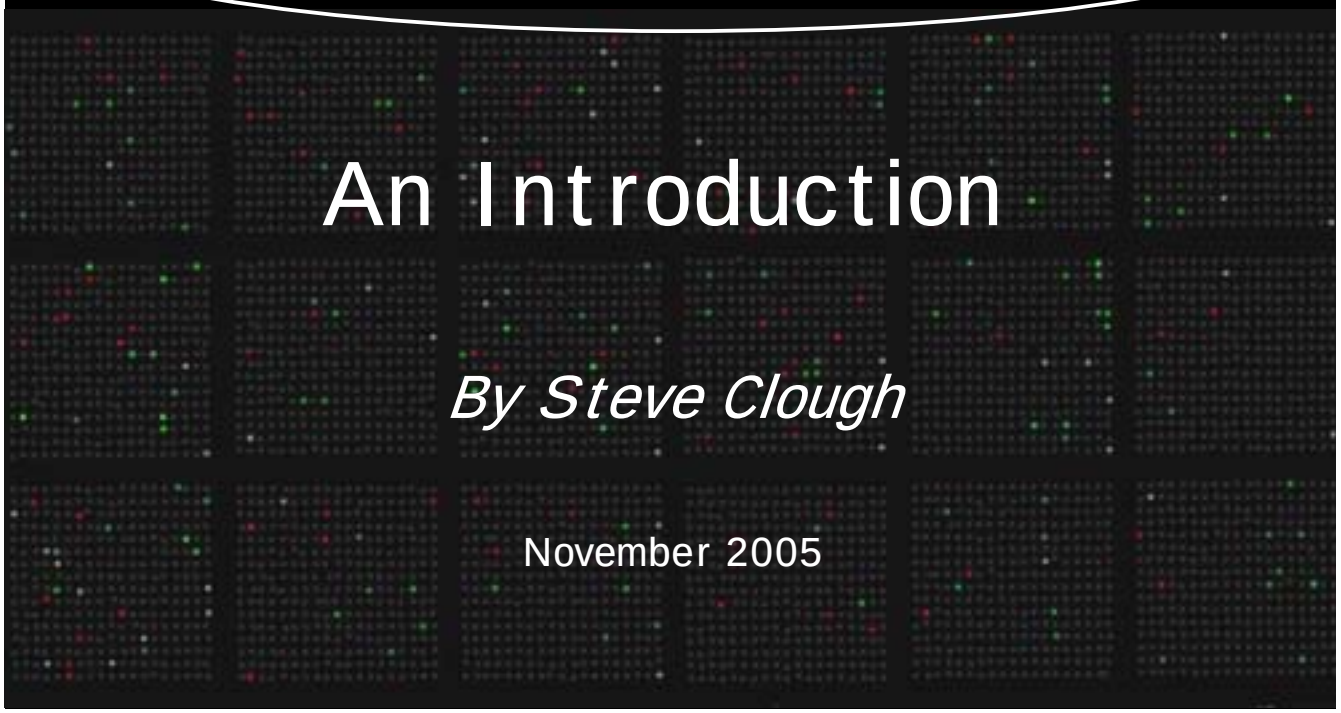
Soybean Microarrays



An Introduction

By Steve Clough

November 2005

Common Microarray platforms

- cDNA: spotted collection of PCR products from different cDNA clones, each representing a different gene
- Oligo: spot collections of oligos, usually 50-70 bp long that span known/predicted ORFs. May have one or more oligos representing each gene.
- Affy: Affymetrix gene chips, 25 bp oligos
  11 per gene predicted to span ORF

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Pros and Cons of cDNA platforms

- cDNA: need to construct cDNA libraries from a variety of tissues and conditions and to sequence to verify lack of duplication.
- Cheapest approach. Do not need to have a sequenced genome
- Hybridization involves strands of hundreds of bases, therefore less specificity in binding and cannot differentiate multigene family members. Good if your organism is closely related but not identical to one used to make the cDNA libraries used to make arrays.

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Pros and Cons of Oligo-based platforms

- Oligo: spot collections of oligos, usually 50-70 bp long that span known/predicted ORFs. Affymetrix chips use 25mers and 11 or so probes per ORF
- Need lots of sequence information from your organism
- Works best if your organism is same or very closely related to the one used to obtain the sequence information
- More costly than cDNA arrays to manufacture

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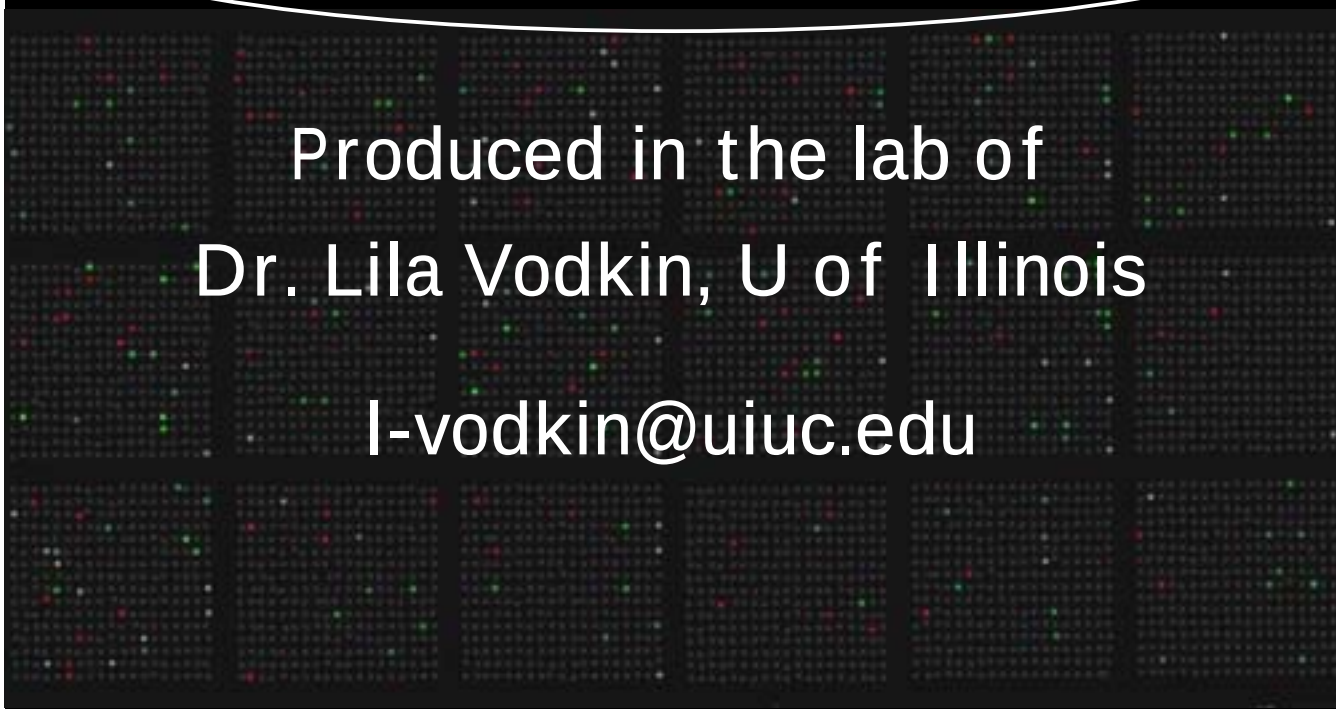
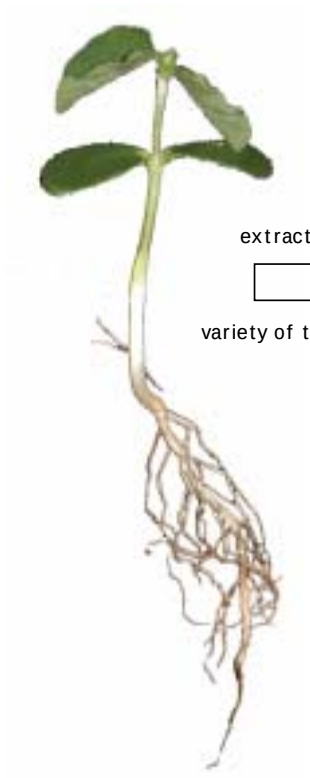
Soybean cDNA Microarrays



Produced in the lab of
Dr. Lila Vodkin, U of Illinois
l-vodkin@uiuc.edu

cDNA Library Synthesis
(represents expressed genes)



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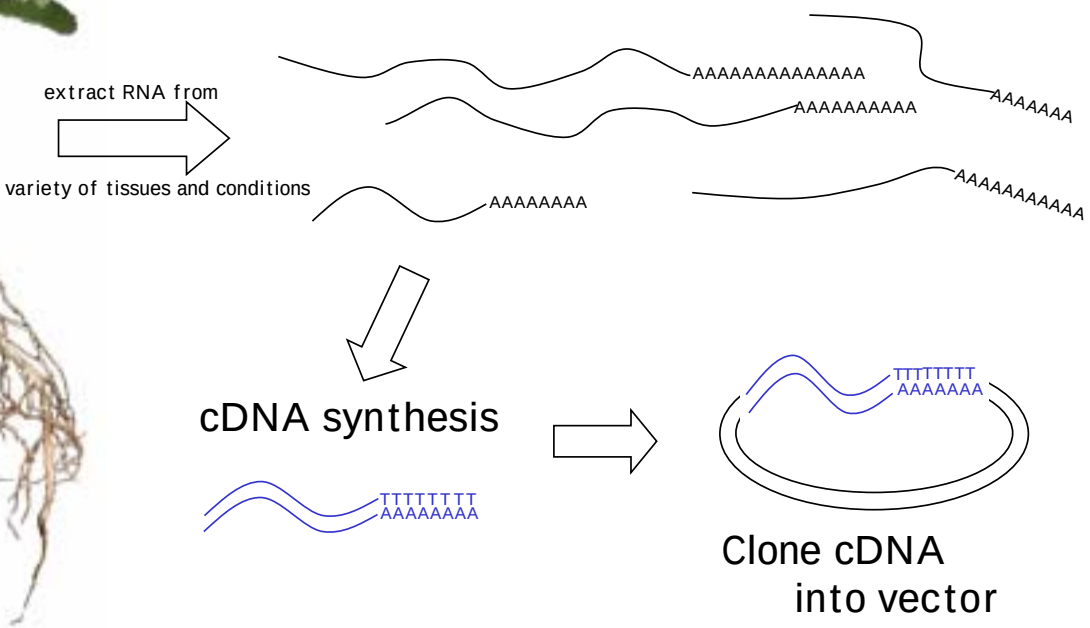
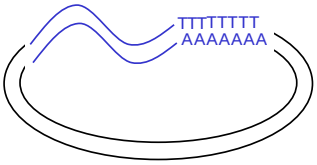
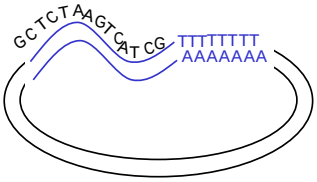




