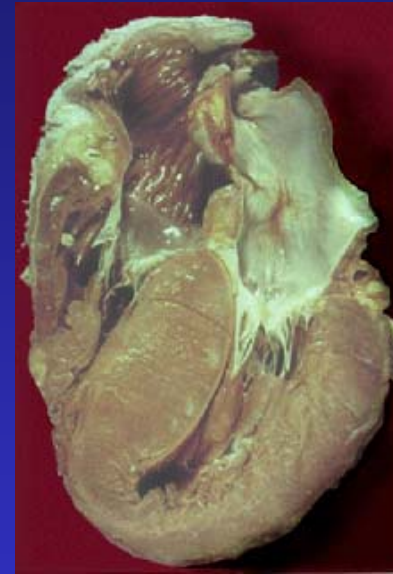
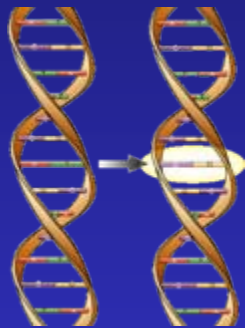


Title: **Cardiomyopathies in mice and men**

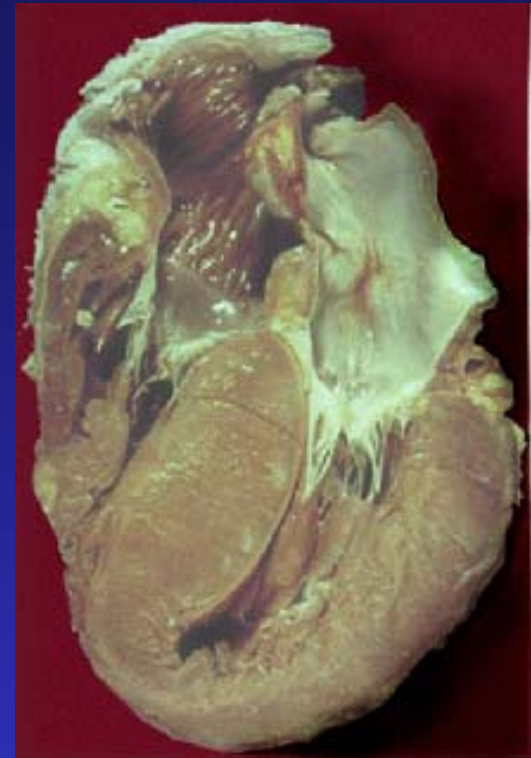
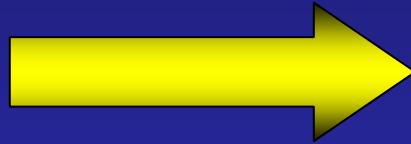
Subtitle: **PMAGE: A Systems Biology approach to Transcriptional Profiling**



Disclosure Information:

J.G. Seidman advises the LMM/HPCGG and is spouse of Dr. C. Seidman, Director of the CVGC (BWH)

Cardiac Hypertrophy



**Independent Risk Factor for Adverse CV Outcomes
Particularly Heart Failure**

Affects: Children, Adults, Aged

Triggers: Hypertension, Diabetes, Valve Disease

Idiopathic: 3% US Population has Unexplained LVH

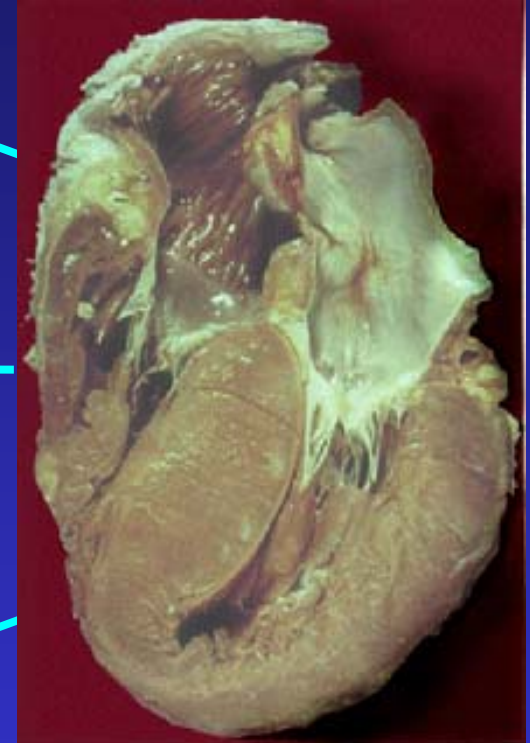
Triggers for Hypertrophic Remodeling



Pressure
overload (BP)

HCM

Glycogen
storage

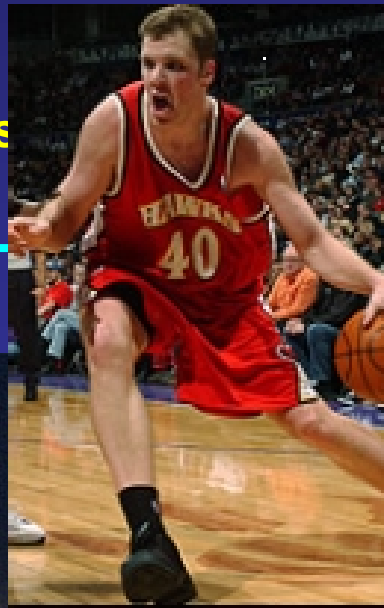


Hypertrophic Cardiomyopathy : Clinical Features

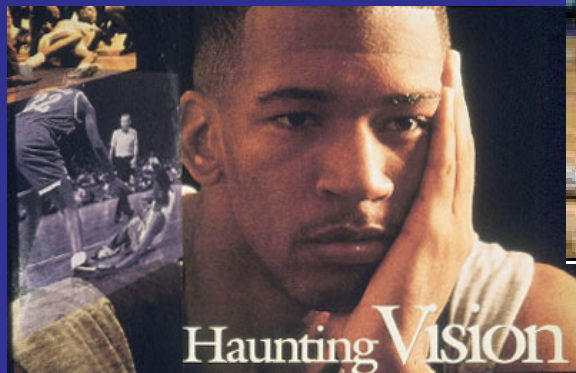
Inheritance: **Autosomal dominant**
Sporadic

Symptoms: **Absent**
Dyspnea
Angina
Palpitations
Syncope

Disease Expression: **Striking histopathology**
Variable hypertrophy
Atrial enlargement
Sudden death
Heart failure



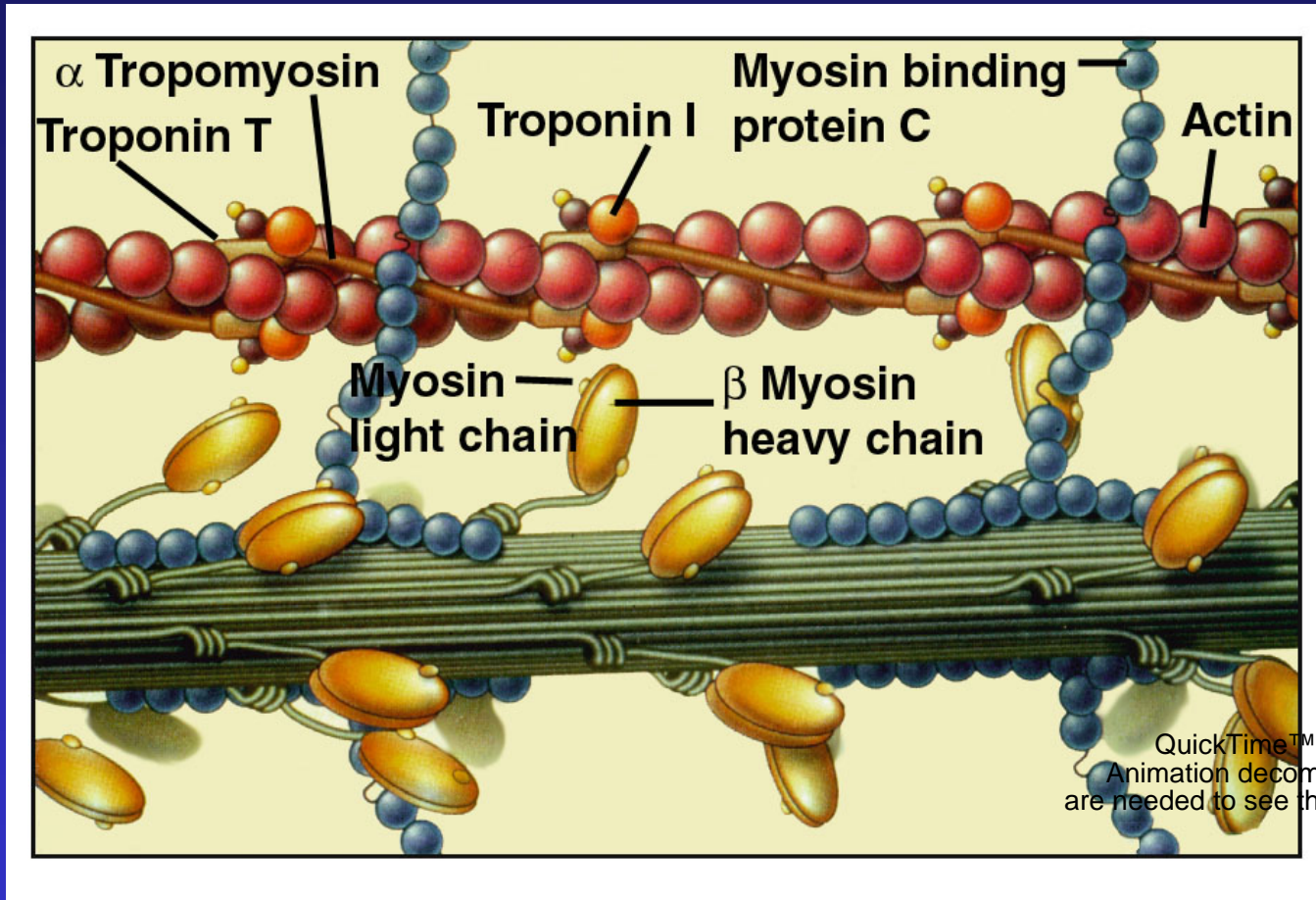
**50% of individuals who die
on the athletic field exhibit
LVH and HCM histopathology**



Sports Illustrated (2001)

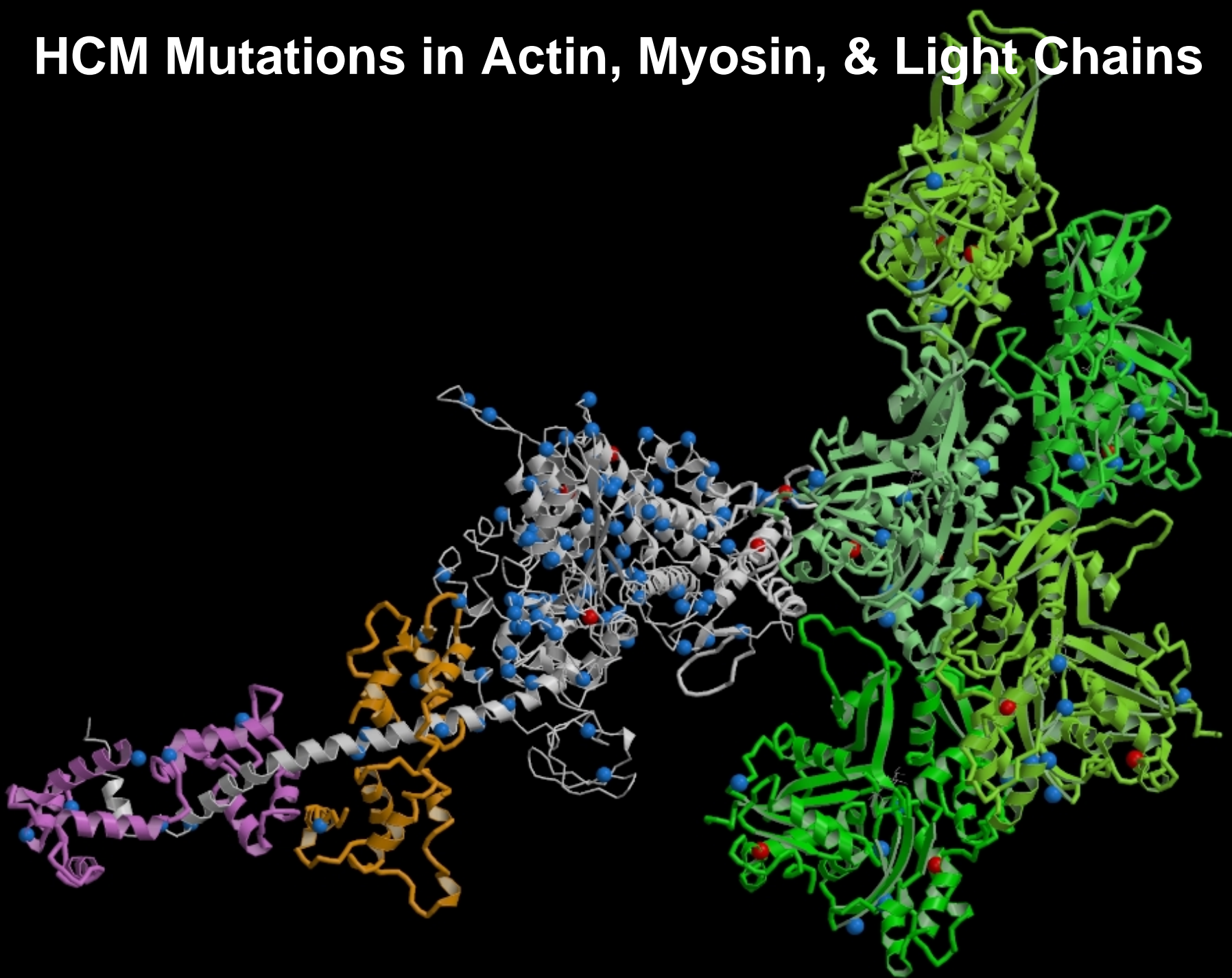
(Collier ESPN, 2005)

