Microarrays

Ralph V. Shohet, M.D.

Department of Internal Medicine, UTSWMC

Outline

1. Types of microarrays

2. How to make one

3. How to use one

Historical perspective

1. Watson-Crick basepairing

2. Southern & Northern analysis

3. Genome project

Types of microarrays

1. Synthesized *in situ*

2. Spotted

Types of microarrays

1. Synthesized *in situ*

    Short oliogonucleotides (25mers)

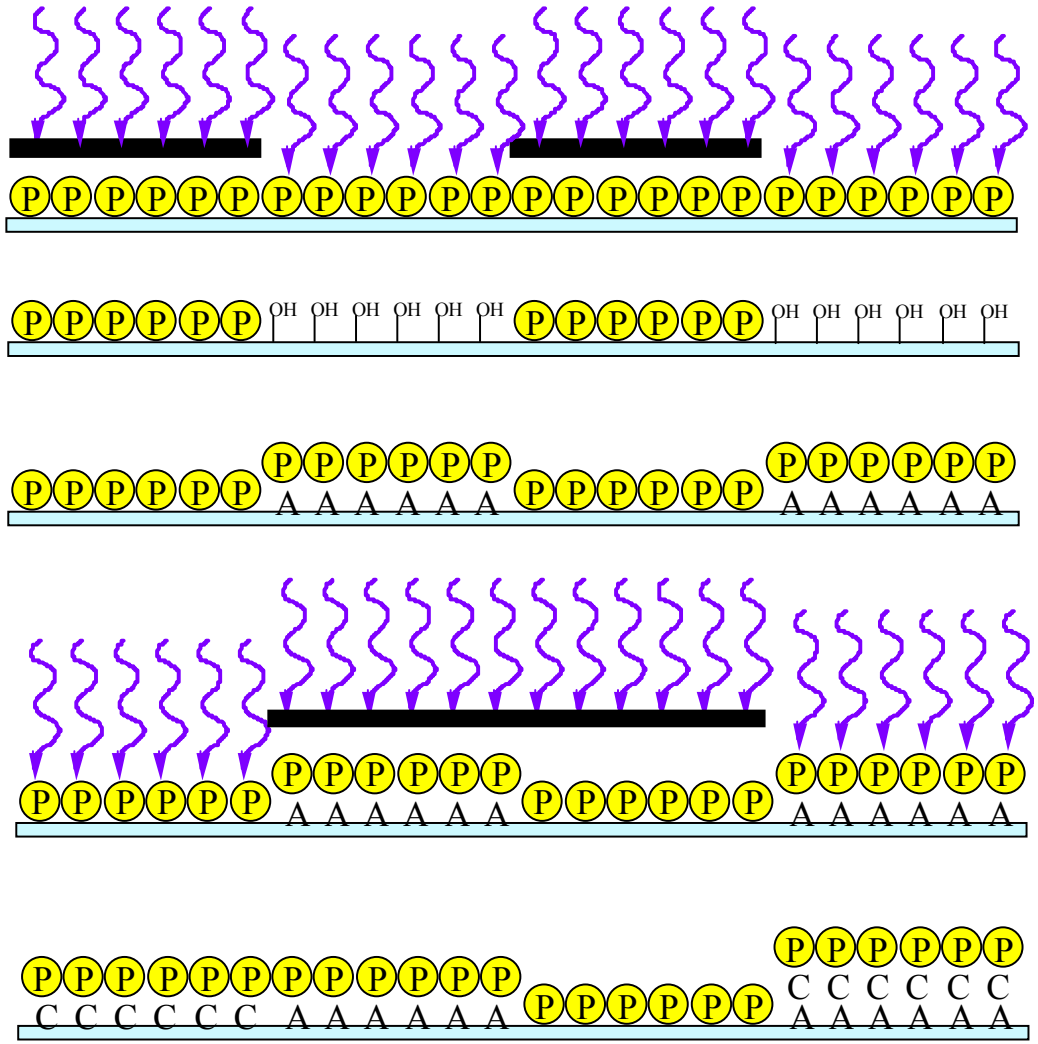
    masked and DOC

particularly useful for interrogating small regions of a gene for sequence variation