cDNA clone



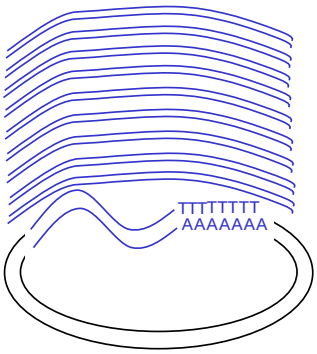
Sequence cDNA

GCTCTAAGTCATCGTACTAGATCT

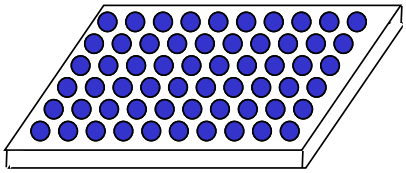
= protein kinase

Compare EST sequence to database to identify

Eliminate duplicates to generate set of unique clones



PCR amplify insert of unique clone set



Pipette PCR products into microtiter plates to print onto slides

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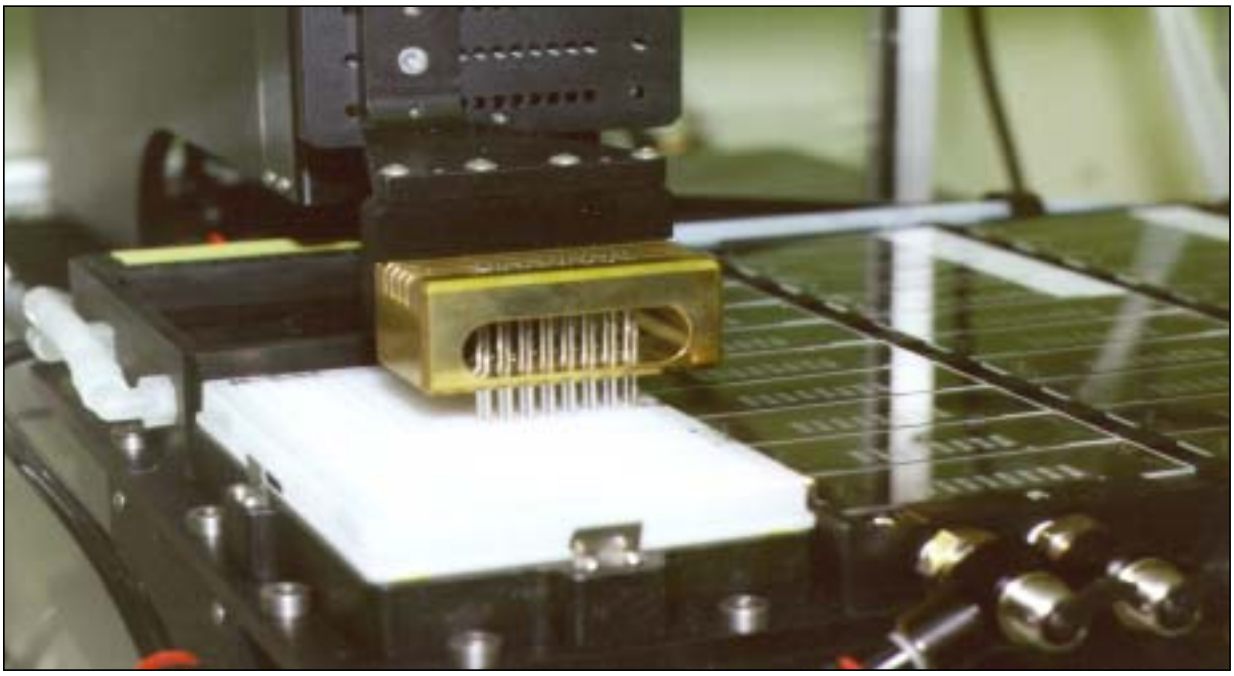
Printing microarrays – picking up PCR samples



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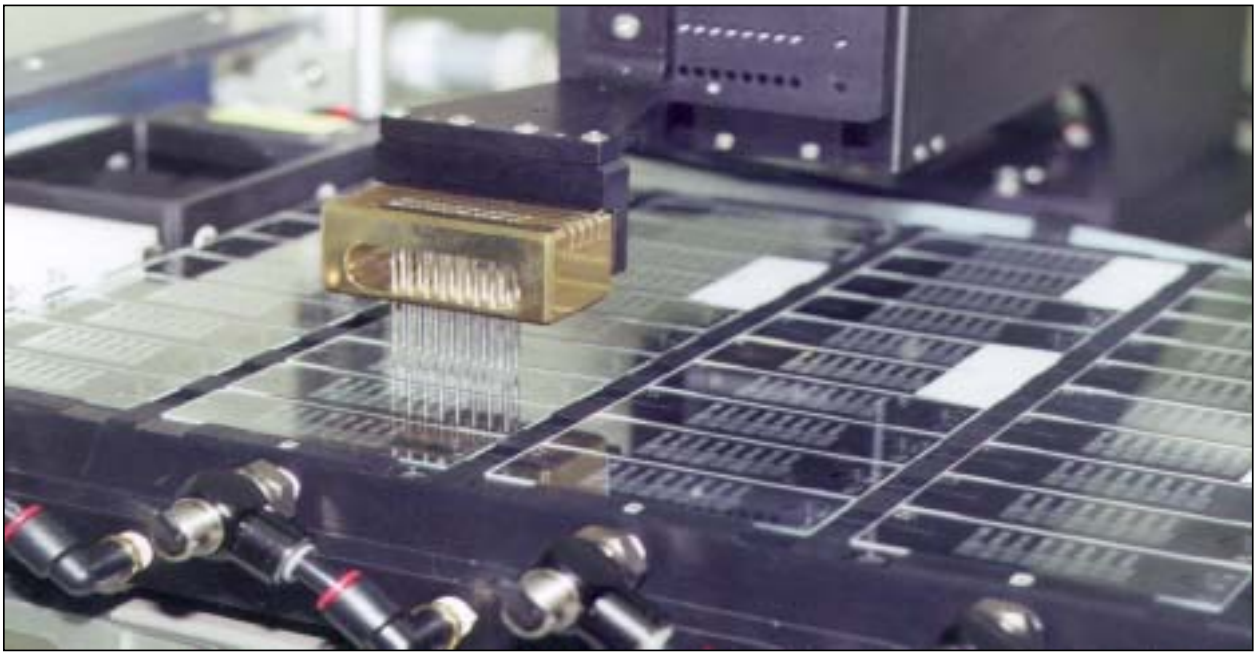
Printing PCR products on glass slides



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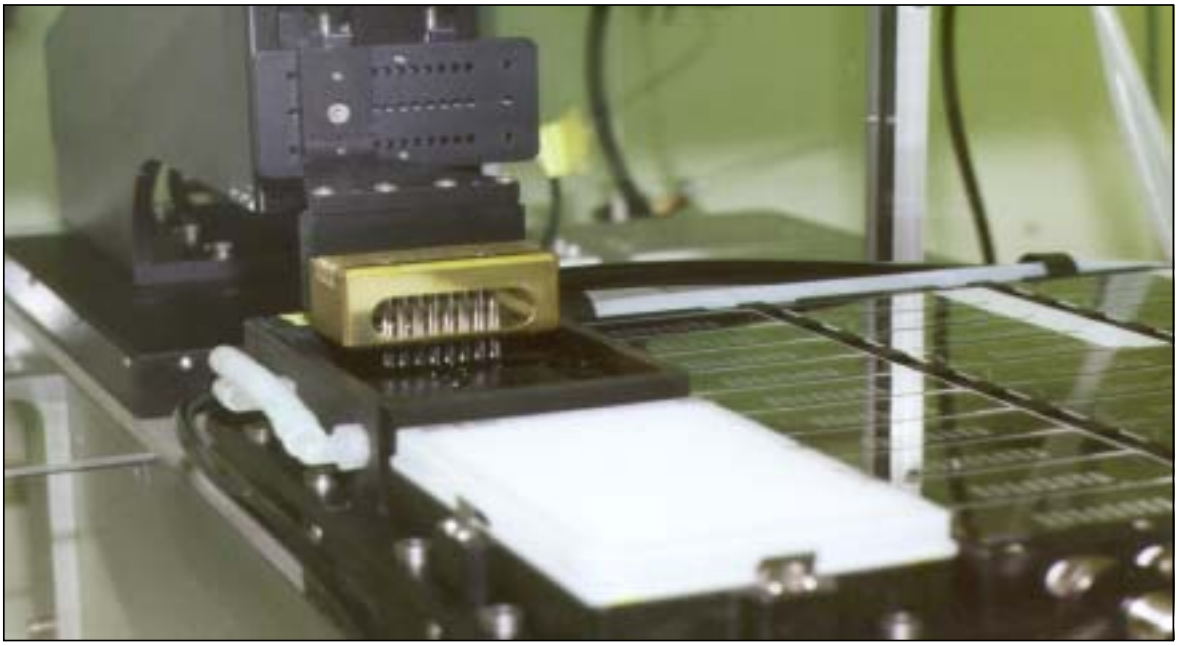
Pin Washing Between PCR Samples



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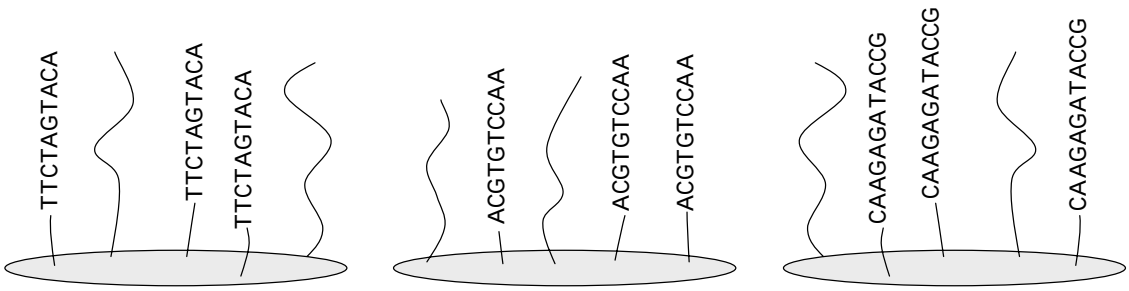
Spots of single-stranded DNA adhered to glass surface

