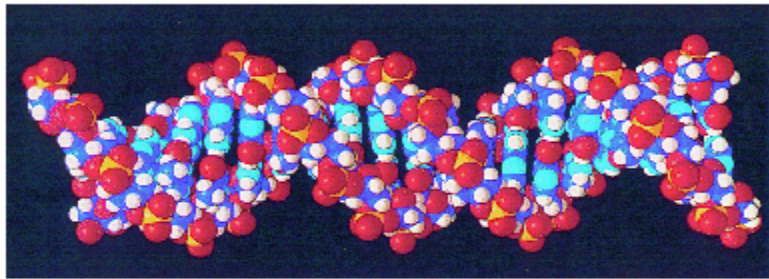


# *Molecular searching techniques*

# *Check out this link!*

<http://web.mit.edu/esgbio/www/rdna/rdna.html>

## **Southerns, Northern, Westerns, & Cloning: "Molecular Searching" Techniques**



*Written by Brian White, MIT. Copyright 1995.*

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These are techniques for analyzing cellular macromolecules: DNA, RNA, and protein. These sections will describe analytical tools.

For Further Reading:

Gel electrophoresis and Southern blotting are described in Purves, Oriens, and Heller pp. 315-8. DNA-RNA h

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## **Theory: Complementarity and Hybridization**

Molecular searches use one of several forms of complementarity to identify the macromolecules of interest among a mixture. Complementarity is the sequence-specific or shape-specific molecular recognition that occurs when two molecules bind. DNA double-helices bind because they have complementary sequences; also, an antibody binds to a region of a protein's shape.

# *Summary of blotting techniques*

1. Westerns

2. Northern

3. Southern

# Complementarity

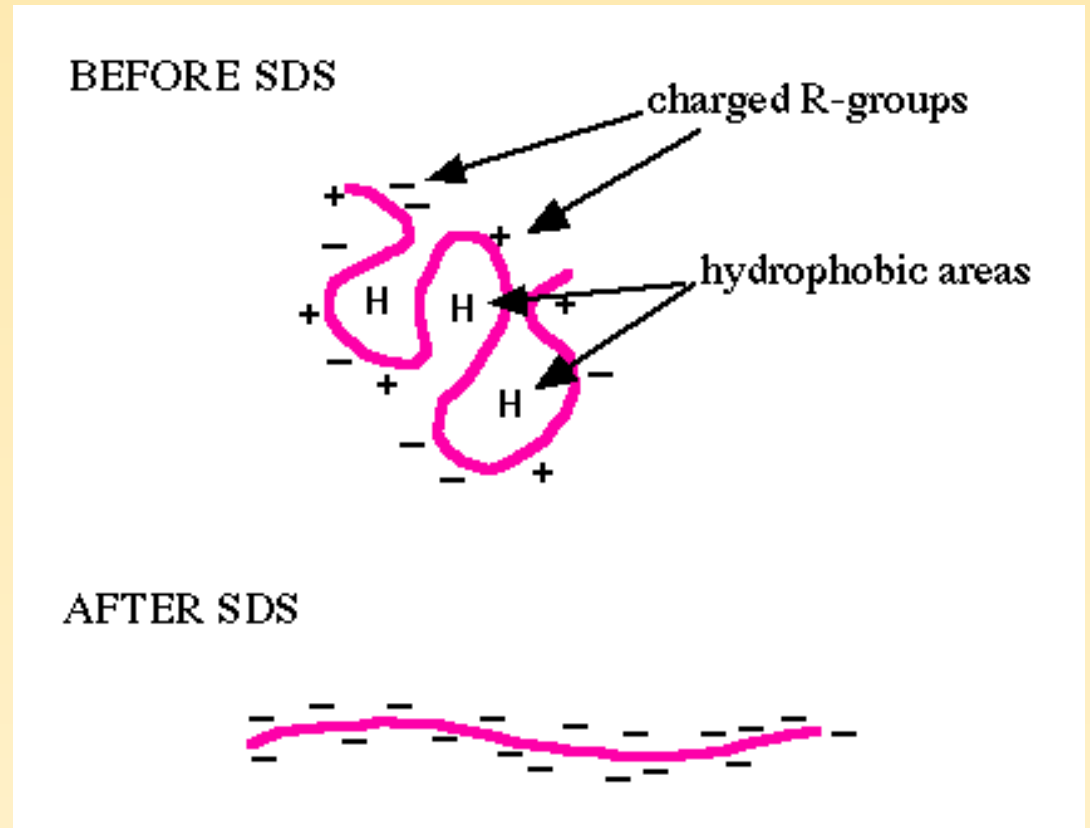
- The sequence-specific or shape-specific molecular recognition that occurs when two molecules bind together.
- Probe-target complex
- Label the probe and detect the target

# *Western Blotting*

- Detects the presence of (and gives us the size of) a specific protein in a crude protein extract.
- Can be used to determine if a gene is expressed (transcribed and translated) or not.

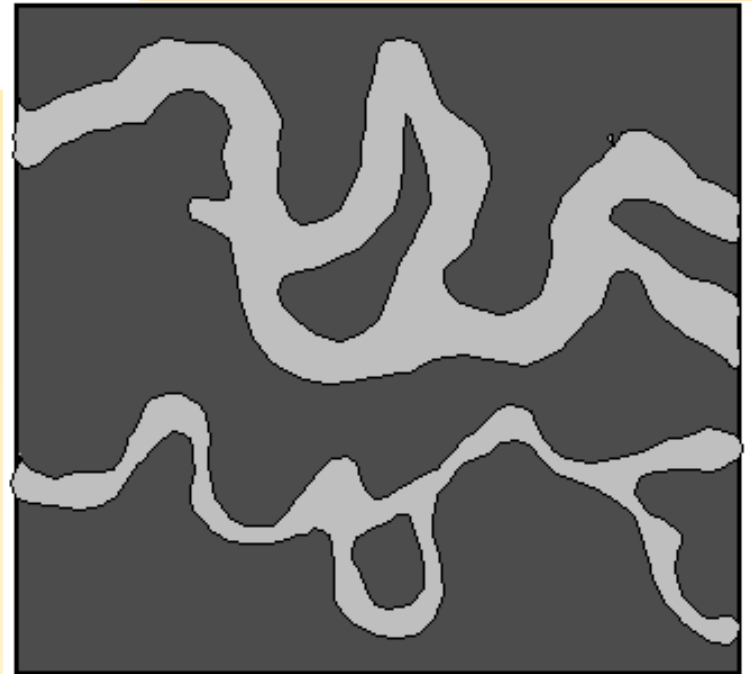
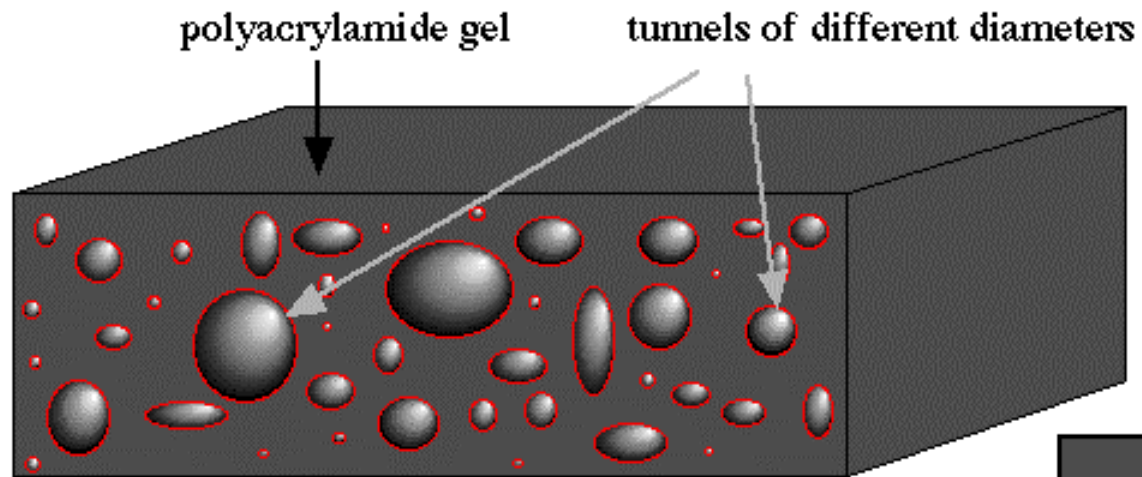
# Separating proteins by size

