

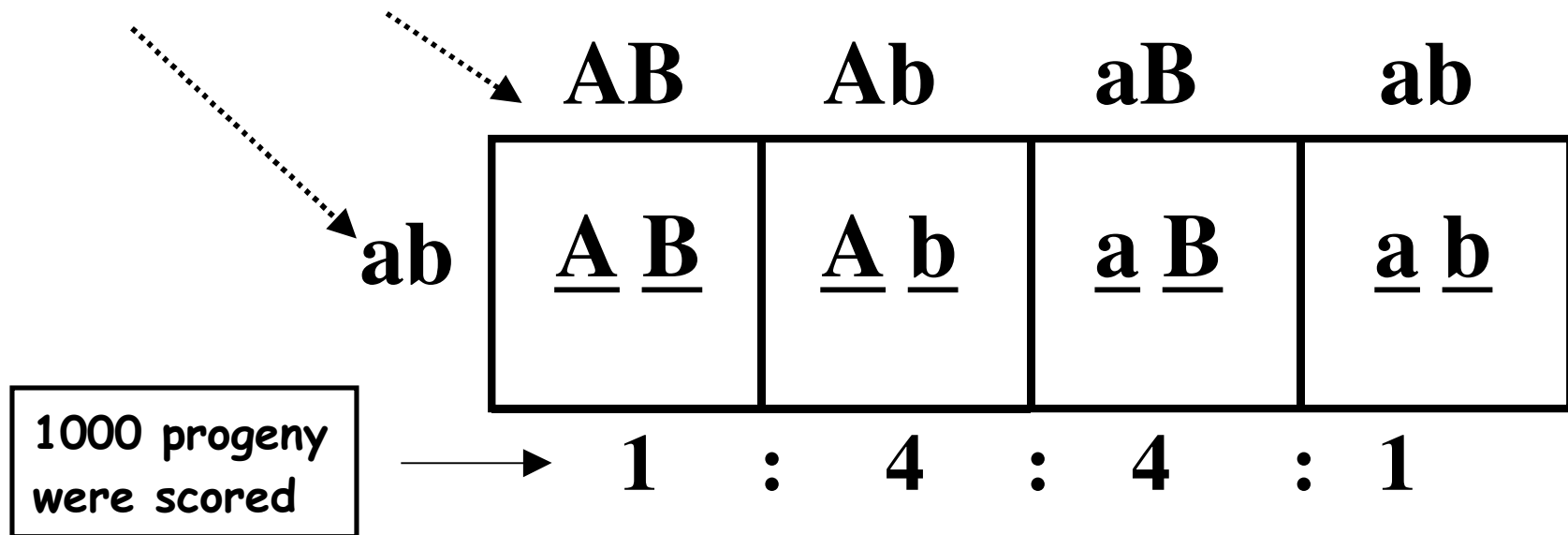
Bio 97

# Human Gene Cloning and more molecular genetics

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# Review Question

A doubly-heterozygous Arrogant Beautiful fly (Nadia) was mated with a meek ugly testcross fly (Fred)



Are the genes linked ? (ignore statistics)

If so, how far apart are they?

Are Nadia's recessive a and b alleles in cis or in trans?

# lecture outline

- How can a clone (recombinant DNA molecule) of interest be found in a library?
- How is DNA analyzed?
  - purifying DNA
  - restriction digest
  - gel electrophoresis
  - visualizing DNA
- What's a polymorphism?
  - How are they used in positional cloning? (not in detail)
  - How are they used in forensics?

# Library

- If you clone restriction-digested human DNA into a plasmid, you get millions of plasmids, each containing a different piece of human DNA --> a library
- We want to find a single book (e.g. a plasmid containing the insulin gene) in this library

# MOVIE

[Movie-steps in making a library \(click here\)](http://highered.mcgraw-hill.com/olc/dl/120078/micro10.swf)

(<http://highered.mcgraw-hill.com/olc/dl/120078/micro10.swf>)

From:

<http://highered.mcgraw-hill.com/sites/dl/free/0072437316/120060/ravenanimation.html>

# How to pick out your gene of interest ?

- Complementation - a gene on a plasmid can complement a mutant phenotype
  - If cells express the phenotype - e.g. DNA repair defects
- Use an antibody (H&J p 368)
  - Antibodies used to detect the bacterial colony expressing the protein of interest from the plasmid of interest

# More ways to pick out your gene of interest

- Hybridization (H&J p. 368)
  - e.g. Cross-hybridization to related sequence
  - Hybridization to a marker (DNA sequence) near the gene of interest (see below)
- Positional cloning - find a marker that is near the gene using human gene mapping techniques

# Purifying nucleic acid from cells

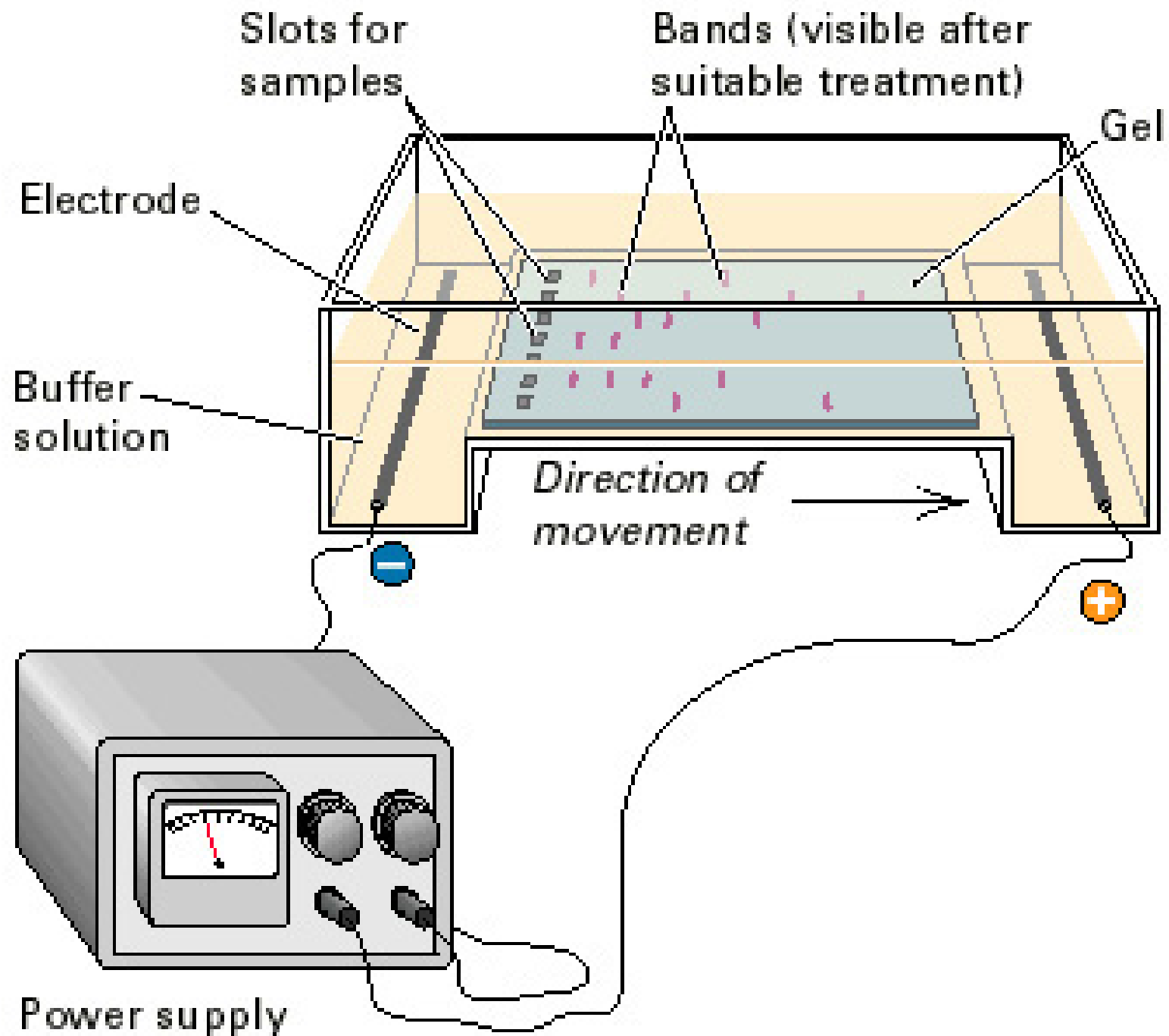
- Lyse the cells
- Extract the proteins and lipids with organic solvent (e.g. phenol)
- Precipitate the nucleic acid with ethanol

