(Mini)Lecture 18 - Molecular Biology Techniques

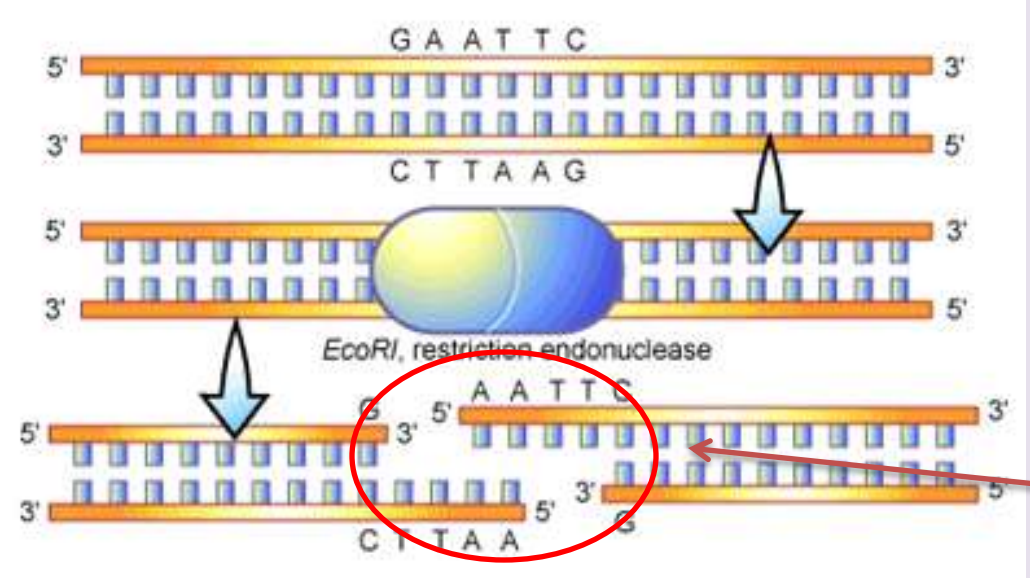
"The honest package you will never see."

Common molecular biology techniques

- Restriction enzymes
- Gel electrophoresis
- PCR
- Western/Southern/Northern blotting

Restriction enzymes

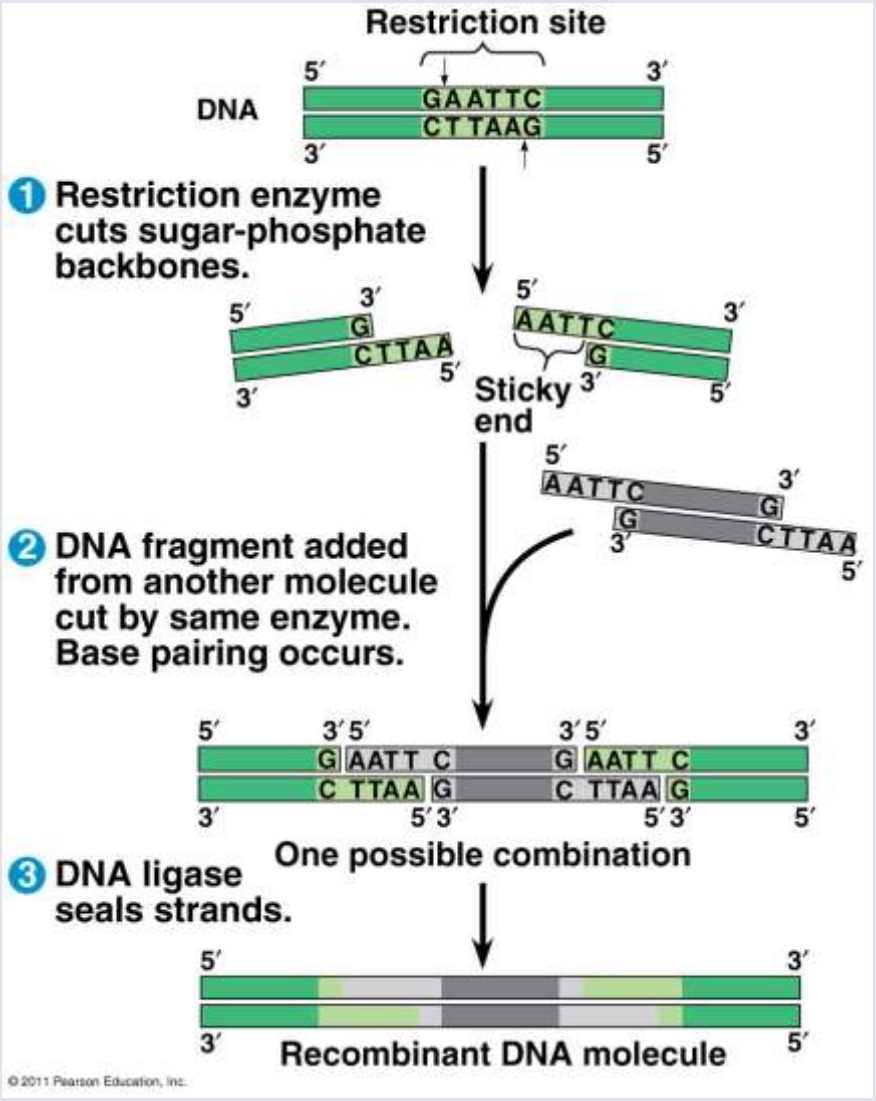
- Likely evolved as a defense against viral infection
- Restriction enzymes recognize specific DNA sequences and chop them up
- Molecular ‘scissors’



