Molecular Biology (2)

Restriction endonucleases, RFLP, and gene cloning

Mamoun Ahram, PhD
Second semester, 2018-2019

Resources

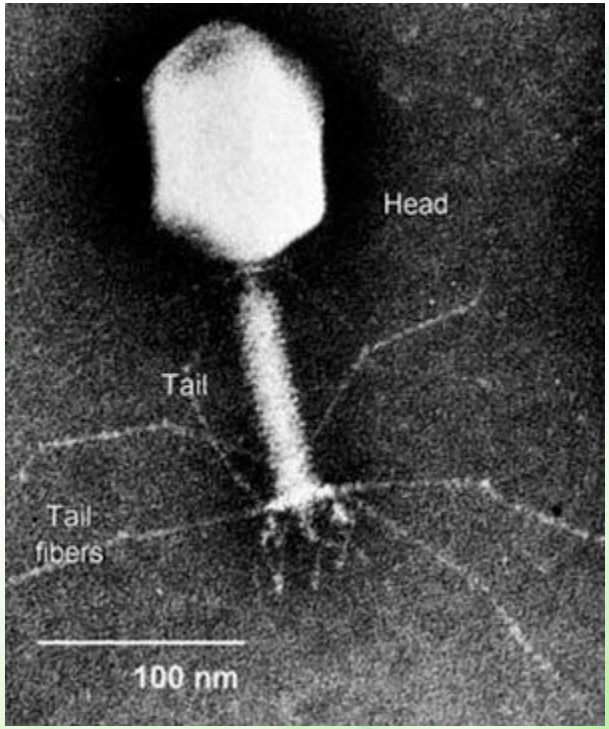
- This lecture
- Cooper, pp 120-124

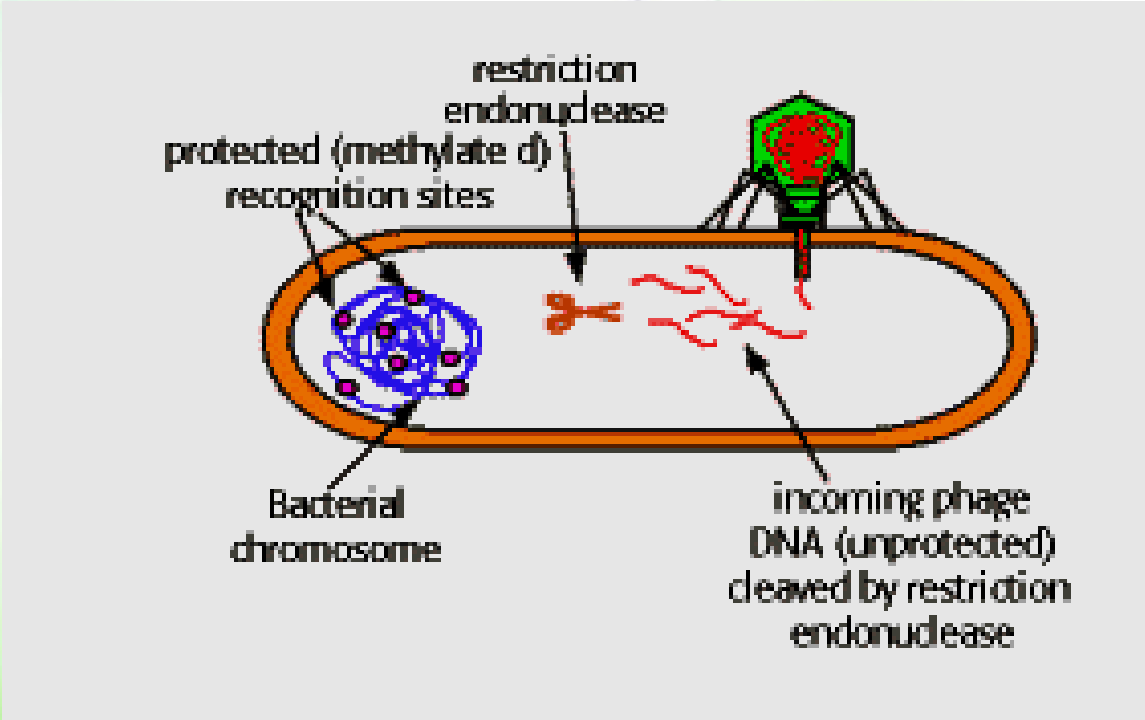
Endonucleases

- Enzymes that degrade DNA within the molecule rather than from either end (exonucleases)
- Restriction endonucleases: Enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at *specific* sequences (4- to 8-bp restriction sites) generating restriction fragments.
- Type II restriction endonucleases: Always cleave always at the same place generating the same set of fragments
  - *Eco*RI (isolated from *E. coli*) cuts at 5'-GAATTC-3‘

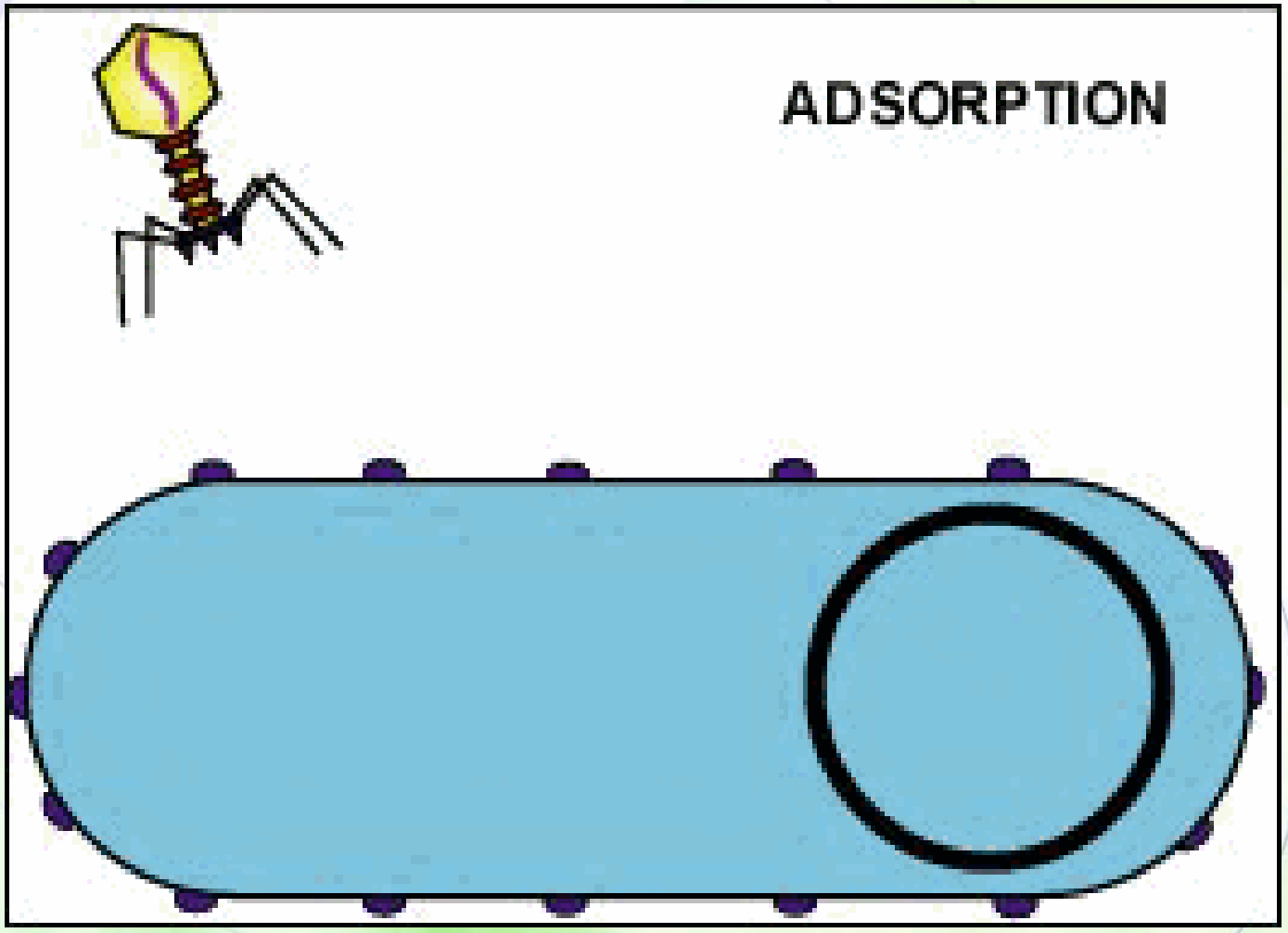
Biological purpose of restriction endonucleases

- They are present in bacteria to protect them from bacteriophages that infect bacteria by transferring their DNA into them restricting their growth.



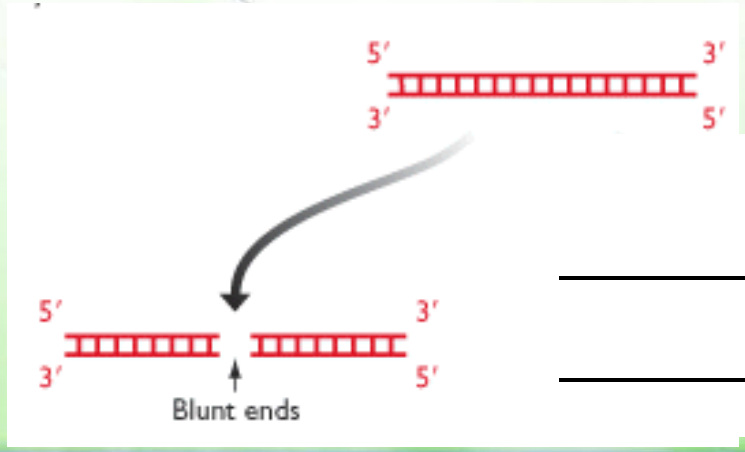


What is transduction?



