Biomedical Genomics Facility

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Microarray Technology
Platform Comparisons

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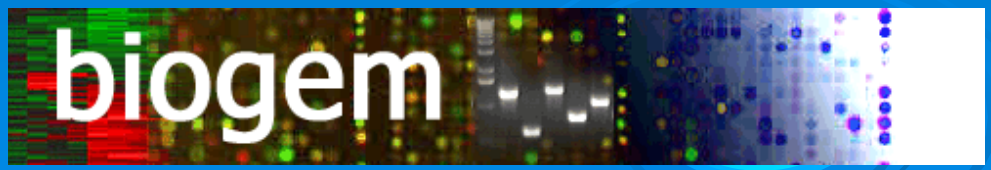
Director Biomedical Genomics Facility
University Of California San Diego



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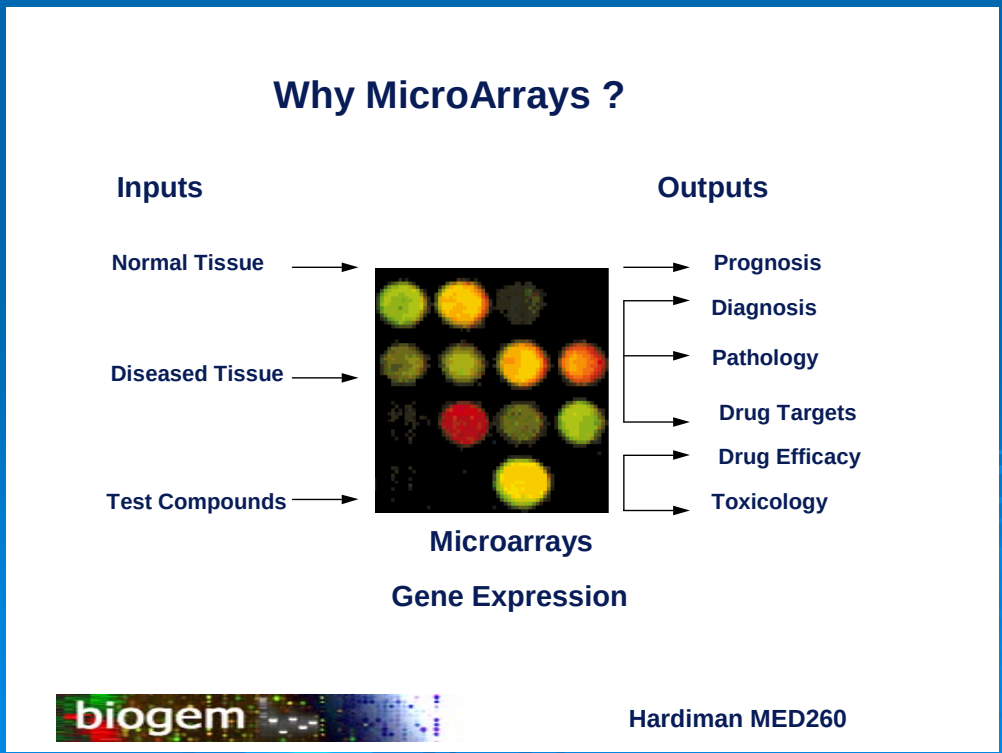
- BIOGEM an organized research unit and core facility created in 2000
- Custom Array Fabrication/Commercial Array Processing
- Fabricated Mouse, Human, Yeast and *Drosophila* Arrays
- UCSD and outside researchers

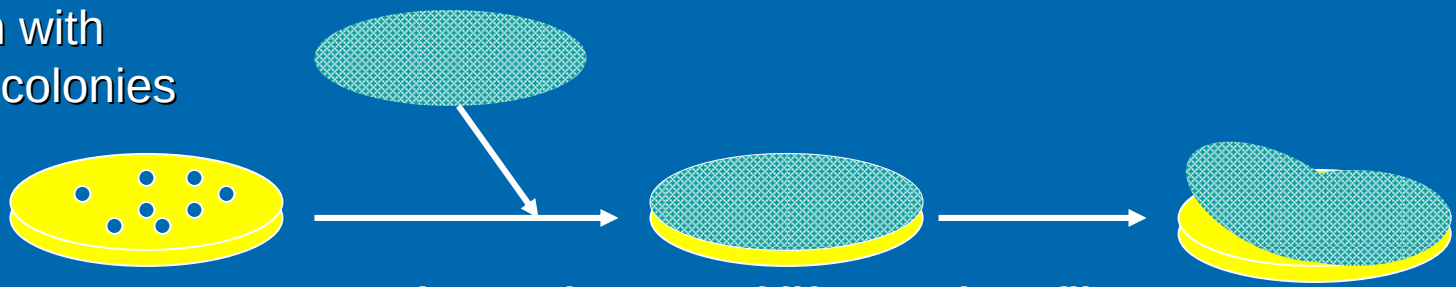
General Applications of Microarrays

- Defining “parts lists” for a cell, tissue or organ
- Determining responses to a perturbation (e.g., a drug, toxin, pathogen, etc.)
- Determining consequences of altering gene expression (e.g., gene knockouts or over expression)

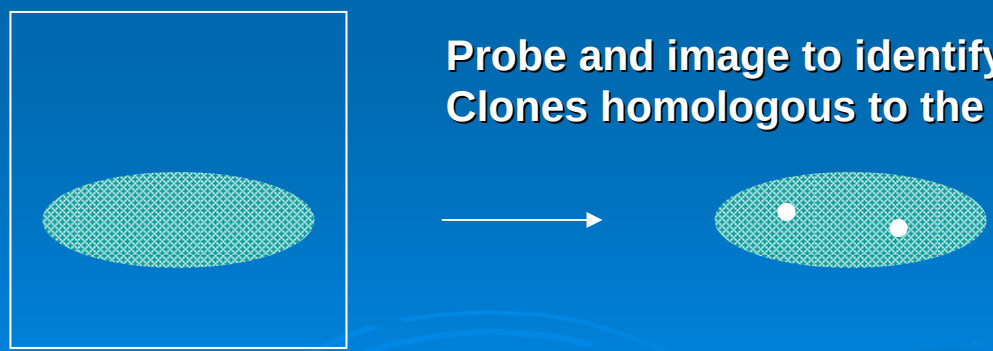


The Original DNA Array

Petri dish with
bacterial colonies



Apply membrane and lift to make a filter containing DNA from each clone.

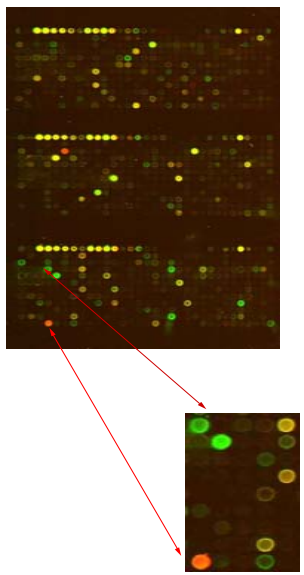


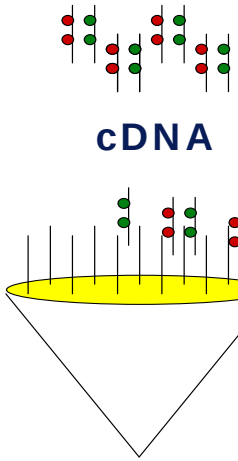
Probe and image to identify Clones homologous to the probe.

Microarrays & Gene Chips

MicroArrays

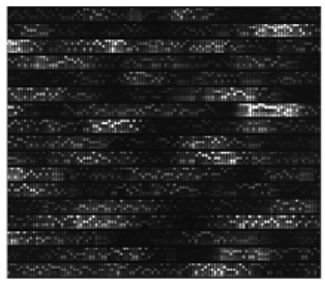
Target DNAs (spotted on arrays) are PCR products Printed on polylysine and aminosilane coated glass slides

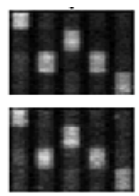




GeneChips

Oligonucleotide probes are synthesized *in situ* on the chip





Hardiman MED260

BIOGEM cDNA arrays retired in 2003

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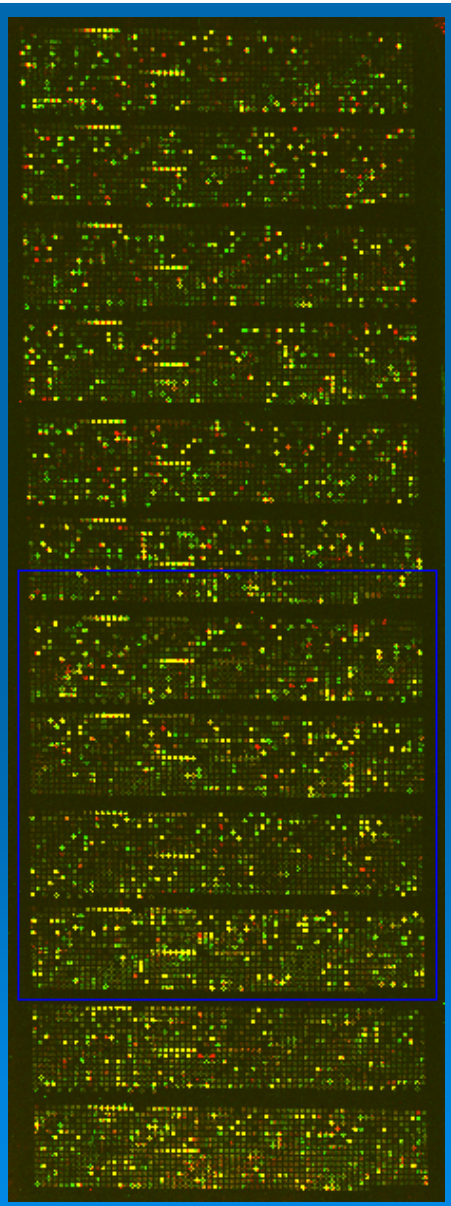

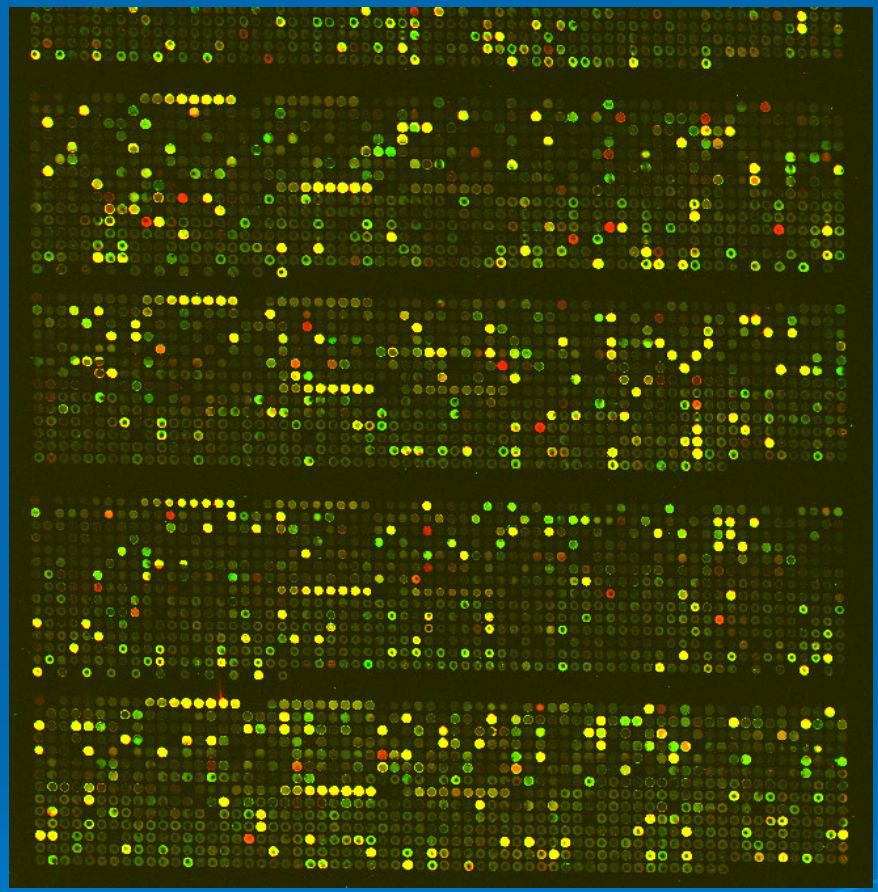

Snapshot and overlay of a BIOGEM human 11520 feature array hybridized with fluorescently labeled cye 3 spleen and cye 5 liver cDNA.

Advantages of Oligonucleotide Arrays

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- Greater Specificity
- Ability to detect Splice Variants
- Cross Hybridization Minimized
- Stable Attachment to Covalent and Non Covalent Surfaces
- Secondary Structure Minimized
- No need for PCR, bacterial growth, can avoid misidentification of features

Microarray Platforms

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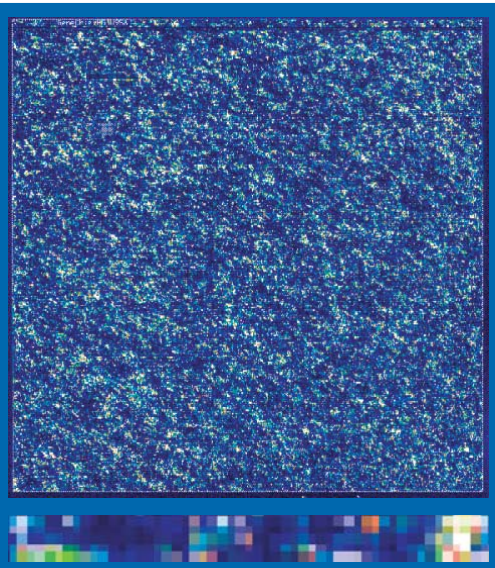
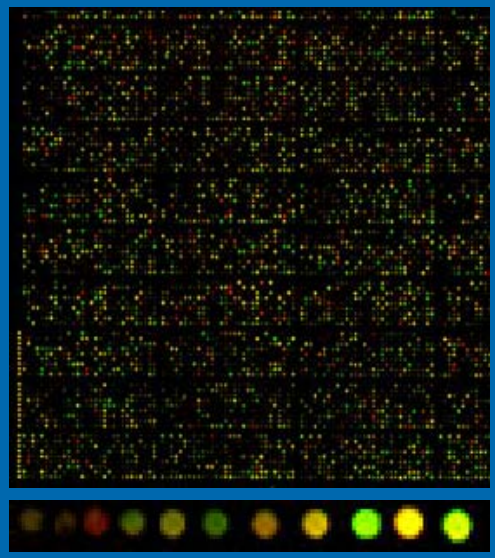
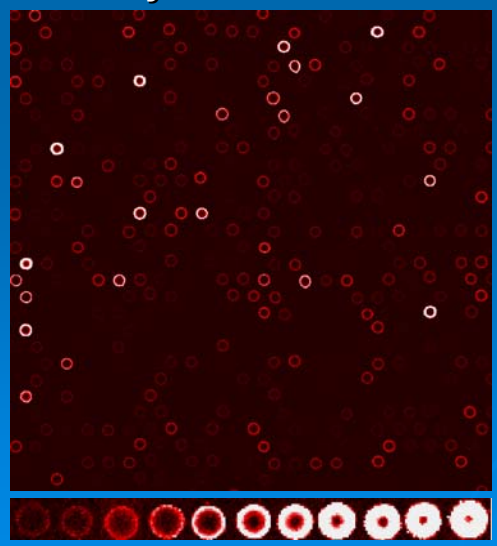


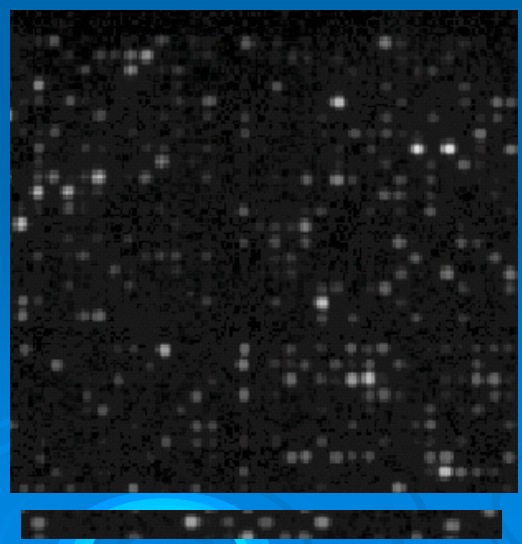
Affymetrix



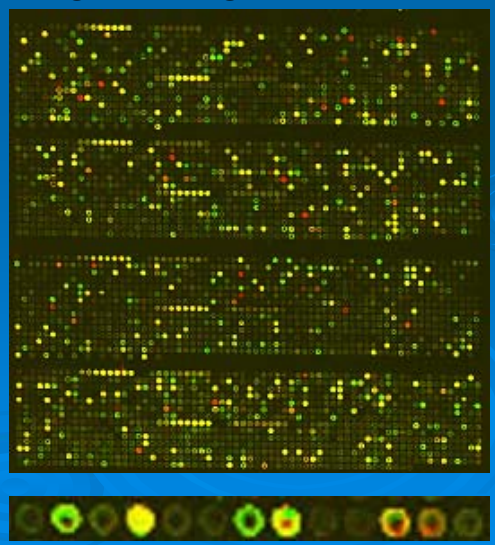
Agilent oligo



Amersham

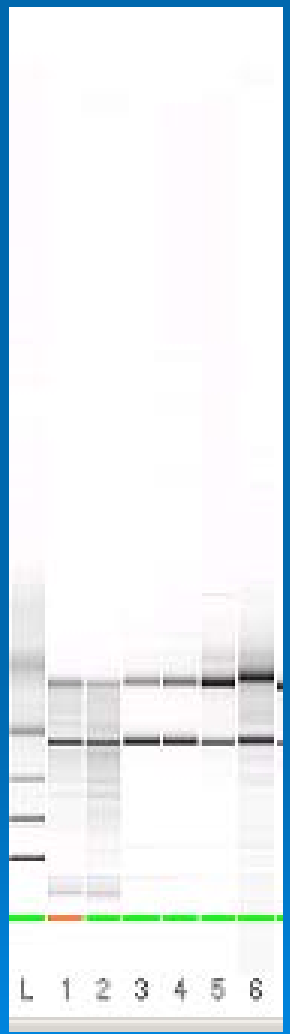


ABI



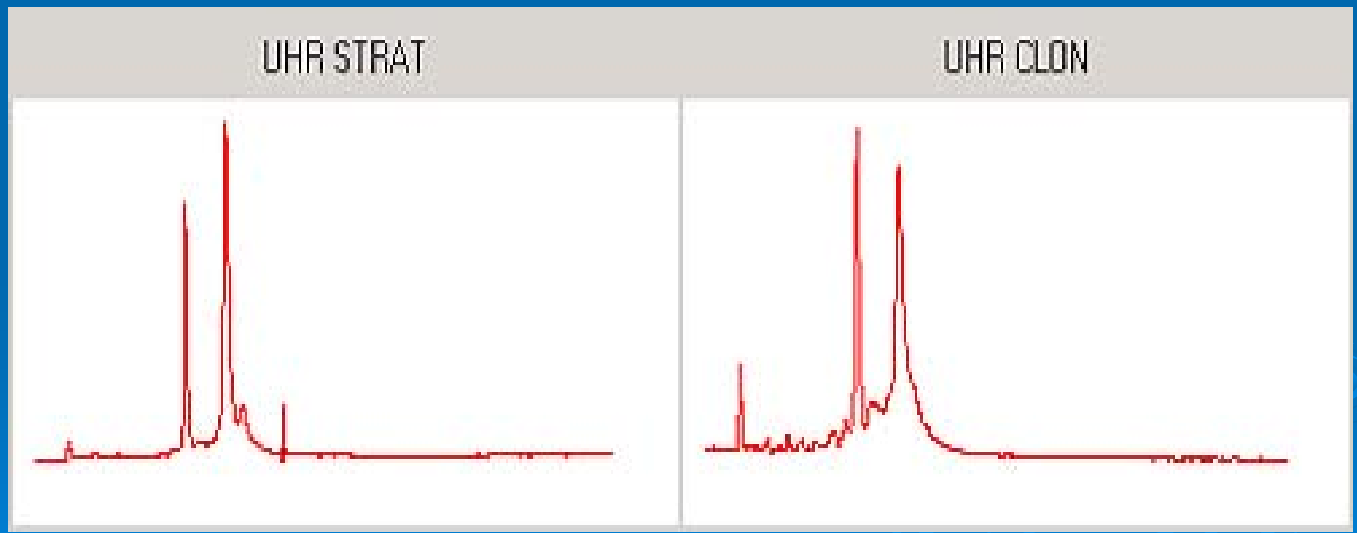
BIOGEM cDNA array

Sample Quality Control is Critical



Microfluidics – Agilent Bioanalyzer

Trace Profiles:



Samples are intact with no genomic DNA contamination.