## To determine the pattern of Restriction Enzyme cleavage in a DNA sample

1. Digest the DNA with the RE
  - Purified DNA
  - Water
  - Buffer
  - RE
2. Run the digested products on a gel to separate them by size
3. Stain the DNA in the gel (or do something else) so that you can visualize the DNA

# Gel Electrophoresis

- Gel electrophoresis separates DNA molecules by size
- An agarose gel is somewhat like jello
- DNA migrates in an electric field
- Larger molecules move slower due to agarose sieving effect



# Visualizing DNA

- DNA bands on a gel can often be visualized by staining with dyes which bind DNA (ethidium bromide)
- Methods used to detect very small amounts of DNA or to identify a single DNA band:
  - Southern blot analysis
  - Polymerase chain reaction (PCR)

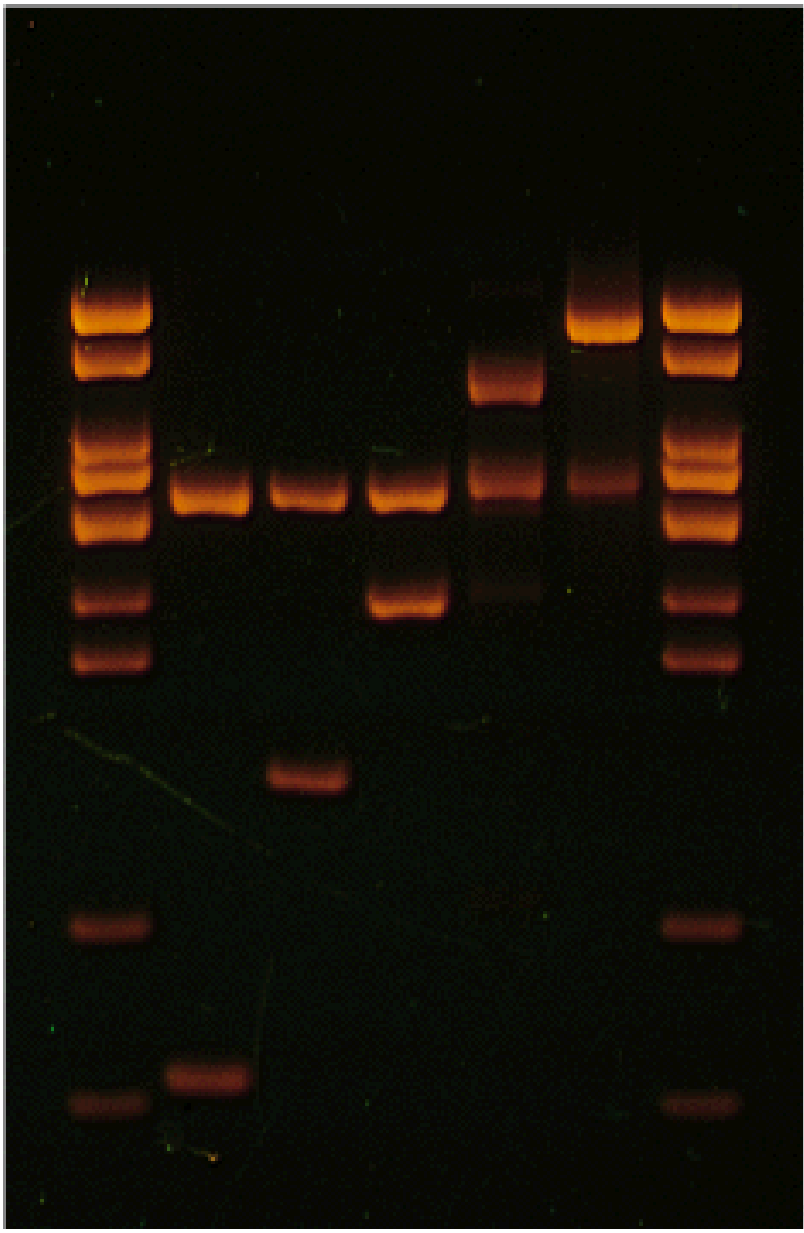
(a)

Clones

M 1 2 3 4 5 M

kb

8  
4  
3  
2  
1



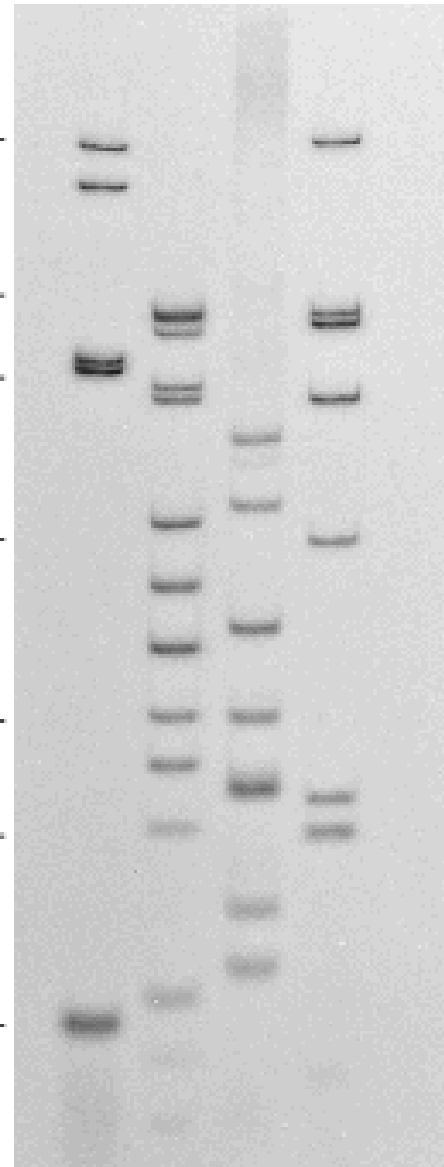
← Vector

(b)