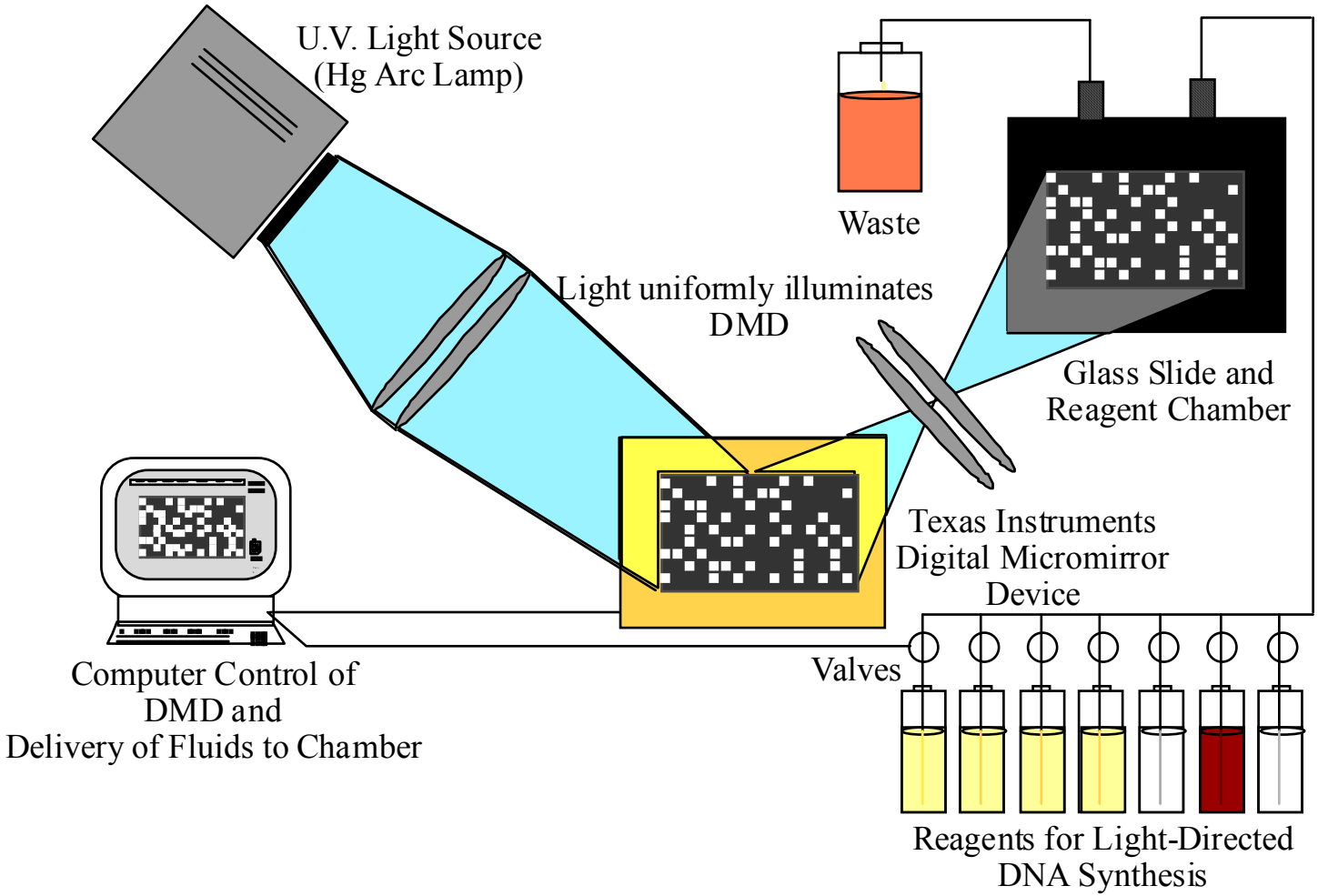
GATTACA

Paramount, 1997

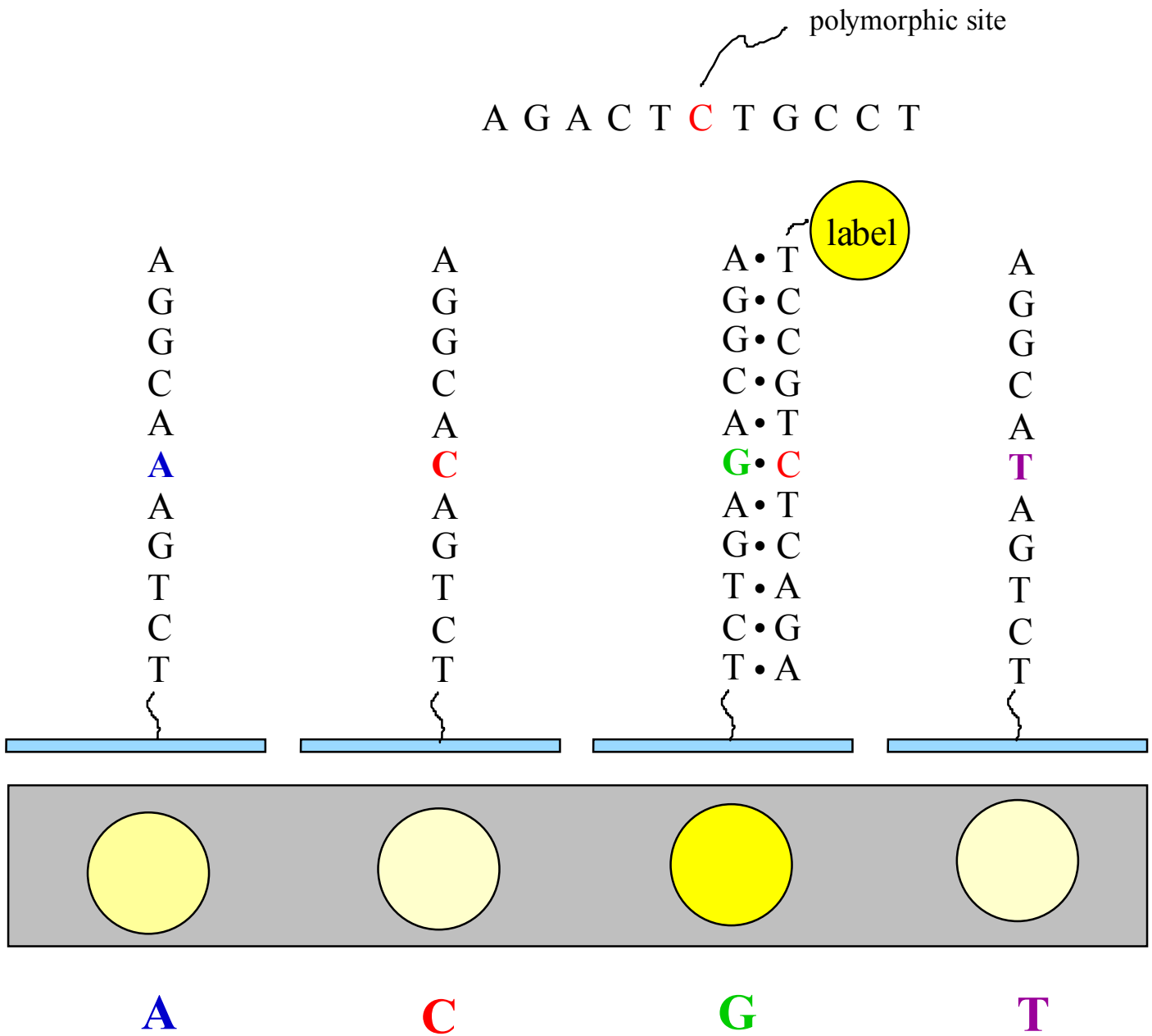
Light-Directed Synthesis of Nucleic Acid Arrays