Types of cleavages

- Restriction enzymes cut DNA in two different ways:
  - Blunt: enzymes cut at the same position on both strands giving a blunt ended fragments.
  - Staggered (off-center): enzymes cut the two DNA strands at different positions generating sticky or cohesive ends.
    - The DNA fragments have short single-stranded overhangs at each end.



Palindromic sequences

- The sequences recognized by restriction endonucleases—their sites of action—read the same from left to right as they do from right to left (on the complementary strand).

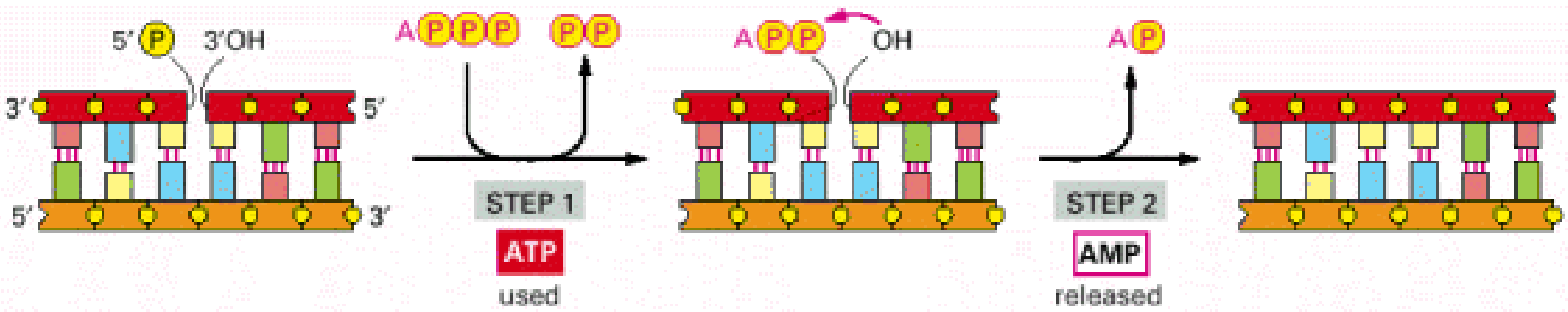
```
EcoRI       5'  GAATTC  3'
            3'  CTTAAG  5'

HindIII     5'  AAGCTT  3'
            3'  TTCGAA  5'

SmaI        5'  CCCGGG  3'
            3'  GGGCCC  5'
```

DNA ligase

- It covalently joins DNA ends (example, restriction fragments).
- It catalyzes the formation of phosphodiester bonds between the 3’-hydroxyl group of one strand and the 5’-phosphate end of another strand.



Advantage of restriction endonucleases

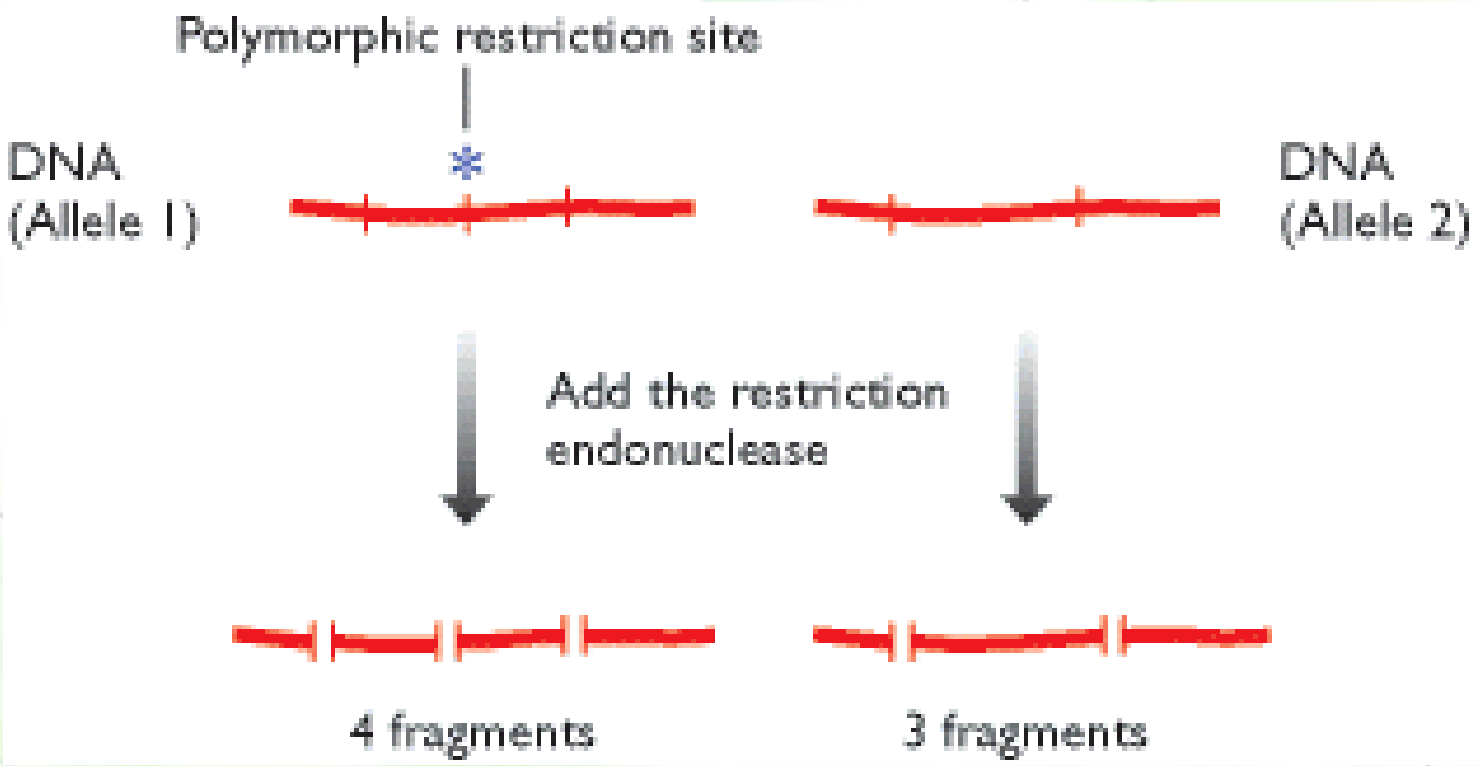
- Restriction fragment length polymorphism (RFLP)
- Cloning

DNA polymorphisms

- Individual variations in DNA sequence (*genetic variants*) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

- Remember: our cells are diploid (alleles can be homozygous or heterozygous)

- What is an allele?


Polymorphic restriction site
DNA
(Allele 1)
DNA
(Allele 2)
Add the restriction
endonuclease
4 fragments
3 fragments

Restriction fragment length polymorphism

- The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP.
- These can be detected by
  - Gel electrophoresis
  - Southern blotting