EcoR1, HINDIII, Xho1, BamH1

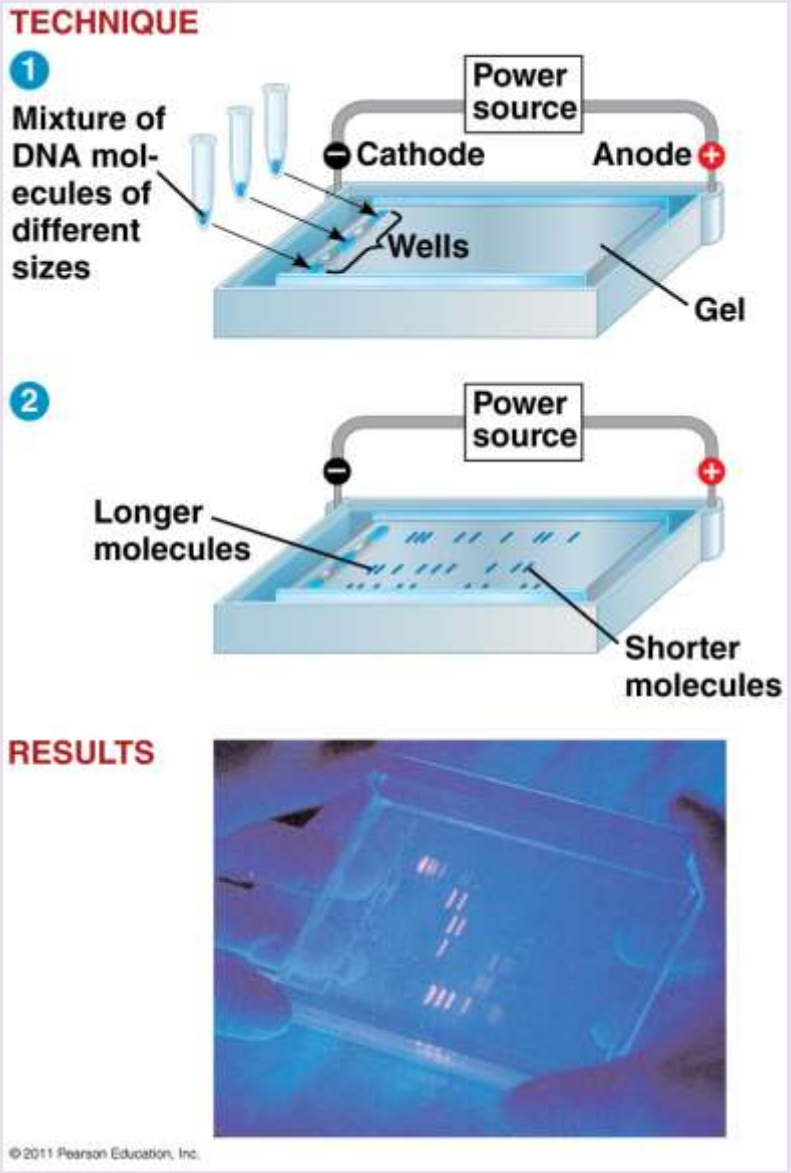
‘sticky ends’