Sample Quality Control is Critical

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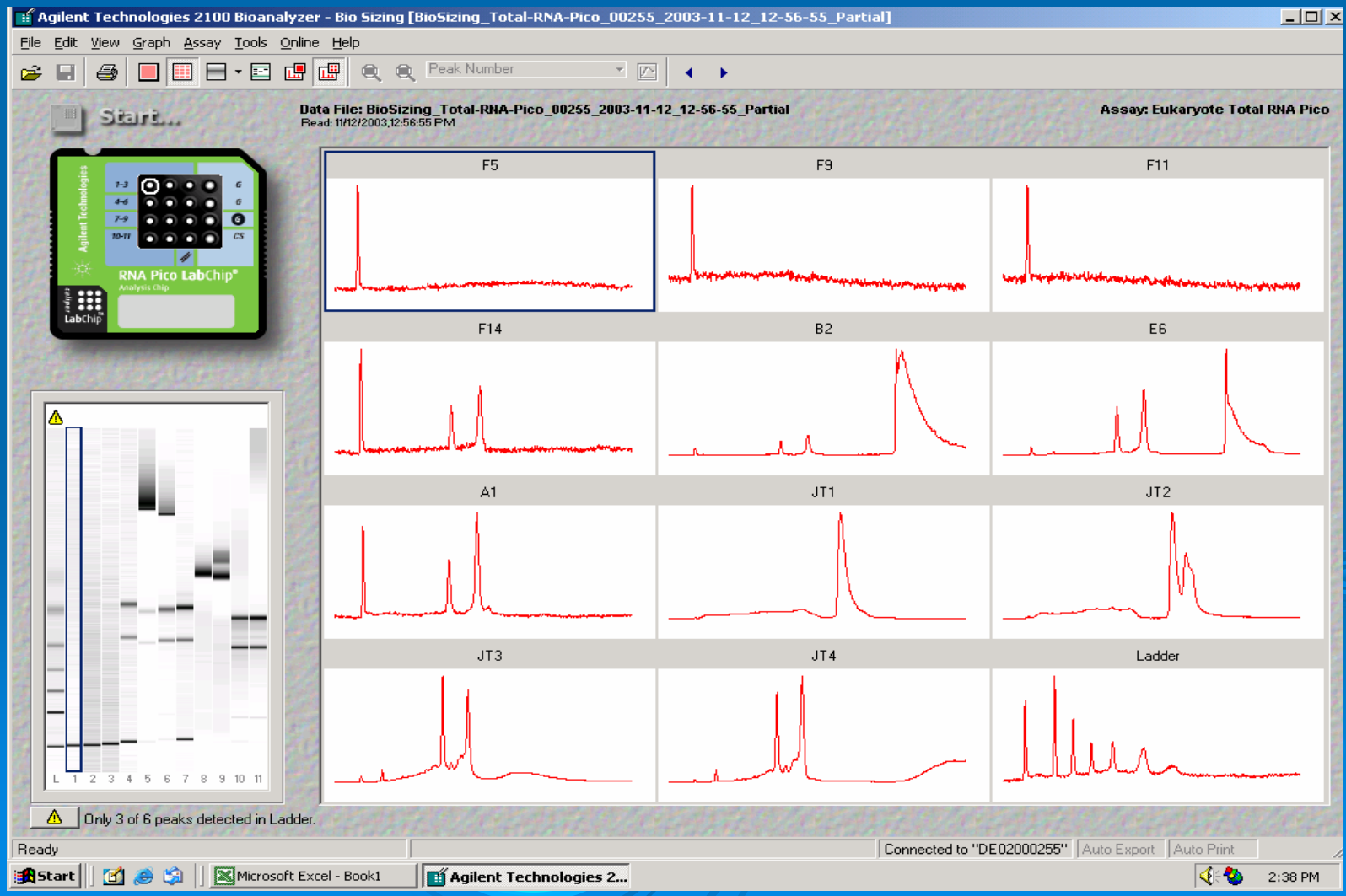
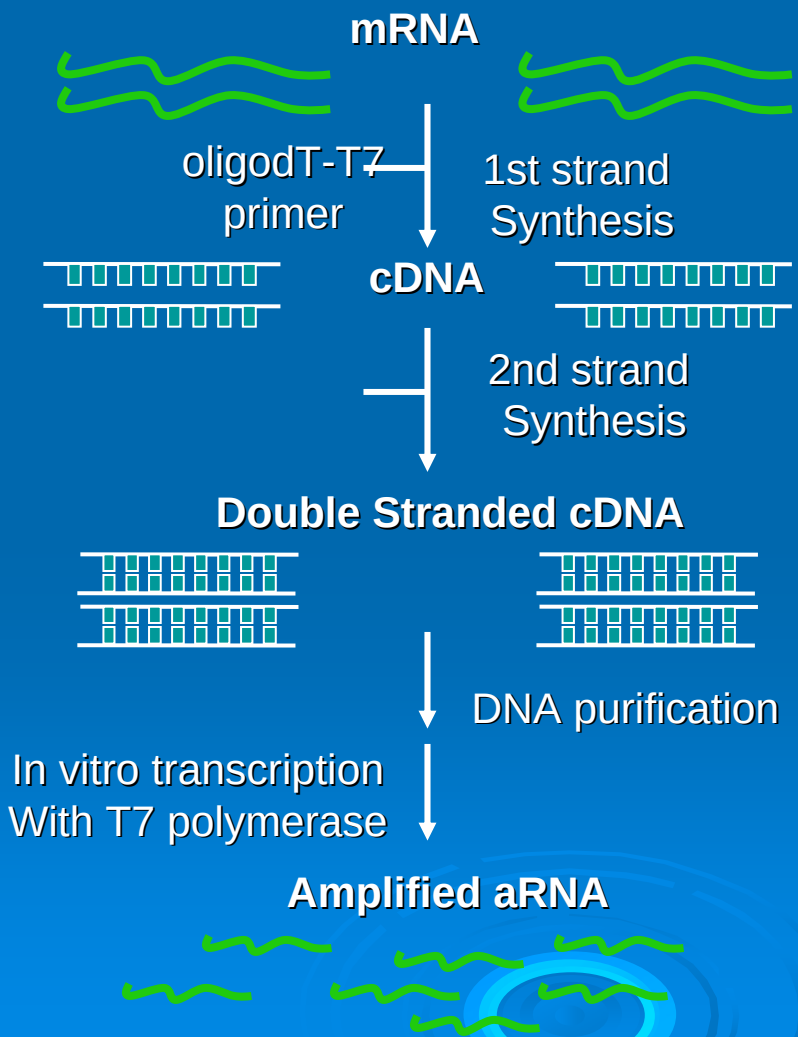
PicoChip – Agilent Bioanalyzer



Quality Control of cRNA

RNA Amplification



Quality Control of cRNA

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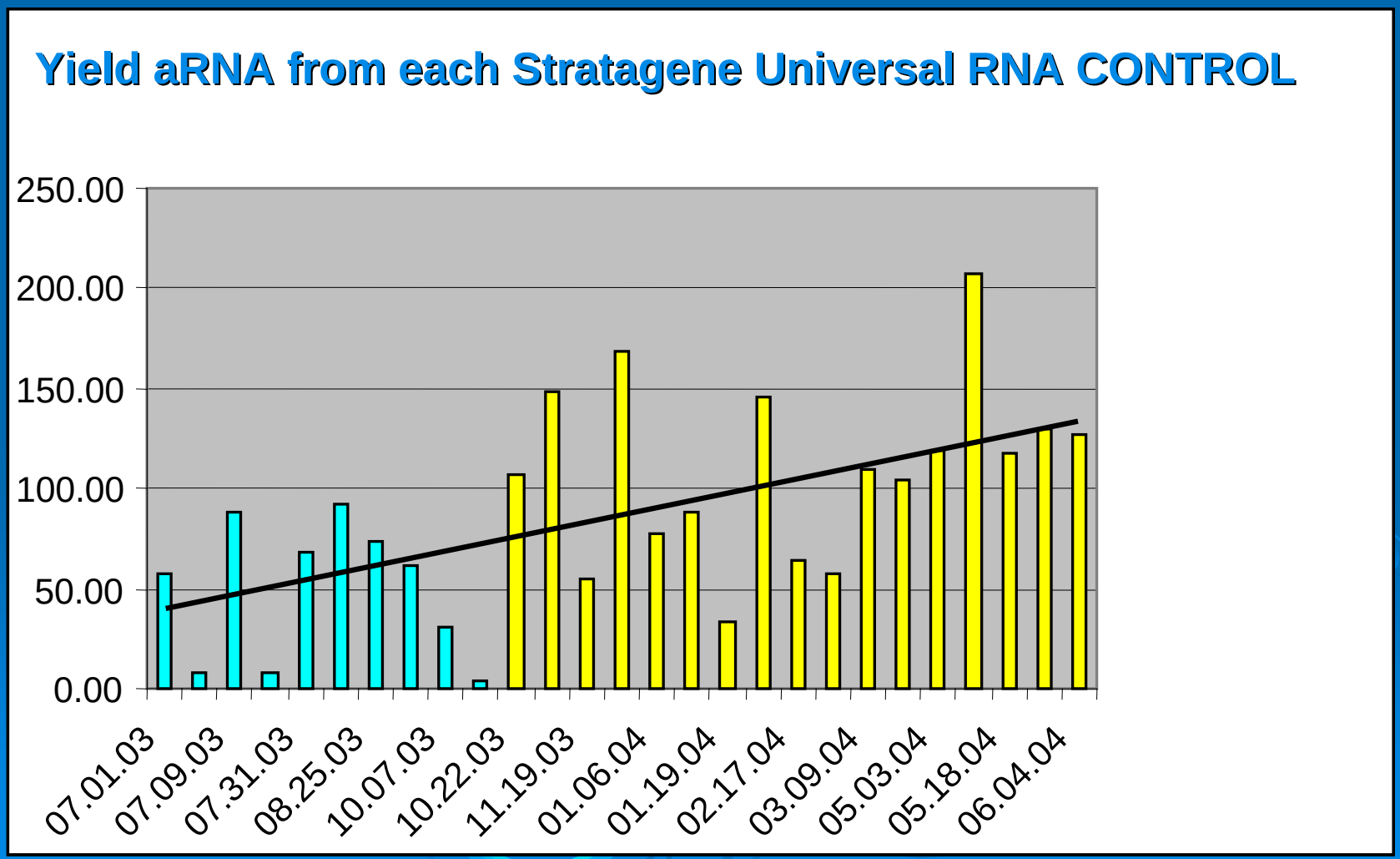
Yield in micrograms



Quality Control of cRNA

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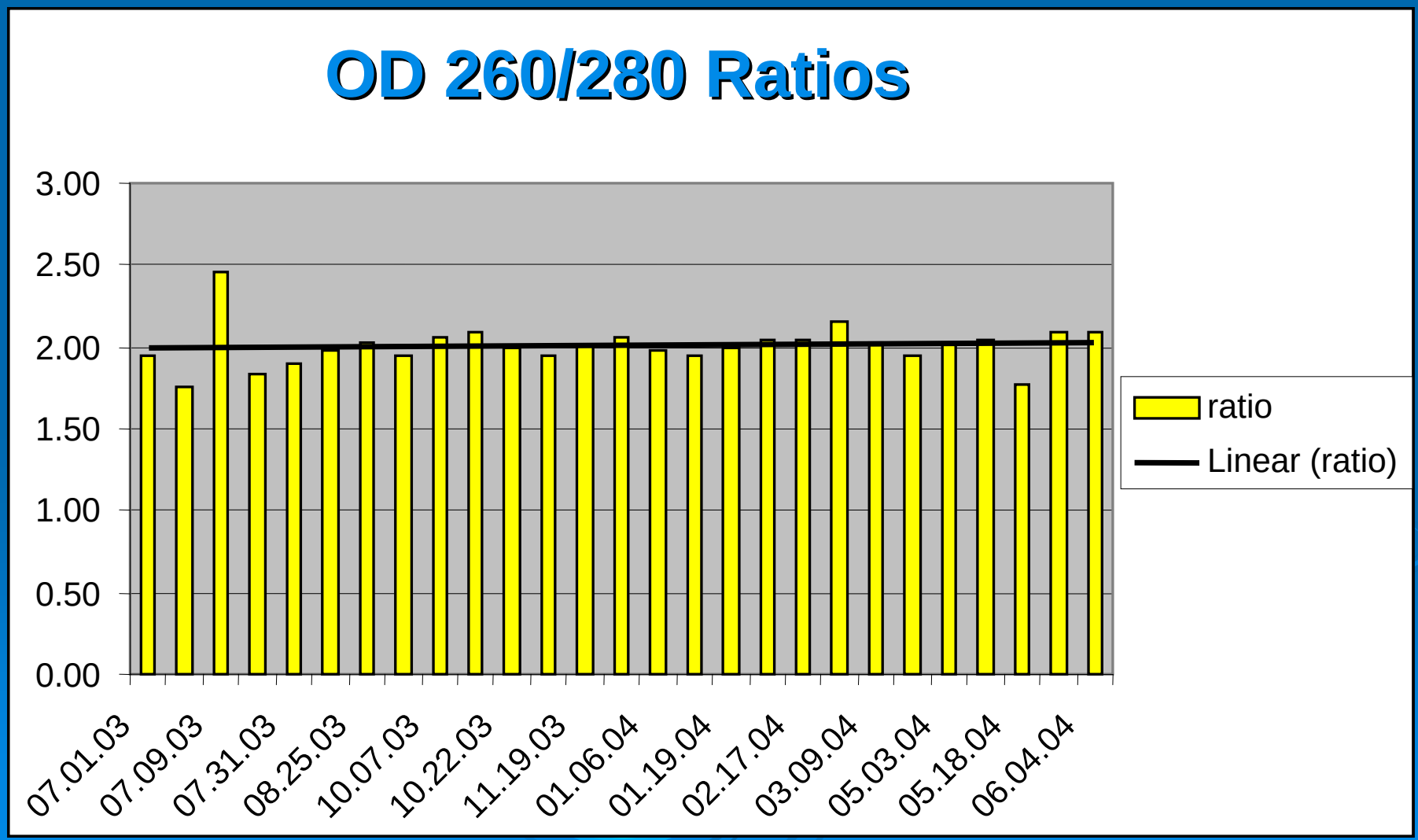

