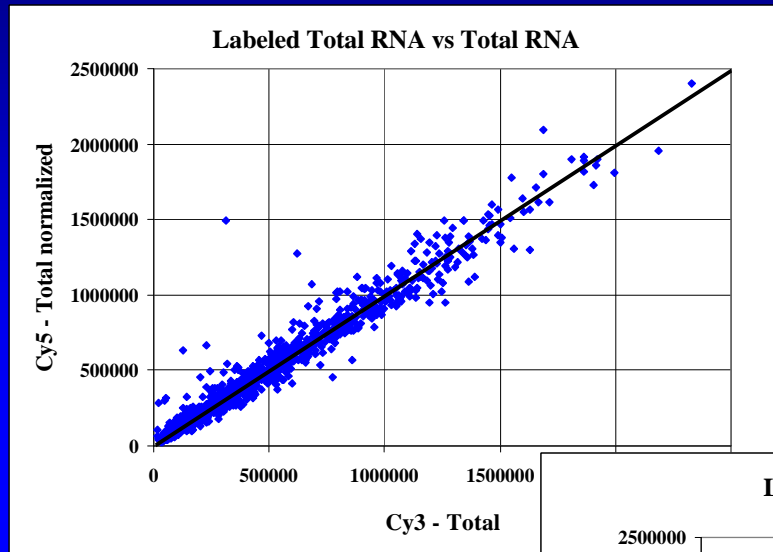
**~1.5µg Poly(A) RNA
(Seradyne Beads)**



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Total and Poly(A) RNA give equivalent results



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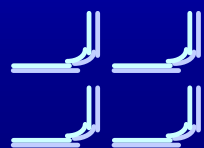
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- **Labeling Protocols**

- **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**



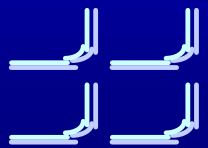
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Confocal Laser Scanner: ScanArray 3000

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

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

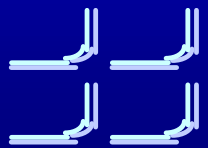


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Image Processing Issues

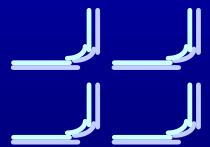
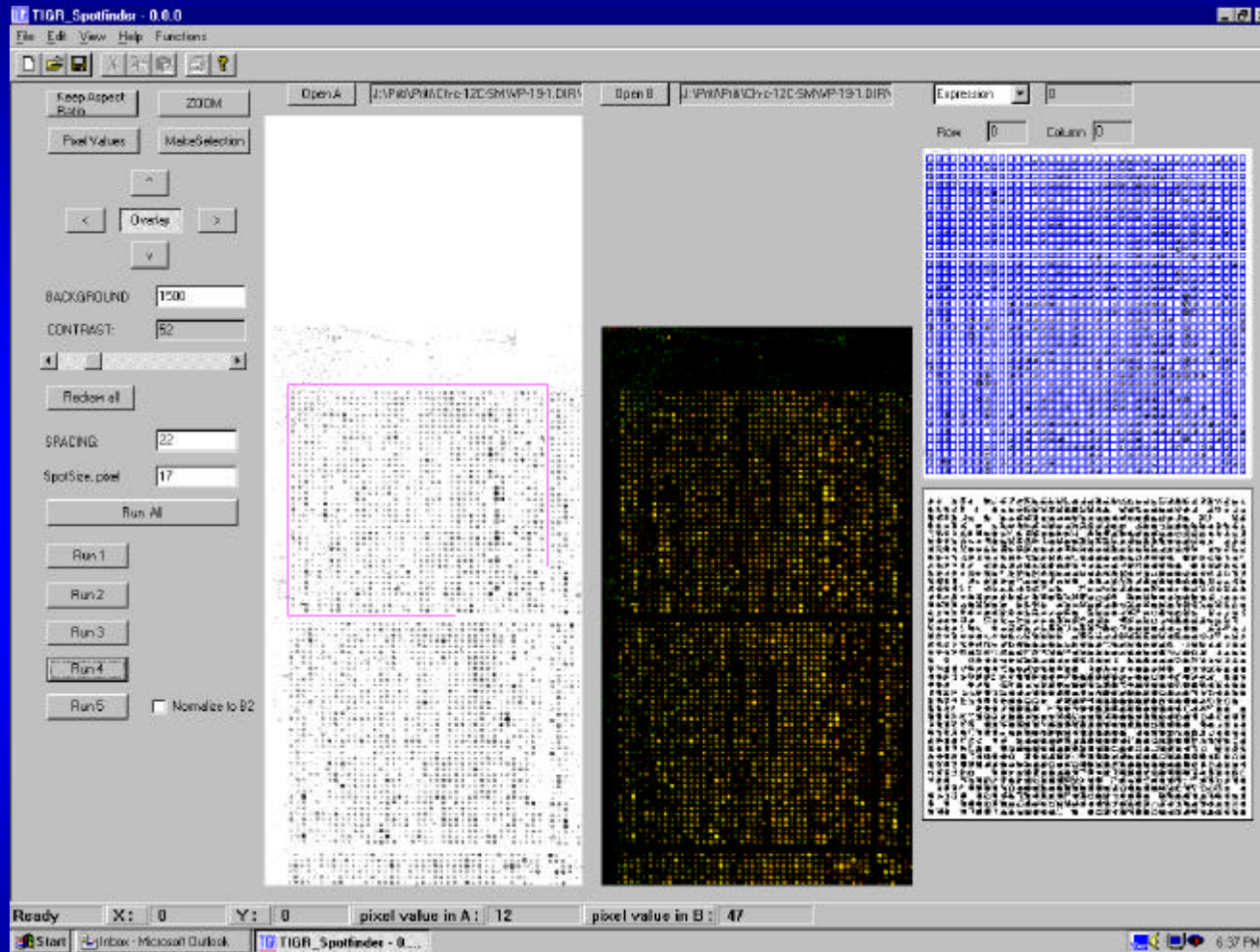
- **Spot Finding**
- **Background Subtraction**
- **Reproducibility**
- **Measure - median *vs.* mean (integrated intensity)**
- **Quality measures**



