Lecture: Biotechnology

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

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Web ref provided on slides
Images: From google image

Biotechnology Applications

- Biotechnology: Principles and Processes
- Processes of Recombinant DNA Technology
- Tools of Biotechnology
- Biotechnology Applications In Medicine
- Biotechnology Applications In Agriculture
- Transgenic Animals and Ethical Issues

Ref: wikipedia

Biotechnology: Principles and Processes

In biotechnology, scientists often need to amplify a small part of DNA or separate out different sized DNA fragments. How do scientists do this? They use specific techniques such as PCR and Gel electrophoresis that allow them to achieve these goals. Let's learn about these techniques in detail.

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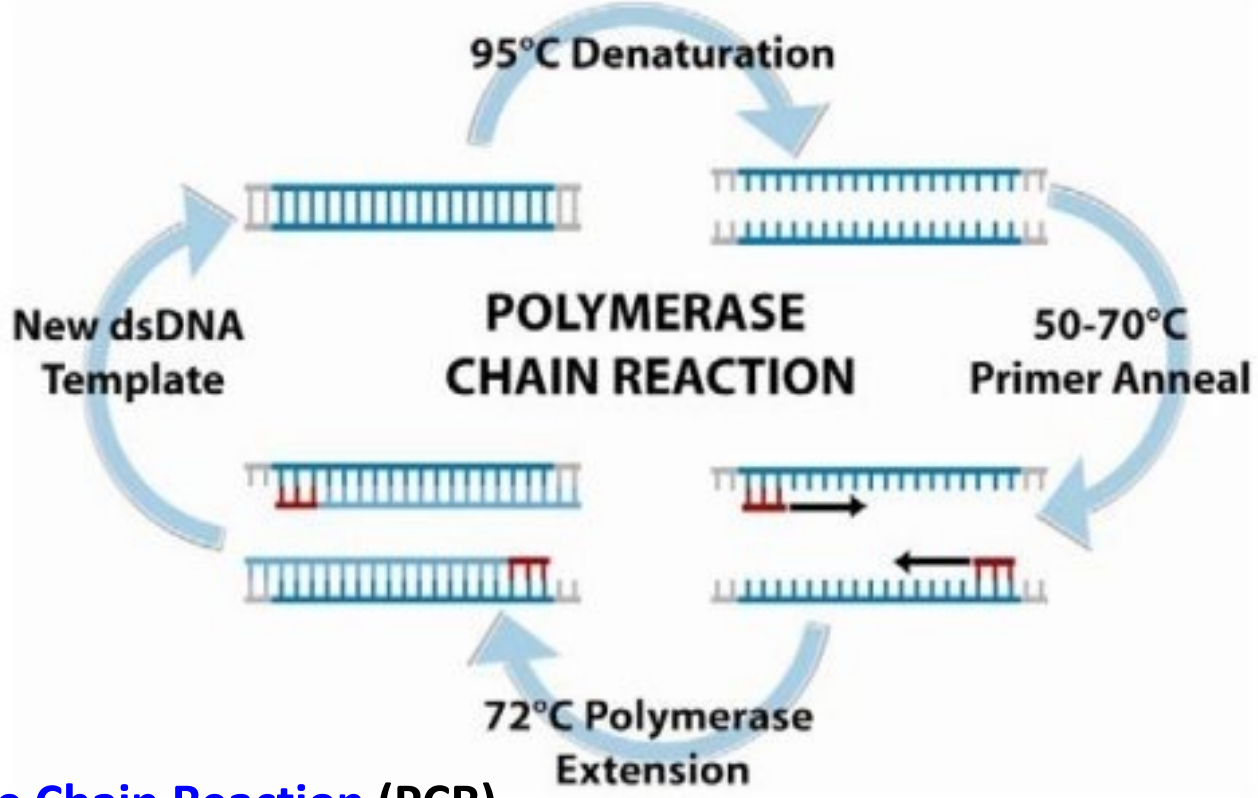
Biotechnology: Principles and Processes



Polymerase Chain Reaction (PCR)

In a breakthrough discovery in 1985, Kary B. Mullis invented the Polymerase Chain Reaction (PCR) technique. This technique allows scientists to make millions of copies of a very small amount of DNA. It is based on the natural process of DNA replication that occurs within a cell. Let's learn about the components and steps of this technique. A PCR reaction needs the following components:

- **DNA Template** – The double-stranded DNA fragment of interest, that is to be amplified.