Quality Control of cRNA

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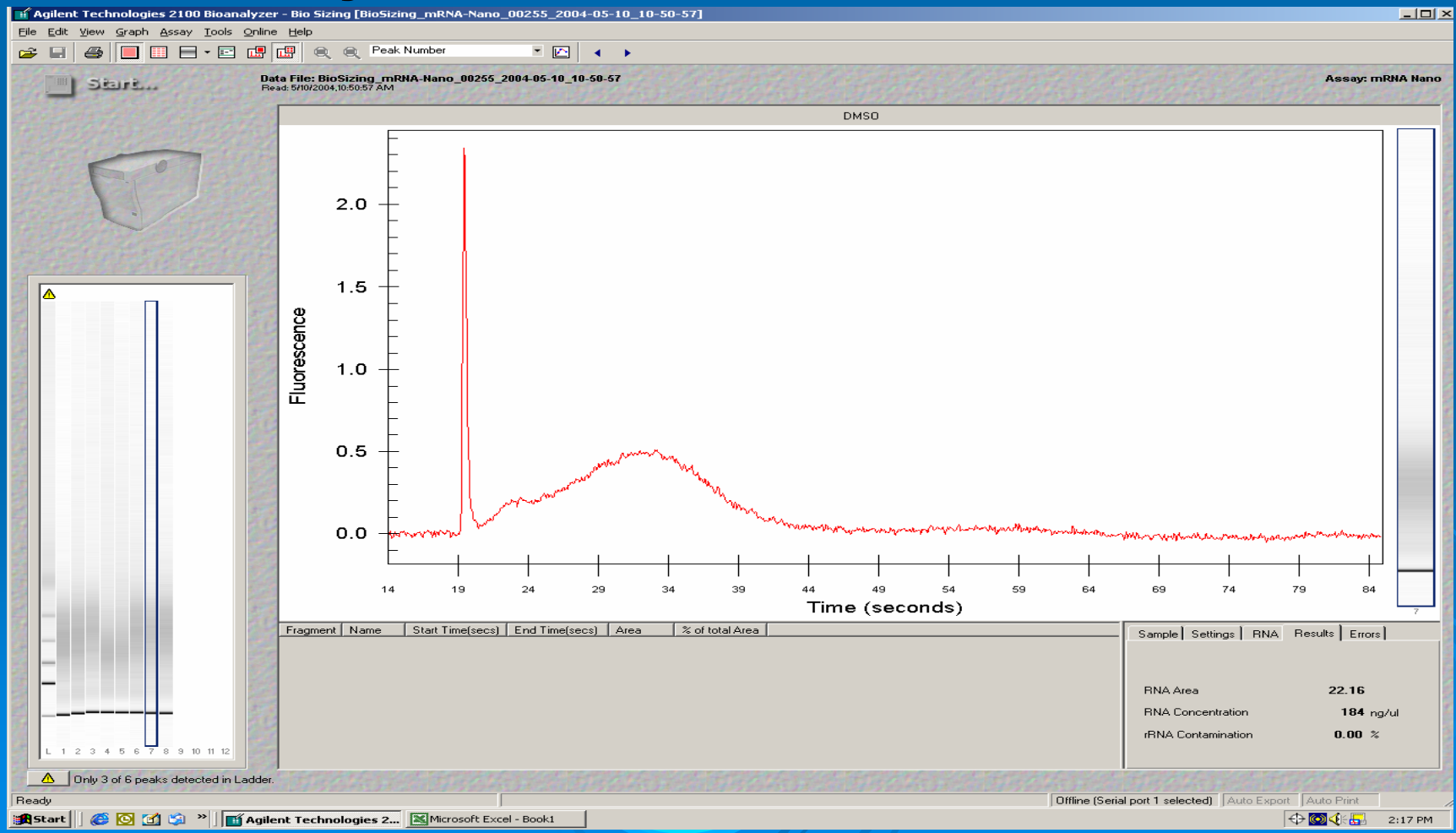
All cRNA samples should be validated on a bioanalyzer



Amersham CodeLink Arrays

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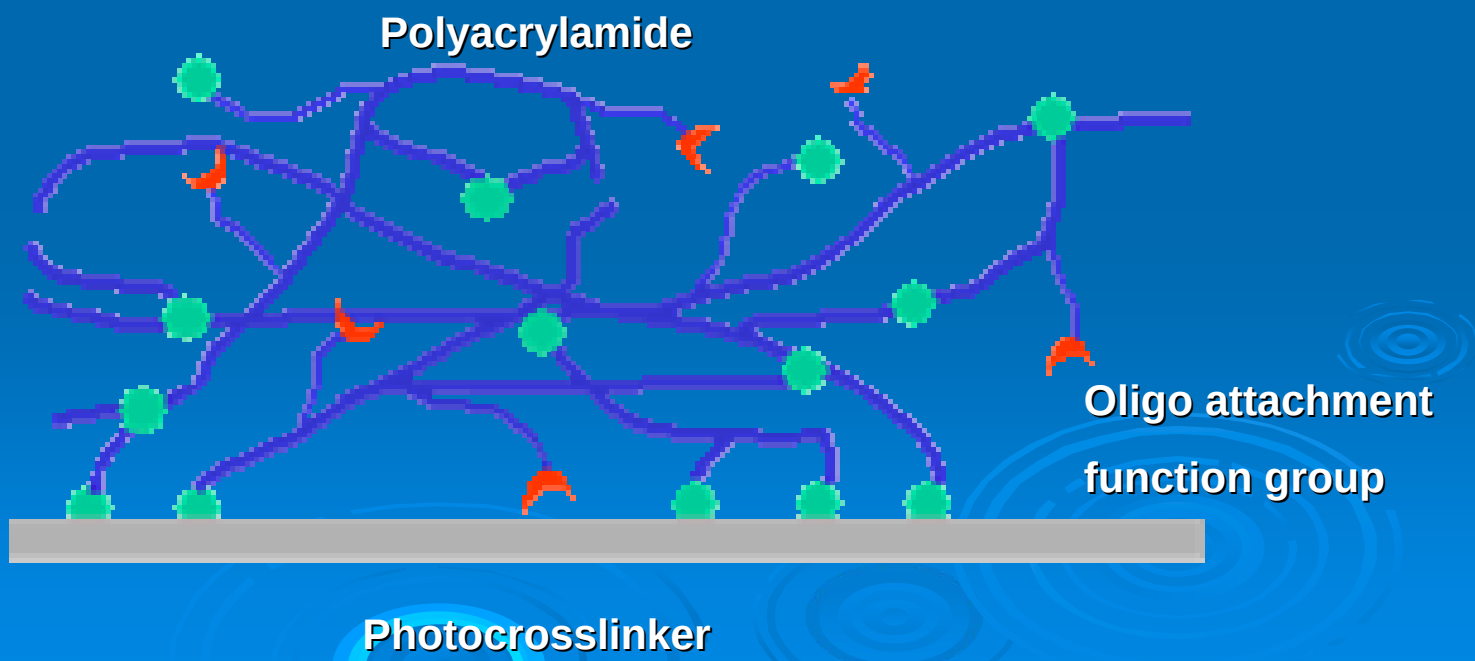
Highly versatile three-dimensional hydrophilic matrix which reduces non-specific binding, resulting in lower background noise.

- **pre-synthesized oligos are printed onto a pre-coated glass substrate Through covalent attachment, the oligos penetrate and are immobilized to active functional groups resulting in high binding capacity**



C. elegans Microarray

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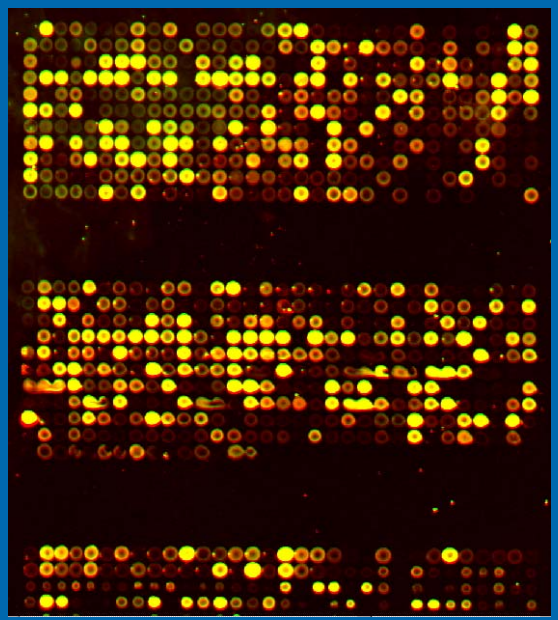
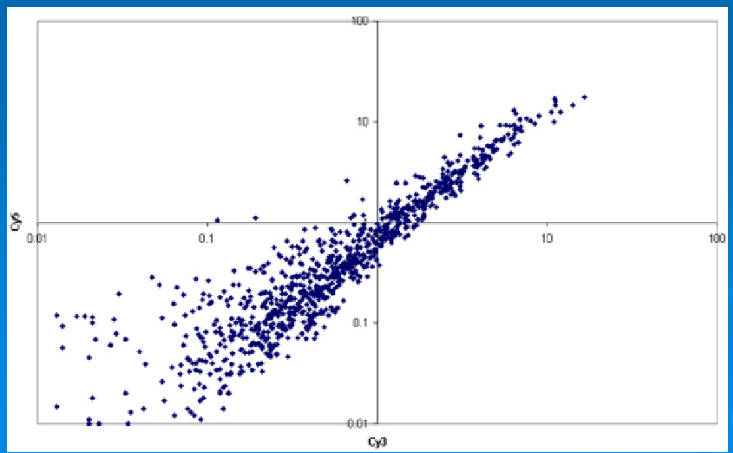
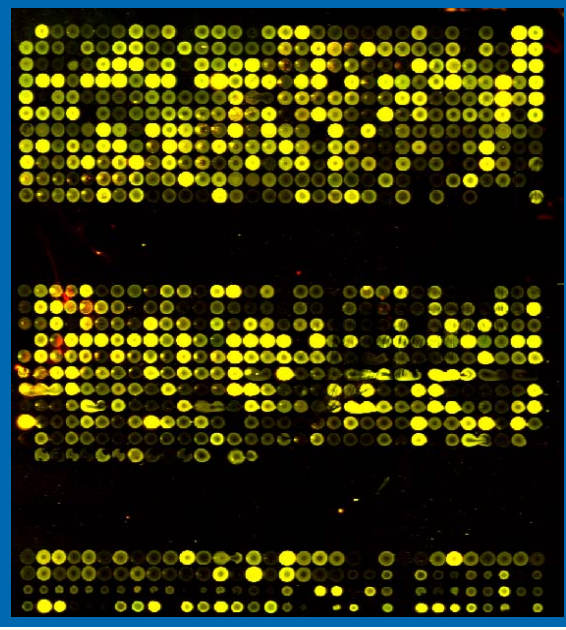
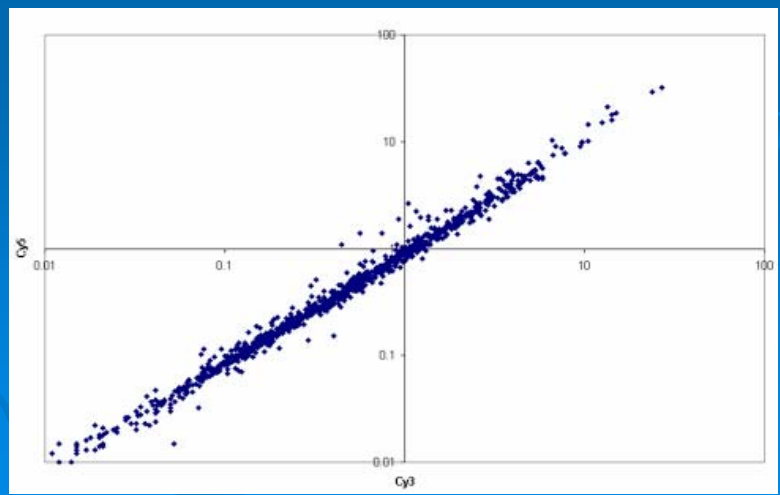
IVERMECTION VS CONTROL





BHA VS CONTROL





C. elegans Microarray

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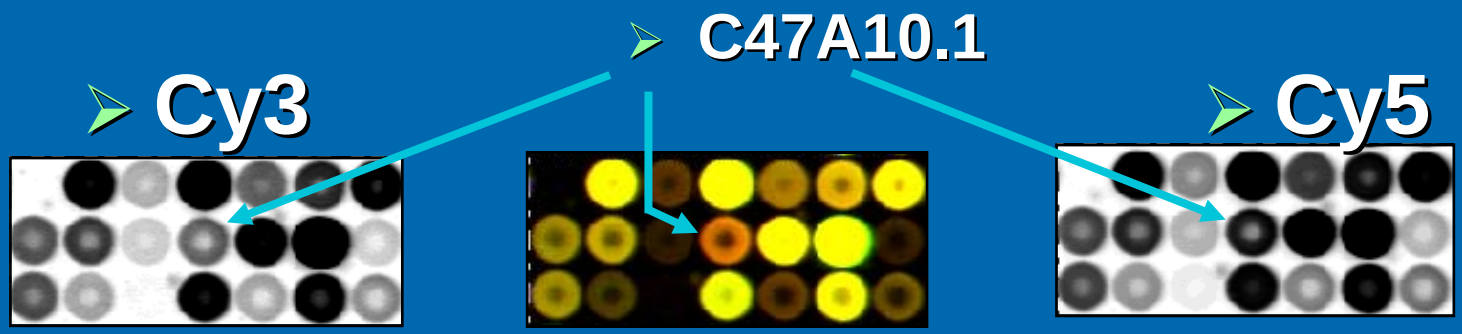
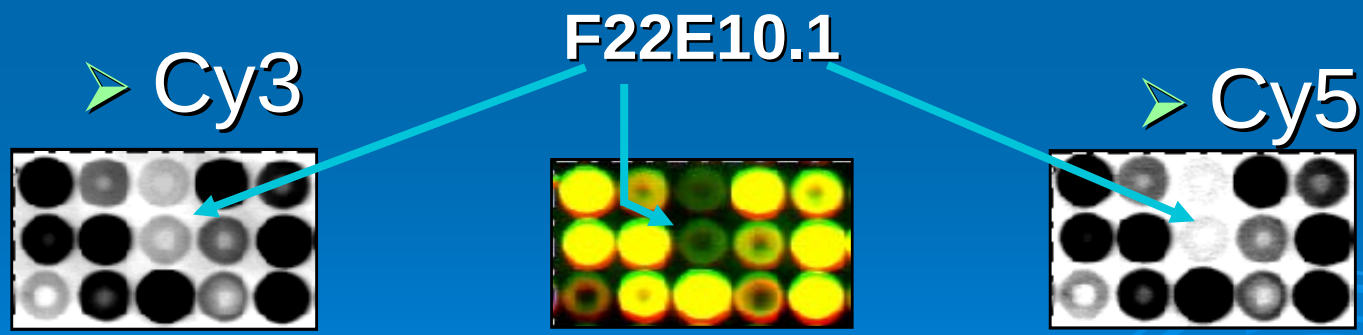
- IVERMECTION VS CONTROL

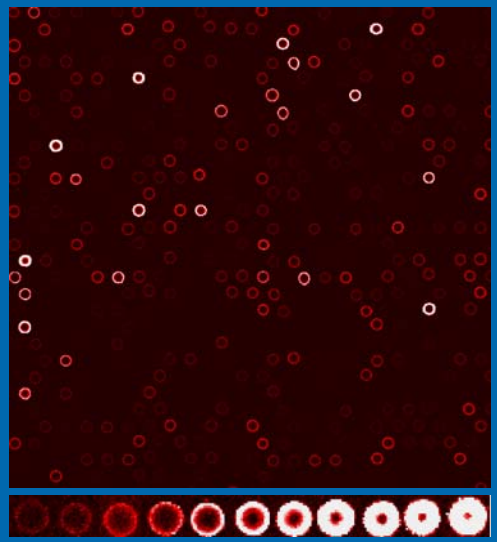


- BHA VS CONTROL



Hardiman et al., 2003

Amersham CodeLink Arrays



Amersham array

Piezo-spotted presynthesized oligonucleotides covalently linked to a proprietary 3D surface. One color.

~10,000 to 20,000 probes & whole mouse, rat and human genome arrays

feature size: 100- 200um

Oligo length: 30-mer

CodeLink BioArray Usage

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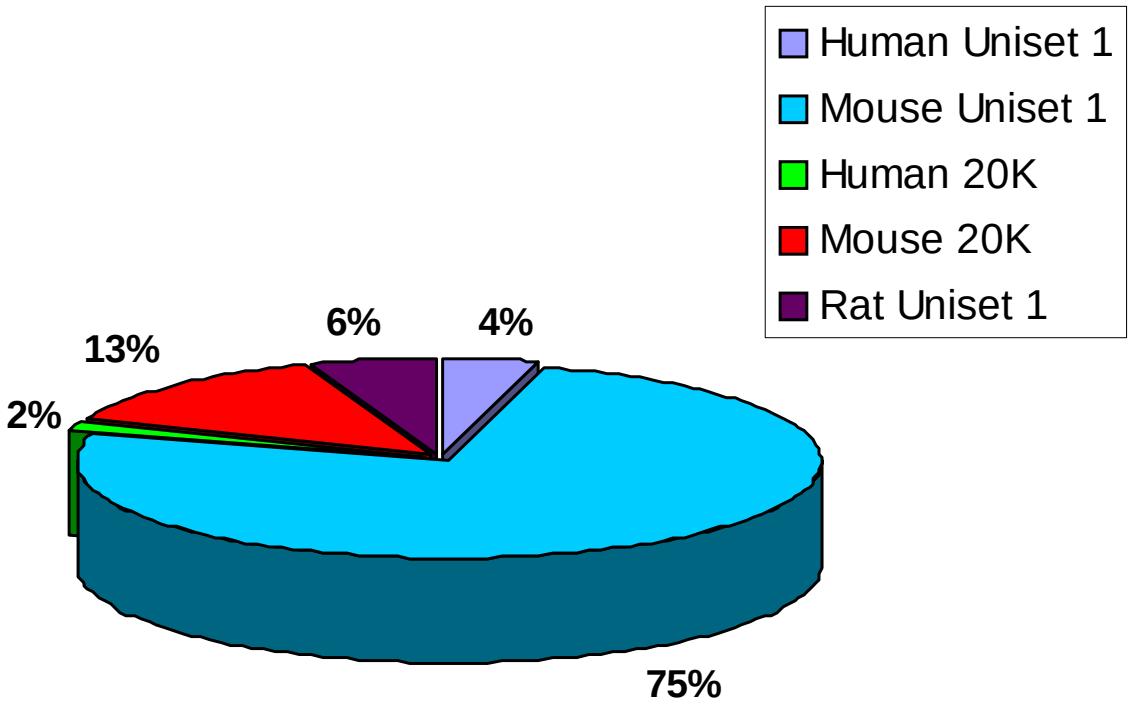

N=372

Experimental Overview

Sample Source: Commercial

Sample Type: Total RNA

Number of Samples: 2

Number of BioArrays Processed:
3 per sample = 6 UniSet Human I Bioarrays

Sample Description: (Commercial Universal RNAS)

1) **Stratagene Human Universal RNA**

2) **Clontech Human Universal RNA**

Target Preparation and Hybridization

Target Preparation: 5 ug of total RNA used for each sample

Sample	cRNA Yield (ug)
UHR STRAT	100
UHR CLON	70

Hybridization: 10 ug of cRNA hybridized to each UniSet Human I Bioarray

Sample Name	Image File Name
UHR STRAT	T00157242
UHR STRAT	T00157272
UHR STRAT	T00157273
UHR CLON	T00157275
UHR CLON	T00157276
UHR CLON	T00157277