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TIGR Spotfinder

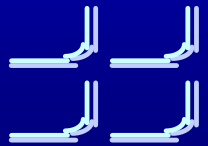


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Data Analysis Issues

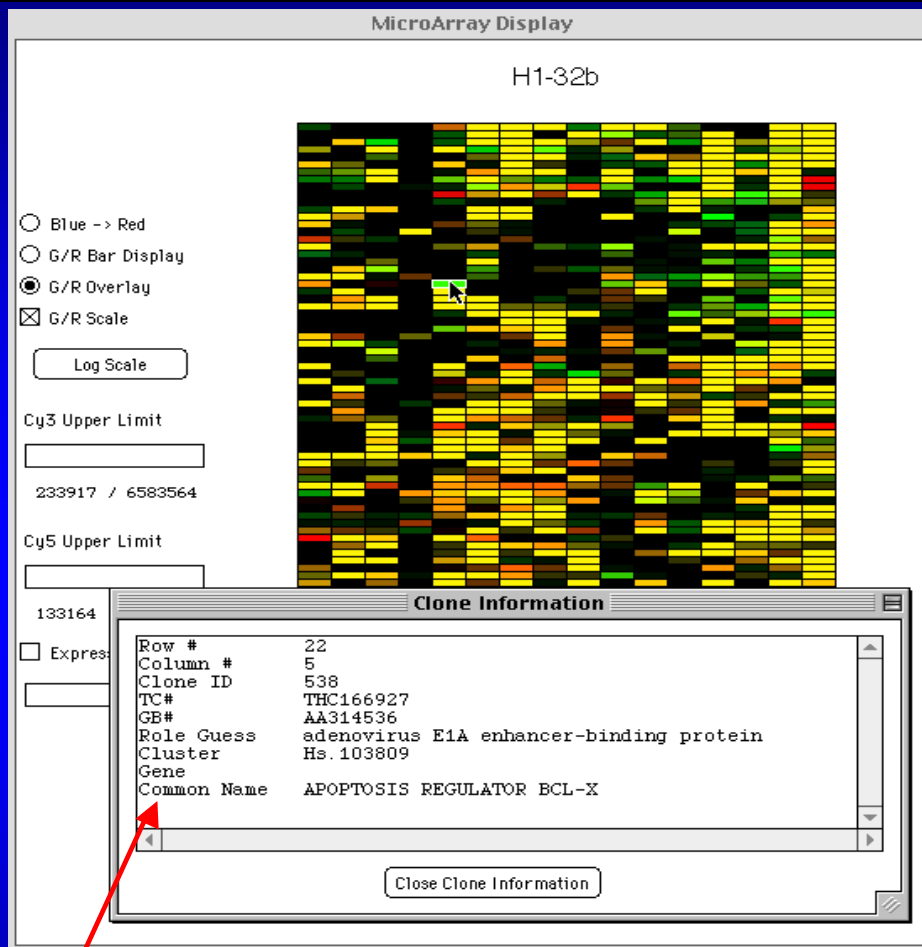
- **Presentation**
- **Multiple Views**
- **Normalization**
- **Identification of Differentially Expressed Genes**
- **Multiple Experiments**



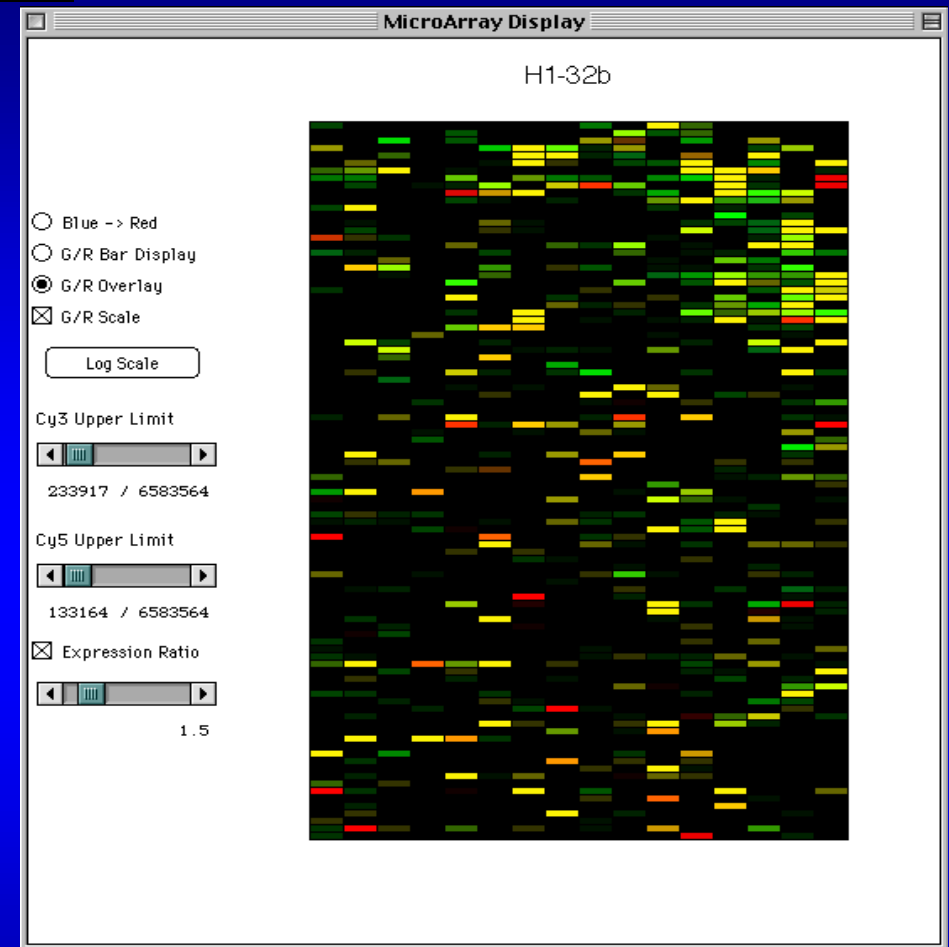
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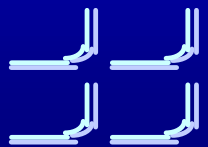
Microarray Data Display Software



