BE 183 Applied Genomics Technologies

Expression Technologies

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Postgenomics: The transcriptome and proteome

- The *transcriptome* is the full complement of RNA molecules produced by a genome
- The *proteome* is the full complement of proteins enabled by the transcriptome

- DNA → RNA → protein
- Genome → transcriptome → proteome
- 30,000 genes → ??? RNAs → ??? proteins?

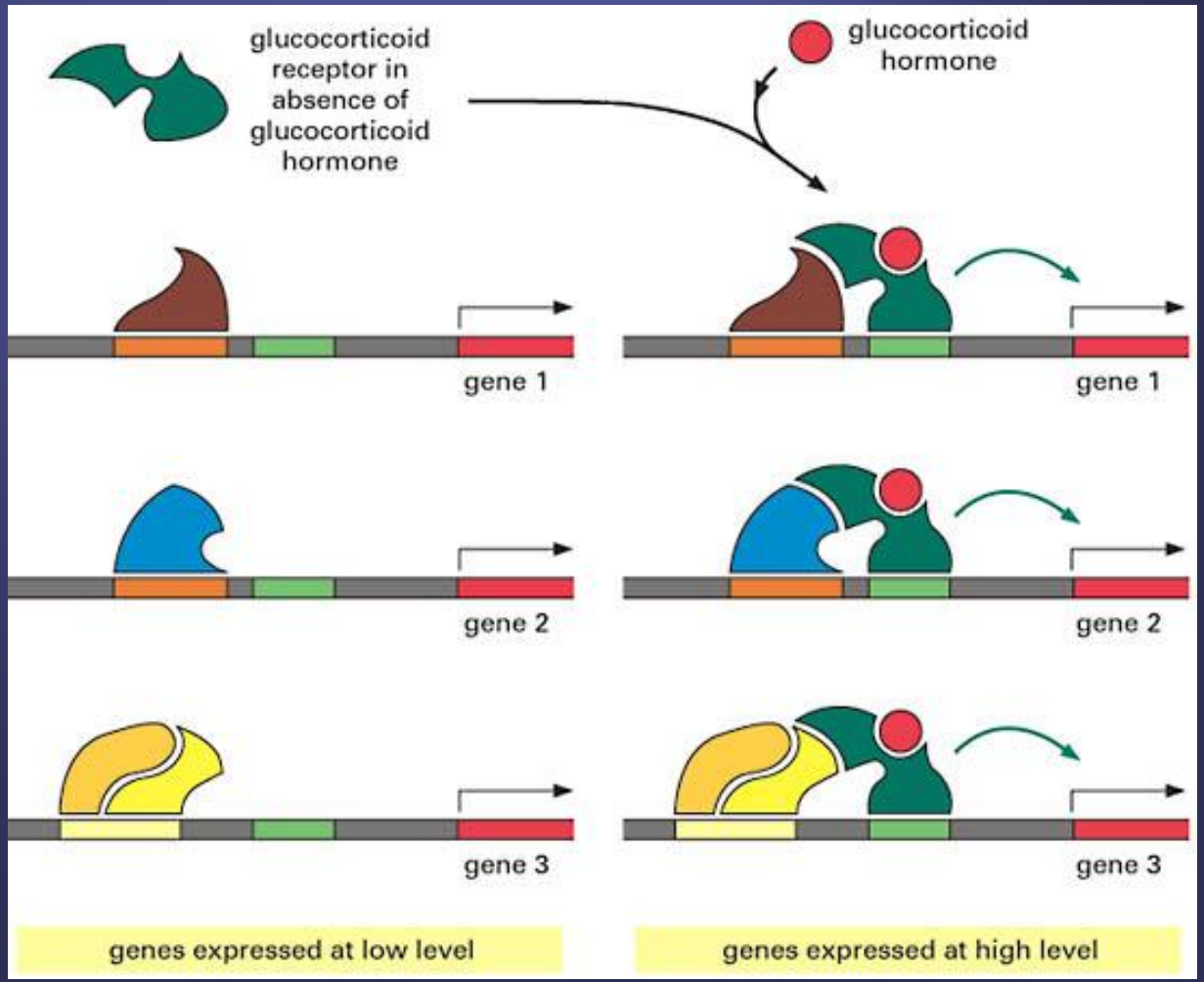
- For example, the drosophila gene *Dscam* can generate 40,000 distinct transcripts through alternative splicing.
- What is the minimum number of exons that would be required?

Expression technologies try to get at the problem of when and where transcription and translation occurs

"*Housekeeping*" genes are always on to enable elementary fns.

"*Luxury*" genes are expressed in a regulated manner

A typical human cell probably expresses ~15,000 genes. Some are *abundant*, but most are *rare*



Classical approaches to expression analysis

RNA

- Northern blot, RNA dot blot, and reverse northern blot
- RNAse protection assay
- Reverse transcriptase polymerase chain reaction (RT-PCR)

Proteins

We'll talk about proteins later…

- Western blot
- Enzyme-linked immunosorbent sandwich assay (ELISA)