Example

Variant 1

*Eco*RI does not cut

GCCGCATTCTA
CGGCGTAAGAT

Variant 2

*Eco*RI does cut

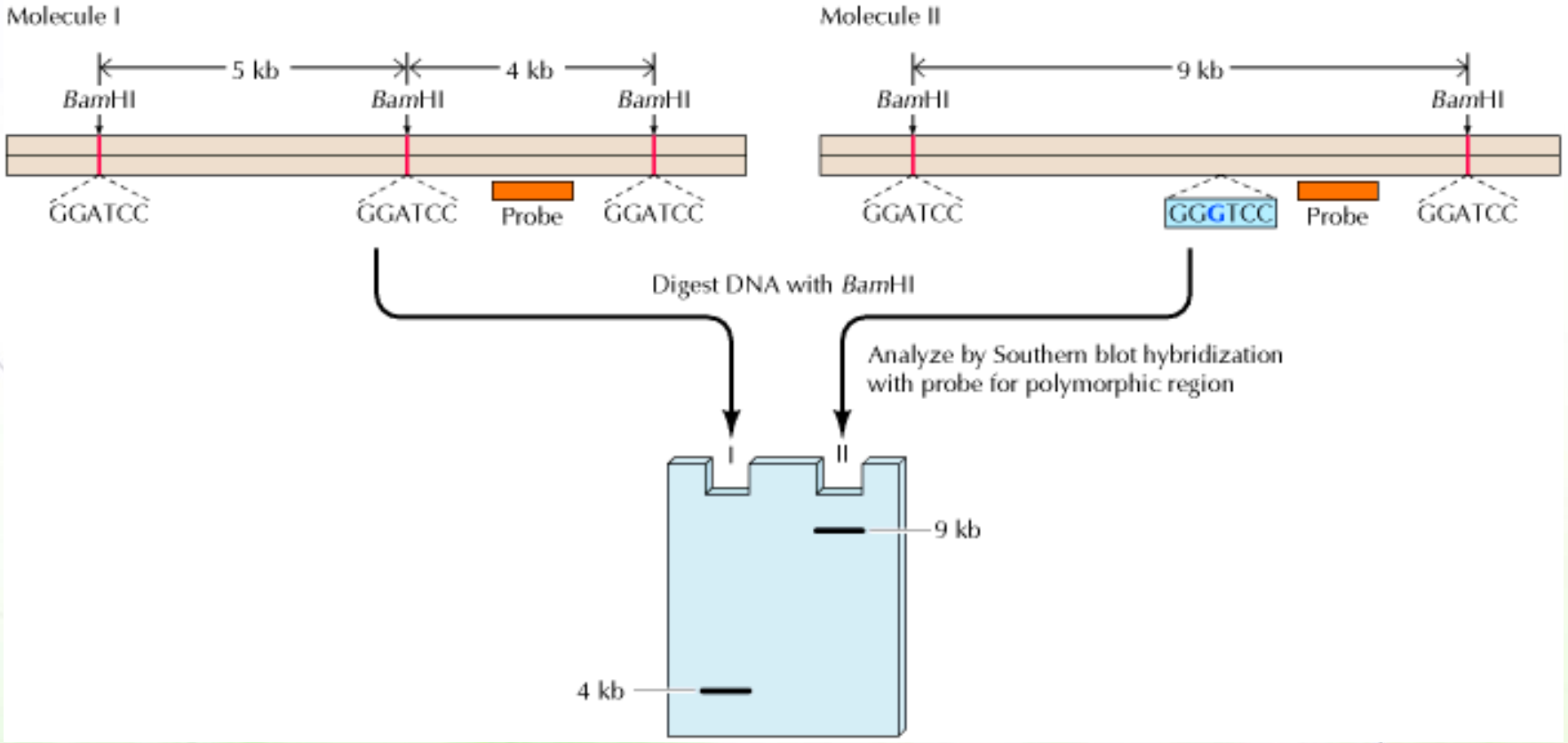
GCCGAATTCTA
CGGCTTAAGAT

RFLP in the clinic

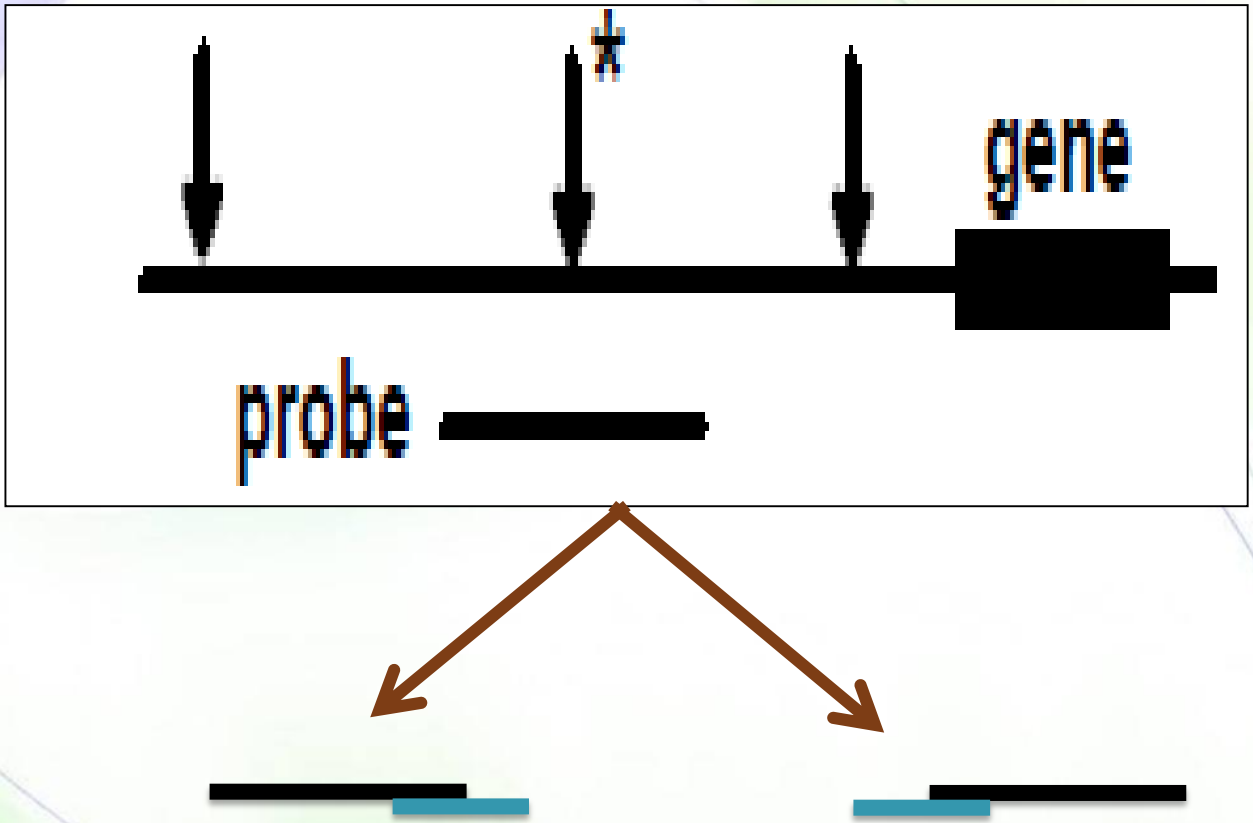
- RFLP can be used as diagnostic tools.

- For example, if a mutation that results in the development of a disease also causes the generation of distinctive RFLP fragments, then we can tell:
  - if the person is diseased as a result of this mutation
  - from which parent this allele is inherited

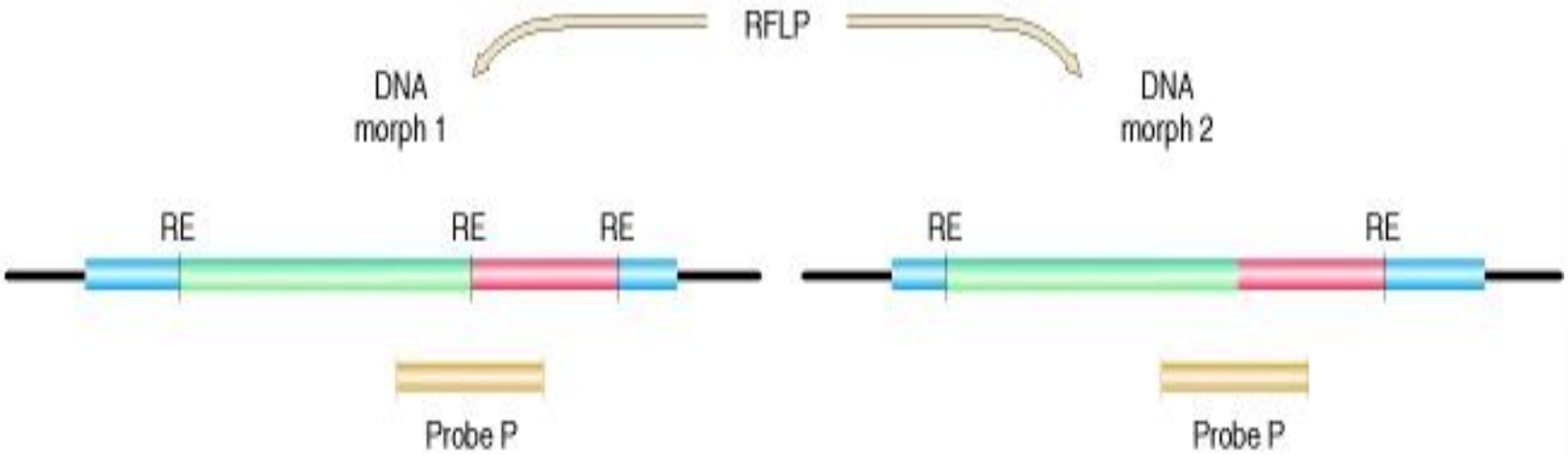
Disease detection by RFLP



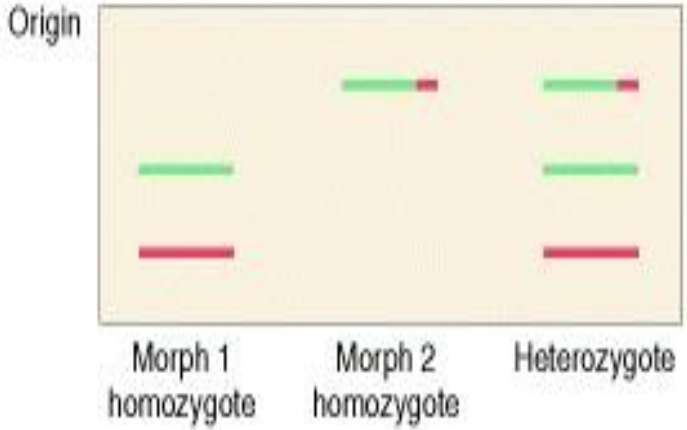
Think!! What would you see in a gel? Why?



As long as the probe can form enough hydrogen bonds with a DNA fragment, it hybridizes.


