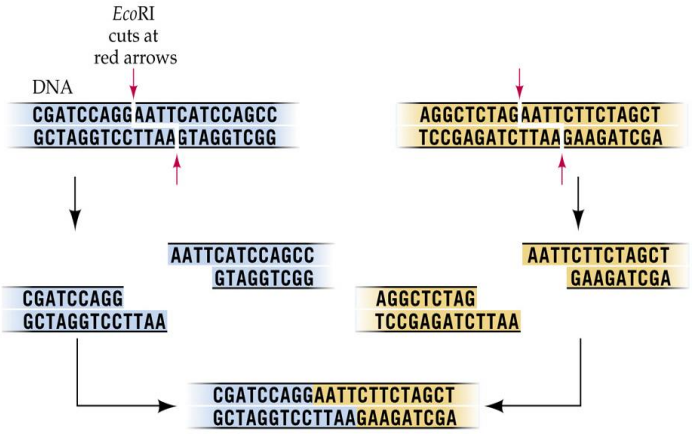
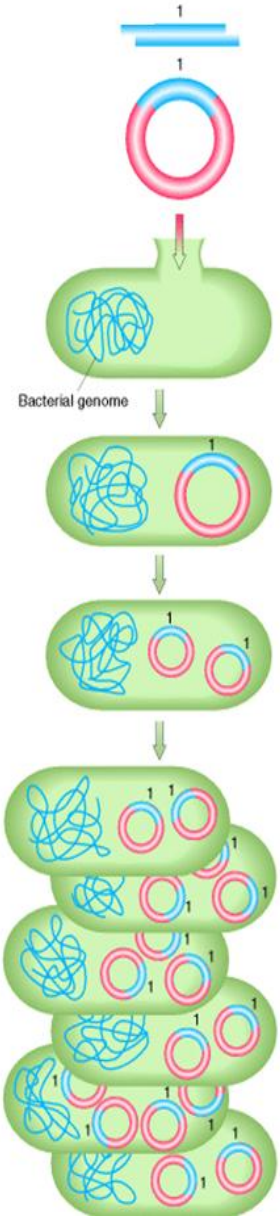
Unit IX – Problem 3 – Genetics: Recombinant DNA

- **What is recombinant DNA technology?**
  - It is the technology which uses genetic engineering to study a specific segment of DNA.
  - Notice that genetic engineering means the manipulation of DNA sequence and the construction of chimeric molecules (molecules containing sequences derived from two different genes: see the image).



- **How is recombinant DNA done technically (see the image)?**
  - It is made by splicing (ربط) a foreign DNA segment into a small replicating molecule (such as bacterial plasmid: it is a small DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently).
  - Plasmid will replicate that foreign DNA fragment along with itself and result in a molecular clone (نسخة مماثلة) of the inserted DNA.



- **What is the importance of studying recombinant DNA technology?**
  - Understanding of molecular basis of inherited diseases (example: sickle-cell disease).
  - Provision of human proteins for therapeutic purposes (examples: insulin and growth hormone).
  - Provision of proteins as vaccines.
  - Diagnostic tests (example: AIDS test).
  - Diagnosis and prediction (التنبؤ) of genetic diseases.
  - Forensic medicine (الطب الشرعي).
  - Gene therapy is promising (example: sickle-cell disease).
- **Review of DNA structure:**
  - It is a double-helix with two anti-parallel strands connected to each other by complementary base-pairing:
    - ✓ (A) – (T)
    - ✓ (G) – (C)

      Note: these bases have hydrogen bonds between them.
- **What are the tools which are used in recombinant DNA?**
  - **Chimeric molecules.**
  - **Restriction enzymes:**
    - ✓ They are enonucleases which cut specific DNA sequences enzymatically, chemically or physically (cutting a sequence from the plasmid of bacteria to result in a linear molecule with single-stranded sticky ends which are useful in constructing chimeric DNA).
      - ❖ Sticky end ligation is easy but may reconnect with each other or may not be in right position.

- Sticky ends are preferable to blunt ends.
- *For blunt ends, problems are solved by:*
  - New ends added by special enzymes.
  - The use of synthetic oligonucleotide linkers that could be ligated to blunt-ended DNA.
  - T4 DNA ligase can be used for direct blunt-end ligation.