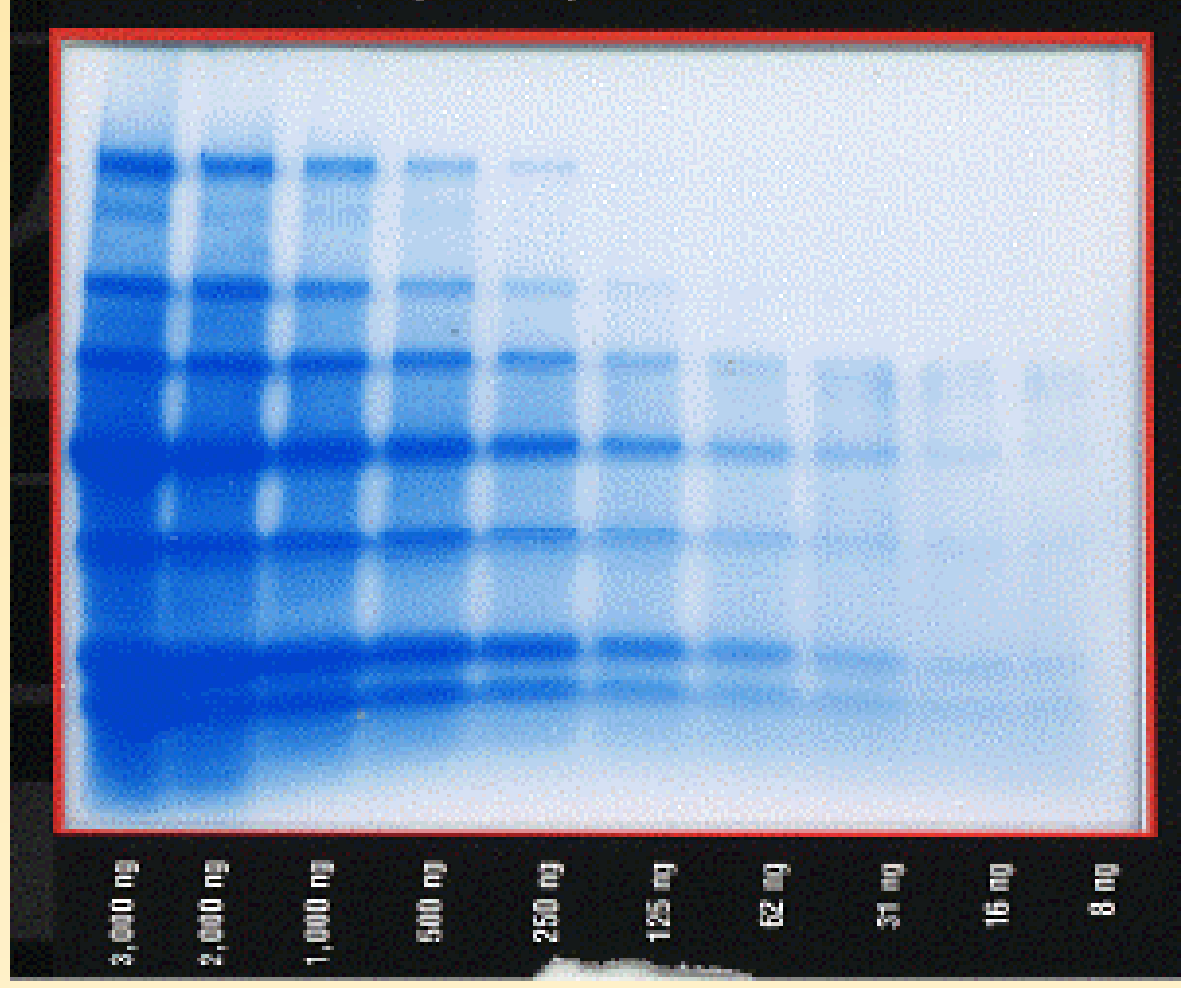
- SDS-PAGE



- Based on size of primary structure

# Acrylamide gel



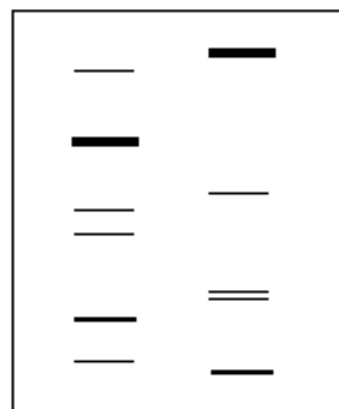




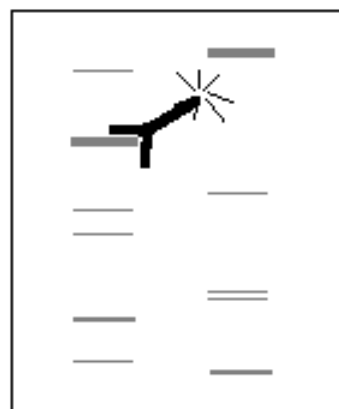
Protein Blot on  
Nitrocellulose



SDS Polyacrylamide  
Gel Electrophoresis



Label with Specific  
Antibody



Detect Antibody



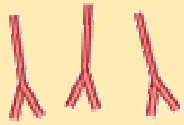
Reveals Protein  
of Interest

# Western Blots based on protein-protein interaction

- ELISA
- Enzyme Linked Immunosorbant Assay



Antigen (e.g HIV)



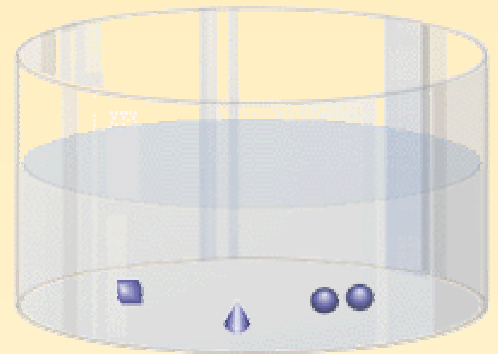
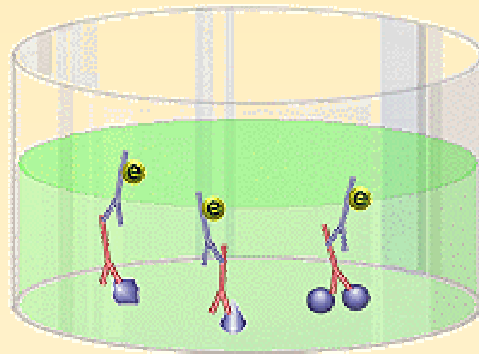
Primary antibody



Secondary antibody



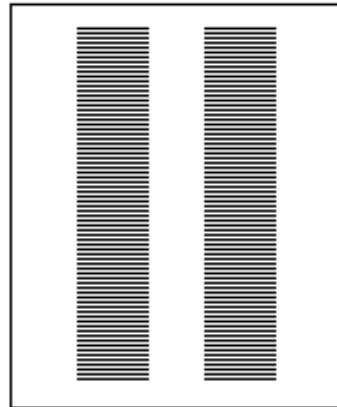
Chromagen



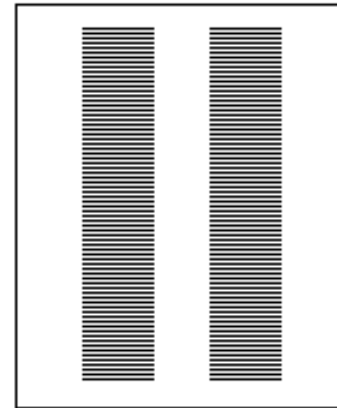
# *Northern Blotting*

- Detects the presence (and gives us the size of) a specific mRNA in a total RNA extract.
- Can determine if a gene is transcribed or not and determine where and when it is transcribed.