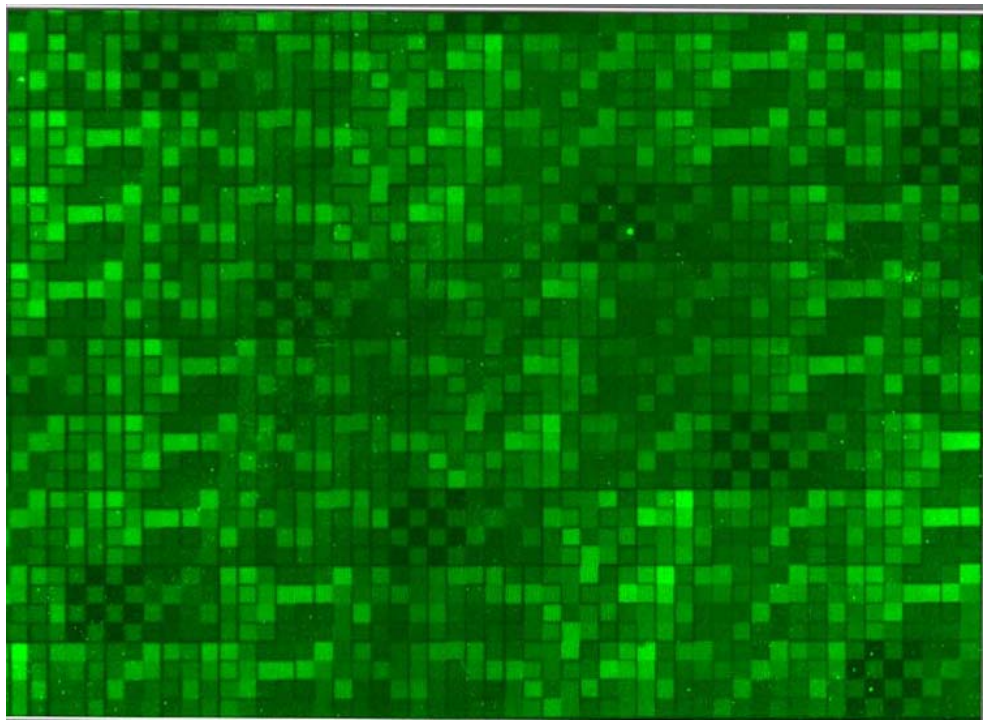
Digital Optical Chemistry



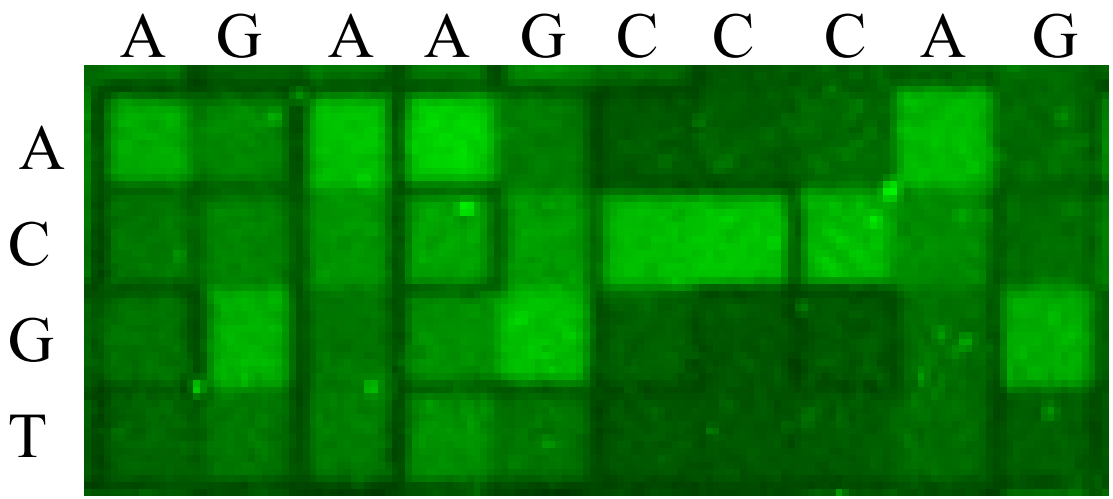
Probing a DNA Sequence by Hybridization



Thousands of Features can be Synthesized



“Resequencing”
60 bp of p53
2300 features



Types of microarrays

2. Spotted
    cDNA
    Long oligonucleotides (70mers)

particularly useful for discovery of gene regulation

Microarray Creation

QuickTime™ and a
Sorenson Video decompressor
are needed to see this picture.