RFLP
DNA
morph 1
DNA
morph 2
RE
RE
RE
RE
RE
Probe P
Probe P

Southern


Origin
Morph 1
homozygote
Morph 2
homozygote
Heterozygote

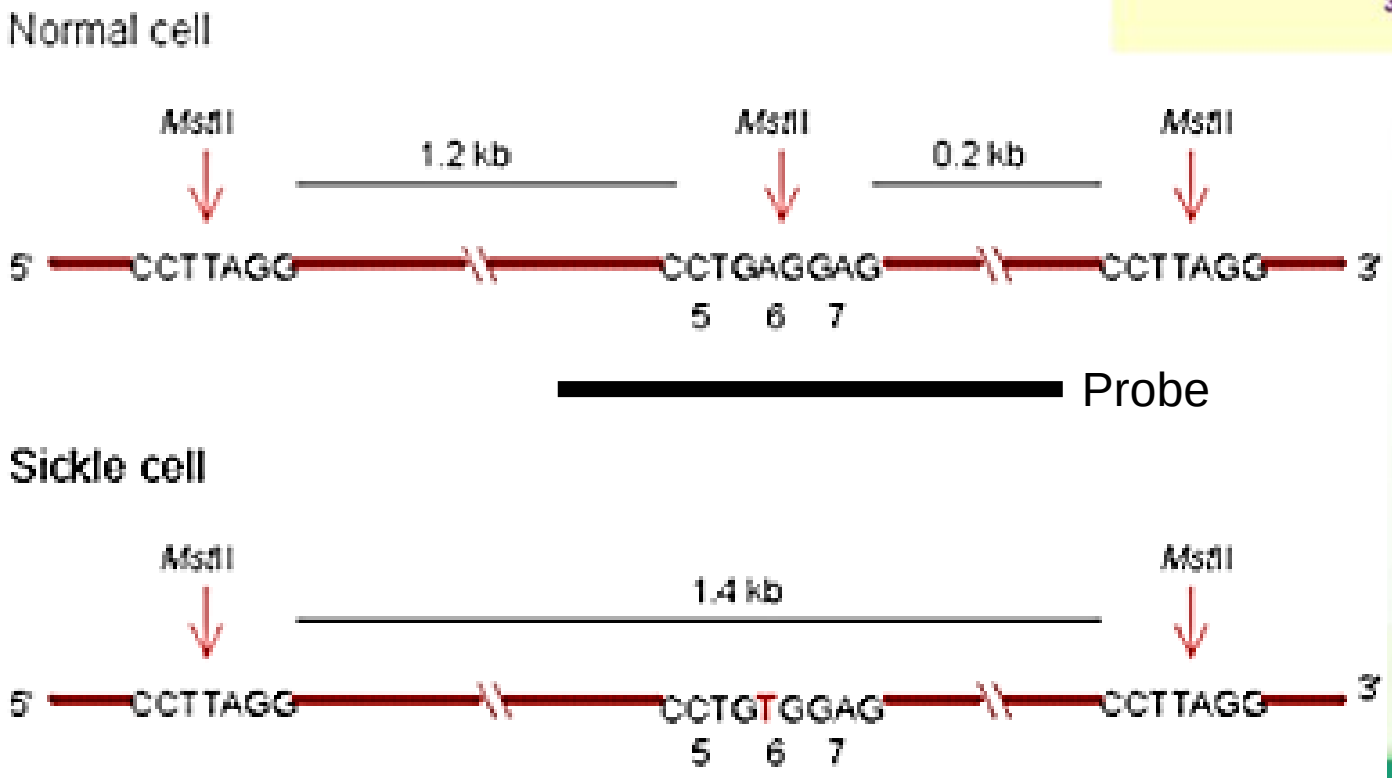
Example 1: Disease detection by RFLP (sickle cell anemia)

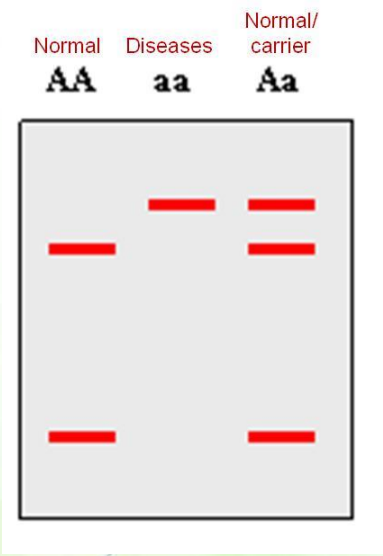
HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

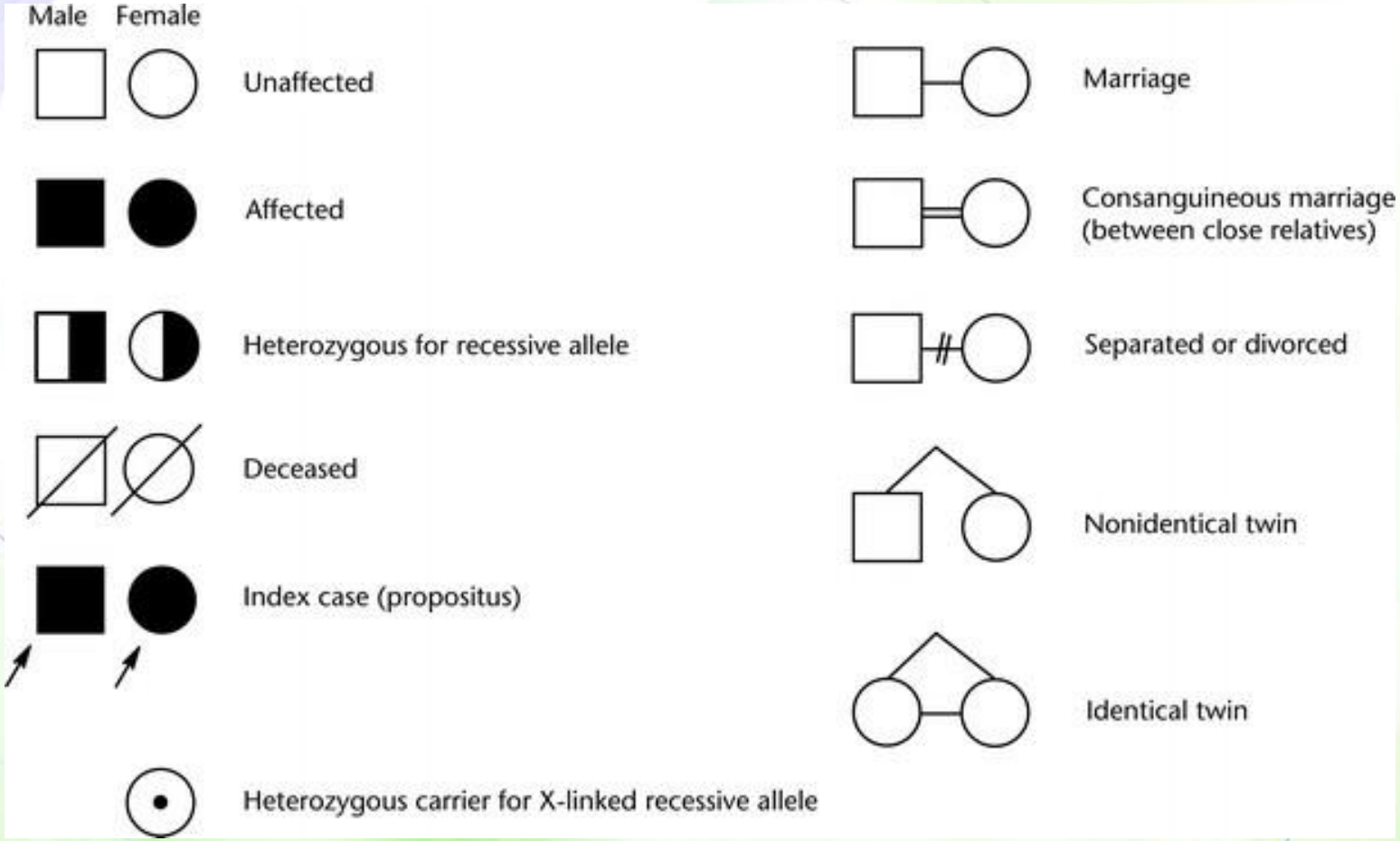
HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9