Typically 10-25,000 spots are printed on a standard 1” x 3” microscope slide



Note: DNA does not bind well to glass so glass is specially coated to allow ionic binding (poly-lysine slides) or covalent binding (amine or aldehyde slides)

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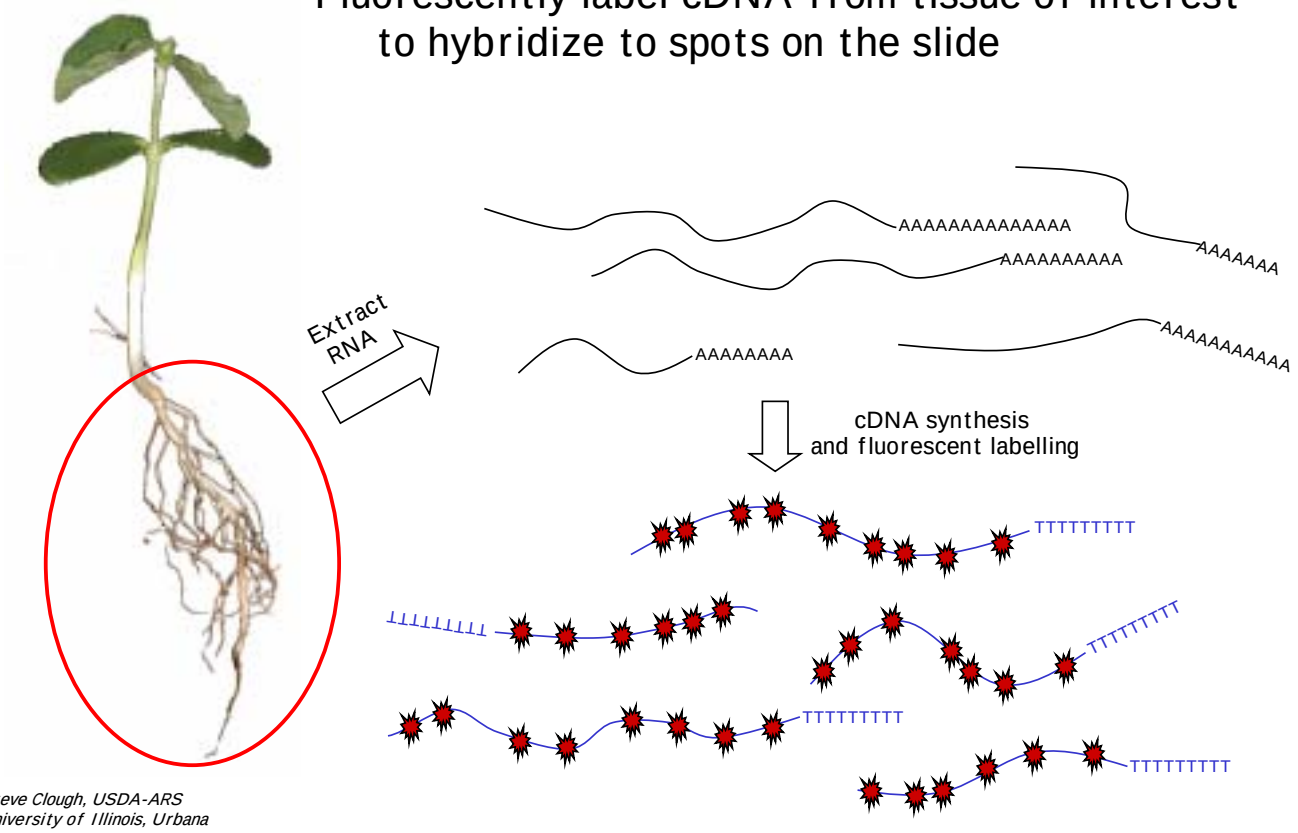
Fluorescently label cDNA from tissue of interest to hybridize to spots on the slide



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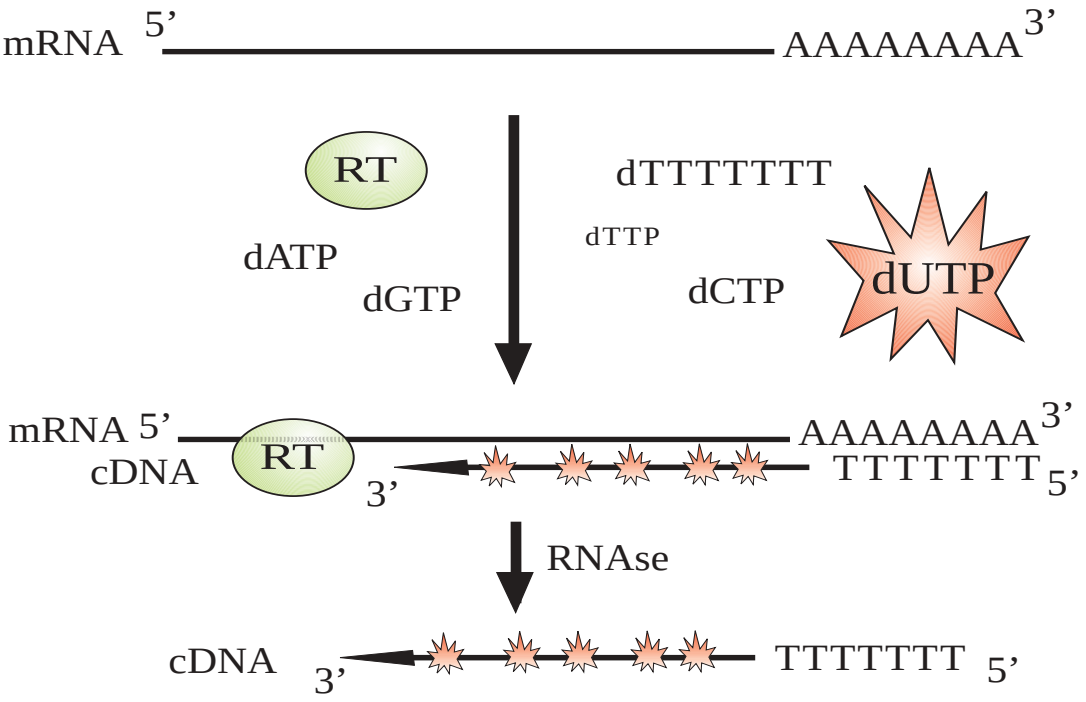
Direct labelling with Reverse Transcriptase



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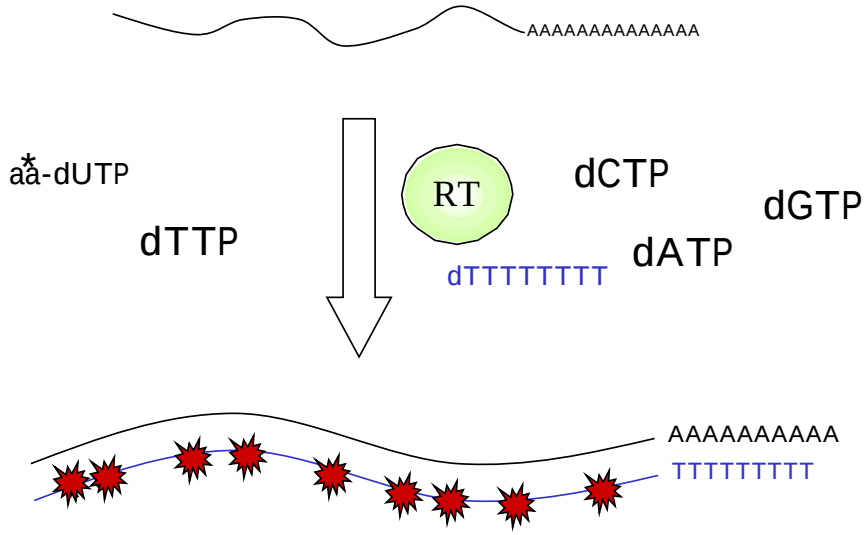
Indirect labelling with aa-dUTP and Reverse Transcriptase



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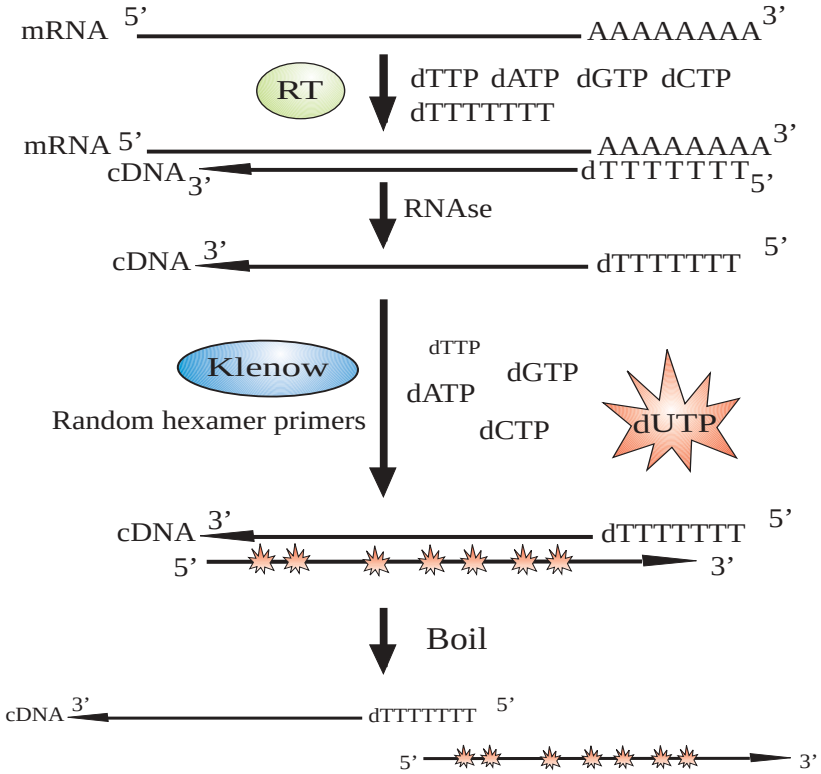
Indirect labelling with Klenow