Limit of Detection

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Limit of Detection of Differential Expression

Sample	ImageFile	ImageFile	%within 2-fold	95% Within x Fold	Correlation Coefficient
UHR STRAT	T00157242	T00157272	98.4	1.6	0.986
	T00157242	T00157273	98.7	1.5	0.991
	T00157272	T00157273	99.4	1.3	0.997
UHR CLON	T00157275	T00157276	99.6	1.3	0.998
	T00157275	T00157277	99.4	1.3	0.997
	T00157276	T00157277	99.5	1.3	0.997

Inspect Images Carefully

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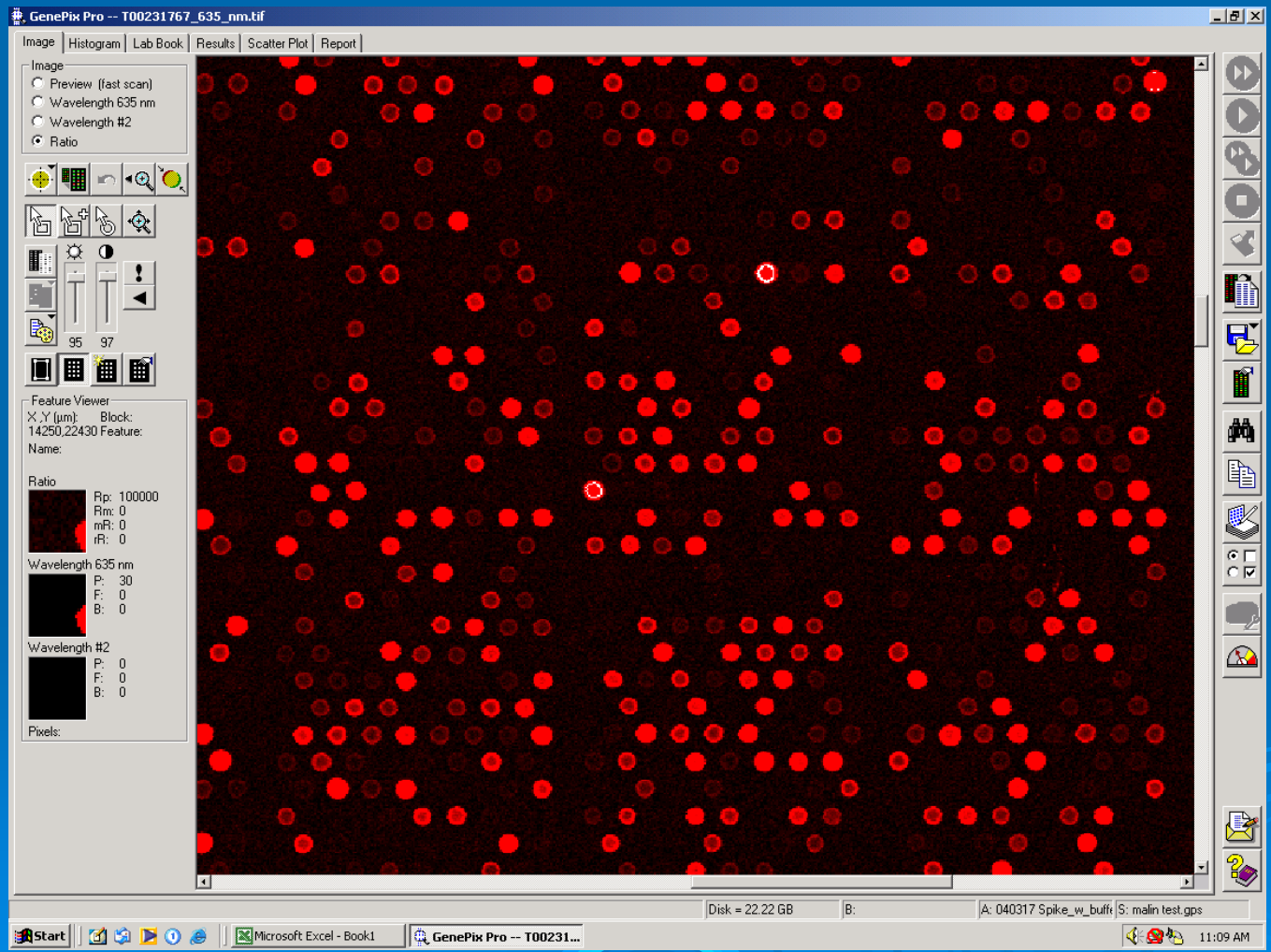
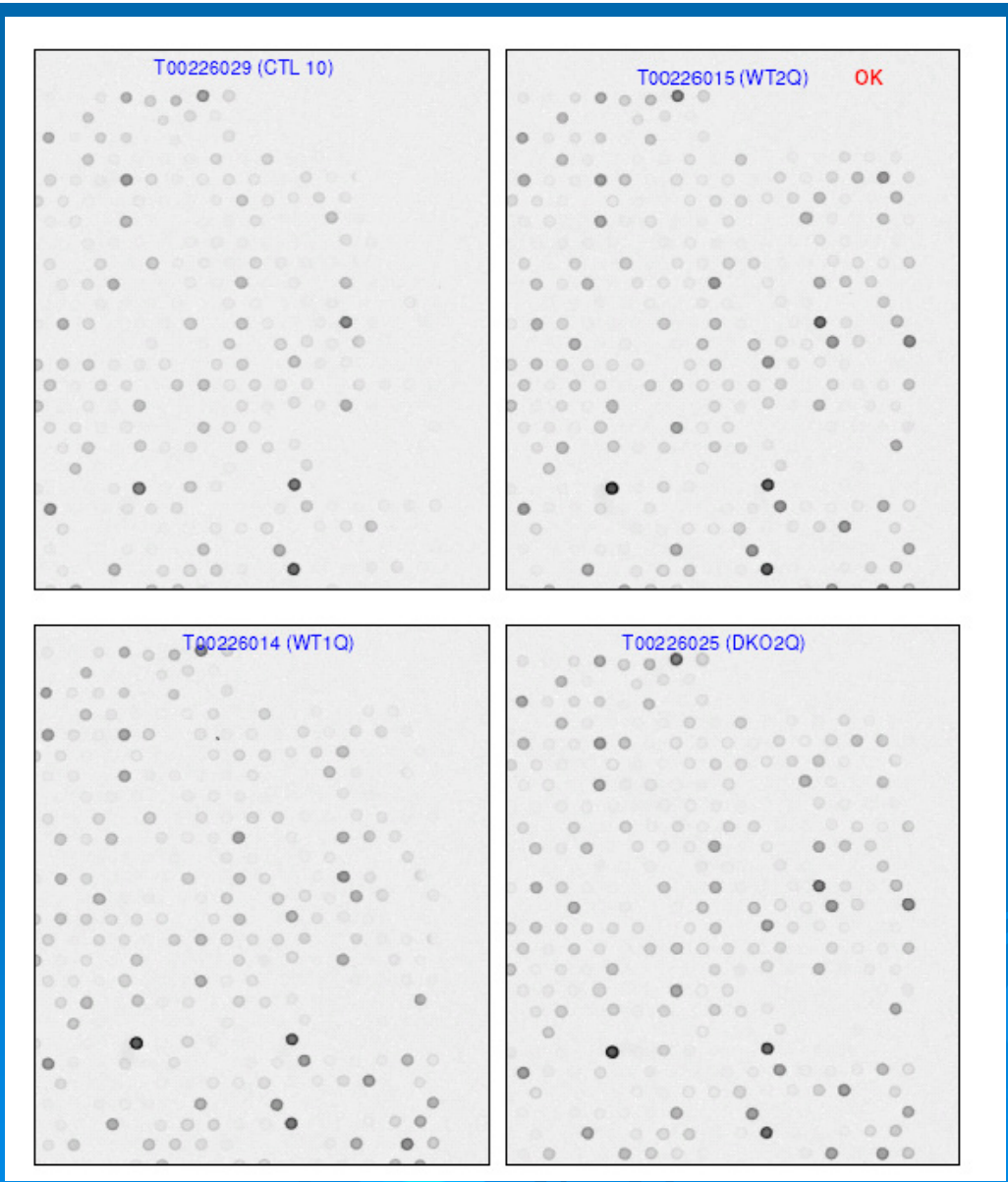


Inspect Images Carefully



Arrays from the Same Batch are great

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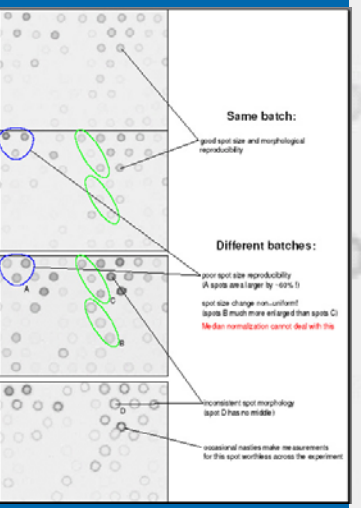

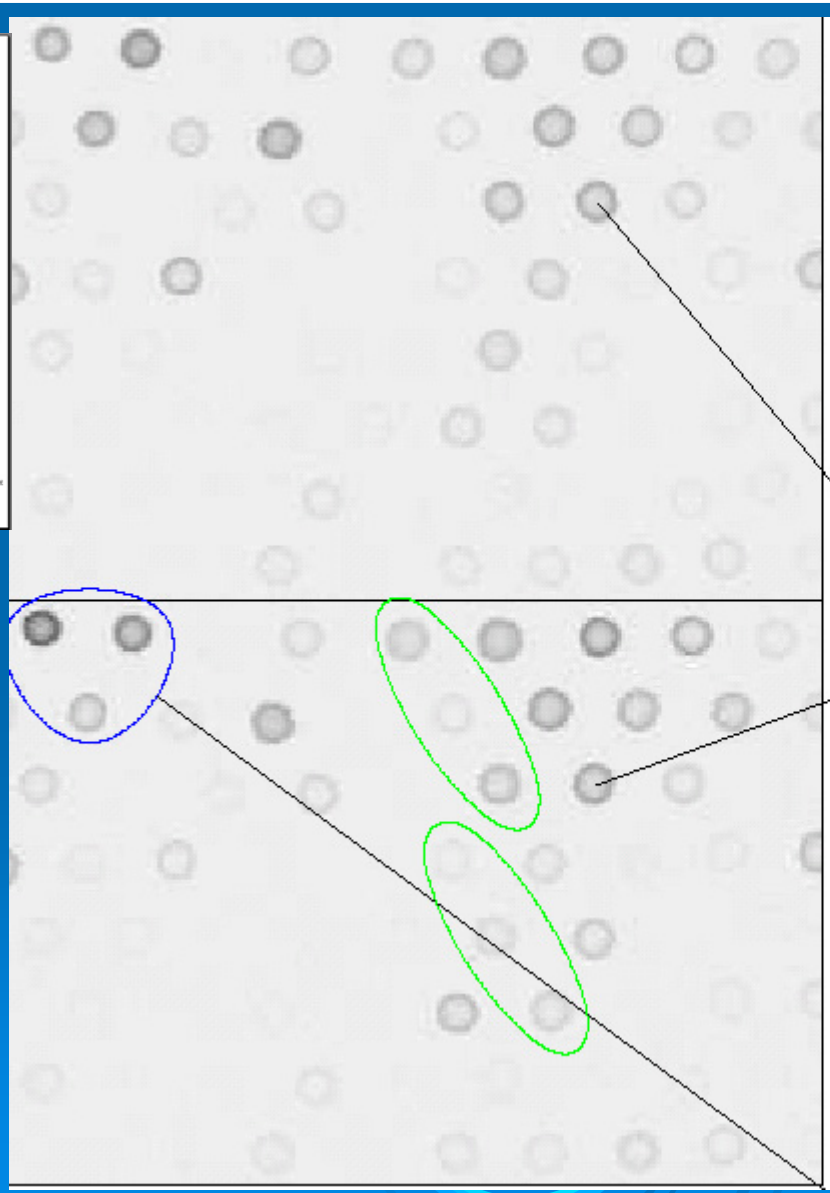

Same batch:

good spot size and morphological reproducibility

Arrays from Different Batches vary

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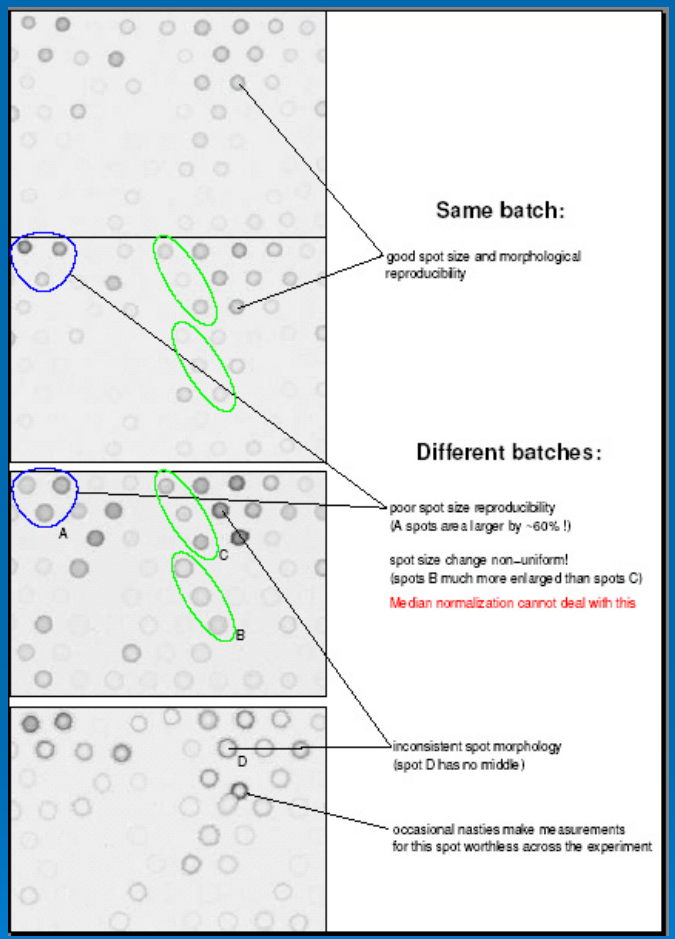

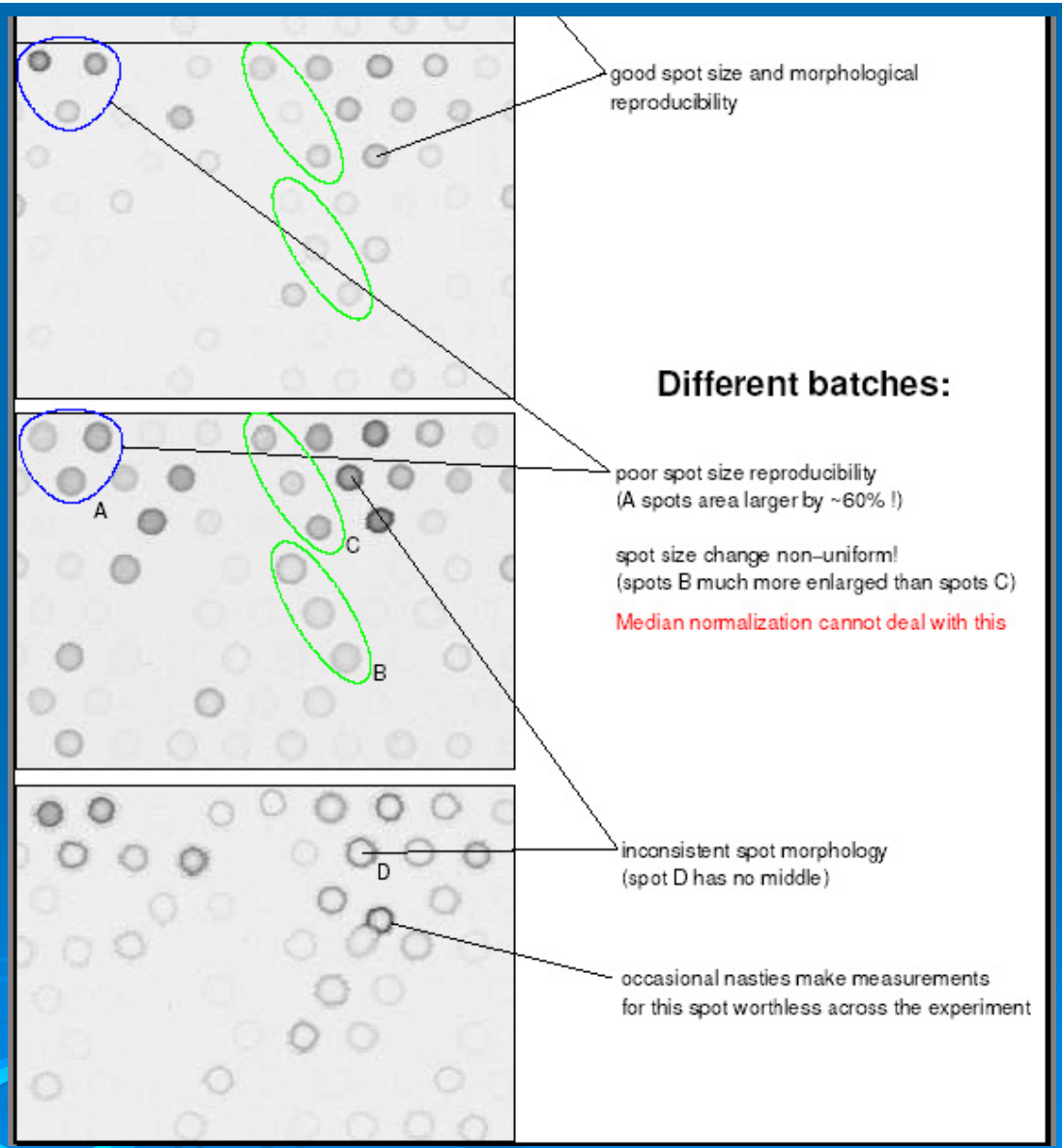

Arrays from Different Batches vary

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- Arrays from Different Batches are not as good

