Lecture 13

Biotechnology

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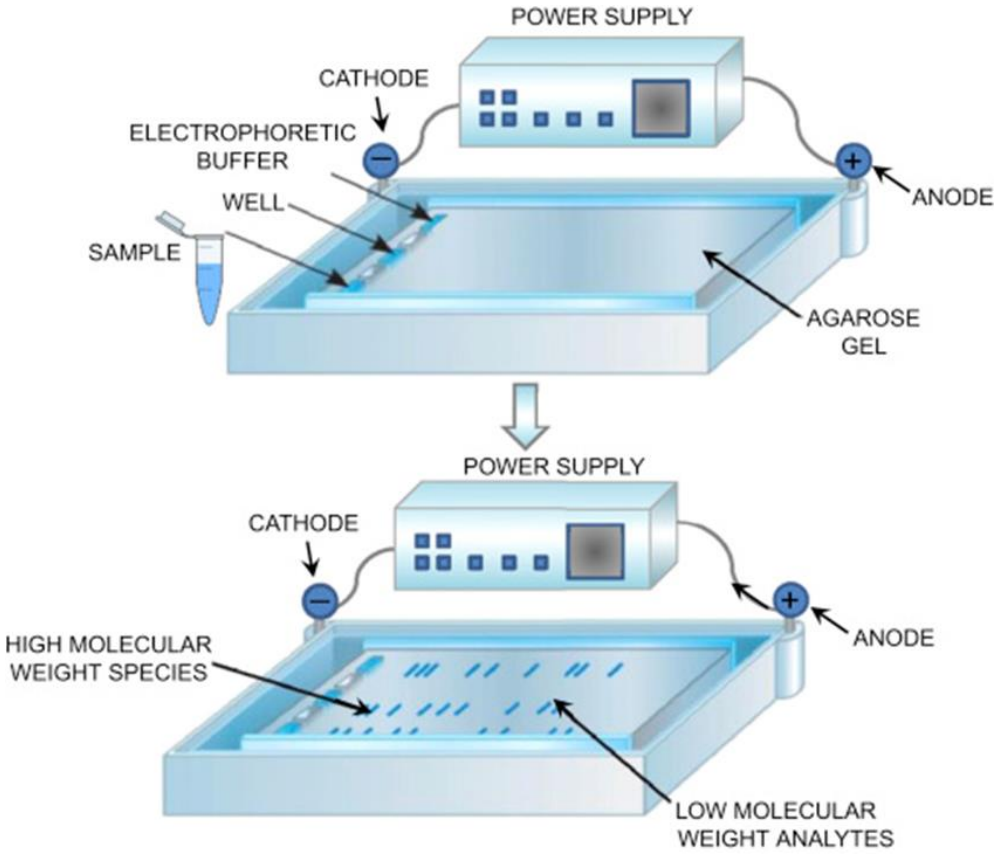
Ref book: Biology for Engineers - Arthur T. Johnson [2nd edition]

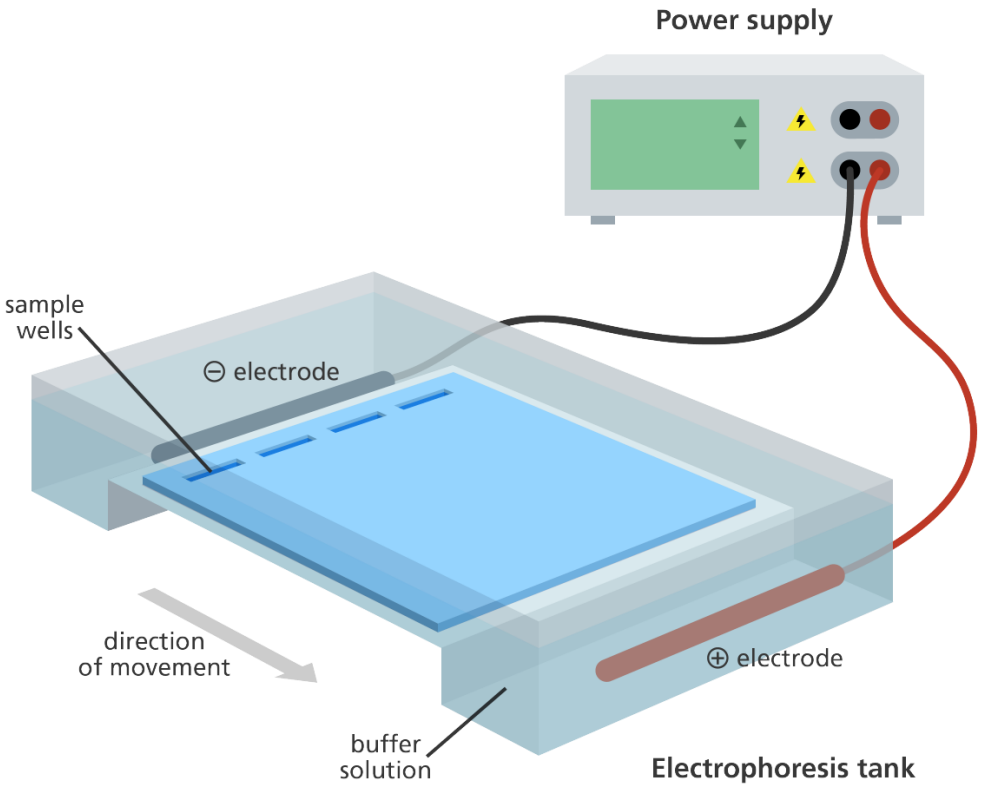
Biology for Engineers – G. K. Suraishkumar