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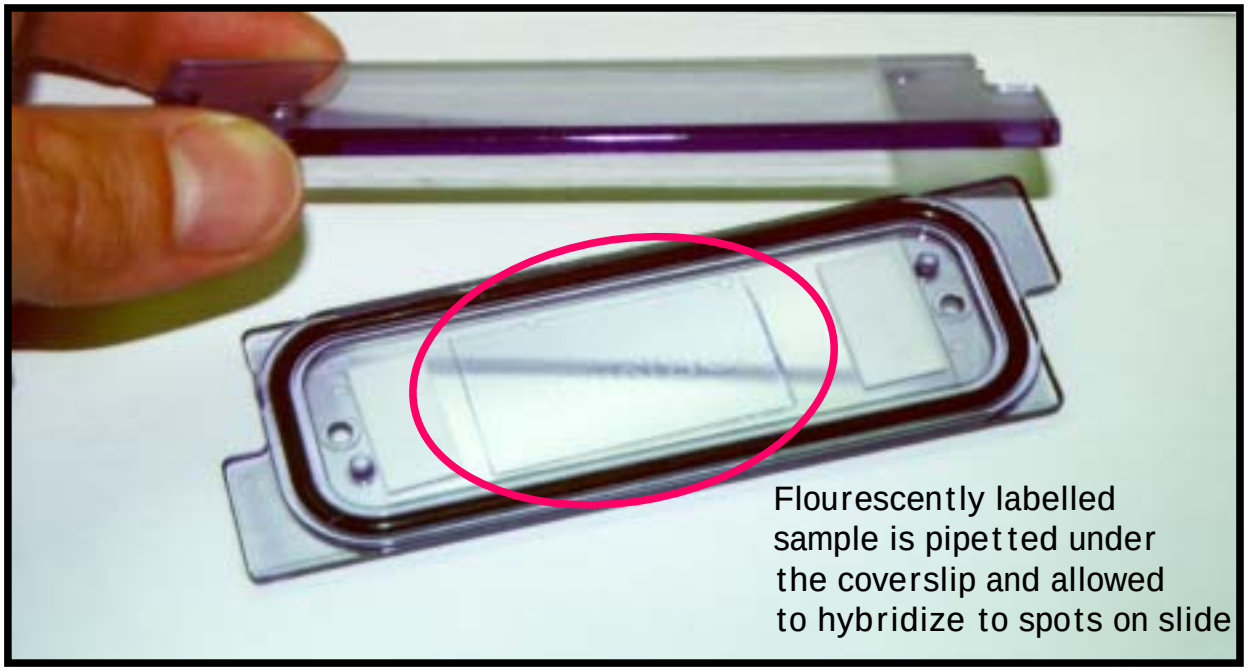
Hybridization Chamber



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Hybridization in Water Bath

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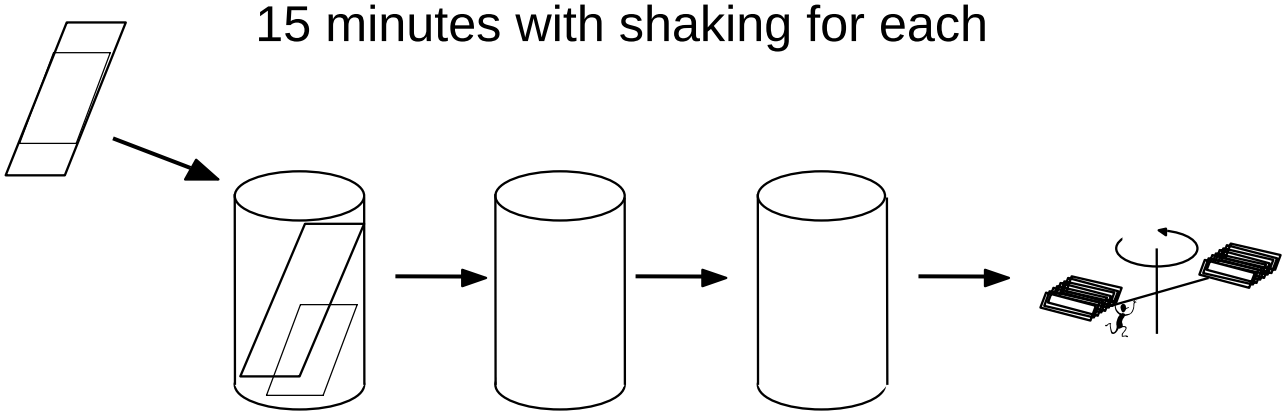
Washing After Hybridization

15 minutes with shaking for each



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Scan on a Fluorescent Scanner

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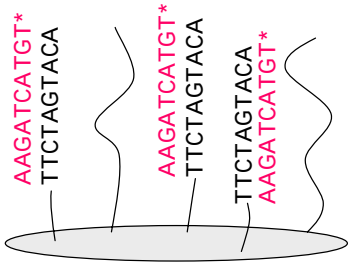
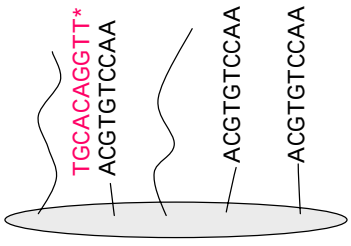
Theory:
Spot A will fluoresce 3 times brighter than Spot B



Spot Gene A



Spot Gene B

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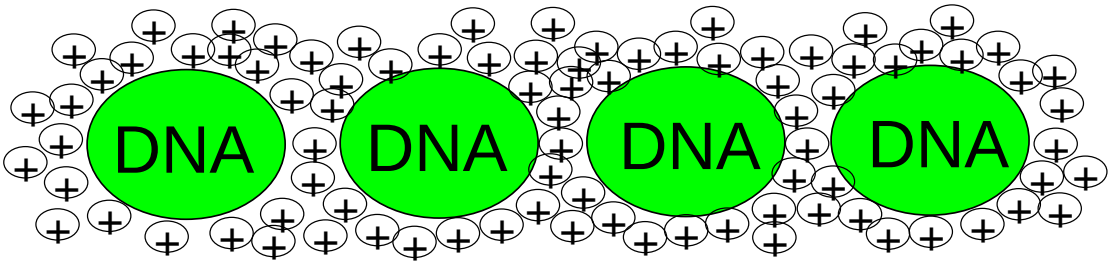
Blocking slides to reduce background. Example, positively charged amine slides.



Wash with SDS to block charges and to remove excess DNA.
Then place in hot water to generate single strands.
Repeat SDS wash.

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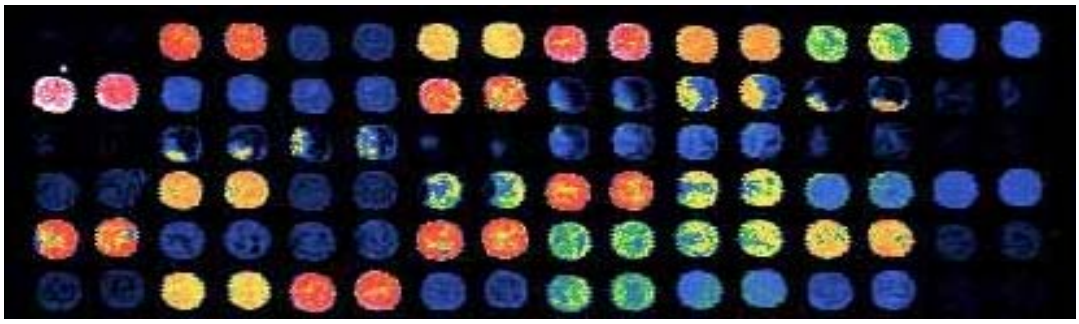
False Coloring of Fluorescent Signal



Scale of increasing fluorescent intensities

2 **65,536**

Stronger signal (16 bit image)

2^1 2^{16}

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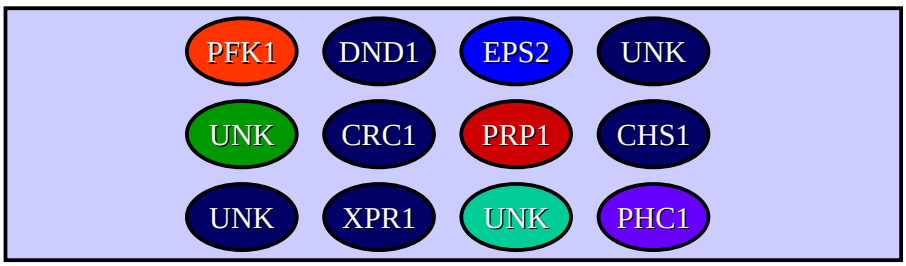
Principles behind gene expression analysis

mRNA ⟶ Fluorescent cDNA

- Hybridized to array of individual spots of different genes
- Fluorescent intensity of spot is proportional to expression level
- Labelled representation of all recently expressed genes