Either

- *In situ* hybridization/immunohistochemistry

DNA Technologies: DNA Hybridization Scheme

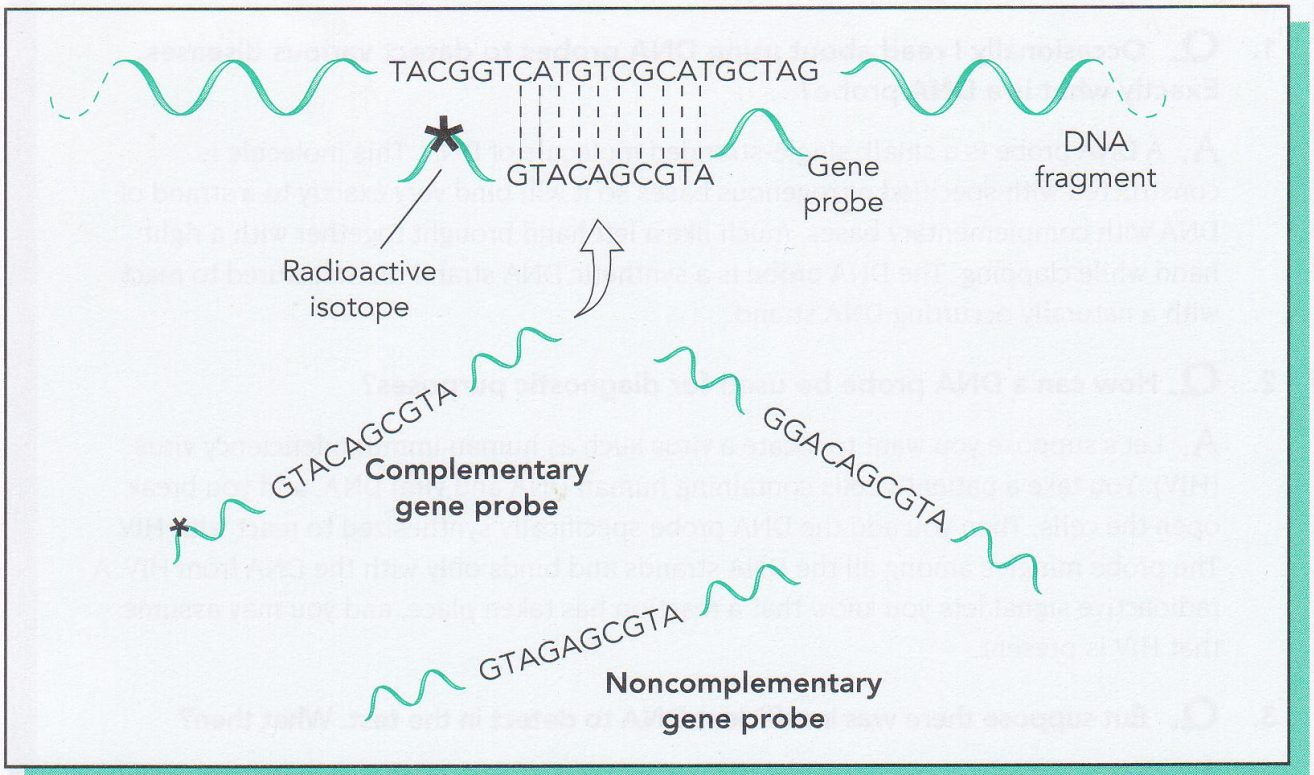
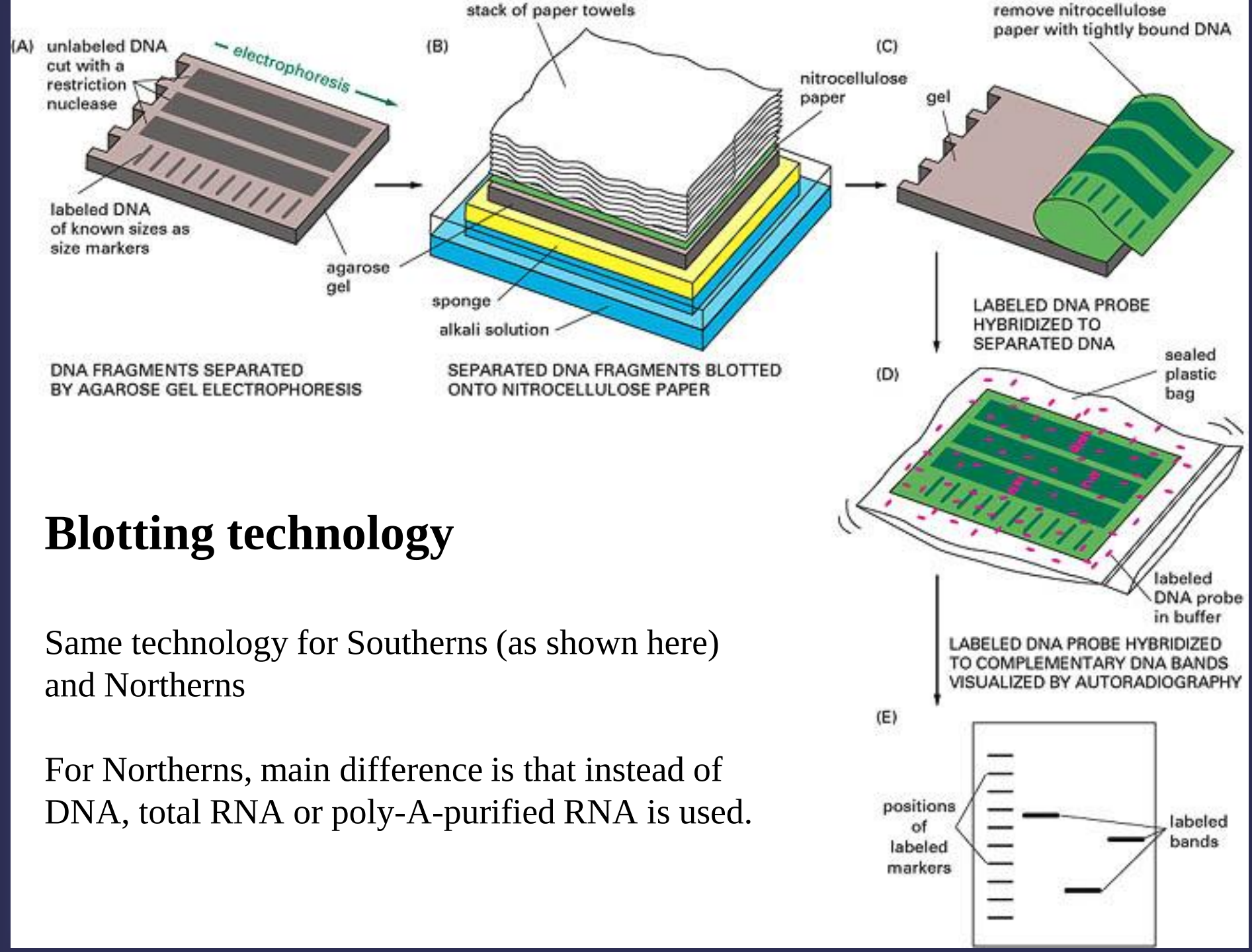