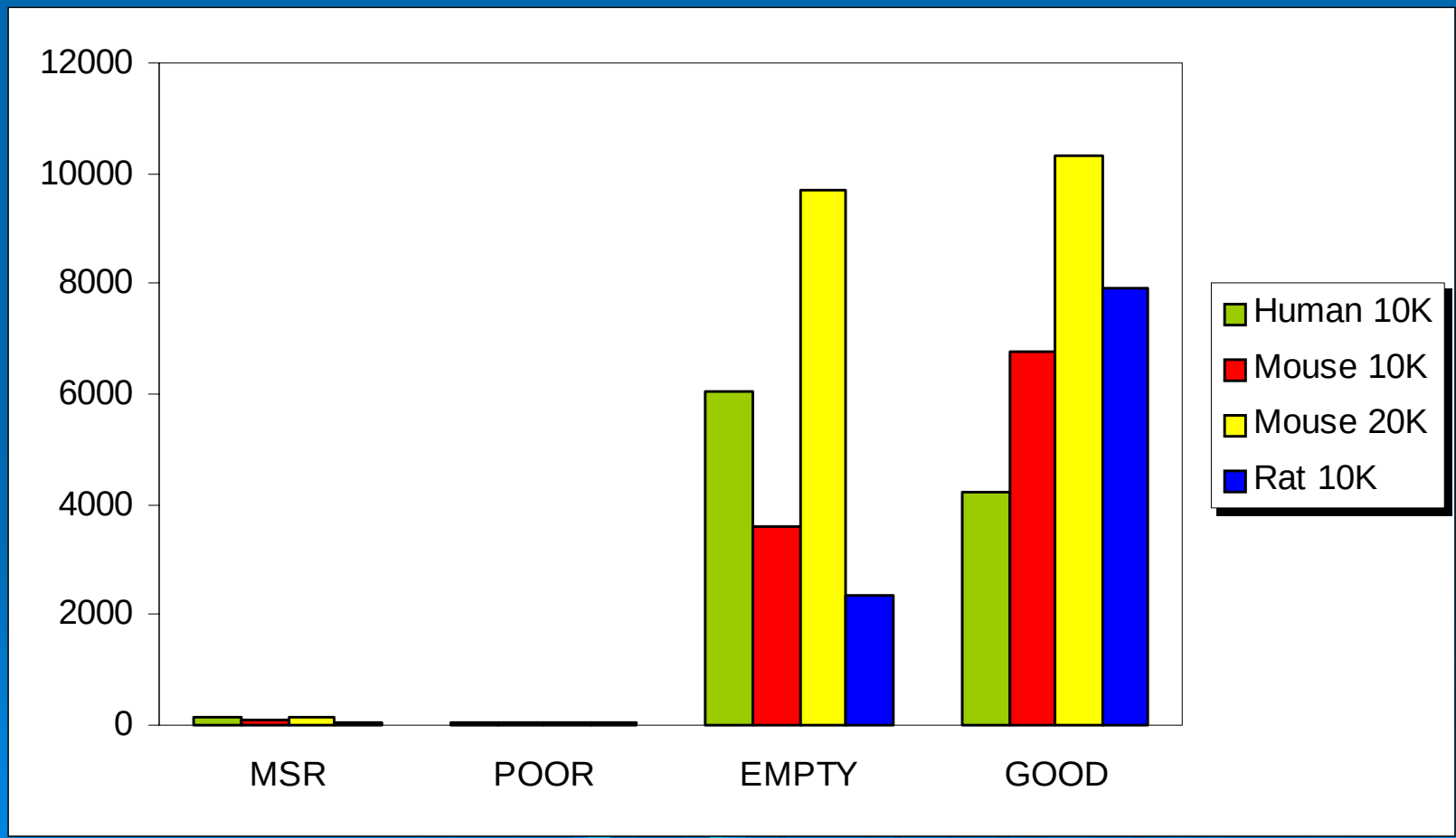
- Since some spots change their size between the batches and others don't, median normalization will not help.

- Some expression levels will show a 60% change for no reason other than irreproducibility of spot sizes across batches!

- Avoid using chips from two different batches for an experiment.

Quality Flags

Quality Flags and CodeLink Arrays at UCSD



Microarray Platforms

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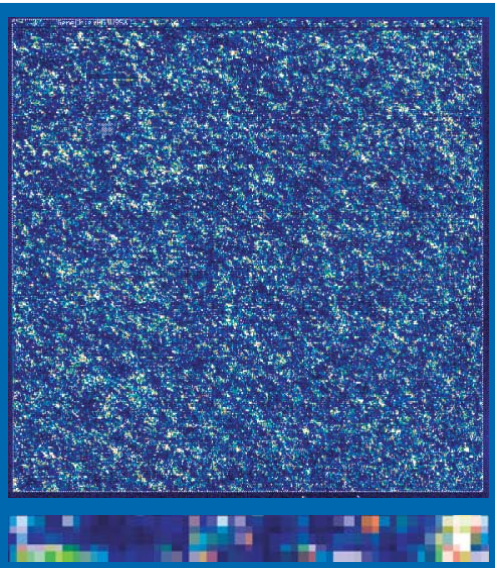
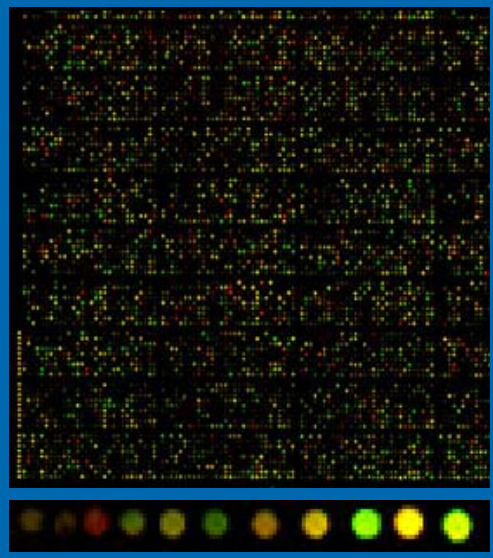
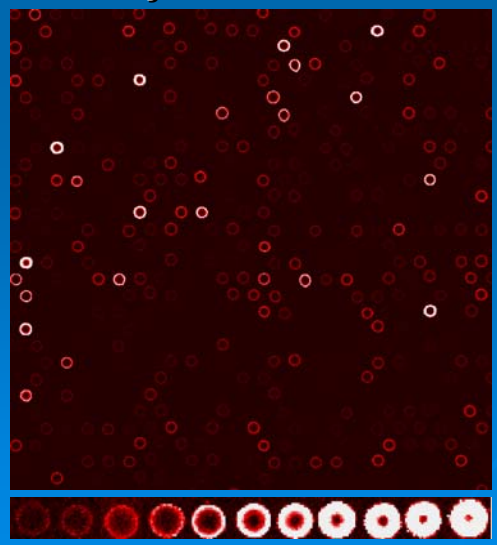


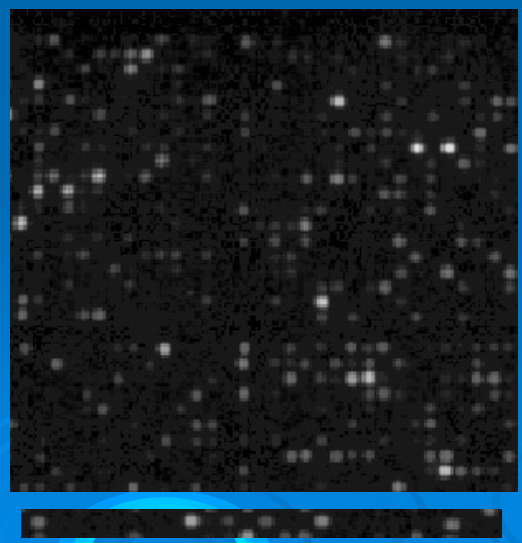
Affymetrix



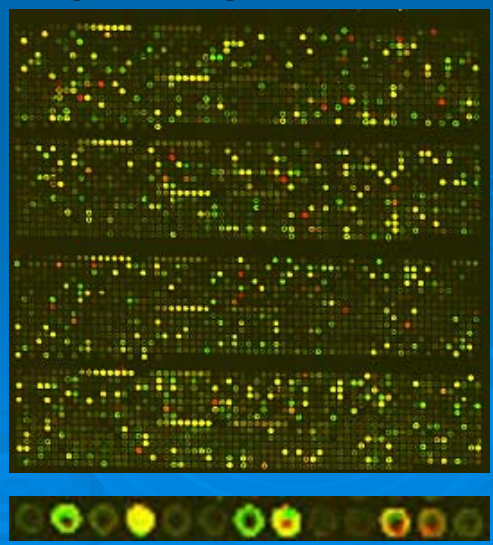
Agilent oligo



Amersham

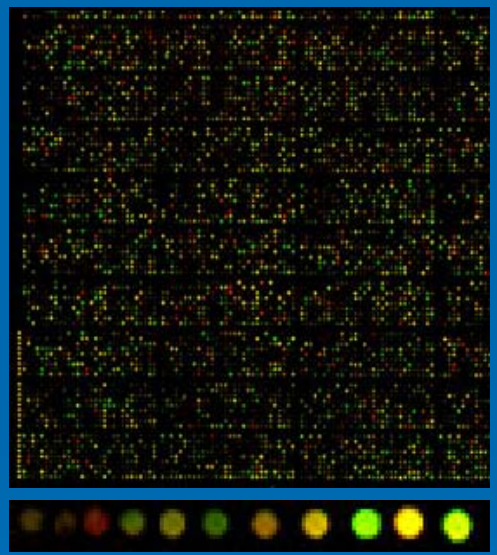


ABI



BIOGEM cDNA array

Agilent Arrays



Agilent oligo array

Agilent Human 1A:

***In situ* ink-jet-deposited phosphoramidite oligonucleotide synthesis of 60-mer probes. Two color.**

~17K to 44K probes

feature size: 170um

Oligo length: 60-mer

Experimental Design Overview

- Control: wild-type Cell
- Treatments (3): Drug Treatment A for 10 hrs, Drug Treatment B for 10 hrs, Drug Treatment C for 10 hrs
- Mode of Action of Drugs A & B well understood
- Mode of Action of Drug C poorly understood
- 2 replicates per treatment (biological)
- Microarray: Agilent Mouse 44k
- Labeling method: Agilent Low Input Fluorescent Linear Amplification kit (50 ng total RNA input)

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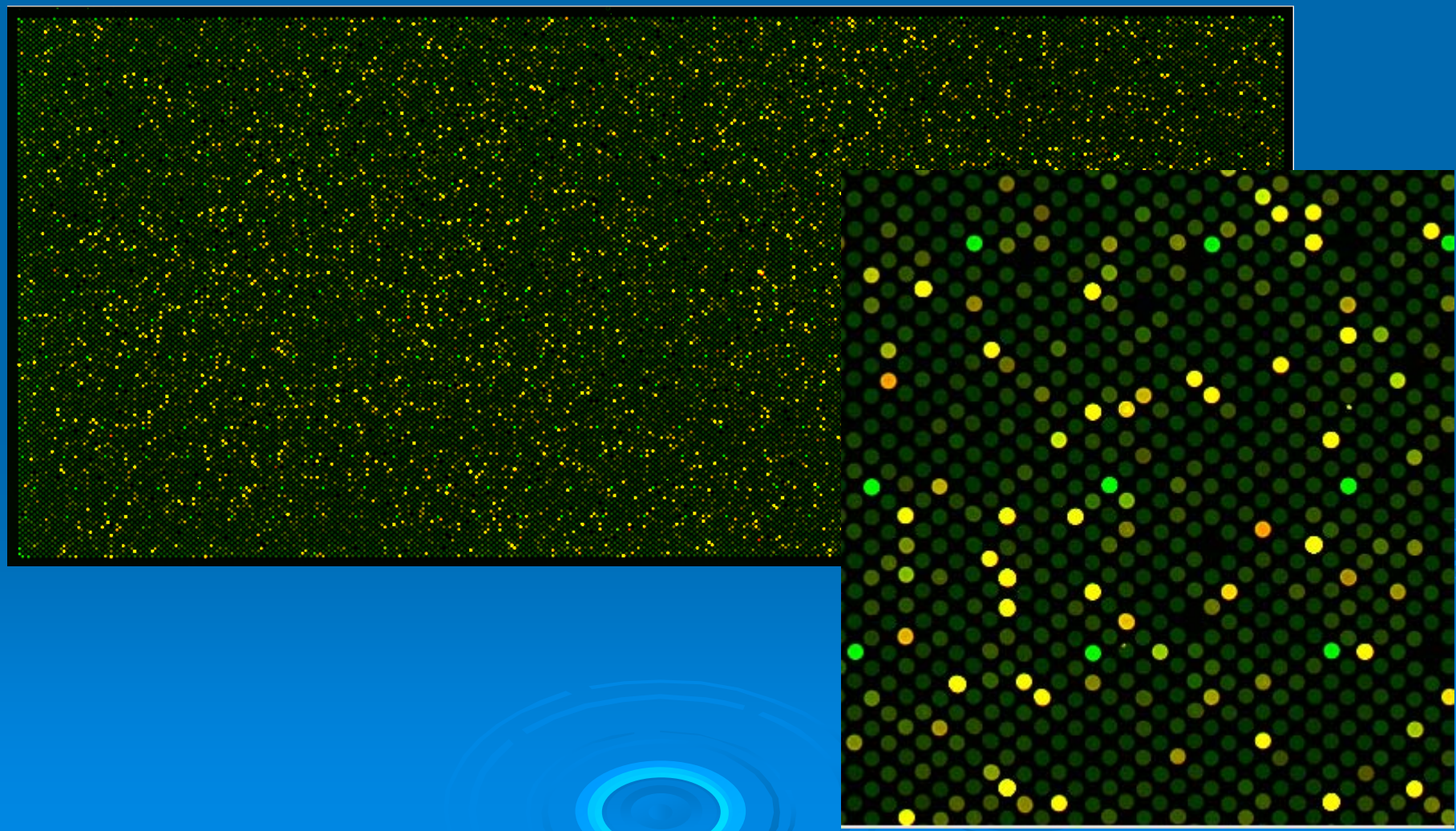
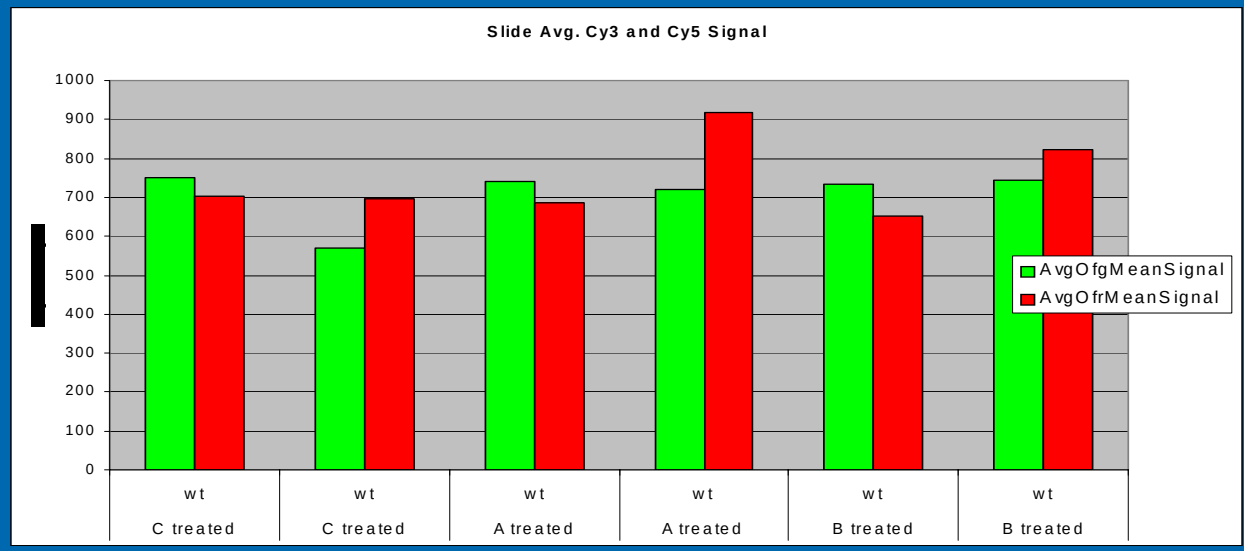
WT vs. Drug Treatment C



