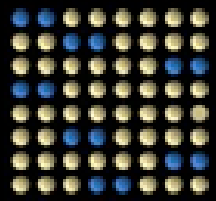
CpG Island Microarrays: Targeting Gene Expression

2nd Annual Canadian Gene Expression Conference

Vancouver BC, March 25th, 2004



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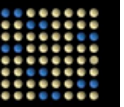
Seminar Overview

- ChIP (Chromatin Immunoprecipitation)
- CpG and Promoter Microarrays for parallel analysis
- Production Issues
- Informatics
- Epigenetics
  - Methylation analysis using CpG and cDNA microarrays



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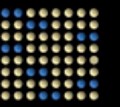
Chromatin Immunoprecipitation (ChIp)

- Typically used to interrogate the occupancy of a particular promoter
- Potentially have the capability to identify novel gene targets associated with specific transcription factors



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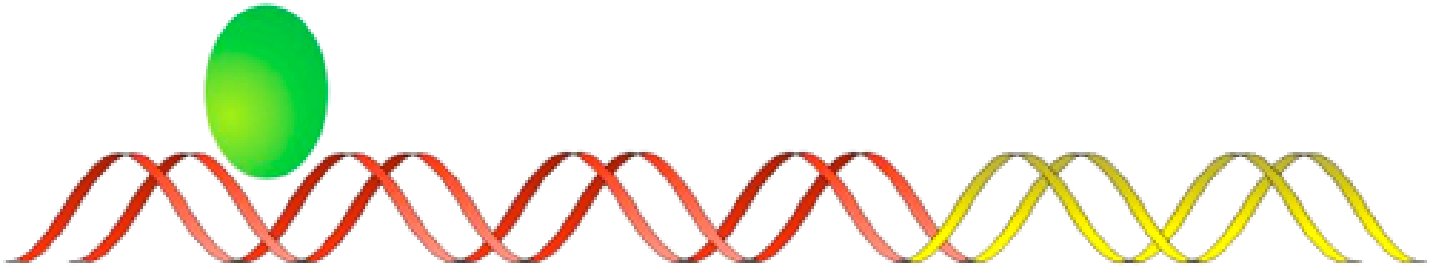
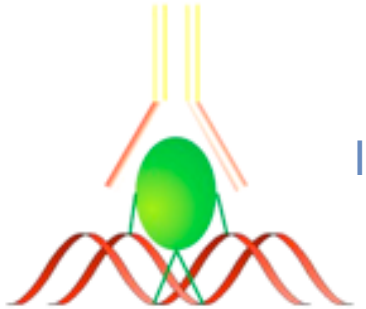
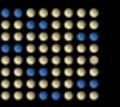


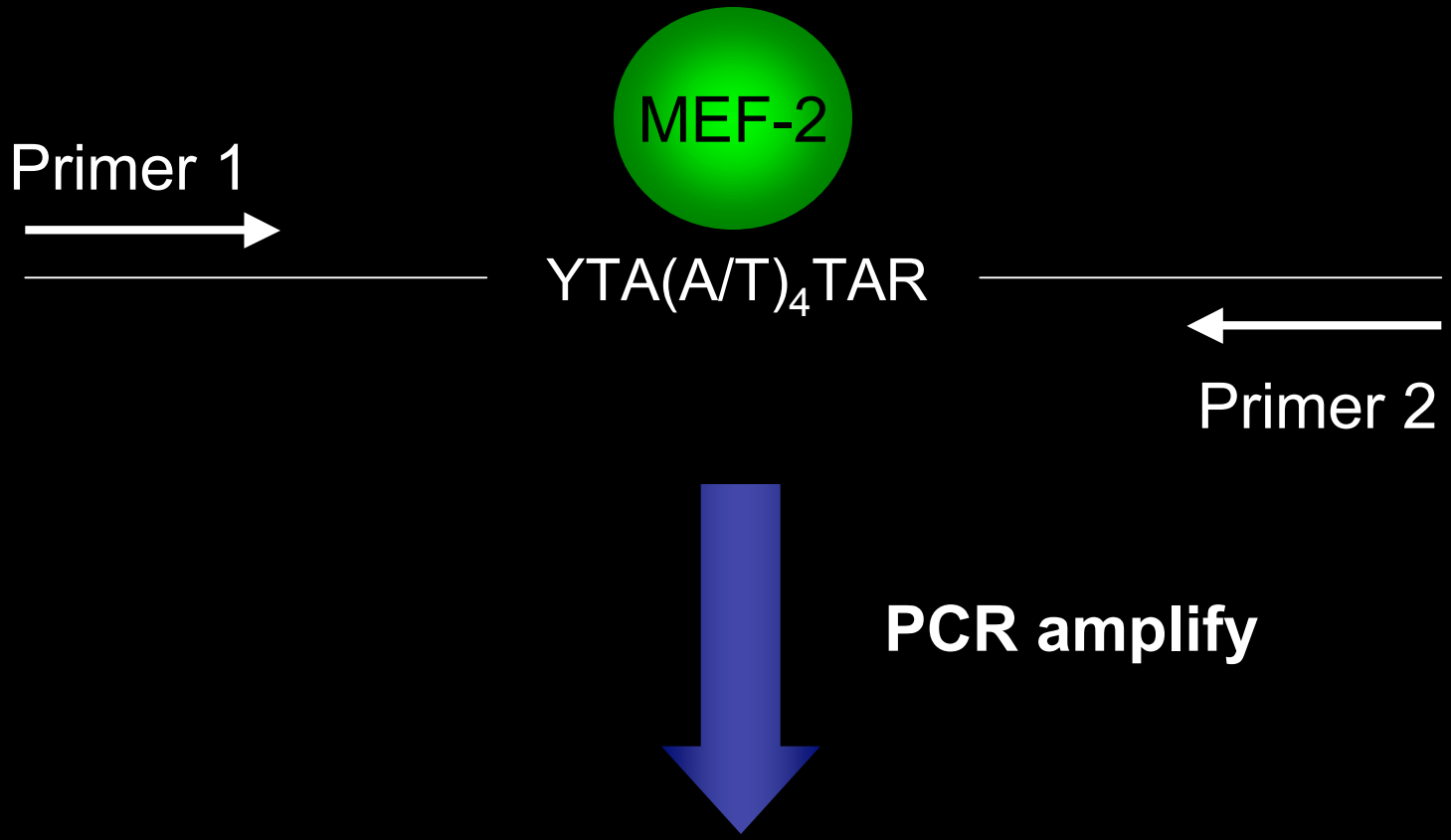


Incubate with specific
1˚ Antibody

Example: MEF-2 (Myocte Enhancing Factor 2)

- Muscle transcription factor known to be a key player in the regulation of muscle differentiation
- Regulates the expression of a number of genes such as muscle creatine kinase, skeletal α-actin, myosin light chain, and myoglobin
- Binding sequence: YTA$(A/T)_4$TAR
- Design primers to flank this region for several regulated genes





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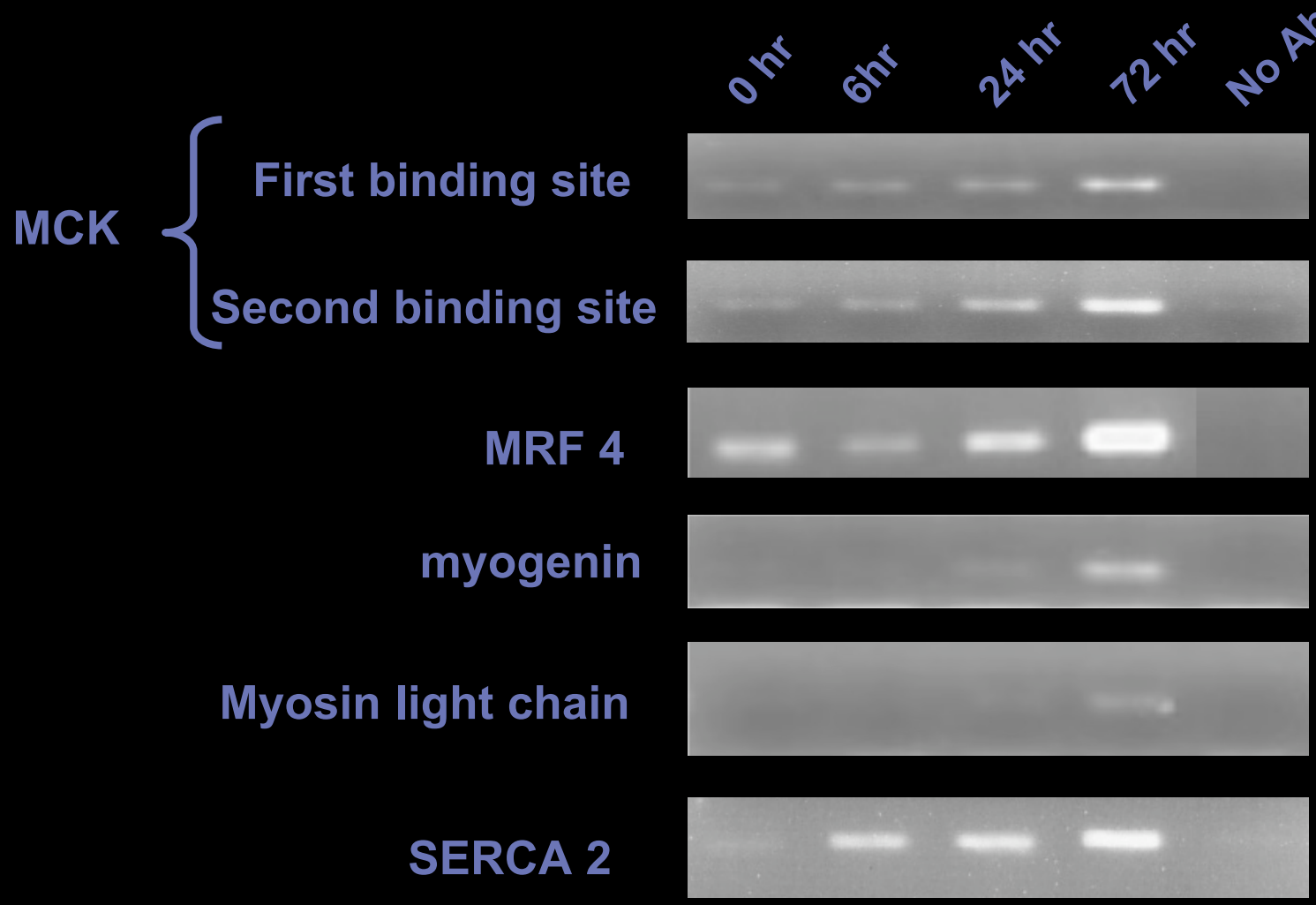
ChIp Analysis of MEF-2 Associated Genes



Courtesy Mark Takahashi, CGC

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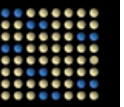
How can we apply this to a high throughput screening method?