Sarcomere Protein Gene Mutations



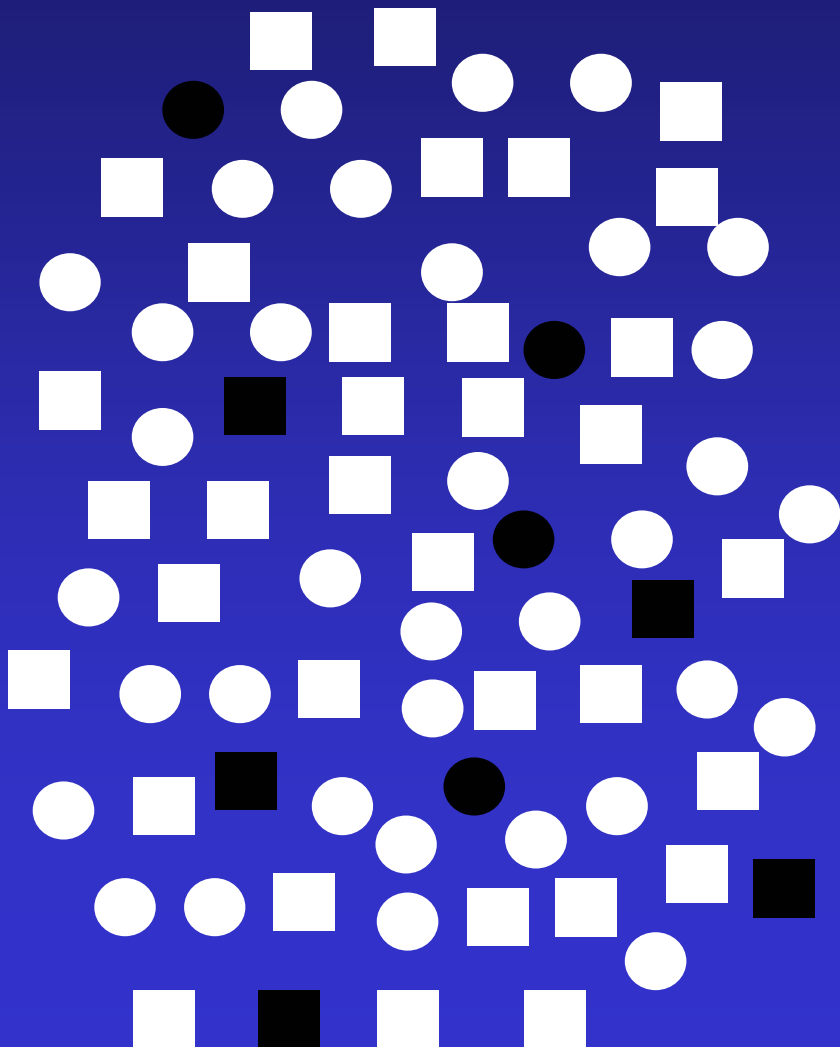
- Dominant negative mutations create defective sarcomeres

HCM Mutations in Actin, Myosin, & Light Chains

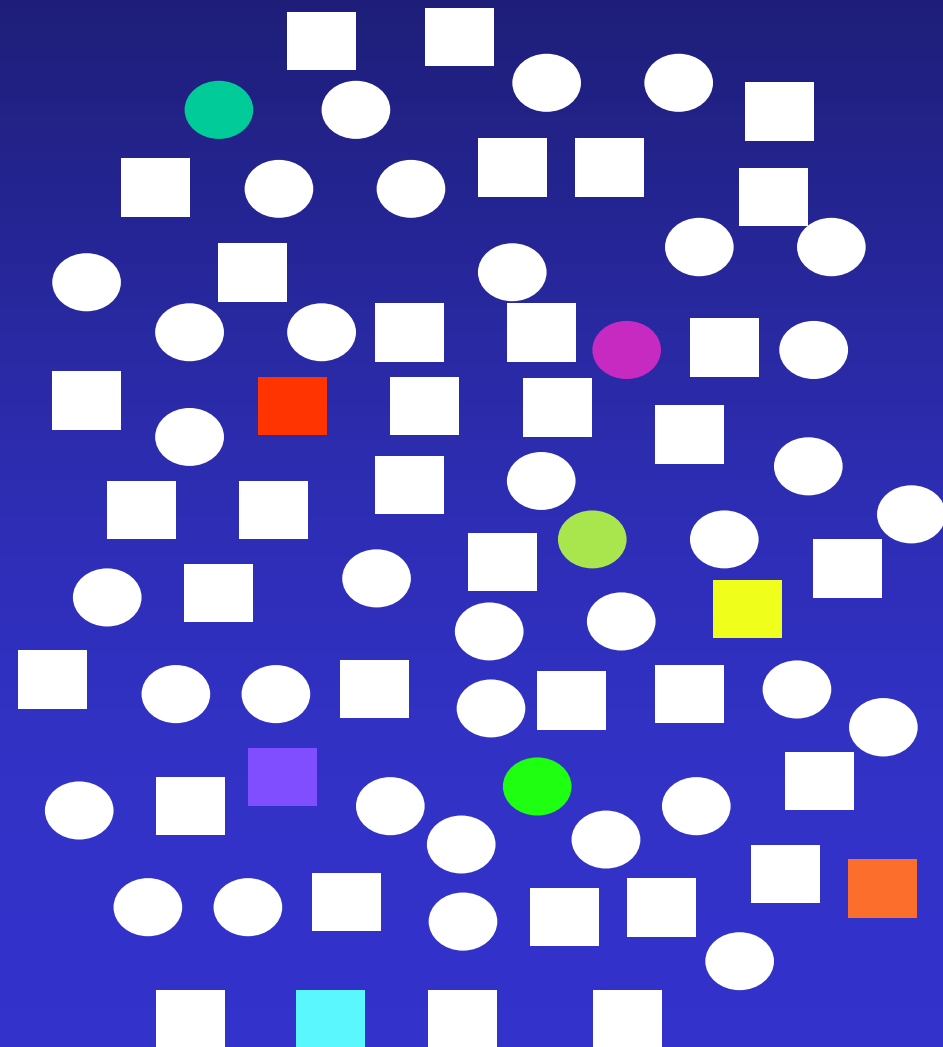


Explaining common heritable disease: Two Common Hypotheses

COMMON variant



Multiple RARE variants



Epidemiology of HCM

Elderly Onset HCM: 20% of subjects have a mutation (Niimura et al., Circ, 2002)

Pediatric HCM cohort: 72 patients age of onset <12 years with unexplained hypertrophy and without family history

Framingham Heart Study

Pediatric Hypertrophy Cohort

**72 patients <10 years old with
unexplained hypertrophy**

33 Mutations found in 72 probands

Mutation	Gene	Reported or novel
H90Y	ACTC	Novel
D605N	MYBPC3	Reported
G1248R	MYBPC3	Novel
G278E	MYBPC3	Reported
G490R	MYBPC3	Reported
Intron31 DS+2t/g	MYBPC3	Novel
R495G	MYBPC3	Novel
R502Q	MYBPC3	Reported
R502W	MYBPC3	Reported
R502W	MYBPC3	Reported
R502W	MYBPC3	Reported
R943X	MYBPC3	Reported
IVS31+2T>G/T1028S	MYBPC3/MYBPC3	Novel/Novel
E924K	MYH7	Reported
E924K	MYH7	Reported
K146N	MYH7	Reported
L1414M	MYH7	Novel
L908V	MYH7	Reported
L908V	MYH7	Reported
L908V	MYH7	Reported
L908V	MYH7	Reported
R719Q	MYH7	Reported
S1836L	MYH7	Novel
V606M	MYH7	Reported
R787C/R97C	MYH7/ACTC	Novel/Novel
R663H/V763M	MYH7/MYH7	Reported/Novel
IVS5+6C>T	MYL3	Novel
H530R	PRKAG2	Novel
K178del	TNNI3	Reported
E96del	TNNT2	Novel
R92Q	TNNT2	Reported

45% of Pediatric cases have an HCM mutation

Collaborators: Jeff Towbin, Neil Bowles

Comparison of the Distribution of Sarcomere Protein Gene Mutations

	HCM	Pediatric onset HCM	Elderly-onset HCM <small>(Niimura H et al.)</small>	LVH in FHS <small>(Morita H et al.)</small>
β-MHC	79 (22.3%)	13 (18.8%)	0	1 (2.0%)
MyBP-C	81 (22.9%)	12 (17.4%)	5 (16.1%)	4 (8.0%)
Troponin T	15 (4.2%)	2 (2.9%)	0	1 (2.0%)
Troponin I	19 (5.4%)	1 (1.4)	2 (6.5%)	1 (2.0%)
Tropomyosin	2 (0.6%)	0	0	0
Essential MLC	2 (0.6%)	1 (1.4%)	ND	1 (2.0%)
Regulatory MLC	5 (1.4%)	0	ND	0
Actin	0	1(1.4%)	ND	0
Total HCM mutations	203 (57.3%)	30 (44.8%)	7 (22.6%)	8 (16.0%)
Storage mutations	8 (2.2%)	3 (4.2%)	N/A	1 (2%)
Total	354	72	31	50

Framingham Heart Study

1948: First Cohort Recruited

1971: Addition of Spouses + Offspring Cohorts
Initial and Periodic Cardiovascular Evaluation
Longitudinal Assessment for CV Disease

3% of Cohort VI have LVH

Table 1. Characteristics of Participants With and Without Increased LVWT (maximum LVWT >13 mm)

	LVWT ² 13 (N=1812)	LVWT>13 (N=50)	P Value*
Clinical			
Age, years	58±9	62±9	0.004
Women, n (%)	958 (53)	9 (18)	<0.001
Body mass index, kg/m ²	27.7±4.8	31.5±5.0	<0.001
Physical exam 3/6 systolic murmur, n (%)	27 (1)	4 (8)	0.01
Systolic BP, mmHg	126±15	132±12	0.22
Diastolic BP, mmHg	75±9	77±8	0.16
BP treatment, n (%)	460 (25)	32 (64)	<0.001
Statin treatment, n (%)	196 (11)	7 (14)	0.98
Heavy physical activity, n (%)	145 (8)	4 (8)	0.78
Prior myocardial infarction or coronary insufficiency, n (%)	80 (4)	4 (8)	0.97
Angina, n (%)	87 (5)	2 (4)	0.35
Syncope, n (%)	129 (7)	8 (16)	0.02
Prior stroke, n (%)	31 (2)	0 (0)	0.98
Heart Failure, n (%)	16 (1)	0 (0)	0.38
NYHA class I or higher, n (%)	150 (8)	5 (10)	0.94
Atrial fibrillation, n (%)	51 (3)	2 (4)	0.78
ECG LV hypertrophy			
With repolarization changes, n (%)	4 (0.2)	3 (6)	<0.001
Without repolarization changes, n (%)	137 (8)	7 (14)	0.27
ECG PR interval <120 msec n (%)	80 (4)	0 (0)	0.24
Estimated glomerular filtration rate, mL/min/1.73 m ²	90 ± 45	83 ± 28	0.58
Chronic kidney disease, n (%)	176 (10)	6 (12)	0.79
Echocardiographic			
Left atrial diameter, mm	39.7±5.3	46.1±6.5	<0.001
LV diastolic diameter, mm	48.0±5.2	48.1±5.5	0.02
LV fractional shortening, %	36.8±5.7	37.9±5.9	0.03
Interventricular septal thickness, mm	9.7±1.2	14.2±1.4	<0.001
LV posterior wall thickness, mm	9.5±1.1	12.6±1.6	<0.001
Maximal LV wall thickness, mm	9.9±1.1	14.3±1.4	<0.001
IVS/PW ratio	1.03±0.09	1.15±0.14	<0.001
Continuous variables, mean±SD; *P values were age- and sex-adjusted.			
Abbreviations: SD, standard deviation; BP, blood pressure; LV, left ventricular; NYHA class, New York Heart Association classification; Wall thicknesses measured at end diastole.			