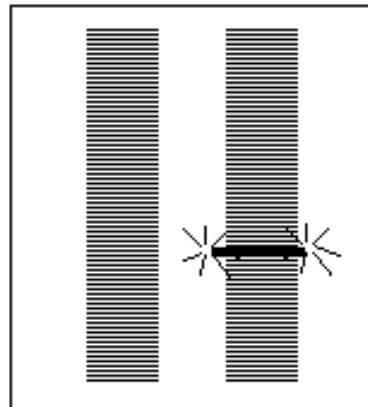
RNA Blot on  
Nitrocellulose



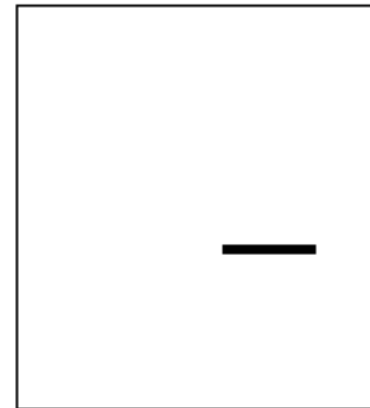
RNA Separation by  
Gel Electrophoresis



Label with Specific  
Nucleic Acid Probe



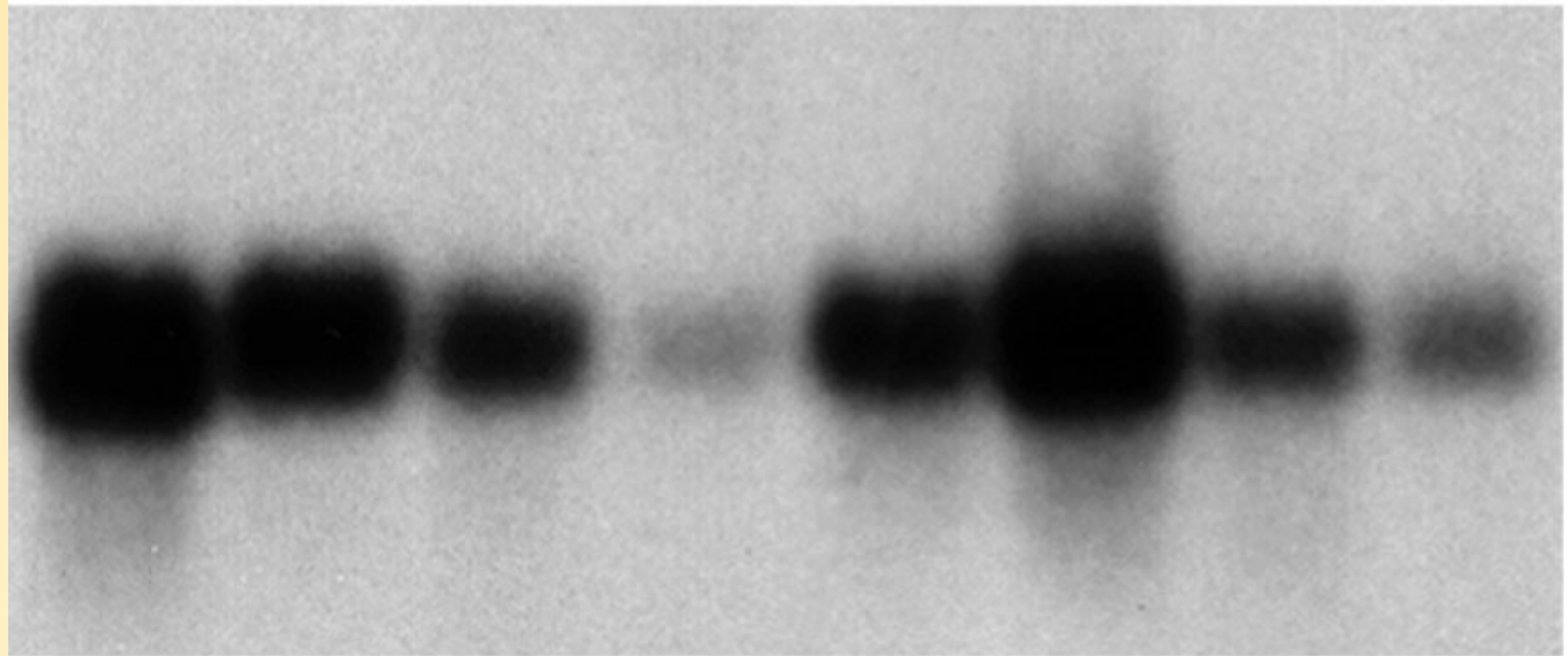
Detect Probe  
(on X-ray film)



Reveals RNA of Interest

# Figure 5.16

Heart      Brain      Spleen      Lung      Liver      Skeletal  
muscle      Kidney      Testis