Need a method of capturing the regulatory sites of genes

- CpG Island Microarrays
  - CpG islands are unmethylated GC rich regions of the genome that are associated with the 5' ends of most house-keeping genes and many regulated genes
  - About 80% of CpG islands are common between human and mouse
  - About 56% of human genes and 47% of mouse genes are associated with CpG islands



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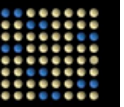
The Sanger 12k Human CpG Set

- 12,288 redundant clones (128 96-well plates)
- Originally there were more clones but due to phage contamination, those plates are no longer available
- Plate 105 also had some phage contamination so it has been excluded from our set
- The Sanger Institute performed 3' and 5' terminal sequencing of each clone
  - Information available at http://www.sanger.ac.uk/HGP/cgi.shtml



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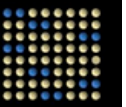
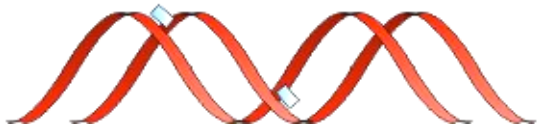
Technical Issues

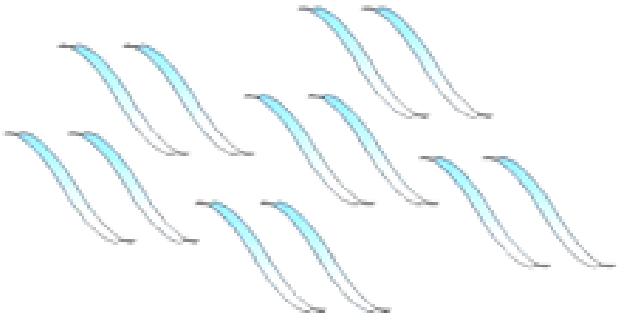
- Due to the relatively low quantities of DNA recovered from ChIP, we require a method of amplification to be able to visualize the immunoprecipitated DNA
  - Employed a modified method from the Pat Brown lab (www.microarrays.org). Our modifications are available on our website at (www.microarrays.ca)
- Annotation of the CpG clones to identify location in the genome and potential genes in proximity with the CpG island.
  - In conjunction with the NCI, we are having all 12,000 clones resequenced.
  - Once data is available, we will use BLAT to align all sequences to the genome and pull out putative regulatory targets.





Degenerate Primers

PCR Amplify ChIp'ed DNA



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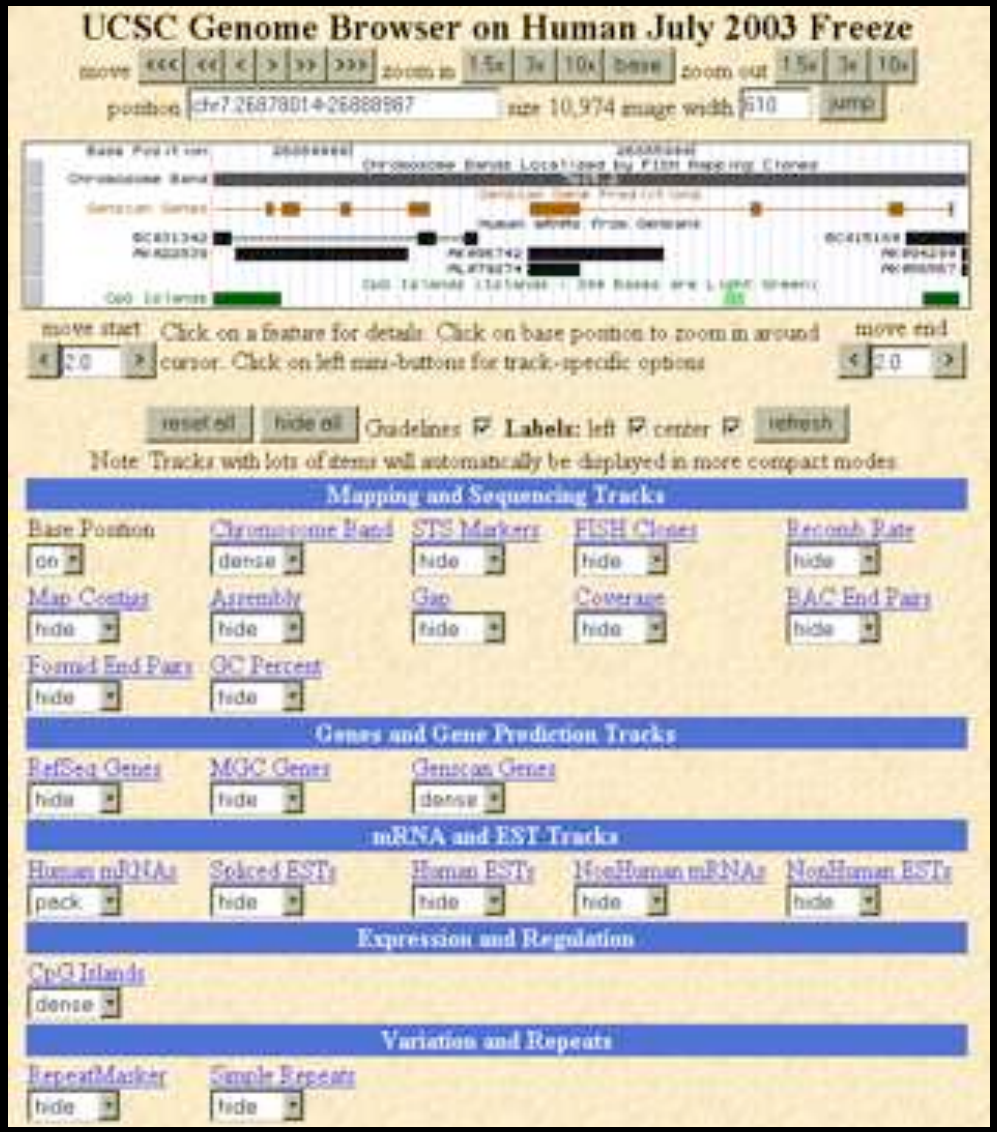
The UCSC Genome Browser
http://genome.ucsc.edu



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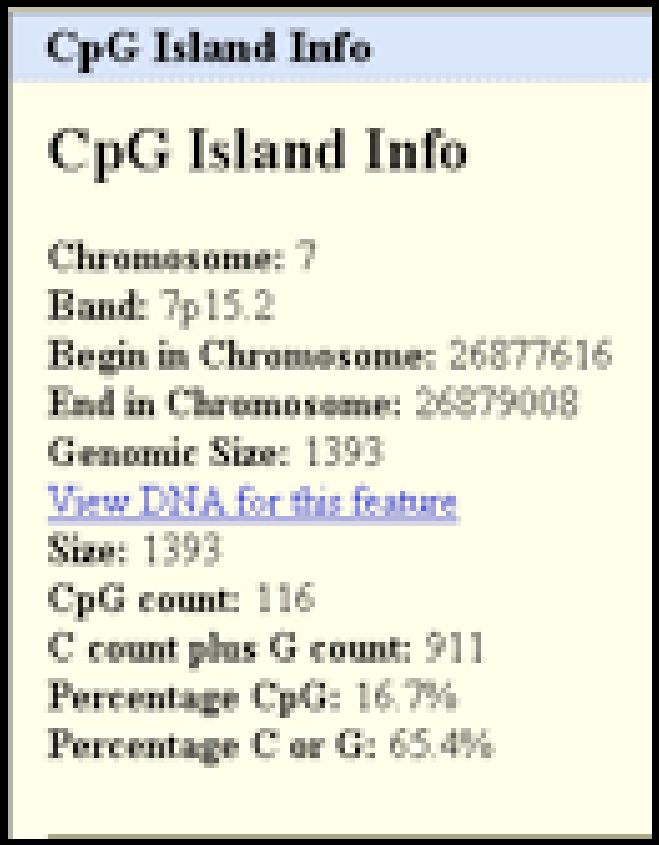
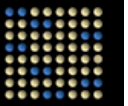
CpG Island Info from UCSC





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Neighbouring Gene Info

AK022839

Information on mRNA AK022839

Description: Homo sapiens cDNA FLJ12777 fis, clone NT2RP2001720
Gene: n/a
Product: n/a
Author: Irie,T., Ota,T., Hayashi,K., Sugiyama,T., Ono,T., Suzuki,Y., Nishikawa,T., Hasa,F., Sugano,S., Arai,K., Yoshikawa,Y., Matsuyama,H., Ishii,S., Kawai,Y., Saito,K., Yamamoto,J., Wakamatsu,A., Nakamura,Y., Nagahari,K., Murakami,Y. and Suzuki,H.
Organism: Homo sapiens
Tissue: n/a
Development stage: n/a
Cell type: NT2
Sex: n/a
Library: NT2RP2
Clone: NT2RP2001720
CDS: n/a
Date: 2002-07-01
GeneLynx AK022839
Standard SOURCE: AK022839

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mRNA/Genomic Alignments

SIZE	IDENTITY	CHROMOSOME	STRAND	START	END	QUERY	START	END	TOTAL
2529	99.9%	7	+	149759210	149805848	AK022839	1	2529	2529

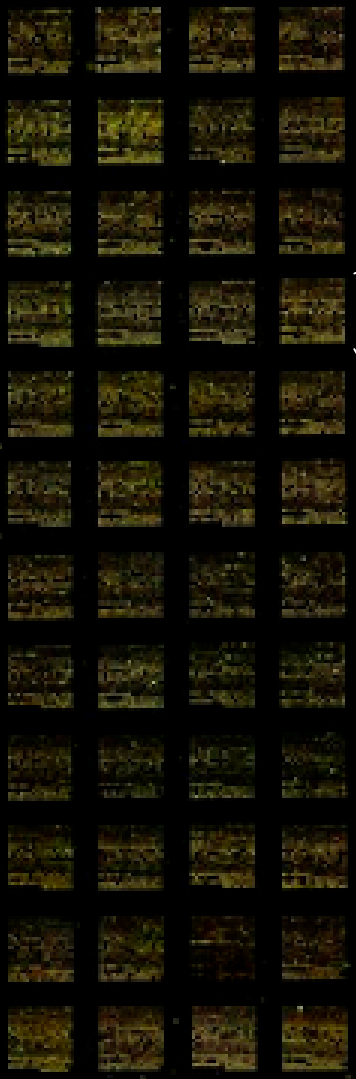
Description

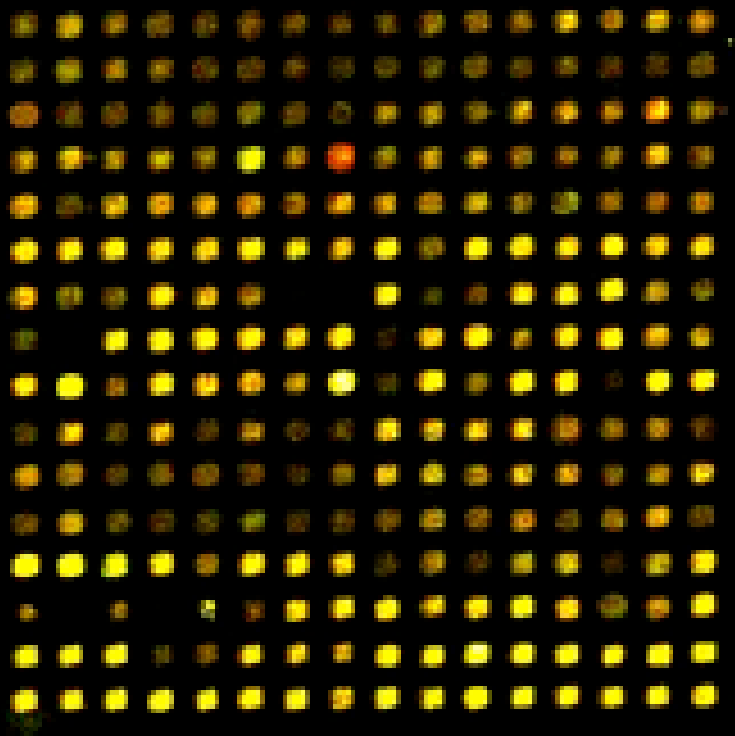
The Human mRNA track shows alignments between human mRNAs in GenBank and the genome. Aligning regions (usually exons) are shown as black boxes connected by lines for gaps (spliced out introns usually). In full display, arrows on the introns indicate the direction of transcription.

Method

GenBank human mRNAs are aligned against the genome using the blat program. When a single mRNA aligns in multiple places, the alignment having the highest base identity is found. Only alignments that have a base identity level within 1% of the best are kept. Alignments must also have at least 95% base identity to be kept.