Comparison of the Distribution of Sarcomere Protein Gene Mutations

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Genetically Engineered Models of HCM

Knock-in models:

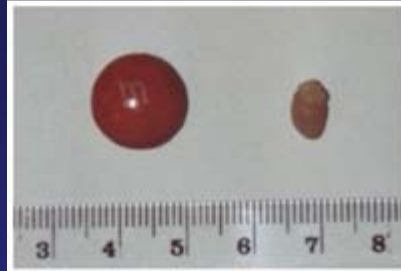
HCM

Arg403Gln

Arg453Cys

Val606Met

Arg719Gln



QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

DCM

Phe764Leu

Ser532Pro

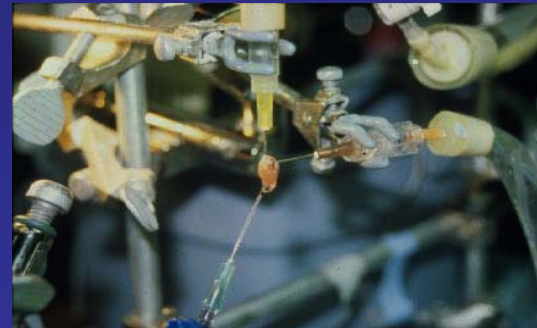
Left ventricular hypertrophy

(LVAWT = 1.15 ± 0.03 ; $p < 0.005$)

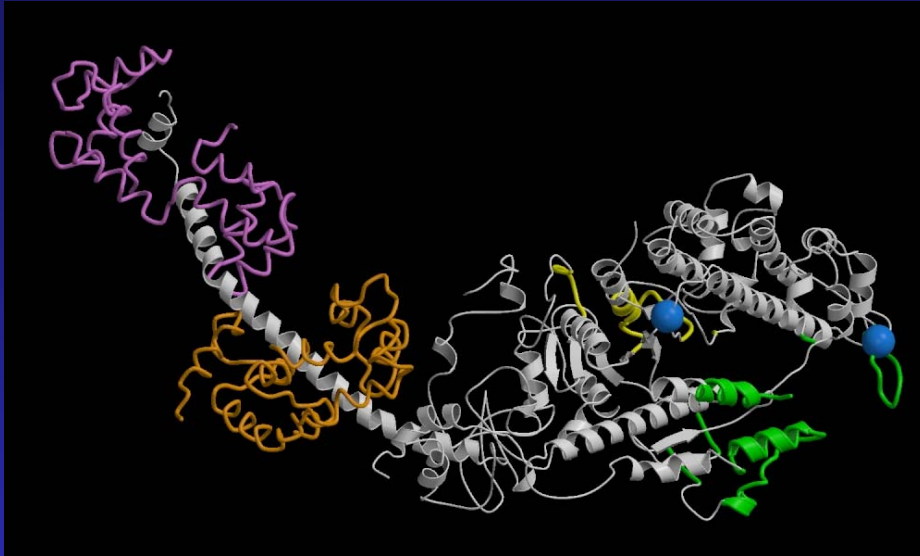
Left atrial enlargement

Myocyte hypertrophy & disarray

Abnormal cardiac function



Insights from Mouse HCM Models



2 Fold > Isometric Force
↑ Actin Filament Sliding
Velocity
2X ATPase Hydrolysis
HCM Mutations
↓
“Super” Myosins

LV wall thickness

Myocyte disarray, fibrosis

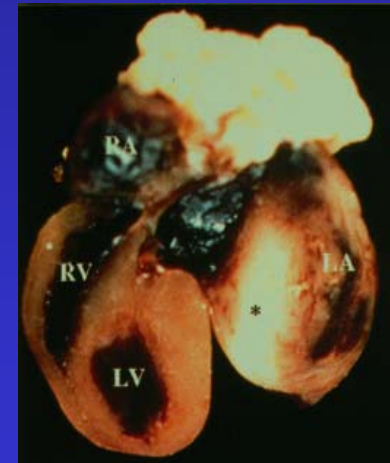
Cardiac dysfunction

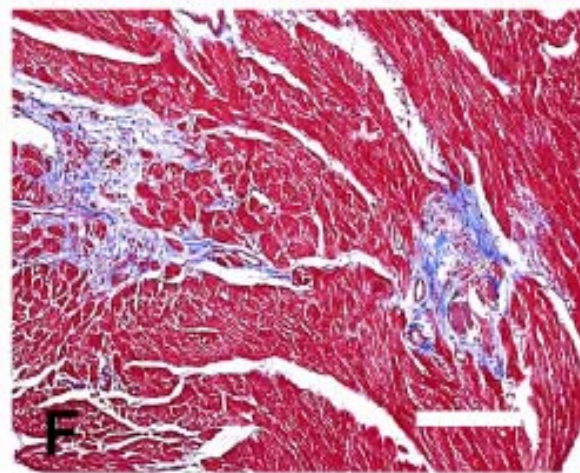
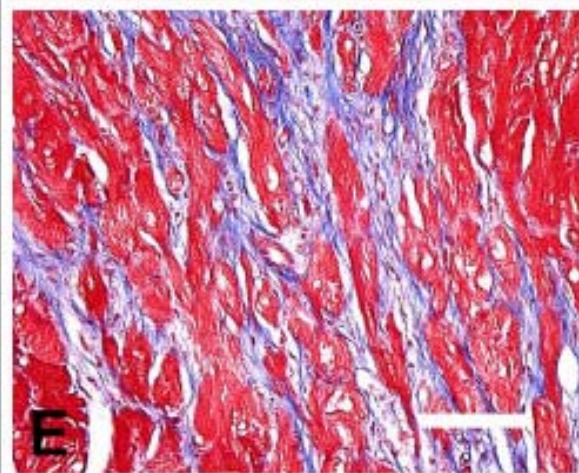
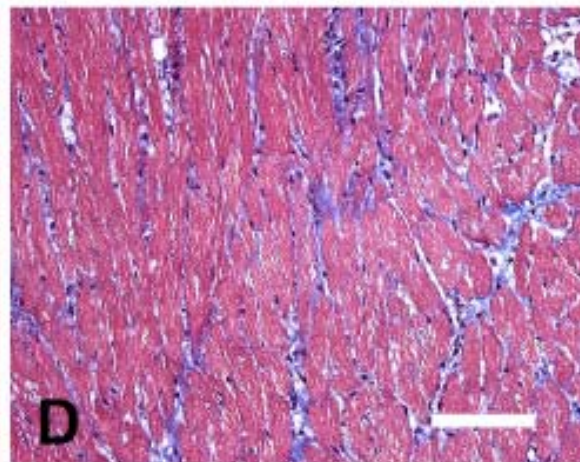
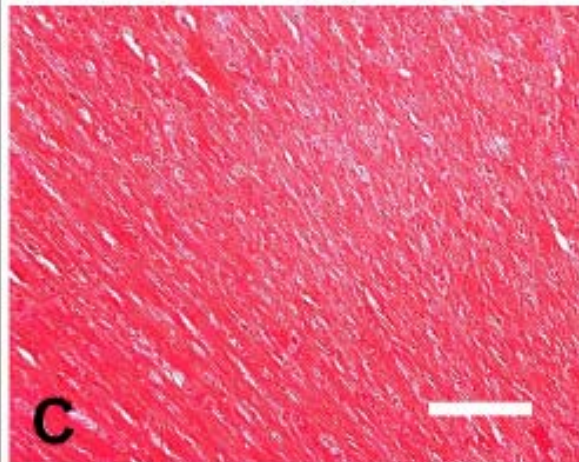
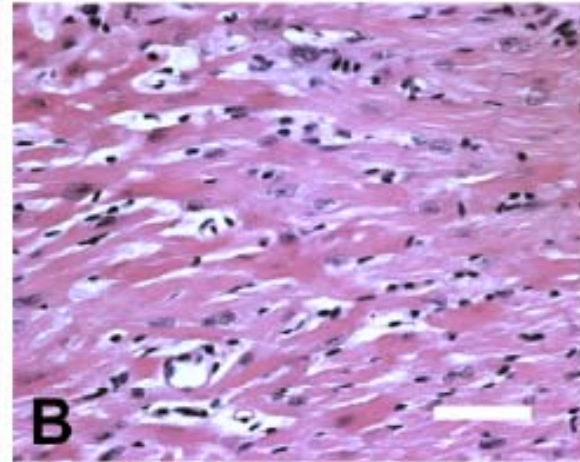
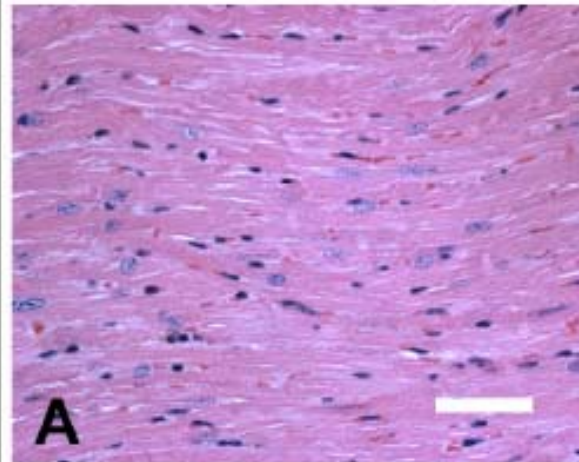
LA enlargement

BIRTH

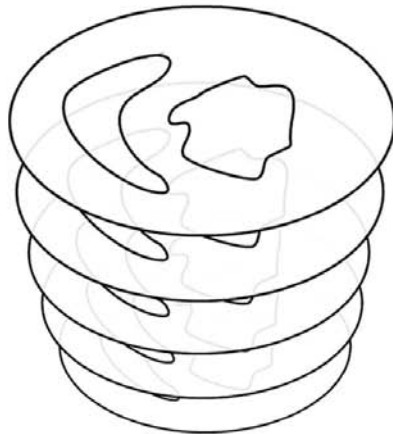
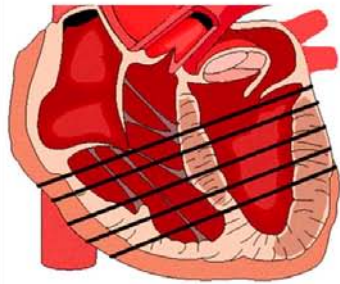
10-20 wks

30-50wks

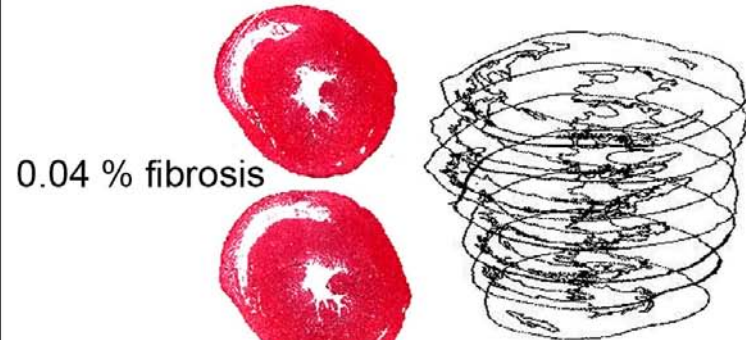




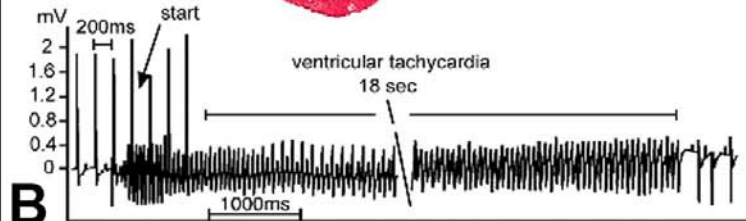
Assesment of Ventricular Fibrosis, Myocyte & Arrhythmogenicity in HCM Mice