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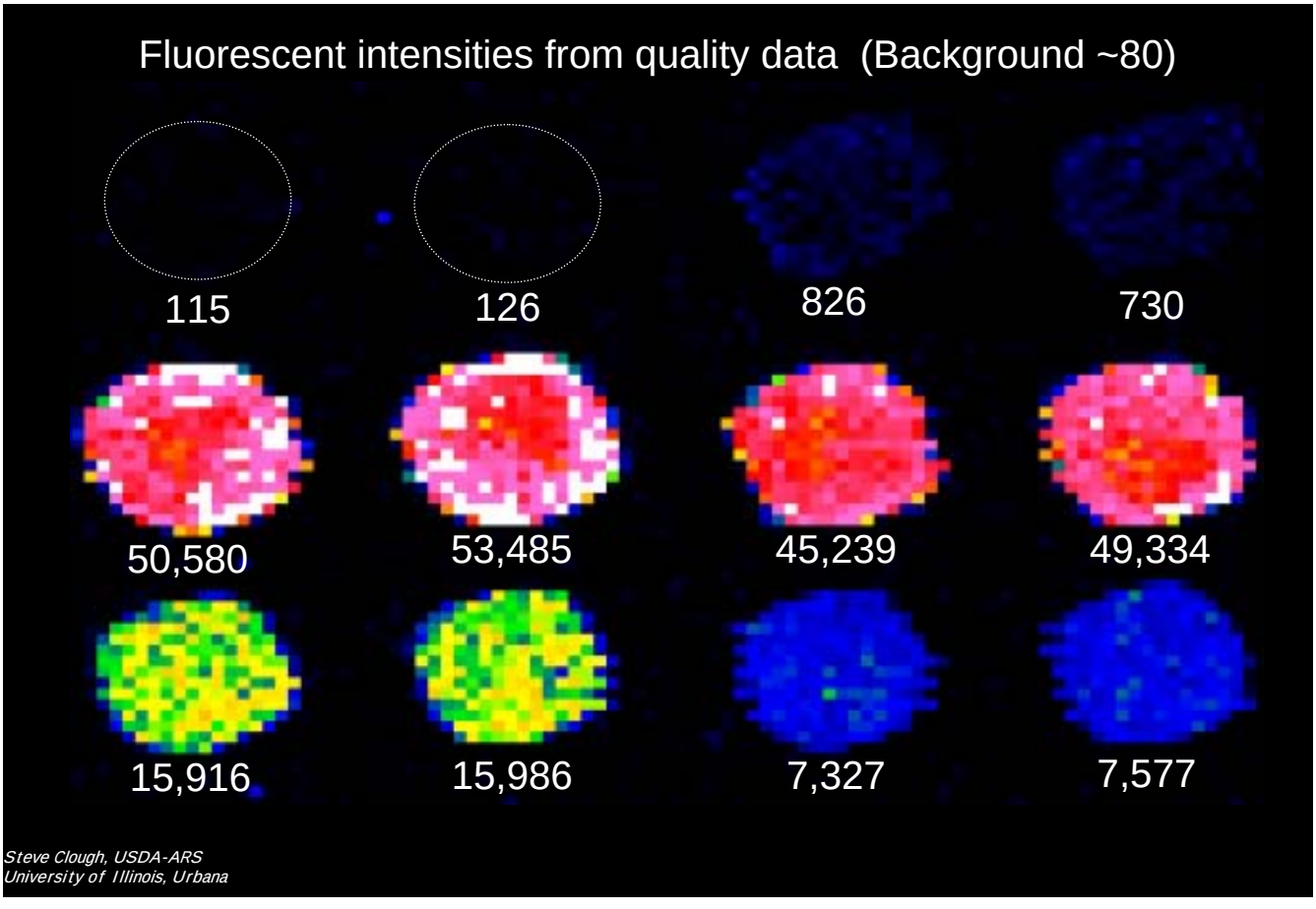
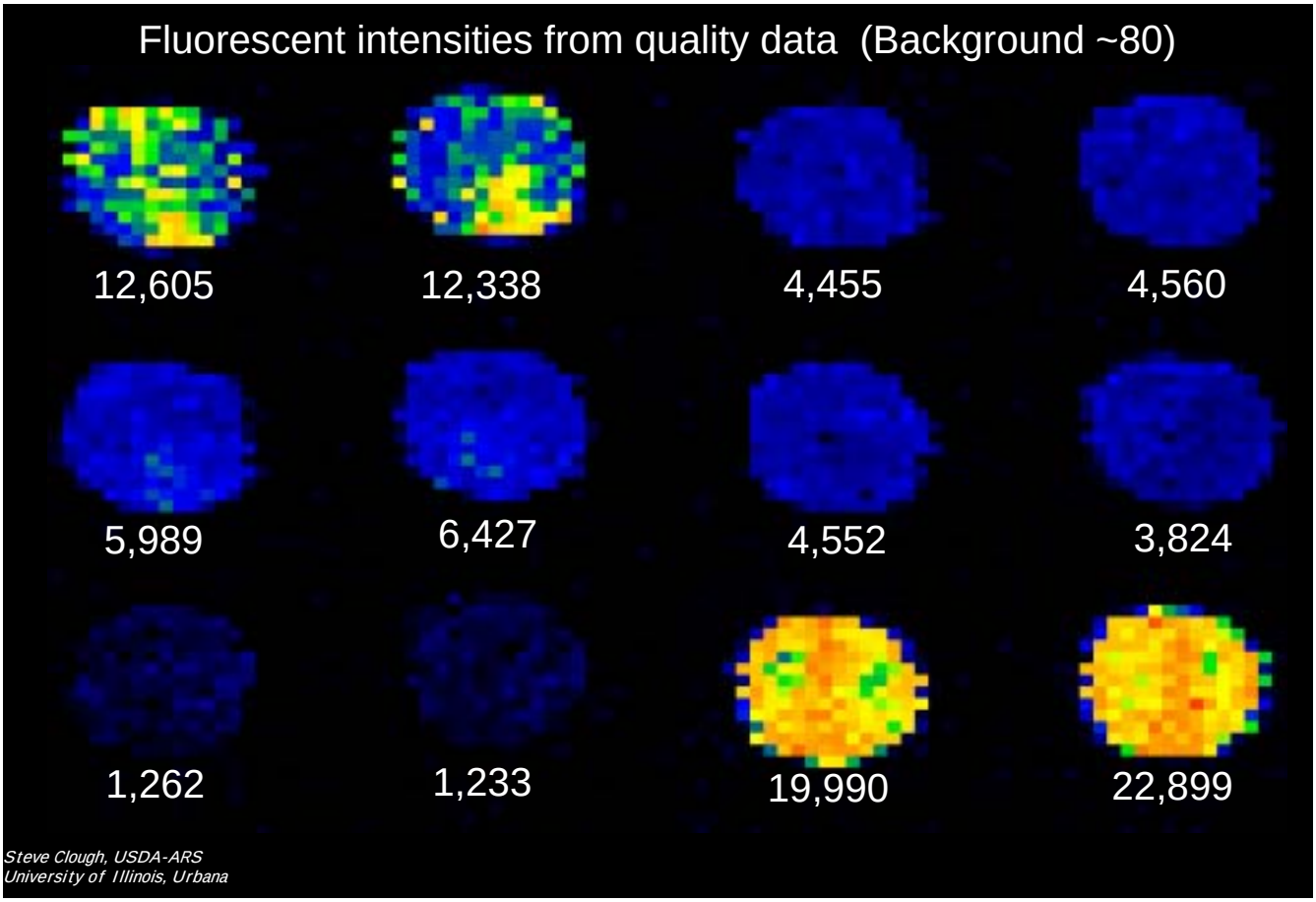
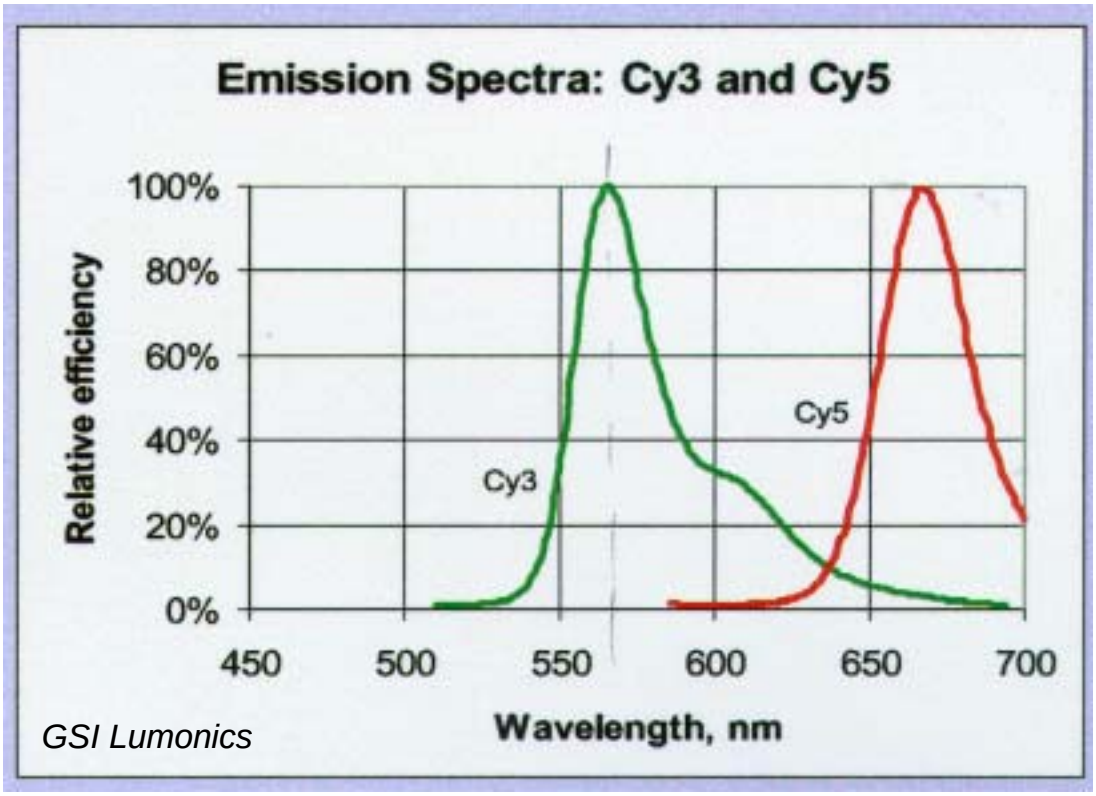
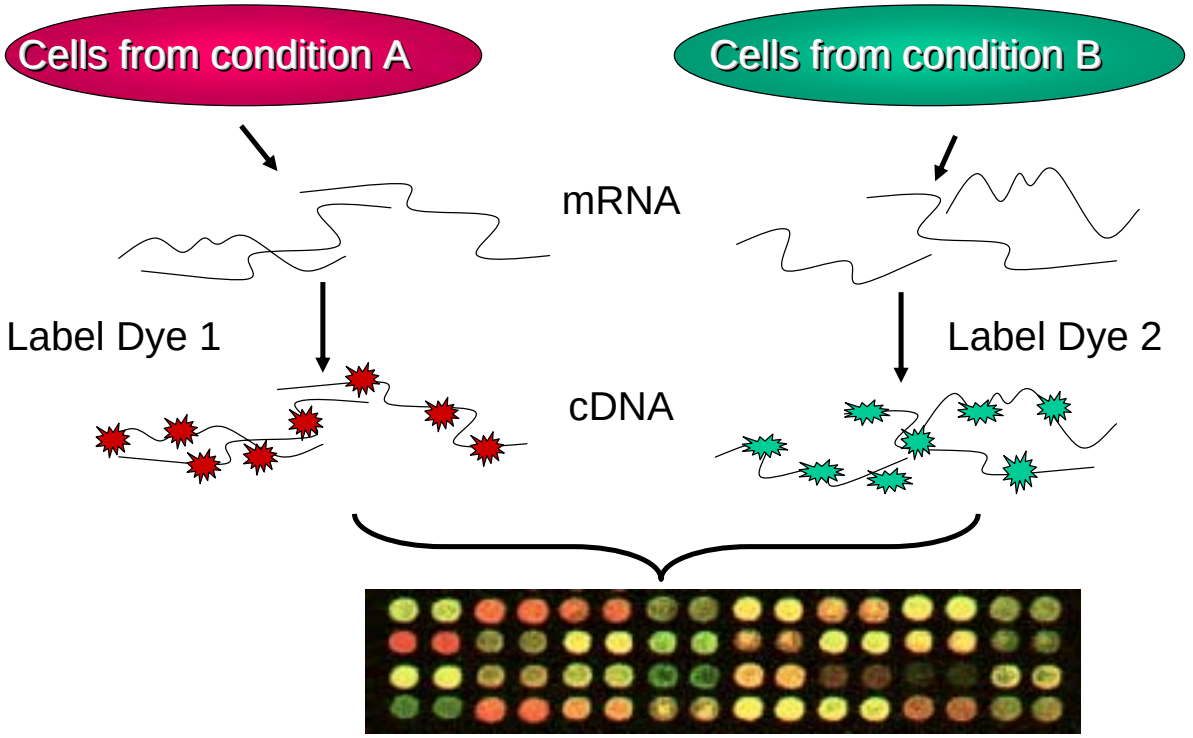