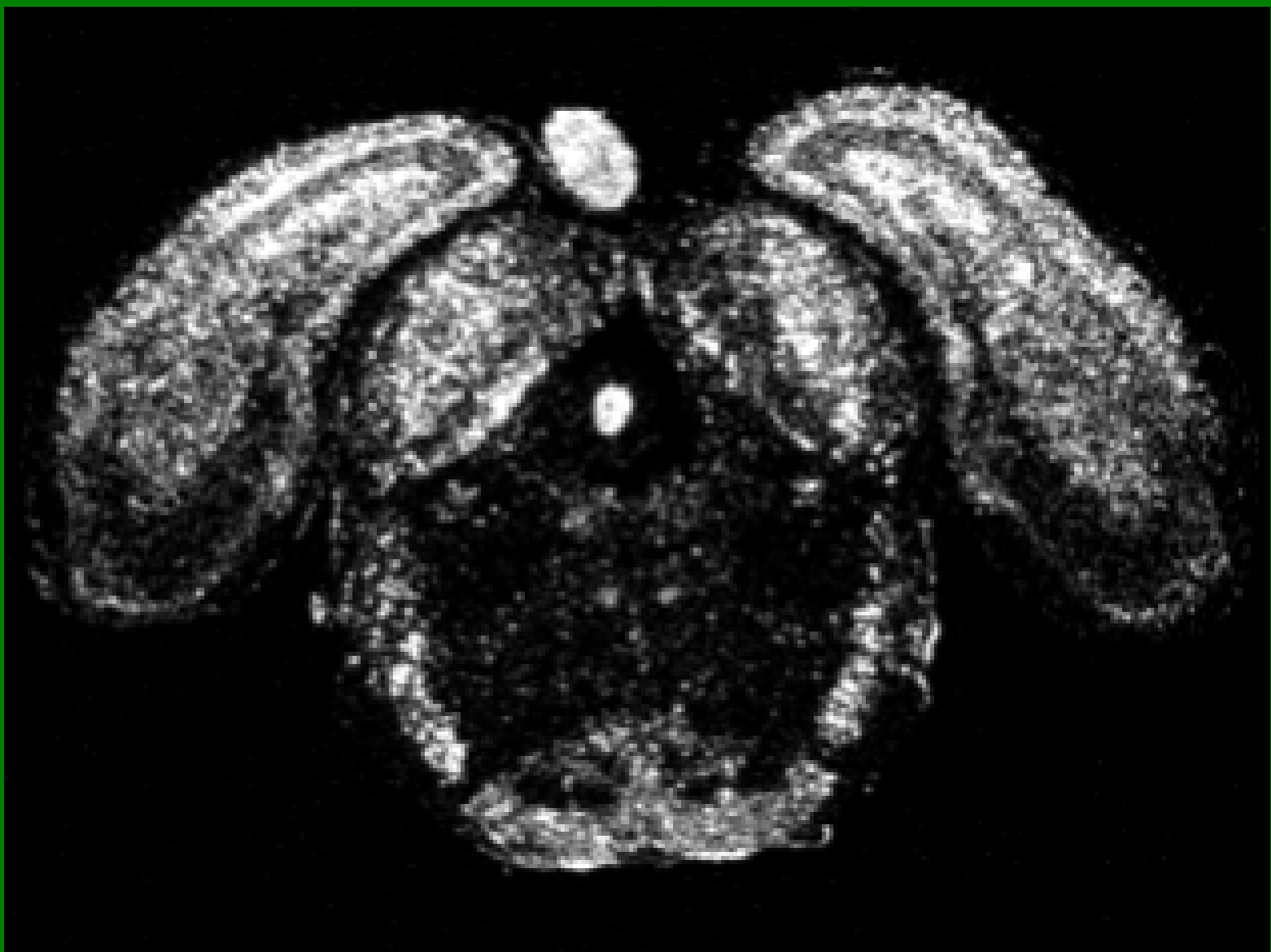


1      2      3      4      5      6      7      8

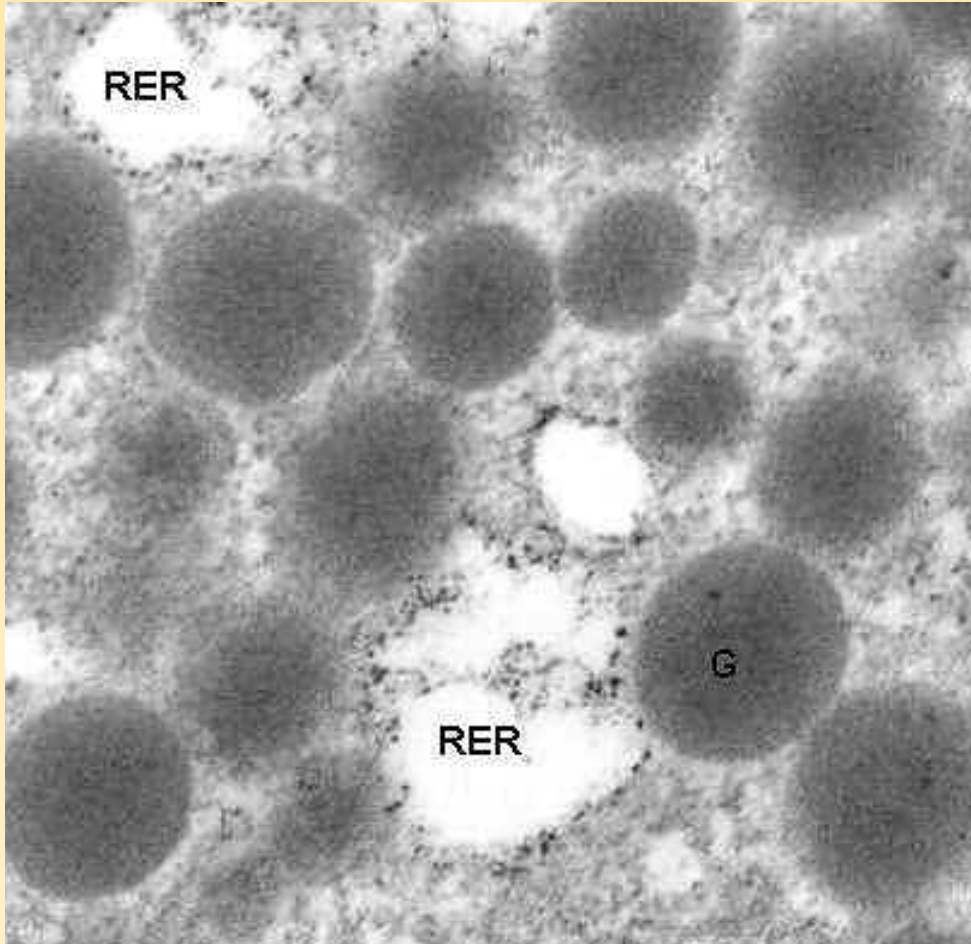
Courtesy Contech.

# *In situ hybridization*

- Detects the presence of a particular mRNA in a tissue sample and allows us to determine which cells the mRNA is expressed in.