From Stratagene™ marketing film, 2001

Library creation for spotted array

Isolate RNA from tissue of interest, reverse transcribe to cDNA

↘

Subclone into plasmid, pick and grow clones

↘

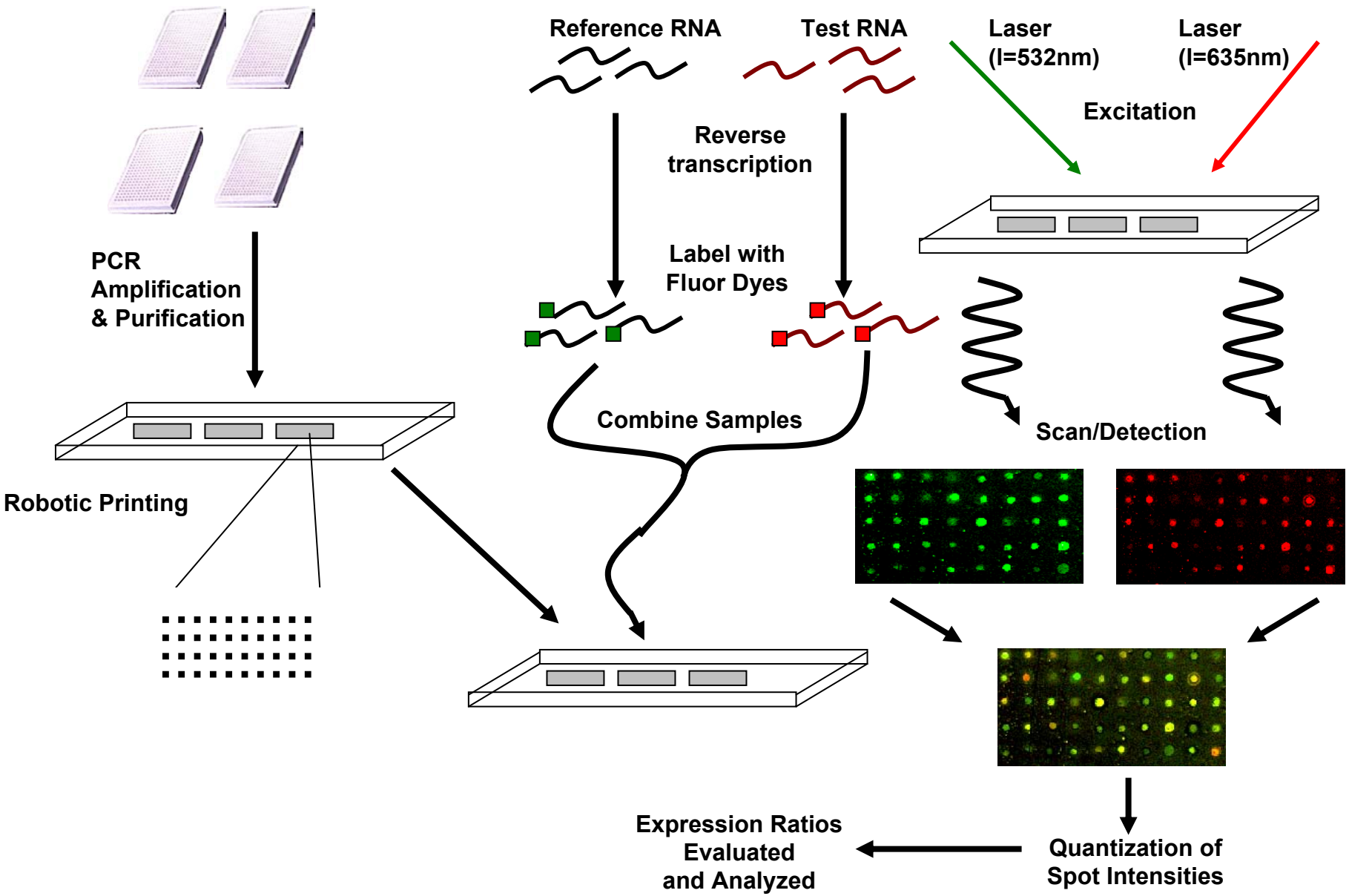
Remove redundancy, sequence

↘

Select clones, amplify inserts

↘

Spot on microarray


Reference RNA
Test RNA
Laser
(l=532nm)
Laser
(l=635nm)
Excitation
Reverse
transcription
PCR
Amplification
& Purification
Label with
Fluor Dyes
Combine Samples
Scan/Detection
Robotic Printing
Expression Ratios
Evaluated
and Analyzed
Quantization of
Spot Intensities

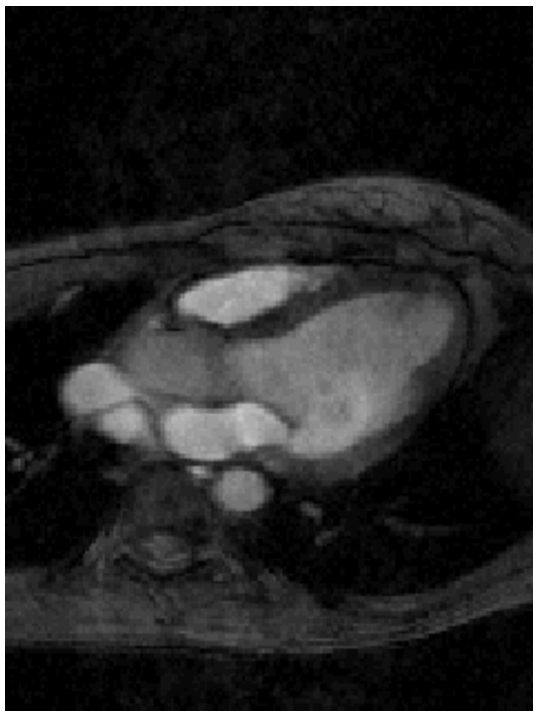
Transcriptional Analysis of the Mouse Heart

Hypotheses

1. Transcriptional regulation in mouse models of cardiac disease implicates genes that participate in the pathology.

2. Genetic variation in these genes contributes to susceptibility to heart disease in humans.

Human ventricular pathology



Normal

HCM

DCM

Strategy for human studies

Mouse Model

↘

Transcriptional Analysis

↘

Candidate Genes

↘

Human Polymorphism

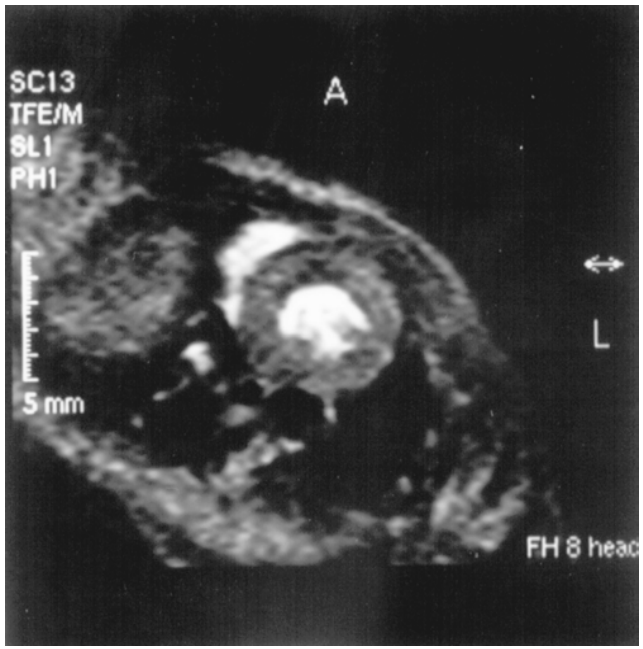
↘

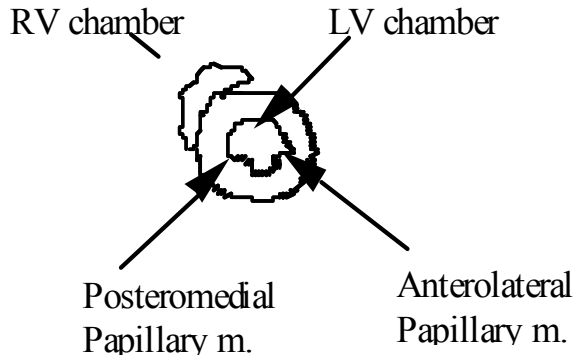
Association Study

Mouse Models of Cardiac Disease

- Dilated Cardiomyopathy - doxorubicin
- Hypertrophy - isoproterenol, aortic banding
- Inflammation - lipopolysaccharide
- Atherosclerosis - diet in “humanized” lipidemia