✓ Why the name? → because they restrict the growth of certain bacterial viruses called bacteriophages. They are defensive enzymes protecting DNA of the host bacteria from foreign DNA of bacteriophages.

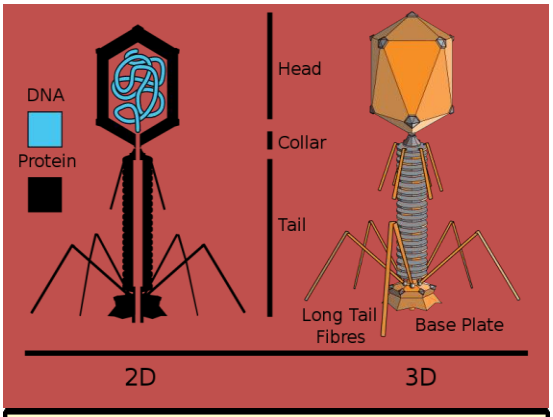
✓ Nomenclature of restrictive enzymes (example): EcoRI

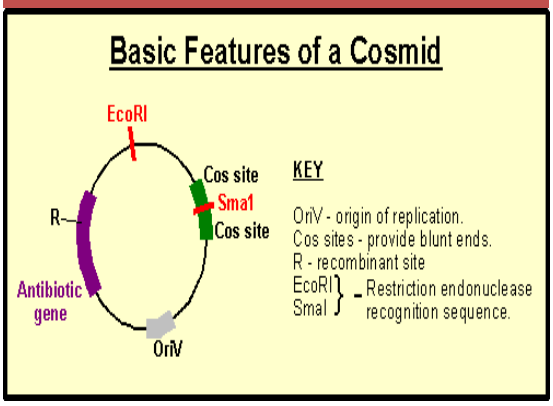
- *First latter*: E – for genus of bacteria from which the enzyme is isolated.
- *Second and third letters*: co – species.
- *R*: for strain.
- *Roman number*: stands for order of discovery.

- **Vectors:**

✓ There are two types of vectors which can be used in recombinant DNA technology:

- *Cloning vectors:*
  - What is a clone? → a large population of identical molecules arising from a common ancestor (السَّلَف نفسه).
  - Cloning: it is the generation of a large number of identical DNA molecules.
  - Cloning vectors (e.g. plasmids) are used to construct chimeric DNA.
  - Host cell: is used to replicate the chimeric DNA.





Cloning vectors	
Plasmids	*Small, circular, duplex DNA molecules. *Normal Function: confer antibiotic resistance to host cell (the bacteria) *Found single or multiple in the same bacterium and can replicate independently from bacterial DNA. *DNA sequences and restriction sites of many plasmids are known. *Accept DNA fragments of 6-8 kb for cloning.
Phages (see the image)	*They have linear DNA. *Chimeric DNA is collected after the phge completes its lytic cycle. *Accept DNA fragments of 10-20 kb. *Example: phage λ
Cosmids (see the image)	*Have features of both: plasmid and phage. *Accept DNA fragment of 35-50 kb *Contain DNA sequences, cos sites, for packaging lambda DNA into phage.
BAC and YAC	*BAC: Bacterial Artificial Chromosome accepts a DNA fragment of 50-250 kb. *YAC: Yeast Artificial Chromosome accepts a DNA fragment of 0.5-3 Mb *Currently replaced plasmids, phages and cosmids.

- *Expression vectors:*
  - Cloning vectors do not permit expression of cloned genes but expression vectors do.
  - Because bacteria cannot process introns, the cloned sequences must be stripped of introns.
  - Cloned genes are inserted next to bacterial transcription and translation start signals.
  - Some expression vectors have restriction sites located just next to a (lac regulatory region).

- These sites permit foreign DNA to be spliced into the vector for expression under the control of the (lac regulatory system).
- Some vectors contain protease inhibitor genes to enhance the final yield of the protein product.

- **Host (bacteria or transgenic animals الحيوانات المعدَّلة وراثياً).**
- **Others (example: ligase).**