- EcoR1 cuts at the sequence GAATTC
  - On average, should cut every $1/4^6$, or 1/4096 bp



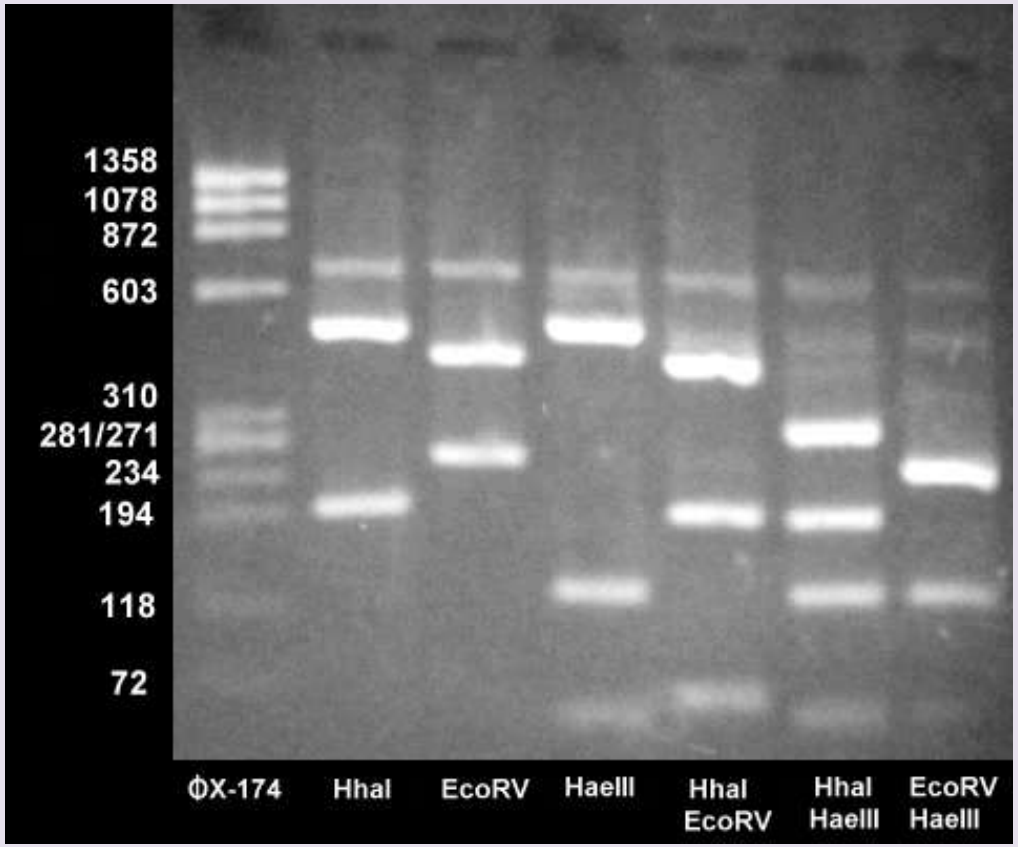
Gel electrophoresis

- Used to separate DNA, proteins, etc. based on size and charge
- The agarose gel acts as a sieve that slows the movement of molecules
- Two electrical nodes are placed on either end – anode and cathode
- Positively charged molecules will move towards the cathode, negative towards the anode
- Smaller molecules will move faster through the gel, and larger molecules will move more slowly
- The molecules produce ‘bands’ where they are concentrated within the gel