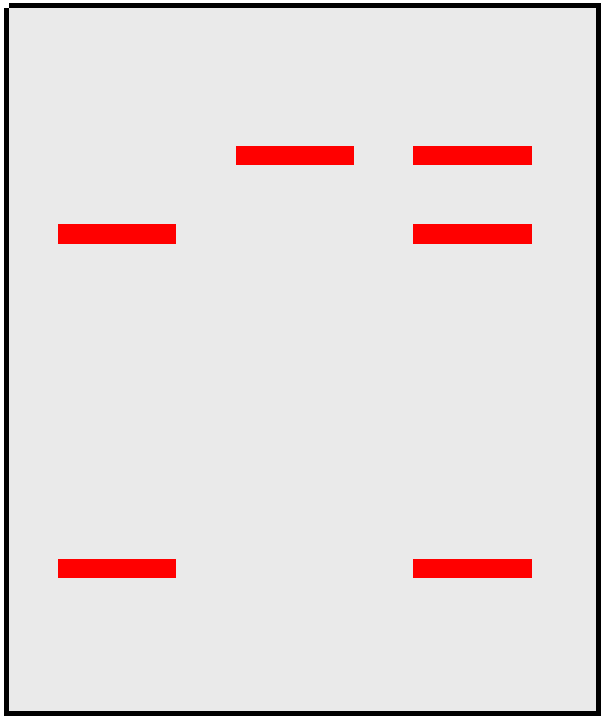


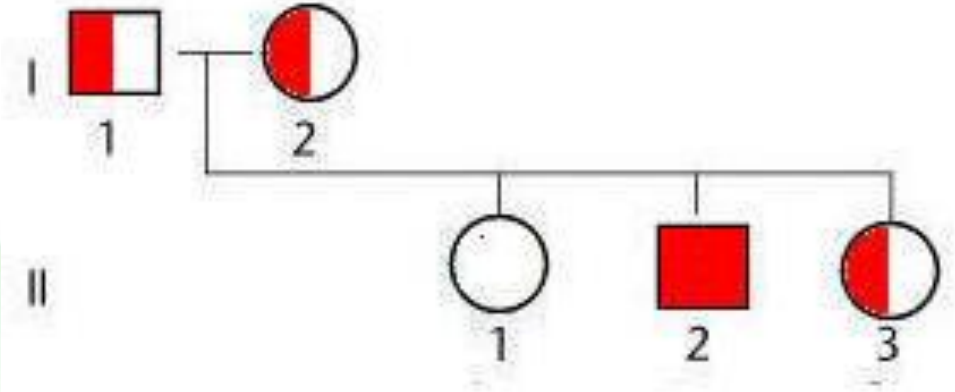


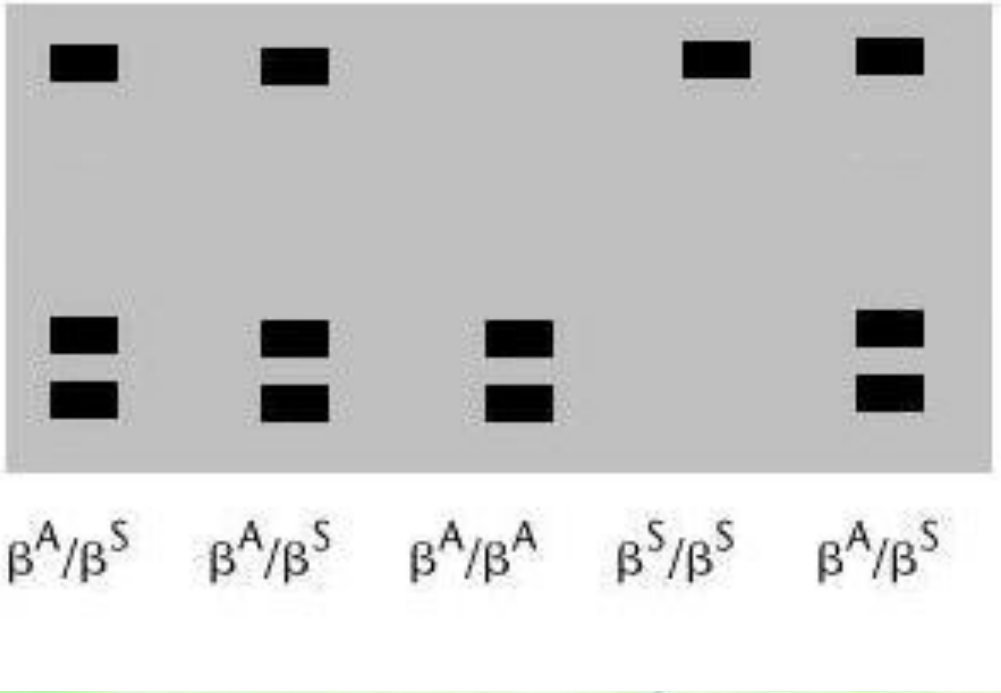
Supplementary information



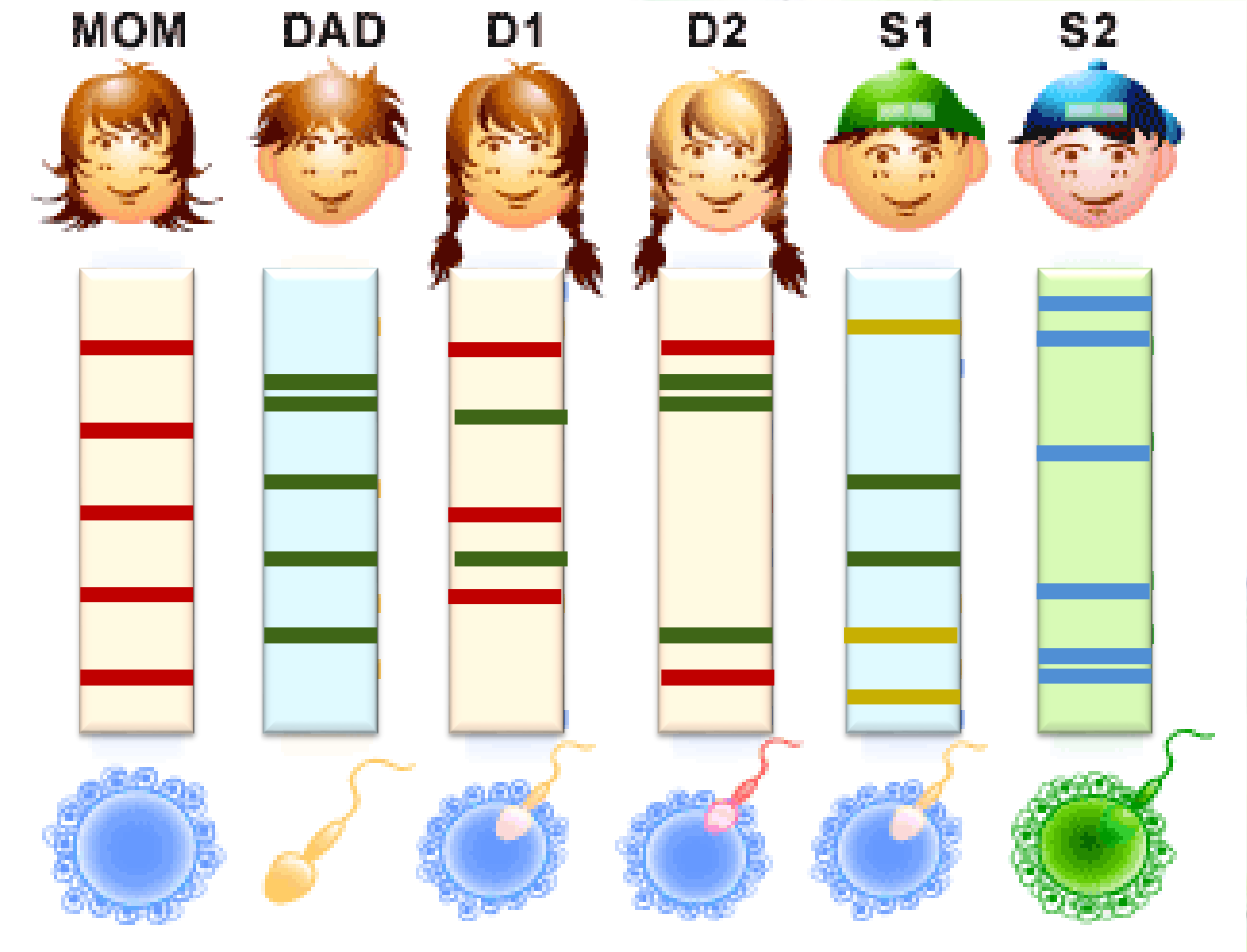
Normal	Diseases	Normal/ carrier
AA	aa	Aa