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



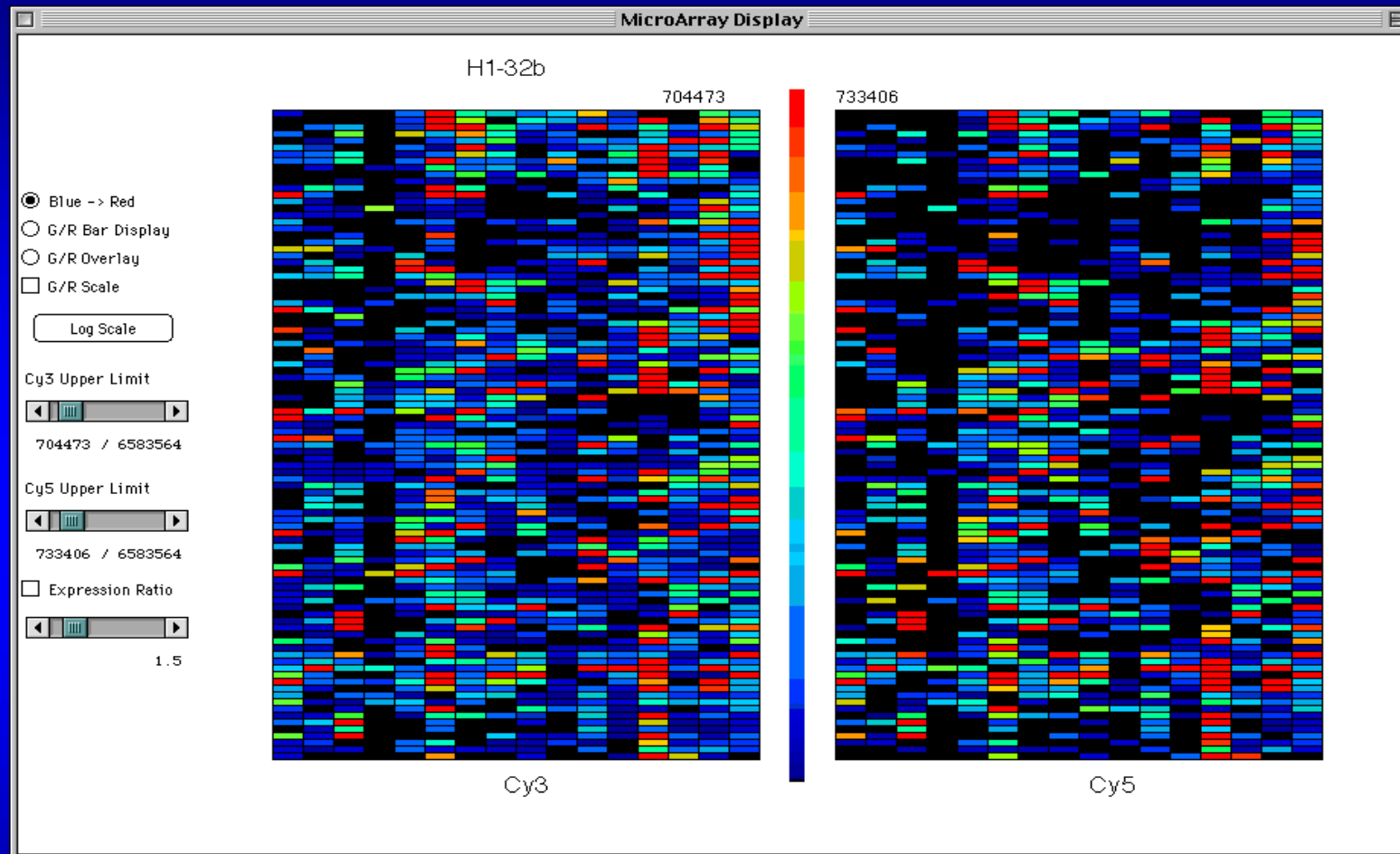
Differentially expressed clones can be selectively displayed



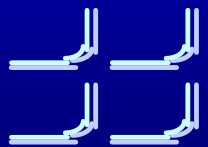
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Microarray Data Display Software



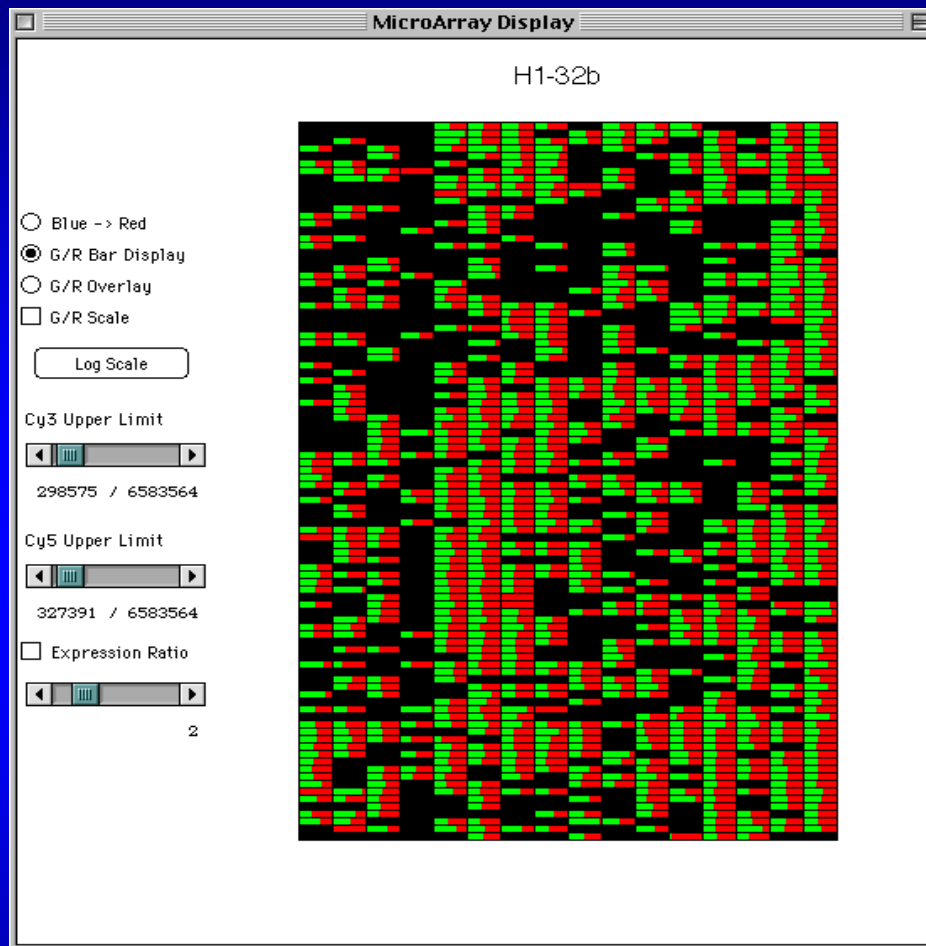
Pseudo-false color display allows assessment of hybridization signal strength