- Apply different restriction enzymes to a DNA sequence
- Several fragments will be produced of varying lengths depending on where and how many times the enzyme cuts

Length in base pairs of fragment



Restriction enzyme used to cut the sequence

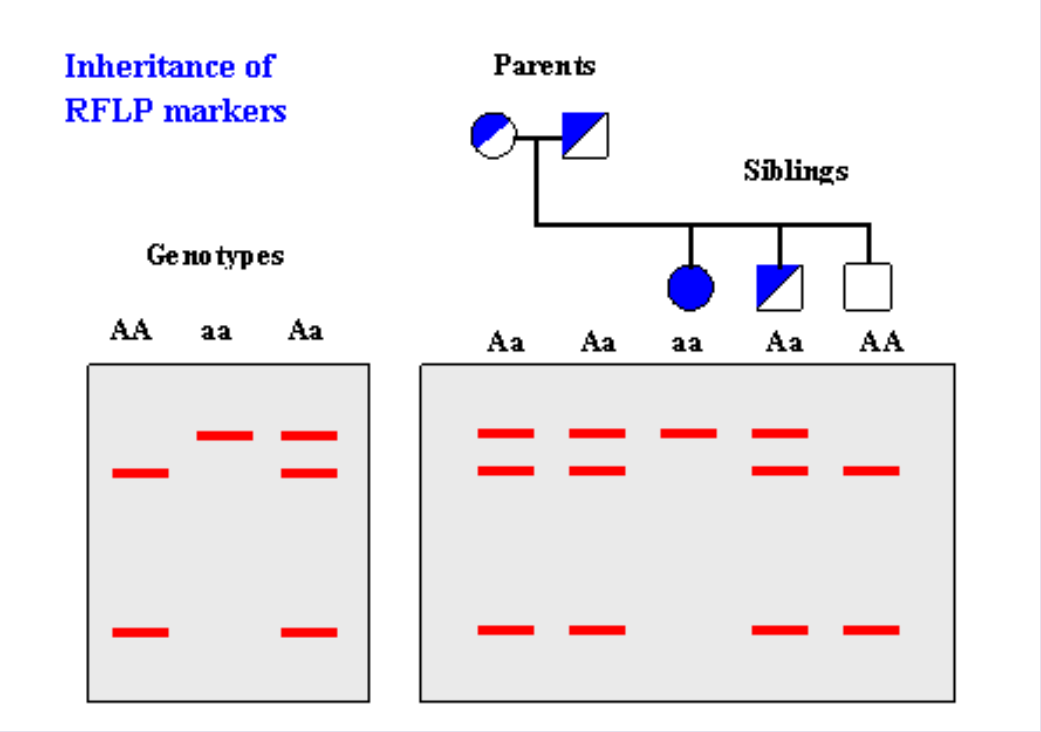
Restriction fragment length polymorphisms