12k Mk1 CpG Array





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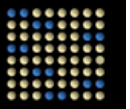
Mouse 7k CpG Island Array

- Mouse CGI library obtained from Sanger Institute (UK)
  - CpG island clones in pGEM-5Zf vector
- Used a 100 µl aliquot (10^7 cells)
- Plated library - approx. 300 colonies/plate
- Used colony picker (Genetix Q-Pix2) to pull clones
- Total of 24 plates
- 7000 viable colonies picked
- Amplied inserts using T7/SP6 primers
- Purified, transferred to 384 well plates and prepared for arraying



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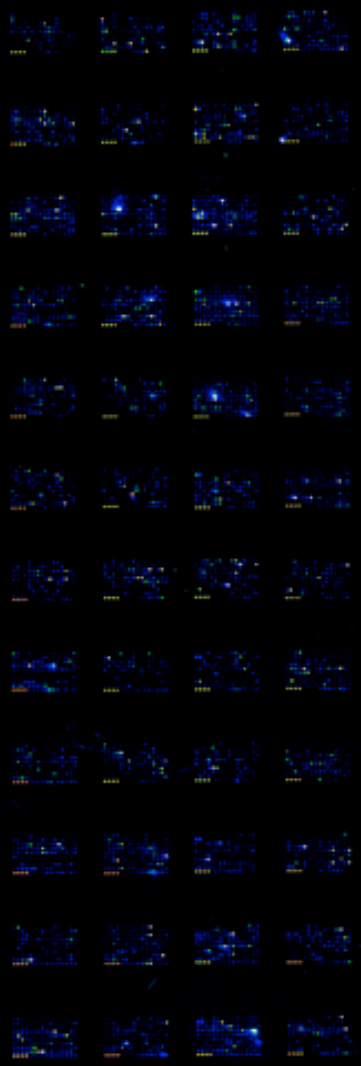
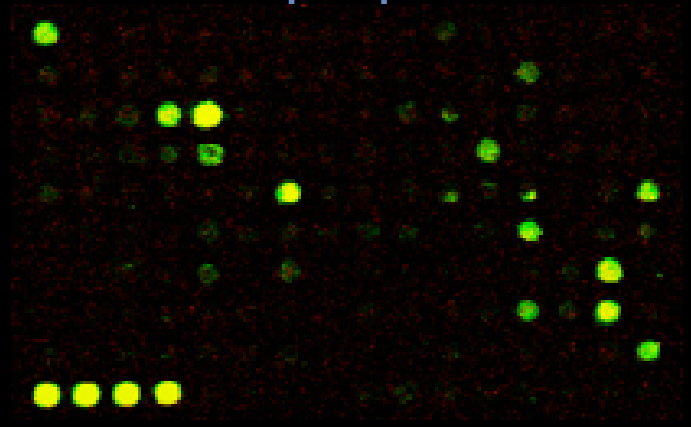


Sample mouse CGI array v.1

Total number of spots: 7680

Arabidopsis controls included in each subarray for normalization purposes



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Methodology (MEF-2 Example)

C_2C_{12} cells were grown to 80% confluence

↓

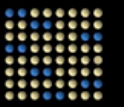
Media was changed to differentiation media
(1% horse serum and 50 ng/ml IGF-1)

↓

Following 0, 6, 24 and 72 hours, cells were treated and harvested

↓

Followed ChIP on CpG microarray protocol



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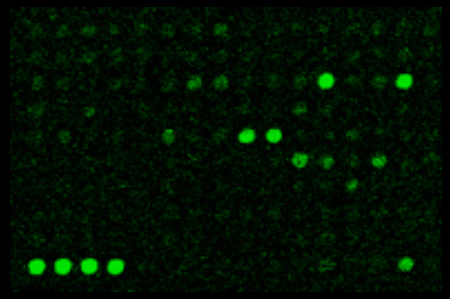
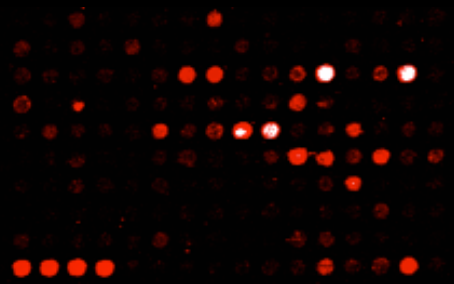
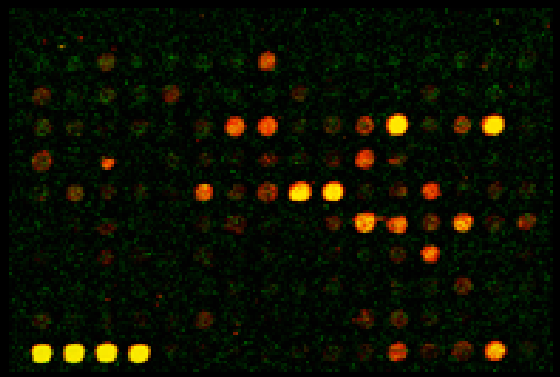
No Antibody



Antibody



Composite



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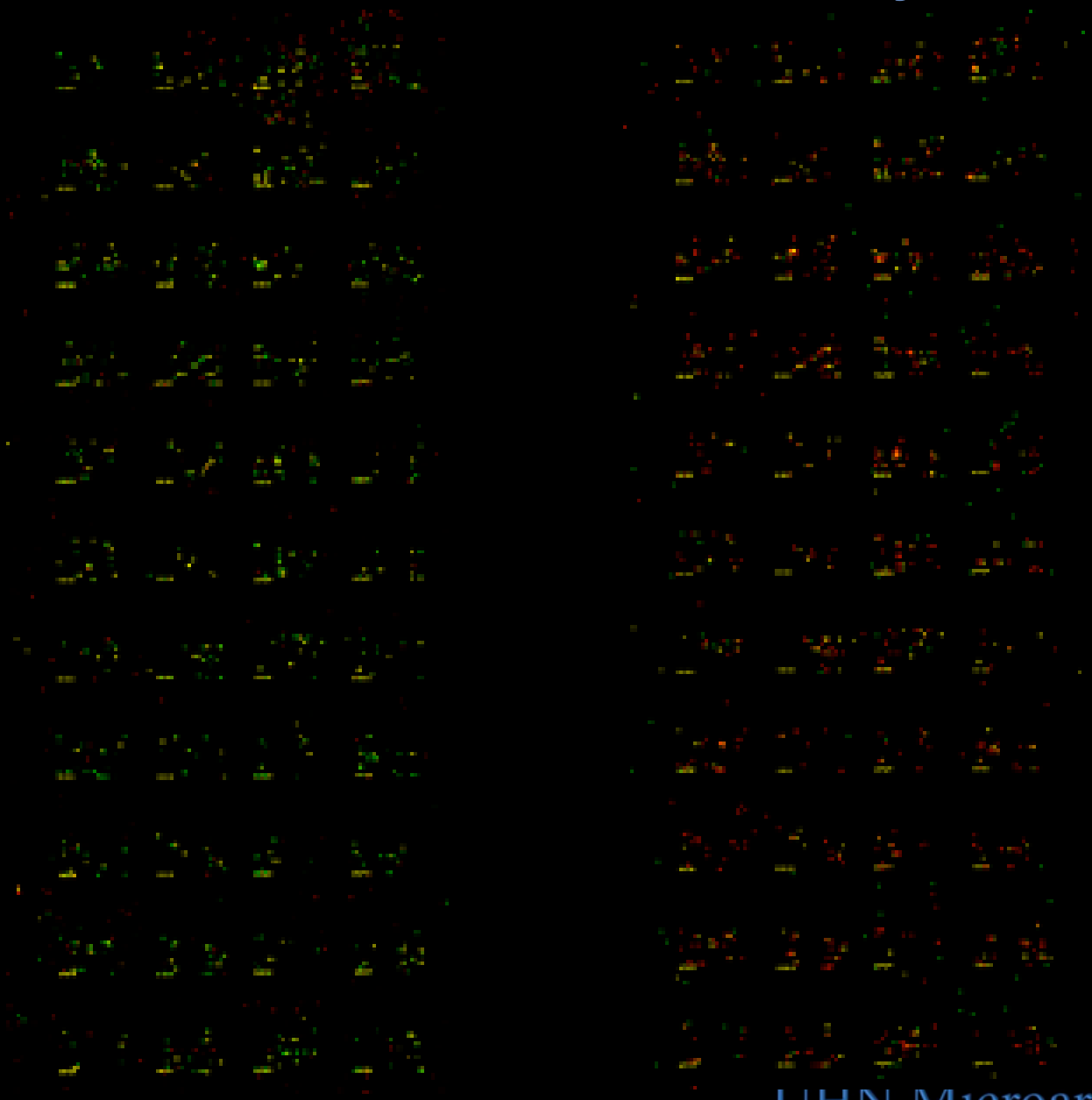

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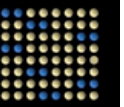
Data Analysis

- All arrays scanned, quantified and entered into GeneTraffic™
- Spot intensities normalised by *Arabadopsis* controls
- Filtered all spots with experimental cannel intensity less than 512
- Selected spots with > 2-fold ratio of +Ab/-AB



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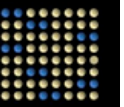
Results

- Total number of positive clones:
  - 0 hours: 0
  - 6 hours: 0
  - 24 hours: 260
  - 72 hours: 0
- 20 clones randomly chosen and sequenced (both directions)
- Sequence data queried against genomic sequence



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Informatics Pipeline

Hits from microarrays → 260 clones

↓

Collect sequences → 20 random

↓

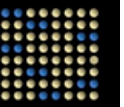
Remove redundancy → 12 remaining

↓

Scan for sequences with repetitive elements → 6 remaining

↓

Submit sequences to BLAT (UCSC)



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Find only those with exact matches (no gaps)

↓

Look for any genes (predicted or known) in region

↓

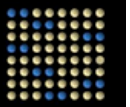
Retrieve large fragment of genomic sequence encompassing the potential promoter, gene and submitted sequence

↓

Use MSCAN to search for potential Mef-2 binding consensus sequence

↓

Extract regulatory region



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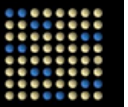
Use BLAT to locate this region on whole genome

Ensure that this sequence is within a reasonable distance of the gene of interest

Design primers to this region

Run ChIP with these specific primers and confirm microarray result



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Sample ID	0 hour FOR	0 hour REV	6 hour FOR	6 hour REV	1 day FOR	1 day REV	3 day FOR	3 day REV
16K01	0.256413942	0.128071129	0.713034687	0.214087724	1.791018318	1.562965508	0.592307149	0.106925116
07H16	0.70023574	-0.103017219	0.9733921	0.194145439	1.70091124	1.26233237	0.795308229	-0.123129723
15G10	0.146715123	0.332615023	0.488551273	0.561857374	1.681257867	1.708030011	0.861299916	0.391230433
03J13	0.473024447	0.381420254	0.737245956	0.567375425	1.676496466	1.519621488	0.52732692	0.383569913
12L24	0.481859942	0.127395987	0.769809207	0.413118907	1.652590398	1.586356043	0.50673634	0.129924325
04B24	0.090128174	0.171141685	0.506018996	0.752638297	1.63963705	1.682808467	0.712043544	0.289281761
02P11	0.37548072	0.410583237	0.388537605	0.577599645	1.63397755	1.691026244	0.445840564	0.410075151
11G22	0.265889717	0.32211013	0.430616757	0.203614646	1.627434286	1.159876808	0.915625876	0.218526916
18G22	0.128237578	0.155283572	0.657384613	0.535431612	1.619981684	1.457295301	0.813558108	0.152732591
14J11	0.302189709	0.326958358	0.449631288	0.622304728	1.617297851	1.787123433	0.343860494	0.430822514
11N01	0.465143407	0.166056566	0.77321046	0.128051847	1.617117254	1.234939723	0.747396268	0.010551712
19K23	0.29633545	0.276918323	0.455015826	0.573781672	1.602261659	1.427559251	0.570072622	0.245716727
13J04	0.75777217	-0.159757551	0.745569792	0.230288466	1.600392978	1.231966923	0.540334694	-0.04314942
05H07	0.146620715	0.434712615	0.697660989	0.36874243	1.563675027	1.593980287	0.755247063	0.138862363
10P15	0.550307985	0.033648846	0.808097901	0.148087902	1.549880514	1.280703094	0.515766324	-0.027965905
19O11	0.139866218	0.528095054	0.117722127	0.654554767	1.532797106	1.390544747	0.797551384	0.114852505
01B02	0.56287742	0.231678632	0.414053947	0.390077023	1.525624877	1.275517159	0.55601813	0.174848198
17F02	0.122234069	0.057376578	0.457740894	0.422462962	1.525354938	1.540102053	0.579401247	0.134821937
11F11	0.347238717	0.011828431	0.588984471	0.216530856	1.524419597	1.485622226	0.84312707	0.130073256