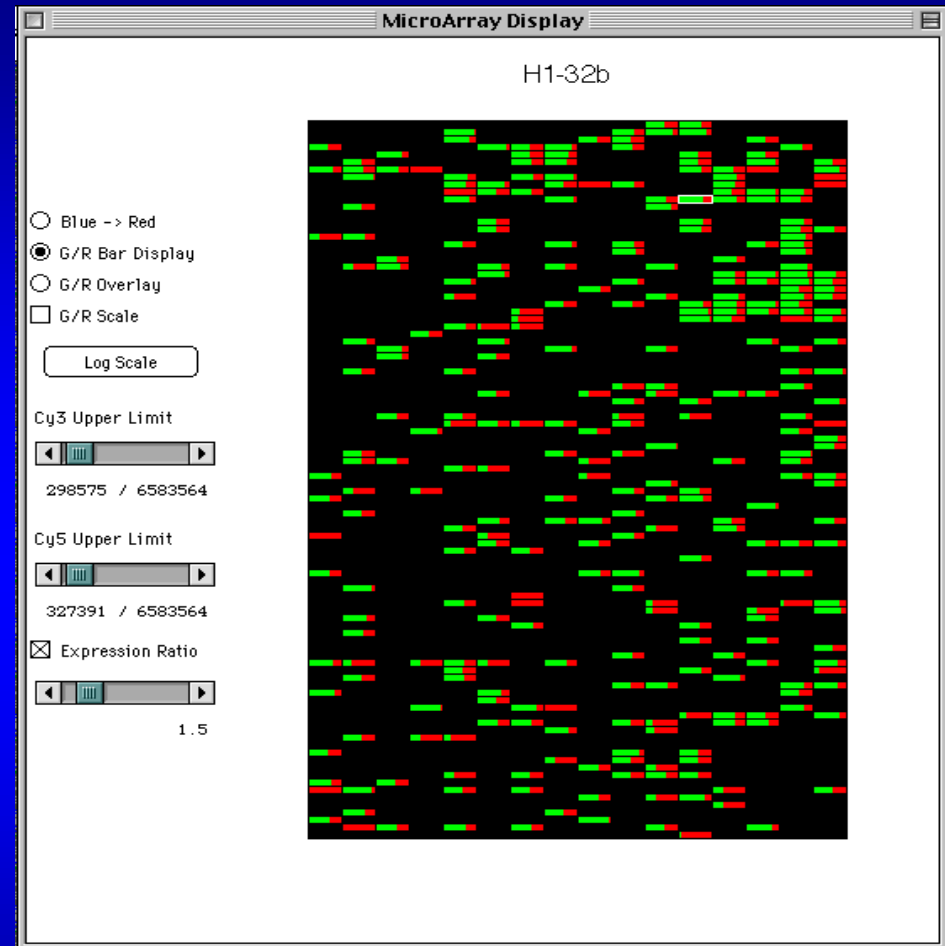
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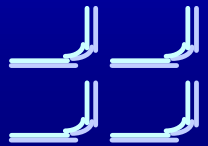
Microarray Data Display Software



Relative Red/Green areas represent
relative expression levels



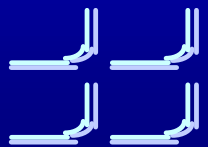
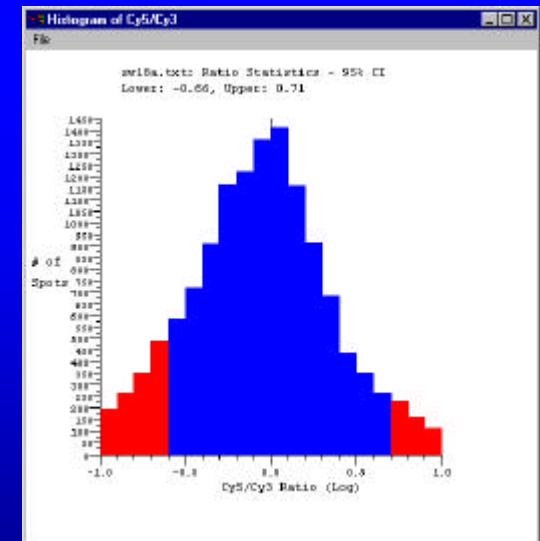
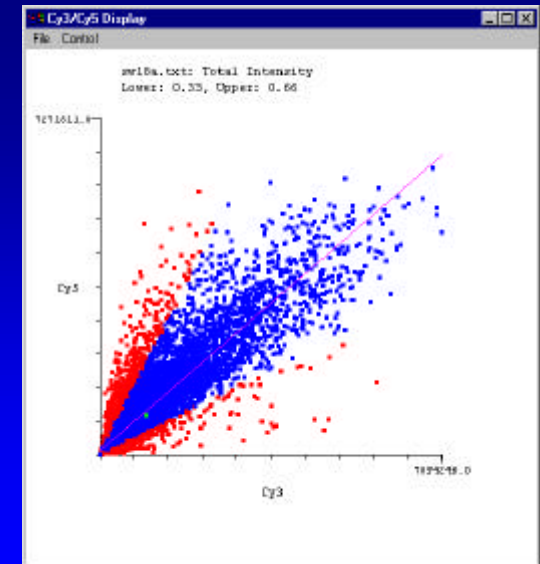
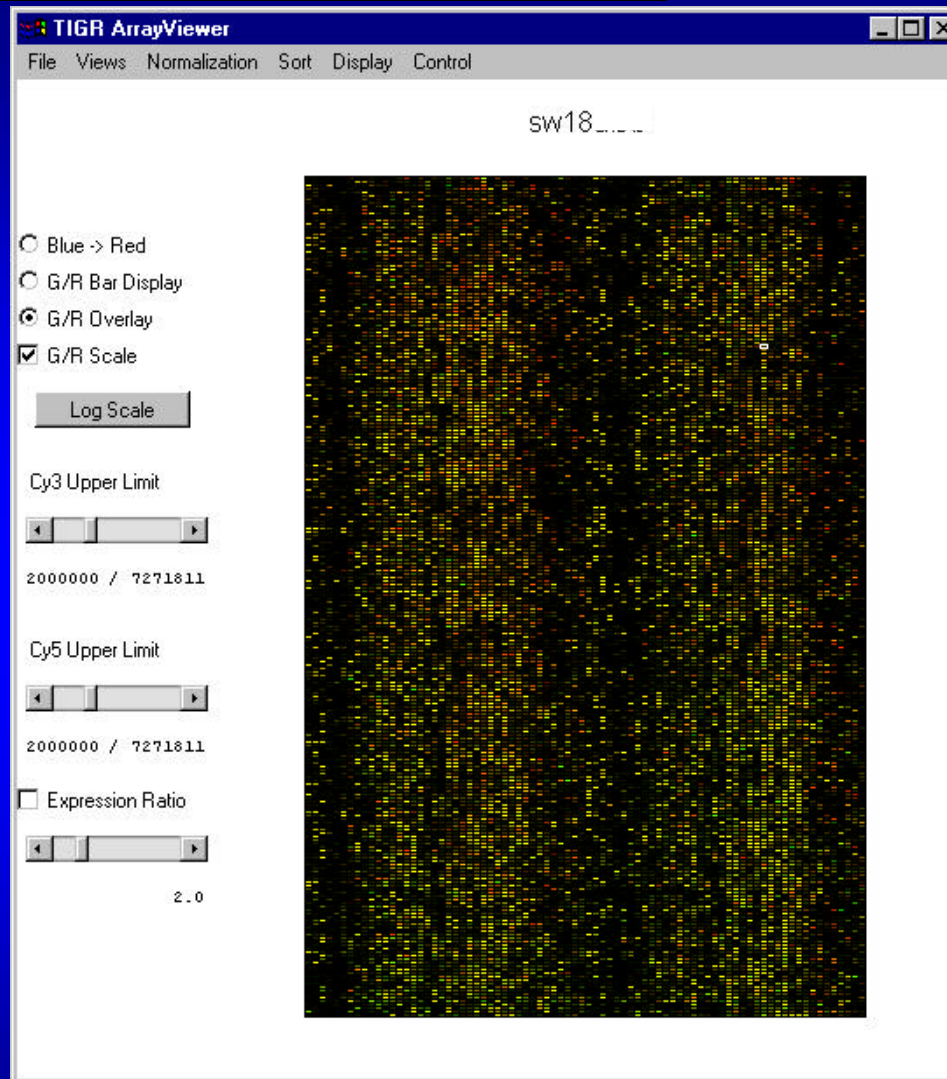
Setting expression ratios show only
over/under expressed genes