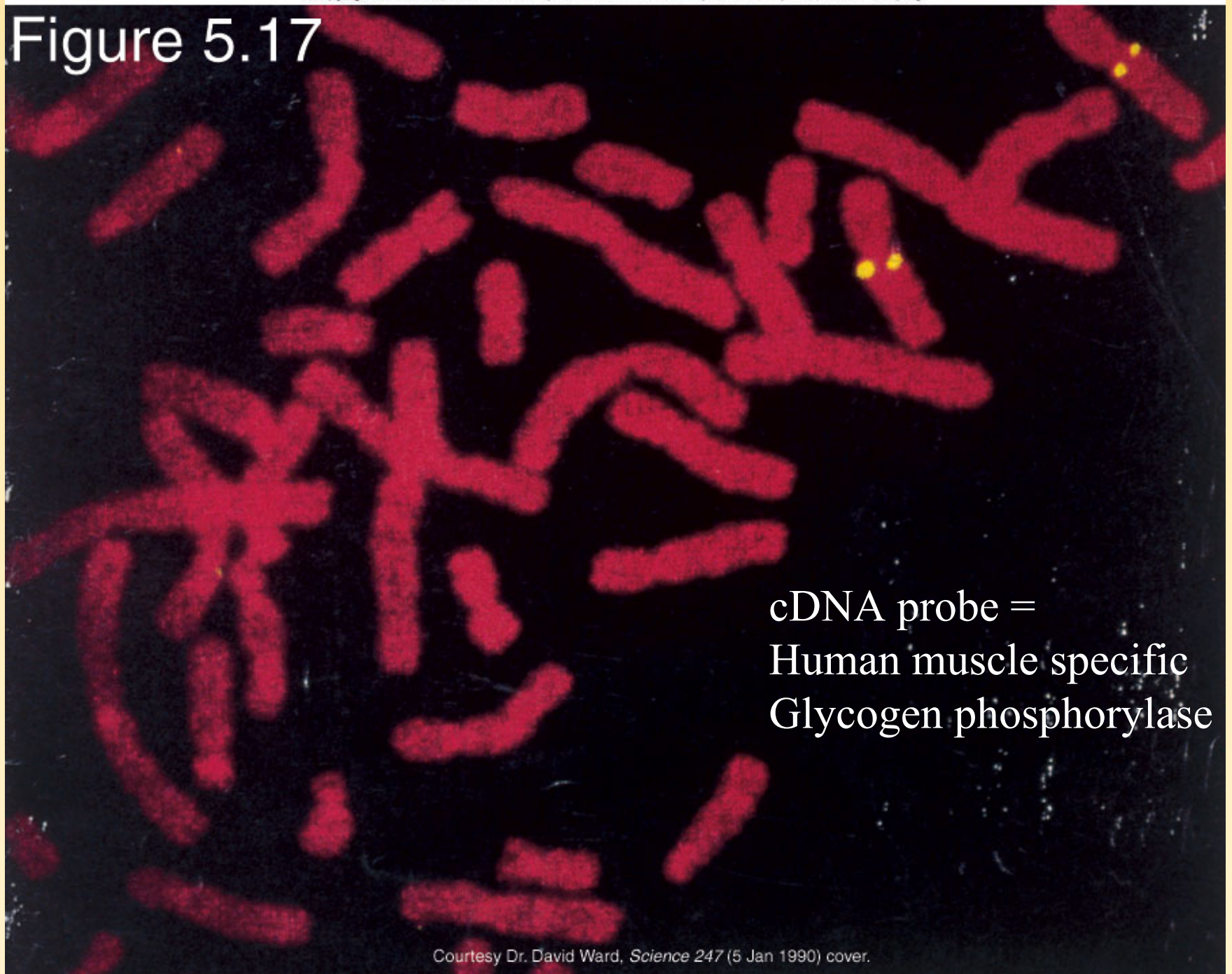


*Electron micrograph showing labeling for Luteinizing hormone beta subunit mRNA on dilated rough endoplasmic reticulum (RER) in a pituitary gonadotrope.*

# *Fluorescence in situ hybridization*

- Determine the location of a gene in a chromosome i.e. map a gene to a chromosome.
- In this case it is called FISH.

# Figure 5.17



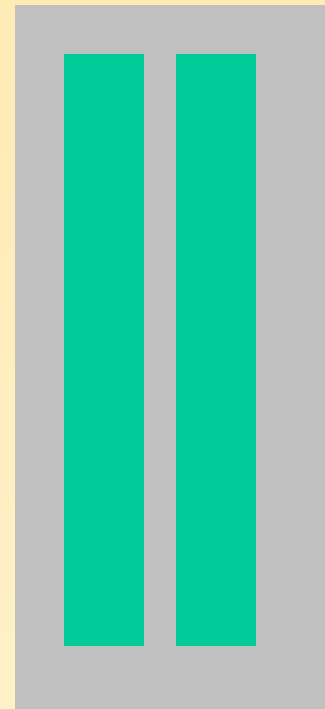
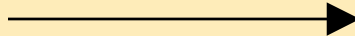
cDNA probe =  
Human muscle specific  
Glycogen phosphorylase

# *Southern Blotting*

- Detects the presence of a particular DNA sequence
- Can be used to determine (approximately) the copy number of a gene.

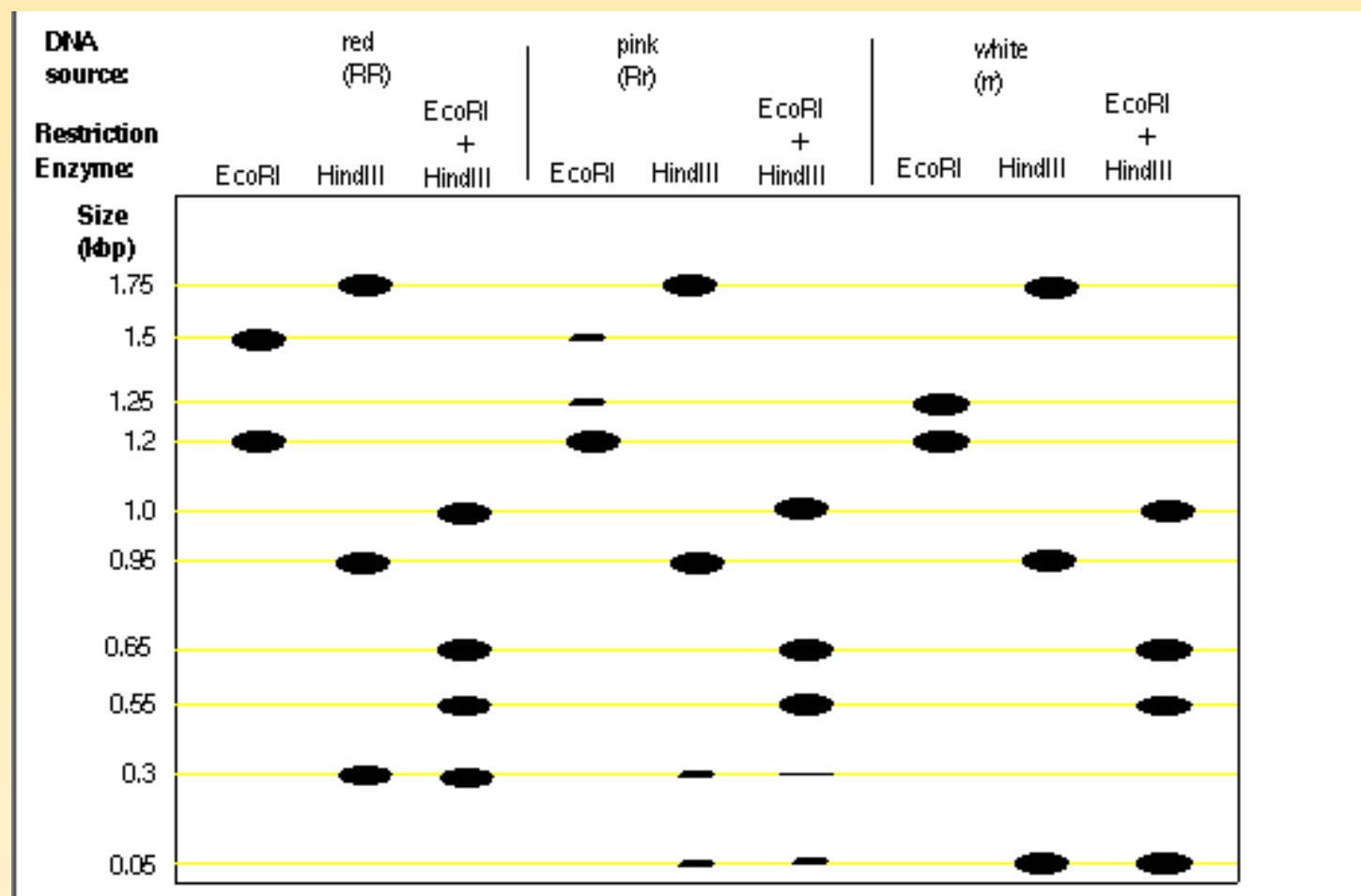
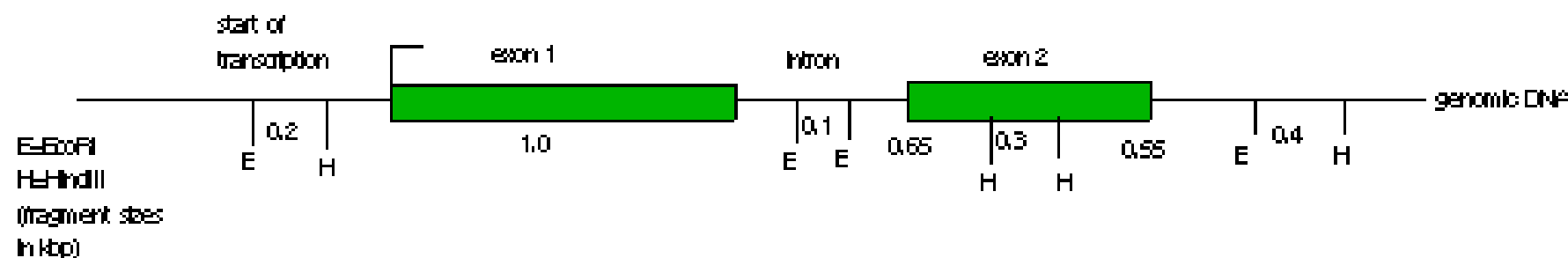
# To do a Southern blot