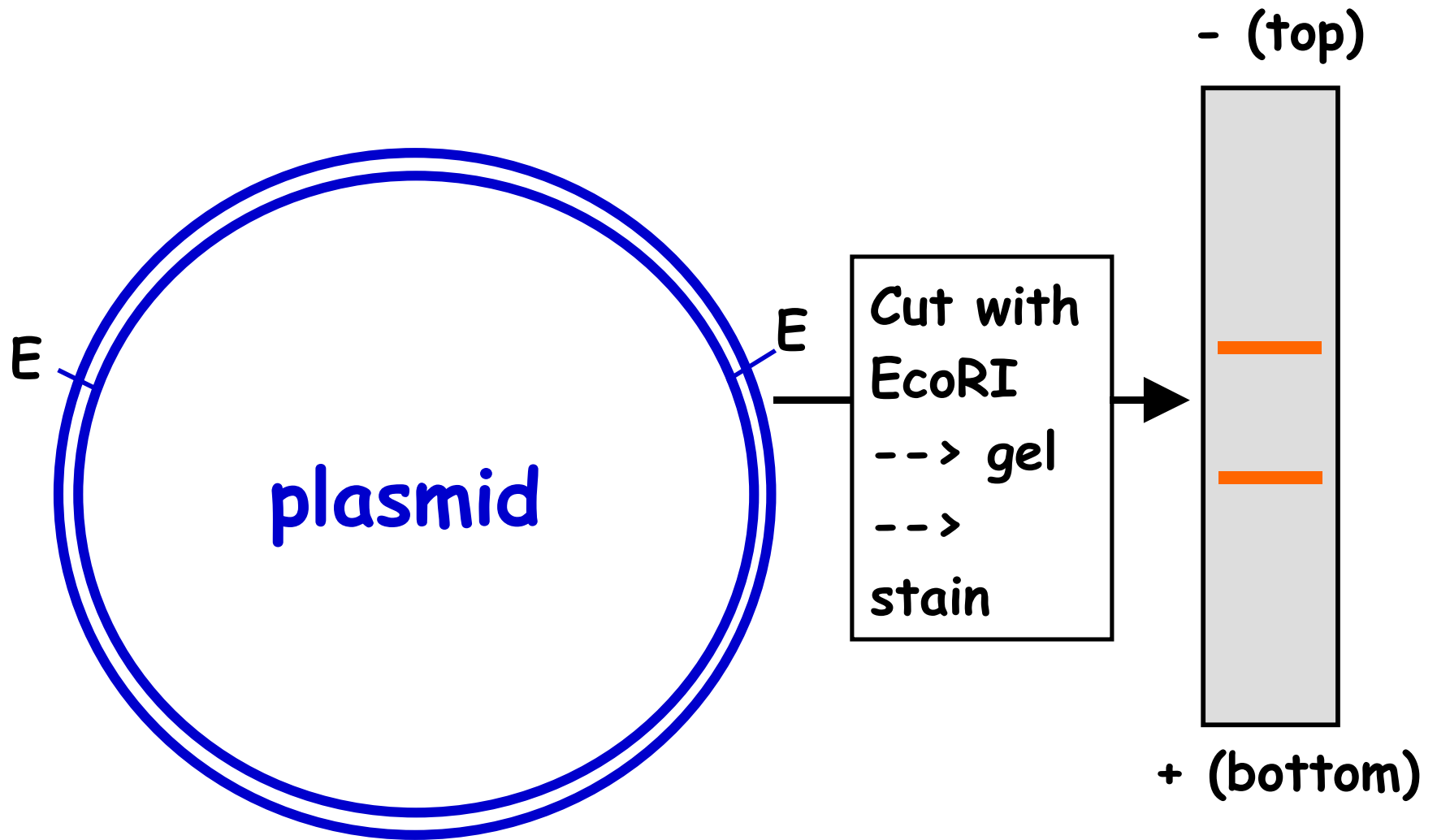
1 2 3 4

bp

1400  
600  
400  
200  
100  
60  
30

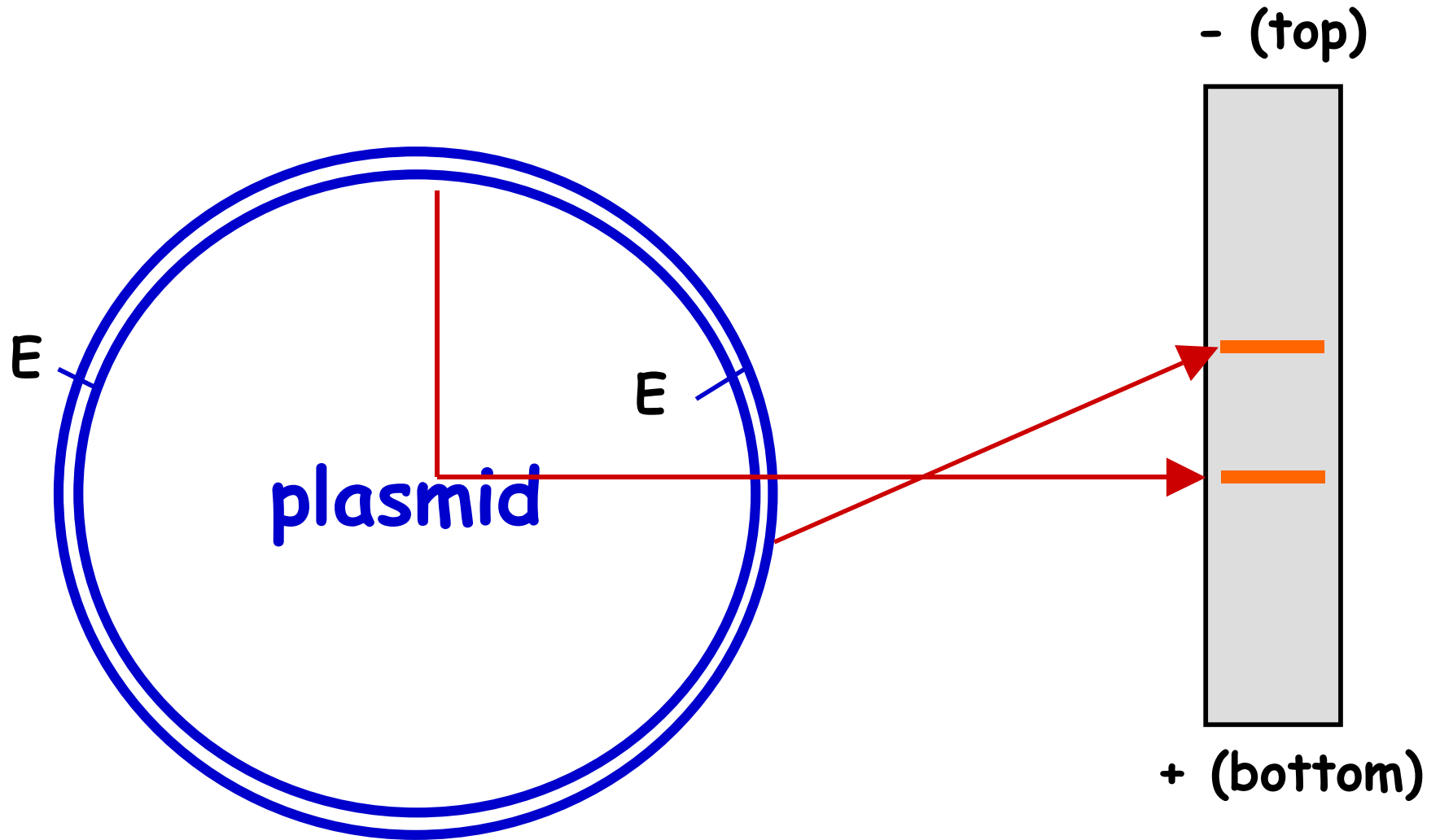


# 5 kb plasmid, 2 EcoRI sites

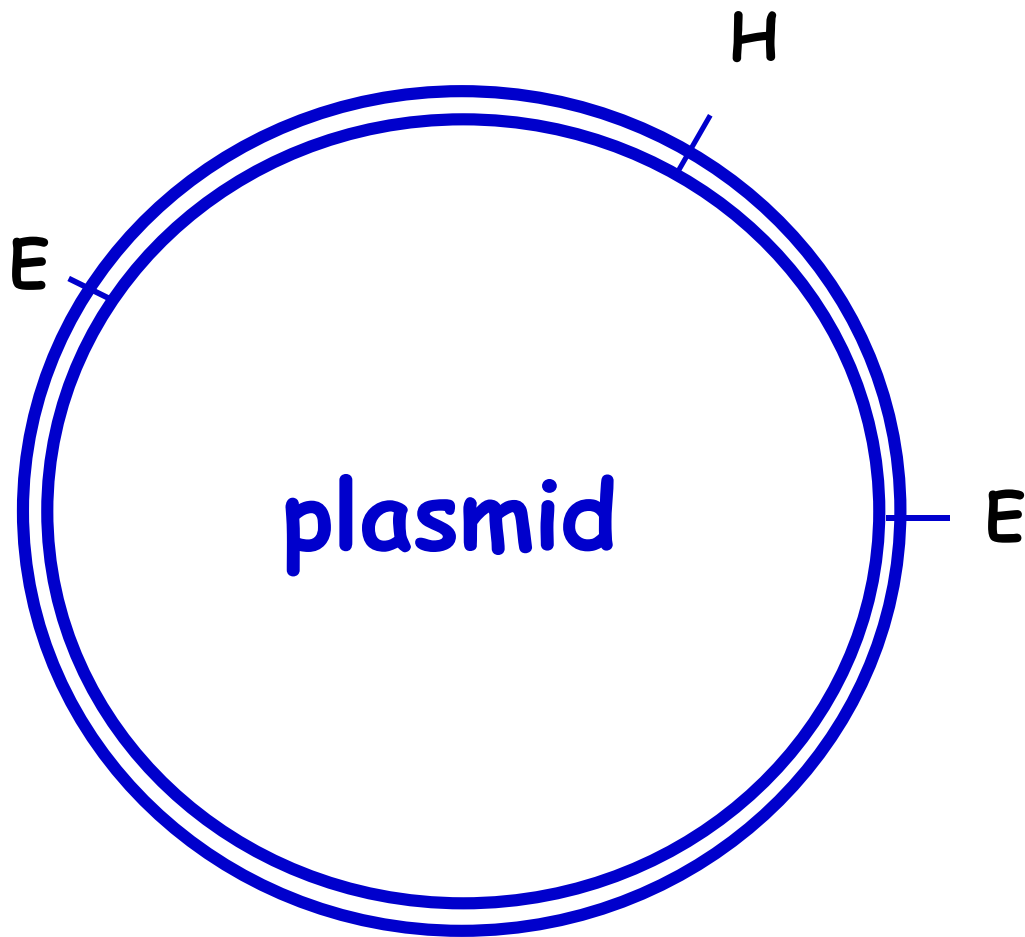


# 5 kb plasmid, 2 EcoRI sites

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Must cut 10 billion plasmid molecules to be able to see in gel

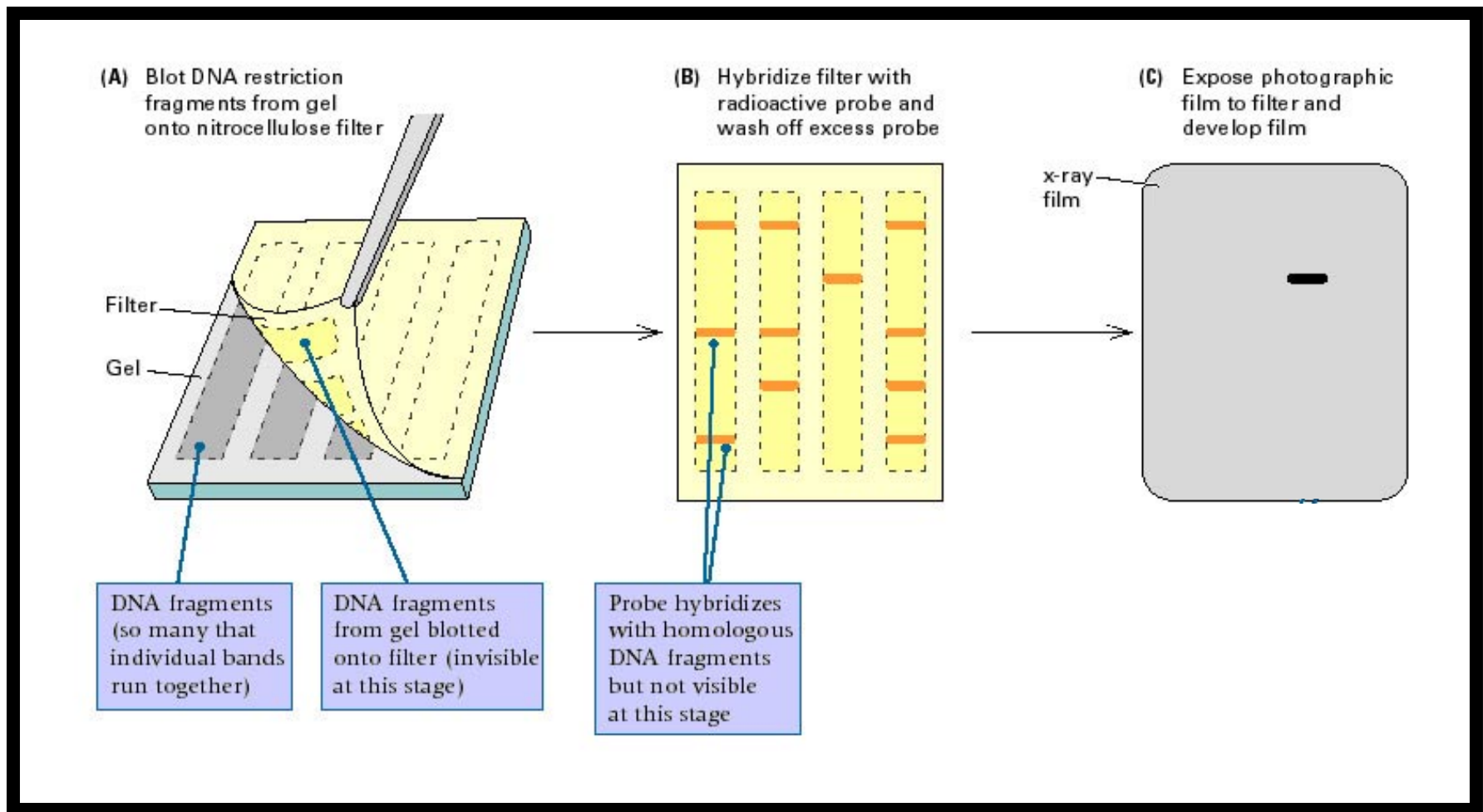


# Southern Blot Analysis

- A way to probe a gel containing a bunch of DNA fragments to try to identify a particular DNA fragment

# Southern Blot Analysis - I

- DNA is cut into pieces by restriction enzymes
- DNA fragments are separated by gel electrophoresis
- DNA is transferred from gel to hybridization filter (blot) and denatured to produce single-stranded bands of DNA