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Data Display/Analysis Software



TIGR

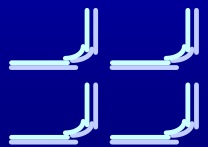
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Normalization Approaches

- 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



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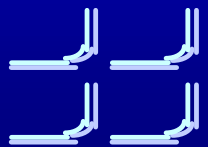
Normalization Approaches (II)

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



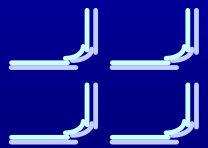
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Normalization Approaches (III)

Solution(?)

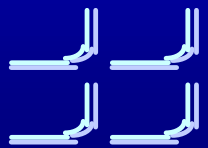
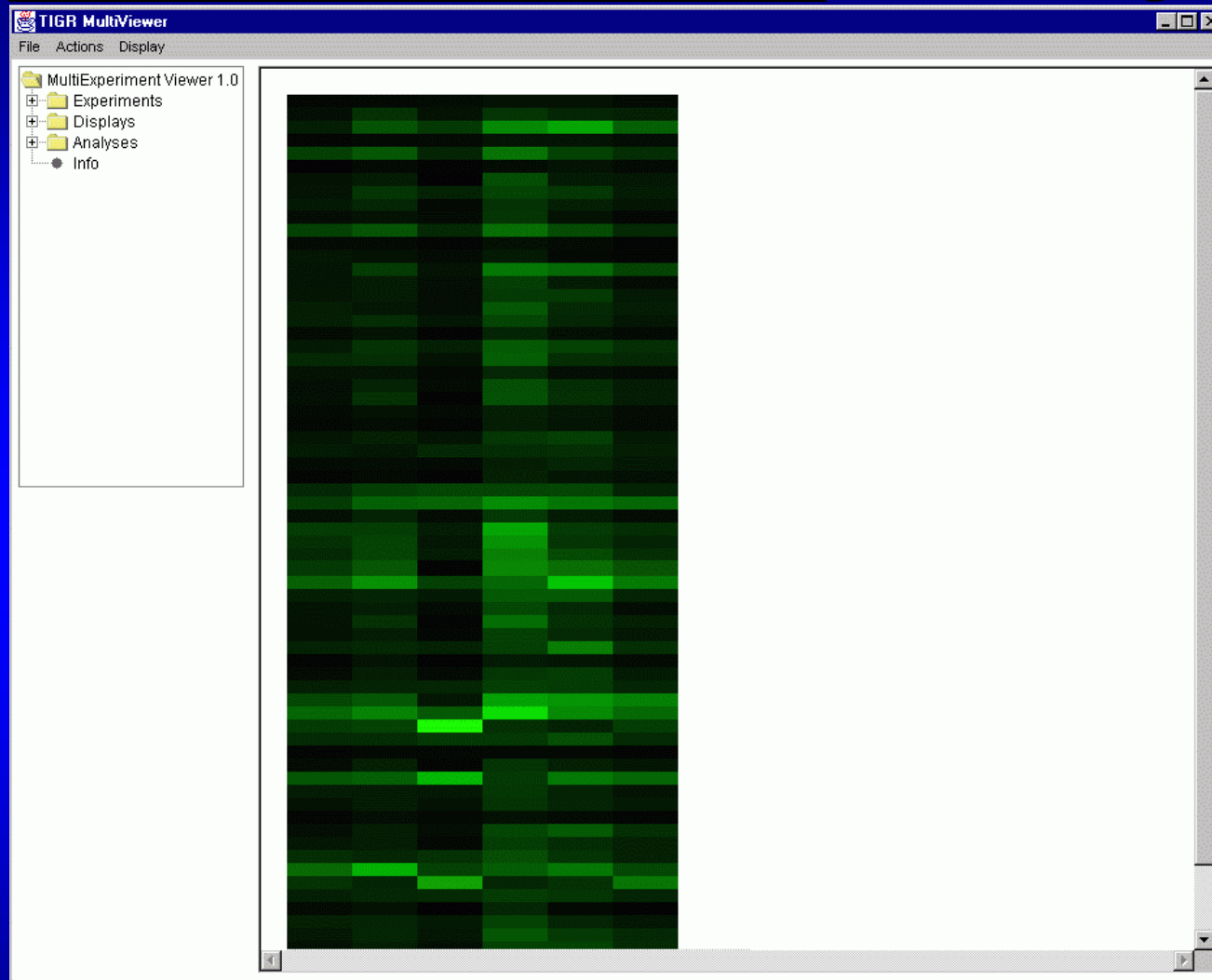
- **Experiment dependent**
- **Use a combination of techniques**