- Cut DNA
- Run on a gel
  - will be a smear
- Transfer to a filter
  - “blotting”
- Hybridize with a probe



# *Southern Blotting problems*

<http://web.mit.edu/esgbio/www/rdna/solvingblots.html>



# *Applications of Southern Blotting*

- DNA fingerprinting
- DNA typing
- Molecular markers



# ***DNA Fingerprinting***

- VNTR analysis = **V**ariable **N**umber **T**andem **R**epeat
- Very short tandem repeats (2-60 bp) minisatellites
- Highly variable in number between individuals.
- Cutting at restriction sites outside repeats gives a fragment of variable size.
- Use probe containing the repeated sequence.
- Will detect many arrays.
- Pattern of bands is different for each individual like a fingerprint.

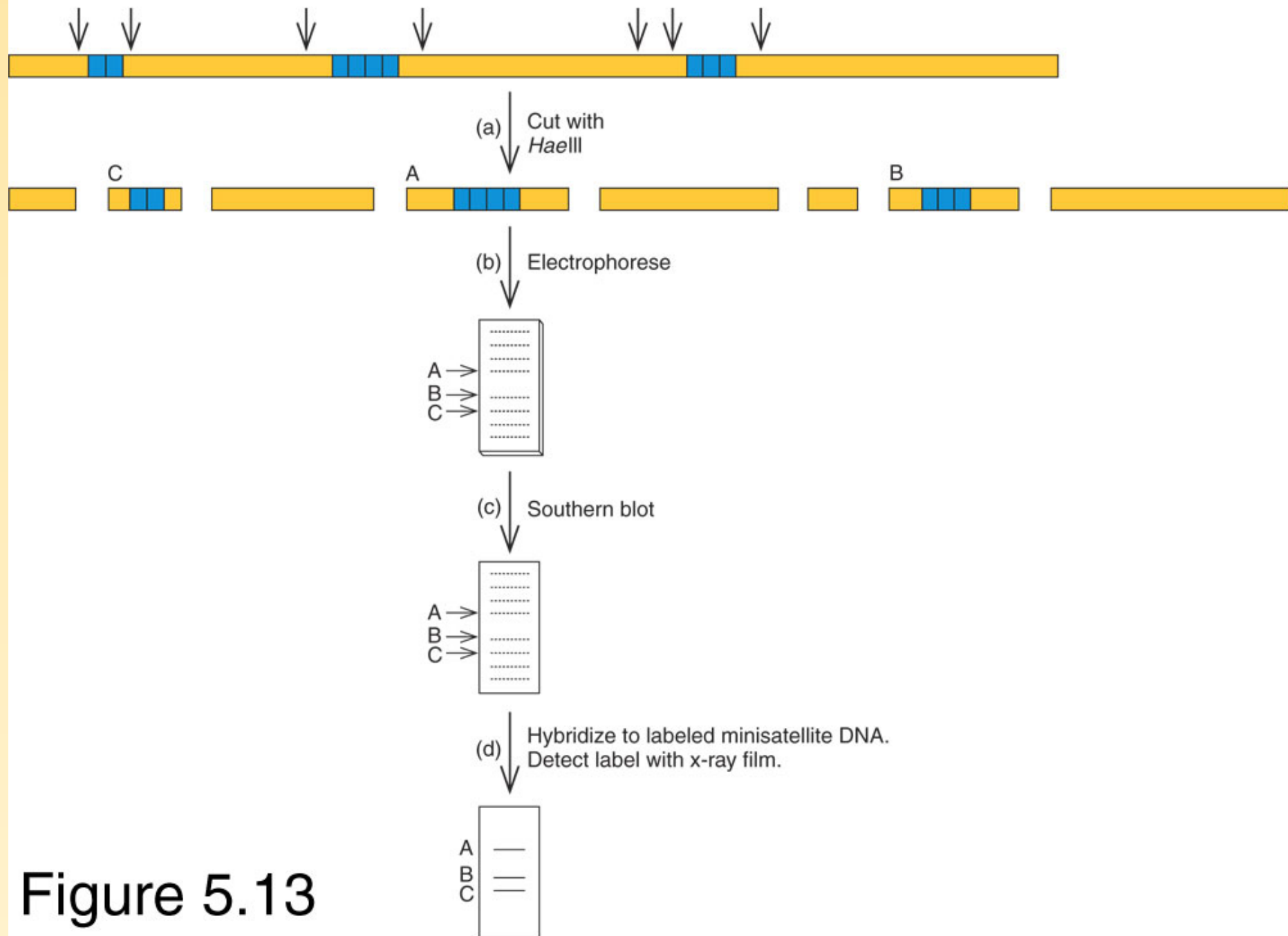
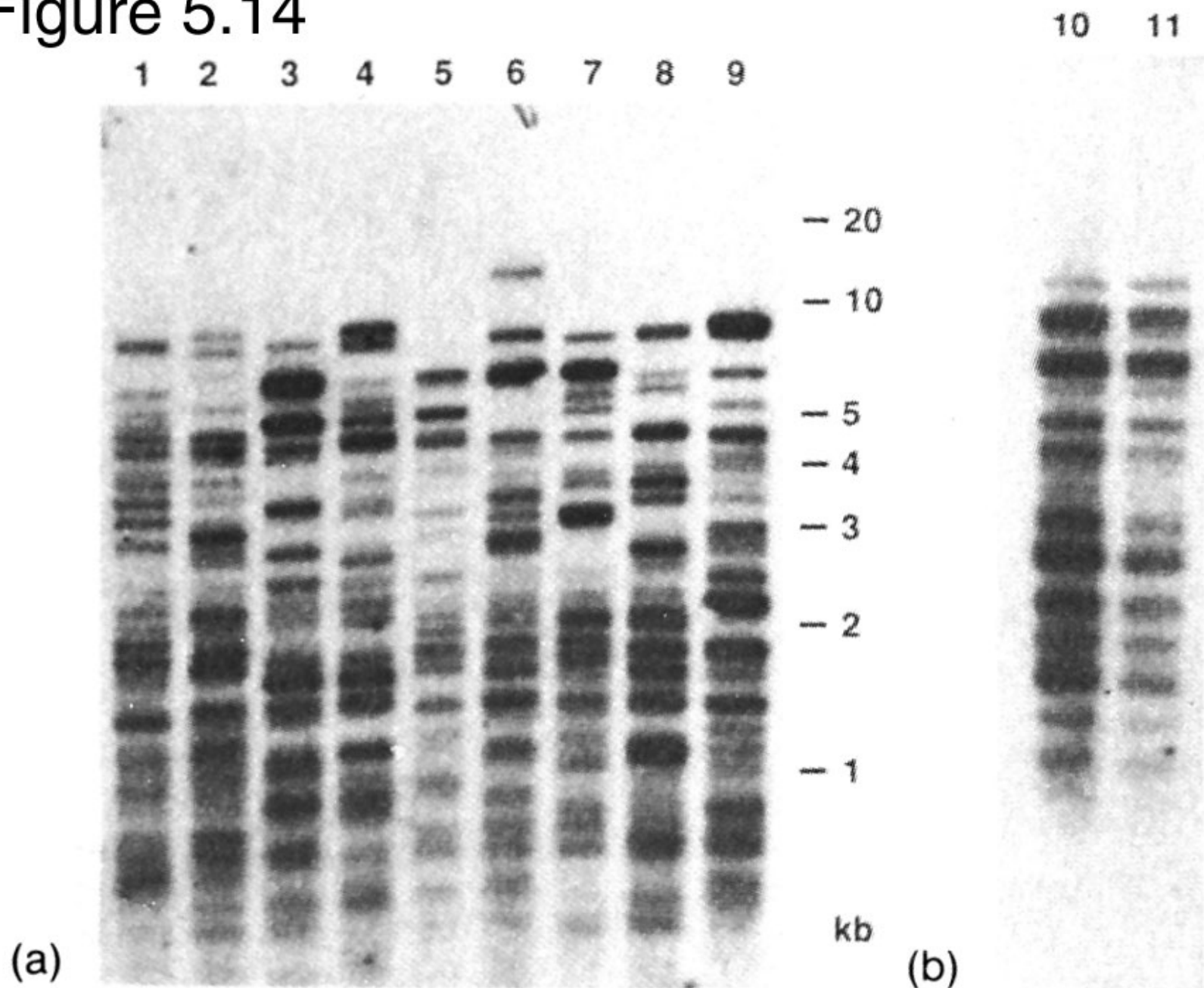