0 6hr 1 3 day

MEF-2

Courtesy Mark Takahashi, CGC

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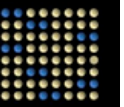
Search Results | Current Page: 1 | Total Pages: 1 | Total Results: 8 |

	Hyb. Group	Hybridization	Gene ID	Sample ID	LEX.E - BG	LEX.R Norm.	Fold Change	Flag	Image
1	0 hours	0 hour FOR	10P15	10P15	39645	27072	1.46		
2	0 hours	0 hour REV	10P15	10P15	16088	15717	1.02		
3	1 day	1 day FOR	10P15	10P15	35601	12159	2.93		
4	1 day	1 day REV	10P15	10P15	28524	11740	2.43		
5	3 day	3 day FOR	10P15	10P15	38542	26957	1.43		
6	3 day	3 day REV	10P15	10P15	24751	25235	-1.02		
7	6 hours	6 hour FOR	10P15	10P15	18083	10328	1.75		
8	6 hours	6 hour REV	10P15	10P15	21749	19627	1.11		



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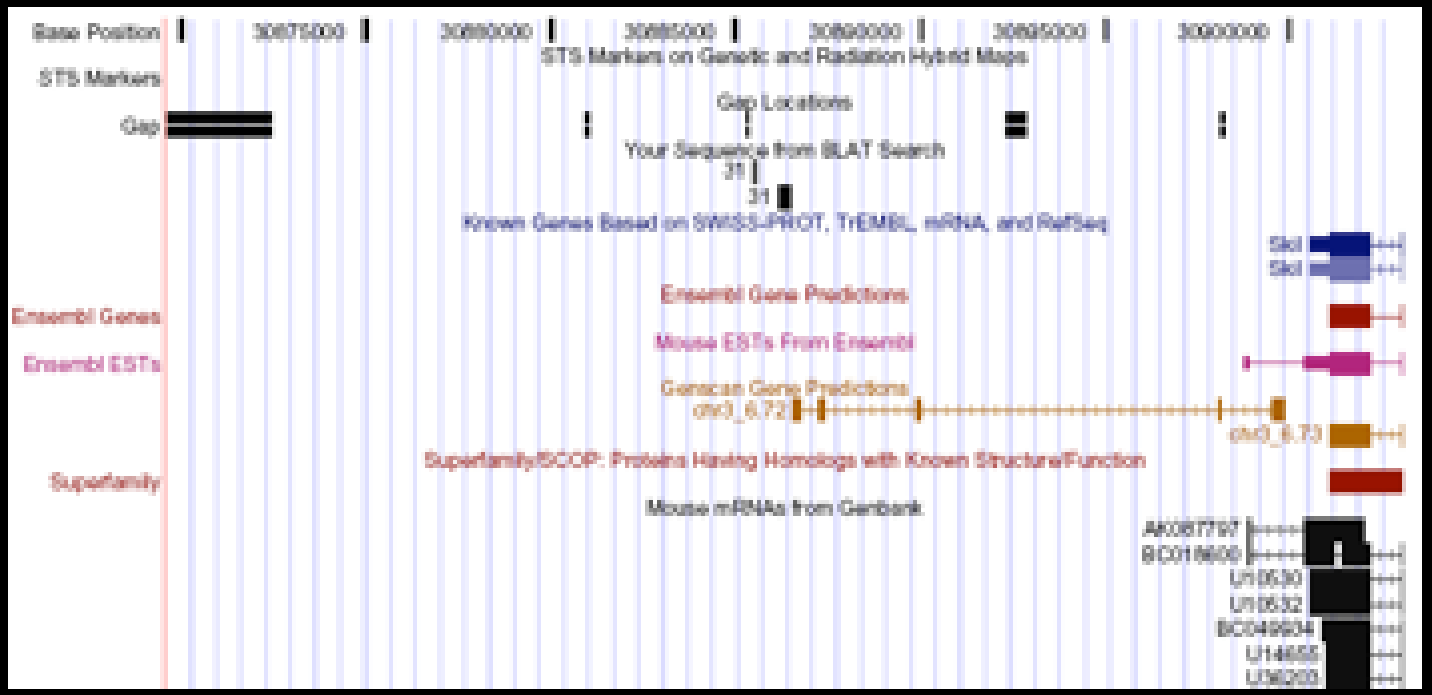

Courtesy Mark Takahashi, CGC

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SOURCE
GeneReport
M. musculus

Ski

Sloan-Kettering viral oncogene homolog

UniGene, LocusLink

Aliases

- 2310012O20Rik; 2610001A11Rik; BC004033; MGC8300; MOC 8300

Chromosomal Location

Chromosome/Cytoband	473.9 cM

Microarray Gene Expression Data

Data available	Show Gene Expression Data

SwissProt Information

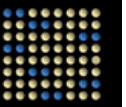
SwissProt Accession No.	Q60698 **Ski oncogene** (*Mus musculus*), 99% similarity over 325 a.a.
Function	may play a role in terminal differentiation of skeletal muscle cells but not in the determination of cells to the myogenic lineage
Developmental Stage	is expressed in a uniform pattern in all embryonic cells prior to skeletal muscle cell formation in the myotomes of somites. expression is first upregulated in skeletal muscle at 12 dpc, this upregulation is evident first in body wall muscle and one day later in limb muscles. at 13.5 dpc a most prominent expression is seen in all skeletal muscles. at this stage expression is seen in all other cells and tissues but at lower levels than in skeletal muscle
Subcellular Location	nuclear
Similarity	to ski protein of sloan-kettering virus and to sno oncogenes
SwissProt Copyright	[illegible]

1Day non-amplified ChIP analysis

Reconfirm the results from the amplified data.

Plated cells onto thirty 150 mm dishes and grew to 80% confluence.

Following 1 day of differentiation cells were fixed and harvested for ChIP analysis.



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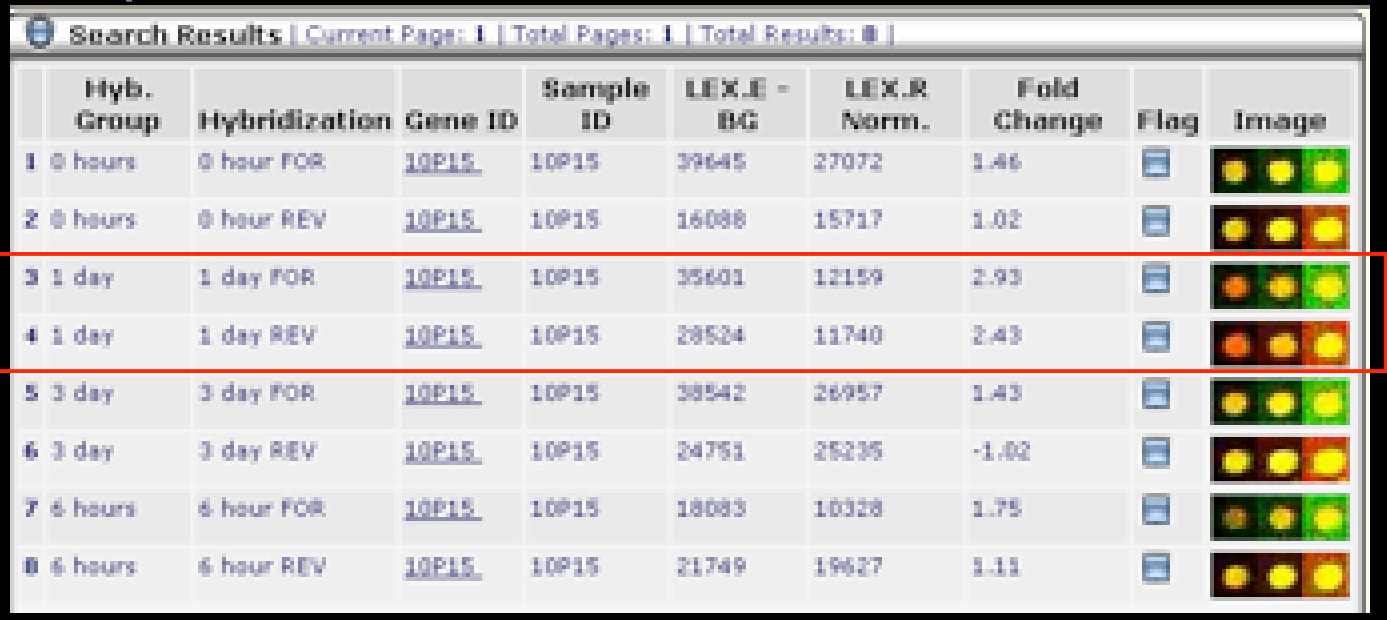
Amplified DNA



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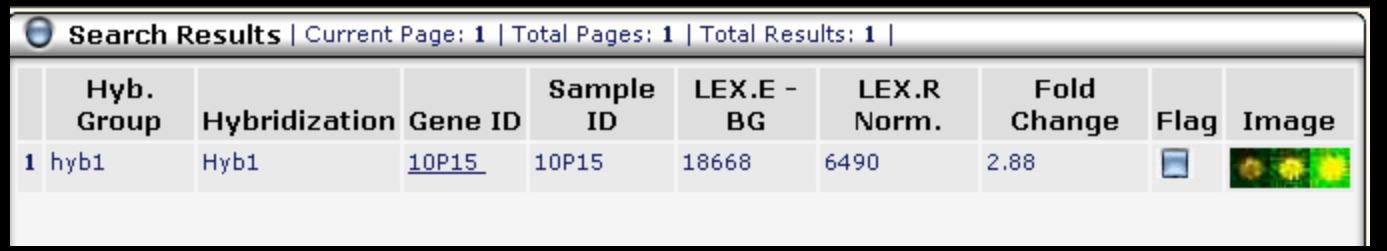
	Hyb. Group	Hybridization	Gene ID	Sample ID	LEX.E - BG	LEX.R Norm.	Fold Change	Flag	Image
1	0 hours	0 hour FOR	10P15	10P15	39645	27072	1.46		
2	0 hours	0 hour REV	10P15	10P15	16088	15717	1.02		
3	1 day	1 day FOR	10P15	10P15	35601	12159	2.93		
4	1 day	1 day REV	10P15	10P15	28524	11740	2.43		
5	3 day	3 day FOR	10P15	10P15	38542	26957	1.43		
6	3 day	3 day REV	10P15	10P15	24751	25235	-1.02		
7	6 hours	6 hour FOR	10P15	10P15	18083	10328	1.75		
8	6 hours	6 hour REV	10P15	10P15	21749	19627	1.11		

Non-amplified DNA



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	Hyb. Group	Hybridization	Gene ID	Sample ID	LEX.E - BG	LEX.R Norm.	Fold Change	Flag	Image
1	hyb1	Hyb1	10P15	10P15	18668	6490	2.88		