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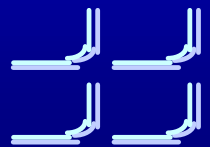
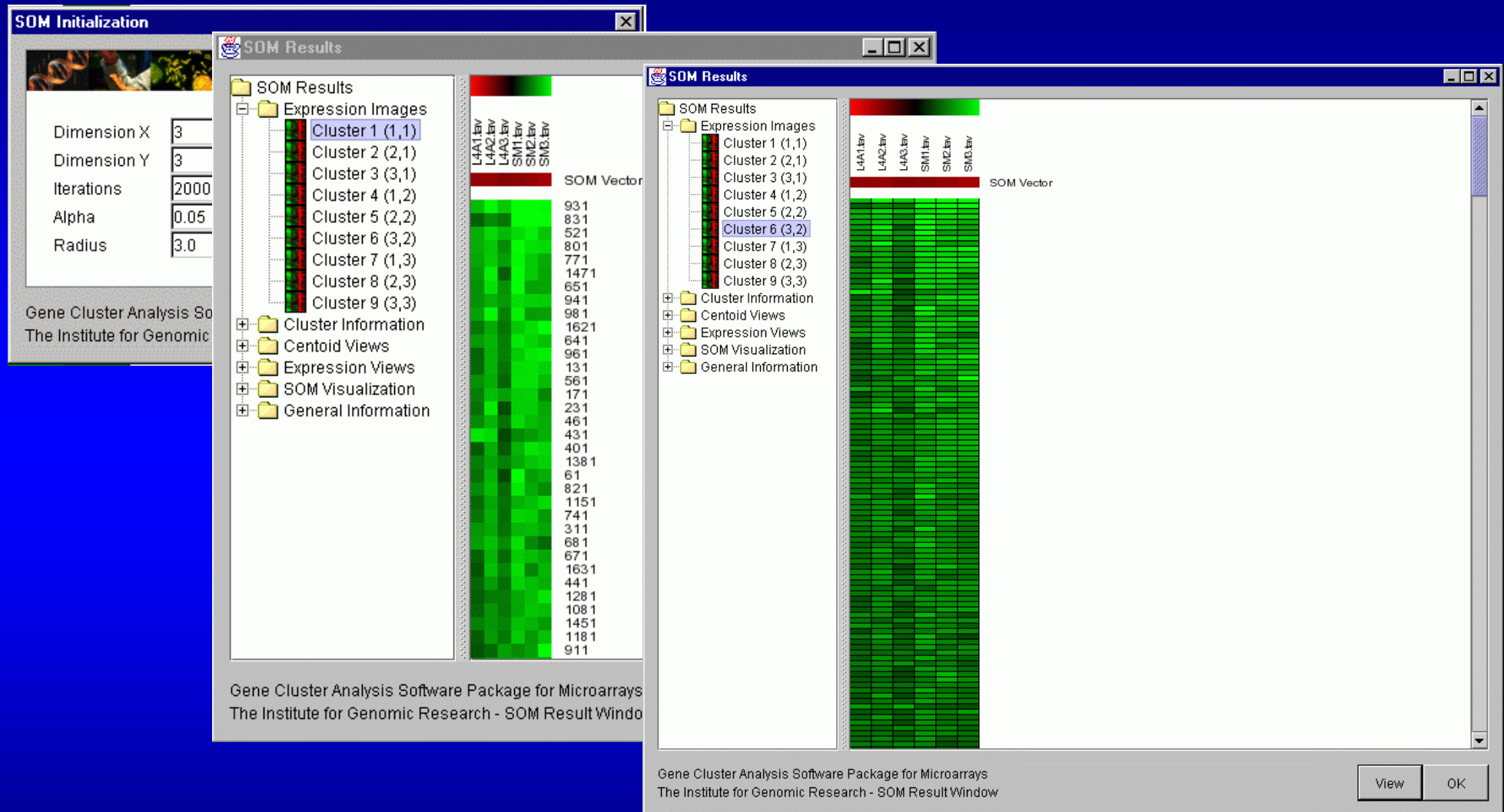
TIGR MultiExperiment Viewer: Data Mining



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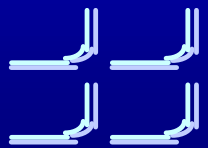
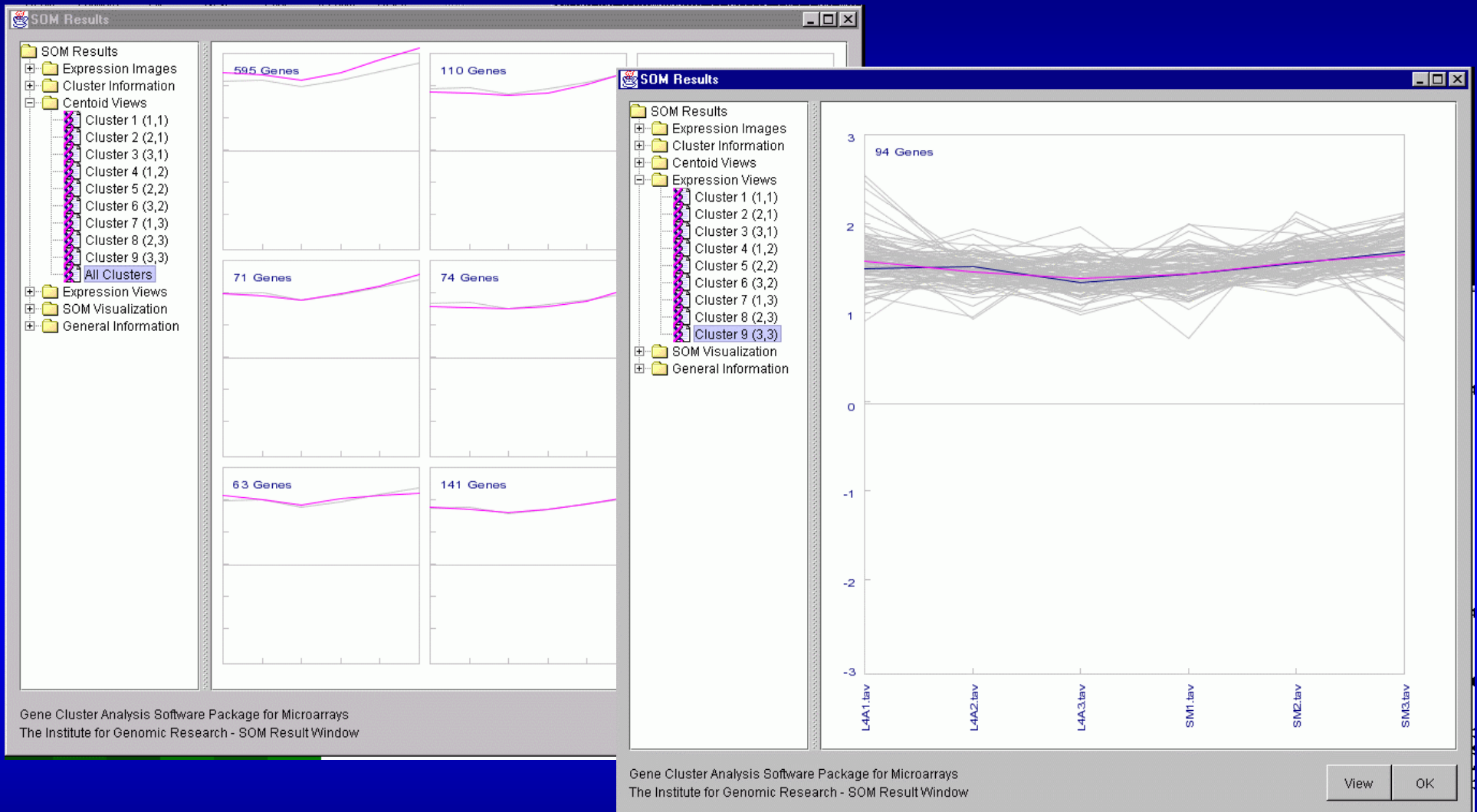
TIGR MultiExperiment Viewer: Self Organizing Maps