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- **Primers** – Short, chemically synthesized, single-stranded pieces of DNA that are complementary to the DNA fragment of interest.
- **DNA Polymerase** – The enzyme that elongates the primers by adding nucleotides to it, using the desired DNA fragment as a template. This enzyme needs to be able to withstand high temperatures used in the PCR reaction. Therefore, scientists isolated a thermostable DNA polymerase from the bacterium –*Thermus aquaticus*. It is known as Taq polymerase.
- **Nucleotides** – Single bases A, T, C, and G are the building blocks of DNA synthesis in a PCR reaction.
- **Buffer system** – The buffer contains potassium and magnesium that are essential for the DNA denaturation and renaturation steps. It also contains other factors important for enzyme activity, fidelity and stability.

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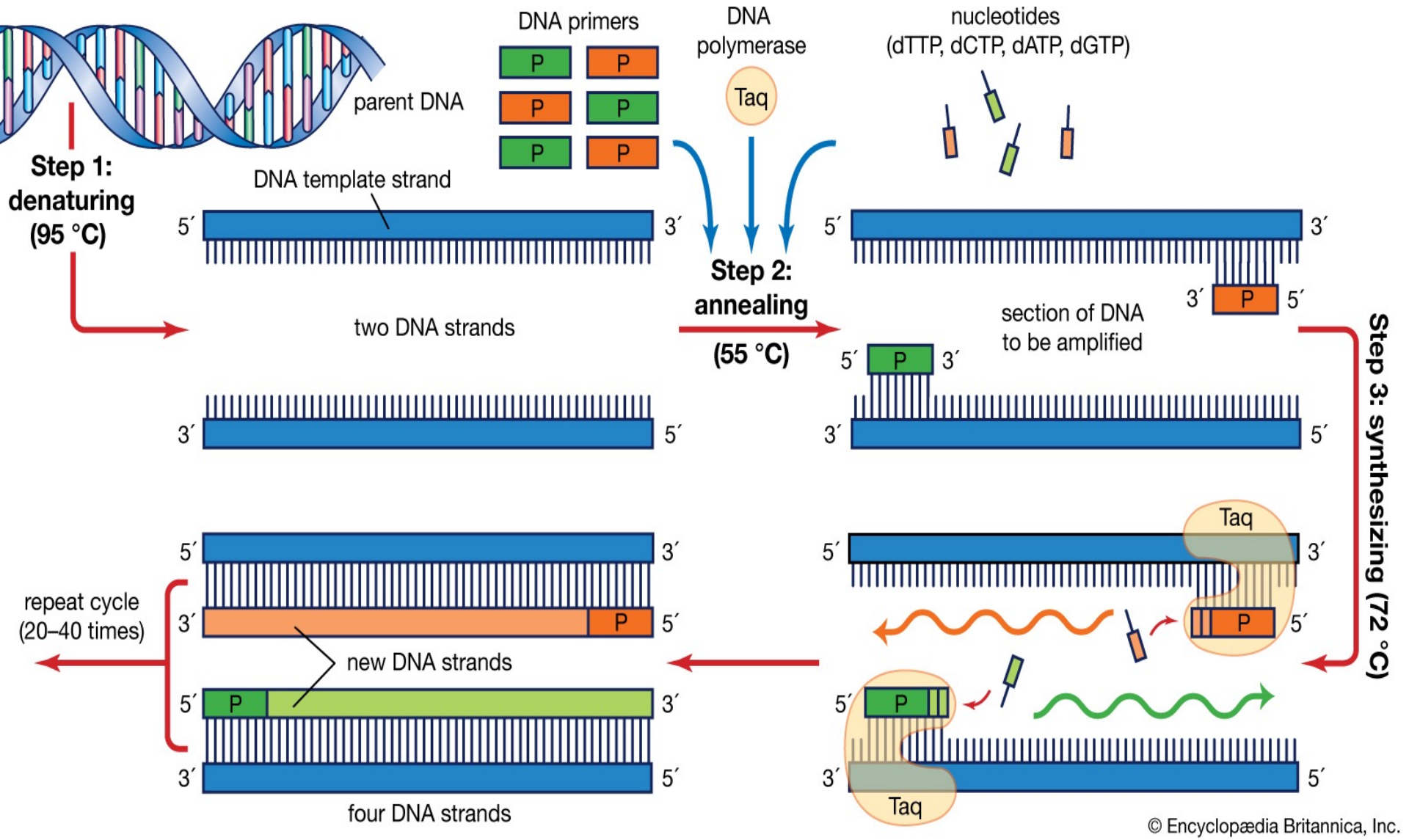