Courtesy Mark Takahashi, CGC

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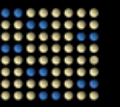
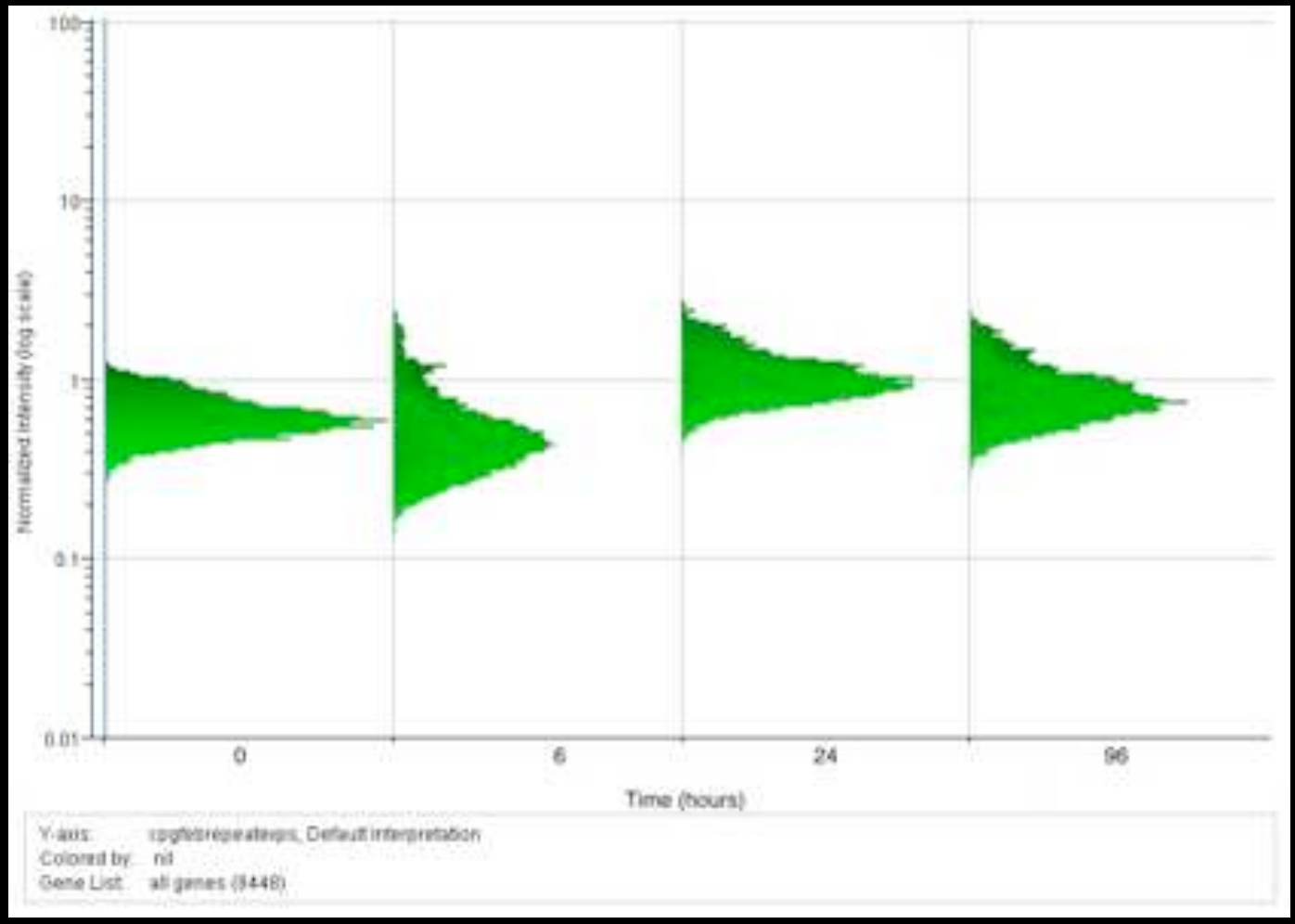
Let's Try that Again

- Repeat experiment, and alter experimental design
- More samples
- Reduce variability at seeding stage
- Reduce variability at labelling, storage stages
- Enhance bioinformatics approaches



Pre-normalised Data



Courtesy Mark Takahashi, CGC

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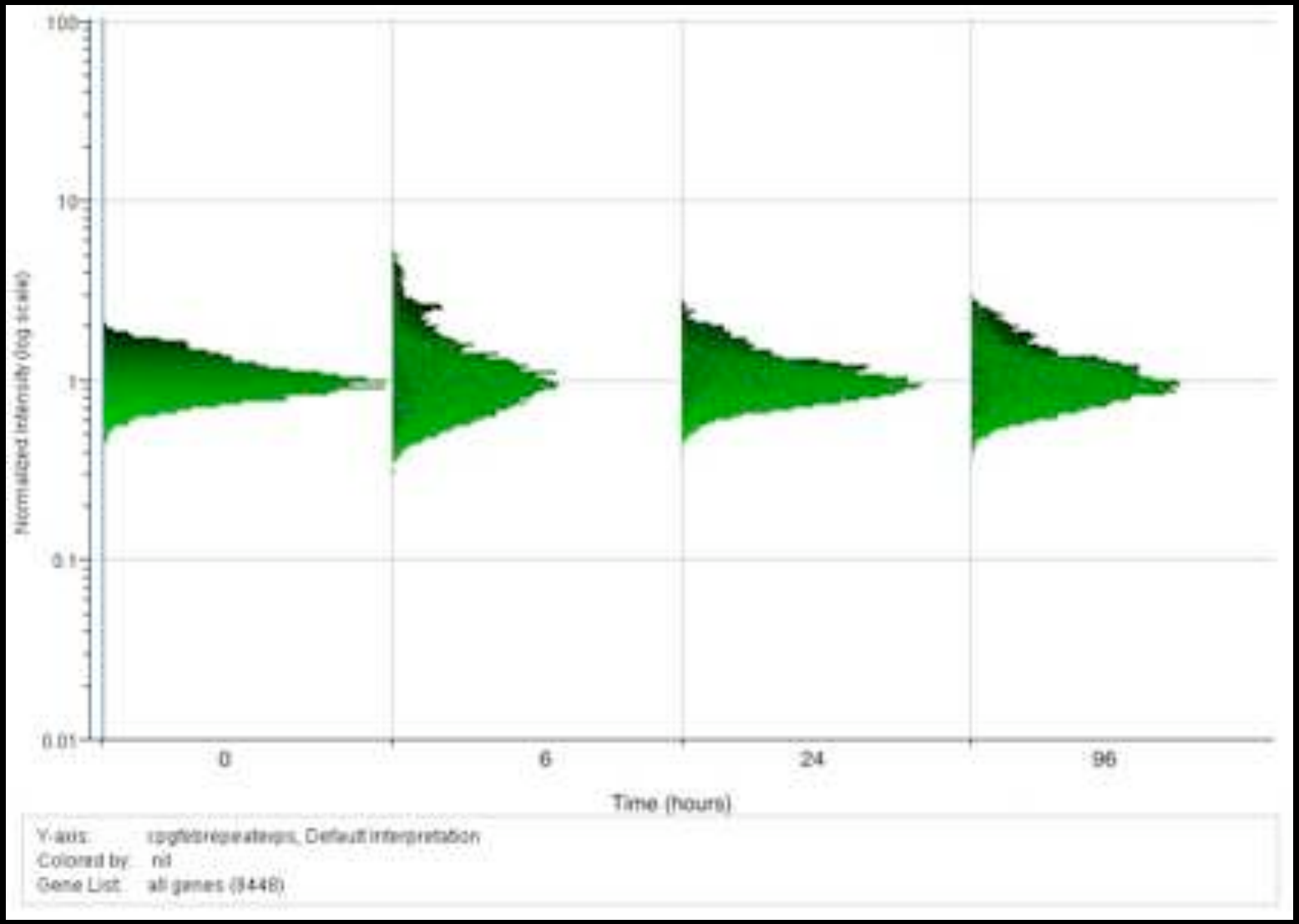
Normalised (Median Intensity)



Courtesy Mark Takahashi, CGC

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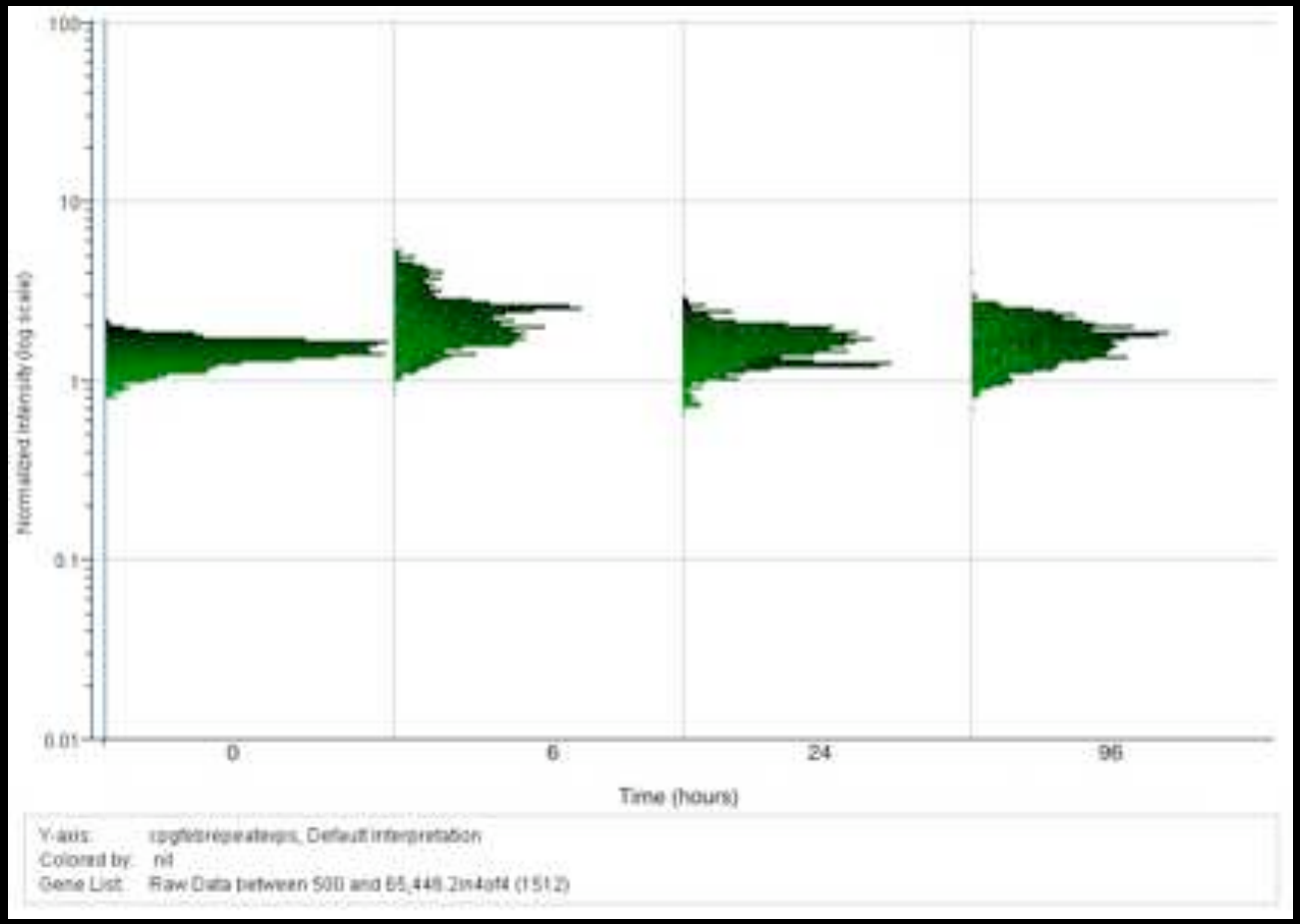
Remove Low Intensity Clones