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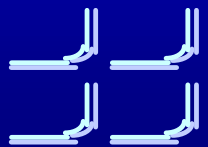
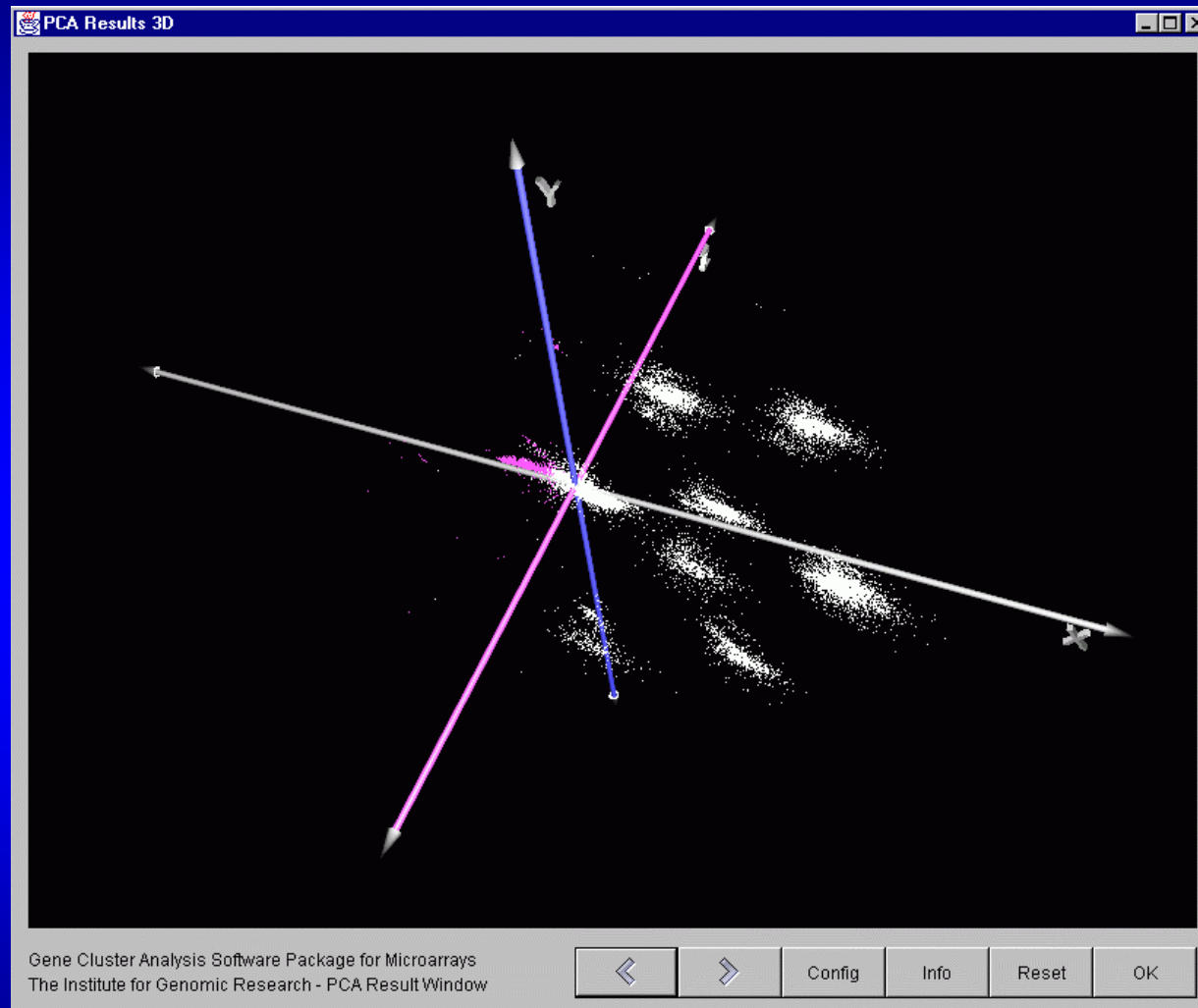
TIGR MultiExperiment Viewer: Self Organizing Map Views



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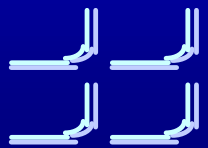
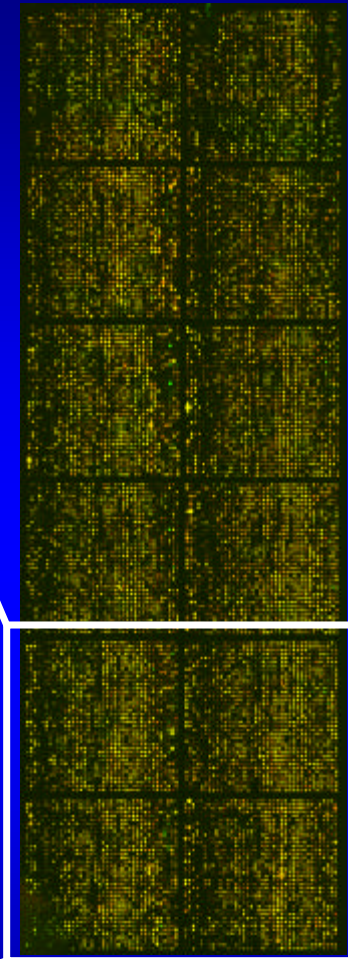
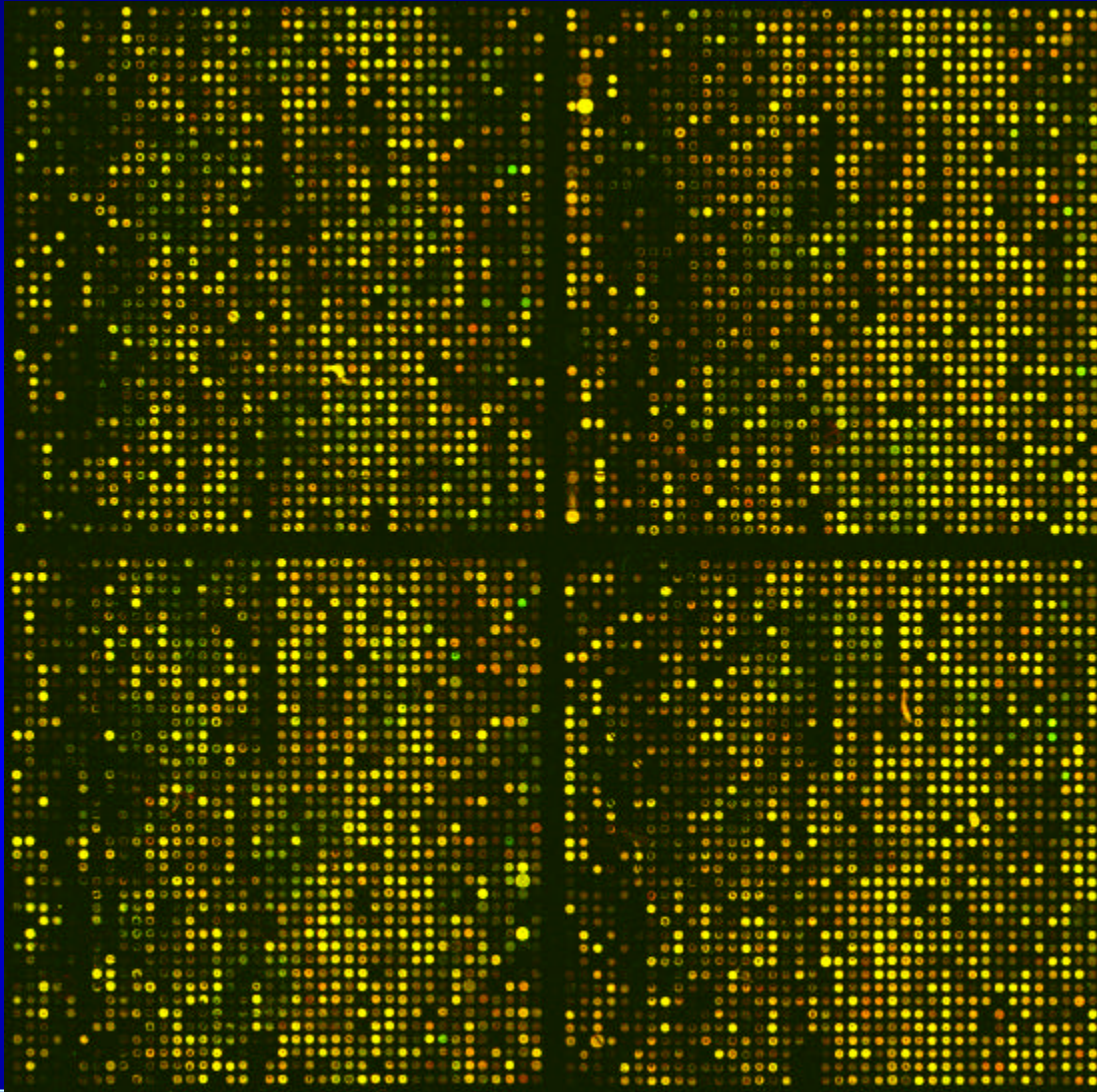
TIGR MultiExperiment Viewer: Principal Component Analysis