FIGURE 7.1

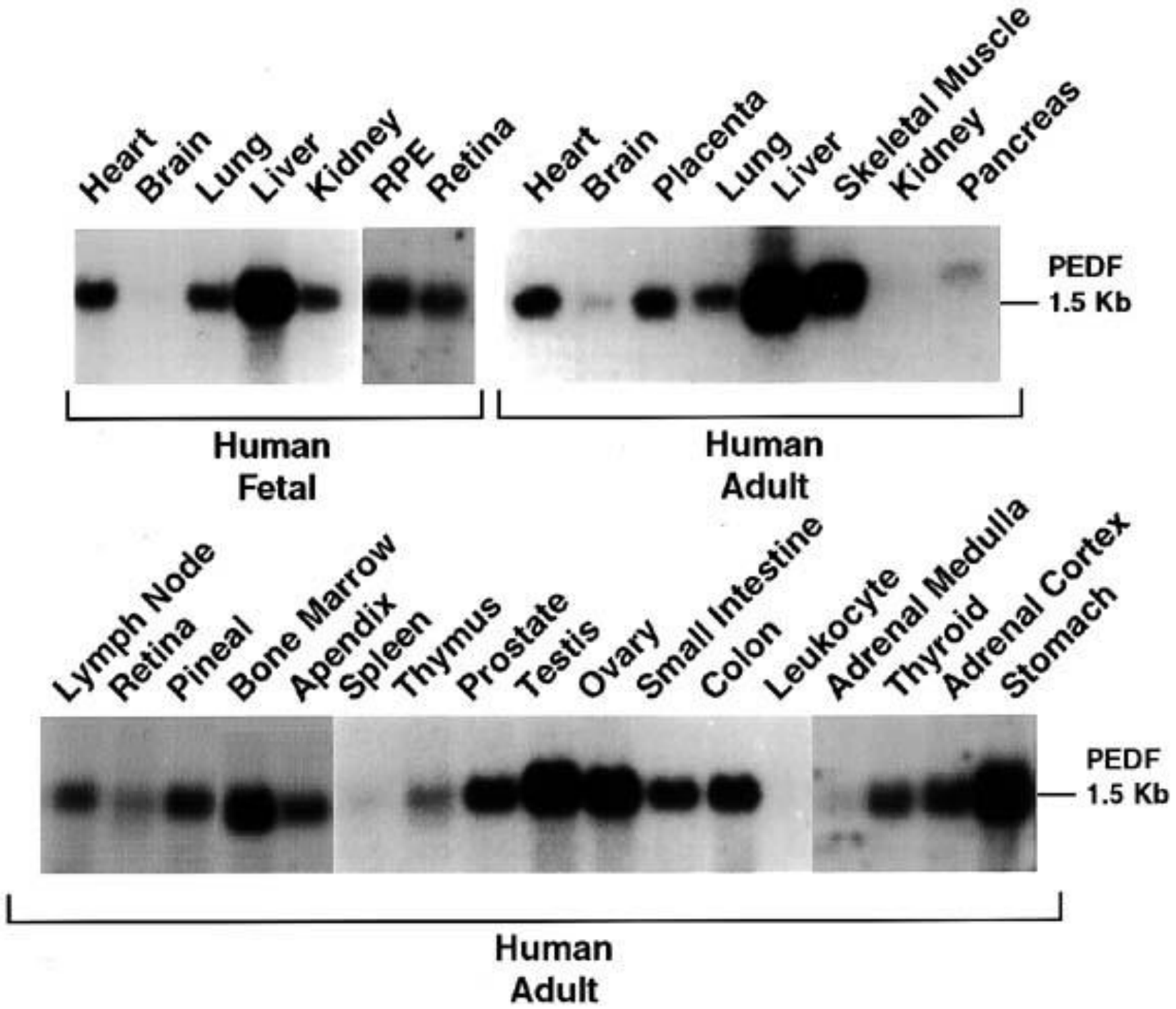
How a gene probe works. A gene probe is a single-stranded segment of DNA. When combined with a DNA molecule containing a complementary site, the gene probe seeks out the site and binds with it. If a radioactive molecule or atom is attached to the probe, the radioactivity accumulates at the binding site and signals that a reaction has taken place. Note in the diagram how the bases of only one probe complement the bases of the DNA fragment.

Blotting technology

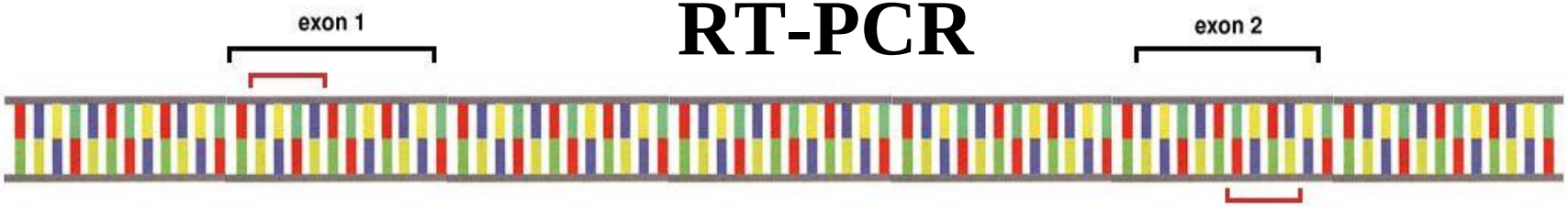


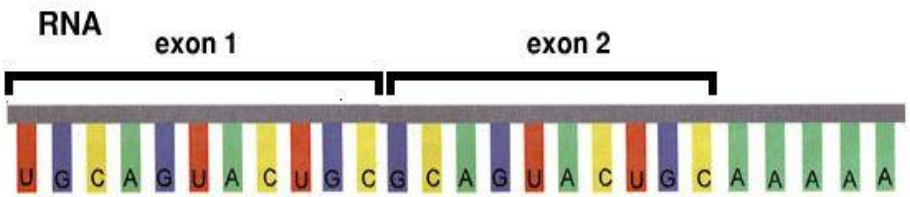
Same technology for Southerns (as shown here) and Northerns

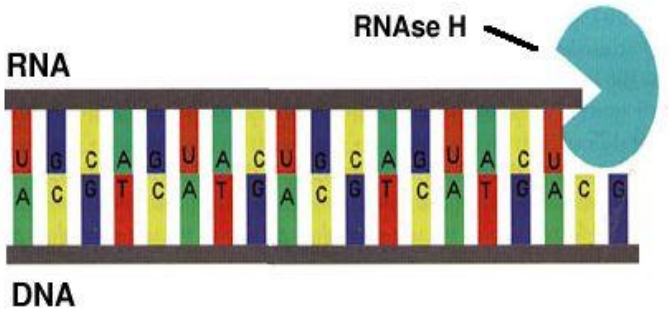
For Northerns, main difference is that instead of DNA, total RNA or poly-A-purified RNA is used.

