

Introduction to cDNA Microarray Technology

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complementary DNA (cDNA)

- cDNA is a strand of DNA that is complementary to part of an mRNA sequence.

```
mRNA      . . . CCUGAUJAGAUGG . . .
cDNA      . . . GGACTATCTACC . . .
```

- cDNA can be formed by extracting mRNA and then using mRNA as a template for formation of cDNA
- cDNA sequences can be copied rapidly using PCR (polymerase chain reaction).
- These sequences can be spotted on glass slides to serve as microarray probes.
- Sequence length varies from a few hundred bases to a thousand or so.

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cDNA Microarrays

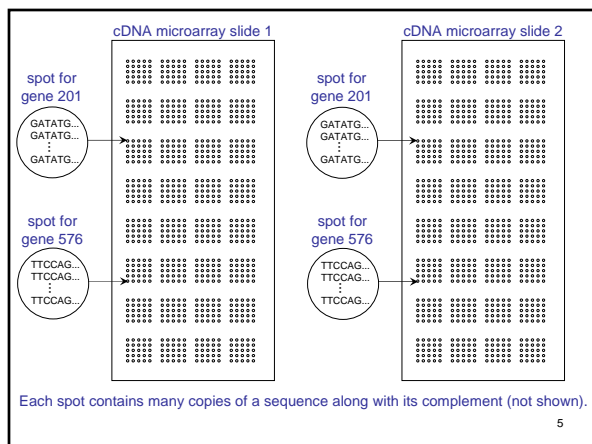
- Glass slides or similar supports containing cDNA sequences that serve as probes for measuring mRNA levels in target samples
- cDNAs are arrayed on each slide in a grid of spots.
- Each spot contains thousands of copies of a sequence that matches a segment of a gene's coding sequence.
- A sequence and its complement are present in the same spot.

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cDNA Microarray (continued)

- Different spots typically represent different genes, but some genes may be represented by multiple spots
- The spotted sequences are known (or can be determined) and their locations on the array are known.
- The sequence locations do not change from slide to slide.
- A single slide typically contains thousands of spots.

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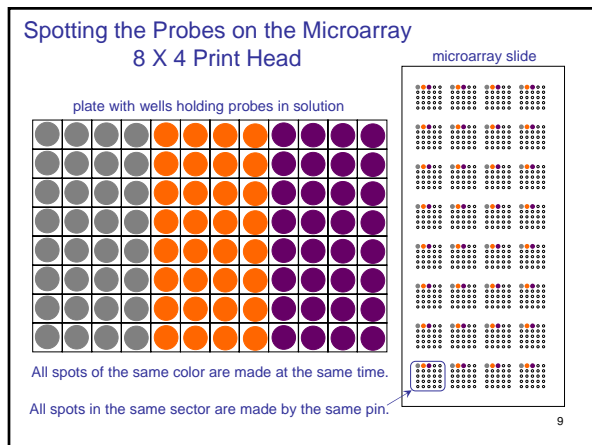
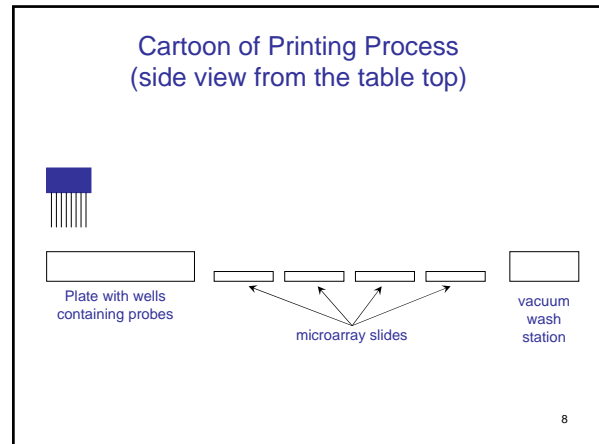


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Spotting cDNA Probes on Microarrays

- Solutions containing probes are transferred from a plate to a microarray slide by a robotic arrayer.
- The robot picks up a small amount of solution containing a probe by dipping a pin into a well on a plate.
- The robot then deposits a small drop of the solution on the microarray slide by touching the pin onto the slide.
- The pin is washed and the process is repeated for a different probe.
- Most arrayers use several pins so that multiple probes are spotted simultaneously on a slide.
- Most arrayers print multiple slides together so that probes are deposited on several slides prior to washing.

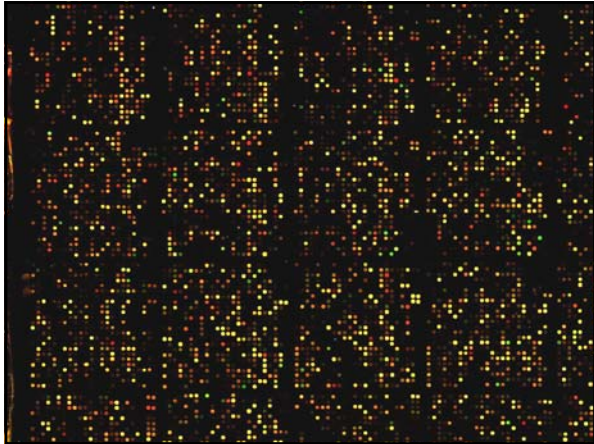
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- ### Using cDNA Microarrays to Measure mRNA Levels
- RNA is extracted from a target sample of interest.
 - mRNA may be reverse transcribed into cDNA.
 - The mRNA or resulting cDNA molecules are dyed with a fluorescent dye.
 - The dyed nucleic acid molecules are placed on a microarray slide.
 - Dyed sequences hybridize to the complementary probes that have been spotted on the array.
 - Any unbound dyed molecules are washed from the slide.
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- ### Using cDNA Microarrays to Measure mRNA Levels
- A laser excites the dye and a scanner records an image of the slide.
 - The image is quantified to obtain measures of fluorescence intensity for each pixel.
 - Pixel values are processed to obtain measures of mRNA abundance for each probe spotted on the array.
 - Usually two samples, dyed with different dyes, are hybridized to a single slide.
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- ### Using cDNA Microarrays to Measure mRNA Levels
- The dyes fluoresce at different wavelengths so it is possible to get separate images for each dye.
 - Cyanine 3 (Cy3) and Cyanine 5 (Cy5) are currently the two most commonly used dyes.
 - The Cy3 dye fluoresces green and the Cy5 fluoresces as red.
 - For viewing purposes, it is common to superimpose the two images using yellow to indicate a mixture of green and red.
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There are many ways to obtain a labeled target sample. Here's a simplified version of one method.