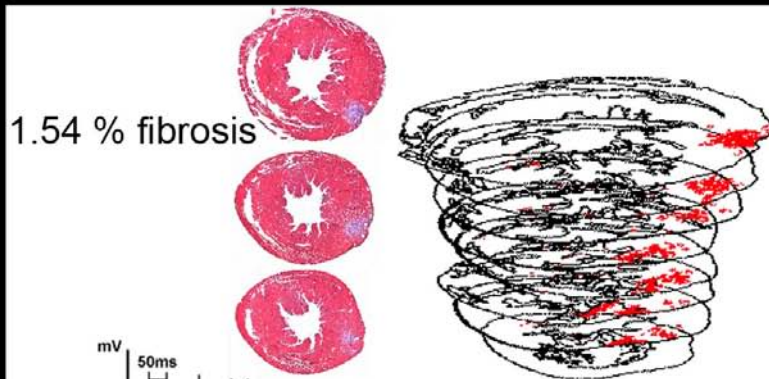
A



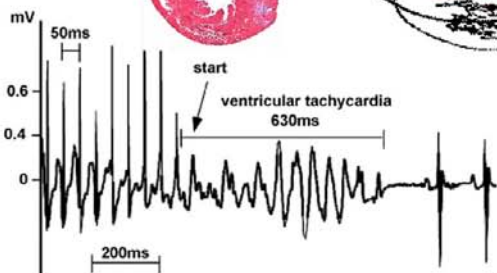
0.04 % fibrosis



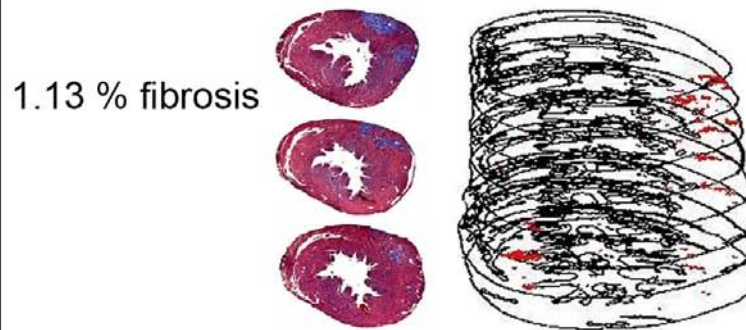
B



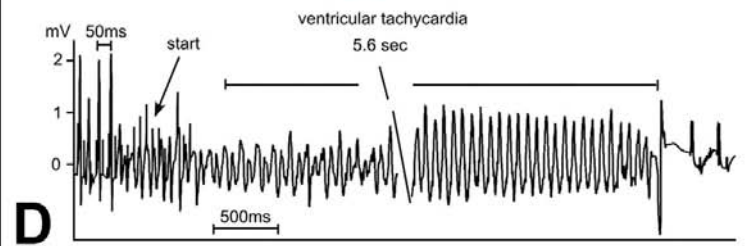
1.54 % fibrosis



C



1.13 % fibrosis



D

Sarcomere Gene Mutation



**Biophysical Defects
Force/Relaxation**



**Pathologic
Remodeling**

Somatic Events

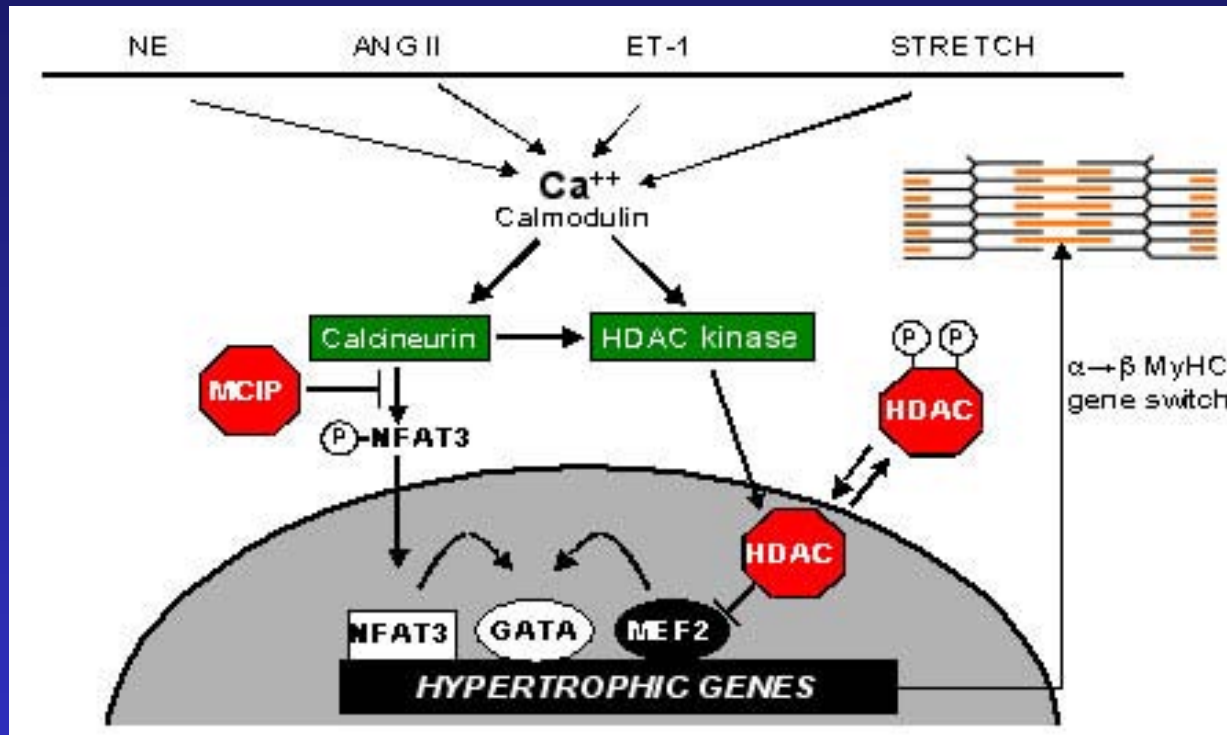


**Disarray
Fibrosis**

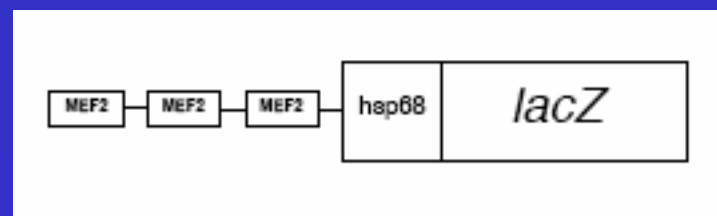


**Hypertrophy
Arrhythmias**

Do Pressure Overload Hypertrophy and HCM activate the same pathways?



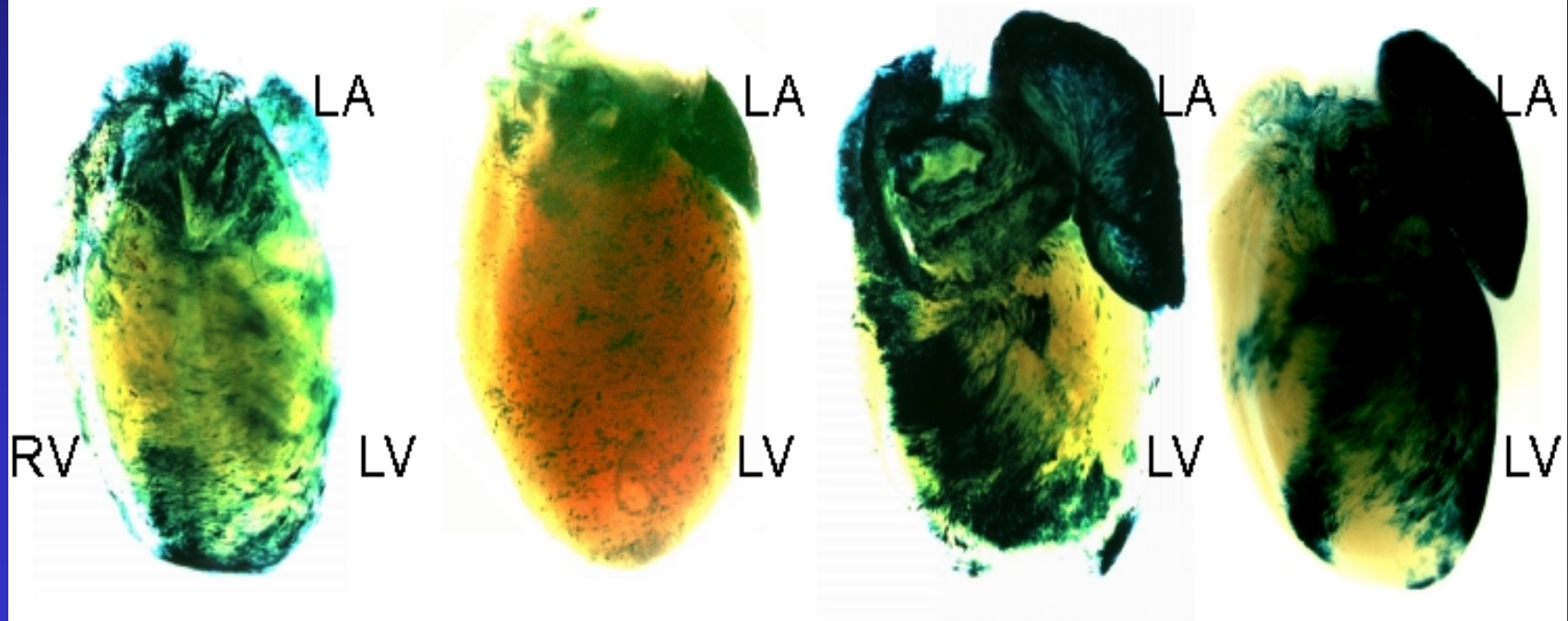
Reporter construct:



Variable Mef2 Induction in HCM Mice

Mef2-LacZ/WT

Mef2-LacZ/MHC⁴⁰³



Age: 12 wk

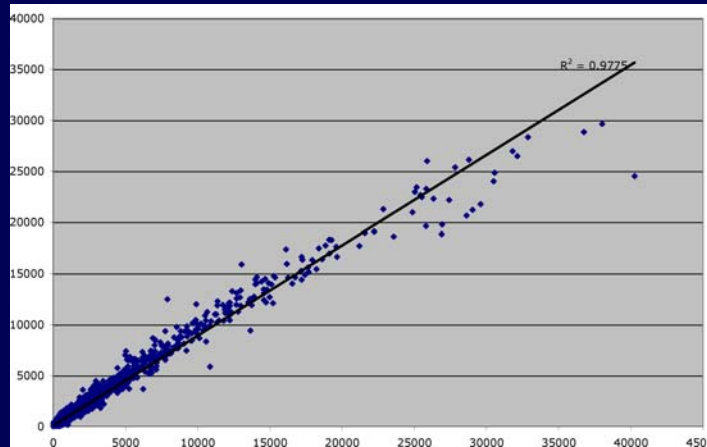
12 wk

30wk

30 wk

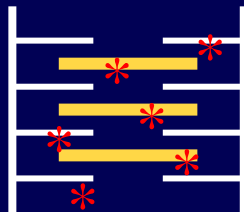
HCM: Transcriptional Profiling Approaches

Microarray Analyses

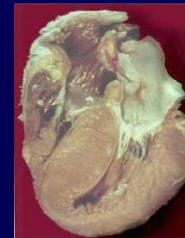


Cardiogenomics.med.harvard.edu

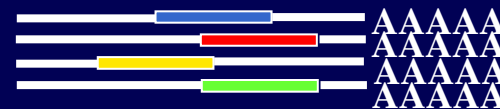
Gene mutation



Hypertrophy phenotype



Serial Analysis of Gene Expression (SAGE)



cDNA



Ditags PCR amplified



Tags concatenated and Sanger sequenced

SAGE: determination of hypertrophy-regulated mRNAs in the mouse left ventricle and septum

Tags: wildtype α MHC^{403/+}
 70,731 69,258

Comparison Unique Tags (p<0.01)
 wildtype vs. α MHC^{403/+} 51

SAGE tag	wildtype	α MHC ^{403/+}	Gene
TAGGGAGATG	68	115	CARP
TAGGGAGATG	32	56	alpha actin
CCCCCGCAGG	15	62	ANP
GATCTCCTGA	0	9	BNP

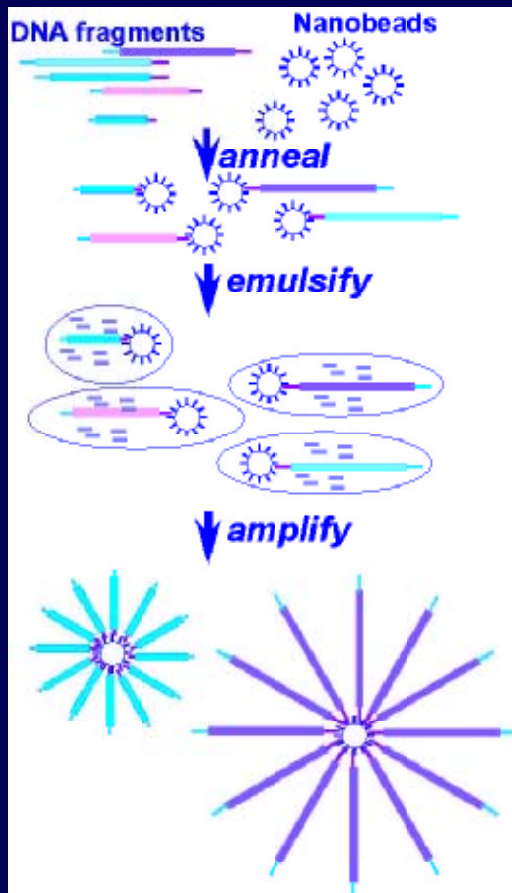
Limitations of SAGE in cardiac expression profiles

TAG sequence	α MHC ^{403/+} tag counts	Identification
CCTGCCCCTC	3	GATA4
CTGGCCGCCT	2	Nkx2.5
TATATTTTAT	0	MEF2A
GGCTCAAGAT	0	NFATc3
TTCCCCGATT	0	Tbx5
ATGGATAAAG	0	Eya2

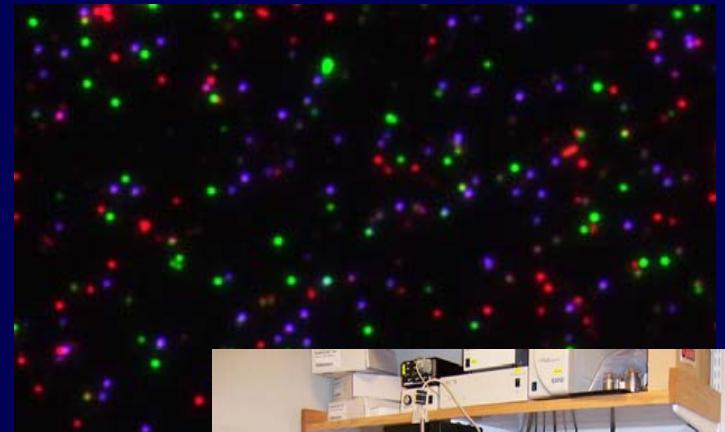
We need new tools to look for rare but important mRNAs such as transcription factors...

PMAGE (Polony Multiplex Analysis of Gene Expression)

Polonies (PCR + colonies)



Sequence by ligation



Polony chemistry

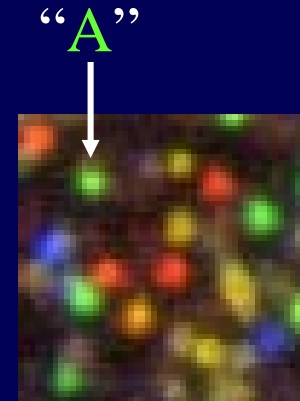


- Anneal anchor oligo
- Ligate query nonamer

Polony chemistry



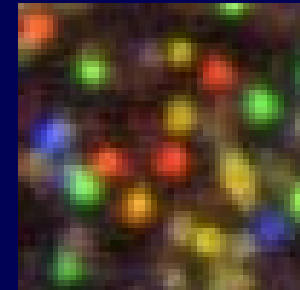
- Epifluorescence image array



Polony chemistry

● ... (5') GGCAGTCGGTGATCTGAAGCTCATGNNNNNNNNNAGAGAATGAGGAACCCGGGGCAG (3')