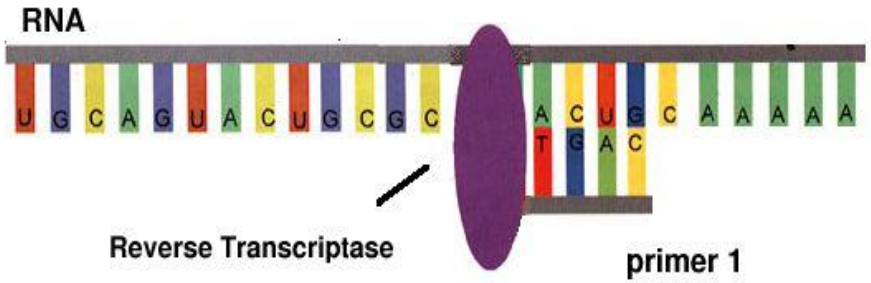

Heart
Brain
Lung
Liver
Kidney
RPE
Retina
Heart
Brain
Placenta
Lung
Liver
Skeletal Muscle
Kidney
Pancreas
PEDF
1.5 Kb
Human
Fetal
Human
Adult
Lymph Node
Retina
Pineal
Bone Marrow
Apendix
Spleen
Thymus
Prostate
Testis
Ovary
Small Intestine
Colon
Leukocyte
Adrenal Medulla
Thyroid
Adrenal Cortex
Stomach
PEDF
1.5 Kb
Human
Adult

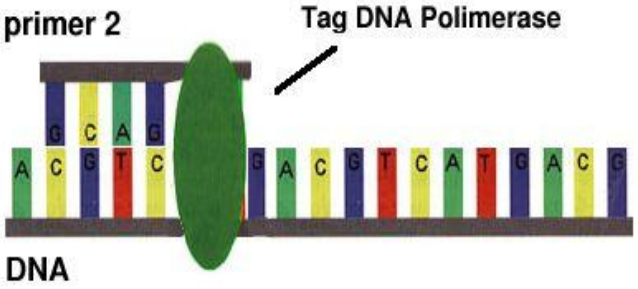
RT-PCR

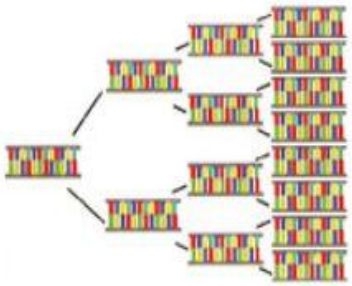






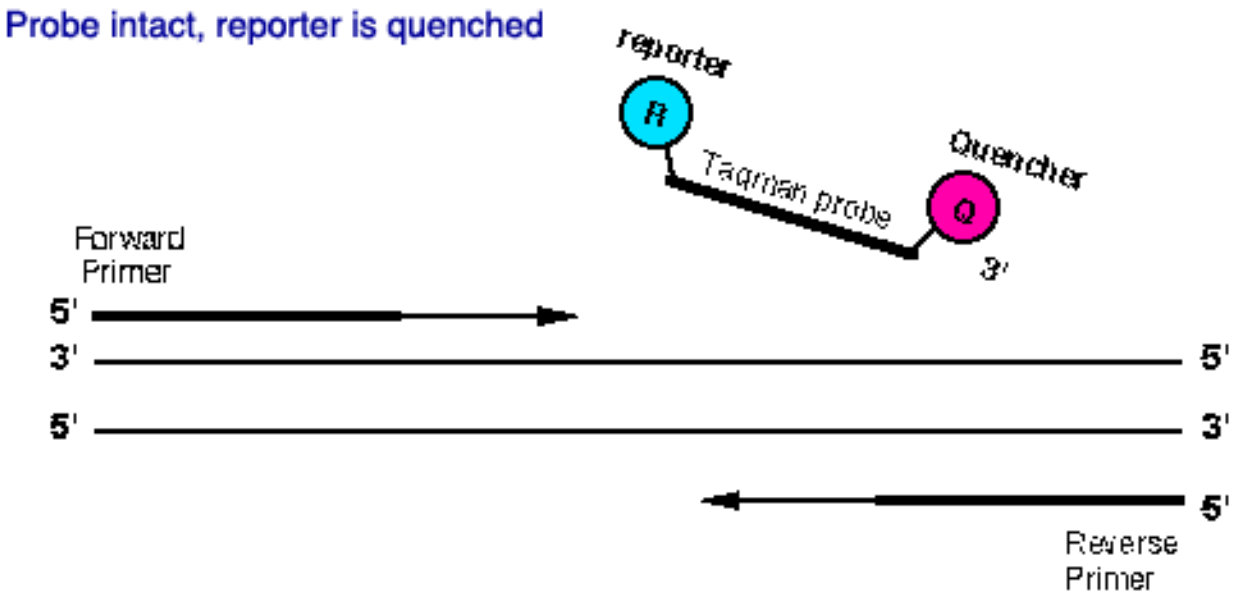






- Also see http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html

Quantitative RT-PCR (qPCR)