Courtesy Mark Takahashi, CGC

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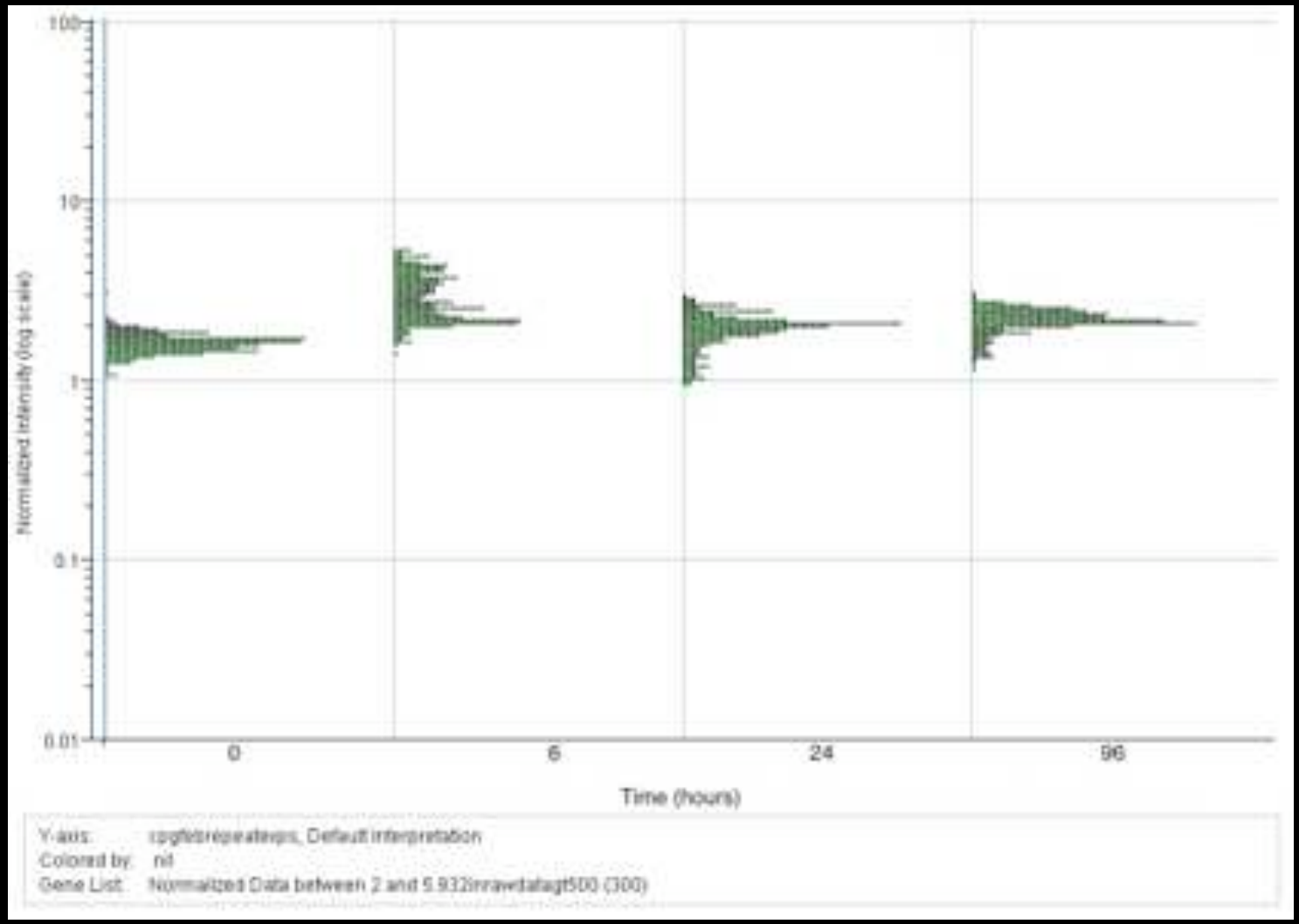
Include only >2 fold ratio



Courtesy Mark Takahashi, CGC

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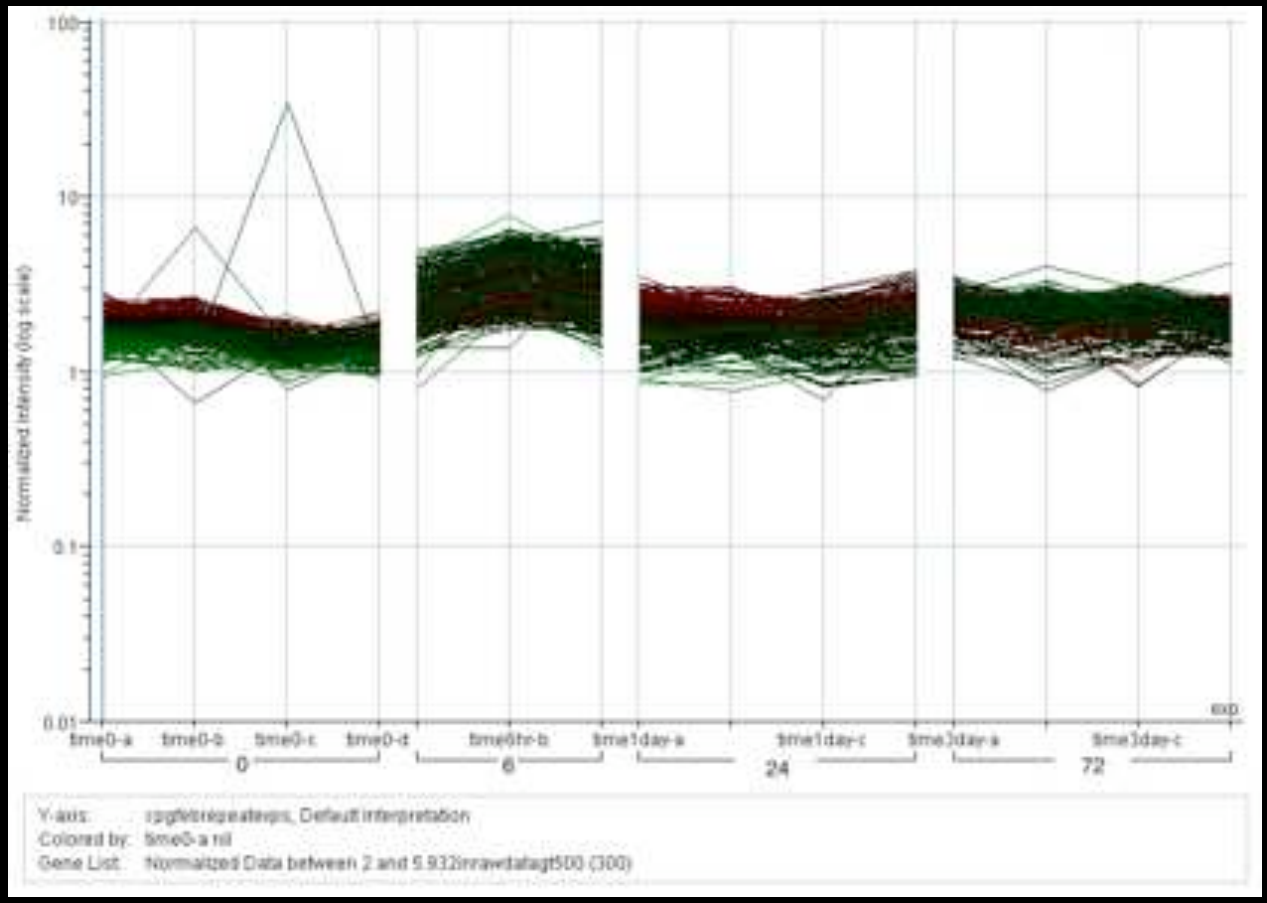
Consistency of Replicates



Courtesy Mark Takahashi, CGC

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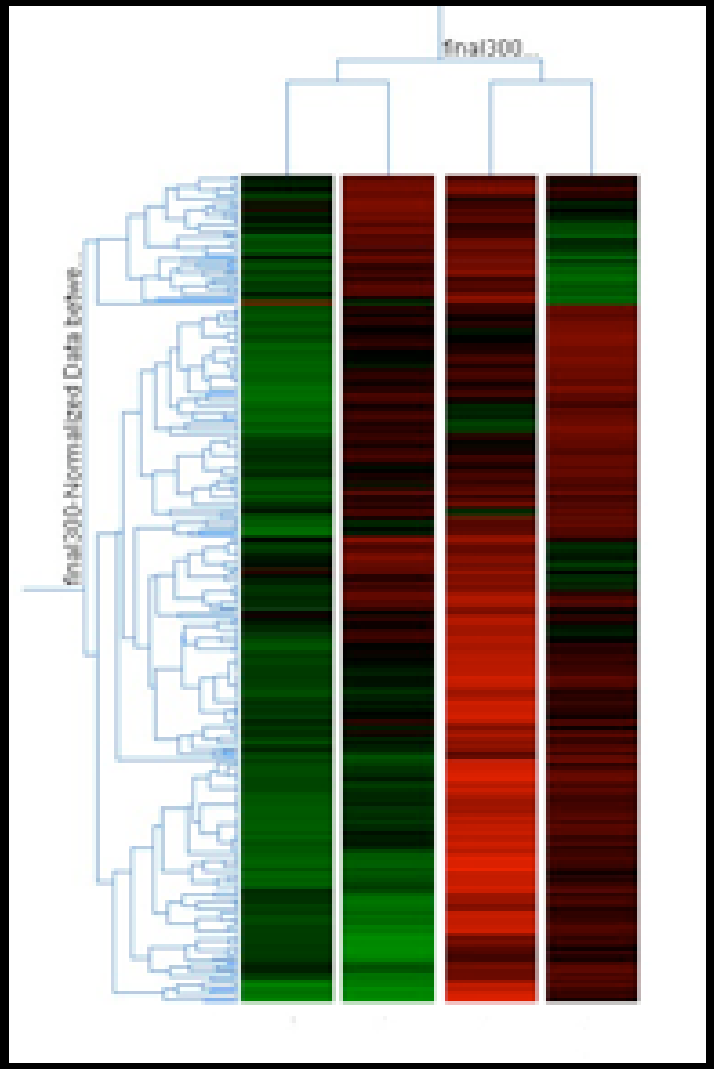
Clustering Positive Clones