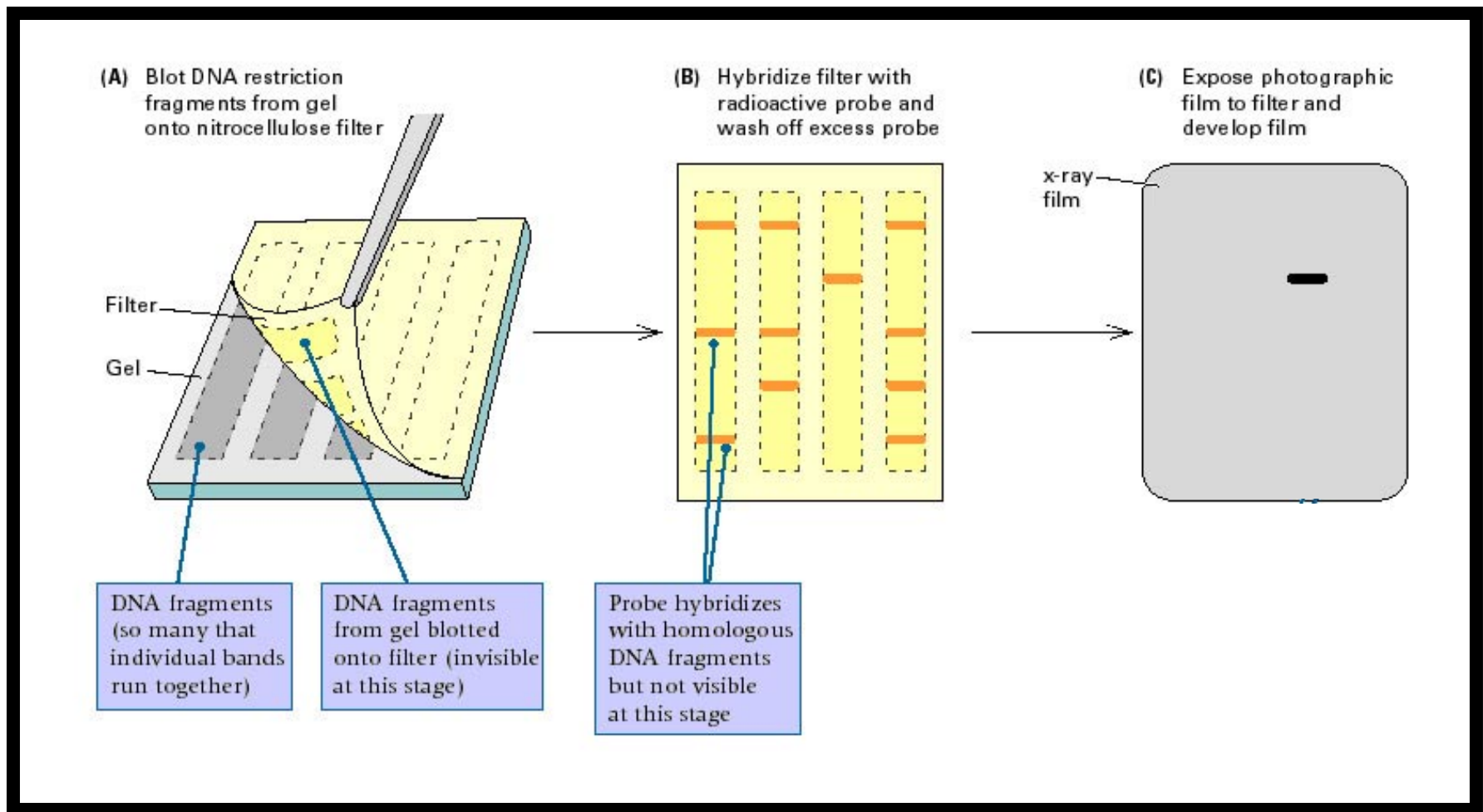


H&J Fig. 6.27 modified

Read H&J section 6.6 & 6.7

# Southern Blot Analysis - II

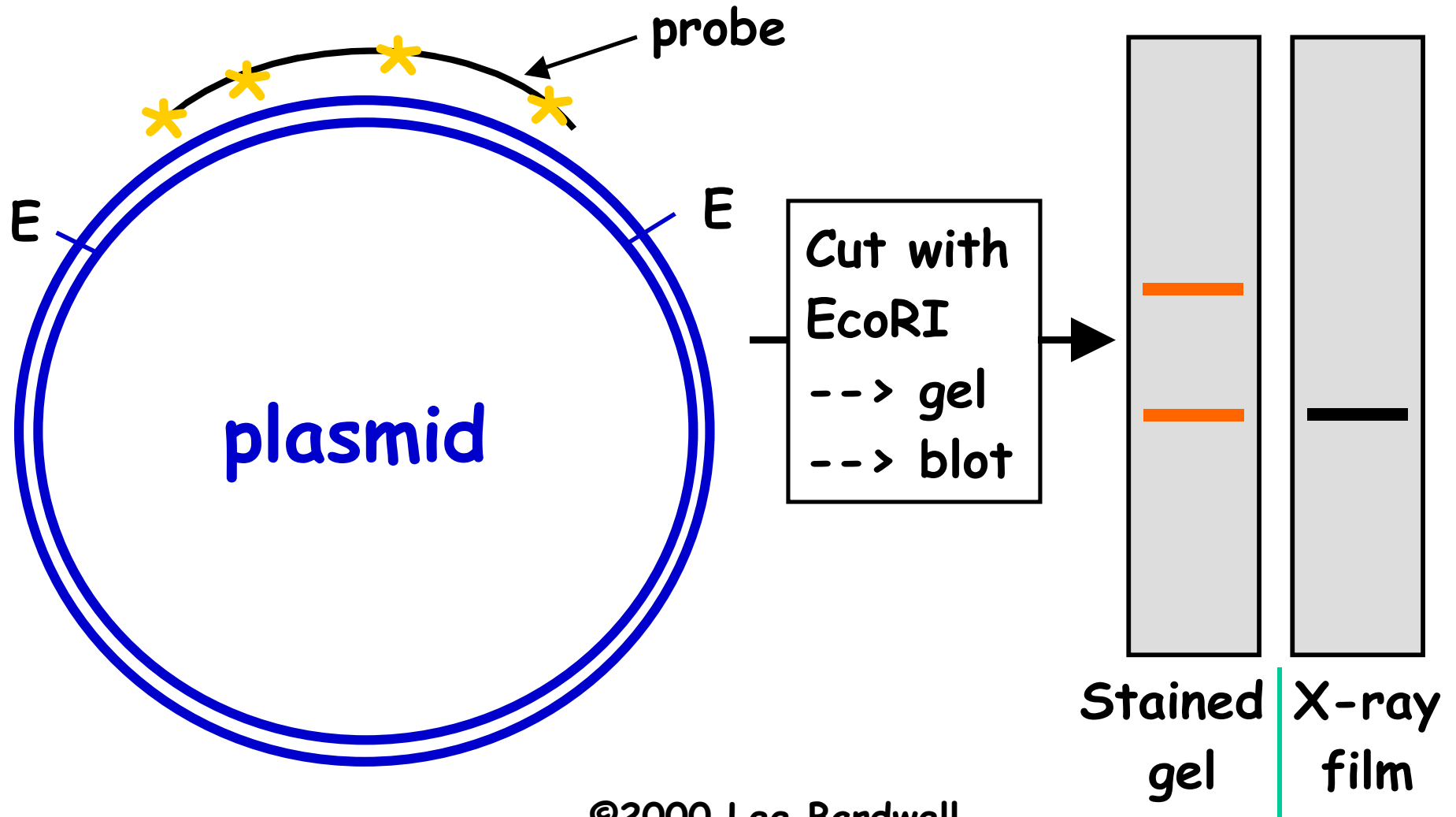
- Filter is mixed with radiolabeled single-stranded DNA probe complementary to the DNA sequence of interest
- hybridization = hydrogen bonds form between complementary base pairs
- DNA bands hybridized to probe are detected by X-ray film exposure