2 dyes with well separated emission spectra allow direct comparison of two biological samples on same slide



Ratio of Expression of Genes from Two Sources



equal higher in A higher in B

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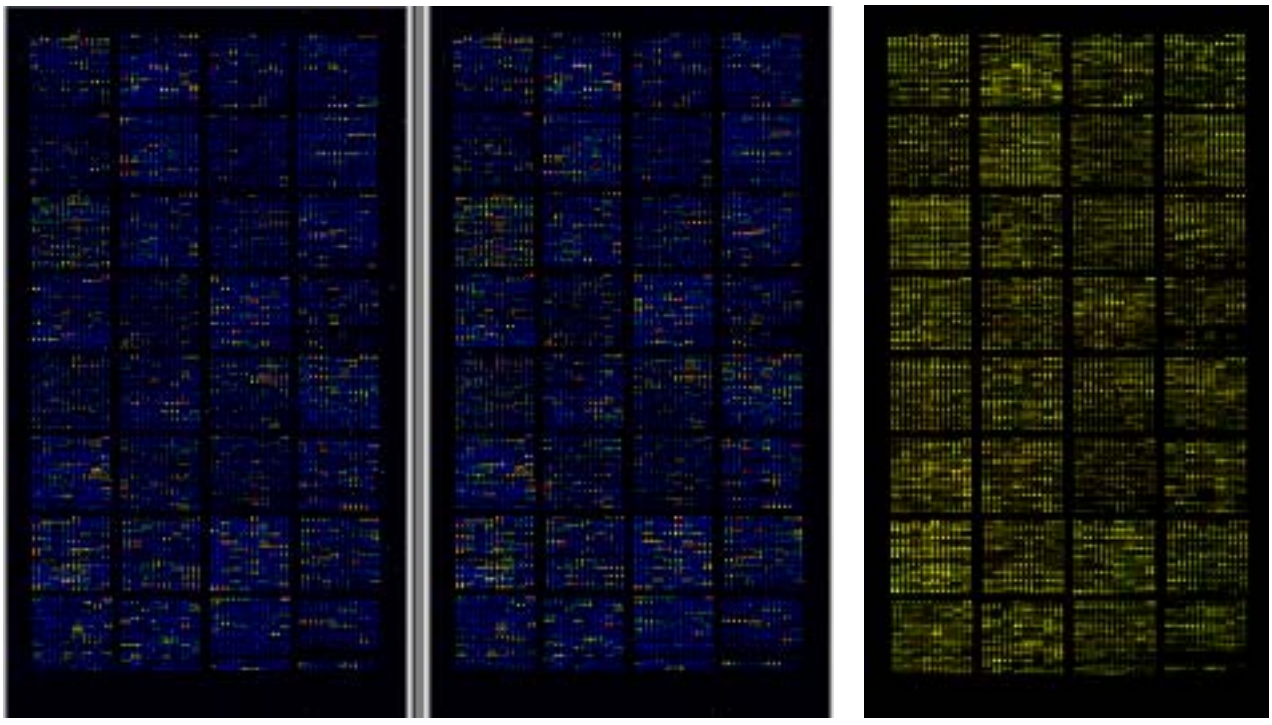
Cy3 Scan Cy5 Scan Overlay



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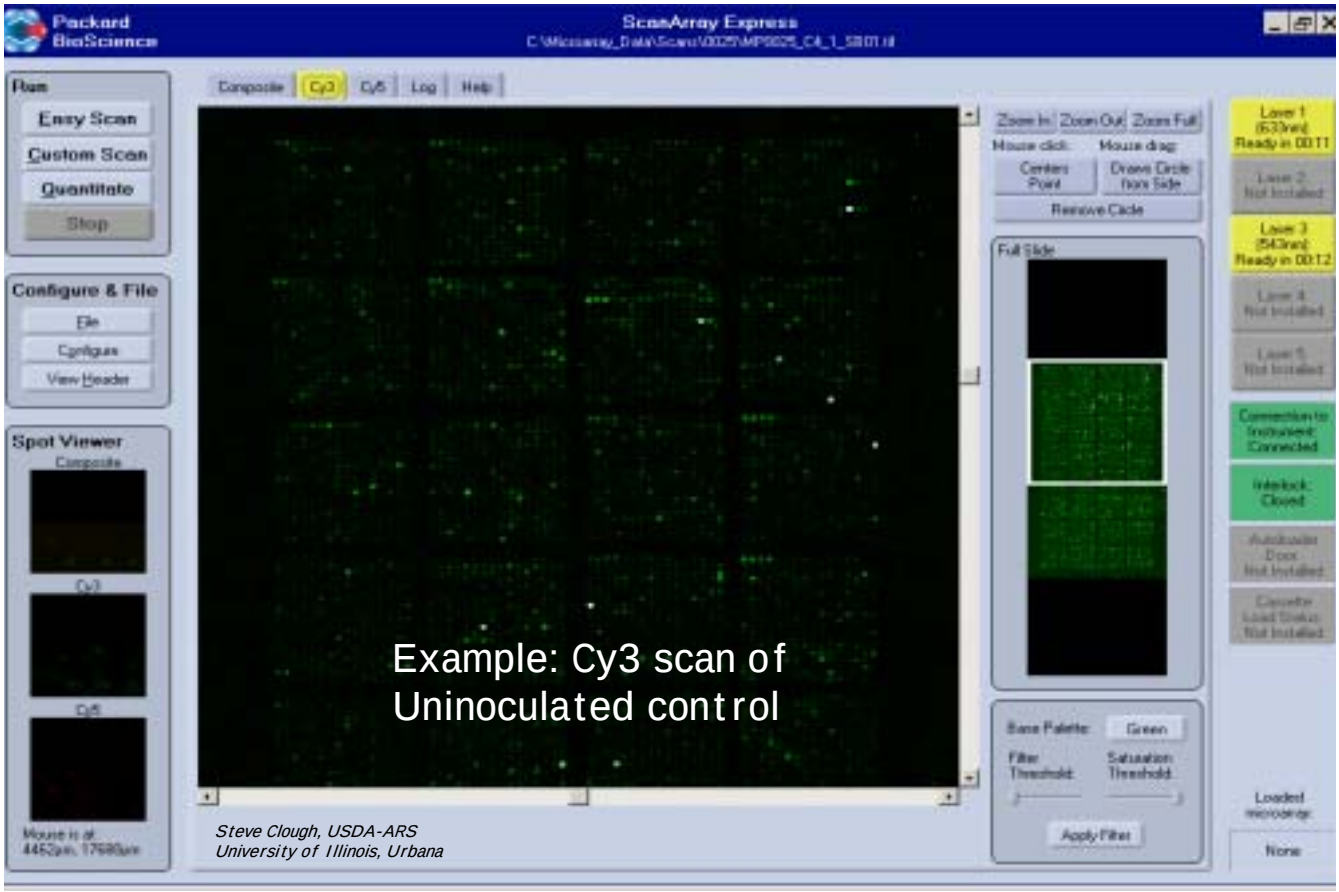
Packard BioScience
ScanArray Express
Run
Easy Scan
Custom Scan
Quantitate
Stop
Configure & File
File
Configure
View Header
Spot Viewer
Composite
Cy3
Cy5
Composite
Cy3
Cy5
Log
Help
Zoom In
Zoom Out
Zoom Full
Mouse click:
Mouse drag:
Centers Point
Draws Circle from Side
Remove Circle
Full Slide
Base Palette:
Green
Filter Threshold:
Saturation Threshold:
Apply Filter
Loaded
None
Example: Cy3 scan of
Uninoculated control
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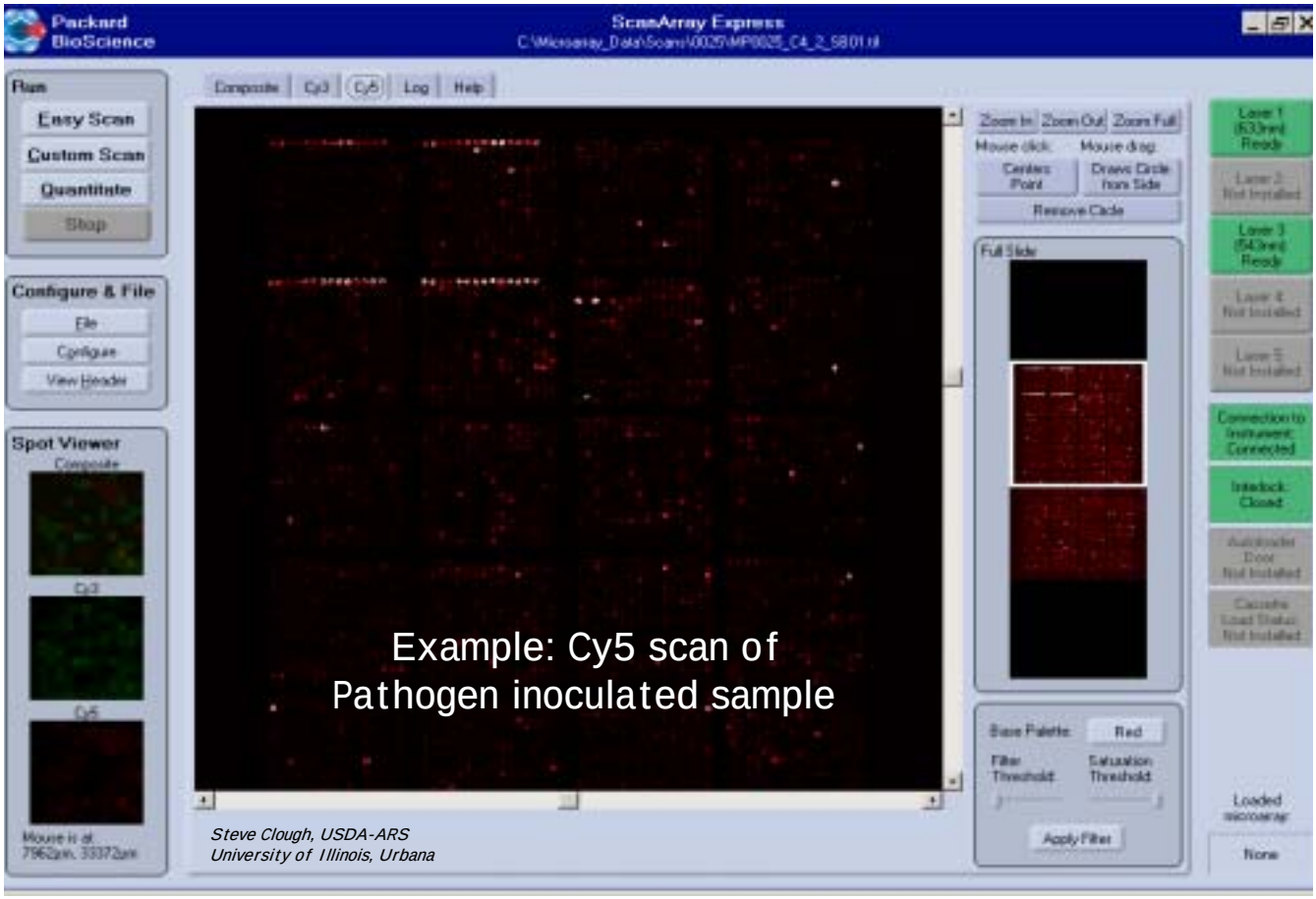
University of Illinois, Urbana


