- **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 letter: 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 (السَّلّف 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.

<table>
<thead>
<tr>
<th>Cloning vectors</th>
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<tbody>
<tr>
<td><strong>Plasmids</strong></td>
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| *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.*

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<th>Phages (see the image)</th>
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| *They have linear DNA.*
| *Chimeric DNA is collected after the phage completes its lytic cycle.*
| *Accept DNA fragments of 10-20 kb.*
| *Example: phage λ.*

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<th>Cosmids (see the image)</th>
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| *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.*

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<th>BAC and YAC</th>
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| *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 لحيوانات المعدلة وراثياً).
- Others (example: ligase).