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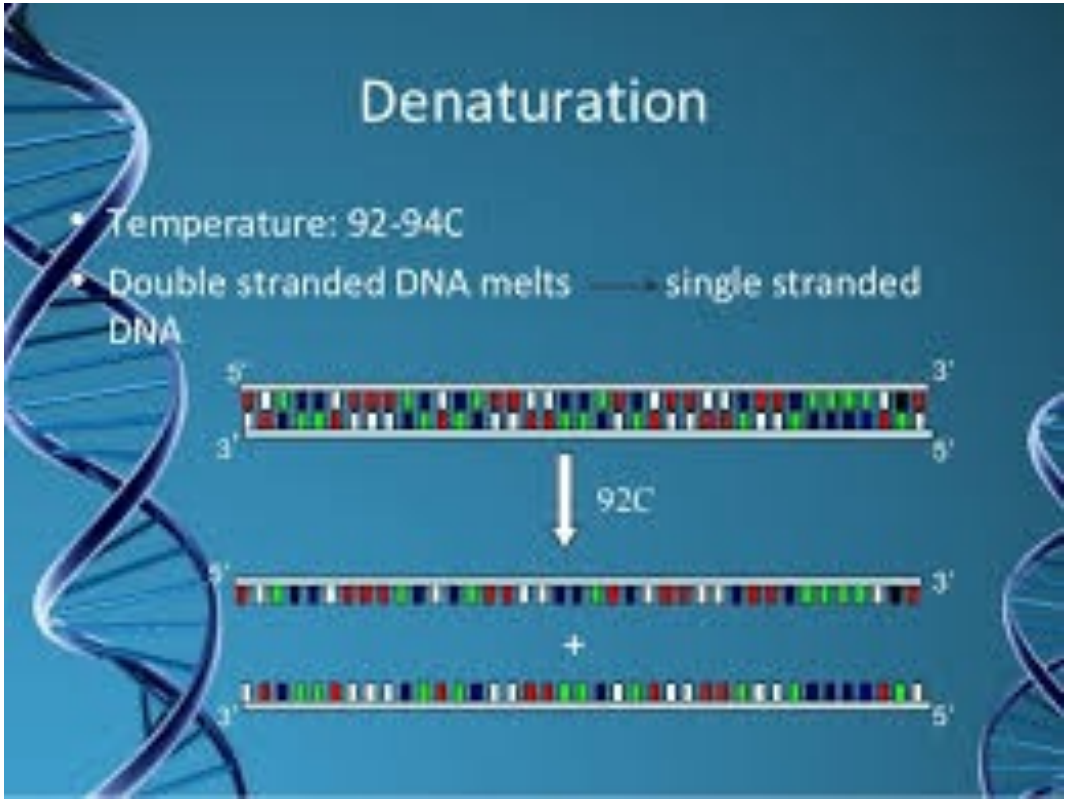
Steps in a PCR Reaction

In a PCR reaction, DNA polymerase synthesizes a new DNA strand complementary to the DNA template by adding nucleotides to the primers. The above components are mixed together in PCR tubes and run on a thermal cycler, where they go through multiple cycles involving the following reactions:

- **Denaturation** – This step involves heating the reaction mixture to a high temperature (94°C). This denatures the double-stranded DNA template into single-strands by breaking the weak hydrogen bonds between the two DNA strands.