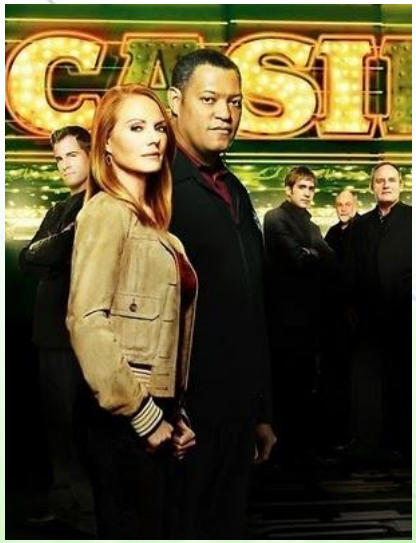


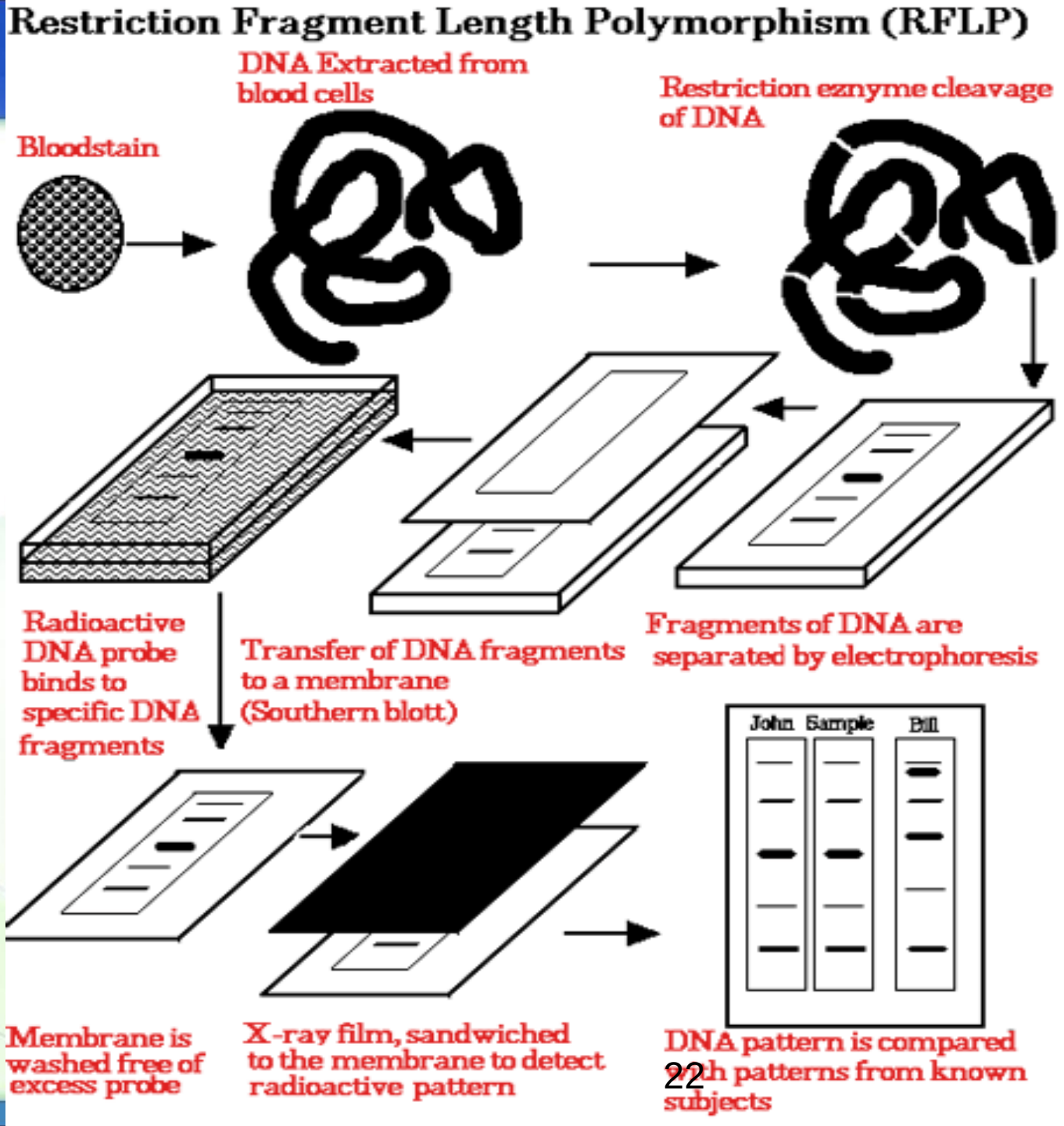


Example 2: Paternity testing



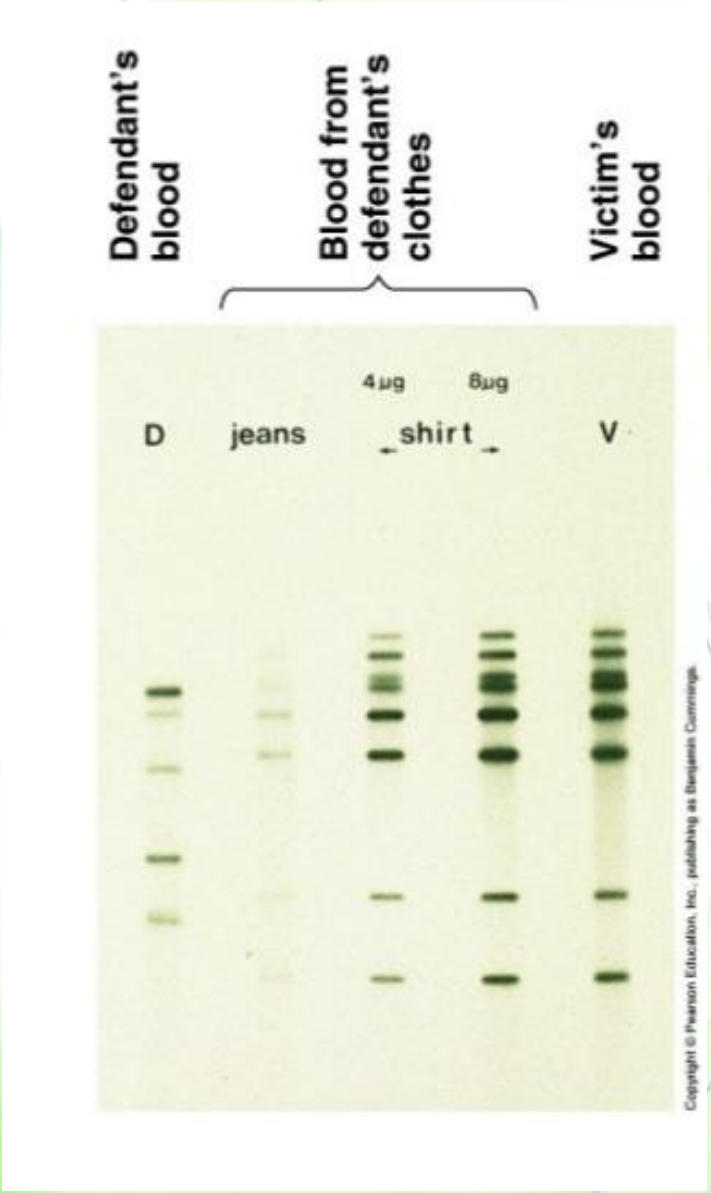
Example 3: Forensics



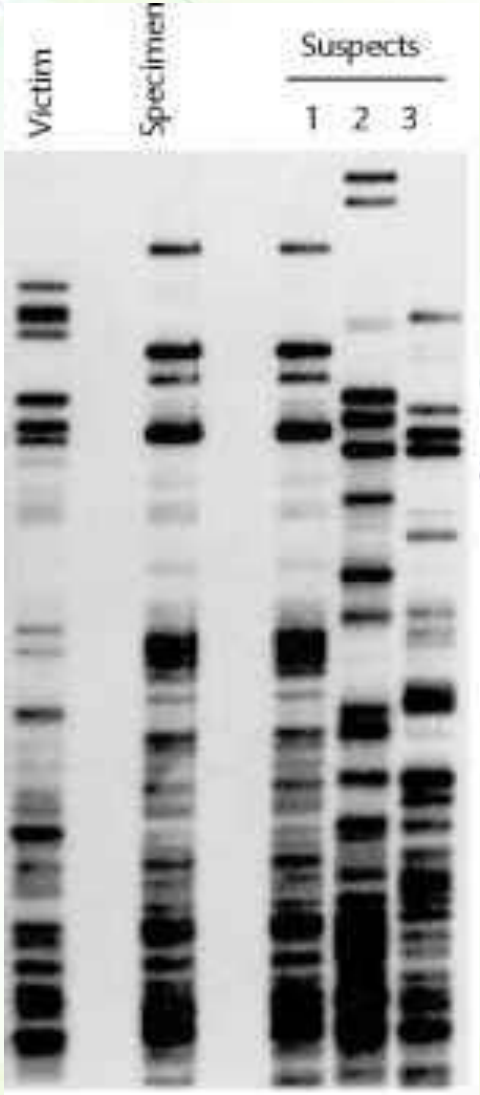


Real case


A

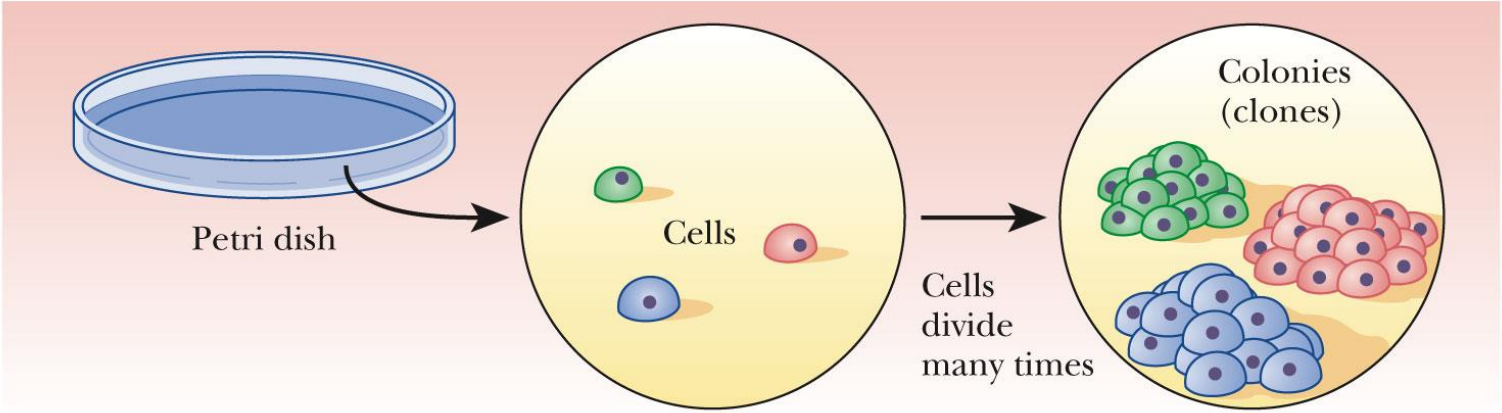

Defendant's blood
Blood from defendant's clothes
Victim's blood
4 µg
8µg
D
jeans
shirt
V
Copyright © Pearson Education, Inc., publishing as Benjamin Cummings.

More real cases



Cloning

- Cloning means that you make several copies of one thing.
- A clone is a genetically identical population, whether of organisms, cells, viruses, or DNA molecules.
- Every member of the population is derived from a single cell, virus, or DNA molecule.



How do we clone a DNA molecule?

- a DNA fragment of interest is inserted into a DNA carrier (called a vector) that can be replicated.
- The resulting DNA molecule is what is known as a recombinant DNA molecule.