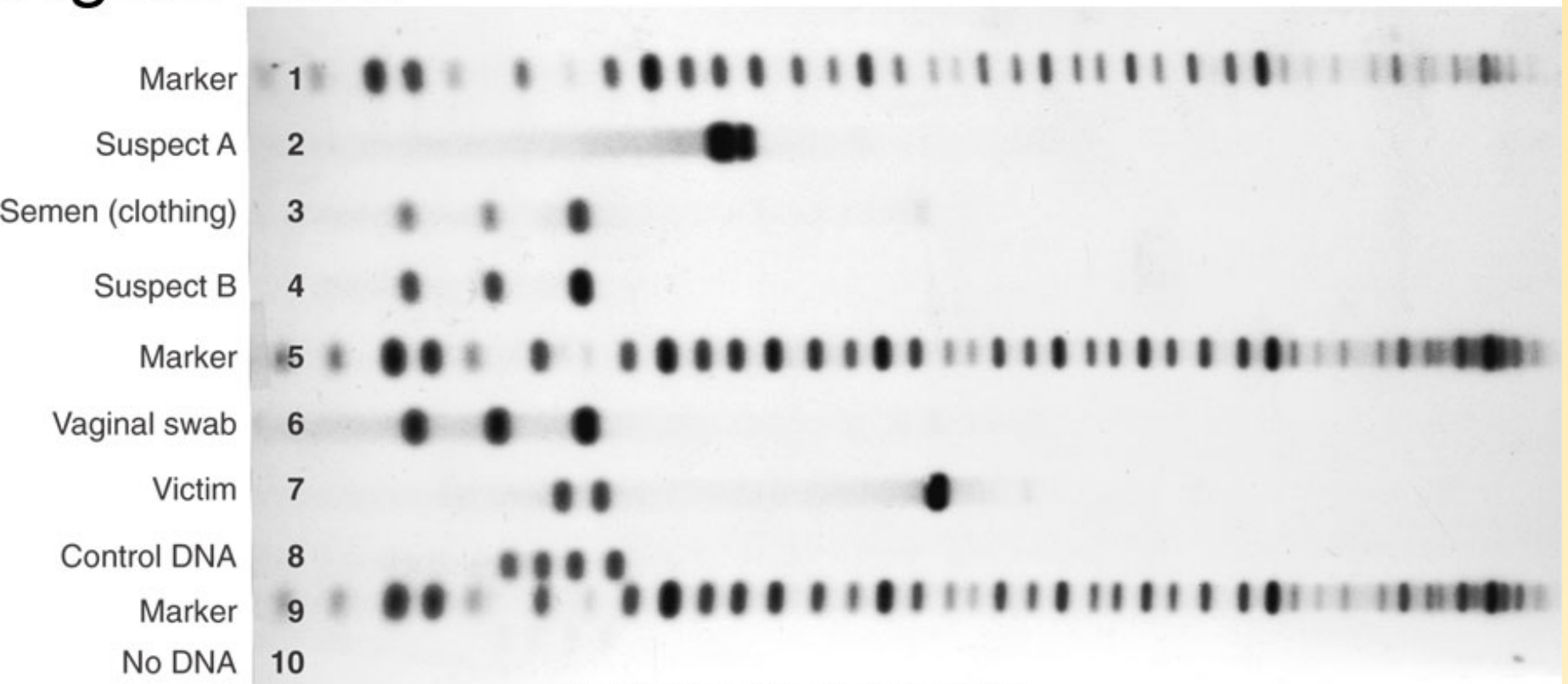
Figure 5.13

# Figure 5.14



G. Vassert et al. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235 (6 Feb 1987) f. 1, p. 683. © AAAS

# Figure 5.15



Courtesy Lifecodes Corporation, Stamford, CT.

# *DNA typing*

- Looks at a single variable locus
  - e.g. CAPS analysis
  - RFLP analysis

# *RFLP analysis*

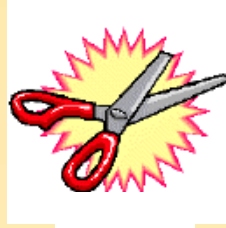
- **Restriction Eragment Length Polymorphism**
- Polymorphism = a variation (natural or induced) in the base sequence of DNA.
- RFLP = variation in the size of DNA fragments generated by restriction enzymes.