- Strip fluorescent oligo

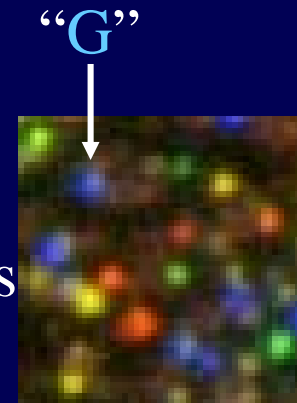


Polony chemistry

● ... (5') GGGCAGTCGGTGATCTGAAGCTCATGNNNNCNNNNNAGAGAATGAGGAACCCGGGGCAG (3')
AGCCACUAGACUUCGAGUACNNNNGNNNN-FITC

NNNNTNNNN-Cy5
NNNNCNNNN-**TexRd**
NNNNANNNN-Cy3

- Repeat for new query position
- Do not build upon previous errors

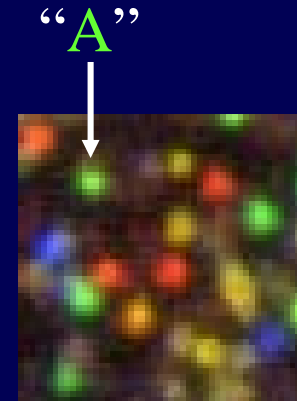


Polony chemistry

● ... (5') GGGCAGTCGGTGATCTGAAGCTCATG **NNTNNNNNNN** AGAGAATGAGGAACCCGGGGCAG (3')
AGCCACUAGACUUCGAGUACNN **ANNNNNN**-Cy3

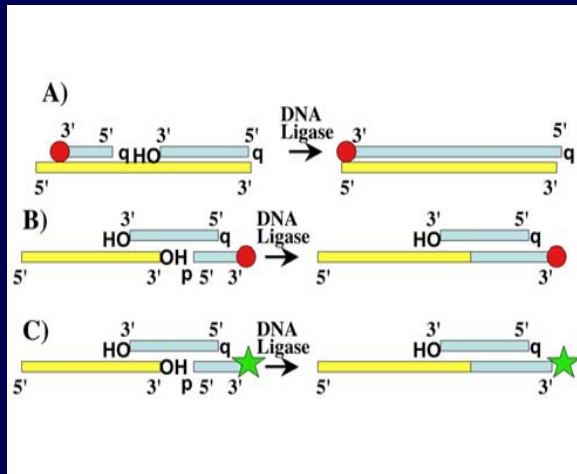
NNTNNNNNNN-Cy5
NNGNNNNNNN-FITC
NN**C**NNNNNNN-**TexRd**

- Repeat for new query position

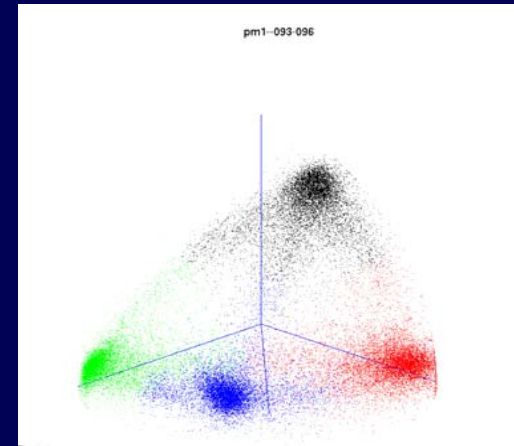


Improving sequencing by ligation

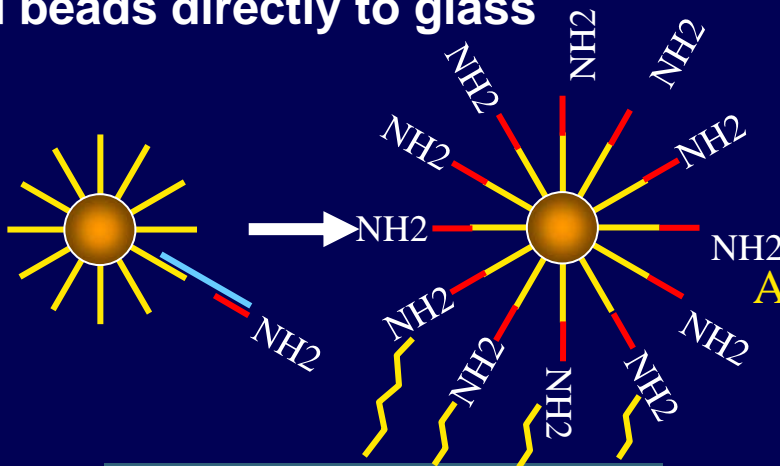
Capping 3' ends by ligation



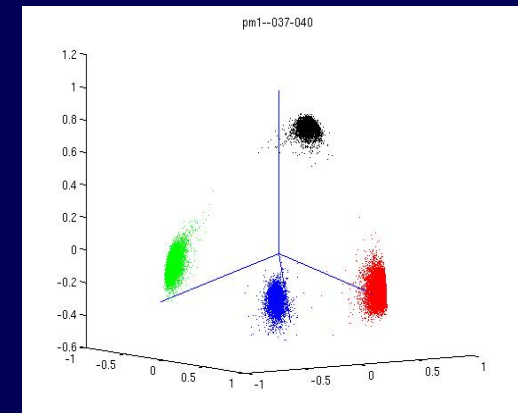
Polony array made in acrylamide gel:



Bind beads directly to glass



Gel-less array:



SAGE vs. PMAGE



cDNA
Digest with frequent cutter
Create adapter-flanked ditags

cDNA
Digest with frequent cutter
Create adapter-flanked unitags



?
PCR amplify ditags
Digest and concatenate



?
Clone concatemers into vector

Sanger sequence

Emulsion PCR to generate polony ePCR beads

Sequence by ligation

duplicate ditags excluded from analysis

1 mRNA molecule → 1 polony amplified bead

PMAGE: Library construct

Emulsion forward **IIs restriction site** **Emulsion reverse**
Acul: cuts 16/14bp

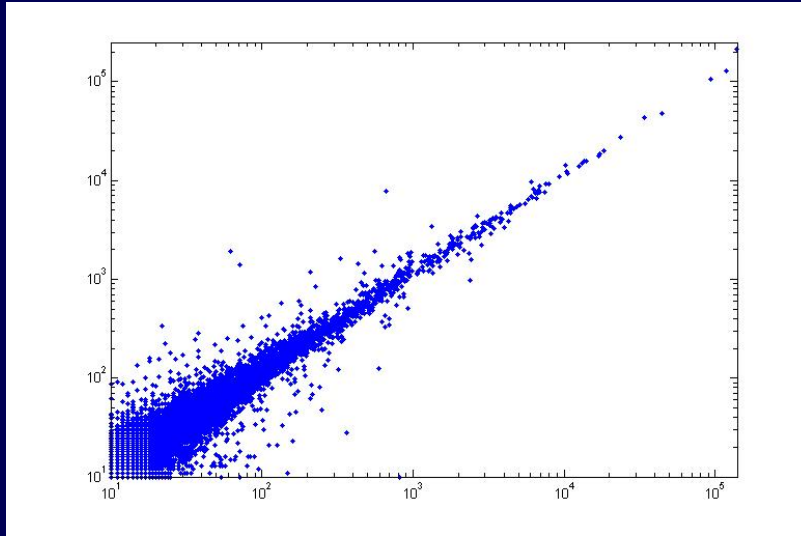
CCACTAGCCTCCGCTTTCTCTCTATGGGCAGTCGGTGATCTGAAGCTCATG ?????????? AGAGAATGAGGAACCCGGGGCAGTT
GGTGATGCGGAGGCGAAAGGAGAGATACCCGTCAGCCACTAGACTTCGAGTAC ?????????? TCTCTTACTCCTGGGCCCCGTCAA

PMAGE tag: 10 bases

- Validate with 10bp SAGE tags
- Utilize a 3bp overlap region (bases 5-7) to confirm tag length

PMAGE: validation

PMAGE expt 2

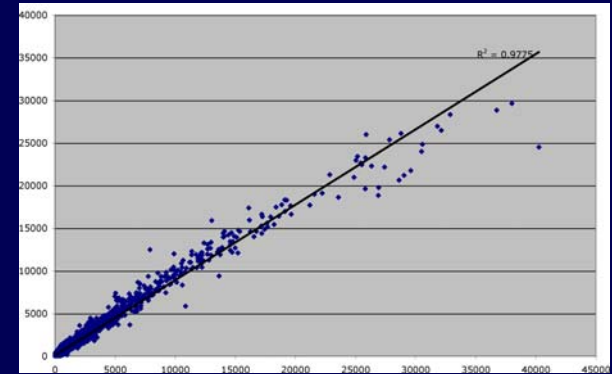


PMAGE expt 1

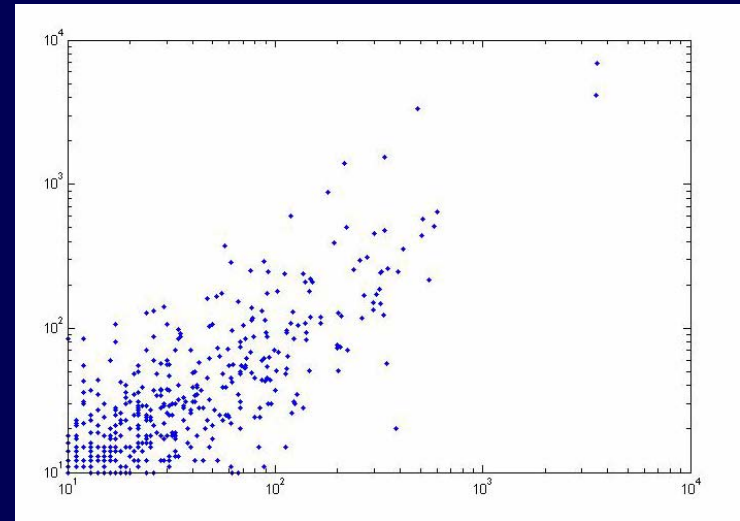
PMAGE gel vs gel-less array

$R^2 = 0.9997$

PMAGE



Microarray comparison



SAGE

$R^2 = 0.89$

PMAGE vs. SAGE

	PMAGE	SAGE
Total tags per library	2,205,288	70,731
Unique tags: total	142,338	17,896
Unique tags: 1 copy	75,871	12,502

Top 12 RNAs in the heart

PMAGE	SAGE	Gene ID	Gene Name
193135	3570	mt-Co3	cytochrome c oxidase III, mito
122787	3524	mt-Atp6	ATP synthase 6, mito
103069	484	mt-Co2	cytochrome c oxidase II, mito
46884	335	mt-Nd4	NADH dehydrogenase 4, mito
41290	215	mt-Nd2	NADH dehydrogenase 2, mito
26815	179	mt-Cytb	cytochrome b, mito
18631	605	mt-Co1	cytochrome c oxidase I, mito
16956	512	mt-Co1	cytochrome c oxidase I, mito
15296	119	Mb	Myoglobin
15141	221	mt-Co1	cytochrome c oxidase I, mito
15299	582	Atp2a2	SERCA, a2
13801	299	Myl3	Myosin, light polypeptide 3

Cardiac RNAs

- 32% mitochondrial
- 3.3% sarcomere
- 5% sarcomere + sarcoplasmic reticulum

Defining ventricular transcription factor networks

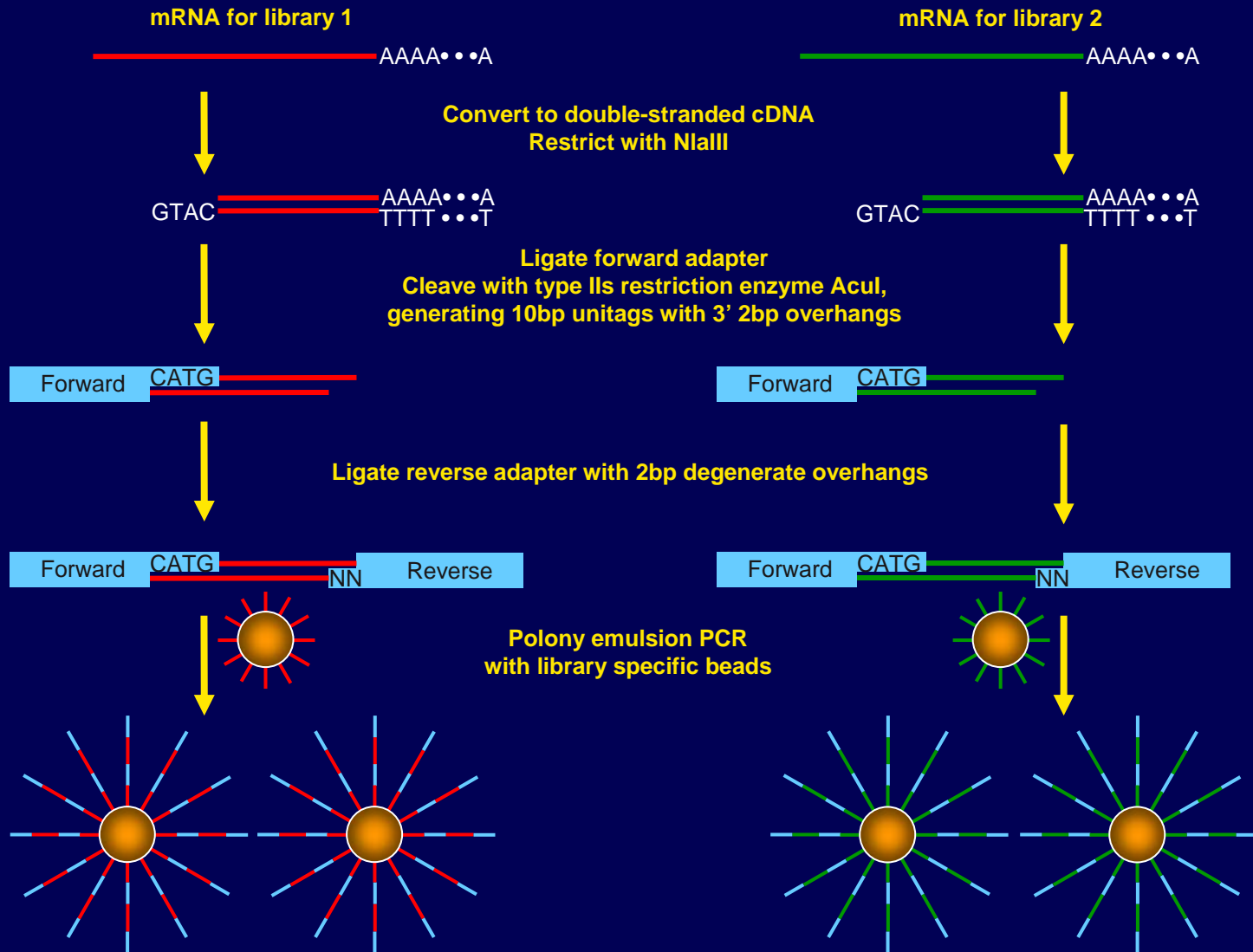
Gene	wt	403	tags	Phenotype
Dach1	44	64	15	x
Dach2	10	8	9	?
Eya1	1	1	2	
Eya2	18	19	15	x
Eya3	29	16	13	x
Eya4	2	1	3	
Gata1	10	5	6	?
Gata2	5	7	1	
Gata3	4	2	5	
Gata4	27	17	5	x
Gata5	1	0	1	
Gata6	42	58	7	?
Irx1	5	6	1	
Irx2	12	12	5	?
Irx3	16	9	6	?
Irx4	36	39	4	x
Irx5	12	7	1	?
Irx6	0	1	1	
Nkx1-2	5	1	2	
Nkx2-2	2	1	2	
Nkx2-3	3	5	1	
Nkx2-5	32	43	3	x
Nkx2-9	2	1	1	
Nkx3-1	3	1	2	
Nkx6-2	22	32	4	?
Nkx6-3	2	3	2	
Tbx1	3	0	1	
Tbx10	3	0	1	
Tbx15	3	3	3	
Tbx18	1	5	3	
Tbx19	4	0	3	
Tbx2	7	6	6	
Tbx20	14	12	6	x
Tbx22	5	1	3	
Tbx3	13	7	5	?
Tbx4	10	8	6	?
Tbx5	17	14	3	x