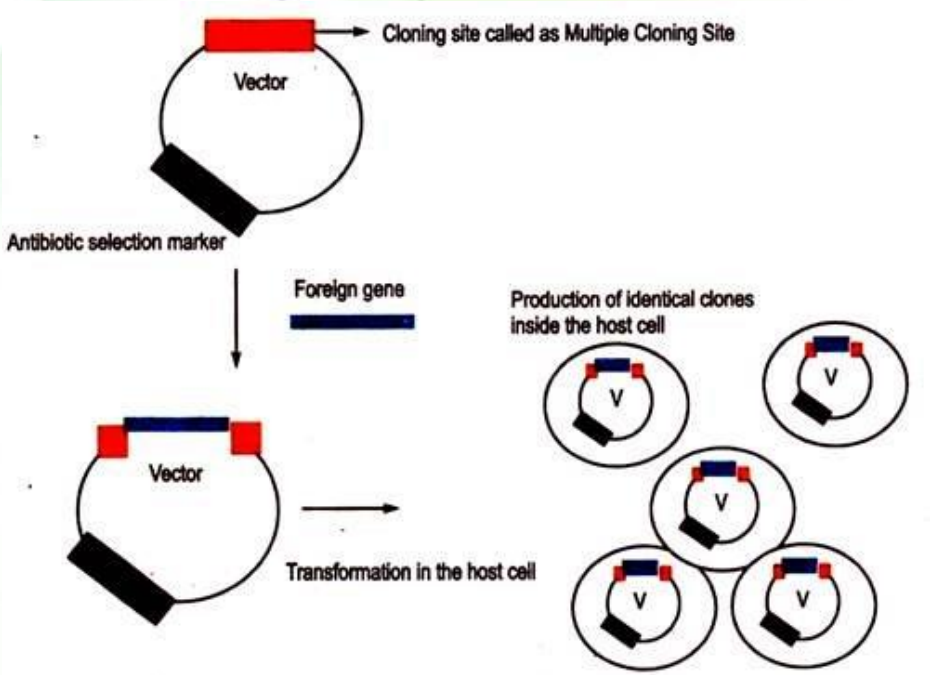
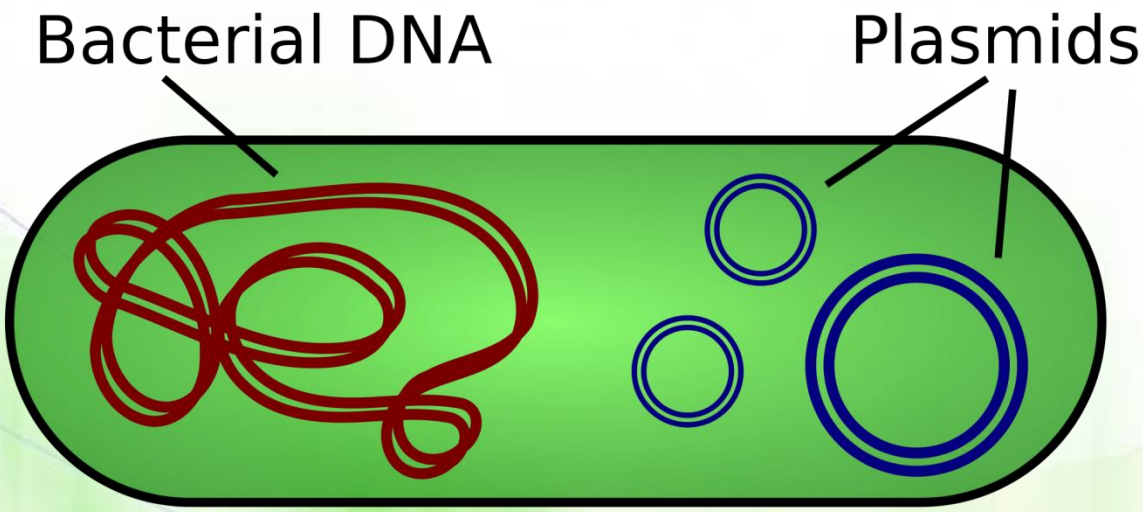


Fig. 8-4 : *Cloning and production of identical DNA Molecules*

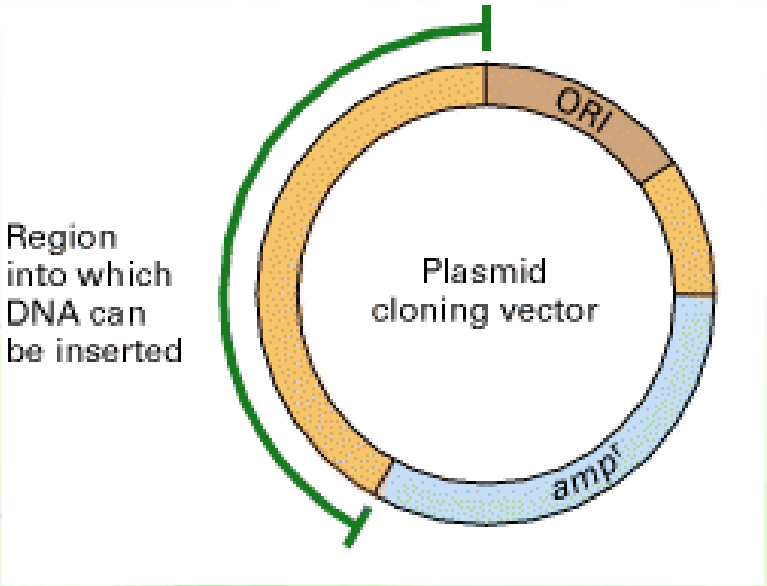
Using plasmids as vectors

- Bacterial plasmids are considered excellent vectors.
- These are bacterial circular DNA that is not part of the main circular DNA chromosome of the bacterium.
- A plasmid exists as a closed circle and replicates independently of the main bacterial genome.



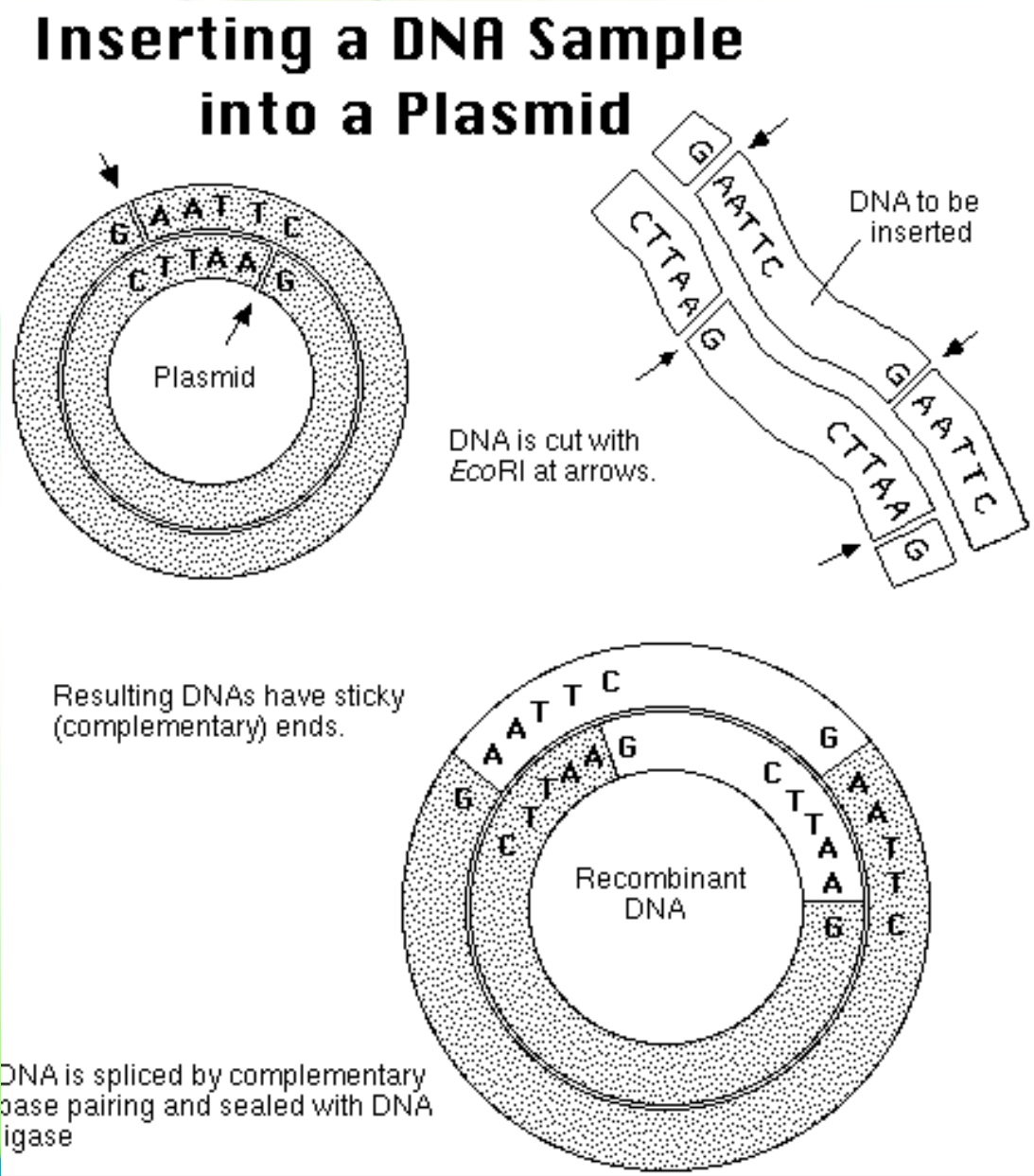
Features of plasmids

- Most plasmid vectors contain at least three essential parts required for DNA cloning:
  - Can replicate
  - Can be selected for/against by an internal drug-resistance gene (selectable marker)
  - Can insert a foreign DNA fragment