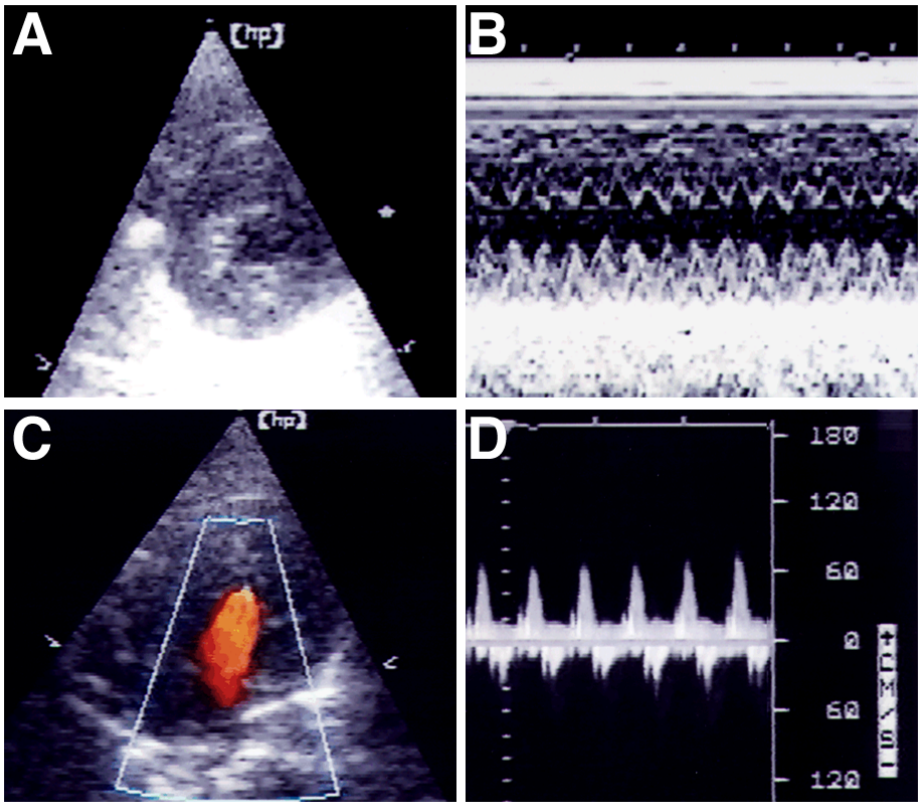
MRI of Mouse Heart - Imaging





MR imaging of mouse heart. Gated 2mm sections were acquired with a 1.5 Tesla instrument, producing 195 micron resolution. A short axis view of the left ventricle is shown at the level of the papillary muscles. A sketch of some of the structures seen is provided to the right.

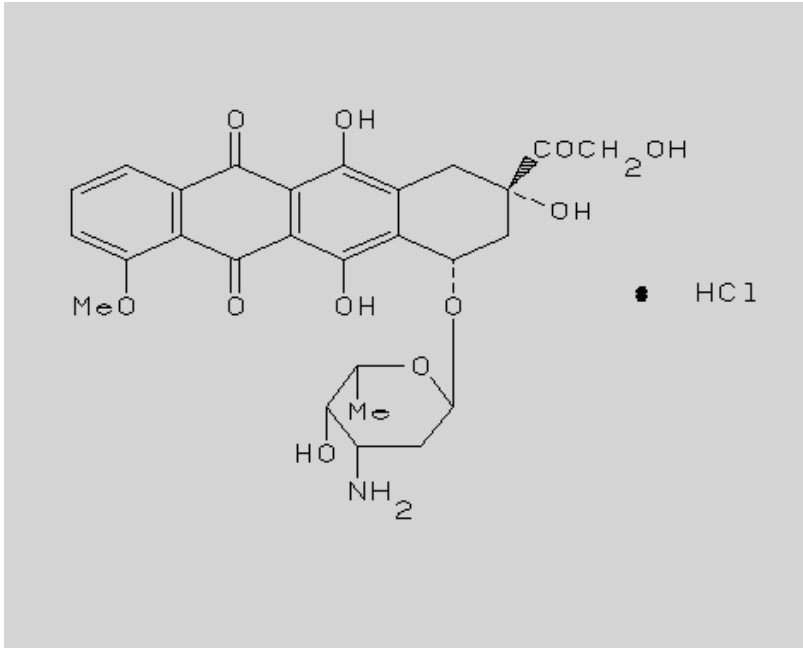
Echocardiography in mice



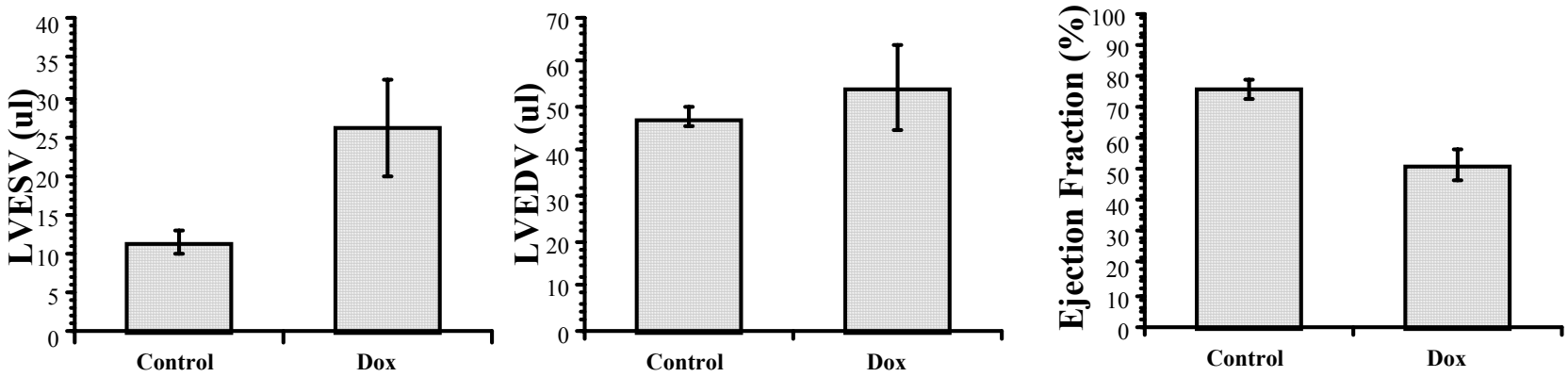
Echocardiography of mouse hearts is performed using a 12 Mhz transducer and brief Avertin anesthesia. Panel A shows a 2D short axis view and panel B the m-mode view at the same level allowing precise temporal resolution of the cardiac cycle as well as fractional shortening and wall thickness. Panel C shows diastolic flow across the mitral valve by color doppler and Panel D the spectral analysis of this flow which provides information about ventricular compliance and left atrial pressure.

Doxorubicin - Clinical details

- Responsive tumors:Breast, esophogus, osteosarcoma, Kaposi's, Hodgkin's and non-Hodgkin's.
- Principal limitation: Cardiomyopathy at doses>550 mg/m^2.
- Risk factors for DCM:Age>70, radiation, other cardiac disease, hypertension, liver disease.