mRNA ...GGCUUAAUGAGCCUUA~~AAAAA~~...A ← poly-A tail
 cDNA target ...CCGAATTAC~~TCGGAA~~TTTTTT...T

↑
poly-T primer

viral enzyme *reverse transcriptase* recognizes poly-T bound to poly-A and begins to add complementary DNA nucleotides. The C nucleotides are dyed.

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Difficult to Make Meaningful Comparisons between Genes

- The measures of mRNA levels are affected by several factors that are partly or completely confounded with genes (e.g., cDNA source plate, cDNA well, print pin, slide position, length of mRNA sequence, base composition of mRNA sequence, specificity of probe sequence, etc.).
- Within-gene comparisons of multiple cell types or across multiple treatment conditions are much more meaningful.

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Using cDNA Microarrays to Measure mRNA Levels (Cartoon Version)

Microarray Slide

ACCTG...G ACCTG...G ACCTG...G	TTCTG...A TTCTG...A TTCTG...A
GGCTT...C GGCTT...C GGCTT...C	ATCTA...A ATCTA...A ATCTA...A
ACGGG...T ACGGG...T ACGGG...T	CGATA...G CGATA...G CGATA...G

Spots (Probes)

Sample 1

Sample 2

Unknown mRNA Sequences (Target)

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Extract mRNA

ACCTG...G ACCTG...G ACCTG...G	TTCTG...A TTCTG...A TTCTG...A
GGCTT...C GGCTT...C GGCTT...C	ATCTA...A ATCTA...A ATCTA...A
ACGGG...T ACGGG...T ACGGG...T	CGATA...G CGATA...G CGATA...G

Sample 1

Sample 2

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Convert to cDNA and Label with Fluorescent Dyes

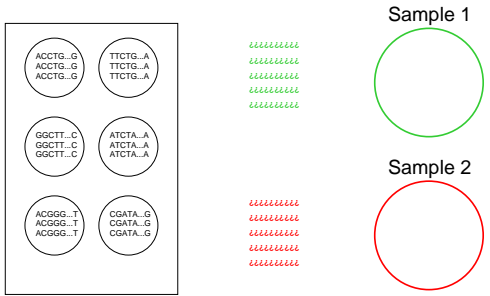
ACCTG...G ACCTG...G ACCTG...G	TTCTG...A TTCTG...A TTCTG...A
GGCTT...C GGCTT...C GGCTT...C	ATCTA...A ATCTA...A ATCTA...A
ACGGG...T ACGGG...T ACGGG...T	CGATA...G CGATA...G CGATA...G

Sample 1

Sample 2

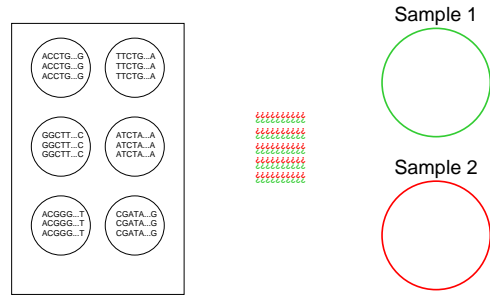
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Mix Labeled cDNA



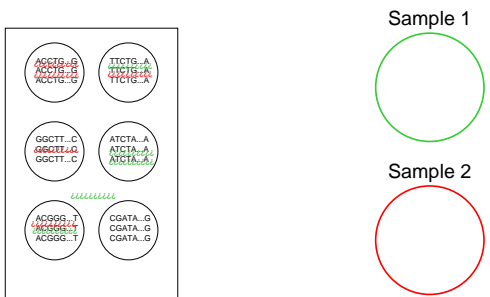
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Hybridize cDNA to the Slide



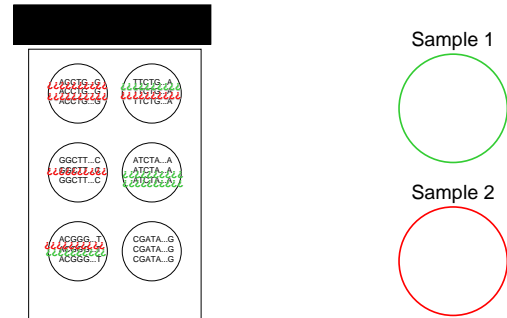
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Wash Off Any Unbound Dyed Molecules



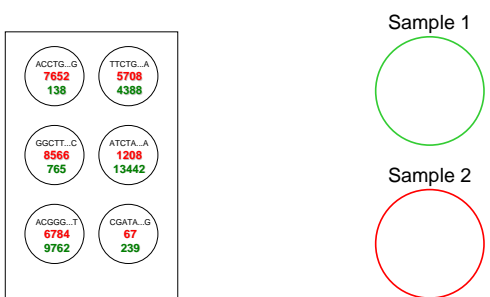
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Excite Dyes with Laser and Scan



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Quantify Signals



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