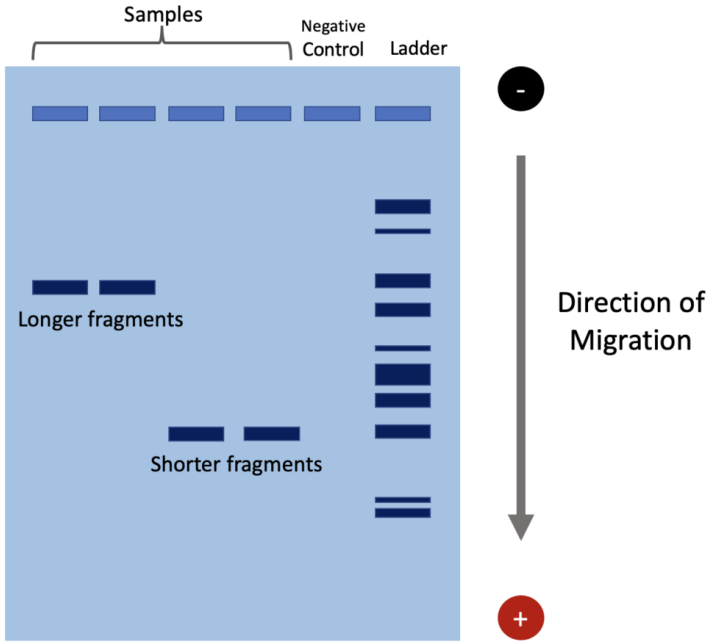
Gel Electrophoresis

- This technique helps to separate DNA, RNA or proteins based on their size.
- For example, if you want to know the sizes of the DNA fragments after restriction enzyme digestion, you can use this procedure.
- Here, the DNA is run through a matrix or a gel and separated out. Agarose, a natural polymer from seaweeds is the most commonly used matrix. Let's understand how this technique works.



Gel Electrophoresis





Gel Electrophoresis

- The DNA to be separated is added to the wells of an agarose gel matrix.
- On the application of current, the negatively charged DNA moves to the positive electrode.
- Since the gel is difficult to move through, the DNA fragments travel at different speeds based on their size.
- Larger fragments move slower, while smaller fragments fit through the gel pores easily and move faster.

Gel Electrophoresis

- The separated DNA fragments are not visible under normal light.
- visualized after staining the bands with a compound – ethidium bromide and exposing them to UV radiation.
- Once they are stained and exposed to UV light, you can see the separated DNA bands and determine their sizes using a DNA marker.
- In this way, we can cut out -
  - ✓ the desired size of DNA fragment
  - ✓ extract it and
  - ✓ further ligate it with cloning vectors.
- The techniques of PCR and gel electrophoresis are crucial in the field of forensics, for genetic fingerprinting and identifying crime suspects.

How?