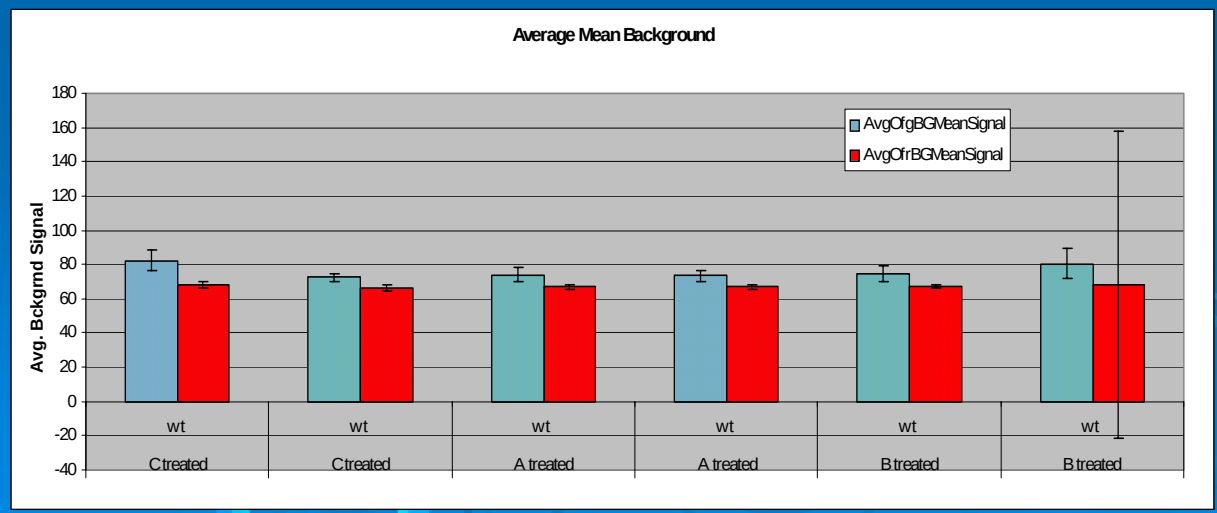
Microarray stats

Feature average signal and local background

Average Mean Signal



Average Mean Local Background Signal



Ratio vs. Avg. Intensity

Combined Replicates – Rosetta Luminator Analysis

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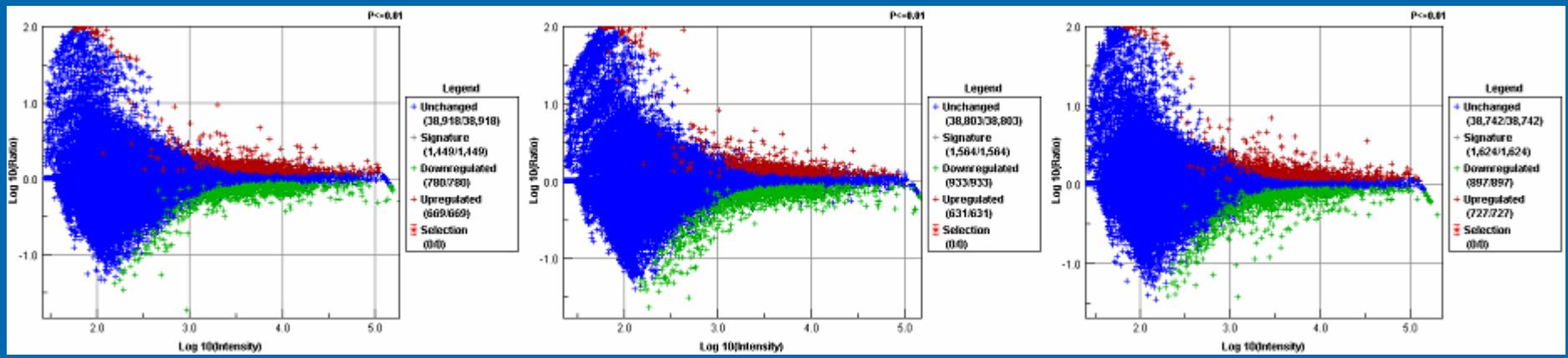
Wt vs. Treatment C

Wt vs. Treatment A

Wt vs. Treatment B



- High noise at low signal
- ~1,500 signature genes for each comparison (P<0.01)

Compare Plots

LogRatio vs. LogRatio, combined replicates

Determine differential expression significance based on error model P-values

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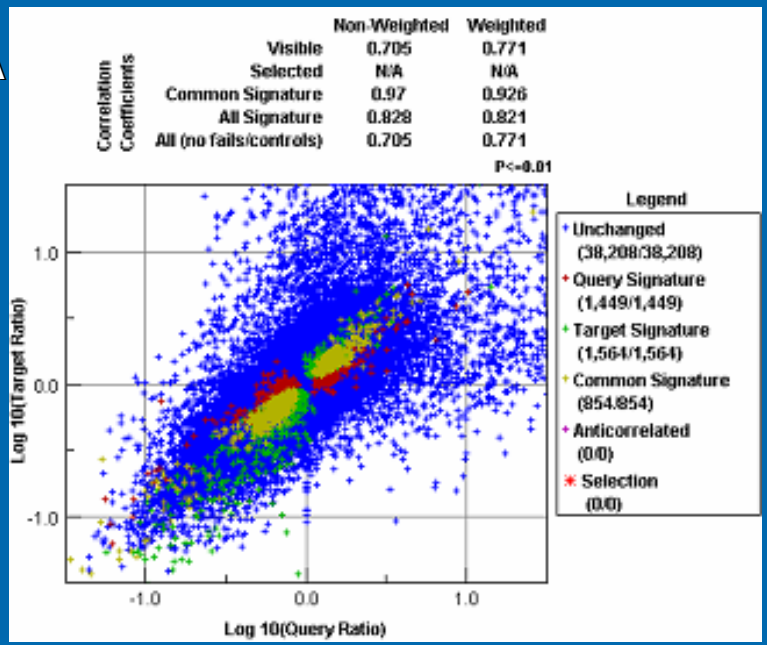
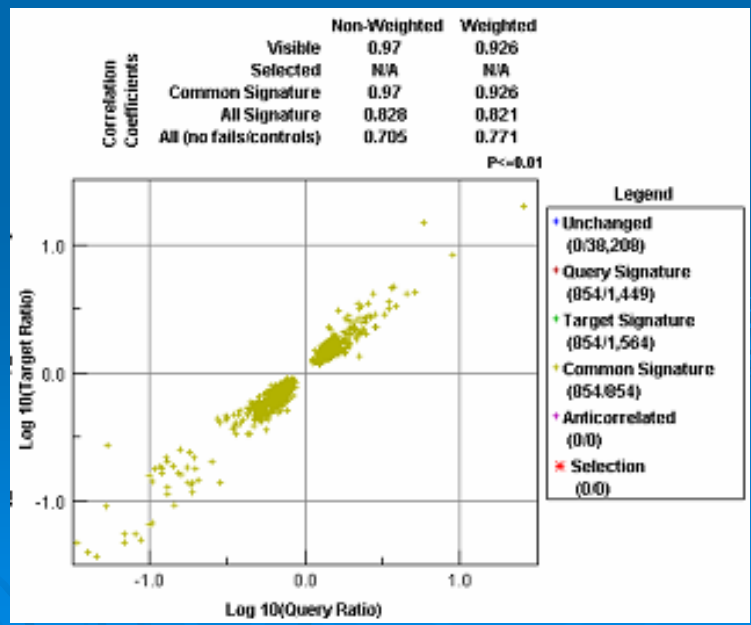
Wt vs. Treatment A



Wt vs. Treatment C

Filter with Illuminator Agilent Error Model P-values



Wt vs. Treatment A

Wt vs. Treatment C

Compare Plots

LogRatio vs. LogRatio, combined replicates

Common signature shown only

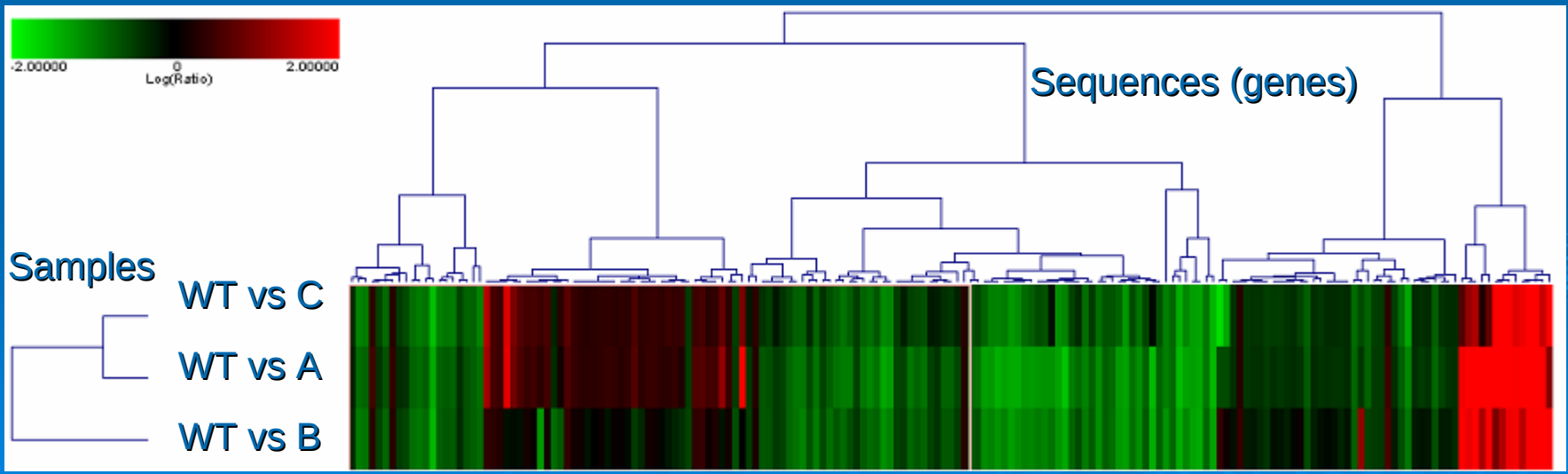
1	2	Correlation Coefficient (weighted)		
		All	All Signature	Common Signature
wt vs. C	wt vs. A	0.77	0.81	0.93
wt vs. C	wt vs. B	0.3	0.198	0.29
wt vs. B	wt vs. A	0.409	0.318	0.509

2-D Cluster Analysis

Agglomerative algorithm
Pearsons correlation

Filter: > 2-fold change, P<0.01, present in 2 of 3 treatments
Results: 179 sequences

Full Cluster View



Agilent 44K Summary

- Microarray hybridizations are of good quality with consistent average feature and background signals with very few outliers(outlier data not shown).
- Higher noise at low end of signal range. May have improved performance by optimizing RNA extraction and labeling.
- Rosetta Illuminator clustering and compare plots suggest that Drug C and A treatment comparisons have most similar differential expression profiles.
- Several groups of genes identified to have similar response between Drug C and A treatments. However, also groups of genes behaving similar between Drug A and B treatments and among the three treatments.

Recommendations

- Dye swap microarray replicate hybridizations
- Self-hyb control to assess error model accuracy
- Possibly optimize RNA preparation and labeling procedure

Platform Comparison

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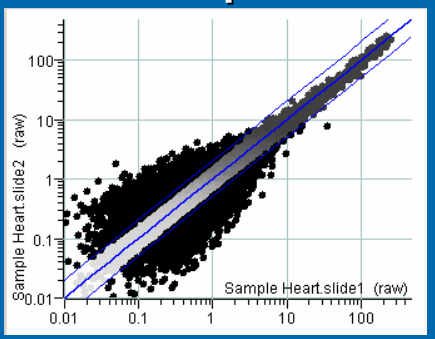
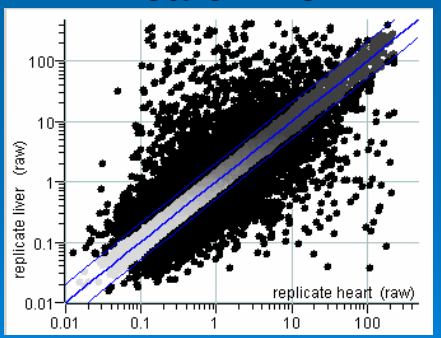
Affymetrix

Heart replicates

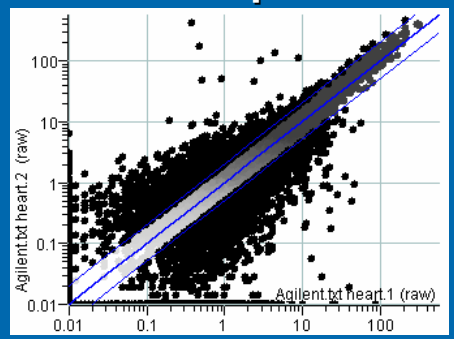


Heart:Liver

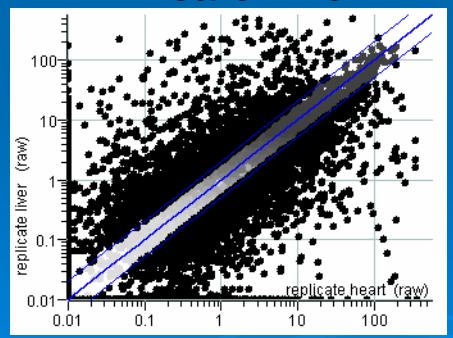


Agilent

Heart replicates

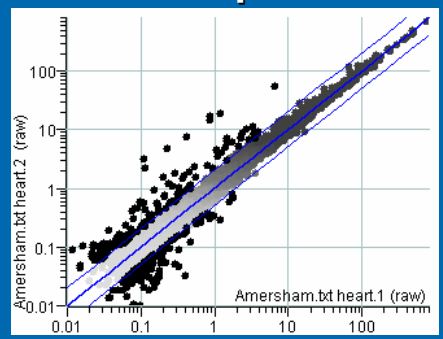


Heart:Liver

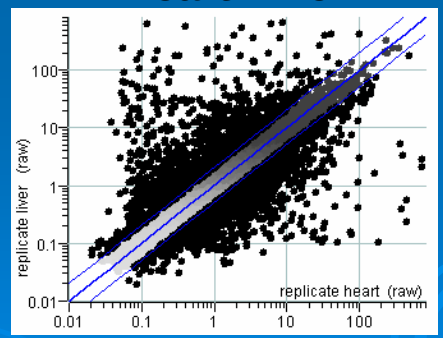


Amersham

Heart replicates

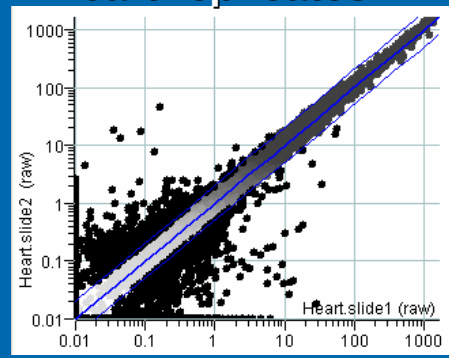


Heart:Liver

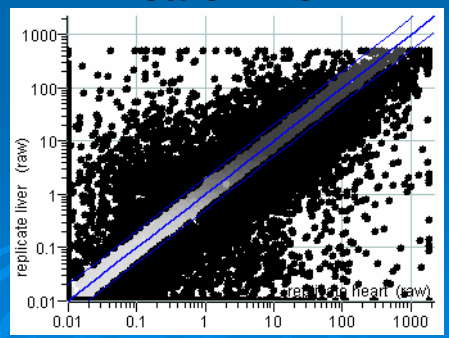


Mergen

Heart replicates



Heart:Liver



Phillip Stafford & Peng Liu 2003

GWLA

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Towards genome-wide location analysis of transcription factors

Method overview

- Experimental design and execution
- Conversion of scanned images to expression values and quality control check
- Secondary analysis - defining gene lists
- Tertiary analysis -hierarchical clustering, gene ontology analysis, etc.

The GeneOntology (GO) database

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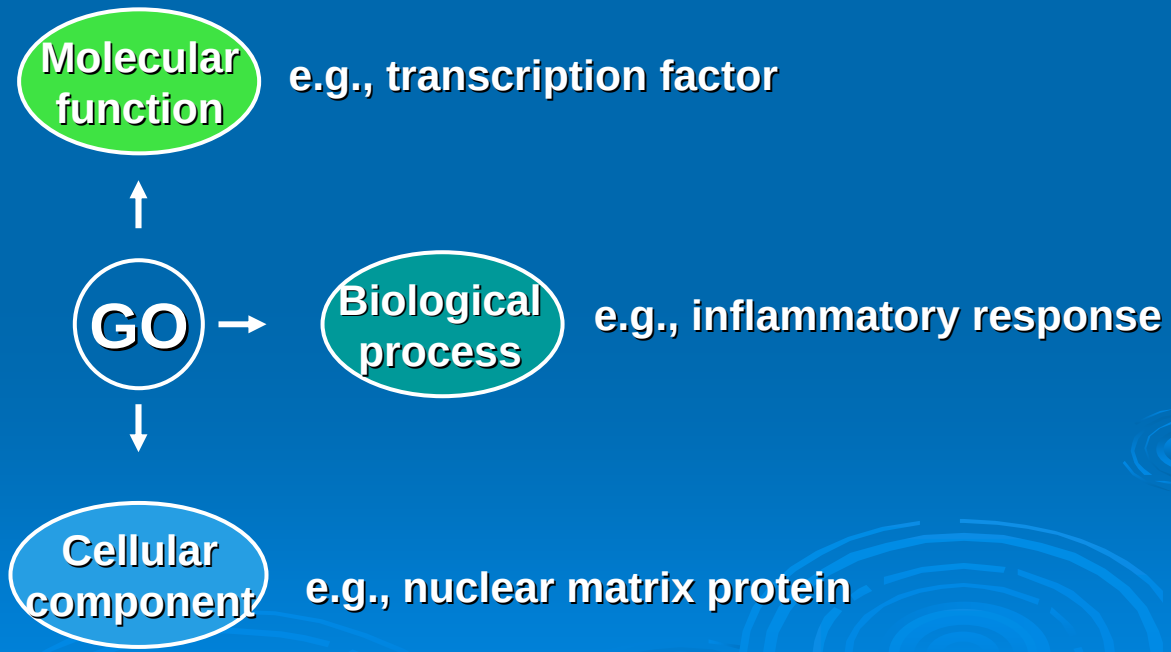
A hierarchical annotation of three main categories, or “kingdoms”



Functional analysis of gene expression

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- Goal: Use gene annotations from curated databases to analyze functionality of differentially regulated genes