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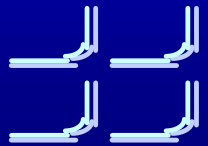
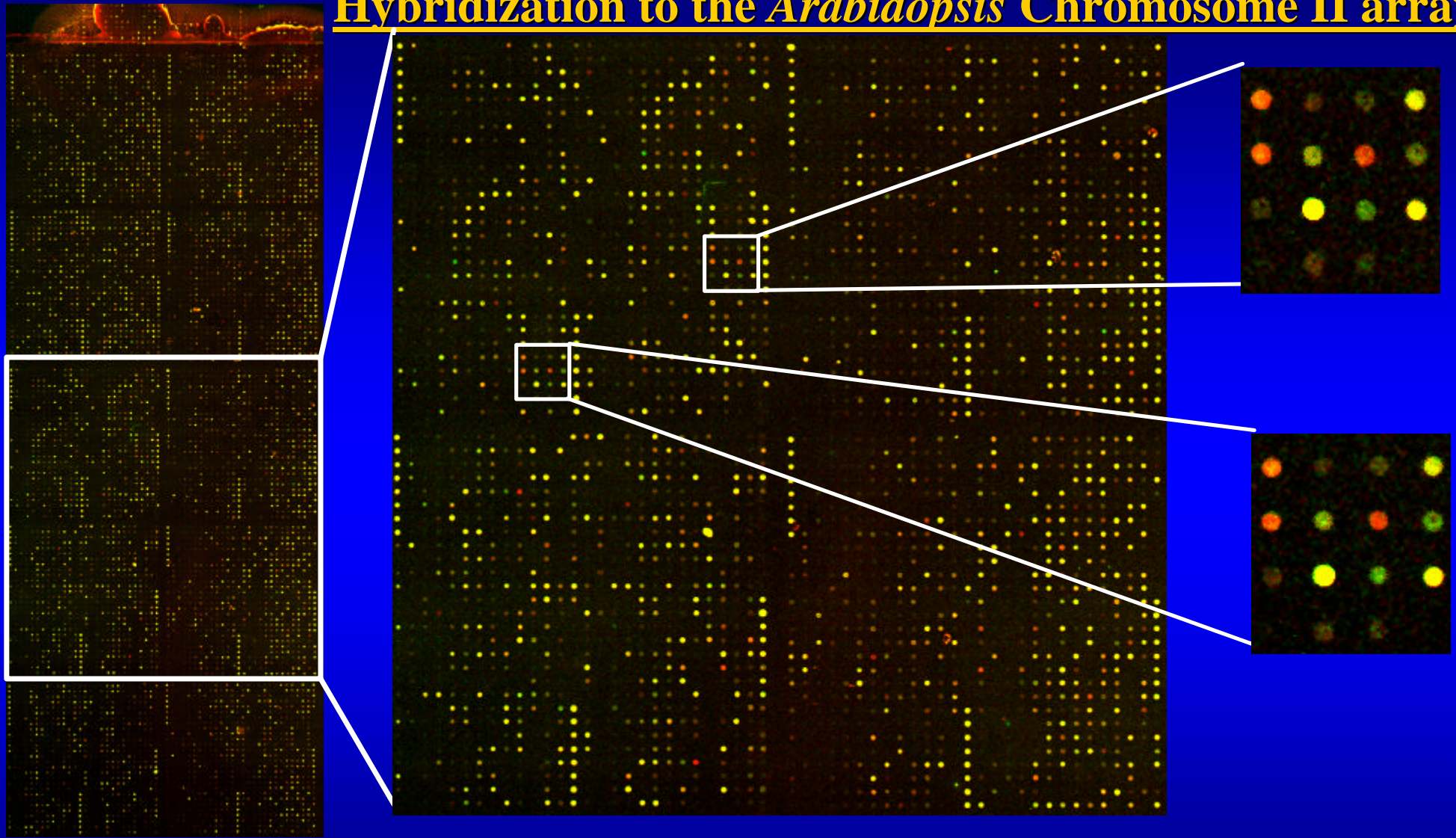
Hybridization to a 19,200 Element Human Array



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Hybridization to the *Arabidopsis* Chromosome II array



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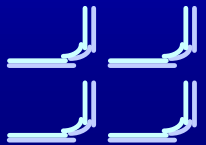
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CMT-GAPS Slides supplied by Corning



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