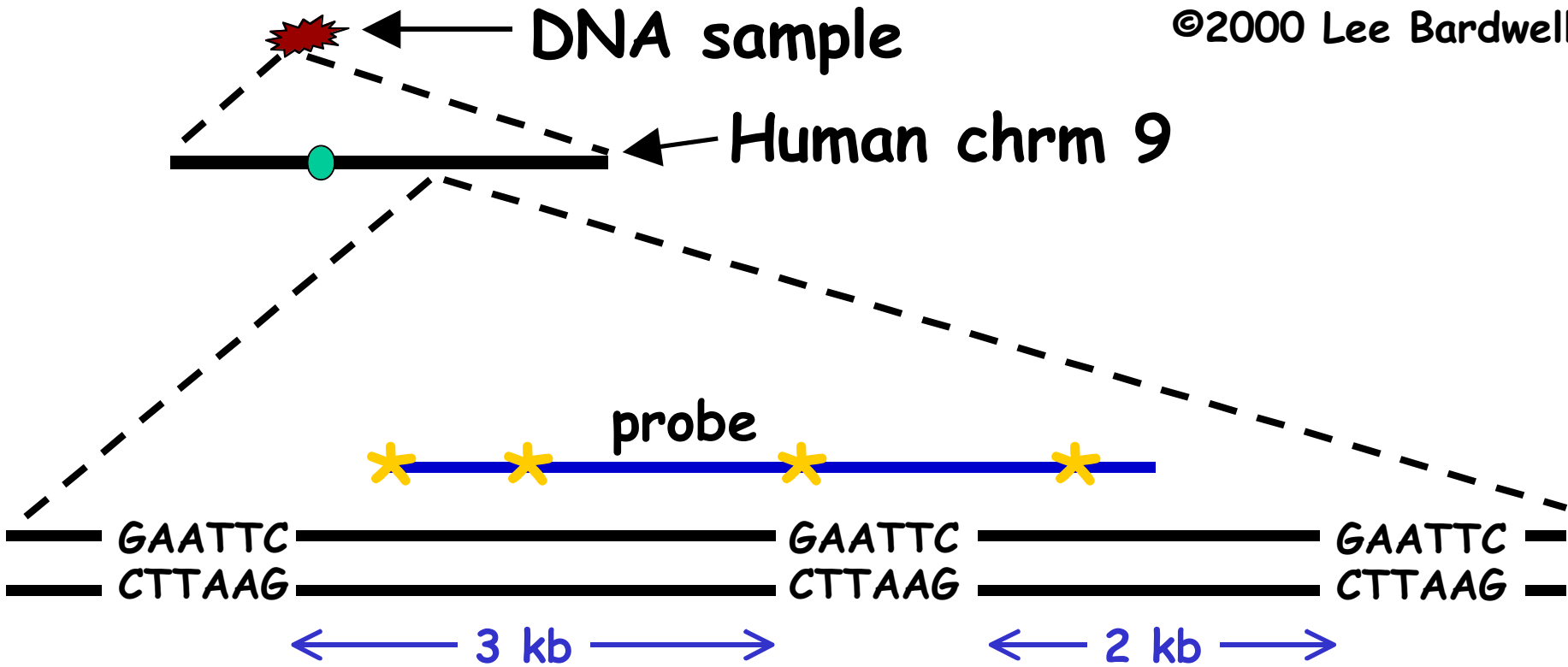


H&J Fig. 6.27 modified

Read H&J section 6.6 & 6.7

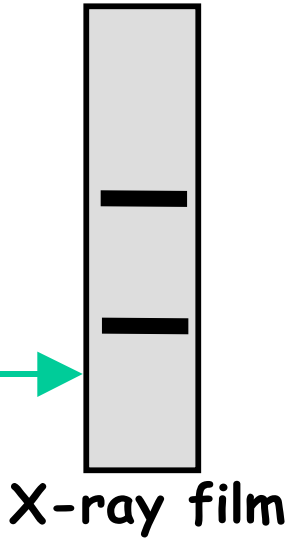
# 5 kb plasmid, 2 EcoRI sites





Cut with EcoRI restriction enzyme  
Gel --> blot

~ 750,000 other bands are not seen because they did not hybridize with the probe



## REVIEW

# Polymerase Chain Reaction (PCR)

- Uses short (oligonucleotide) primers flanking the region of interest
- Repeated rounds of DNA polymerization in an *in vitro* (test-tube) reaction result in the exponential amplification of the region of interest
- (H&J section 6.7)

# Net Result...

**Of Southern blot or PCR**

- detection one/few bands in a background of hundreds of thousands of bands**

# Things human geneticists can't do

- Establish true-breeding lab strains
- Perform testcrosses or backcrosses
- Score lots of progeny from the same mating

# How to map a human disease gene

- Find a large, multigenerational, affected family
- Test linkage of the disease to a mapped polymorphism
- Do this for many, many markers until you find a closely linked one

# We're all different

- On average, any two people differ at 1 in every 1000 basepairs
- Human vs. chimp...

# Polymorphism

Relatively common\*  
genetic difference in a  
population

\*Frequency of heterozygous genotypes is 10% or more

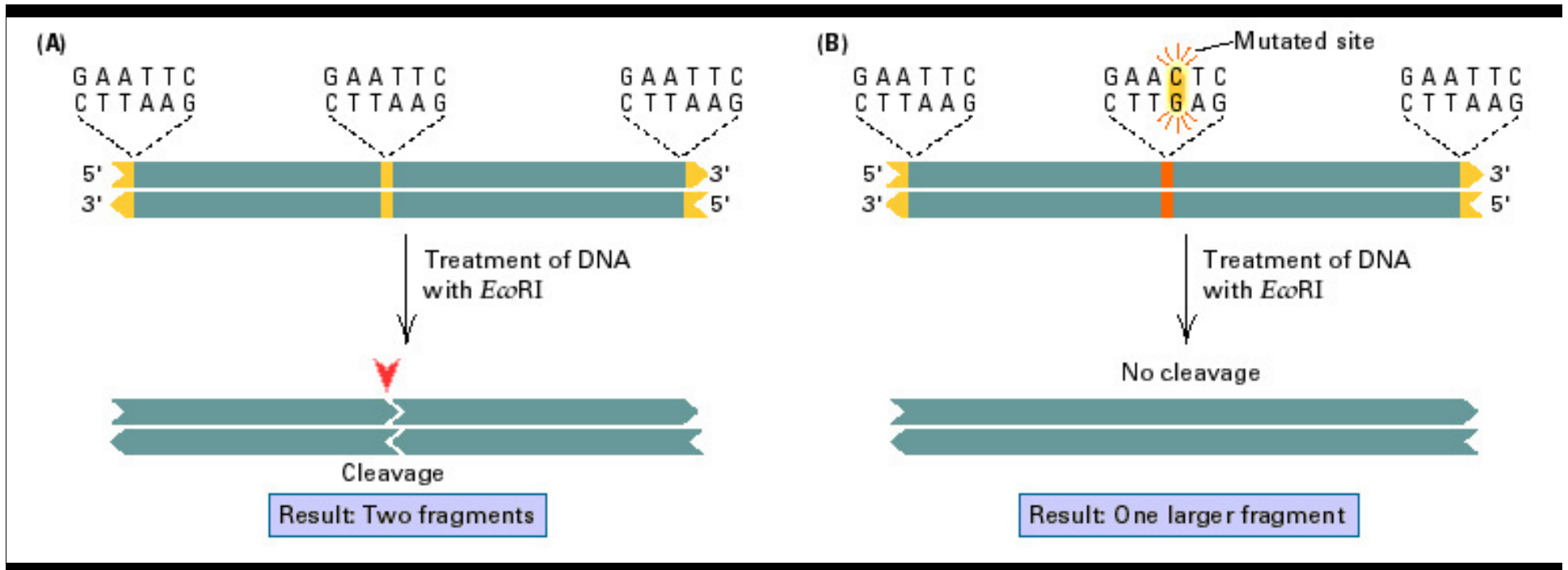
# RFLPs

restriction fragment length polymorphisms

polymorphisms that alter the length of restriction fragments

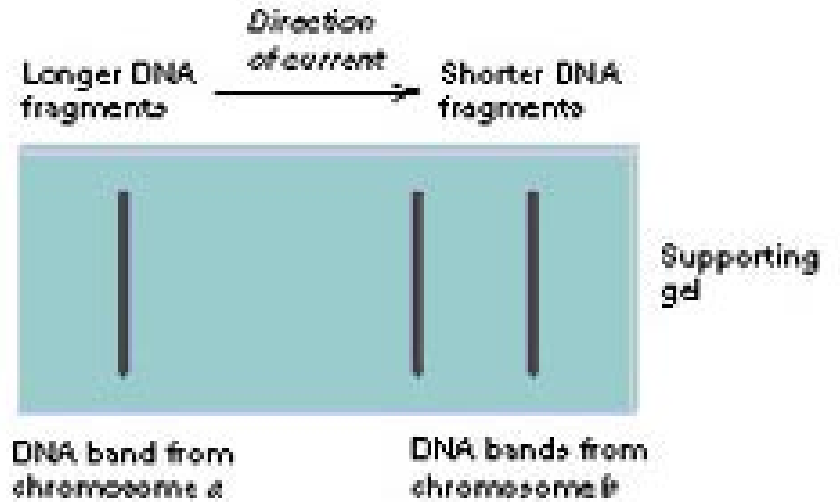
Can result from...