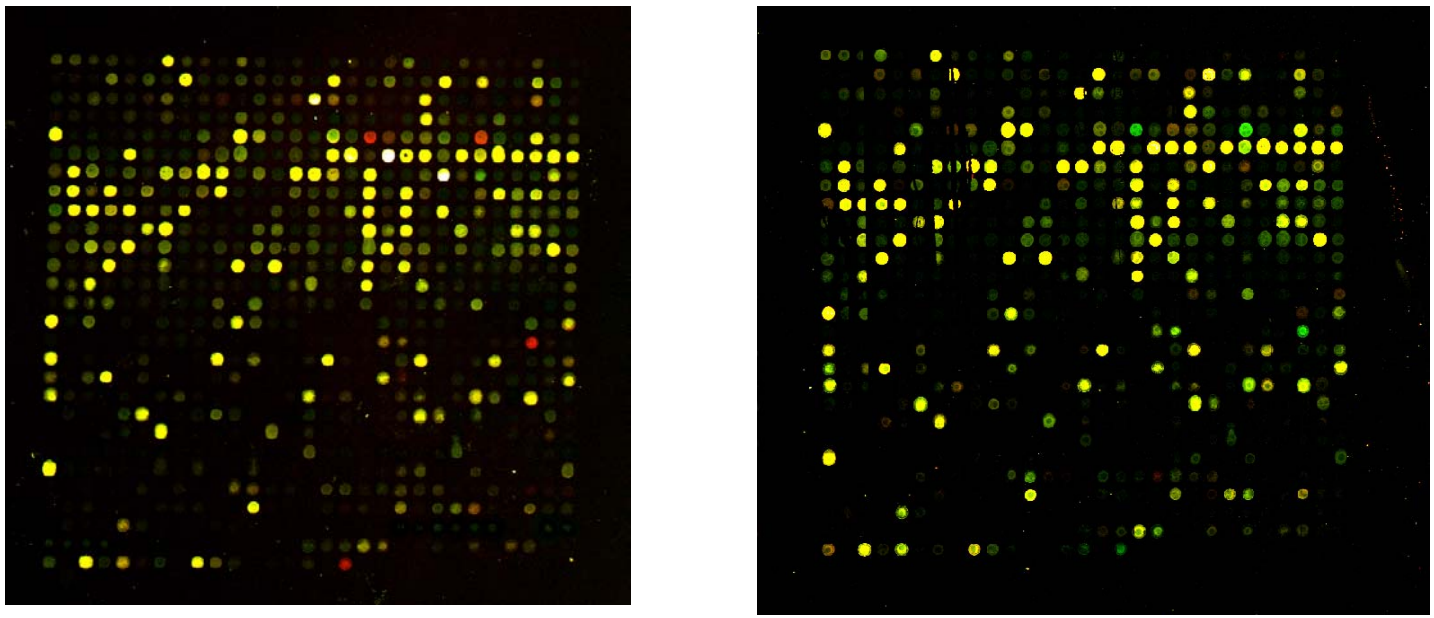


Murine Doxorubicin Cardiomyopathy



Left ventricular end-diastolic volume (LVEDV), end-systolic volume (LVESV), and ejection fraction as determined by MRI after 12 weeks of adriamycin (ADR) at 3 mg/kg ip/wk. Control n=3, ADR n=12

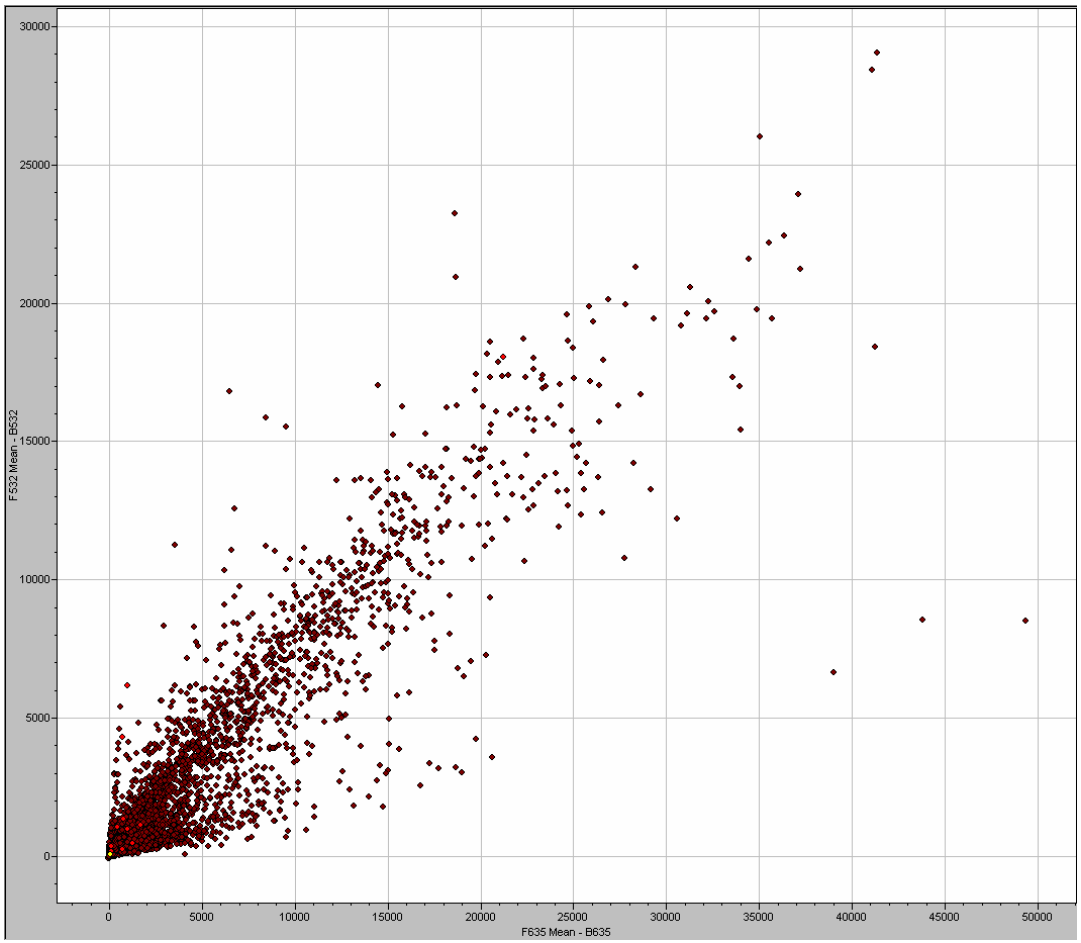
Microarray Hybridization



On the left panel RNA from the heart of a mouse treated with 15 mg/kg doxorubicin was labeled with Cy3 and hybridized simultaneously with Cy5 labeled RNA from an untreated mouse. Upregulated genes are red and downregulated genes are green. On the right the same portion of the array is shown with the samples labeled in the opposite manner.