DNA fingerprinting

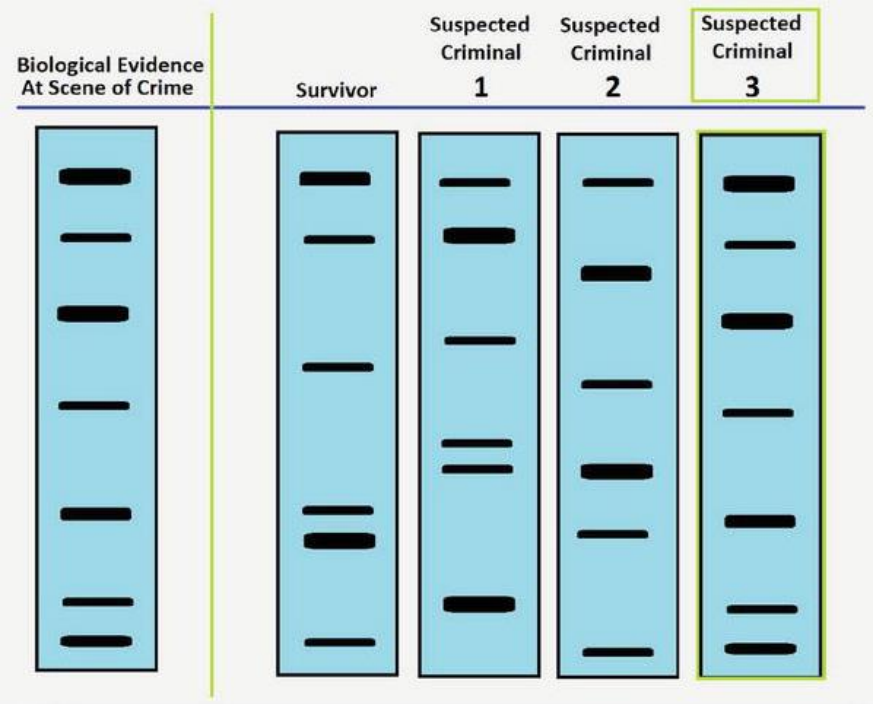
Restriction Fragment Length Polymorphism (RFLP) is a DNA fingerprinting technique that involves the following steps:

DNA extraction: DNA is extracted from a sample, such as blood or saliva, and purified.

DNA fragmentation: The purified DNA is cut into fragments using restriction endonucleases.

Gel electrophoresis: The DNA fragments are separated by size using gel electrophoresis.

Visualization: A labeled DNA sequence, called an RFLP probe, is used to reveal a unique pattern that identifies the DNA.



Recombinant DNA technology

Recombinant DNA, a segment of DNA that is generated by combining genetic material from at least two different species. Such new genetic combinations are of value to science, medicine, agriculture, and industry. (*Ref: Britannica*)

In standard cloning protocols, the **cloning of any DNA fragment** essentially involves **few steps:**

(1) Choice of host organism and cloning vector,

(2) Preparation of vector DNA,

(3) Preparation of DNA to be cloned,

(4) Creation of recombinant DNA,

(5) Introduction of recombinant DNA into the host organism,

(6) Selection.

Applications of rDNA technology

Recombinant DNA (rDNA) technology has many applications, including:

Medicine: rDNA technology has been used to develop **new drugs, vaccines, and pharmaceuticals.** It can also be used to treat genetic disorders by repairing or replacing faulty genes. For example, rDNA technology has been used to produce synthetic human insulin and erythropoietin.

Agriculture: rDNA technology has been used to create genetically modified crops that are resistant to herbicides and pests. For example, Bt-cotton has been genetically modified to protect the plant from ball worms.

Food industry: rDNA technology can be used to improve the nutritional quality of food. For example, the nutritional value of soybeans has been improved by introducing a gene from the Brazil nut.

Applications of rDNA technology

Bioremediation: rDNA technology can be used to clean up polluted soil and water by introducing genetically modified organisms (GMOs) into the site.

Diagnostics: rDNA technology can be used to diagnose infectious diseases and genetic diseases. For example, DNA fingerprinting can be used to determine paternity.