Courtesy Mark Takahashi, CGC

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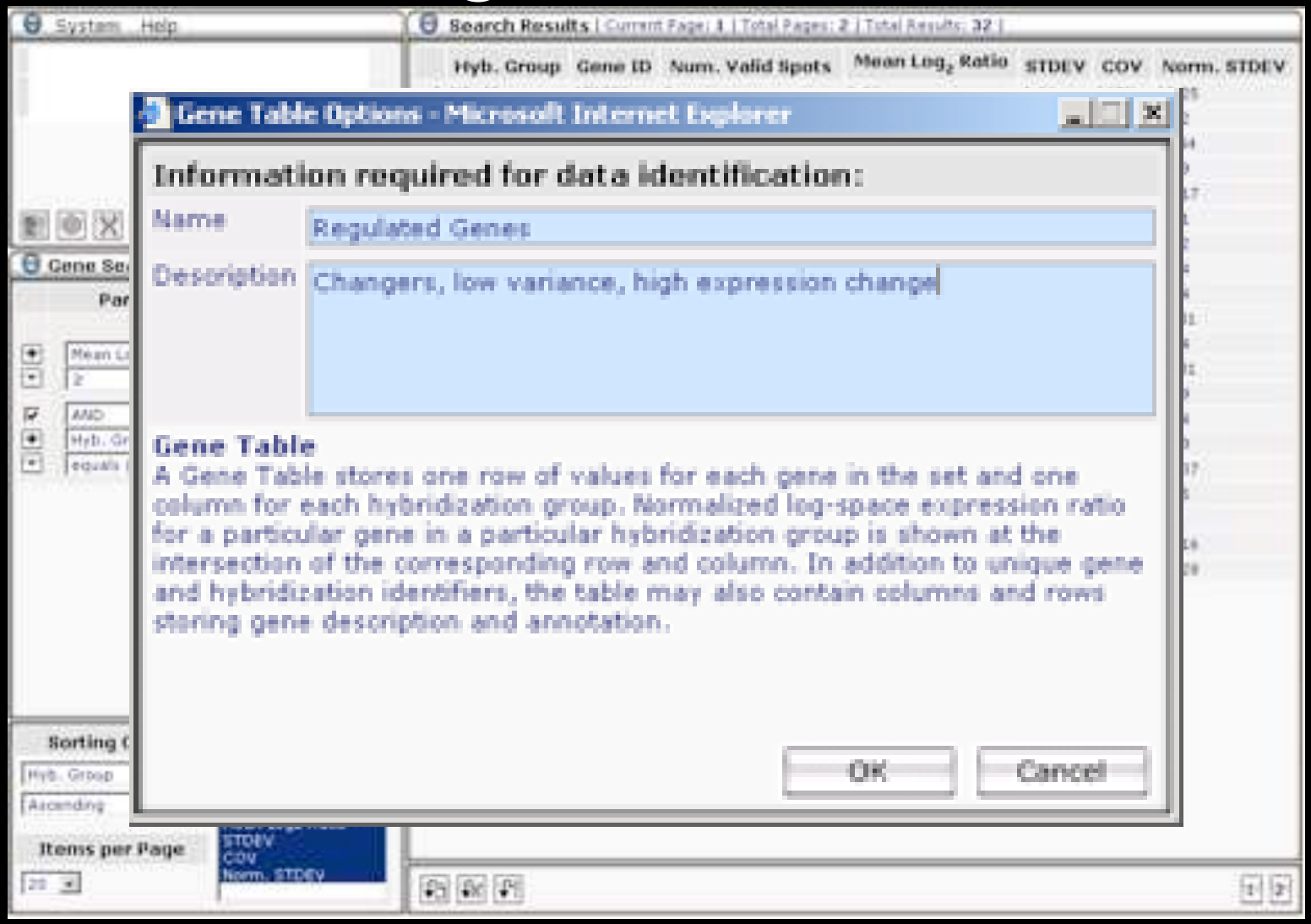
Selecting Positive Clones



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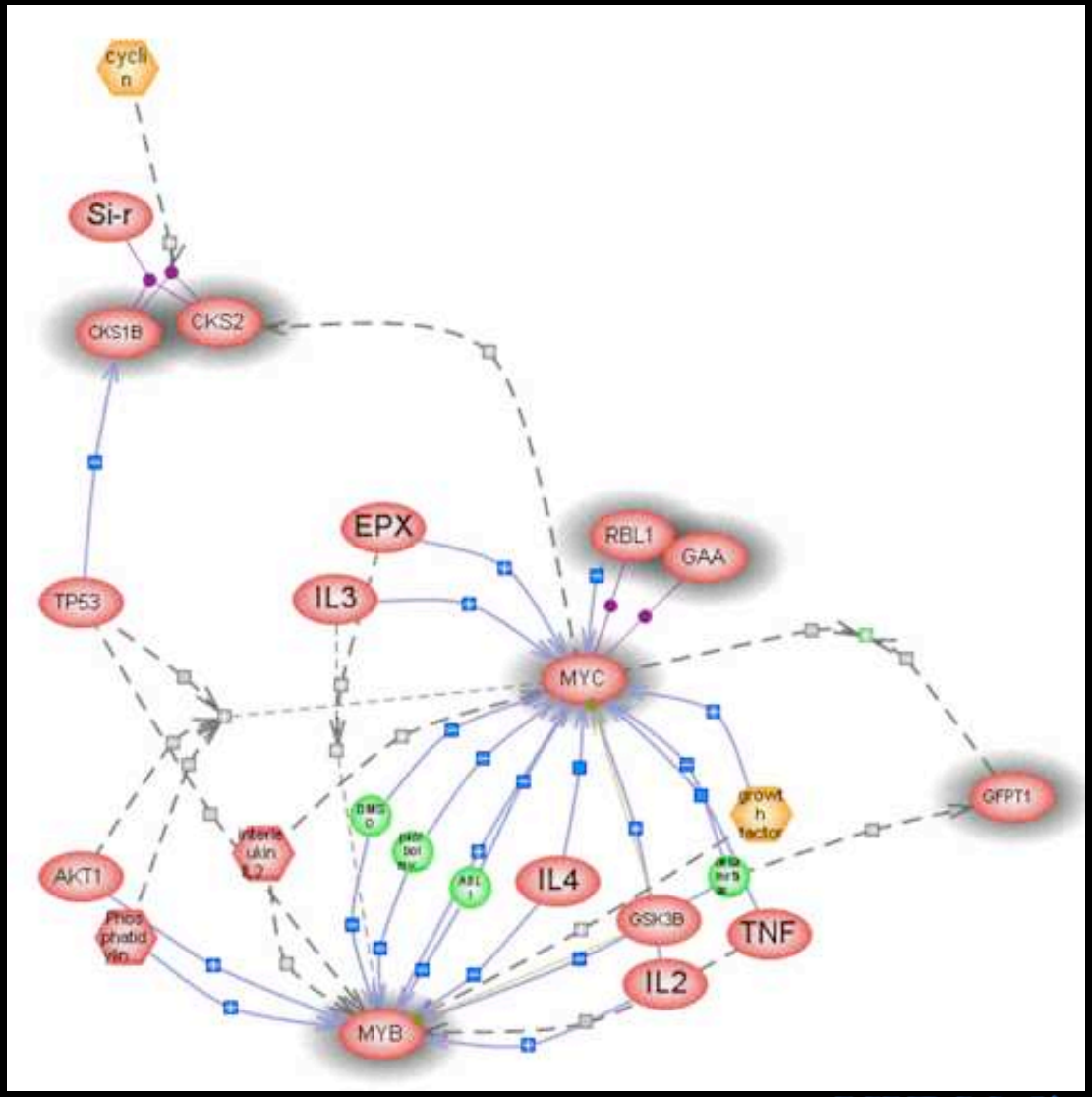
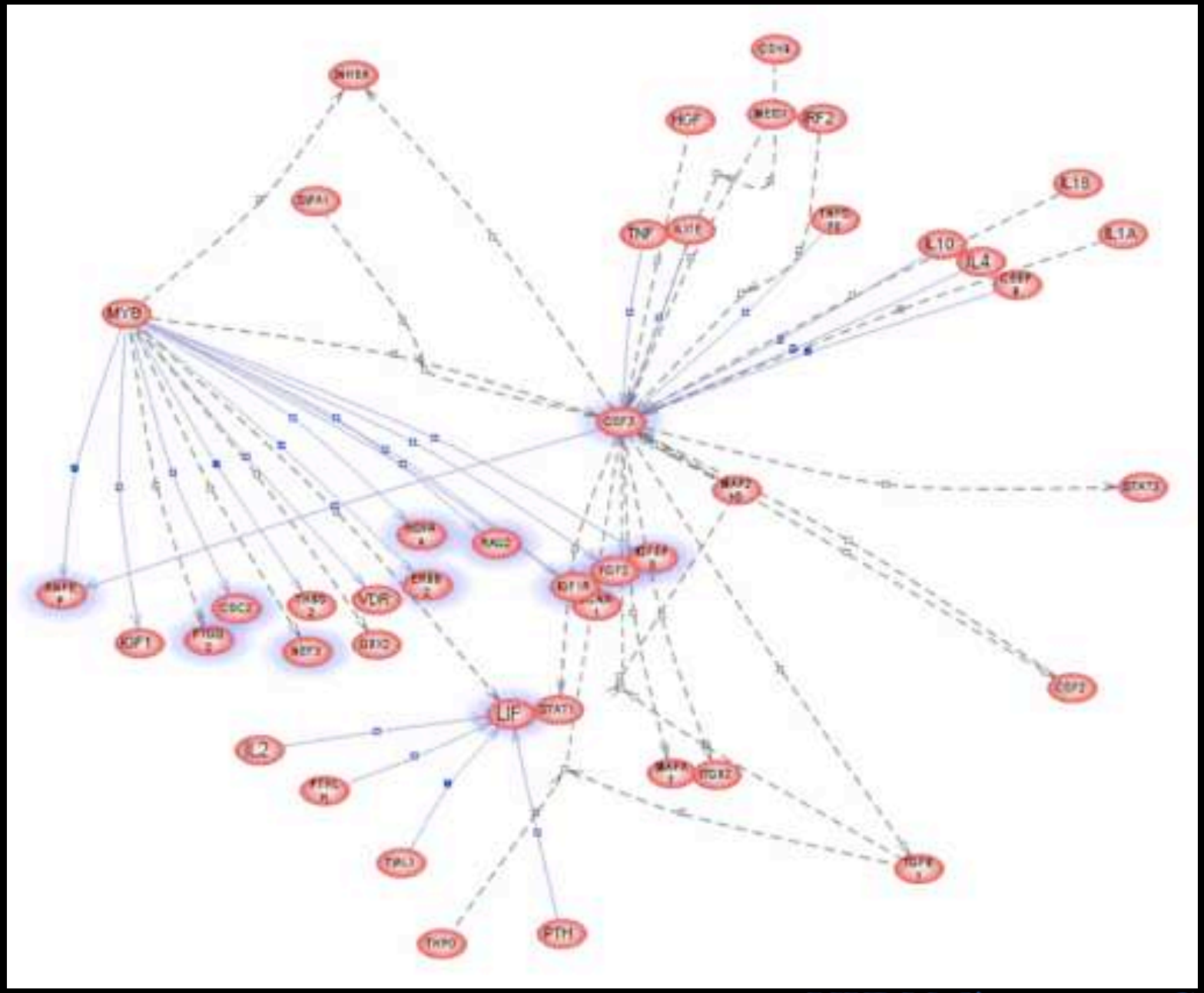
Pathway Analysis (with PathwayAssist™)