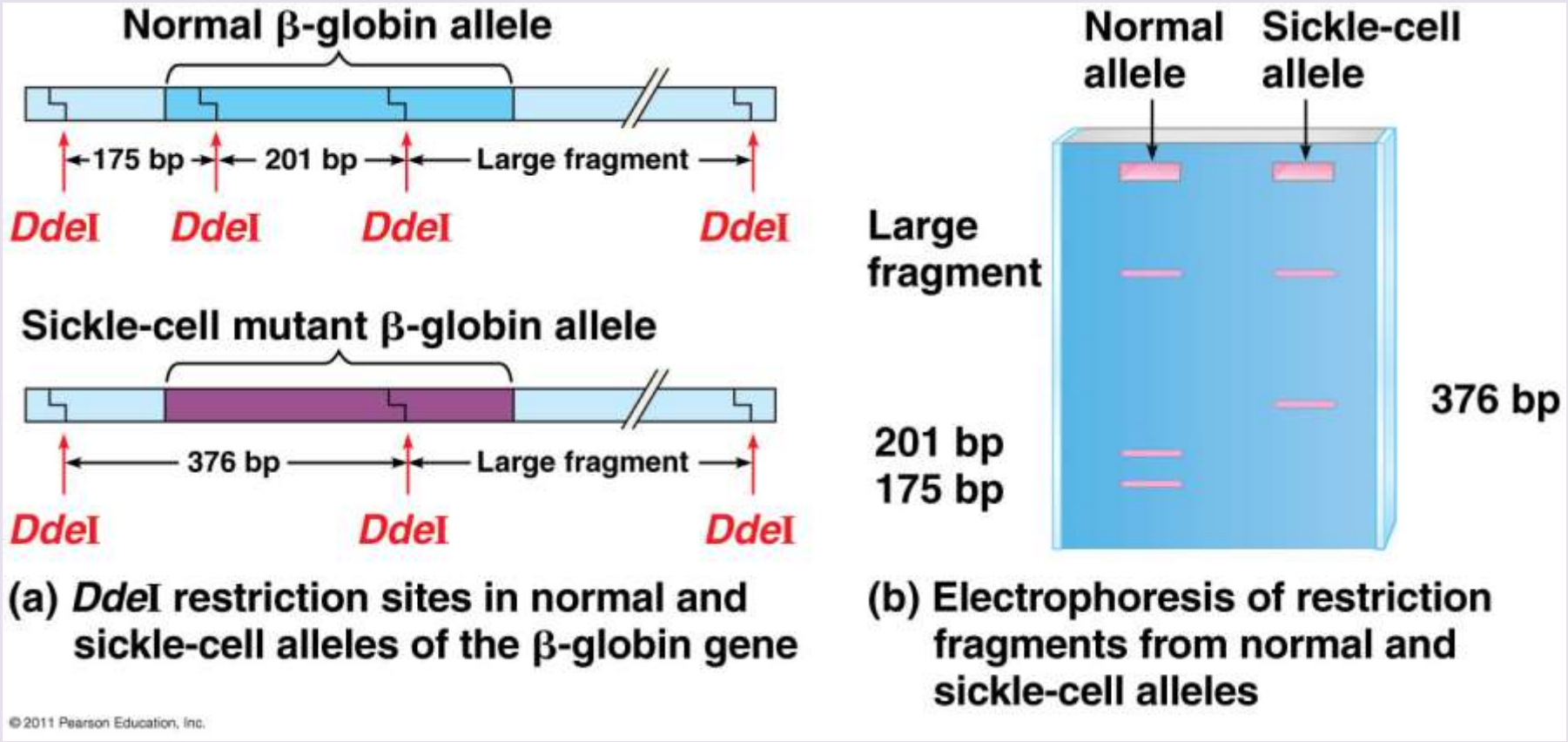
- Used for paternity tests, crime scenes, etc.