Multiple tags per gene

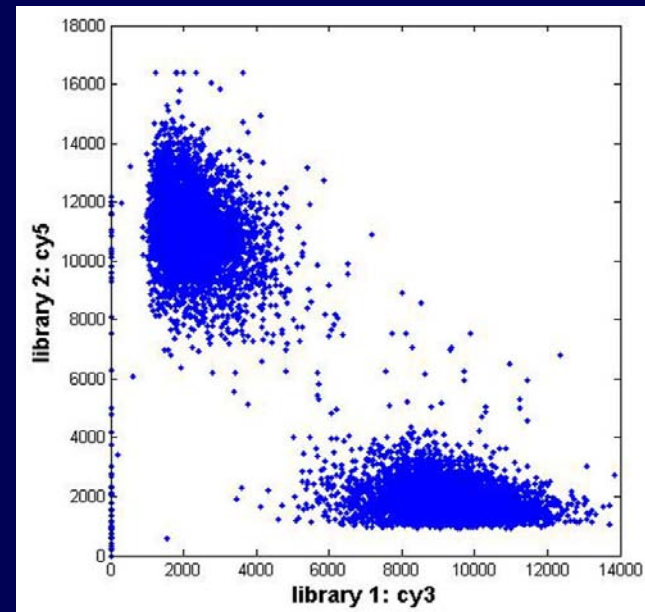
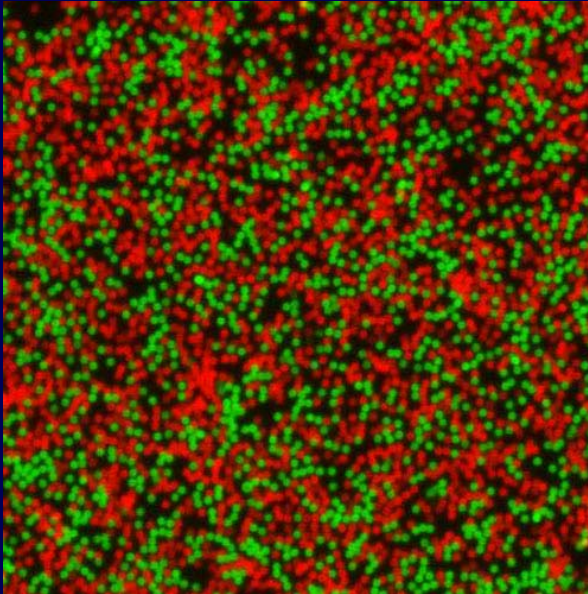
Gene	Tag	P _{image} wt	P _{image} 403	Sage wt
Gata4	ATAGCAGCTG	6	8	0
Gata4	CCTGCCCTC	8	16	3
Gata4	GGGACATAAT	2	2	0
Gata4	TCCCAGACTT	1	0	0
Gata4	TCCTCTGCAA	0	1	1
Nkx2-5	CGGGGAGGCT	0	1	0
Nkx2-5	CTGGCCGCCT	34	24	1
Nkx2-5	TTCCCCAGCC	9	7	0

- 37 Transcription factors in 6 families
- At least 17 are expressed in the LV
- 7 (41%) were not known to be expressed

PMAGE: Multi-Library Construction



PMAGE: multiple library comparisons



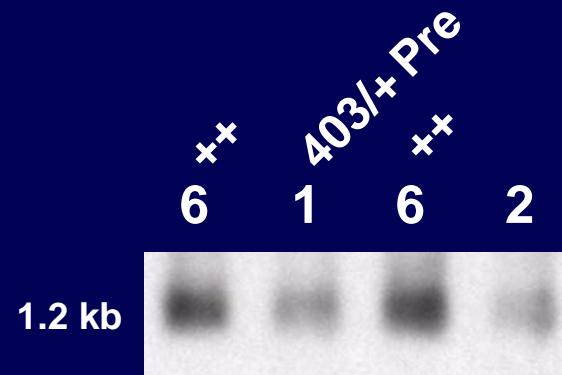
Transcriptional regulators

PMAGE

SAGE

Tag	wildtype	α MHC ^{403/+}	wildtype	α MHC ^{403/+}	Sybmol	Title
CTGGCCGCCT	34	24	1	2	Nkx2-5	NK2.5 transcription factor
TATATTTTAT	18	8	1	0	Mef2a	Myocyte enhance factor 2A
CCTGCCCCTC	8	16	3	3	GATA4	GATA binding protein 4
TATATTTTAT	18	8	1	0	Mef2a	Myocyte enhance factor 2A
TTCCCCGATT	14	15	1	0	Tbx5	T-box 5
TCGGCGCAGA	364	261	6	1	Hod	Homeobox only domain

$p = 1.86E-05$



Tg^{HOP} Exacerbates MHC⁴⁰³ & MHC⁴⁵³ Phenotypes

α MHC promoter

HOP ORF_{73 aa}

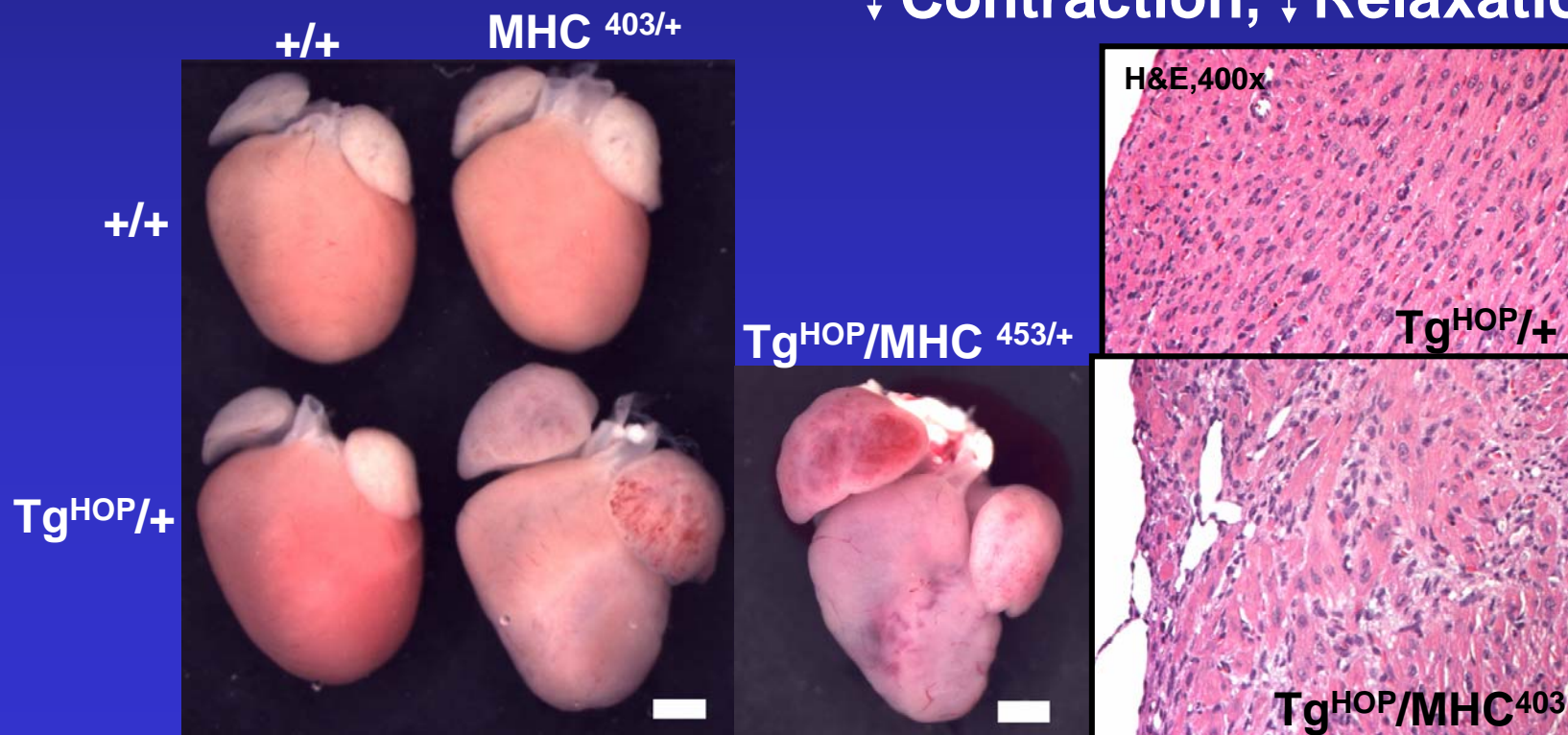
hGh 3' UTR

Tg^{HOP} Lines

- Normal survival
- No hypertrophy
- Normal contractile function

Tg^{HOP}/MHC⁴⁰³ & Tg^{HOP}/MHC⁴⁵³

- Neonatal death < 2wks
- Neonatal hypertrophy
- Isolated Myocytes: Elongated
↓ Contraction, ↓ Relaxation



Advantages of PMAGE

- At current iteration, ~5,000,000 tags per sequencing run
- Separate experiments/patients can be sequenced simultaneously
- Deep sampling of rare transcripts
- Can investigate genome-wide transcriptional networks
- Can detect less than 2 fold changes in gene expression
- Cost-effective

Cost Benefit Analysis of SAGE vs PMAGE

	PMAGE	SAGE/Sanger	Factor Improvement over SAGE/Sanger
Tags per Sanger read	N/A	~40	N/A
Price per Sanger read	N/A	\$5	N/A
Tags per experiment	~5,000,000	100,000	25
Price per experiment	\$1,299*	\$12,500	~10
Price per tag	\$ 0.00026	\$0.125	481

* Multiple libraries can be combined in one array
for simultaneous sequencing

Seidman Laboratory

■ Hiroyuki Morita
Michael Arad
Carolyn Ho
Cordula Wolf
Jae Kim

Collaborators

cRyR: Craig Basson

FHS: Emelia Benjamin

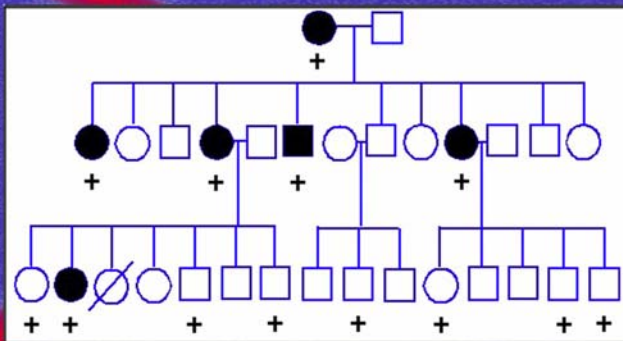
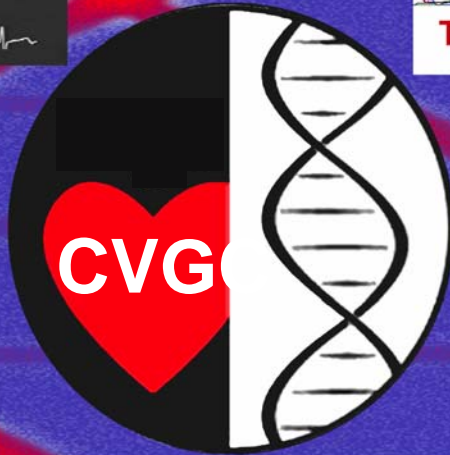
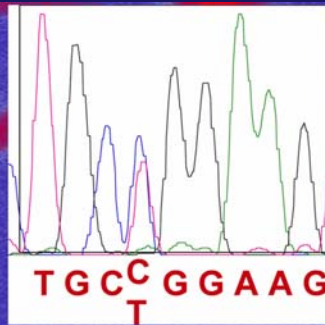
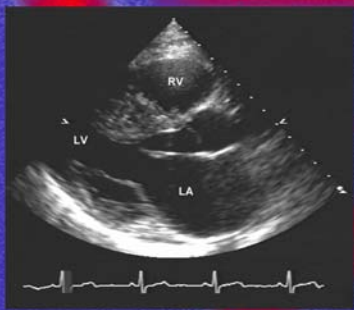
Pediatric HCM: Jeff Towbin