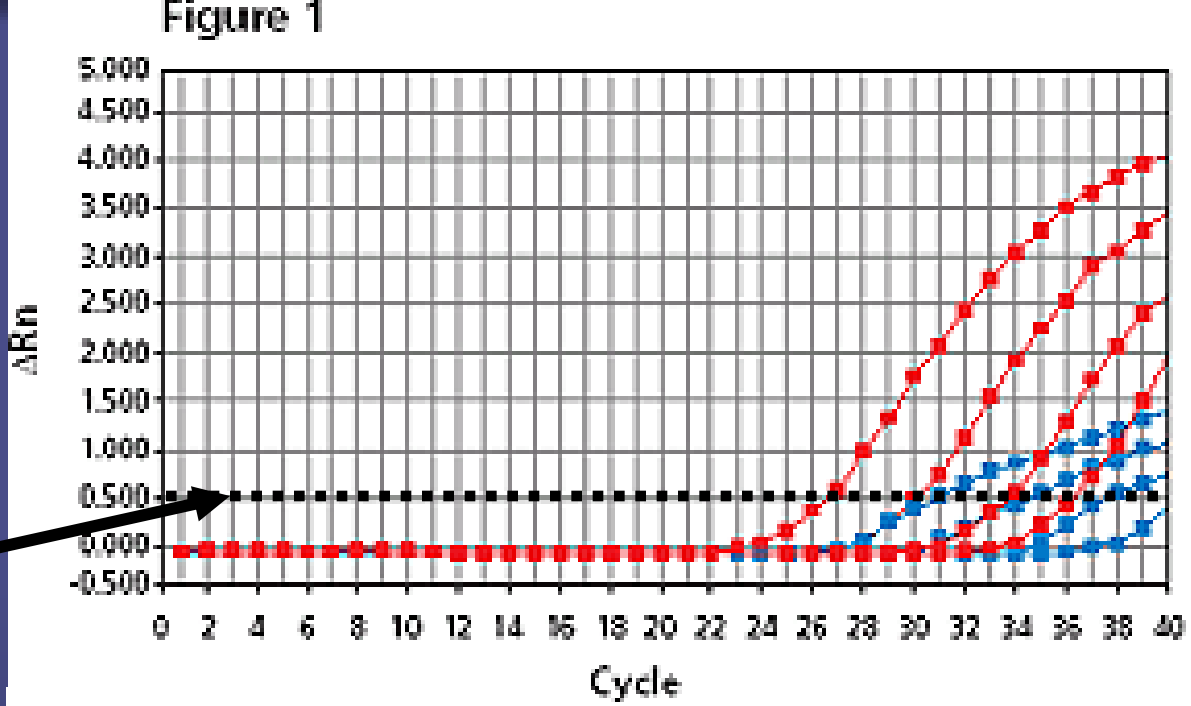


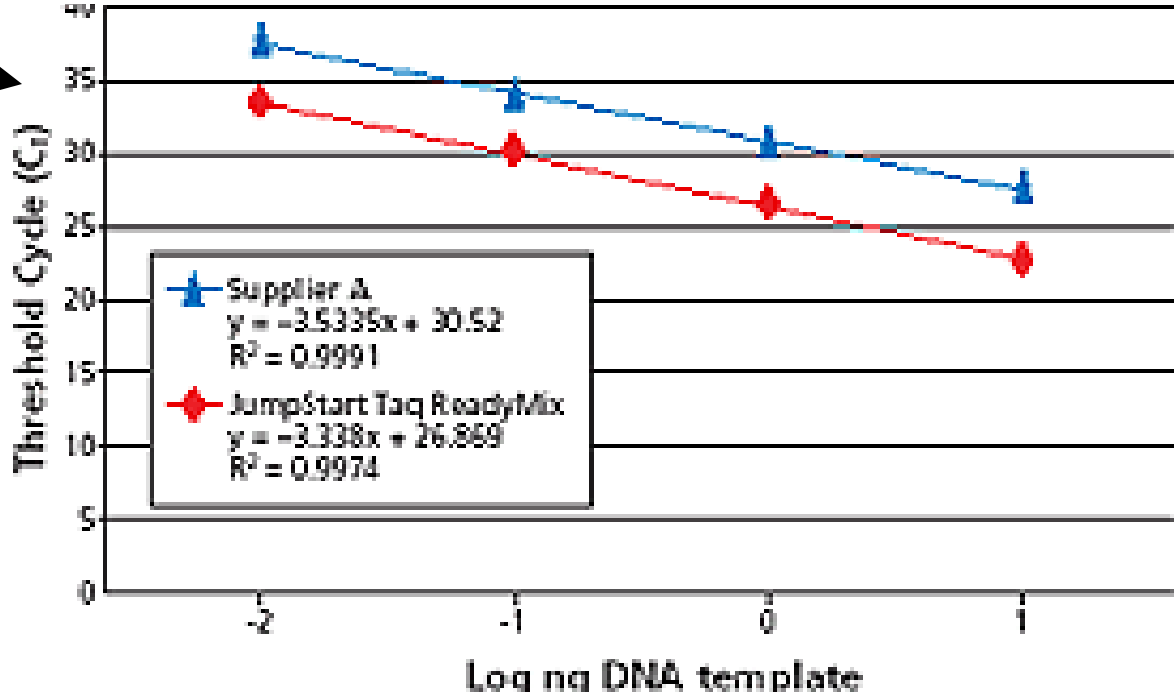
- **Releases fluor. dye as strand is copied**
- **Records fluorescence at every amplification step using laser and CCD camera**

Quantitative RT-PCR

The threshold cycle is the PCR cycle at which a significant increase in reporter fluorescence above baseline can be detected.

This is later used for quantitation versus a calibration curve





High-throughput approaches

RNA

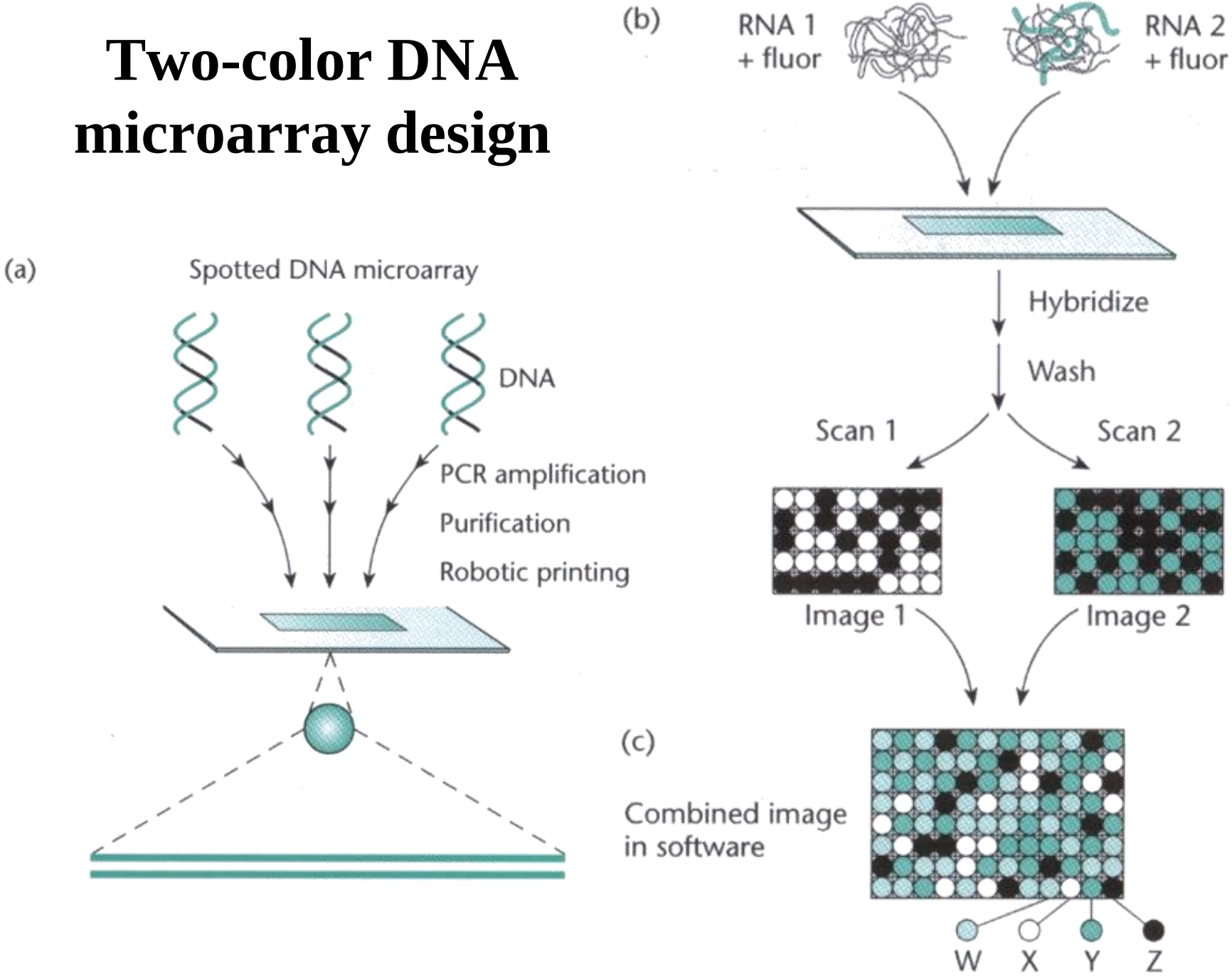
- DNA Microarrays
- cDNA / EST sequencing
- Differential display
- SAGE
- Massively parallel signature sequencing (MPSS)