View of the Gene Ontology: inflammatory response

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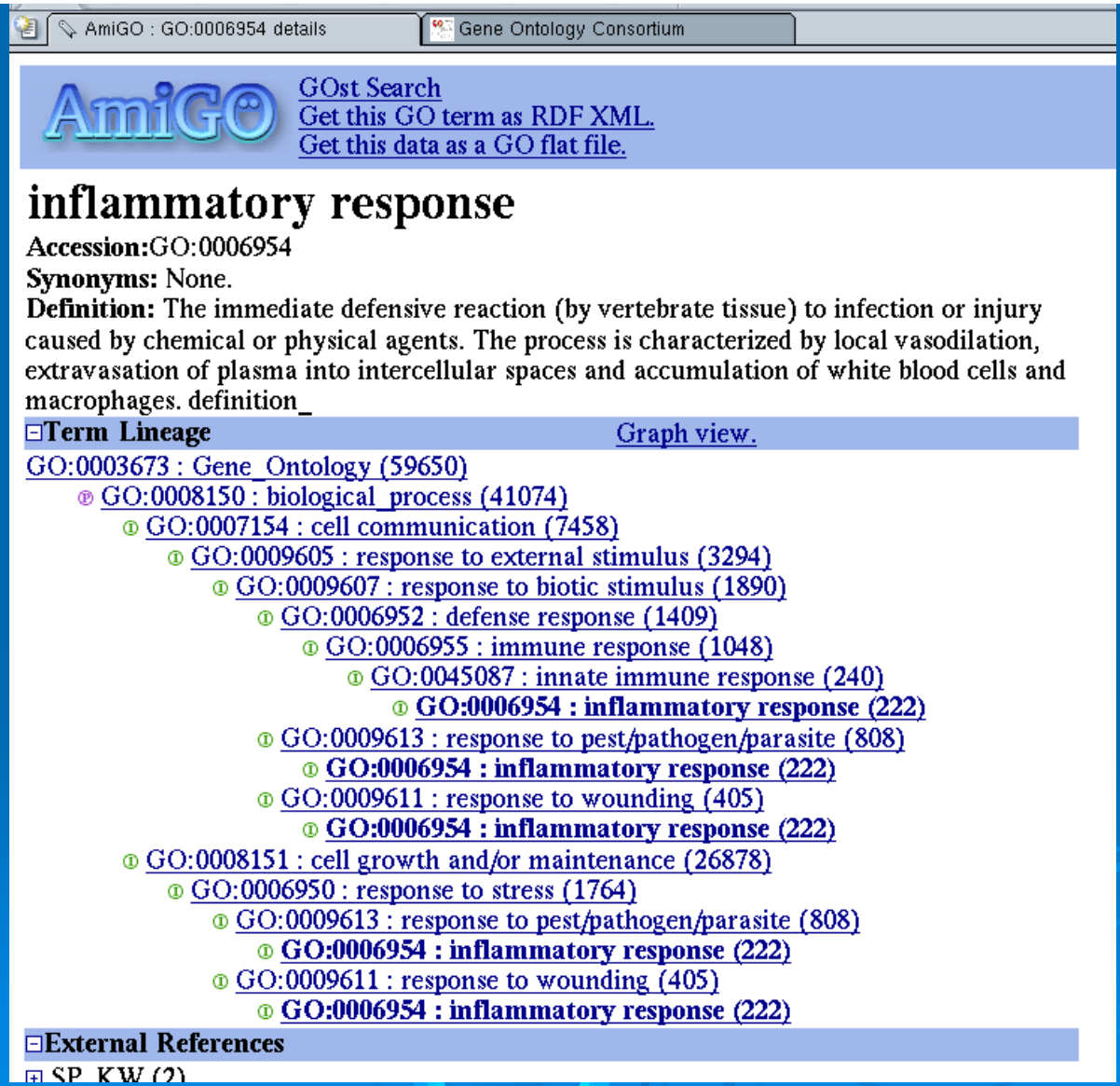

Genome-wide Location Analysis

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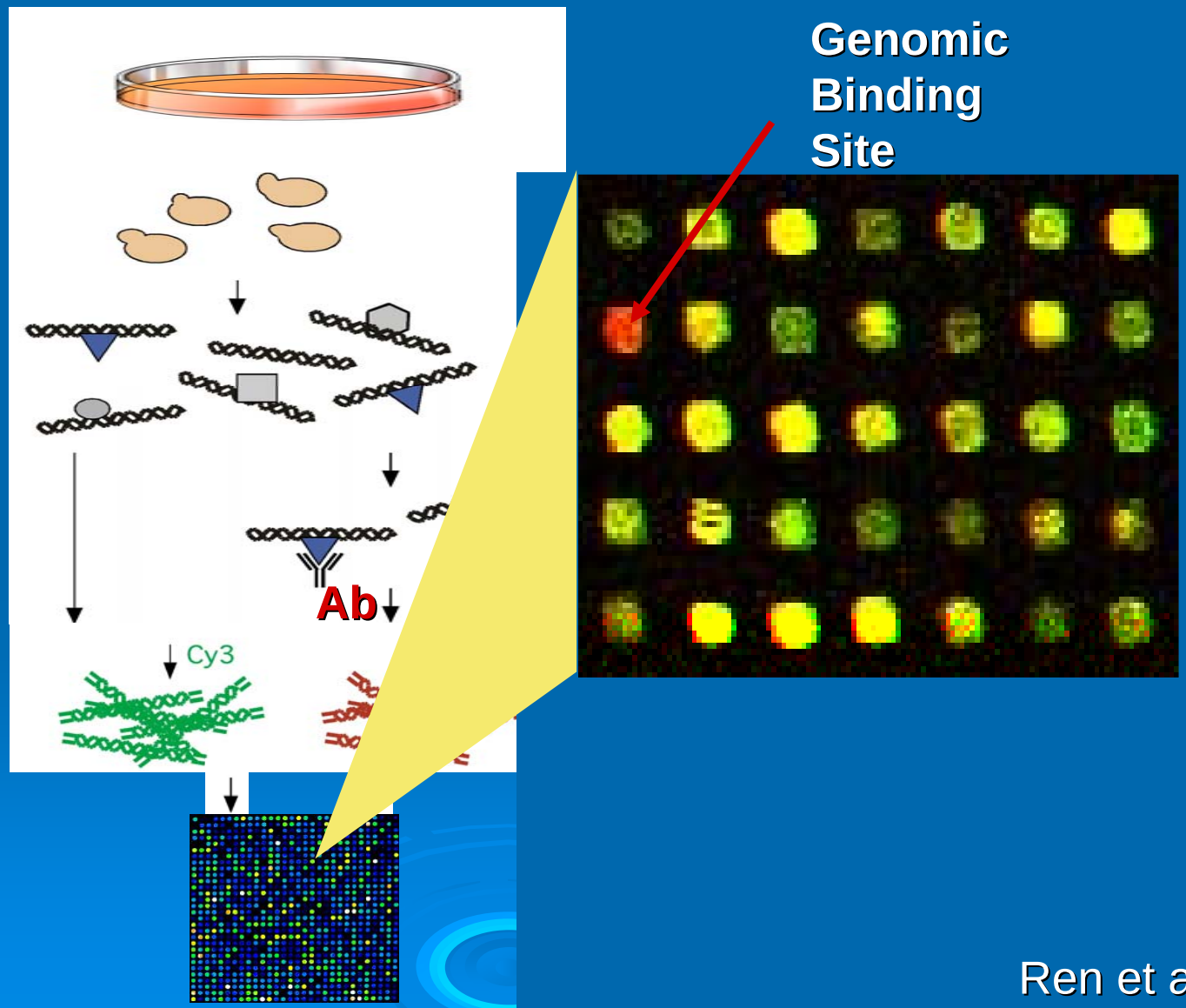

Ren et al., 2000

Summary

- A high throughput method to identify the genomic binding sites for transcription factors in yeast and human cells
- Widespread DNA binding and transcriptional regulation by c-myc in Burkitt's lymphoma cells - implying a much broader biological role for c-myc than previously appreciated

Mouse promoter array – Version 2.0

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List of 1500 genes

Celera Database
GeneBank
Alignments of mRNA

Extraction of ~ 2000 promoters

Design of primers to amplify 1 kb sequences [-800 bp;+200 bp]

PCR

Verification of PCR products

Sequencing

Printing to Glass Slides

Promotor Array Development

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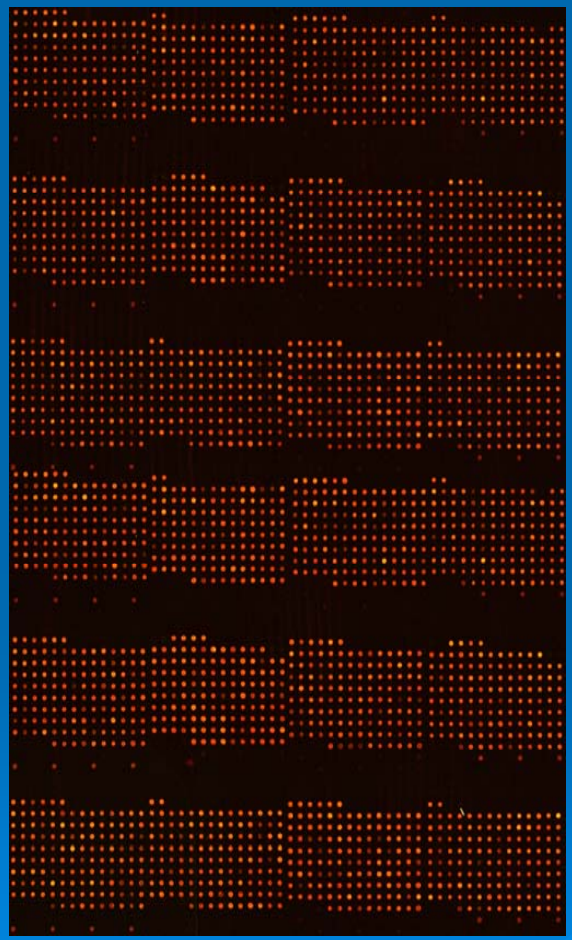
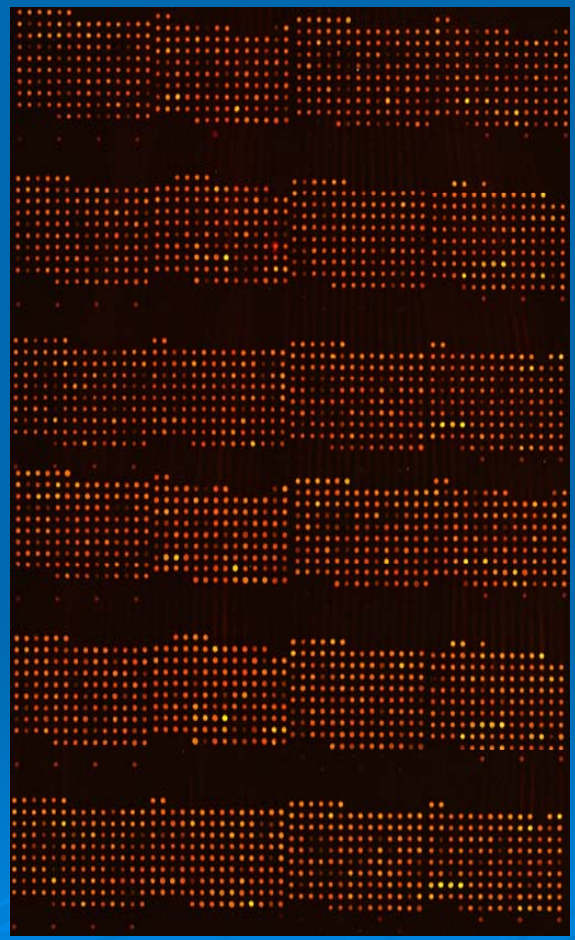
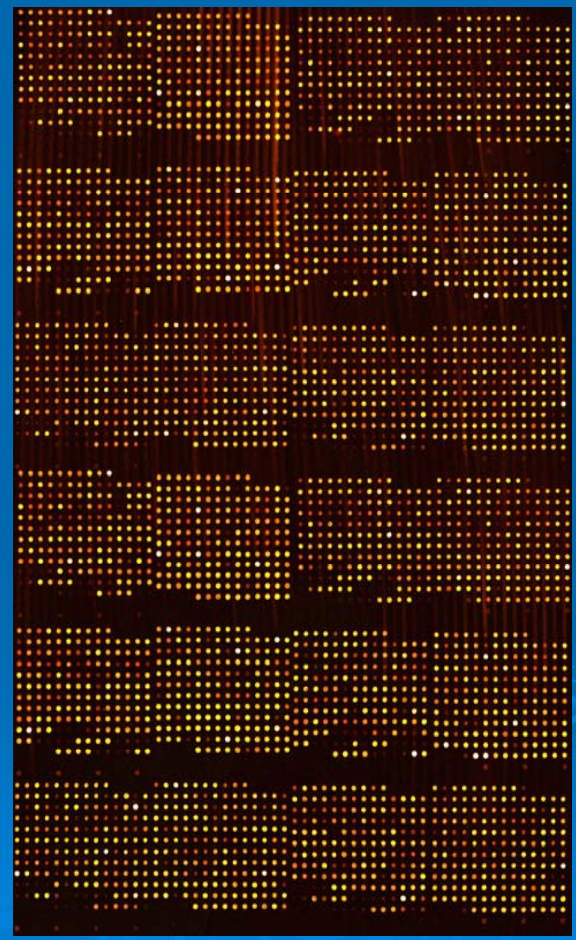
V1.0



V1.1



V 1.2



Promotor Array Development

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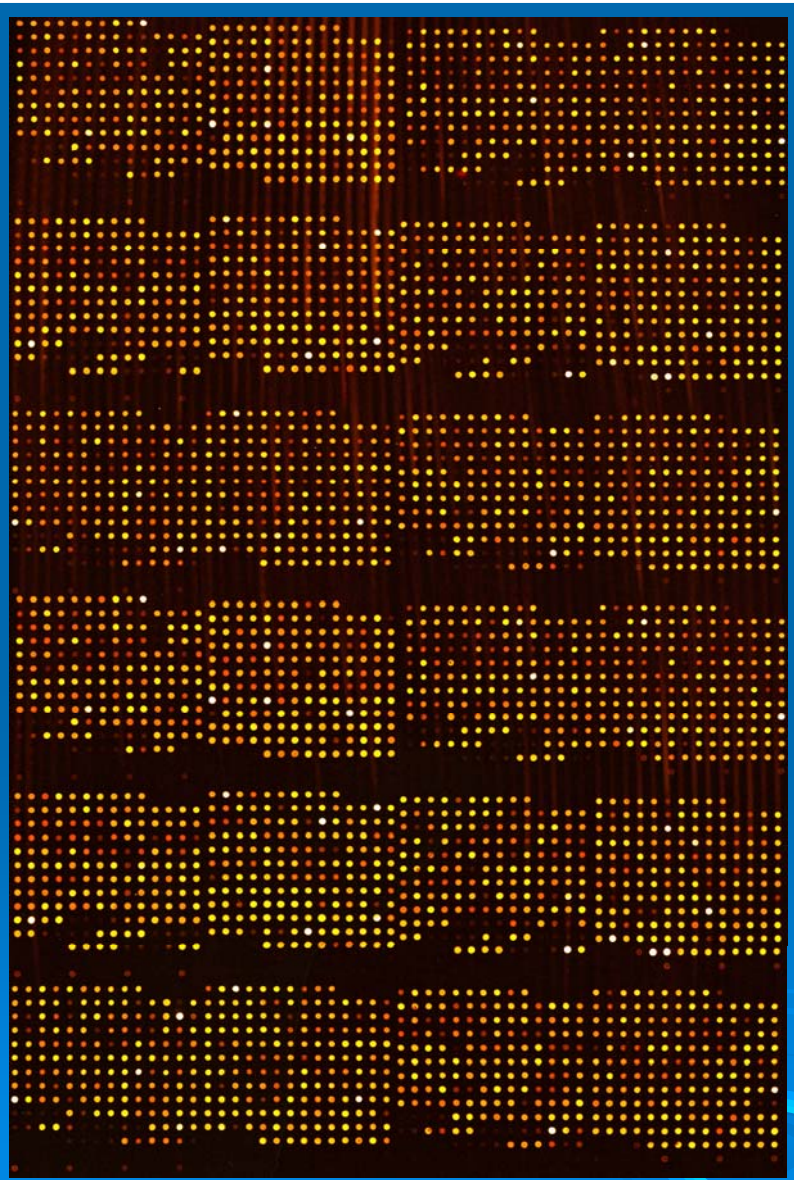