Neil Bowles

Polony Sequencing: George Church

Greg Porreca

Jay Shendure



wildtype vs. α MHC^{403/+} PMAGE

Tag	PMAGE		SAGE		Sybmol	Title
	wildtype	α MHC ^{403/+}	wildtype	α MHC ^{403/+}		
ATACTGACAT	193135	208799	3570	3338	mt-Co3	cytochrome c oxidase III, mito
ATAATACATA	122787	103654	3524	3823	mt-Atp6	ATP synthase 6, mito
AGCAGTCCCC	103069	102773	484	464	mt-Co2	cytochrome c oxidase II, mito
ATGACTGATA	46884	42665	335	297	mt-Nd4	NADH dehydrogenase 4, mito
AGGAGGACTT	41290	40545	215	226	mt-Nd2	NADH dehydrogenase 2, mito
AGGACAAATA	26815	23886	179	215	mt-Cytb	cytochrome b, mito
GCTGCCCTCC	18631	22425	605	518	mt-Co1	cytochrome c oxidase I, mito
TAGTTACTTA	16956	14729	512	437	mt-Co1	cytochrome c oxidase I, mito
CTCAGGTCTC	15296	15461	119	118	Mb	Myoglobin
AGCAAAGCC	15141	13549	221	240	mt-Co1	cytochrome c oxidase I, mito
ATTTCAGGTA	15299	13279	582	530	Atp2a2	SERCA, a2
TCTGGAGCTT	13801	14263	299	238	Myl3	Myosin, light polypeptide 3

wildtype vs. α MHC^{403/+} PMAGE

		PMAGE	SAGE
PMAGE	SAGE	Gene ID	Gene Name
193135	3570	mt-Co3	cytochrome c oxidase III, mito
122787	3524	mt-Atp6	ATP synthase 6, mito
103069	484	mt-Co2	cytochrome c oxidase II, mito
46884	335	mt-Nd4	NADH dehydrogenase 4, mito
41290	215	mt-Nd2	NADH dehydrogenase 2, mito
26815	179	mt-Cytb	cytochrome b, mito
18631	605	mt-Co1	cytochrome c oxidase I, mito
16956	512	mt-Co1	cytochrome c oxidase I, mito
15296	119	Mb	Myoglobin
15141	221	mt-Co1	cytochrome c oxidase I, mito
15299	582	Atp2a2	SERCA, a2
13801	299	Myl3	Myosin, light polypeptide 3

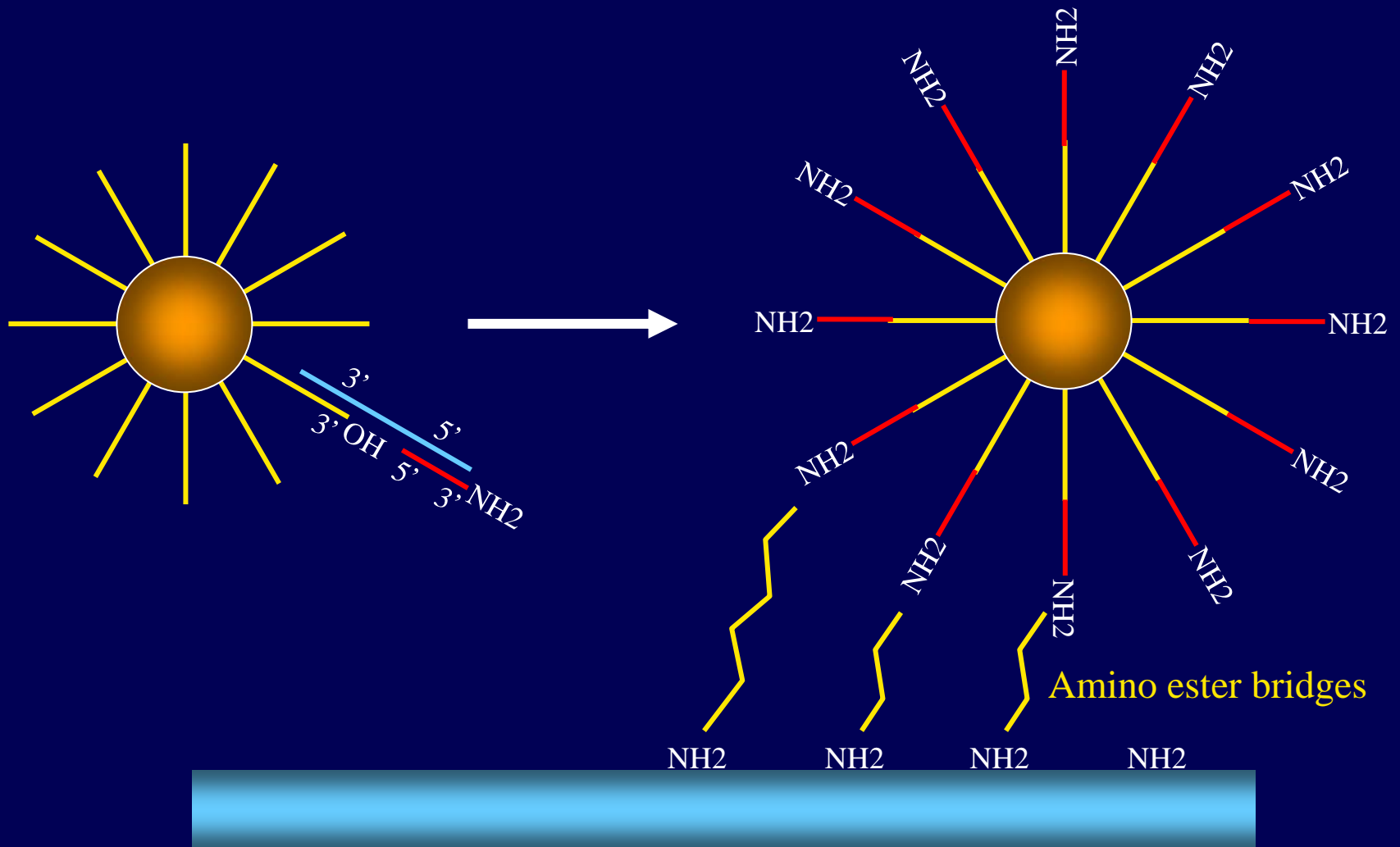
Most abundant mRNAs in α MHC^{403/+}

TAG sequence	α MHC ^{403/+} tag counts	Identification
ATAATACATA	3823	mt-Atp6 ATP synthase 6, mitochondrial
ATACTGACAT	3338	mt-Co3 cytochrome c oxidase III, mitochondrial
AGCAATTCAA	547	mt-Nd3 NADH dehydrogenase 3, mitochondrial
ATTCAGGTA	530	Atp2a2 ATPase, Ca ⁺⁺ transporting, cardiac muscle, slow twitch 2
GCTGCCCTCC	518	mt-Co1 cytochrome c oxidase I, mitochondrial
AGCAGTCCCC	464	mt-Co2 cytochrome c oxidase II, mitochondrial
GCTCCCACAC	445	Tnni3 troponin I, cardiac
TAGTTACTTA	437	mt-Co1 cytochrome c oxidase I, mitochondrial
CAGGCCACAC	359	Atp5b ATP synthase, H ⁺ transporting mitochondrial F1 complex, beta subunit

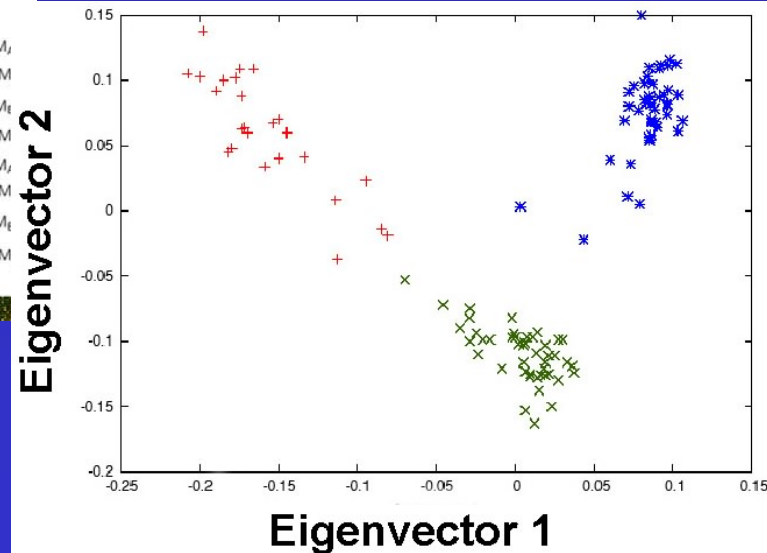
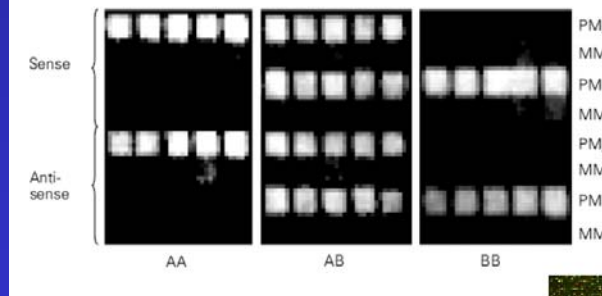
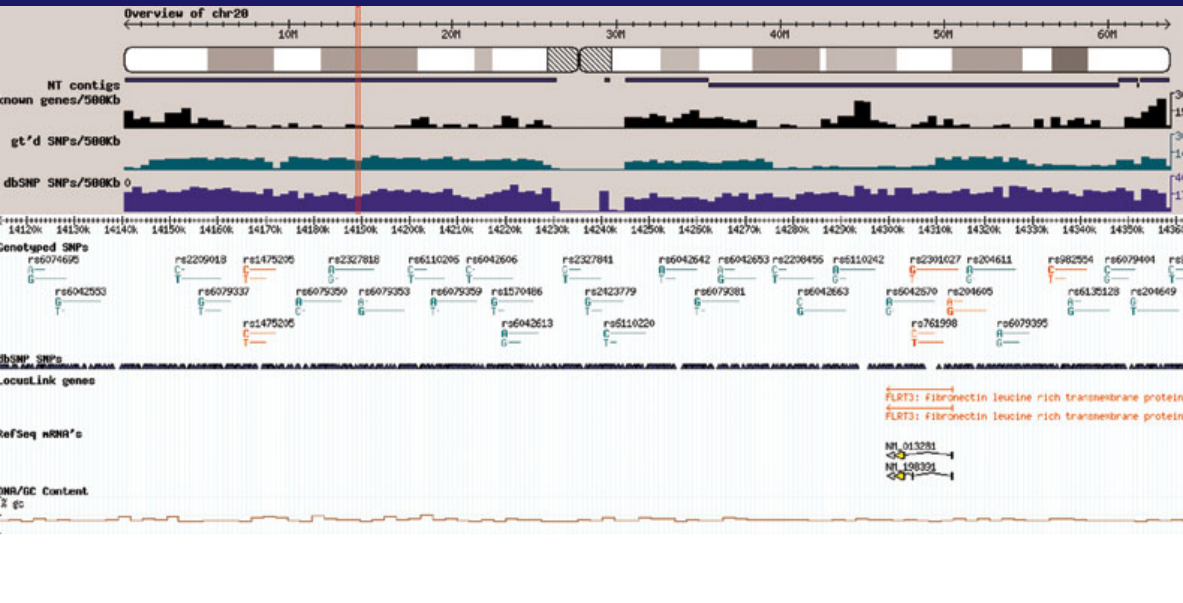
Improving sequencing by ligation

- **Address sequence-specific systematic biases**
(Tms of degenerate query nonamers vary widely)
 - Perform “stepped temperature” ligations
- **Need to improve overall sequencing quality**
 - **Macromolecular exclusion to increase ligation efficiency**
 - **Enhanced signal to noise by improved capping of reactive 3' DNA ends**
 - **Improved chemistry, imaging, and data volume with gelless arrays**