We'll talk about proteins later…

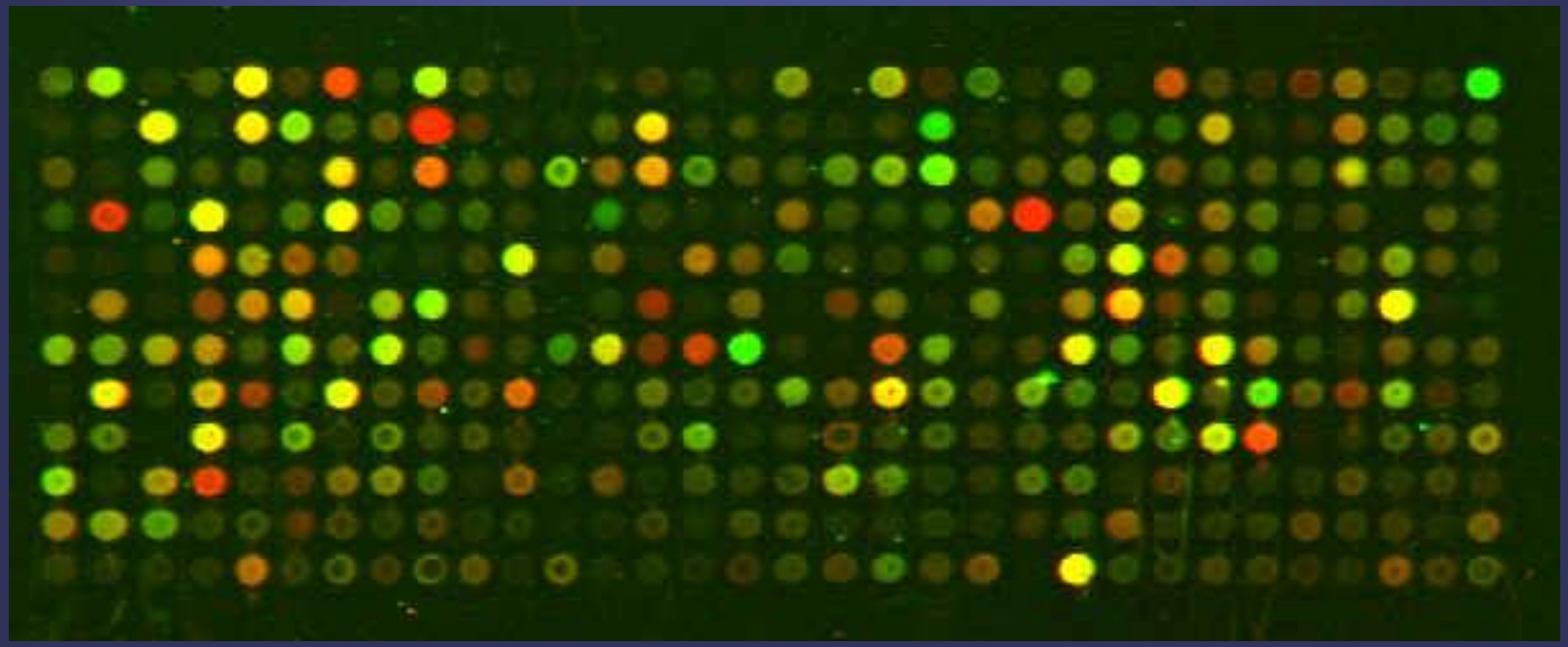
Proteins

- 2D PAGE
- Mass spectrometry

Two-color DNA microarray design



cDNA-chip of brain glioblastoma tumor



Types of microarrays

- Spotted
  - Robotic transfer of clones or PCR products
  - Spotting on nylon membranes or glass slides coated with poly-lysine
- Synthetic
  - Direct oligo synthesis on solid microarray substrate
  - Uses photolithography (Affymetrix) or ink-jet printing (Agilent)
- All configurations assume the DNA on the array is in excess of the hybridized sample—thus the kinetics are linear and the spot intensity reflects that amount of hybridized sample.
- Labeling can be radioactive, fluorescent (one-color), or two-color

Microarrays (continued)

Imaging

- Radioactive ^{32}P labeling: Autoradiography or phosphorimager
- Fluorescent labeling: Confocal microscope (invented by Marvin Minsky!!)