- changes that introduce or delete a restriction enzyme site
- differences in copy number of tandem DNA repeats

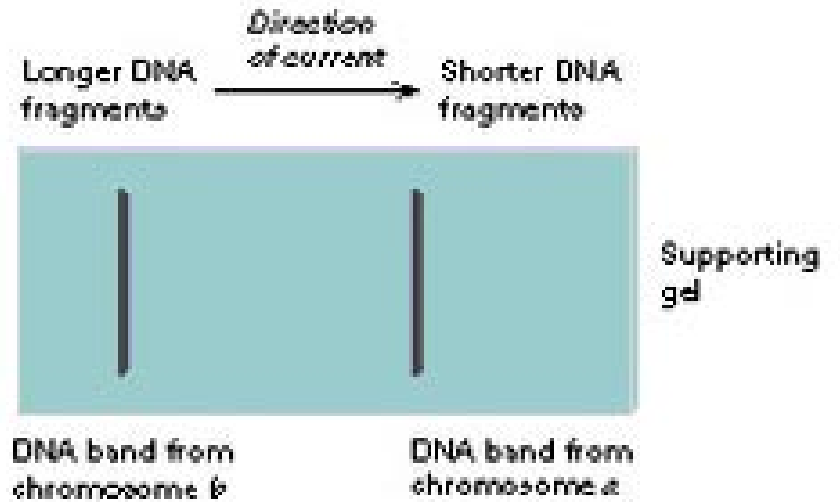
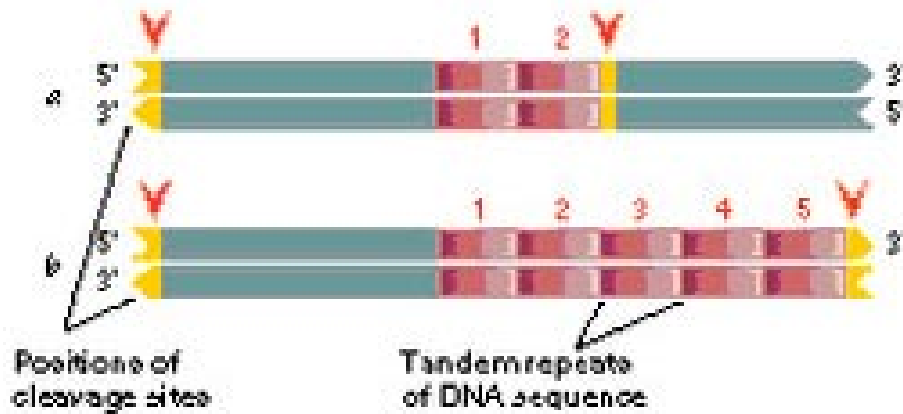


H&J Fig. 4.19

**(A) DNA in chromosomes**



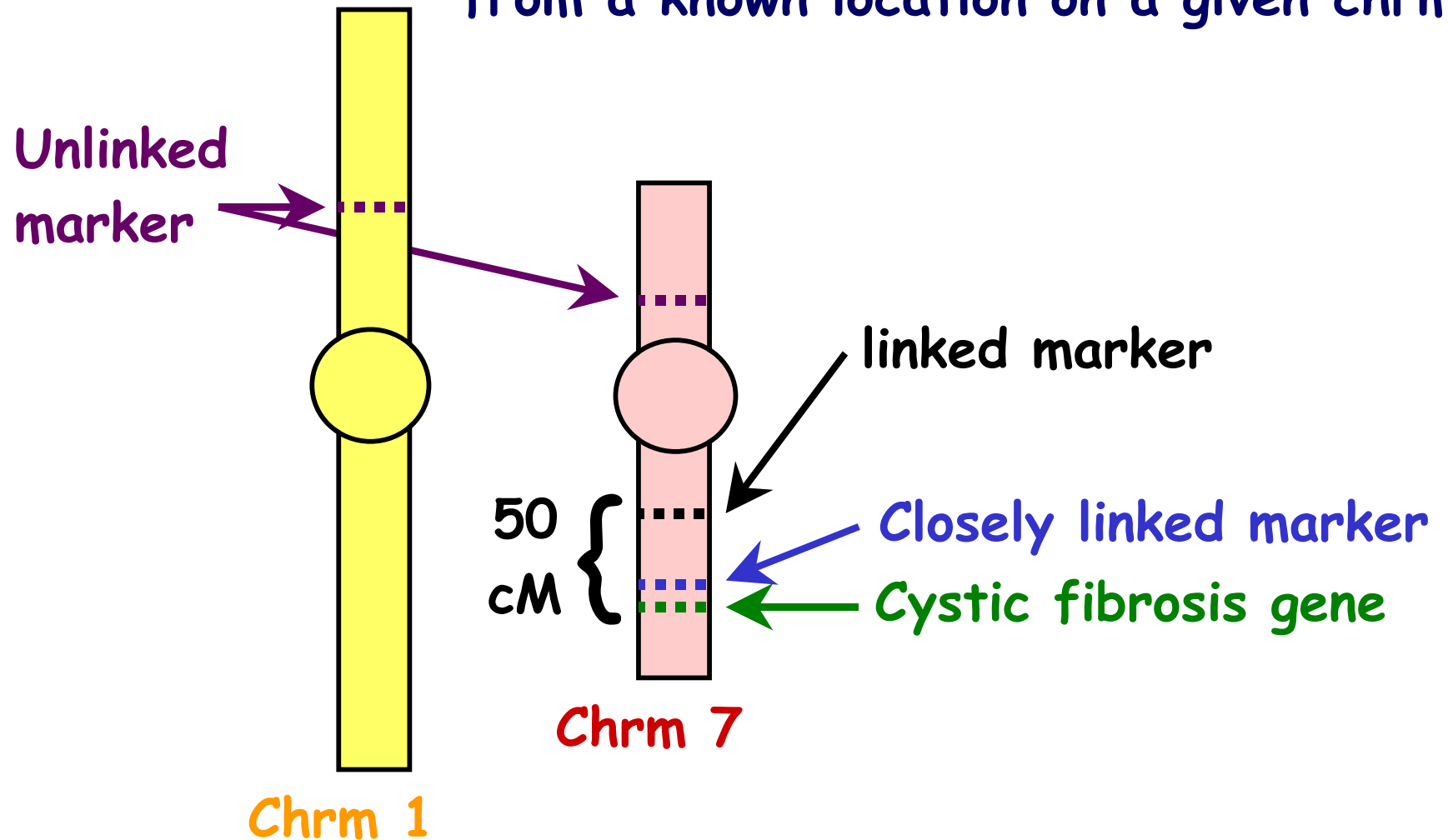
**(B) DNA in chromosomes**



H&J Fig. 4.20

# Marker

A small (200-1000 bp) region of DNA from a known location on a given chrm





## To look for linkage of a polymorphism to a disease gene

- Type each family member for the polym (=marker) and for the disease phenotype --> pedigree
- A closely linked marker will segregate with the disease