Immunotherapy:

rDNA technology can be used to develop immunotherapies, such as T-cell therapy, which uses the body's immune system to destroy cancer cells

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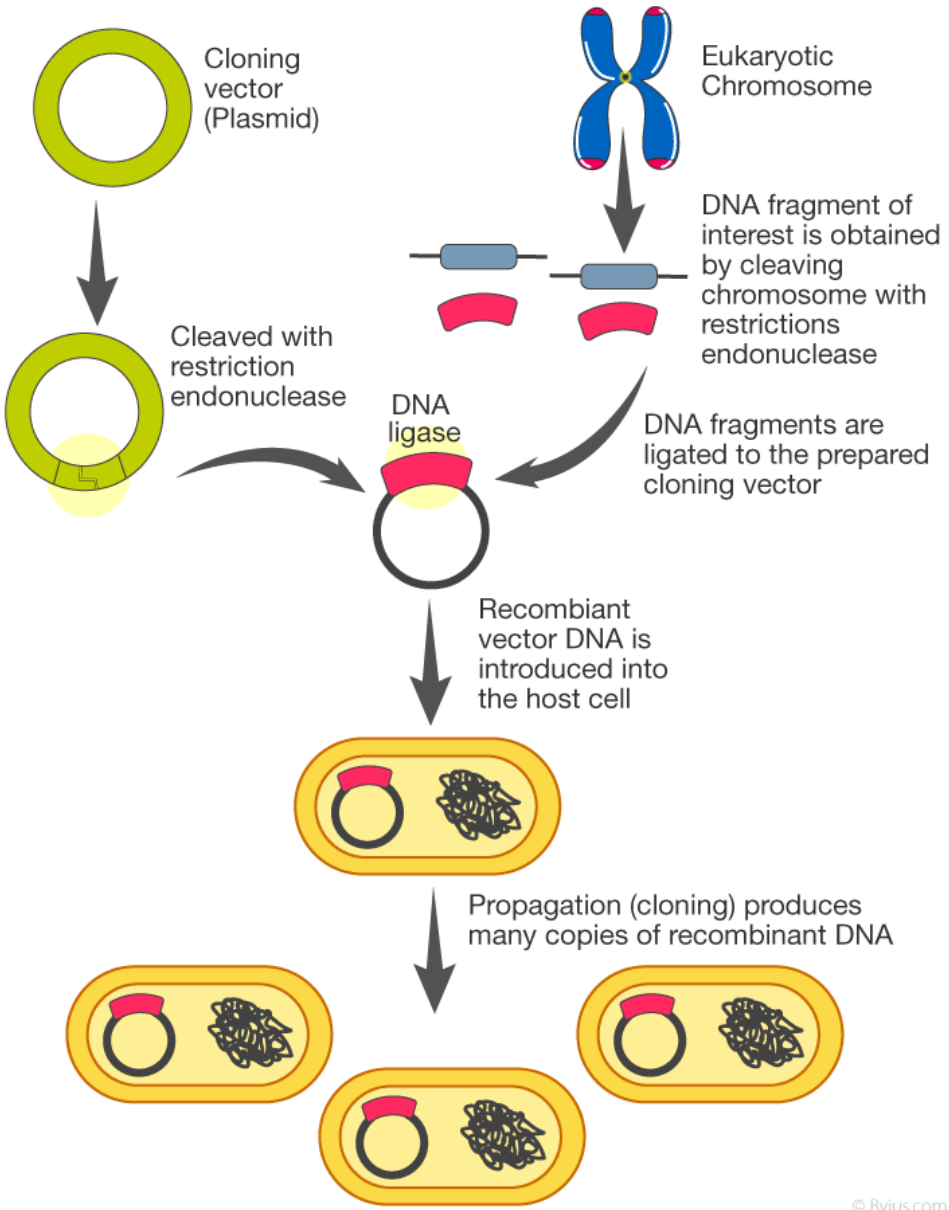
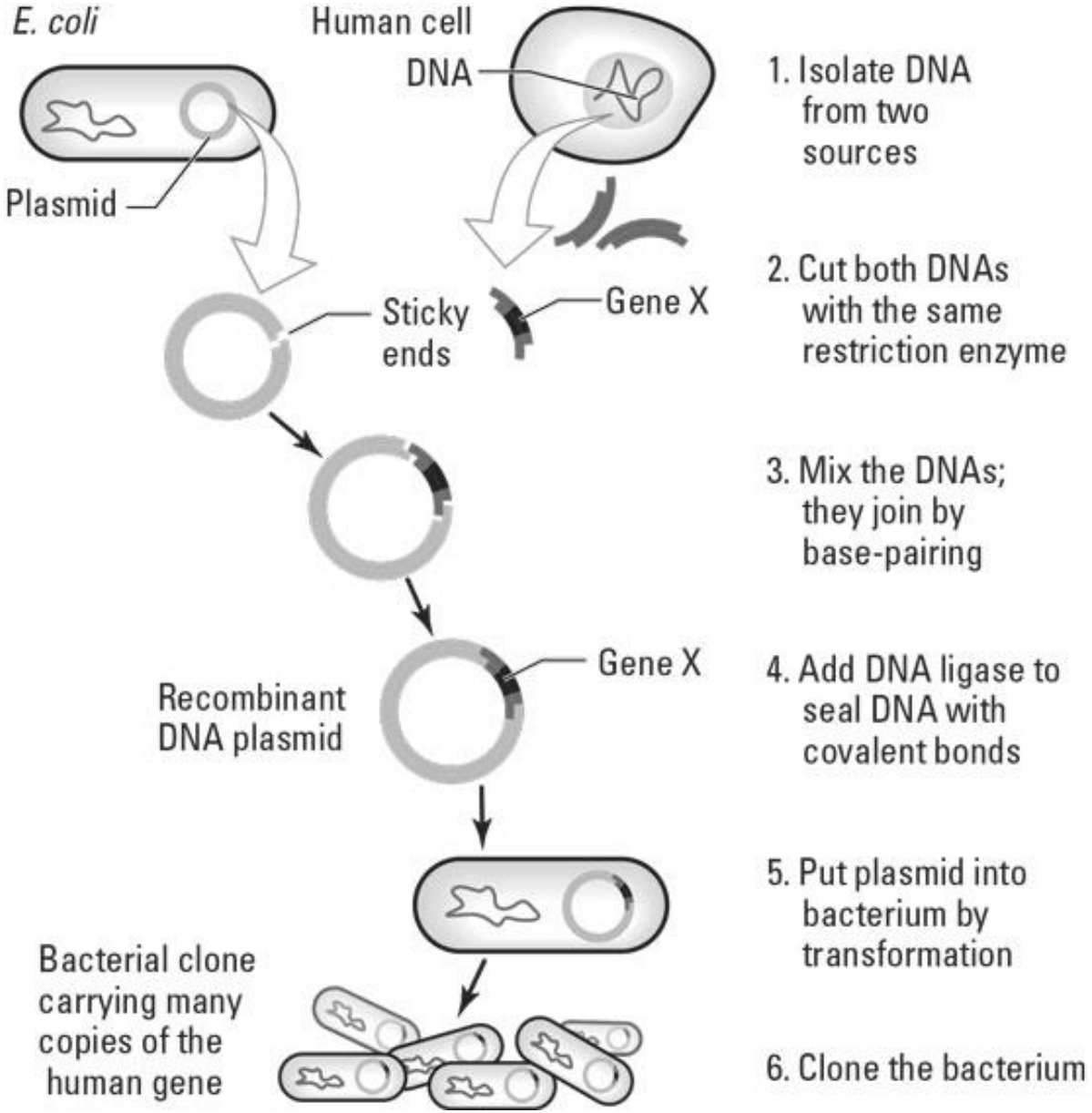
Some products of rDNA technology