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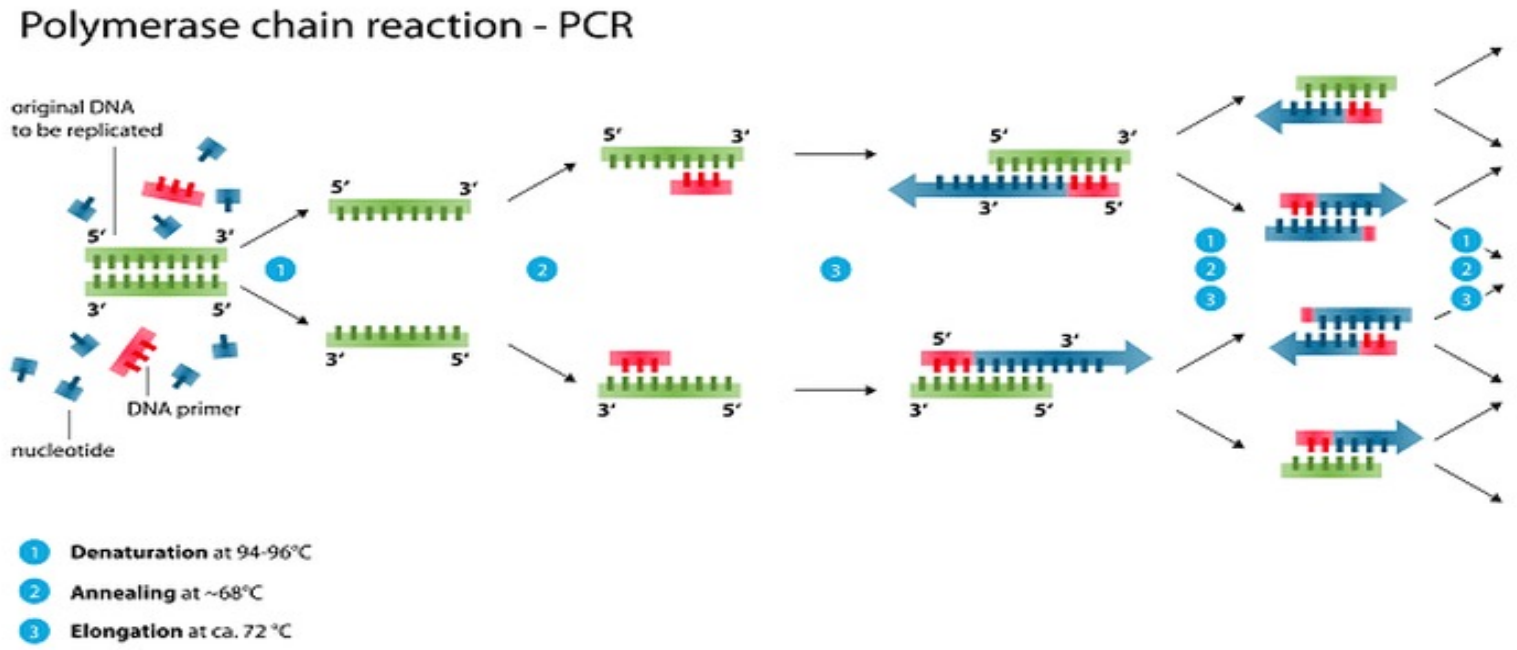

- **Annealing** – In this step, the reaction mixture is brought to a temperature of 54-60°C, to allow the primers to bind (anneal) to their complementary sequence in the template DNA.
- **Elongation/Extension** – In this step, the DNA polymerase sequentially adds nucleotides to the primers and extends it in the 5′ to 3′ direction.

One cycle of denaturation, annealing, and elongation amplifies the double-stranded DNA template to give two pieces of double-stranded DNA. The cycles keep repeating to produce more and more copies, increasing the number of copies exponentially.

Today, PCR is very important in many fields. In medicine, it is useful to detect genetic mutations and to detect HIV, malaria etc. in clinical samples.