Results: Doxorubicin 15 mg/kg Day 1

Cy3 channel intensity

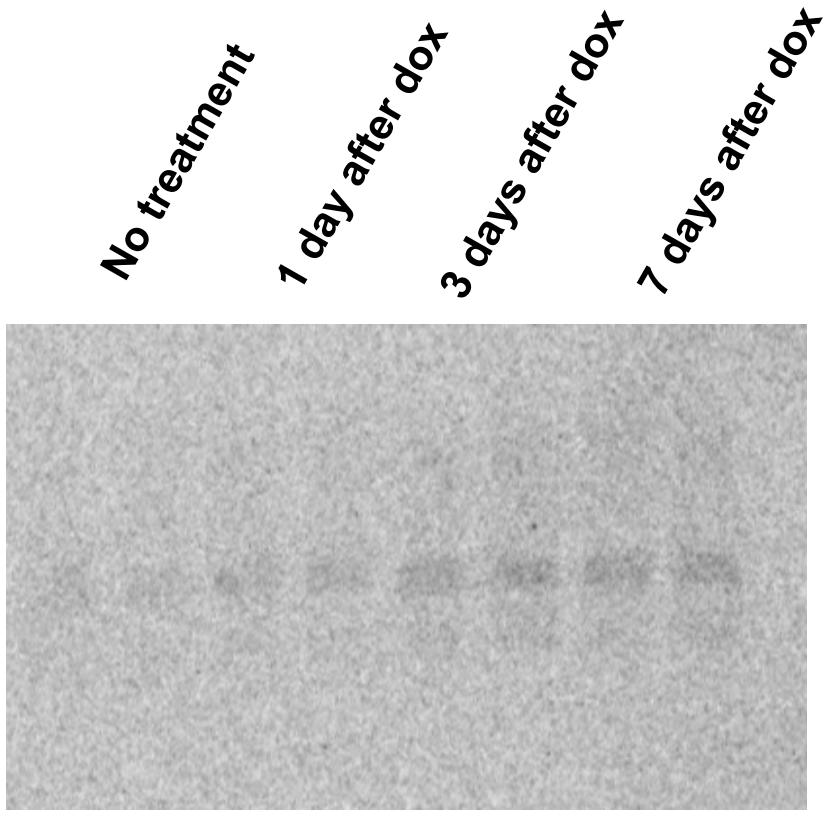


Cy5 channel intensity

Regulated Genes

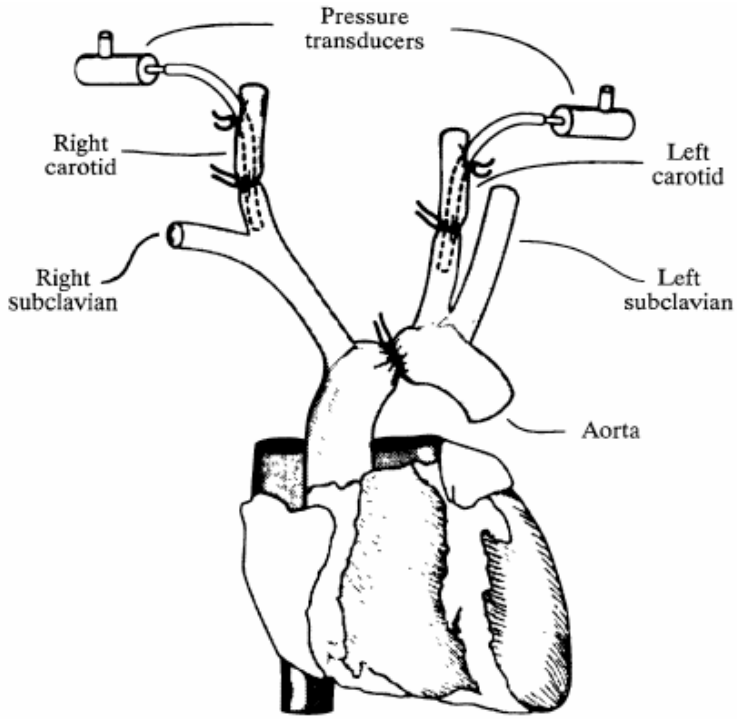
Clone ID	*Sequence Homology*	*Log_{10} Regulation* *Exp 1*	*Exp 2*
H14B7	**Mus musculus N-myc downstream r...**	1.19	0.93
SM15F11	**Zinc finger protein [Homo sapiens]**	1.20	1.12
SM15E8	**Mus musculus carbonic anhydrase III**	-0.46	-1.14
SM32C1	**Mouse mRNA for 21 kd polypep**	-0.32	-0.35

Confirmation of Array Results



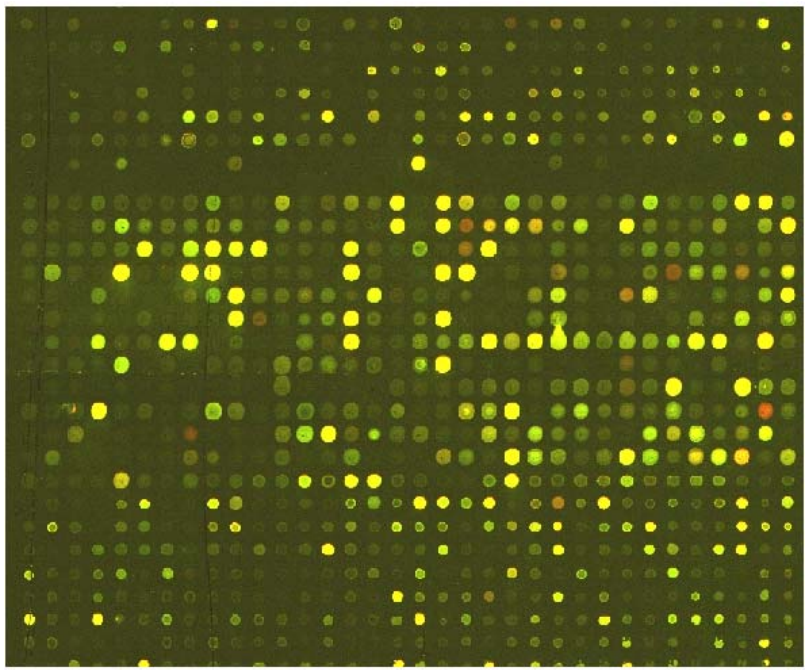
Northern analysis of clone SM15F11, a zinc finger protein of unknown function, shows progressive upregulation in the hearts of mice treated with 15 mg/kg doxorubicin

Aortic banded cardiac hypertrophy



Aortic banding was maintained for periods of one, four, and eight weeks (by H. Rockman, Duke Univ.). Previous analysis of this model has shown rapid and transient expression of an immediate-early gene program, an increase of heart weight/body weight ratio, and an up-regulation of the ANF gene. The aortic gradient was measured, as depicted, prior to sacrifice. Total RNA was isolated from the left ventricles of 3 mice with gradients between 60-70 mmHg and pooled for analysis.