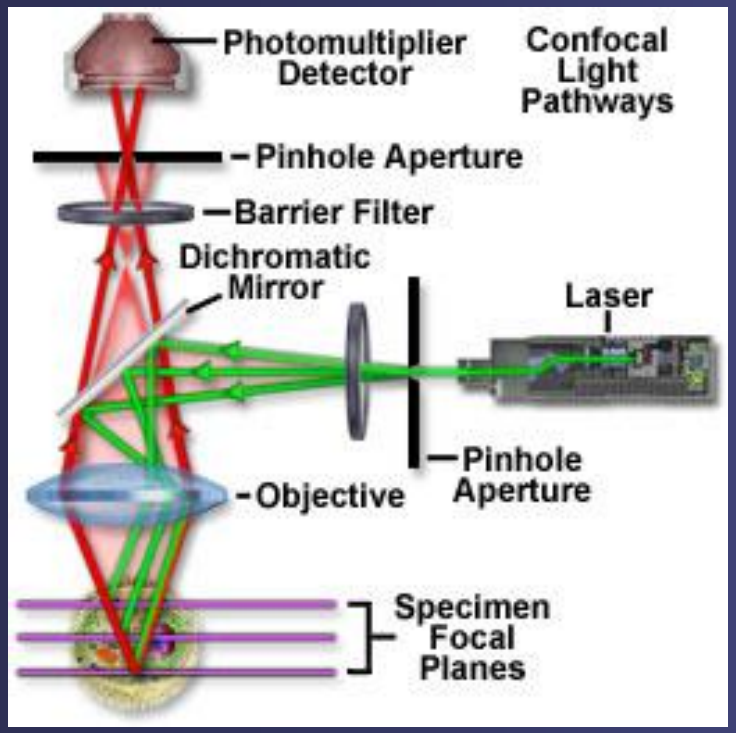
Feature density

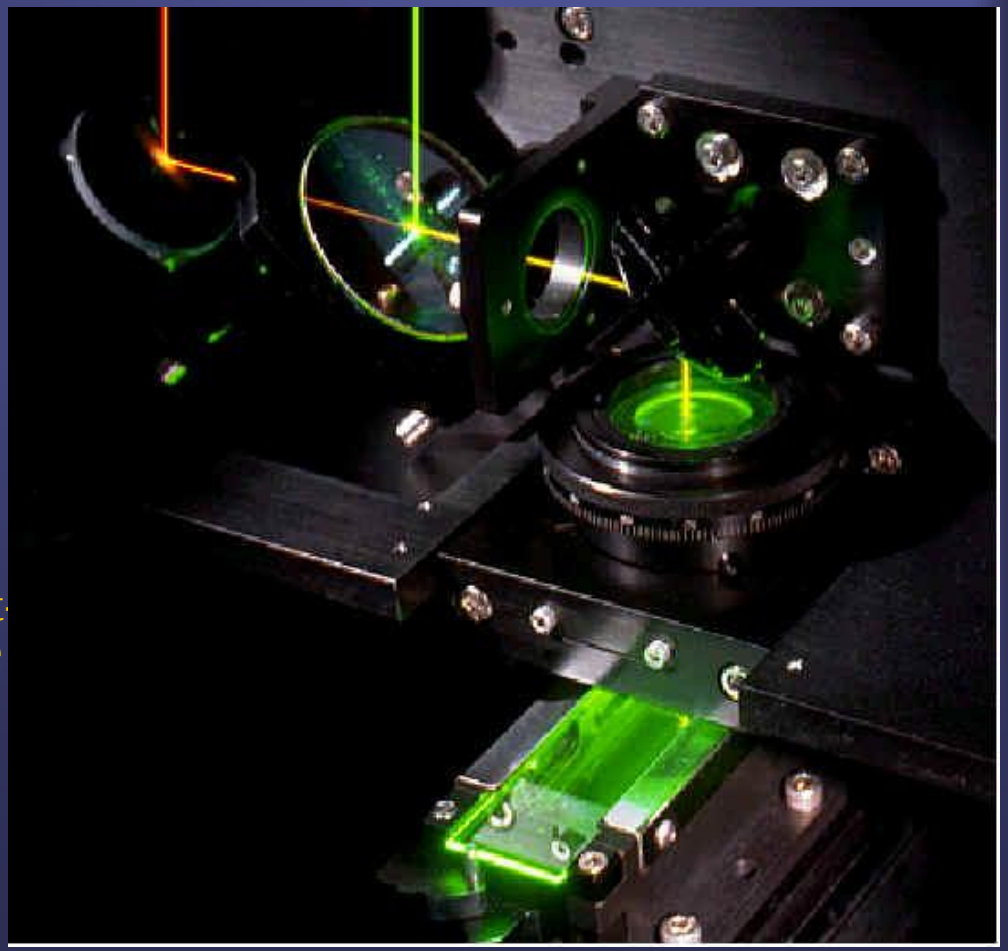
- Nylon membrane macroarrays → 100-1000 features
- Glass slide spotted array → 5,000 features / cm^2
- Synthesized arrays → 50,000 features / cm^2

Microarray robotics

Uses principle of capillary contact printing or else “ring and pin” non-contact design