TCGTCAGTAACTGAATTCATCGCAATGAATTCACTGCTAC = three fragments

Mutation eliminates an EcoR1 site

TCGTCAGTAACTGAATTCATCGCAATGAATTCACTGCTAC = two fragments



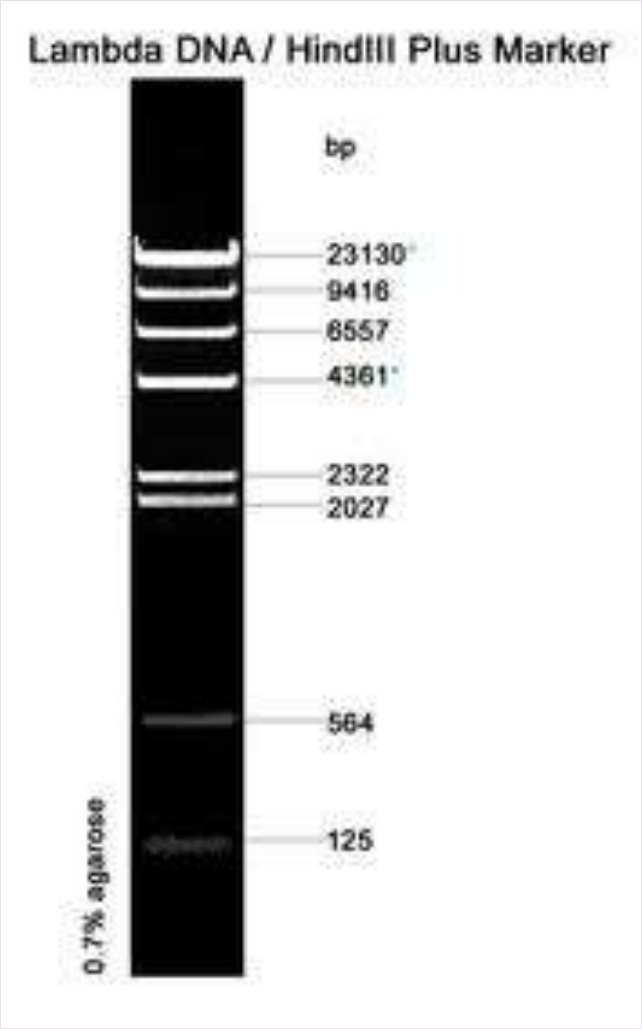


(a) *Dde*I restriction sites in normal and sickle-cell alleles of the β-globin gene

(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

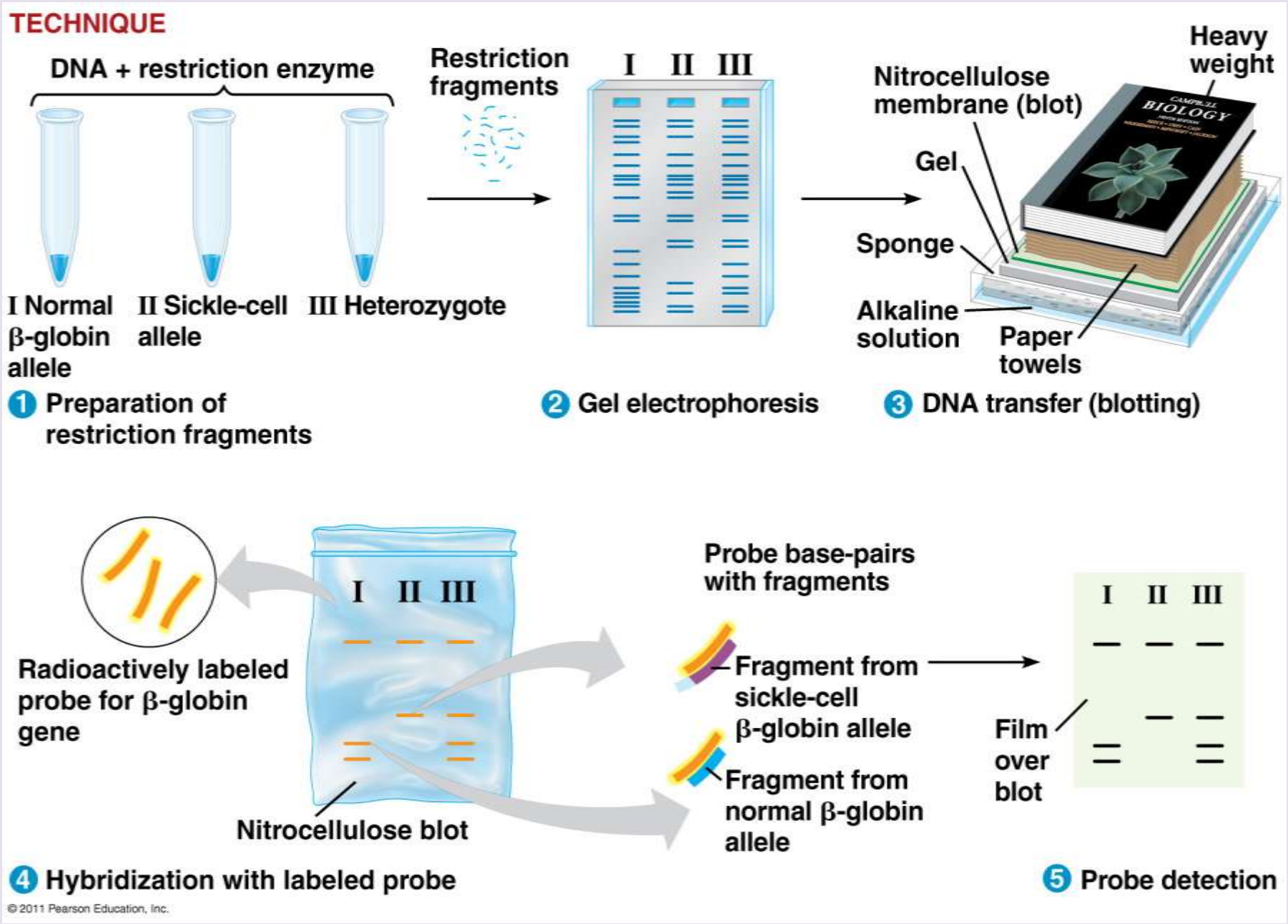
How do you know the size of your fragments?