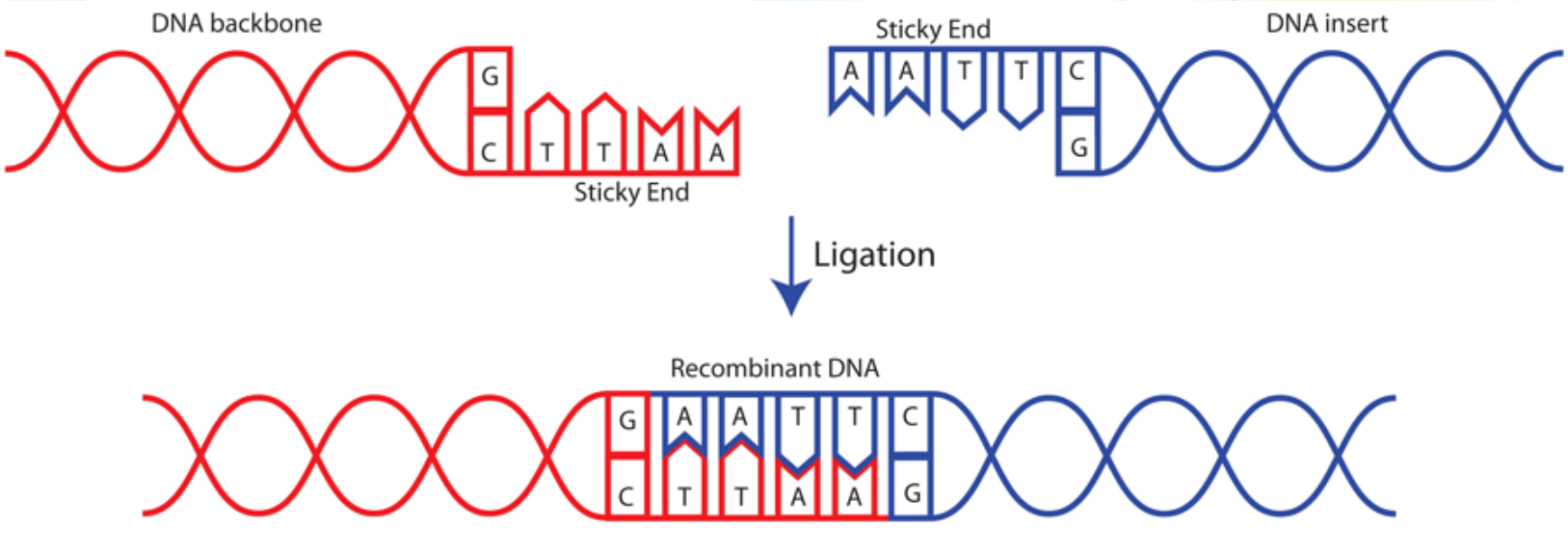


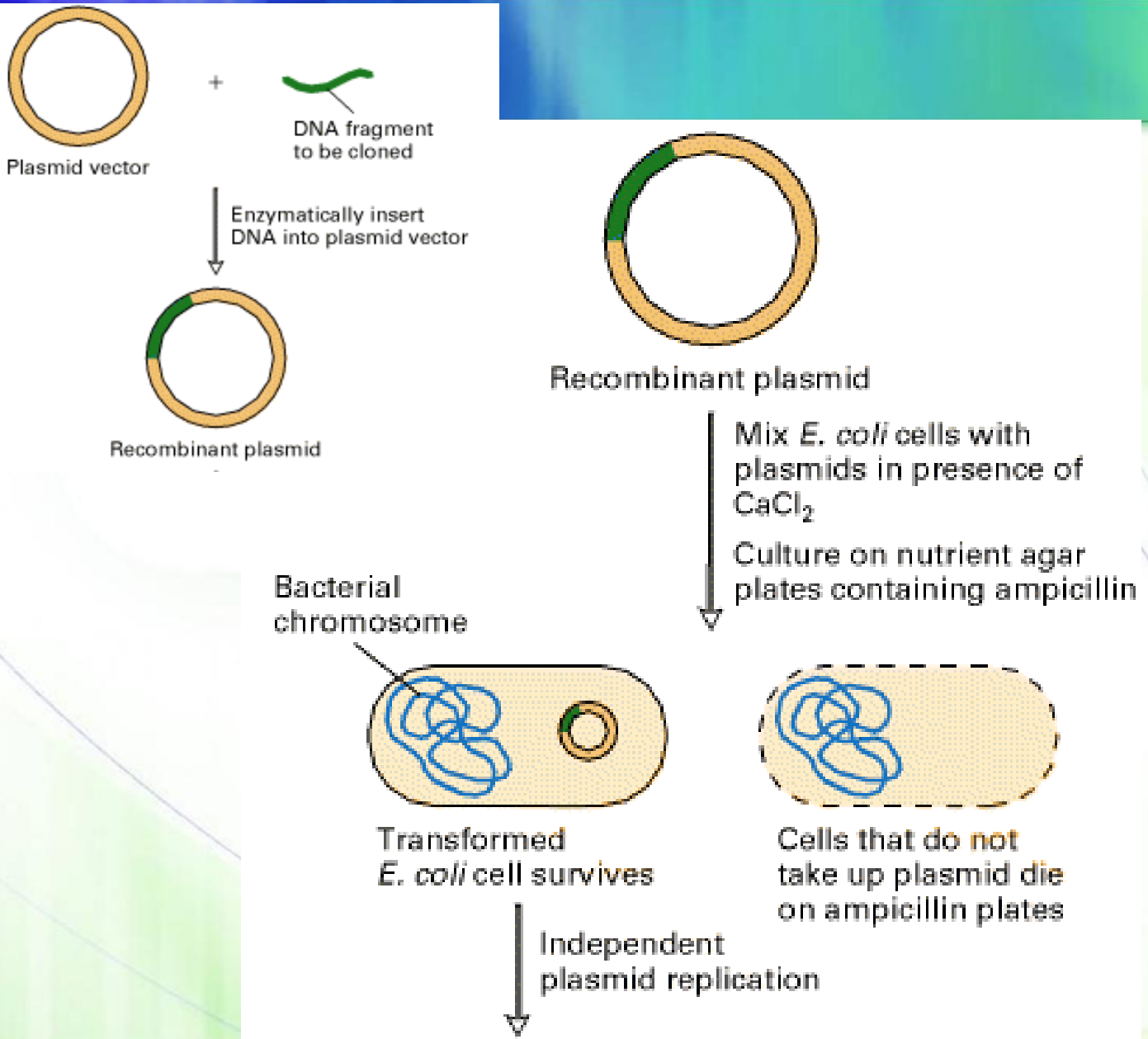
Making of recombinant DNA

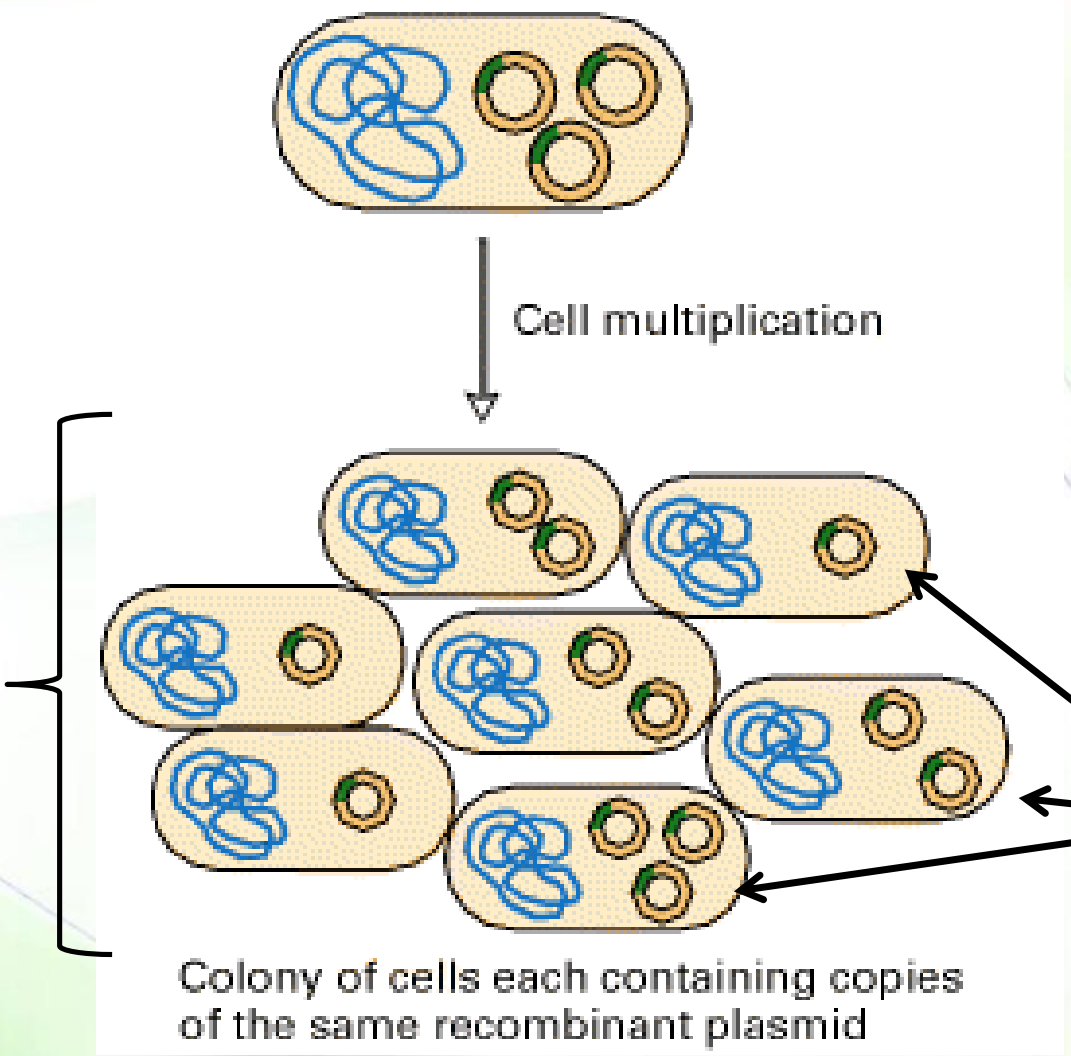
- Both DNA fragments (the DNA to be cloned and a vector) are cut by the same restriction endonuclease that makes DNA fragments with same sticky-ends that hybdridize to each other, when mixed.



Zoom into the sticky ends




Plasmid vector
+
DNA fragment to be cloned
Enzymatically insert DNA into plasmid vector
Recombinant plasmid
Recombinant plasmid
Mix E. coli cells with plasmids in presence of $CaCl_2$
Culture on nutrient agar plates containing ampicillin
Bacterial chromosome
Transformed E. coli cell survives
Cells that do not take up plasmid die on ampicillin plates
Independent plasmid replication


Cell multiplication
Cell clones
Cloned DNA
Colony of cells each containing copies of the same recombinant plasmid