Packard BioScience
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Custom Scan
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Configure & File
File
Configure
View Header
Spot Viewer
Composite
Cy3
Cy5
Composite
Cy3
Cy5
Log
Help
Zoom In
Zoom Out
Zoom Full
Mouse click:
Mouse drag:
Centers Point
Draws Circle from Side
Remove Circle
Full Slide
Base Palette:
Red
Filter Threshold:
Saturation Threshold:
Apply Filter
Loaded
None
Example: Cy5 scan of
Pathogen inoculated sample
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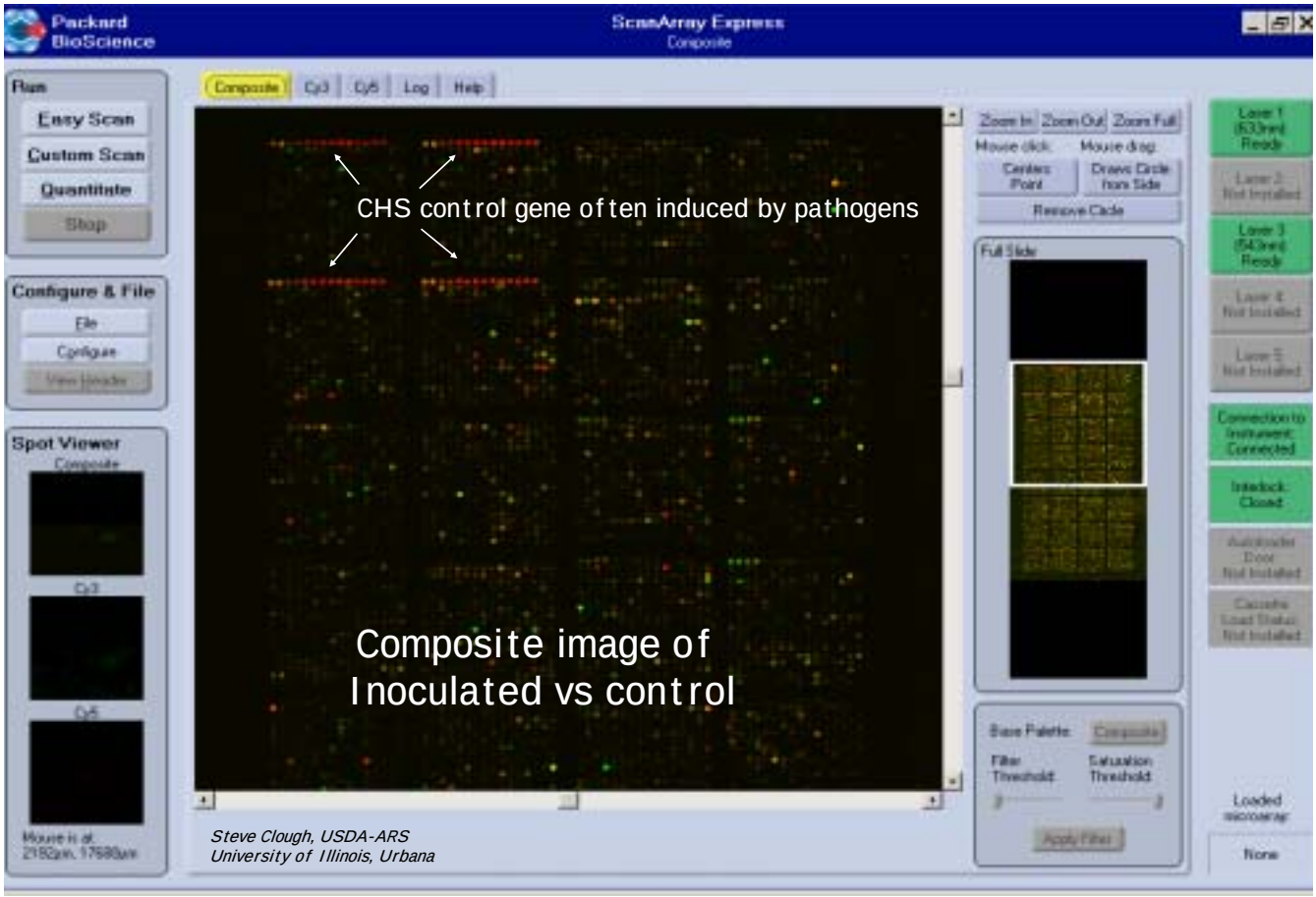
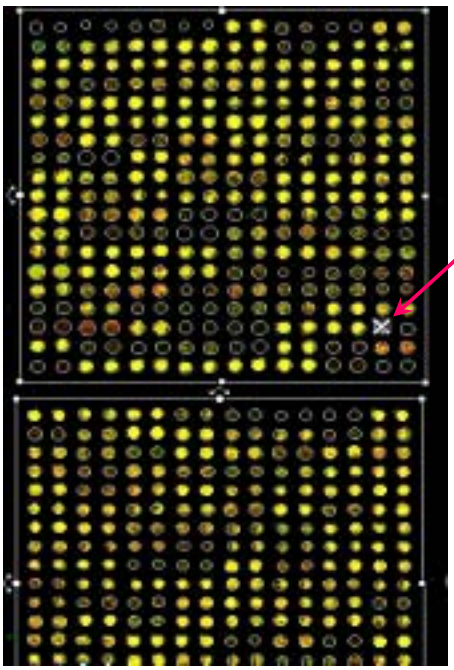


Use software such as GenePix to extract data from image



1. Locate spots, define spot area, collect data from pixels within spots
2. Flags bad spots (ex: dust in spot)
3. Calculates ratio Cy5 fluorescent intensity over Cy3 intensity for each spot
4. Produces tab-delineated tables for import to analysis programs

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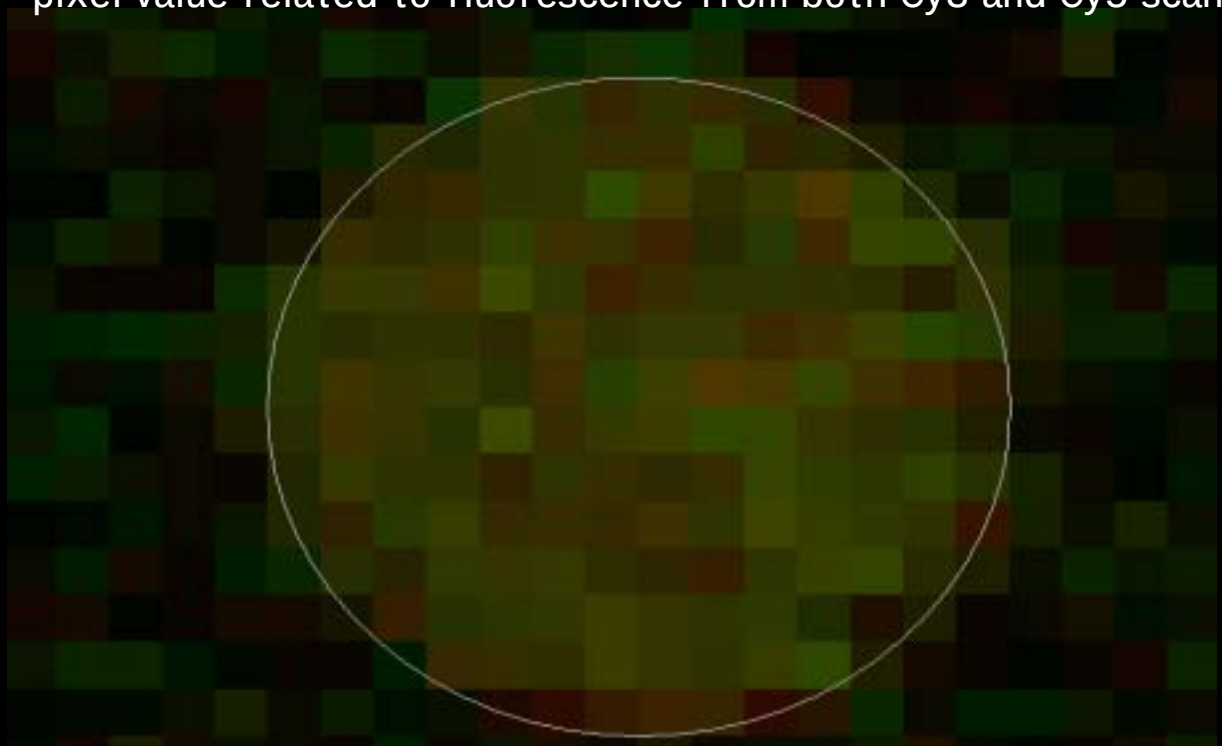
Value of pixels within spot equals the raw data. Software will give pixel value related to fluorescence from both Cy3 and Cy5 scans