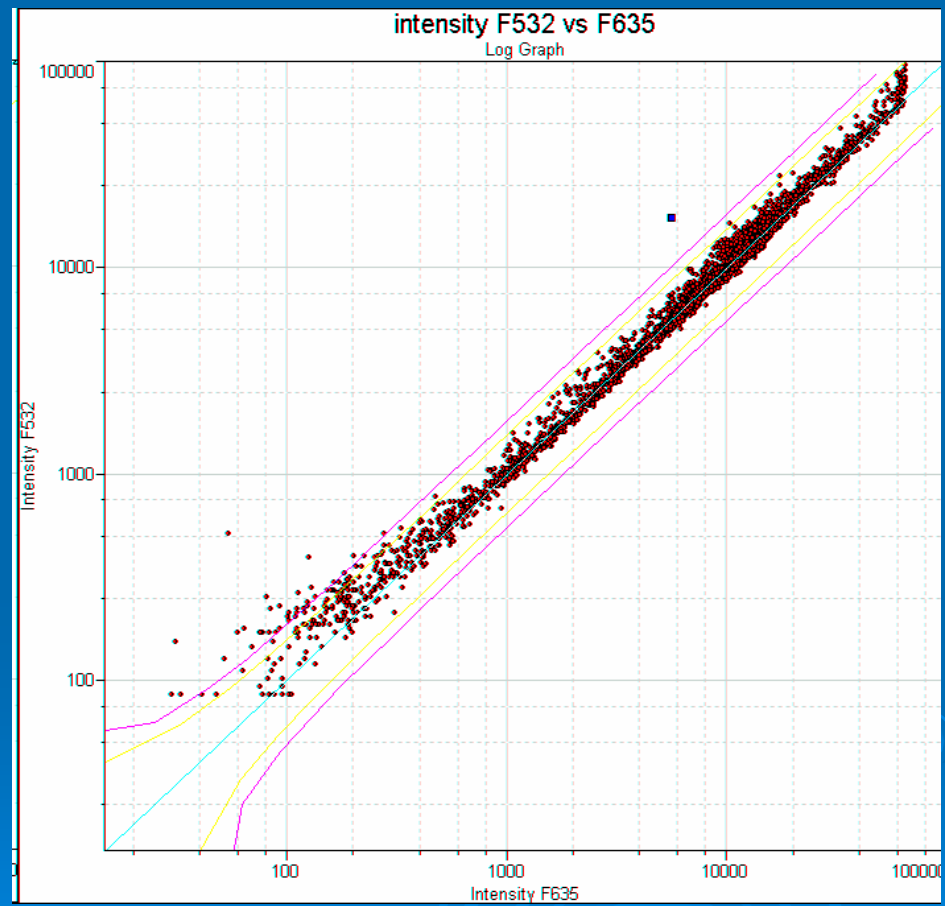

Speed, High throughput, Accuracy

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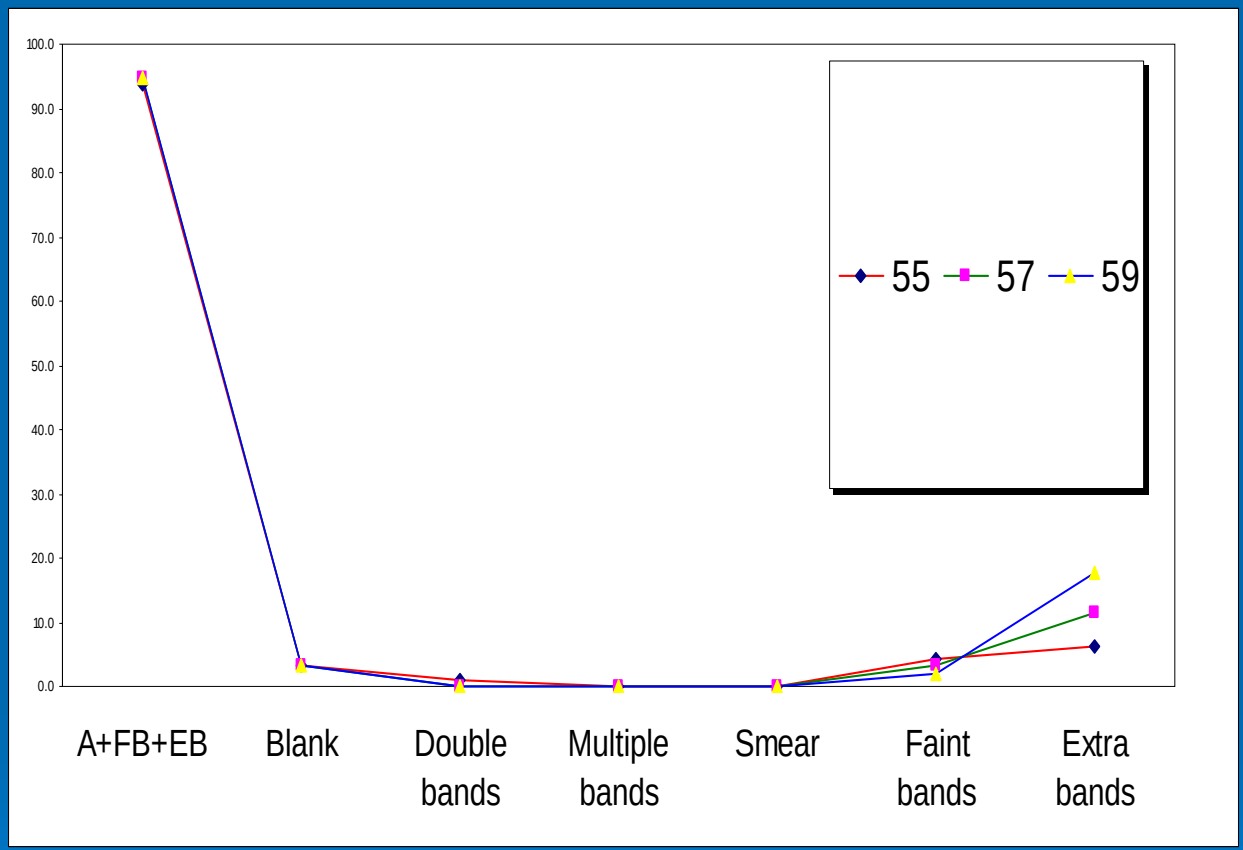
4 components

- Process
- Enzyme
- Length/Conditions of PCR
- Purification

Promotor Array Development



	55	57	59
Acceptable	93.8	94.8	94.8
Blank	3.1	3.1	3.1
Double bands	1.0	0.0	0.0
Multiple bands	0.0	0.0	0.0
Smear	0.0	0.0	0.0
Faint bands	4.2	3.1	2.1
Extra bands	6.3	11.5	17.7

55^{0}C Optimal Annealing Temp

Promotor Array Development

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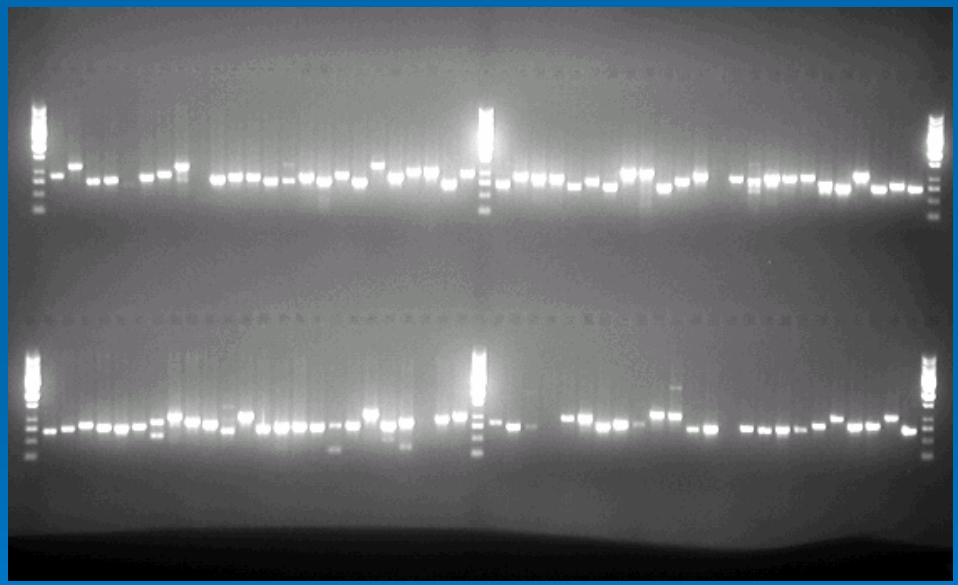
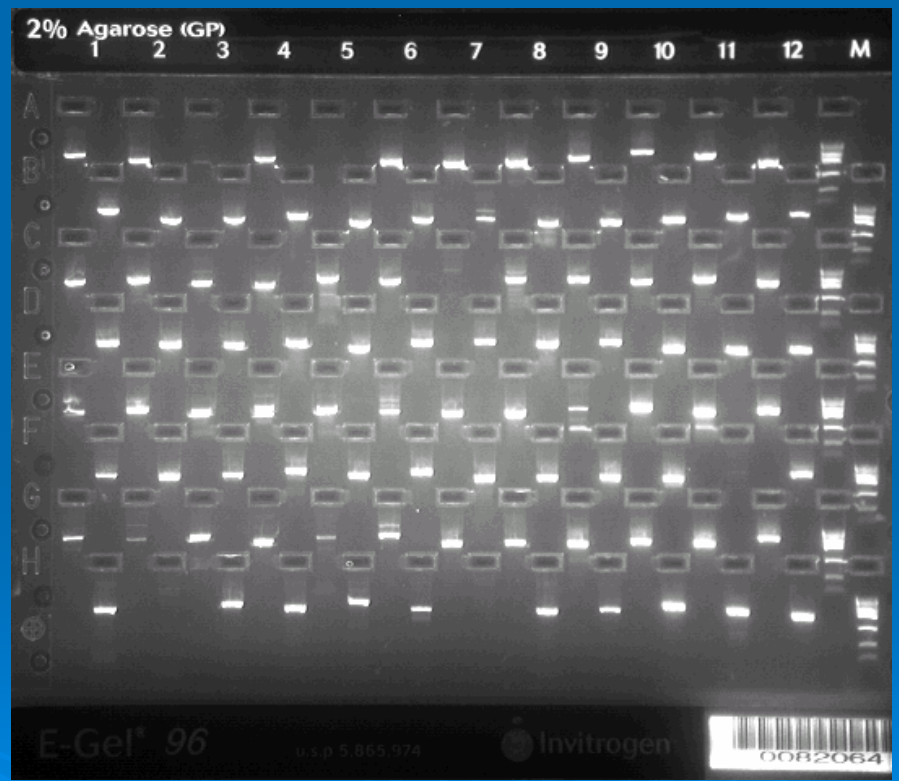
Comparison of E-Gel and Conventional gels

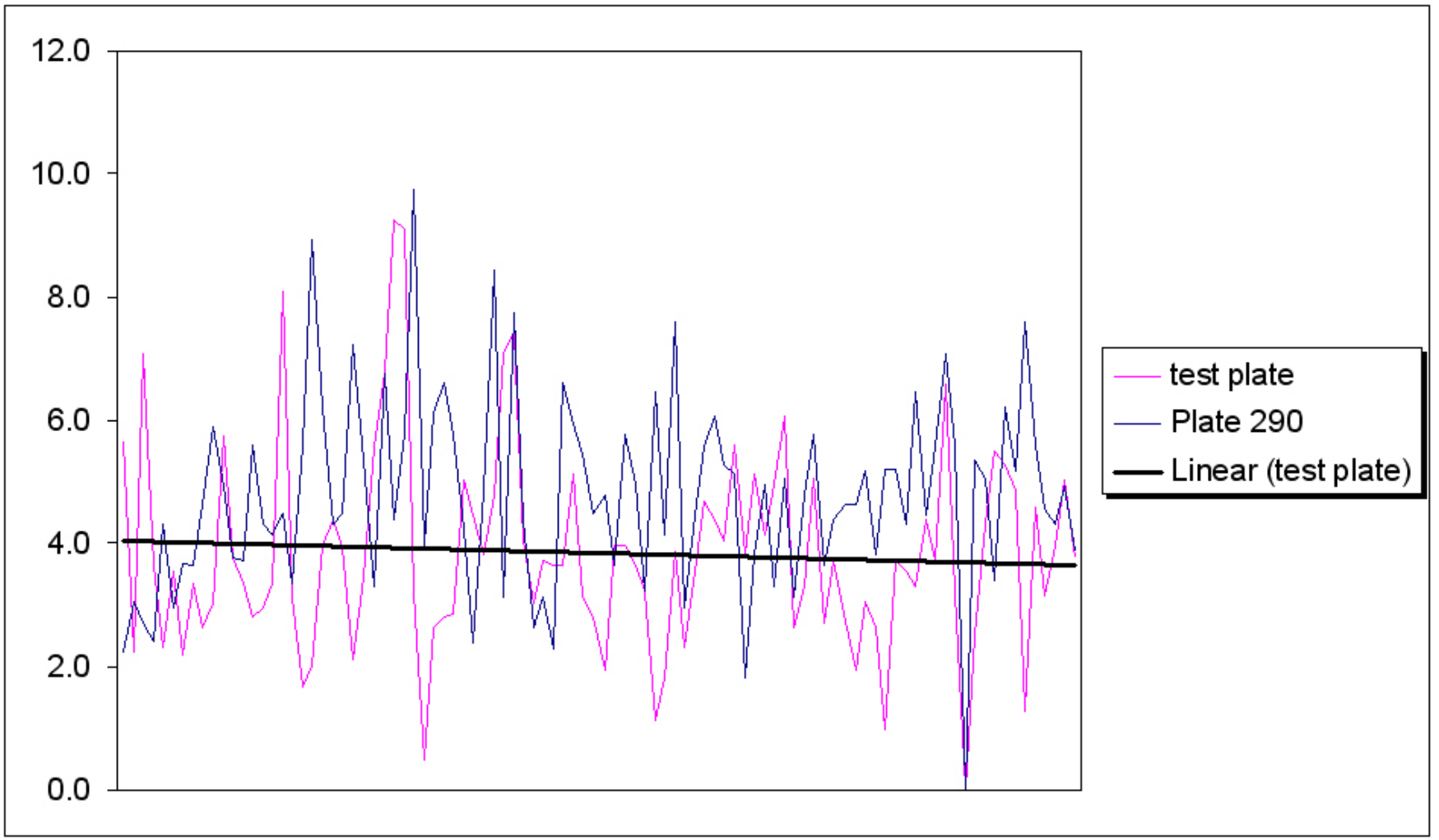
2-3 hours



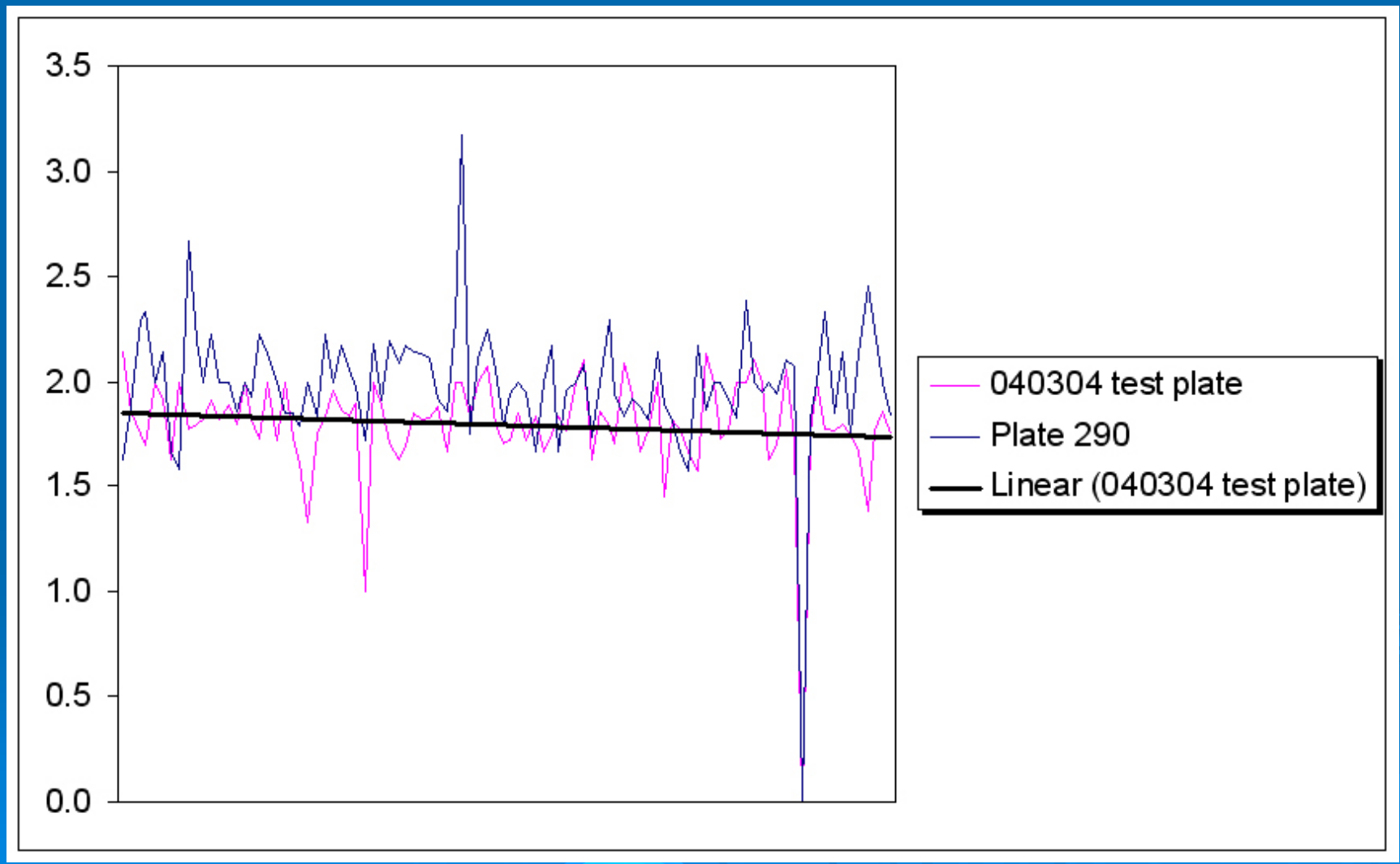
8 minutes



PCR Purification with Eppendorf – Yields are good



PCR Purification with Eppendorf – OD 260/280 ratios are good



Microarrays Methods and Applications

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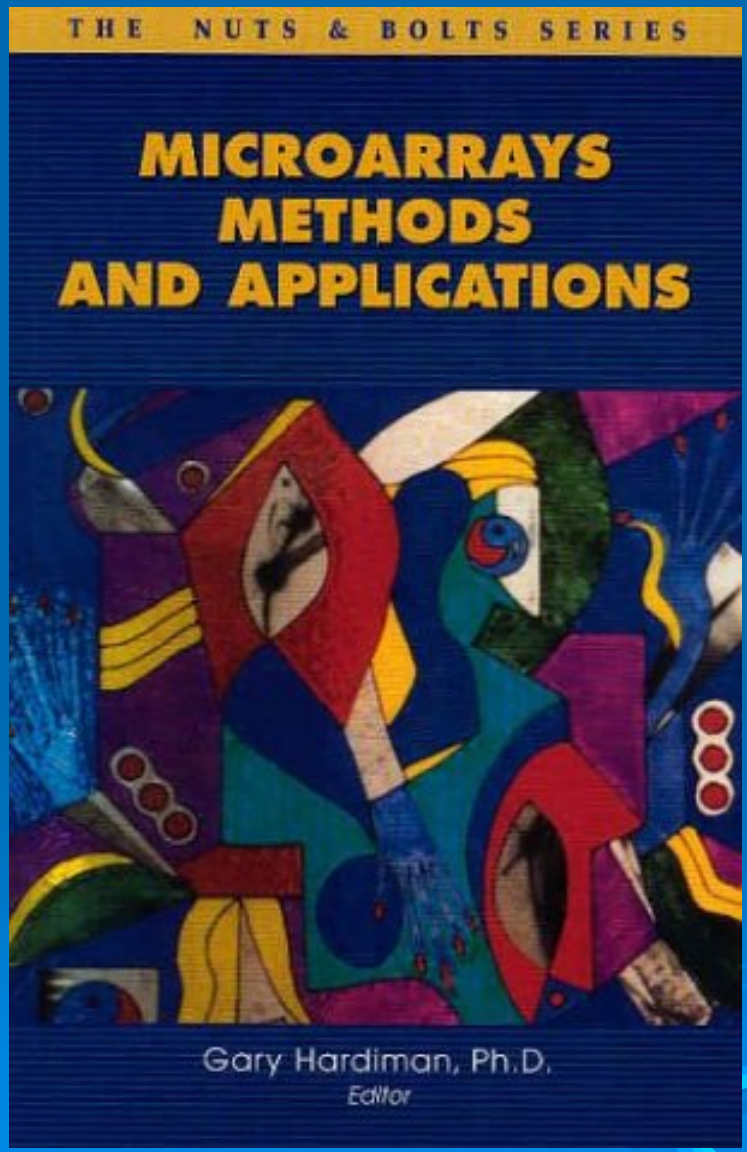

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Nuts&Bolts

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