Microarray hybridization of hypertrophic RNA



Probes were prepared from 20 μg of pooled total RNA by reverse transcription incorporating Cy3 or Cy5. The labeled probes were combined and hybridization was carried out overnight. Slides were read in a two-color fluorescent scanner. Each pooled RNA sample was analyzed in at least two array hybridizations including at least one in which the labeling dyes were reversed. Agreement between replica experiments was high with Pearson coefficients >.8 for the entire array and >.9 for genes showing >2 fold regulation.

Named genes > 3-fold upregulated in aortic banding

Unigene Number	Unigene Name	Acc. Number	Week 1	Week 4	Week 8
Mm.19961	Natriuretic peptide precursor type A	AA139662	1.695	1.002	0.954
Mm.87854	ATPase, Cu++ transporting, beta polypeptide	BG795233	1.091	0.635	0.516
Mm.10279	Ankyrin-like repeat protein	BG791506	0.672	0.600	0.491
Mm.29189	Adenylate kinase 1	BG795229	1.092	0.684	0.417
Mm.196608	Actin, alpha 1, skeletal muscle	BG794199	0.849	0.775	0.436
Mm.41326	G protein beta subunit-like	NM_019988	0.842	0.581	0.300
Mm.142368	Hemoglobin, beta adult major chain	BG795799	0.737	0.770	0.298
Mm.39744	Small EDRK-rich factor 2	BG794287	0.722	0.878	0.263
Mm.147387	Procollagen, type III, alpha 1	AA855884	1.137	0.782	0.067
Mm.30145	Ectonucleotide pyrophosphatase/phosphodiester	AA915069	0.886	0.581	-0.041
Mm.695	Abl-interactor 1	BG796249	0.621	0.403	0.357
Mm.18709	Sarcoglycan, alpha (50kDa dystrophin-associate	BG795635	0.623	0.295	0.262
Mm.116715	Myocyte enhancer factor 2A	NM_013597	0.534	0.341	-0.194
Mm.142866	Mus musculus pigpen protein mRNA, complete c	AA914803	0.560	0.323	-0.082
Mm.3126	Four and a half LIM domains 1	BG795521	0.856	0.026	0.058
Mm.83615	Transient receptor protein 2	NM_011644	0.673	0.026	0.005
Mm.4069	ATP synthase, H+ transporting, mitochondrial F1	BG794143	0.601	0.026	-0.010
Mm.6712	Desmin	AA433773	0.568	0.210	-0.023
Mm.22753	Cathepsin B	BG792886	0.558	0.111	-0.118
Mm.28687	Moesin	AA139641	0.548	0.065	-0.002
Mm.156892	Heterogeneous nuclear ribonucleoprotein D	NM_007516	0.537	0.074	0.017
Mm.196344	Clusterin	BG792204	0.534	0.201	0.008
Mm.584	Annexin A2	NM_007585	0.507	0.027	-0.052
Mm.7	Vimentin	BG794141	0.493	0.039	0.059
Mm.9745	Lactate dehydrogenase 2, B chain	BG792776	0.368	0.634	0.286
Mm.35439	Secreted acidic cysteine rich glycoprotein	BG793684	0.333	0.628	0.165
	NOVEL	BG796096	0.461	0.512	0.224
Mm.2740	Natriuretic peptide precursor type B	NM_008726	0.239	0.269	0.513

Named genes > 3-fold downregulated in aortic banding

Unigene Number	Unigene Name	Acc. Number	Week 1	Week 4	Week 8
Mm.196173	Actin, gamma, cytoplasmic	BG795136	-0.621	-0.461	-0.508
Mm.2112	Enoyl coenzyme A hydratase 1, peroxisomal	BG793116	-0.541	-0.377	-0.306
Mm.604	Troponin I, cardiac	AA839930	-0.511	-0.360	-0.305
Mm.19154	Succinate-Coenzyme A ligase, ADP-forming, bet	AA919318	-0.491	-0.370	-0.181
Mm.157893	Major urinary protein 1	BG796085	-0.638	-0.441	-0.154
Mm.34145	Phospholamban	BG817275	-0.486	-0.320	-0.087
Mm.2287	Proteasome (prosome, macropain) subunit, alpha	NM_011967	-0.603	-0.298	-0.187
Mm.1639	Myeloid cell leukemia sequence 1	BG796271	-0.603	-0.252	-0.190
Mm.16228	Solute carrier family 25 (mitochondrial carrier; ad	BG792403	-0.508	-0.259	-0.065
Mm.142822	Ewing sarcoma homolog	BG795113	-0.602	-0.084	0.039
Mm.3894	FK506 binding protein 10 (65 kDa)	NM_010221	-0.533	-0.184	-0.233
Mm.2966	Isocitrate dehydrogenase 2 (NADP+), mitochond	BG795948	-0.530	-0.027	-0.099
Mm.3193	Protein kinase inhibitor, alpha	BG795171	-0.495	-0.235	-0.232
Mm.16766	Protein kinase, cAMP dependent, catalytic, beta	NM_011100	-0.492	-0.248	-0.124
Mm.597	Platelet-activating factor acetylhydrolase, isoform	NM_008776	-0.490	-0.130	-0.227
Mm.22257	FTSJ-like protein (E. coli)	AA117637	-0.489	-0.023	0.007
Mm.10530	Acetyl-Coenzyme A dehydrogenase, medium cha	NM_007382	-0.483	-0.124	-0.230
Mm.16323	Eukaryotic translation initiation factor 4A2	BG796047	-0.481	-0.202	-0.141
Mm.14543	Endothelin 1	NM_010104	-0.480	-0.215	-0.160
Mm.2820	Galactokinase	NM_016905	-0.479	-0.178	-0.112

Results of human studies

1. Genes examined 180
2. Polymorphisms detected >1000
3. Nonsynonymous polymorphisms 186
4. Subjects enrolled
    - Reynolds cohort 4000
    - Cath lab 3500
    - Families 200

Acknowledgements
“Art is I, science is we” (Claude Bernard)

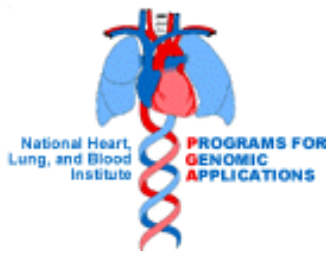
Teresa Gallardo - array creation

Eduardo Fernandez - developed dox model

Zakir Siddiquee - made mice, array hybs

Jeff Schagemann - informatics

Support from an Advanced Technology Grant of the Texas Higher Education Coordinating Board and the NIH Program for Genomic Applications



quidquid latine dictum sit, altum viditur

("Anything is more impressive if you say it in Latin")