Cross-linking polony beads directly to glass solid support



Genome wide >500,000 SNP analyses allow association studies



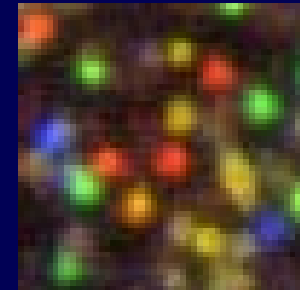
Polony chemistry

...(5') GGGCAGTCGGTGATCTGAAGCTCATGNNNNNNNNNAGAGAATGAGGAACCCGGGGCAG (3')
3'-AGCCACUAGACUUCGAGUAC

Polony chemistry



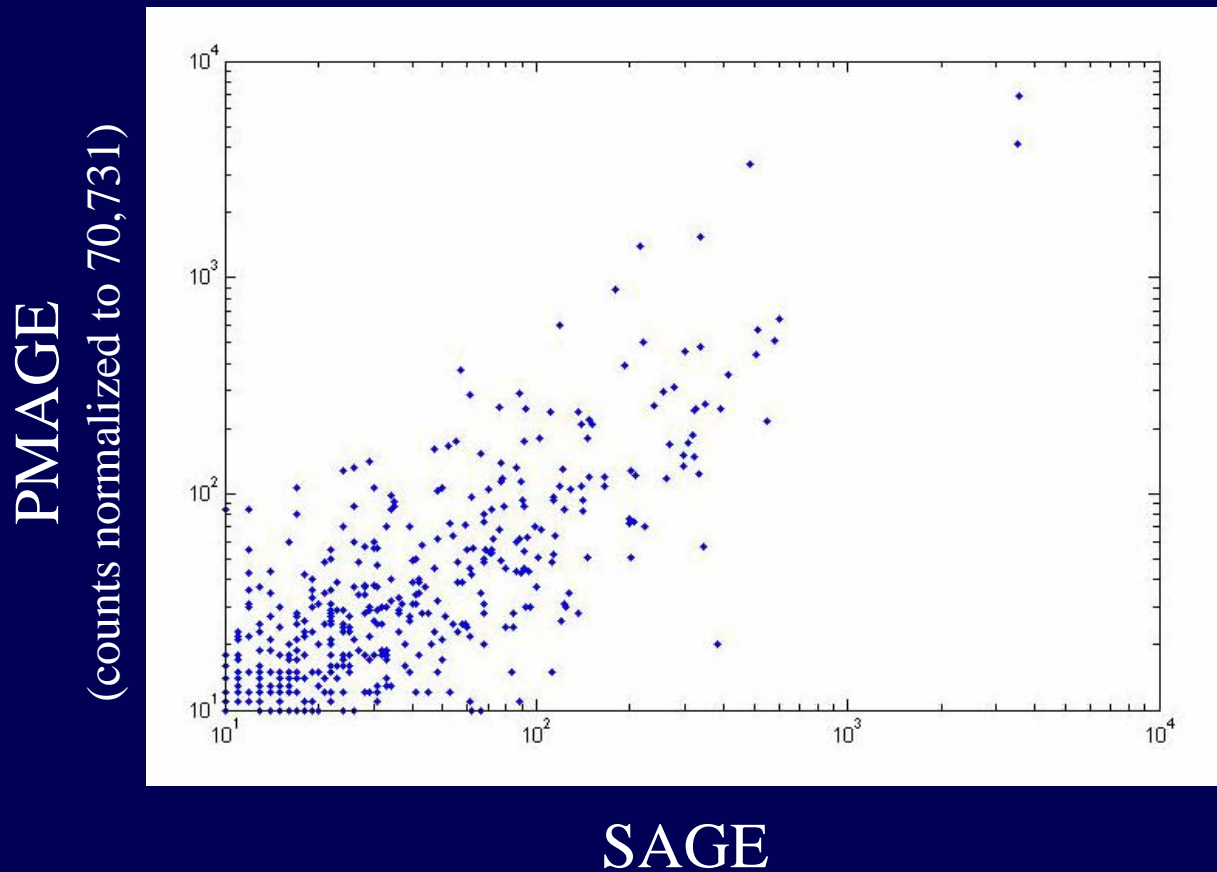
- Anneal anchor oligo
- Ligate query nonamer
- Epifluorescence image array



Compendium of LV Transcription Factors

Gene	Tag	Pmage wt	Pmage 403	Sage wt
Gata4	ATAGCAGCTG	6	8	0
Gata4	CCTGCCCTC	8	16	3
Gata4	GGGACATAAT	2	2	0
Gata4	TCCCAGACTT	1	0	0
Gata4	TCCTCTGCAA	0	1	1
Nkx2-5	CGGGGAGGCT	0	1	0
Nkx2-5	CTGGCCGCCT	34	24	1
Nkx2-5	TTCCCAGCC	9	7	0

PMAGE vs. SAGE



Correlation coefficient: $R = 0.89$

Advantages of PMAGE

- **One sequence tag generated from each mRNA molecule.**
- **No amplification in cloning process (as with SAGE)**
- **Currently can sequence ~5,000,000 polonies per run**
- **Results of multiple sequencing runs can be combined**
- **Costs: < 1/20 of SAGE**
- **Great potential for sampling rare transcripts**
- **Can provide transcriptional network in heart disease**

Does Histopathology Correlate with Arrhythmias ?

	WT	MHC ^{403/+}	p value*	MHC ^{403/+} Arrhythmia		p value**
				Inducible	Not Inducible	
Number of mice	5	16		11	5	
Age (weeks)	71±23	57±21	ns	56±15	59±32	ns
Total fibrosis	0.17±0.24	0.98±0.75	0.001	1.10±0.86	0.72±0.39	ns
Max fibrosis	0.66±0.68	2.58±1.85	0.002	2.85±2.0	1.99±1.6	ns
Myocyte disarray	8.07±4.28	40.07±11.02	<0.0001	38.89±10	41.01±13	ns
Max LVWT (mm)	0.85±0.05	1.17±0.13	<0.001	1.29±0.09	1.1±0.09	0.008
LVEDD (mm)	3.29±0.3	2.70±0.33	0.002	2.37±0.29	2.87±0.20	0.005
FS (%)	51.6±6.7	64.25±10.88	0.005	73.8±11	59.5±8	0.024
CV min (m/sec)	0.43±0.10	0.41±0.10	ns	0.34±0.13	0.46±0.11	ns
CV max (m/sec)	0.69±0.14	0.76±0.13	ns	0.75±0.10	0.85±0.13	ns
Anisotropic ratio	1.63±0.22	1.99±0.56	ns	2.37±0.61	1.98±0.68	ns

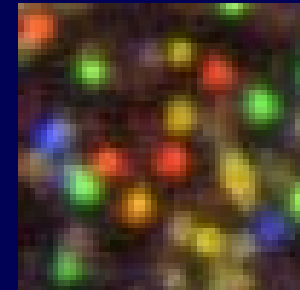
What features do we want to investigate transcription factors in a genome-wide approach?

- Digital quantification of mRNA molecules
 - Superior statistical rigor over hybridization based ratio comparisons
- Highly sensitive
 - Need to quantitate millions of mRNA molecules
- Accurate sequences and reproducible results
- Identify previously unidentified RNAs
- Cost-effective

Polony chemistry

● ... (5') GGCAGTCGGTGATCTGAAGCTCATGNNNNNNNNNAGAGAATGAGGAACCCGGGGCAG (3')

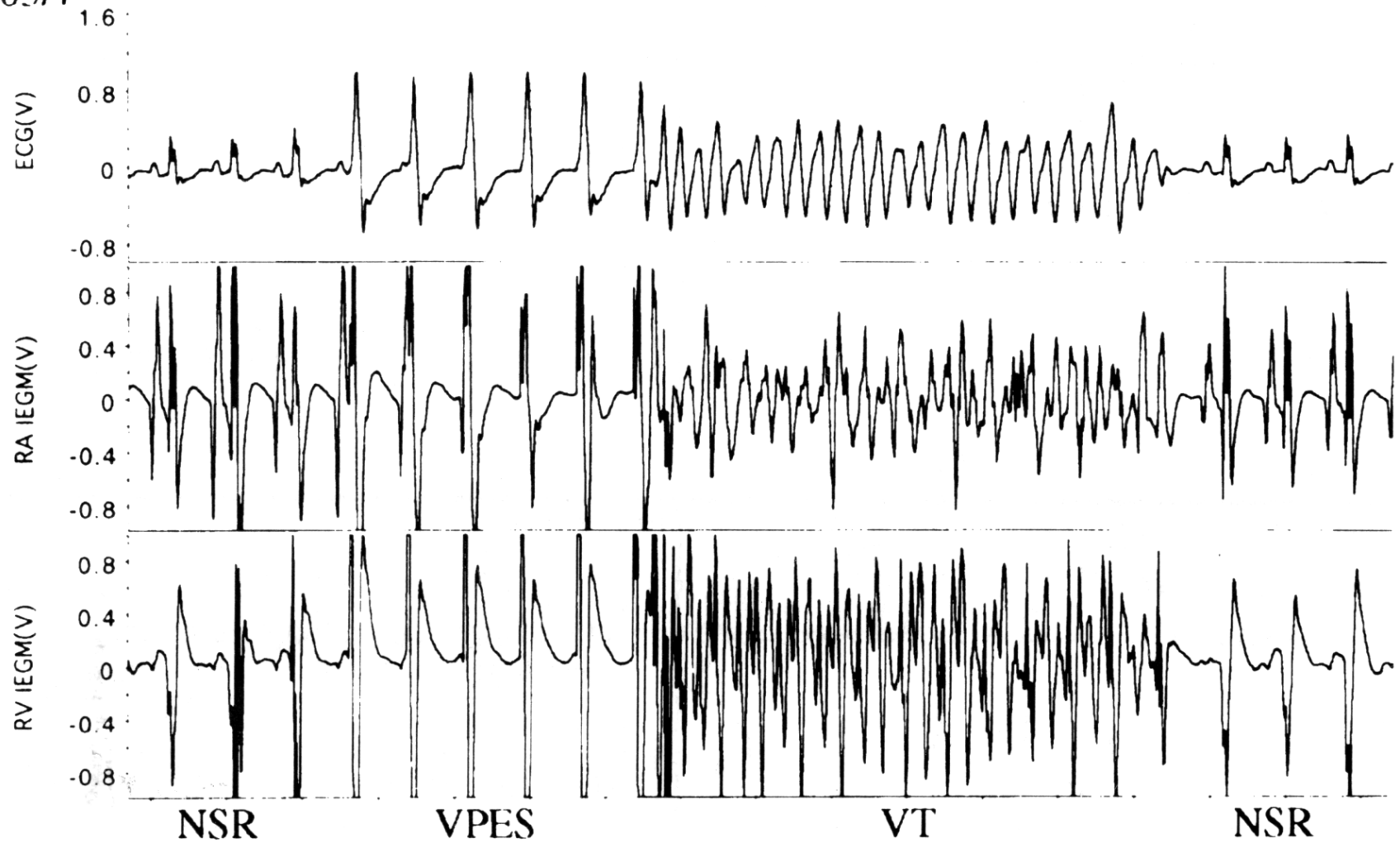
NNANNNNNN-Cy3
NNTNNNNNN-Cy5
NNGNNNNNN-FITC
NNCNNNNNN-*TexRd*



Ventricular Arrhythmias in HCM Mice

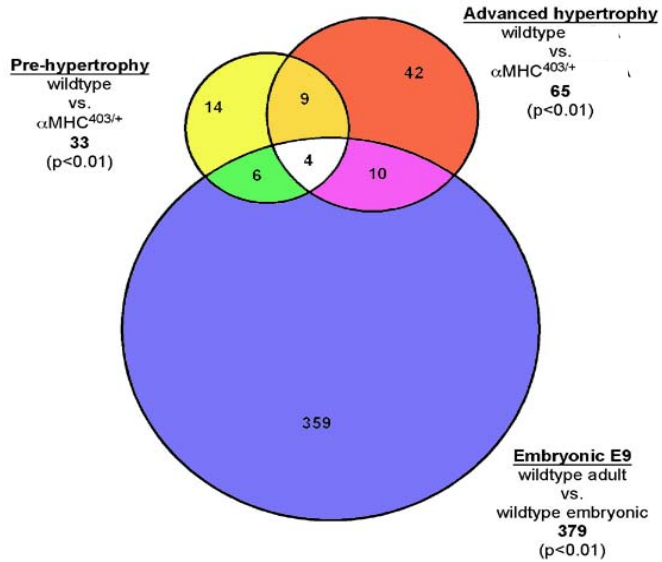
Female

403/+

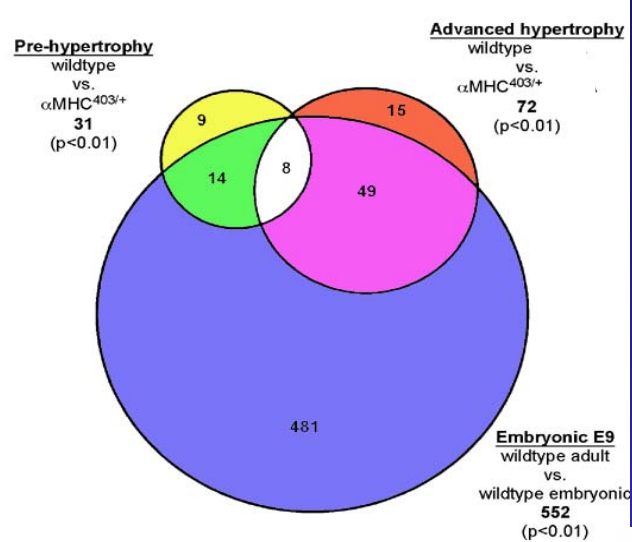


Molecular Consequences of Sarcomere Mutations: SAGE

Upregulated

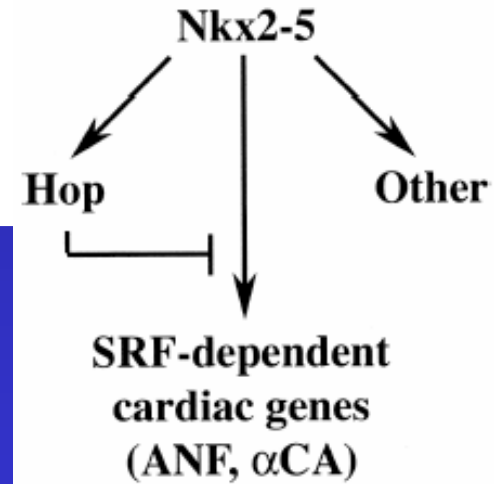


Downregulated



- ↑ Prototypic Hypertrophic Genes (ANF, CARP)
- ↓ Mitochondrial & Genes Involved in Energetics
- No Global Induction of Fetal Gene Expression

HOP inhibits SRF mediated gene expression

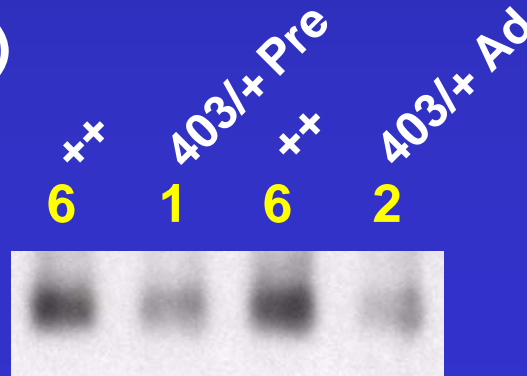


Homebox Only Protein (HOP)
↓ by Sarcomere Mutation

SAGE tag count:

Total ++ (12) vs. 403/+ (3) p = 0.0014

1.2 kb



(Shin et. al., Cell, 2002)
(Chen et. al., Cell 2002)

Ventricular Ectopic Activity in HCM Mice