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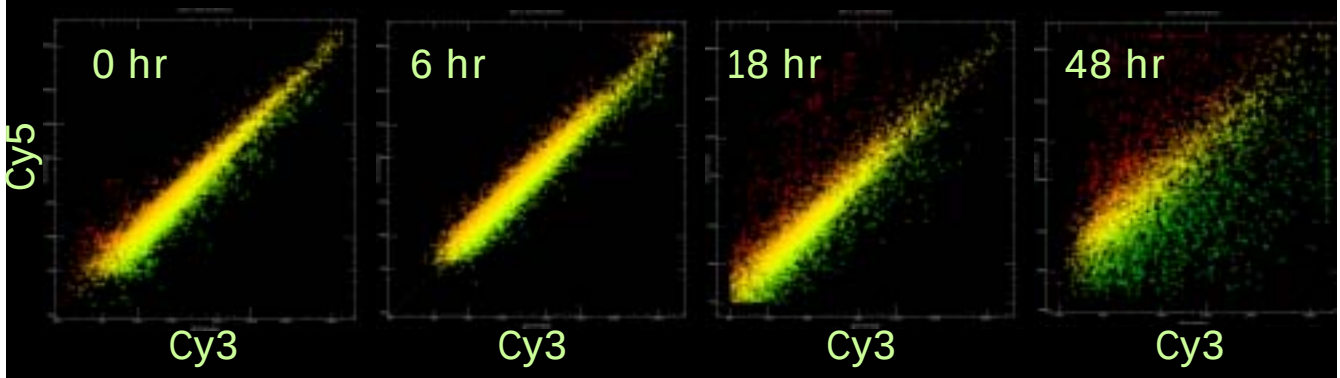
Quick view of expression results per slide can be seen by examining scatter plots of Cy5/3 intensity ratios per spot



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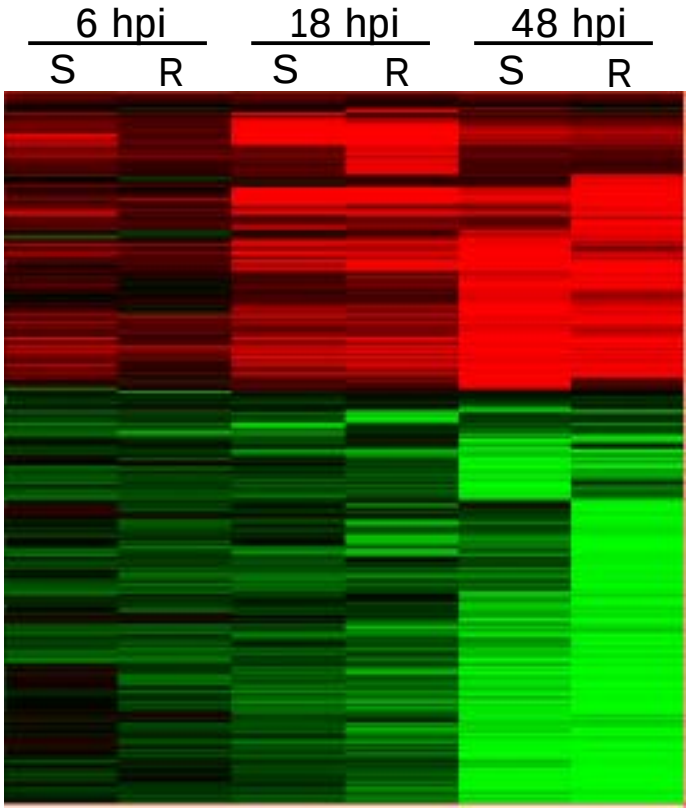
Data analysis

One can identify genes with common expression patterns by hierarchical clustering.

Each horizontal line represents on gene.



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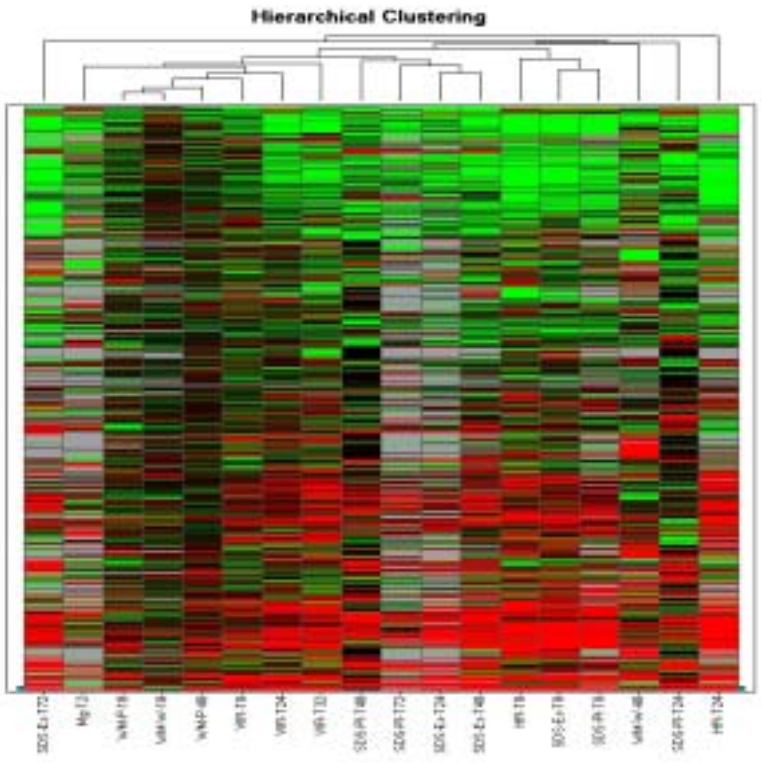
Clustering across experiments



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Plant Microarray Controls

- Negative: mammalian genes
- Positive: high, medium, low expressers
  tissue specific
  ubiquitous
- Miscellaneous: transgenes
  bacterial
- Labelling efficiency: spiked control mRNA--
  genes that are non-homologous to plants.

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Negative Plant Controls

- Spotting solution
- Genes not present in plant
  - Mammal-specific:
    - antibody / immunoglobulin
    - neuro-related
    - myosin
    - etc.
- Verify 'plant negative' by BLAST against plant databases

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Positive Plant Controls

- Tissue specific, high expressers:
  - ex: cotyledon: conglycinin
    roots: auxin down regulated gene 12
    leaves: RUBISCO (small chain)
- Ubiquitous:
  - ex: ubiquitin (med-high)
    EF1 (med-high)
    DAD1 (low-med)
    tubulin (med-high)

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Labelling Efficiency Controls

- Spiked mRNAs:
  - Mammalian genes, non-homologous to plant genome.
    Select several spiking controls (ex: 4).

<u>To use</u>:

- Include them on the array.
- Clone (with polyT tail) into a T7 or T3 expression vector
  - Or PCR with T7 or T3 promoter attached to 5' primer and a poly (dT) to the 3' primer.
- Invitro transcribe with T7 or T3 RNA polymerase.
- Add this 'mRNA' to your labelling reactions--
  - each one at a different concentration level
    to span the dynamic range of fluorescent intensities

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cDNA Arrays vs Oligo Arrays

- cDNA: spot a collection of ESTs
- Oligo: spot collections of oligos that span known/predicted ORFs
  - need sequence info
  - only option for prokaryotes
  - ‘shagged rug’ spots

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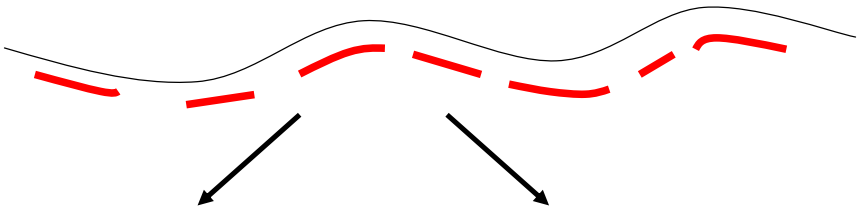
Oligo-based Microarrays

Design specific oligos for every ORF



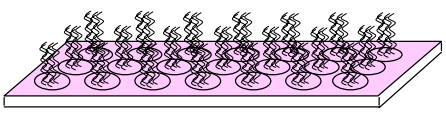
Spotted Microarray

Synthesize with 5’-amino linker

Design one to multiple oligos/ORF

Collect in 384-well plates

Spot on aldehyde coated slides



Affymetrix Gene Chips

Synthesize oligo directly on chip

Proprietary photolithography synthesis

11 oligo/ORF plus mismatches

Spotted oligo termed the ‘probe’

Perfect match oligos

1-base mismatch oligos

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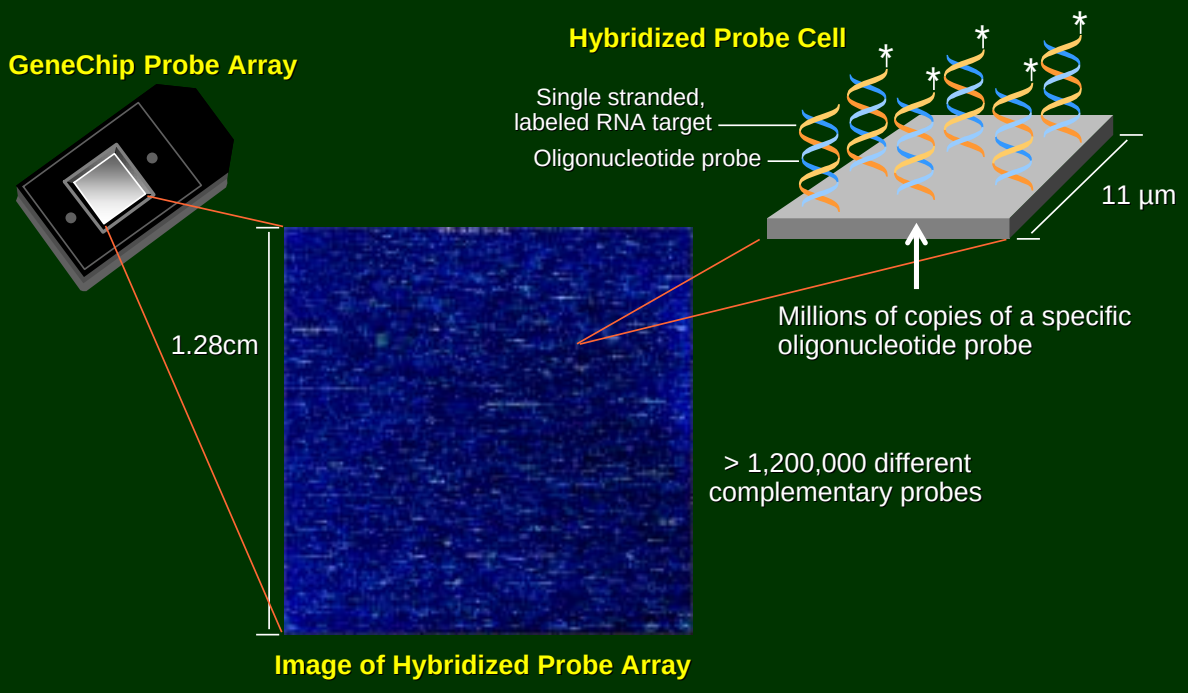
GeneChip® Probe Arrays



Courtesy of Mike Lelivelt