TTCGTCGTATCCGTTATGCGAATTCCTGCATAATGGTC



TTCGTCGAATCCGTTATGCTAATTCTGCATAATGGTC





Individual I



1 kb

3 kb



Probe



Individual II



1.5 kb

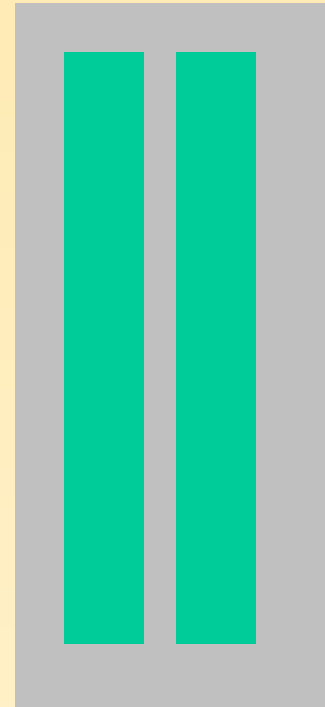
2.5 kb





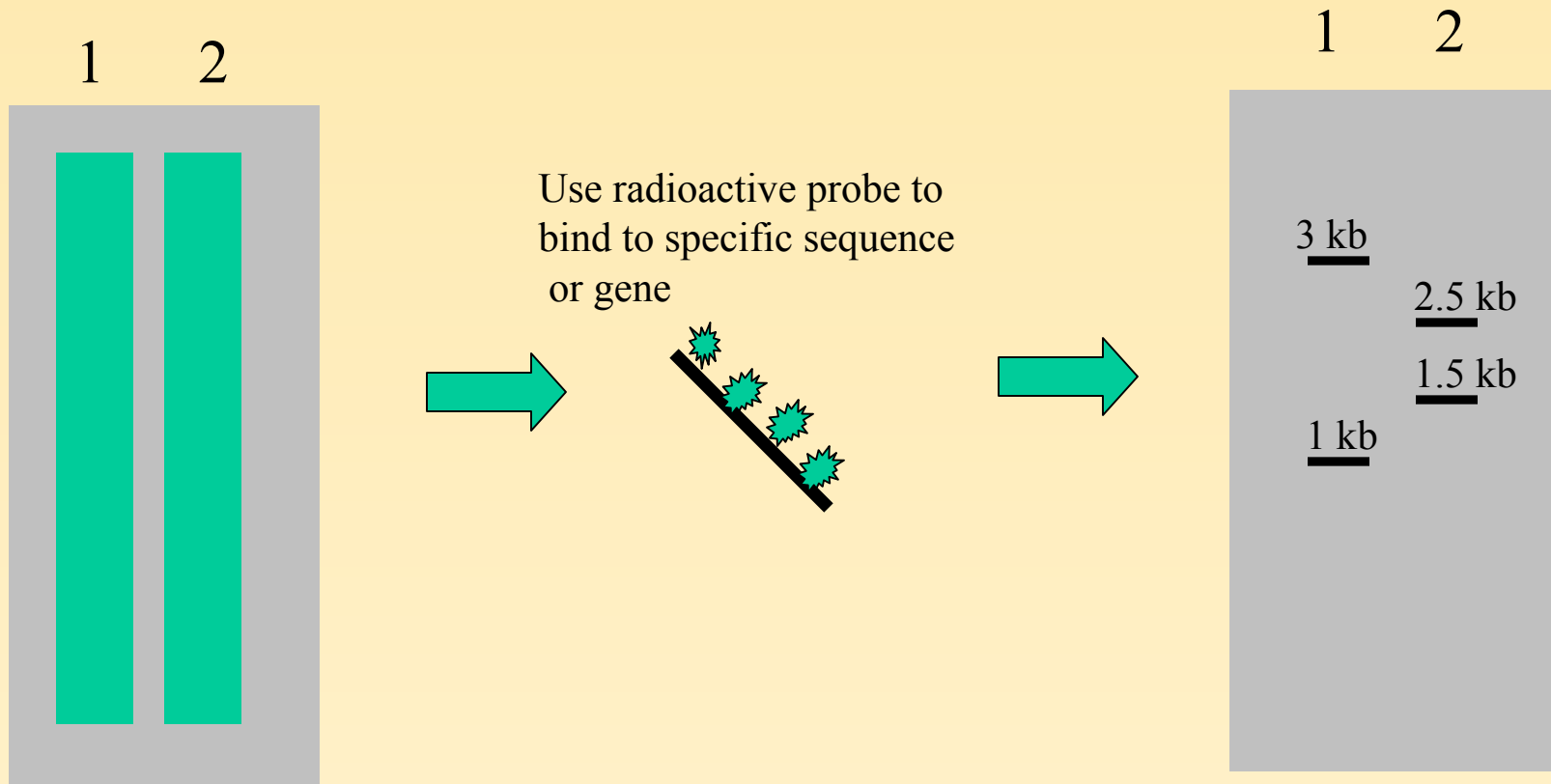
# To do a Southern blot

- Cut DNA
- Run on a gel
  - will be a smear
- Transfer to a filter
  - “blotting”
- Hybridize with a probe



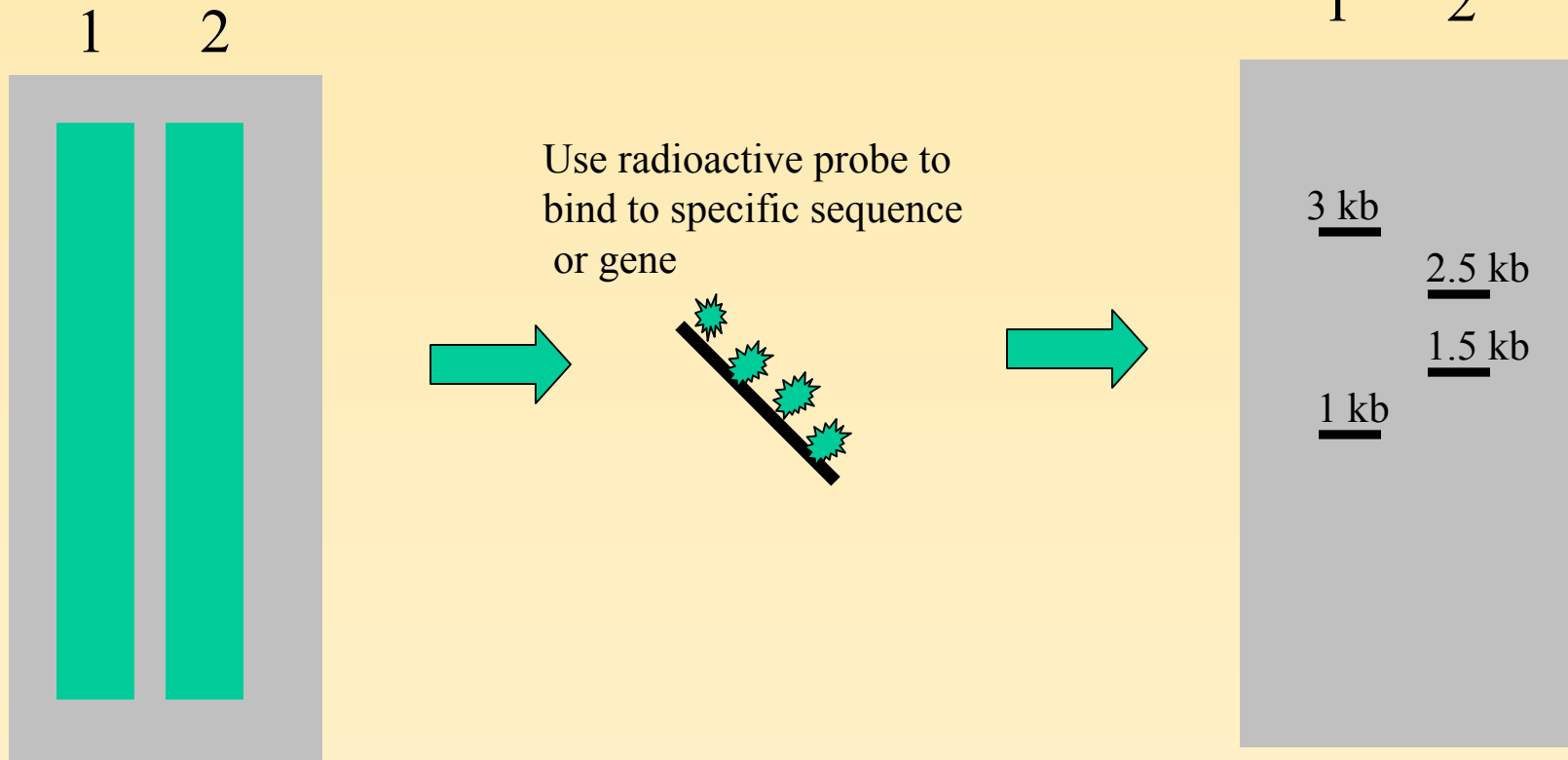
Gel electrophoresis  
of entire set of genome  
fragments transferred to paper

X-ray shows specific  
set of bands



Gel electrophoresis  
of entire set of genome  
fragments

X-ray shows specific  
set of bands



# Paternity test example: RFLP analysis with a single fragment