- We will often run a well with a known DNA sample alongside the unknowns
- Lambda virus DNA cut by the restriction enzyme HindIII produces very distinct, defined fragment sizes every time
- You can then compare the length the known sequences travel to your unknowns



Blotting

- What happens when something like a lambda ladder is not available?
- You can identify the presence of a certain DNA fragment or protein using special detection chemicals
  - These chemicals will bind only to a certain protein/DNA sequence
- However…you have to extract your DNA fragments from your gel first
- This is called blotting


TECHNIQUE
DNA + restriction enzyme
Restriction fragments
I Normal β-globin allele
II Sickle-cell allele
III Heterozygote
I II III
Nitrocellulose membrane (blot)
Heavy weight
Gel
Sponge
Alkaline solution
Paper towels
1 Preparation of restriction fragments
2 Gel electrophoresis
3 DNA transfer (blotting)
Radioactively labeled probe for β-globin gene
Nitrocellulose blot
Probe base-pairs with fragments
Fragment from sickle-cell β-globin allele
Fragment from normal β-globin allele
Film over blot
4 Hybridization with labeled probe
5 Probe detection
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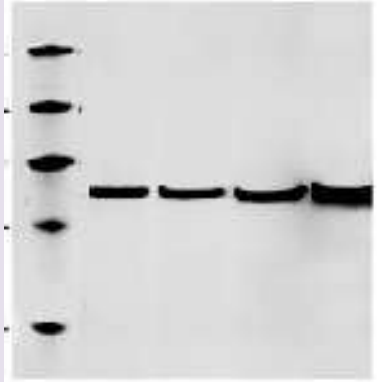
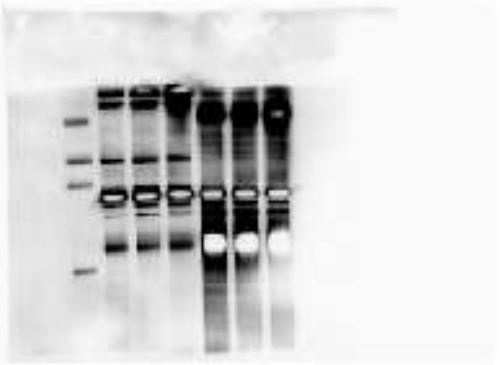
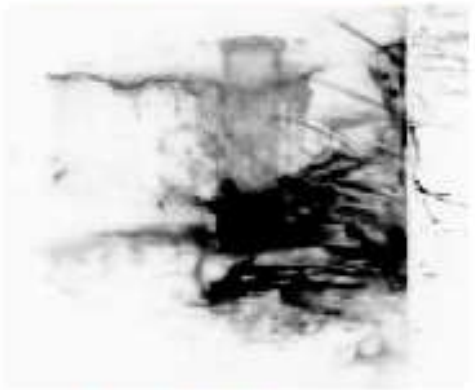
Types of blots

- Western blotting: used to detect specific proteins in a sample
- Northern blotting: used to detect specific RNA sequences in a sample
- Southern blotting: used to detect specific DNA sequences in a sample

THERE ARE THREE KINDS OF WESTERNS...







THE GOOD THE BAD AND THE UGLY

Vocabulary

- Western/Northern/Southern blotting
- Restriction enzymes
- Restriction site
- Gel electrophoresis
- RFLP