complementary DNA (cDNA)

- cDNA is a strand of DNA that is complementary to part of an mRNA sequence.
  - mRNA: \ldots CCUGAUAGAUGG \ldots
  - cDNA: \ldots GAACATCTCTAC \ldots
- 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.

**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.

**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.

**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.
**The PixSys 5500 Arraying Robot (Cartesian Technologies)**

- Robotic arm
- Vacuum wash station

*The print head holds up to 32 pins in a 8x4 format*

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**Cartoon of Printing Process (side view from the table top)**

- Plate with wells containing probes
- Microarray slides
- Vacuum wash station

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**Spotting the Probes on the Microarray 8 X 4 Print Head**

- Plate with wells holding probes in solution
- Microarray slide

*All spots of the same color are made at the same time.*
*All spots in the same sector are made by the same pin.*

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**Using cDNA Microarrays to Measure mRNA Levels**

- RNA is extracted from a target sample of interest.
- mRNA are reverse transcribed into cDNA.
- The resulting cDNA are labeled with a fluorescent dye.
- The dyeed cDNA are placed on a microarray slide.
- Dyed cDNA sequences hybridize to complementary probes spotted on the array.
- 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.

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**Using cDNA Microarrays to Measure mRNA Levels (ctd.)**

- Usually two samples, dyed with different dyes, are hybridized to a single slide.
- 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.
- Images from the scanner are black and white, but it is typical to display Cy3 images as green and Cy5 images are displayed as red.
- It is common to superimpose the two images using yellow to indicate a mixture of green and red.
There are many ways to obtain a labeled target sample. Here’s a simplified version of one method.

\[
\text{mRNA} \quad \ldots \text{GGCUUAUGAGCCUUAAAAAA}\ldots \text{poly-A tail}
\]

\[
\text{cDNA target} \quad \ldots \text{CGGAATTCTGGA}\ldots \text{poly-T primer}
\]

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

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.
Hybridize cDNA to the Slide

Excite Dyes with Laser

Scan

Quantify Signals