Microarray confocal scanner



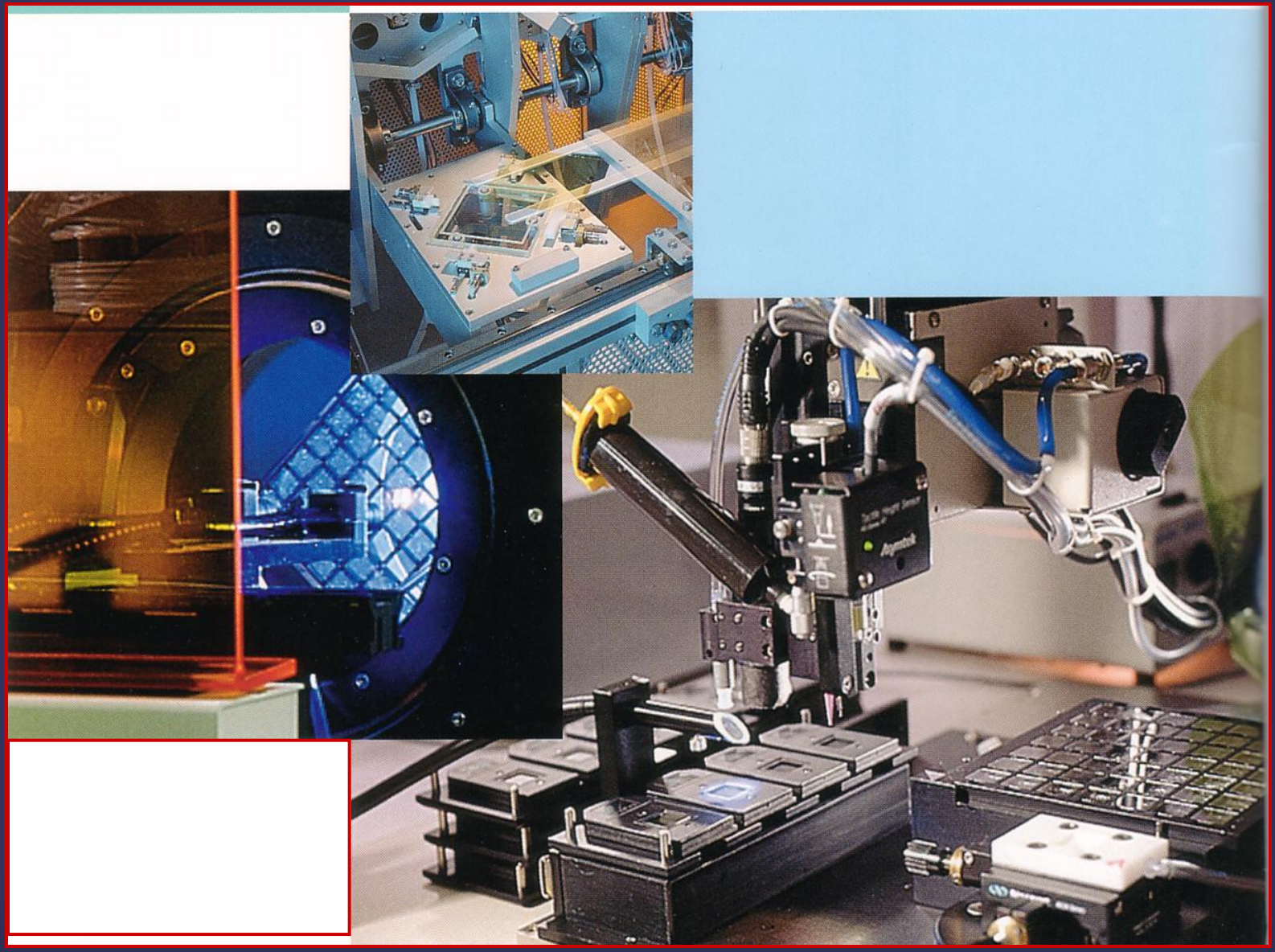


- Collects sharply defined optical sections from which 3D renderings can be created
- The key is spatial filtering to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus.
- Two lasers for excitation
- Two color scan in less than 10 minutes
- High resolution, 10 micron pixel size

Synthesized oligo arrays – Affymetrix High Density DNA Arrays

Affymetrix Gene Chip System

New DNA Technologies – Affymetrix Photolithographic Equipment and Wafers



New DNA Technologies – Affymetrix High Density Arrays

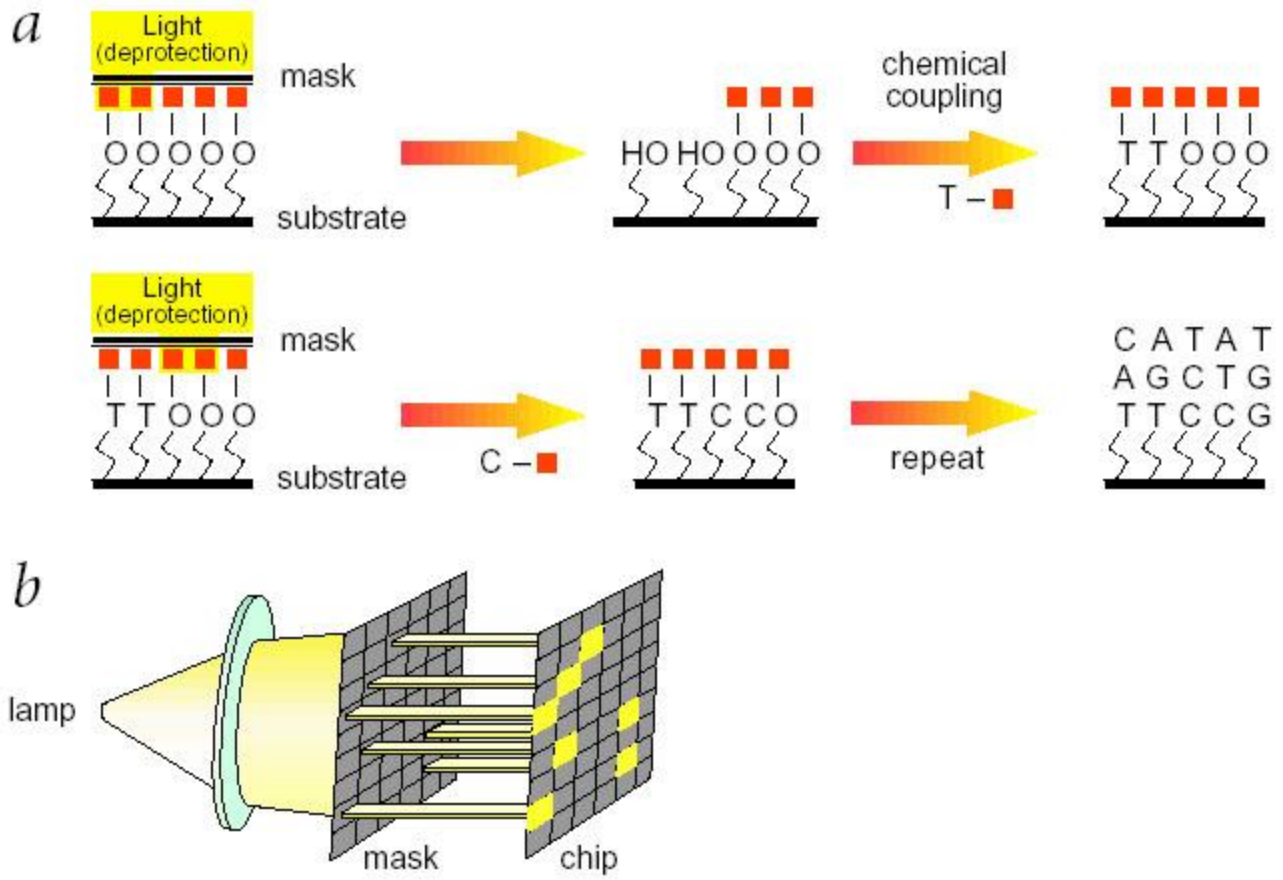


Fig. 1 ***a***, Light directed oligonucleotide synthesis. A solid support is derivatized with a covalent linker molecule terminated with a photolabile protecting group. Light is directed through a mask to deprotect and activate selected sites, and protected nucleotides couple to the activated sites. The process is repeated, activating different sets of sites and coupling different bases allowing arbitrary DNA probes to be constructed at each site. ***b***, Schematic representation of the lamp, mask and array.