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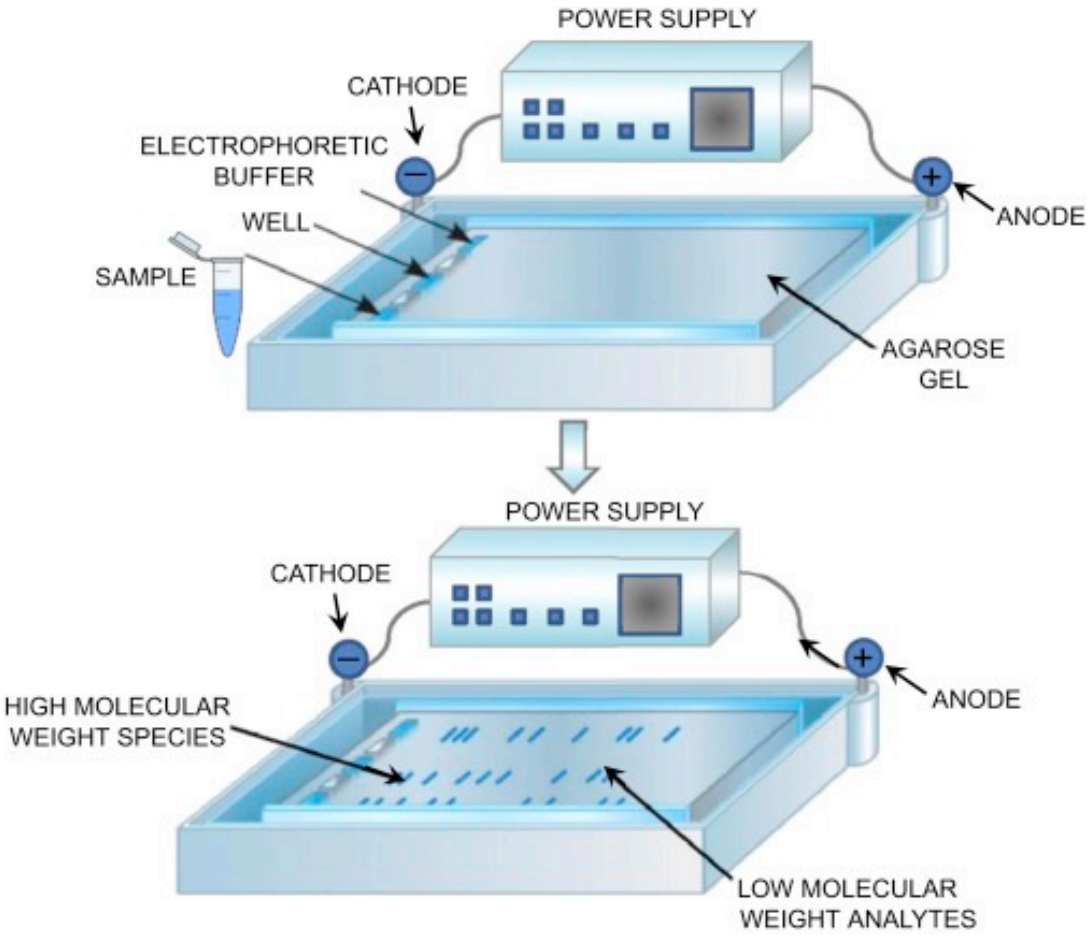
Gel Electrophoresis

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.



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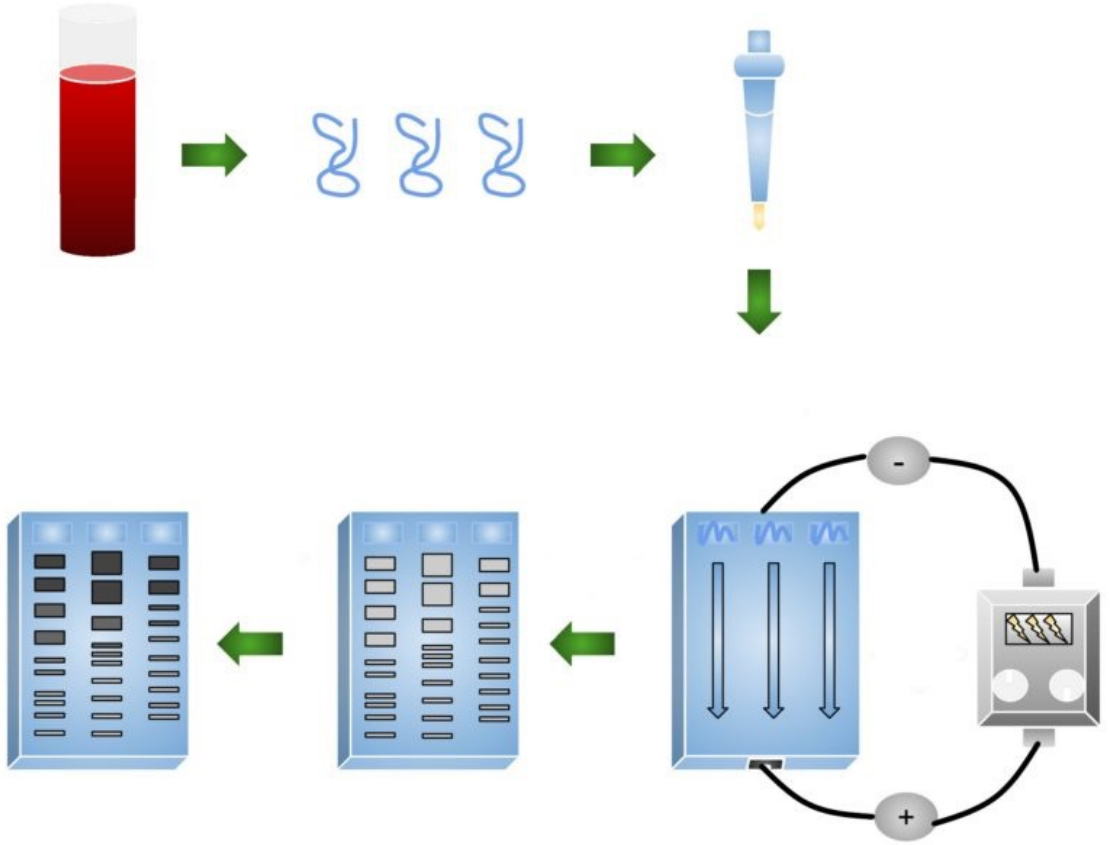
Working of Agarose Gel Electrophoresis