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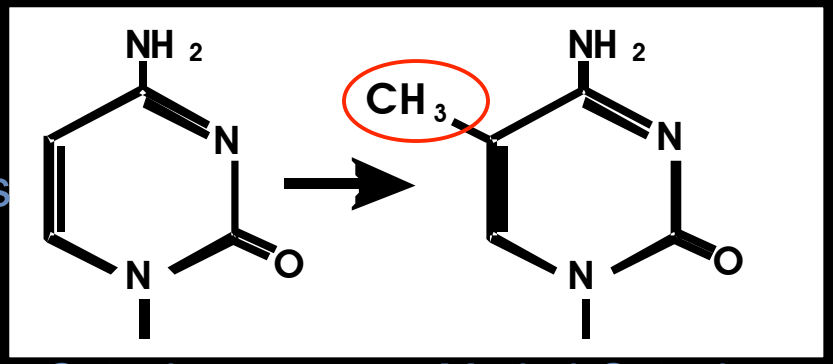
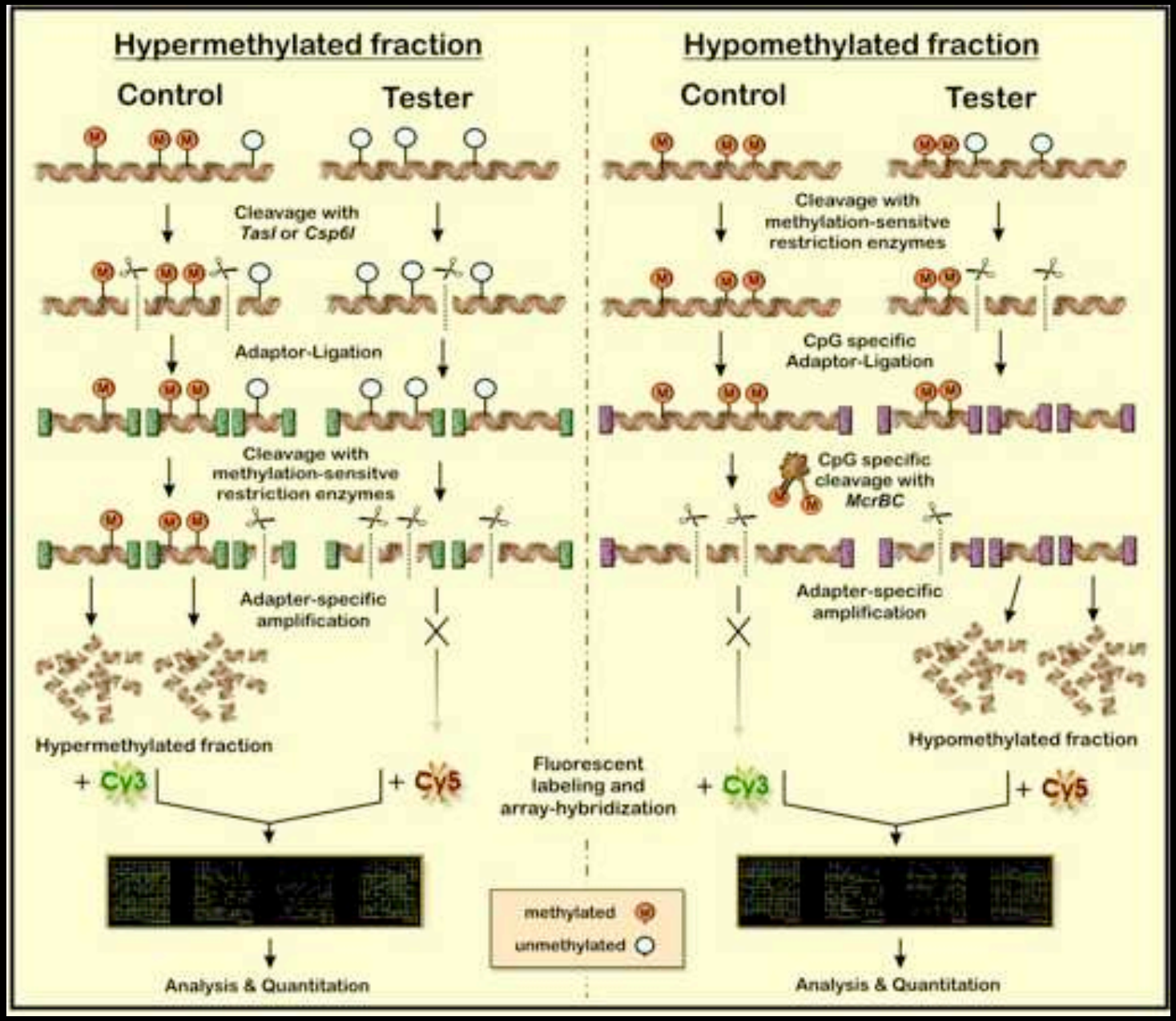
Epigenetics

- **Epigenomics** is the genomwide study of methylation and other epigenetic phenomena - that is, the study of the epigenome.
- The **epigenome** is the collection of biochemical modifications to chromatin that indexes genetic information.
- This collection of modifications includes DNA and protein modifications like:
  - Histone-Acetylation
  - Histone-Methylation
  - Matrix attachment sites
  - DNA-Methylation



Cytosine 5-Methyl-Cytosine



Courtesy Axel Schumacher & Art Petronis, CAMH

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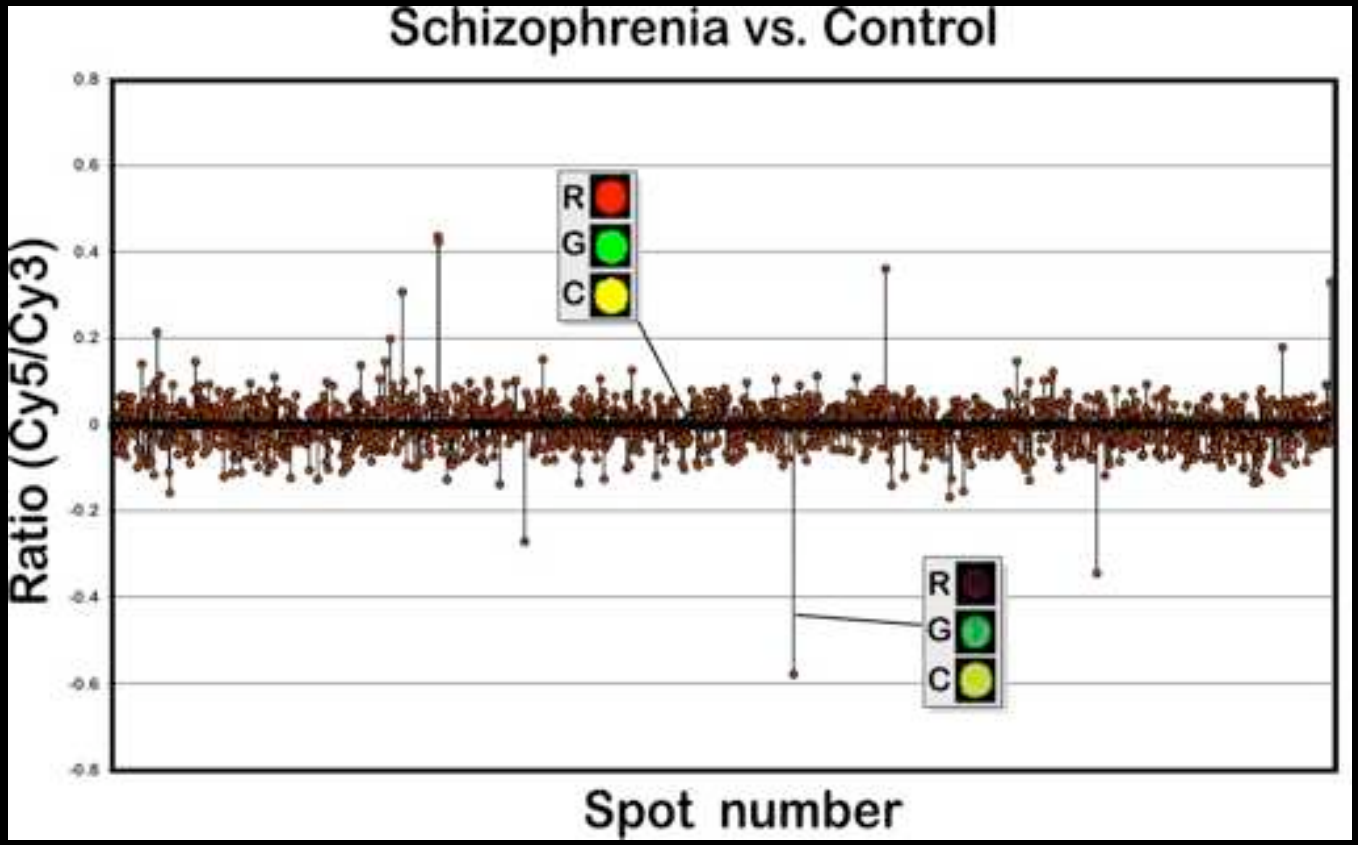
1.7k Human cDNA Microarray



Courtesy Axel Schumacher & Art Petronis, CAMH

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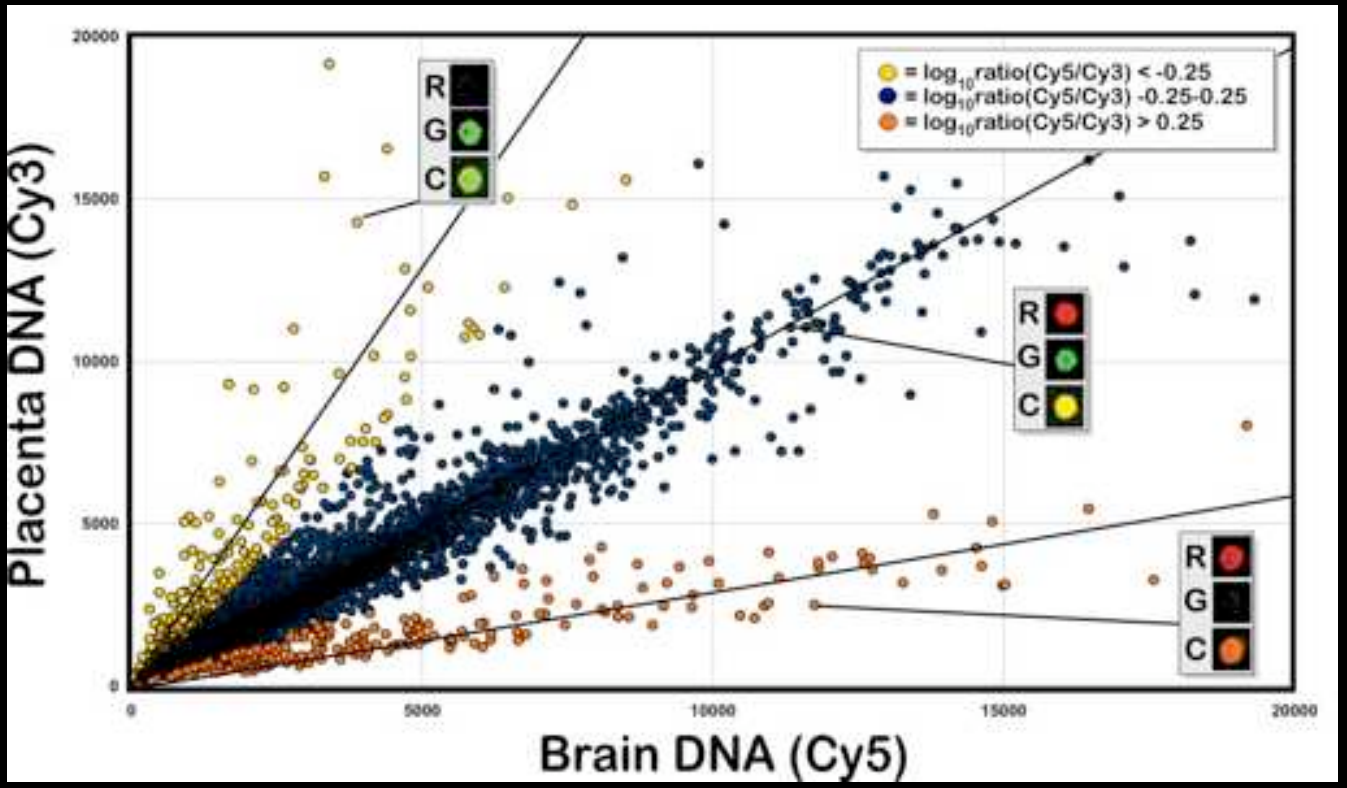
12k CpG Island Microarray



Courtesy Axel Schumacher & Art Petronis, CAMH

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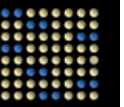
Plans For the Future

- Sequence entire human and mouse clonesets
  - Currently being done by UBC sequencing facility
- Clean up clones
  - Eliminate contaminated wells if any, chimeric clones etc…
- Eliminate redundancy
  - Bioinformatics
- Develop clones for additional promoter sites
  - Look at Mouse - Human regions of similarity
- Correlate CpG clones to ESTs on expression arrays
  - Bioinformatics



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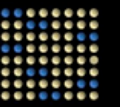
- Carl Virtanen
- Zihibin Lu



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Thank-You
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