Using procedures like this, many human genes have been cloned in *E. coli* or in yeast. Cultured cells (*E. coli*, yeast, mammalian cells) transformed with a human gene are being used to manufacture more than 100 products for human therapy. Some examples:

- **insulin** for diabetics
- **factor VIII** for males suffering from hemophilia A
- **factor IX** for hemophilia B
- **human growth hormone** (**HGH**)
- **erythropoietin** (**EPO**) for treating anemia
- several types of **interferons**
- several **interleukins**
- **granulocyte-macrophage colony-stimulating factor** (**GM-CSF**) for stimulating the bone marrow after a bone marrow transplant
- **granulocyte colony-stimulating factor** (**G-CSF**) for stimulating neutrophil production (e.g., after chemotherapy) and for mobilizing hematopoietic stem cells from the bone marrow into the blood.
- **tissue plasminogen activator** (**TPA**) for dissolving blood clots
- **adenosine deaminase** (**ADA**) for treating some forms of **severe combined immunodeficiency** (**SCID**)
- **parathyroid hormone**
- many **monoclonal antibodies**
- **hepatitis B surface antigen** (**HBsAg**) to vaccinate against the hepatitis B virus
- **C1 inhibitor** (**C1INH**) used to treat hereditary angioedema

Recombinant DNA technology





Ref:Molecular & Cell biology for dummies