# Highly polymorphic markers\* ...

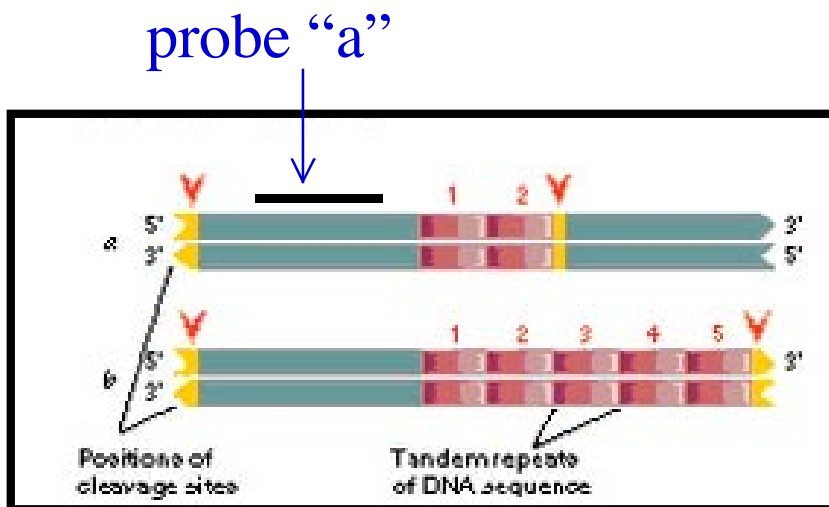
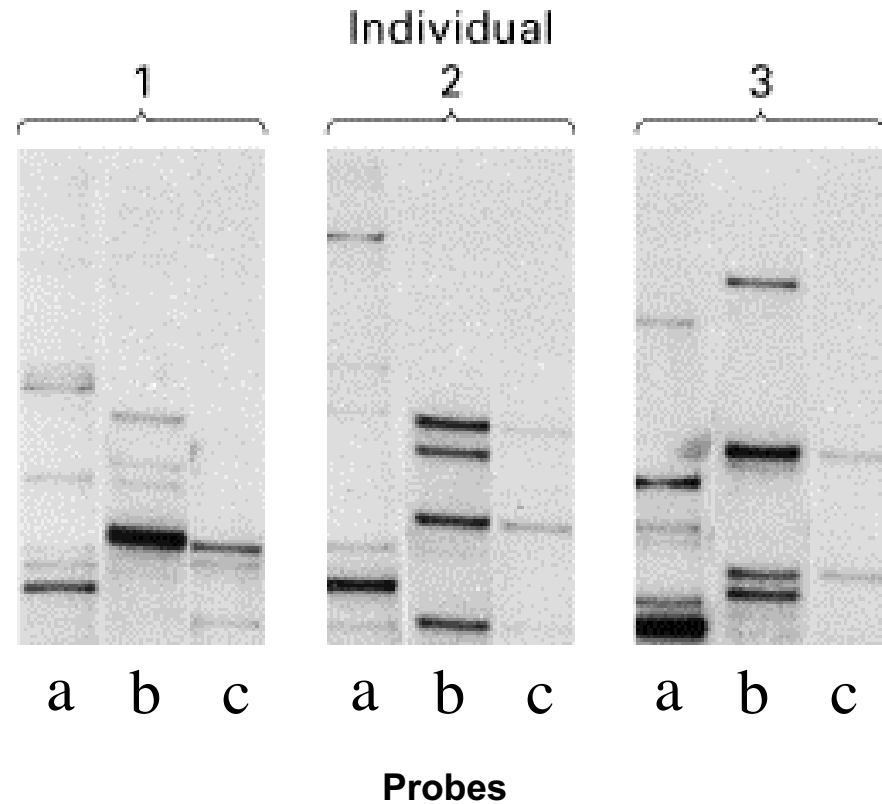
- Are also useful at the scene of a crime
- And in paternity cases
- DNA typing, or “DNA fingerprinting”
- (H&J Section 14.4)

\*Markers that can be used as probes to detect highly polymorphic regions

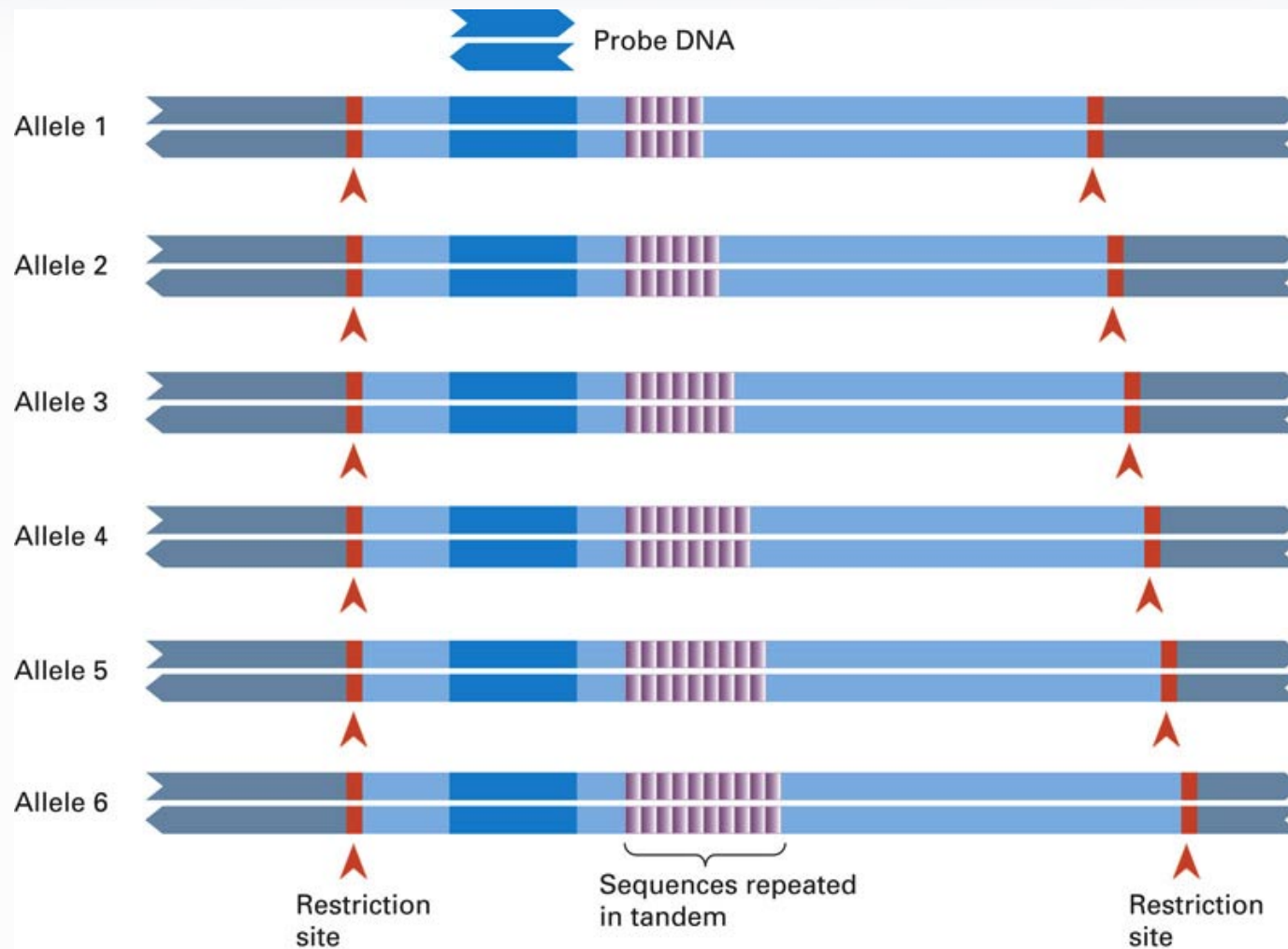
# DNA Fingerprinting

- 3 markers/probes
- "minisatellite" SSRs\*

\*simple sequence repeats



Check out figures 14.18 and 14.19



**H&J Fig. 14.17. Allelic variation resulting from a variable number of units repeated in tandem in a nonessential region of a gene**

# Forensics

Crime scene  
Sample:  
Victim + perp



## Suspects

Victim

AJ

OJ

DJ

—	—	—	—	
—	—	—		
—			—	—
				—