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.



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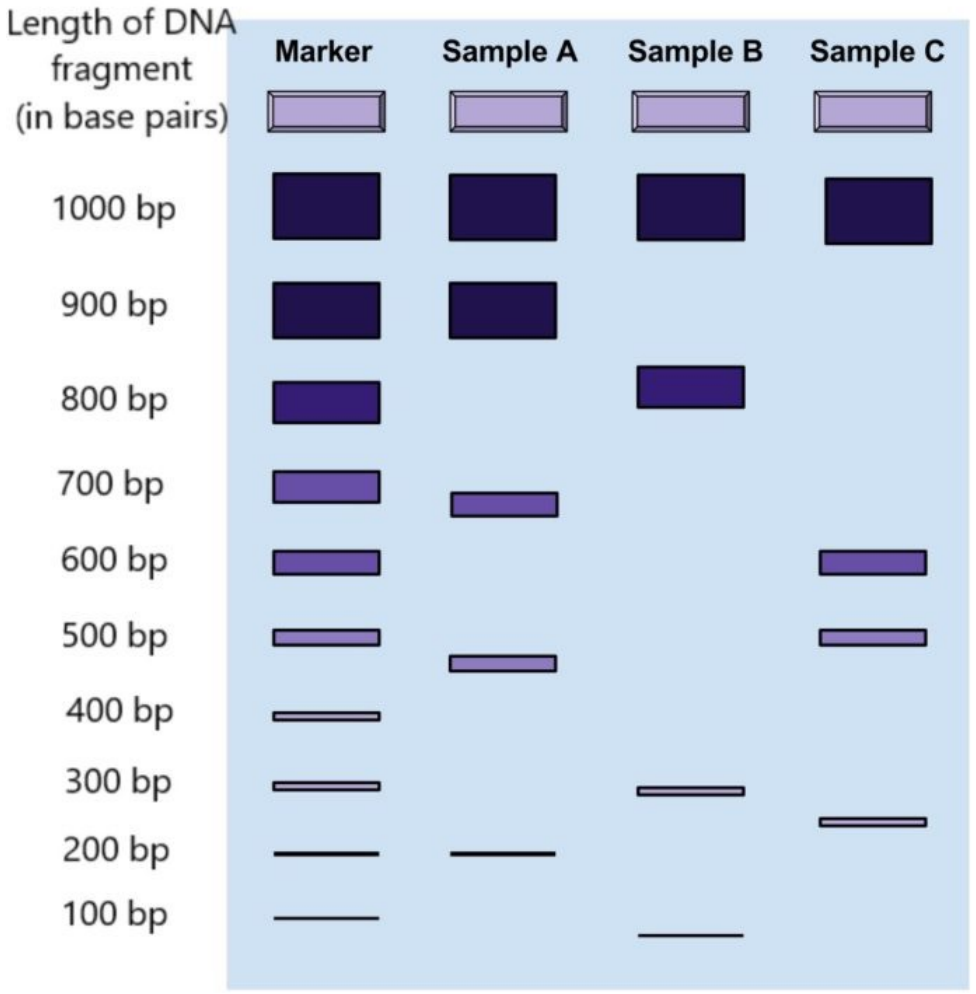
The separated DNA fragments are not visible under normal light. Therefore, they are visualized after staining the bands with a compound – ethidium bromide and exposing them to UV radiation. Once they are stained and exposed to UV light, you can see the separated DNA bands and determine their sizes using 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.



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Processes of Recombinant DNA Technology

Recombinant DNA (rDNA) technology refers to the process of joining DNA molecules from two different sources and inserting them into a host organism, to generate products for human use. Can you put the DNA molecules in the host organism first and then cut and join them? No! This process involves multiple steps that have to proceed in a specific sequence to generate the desired product. Let's understand each step in detail.

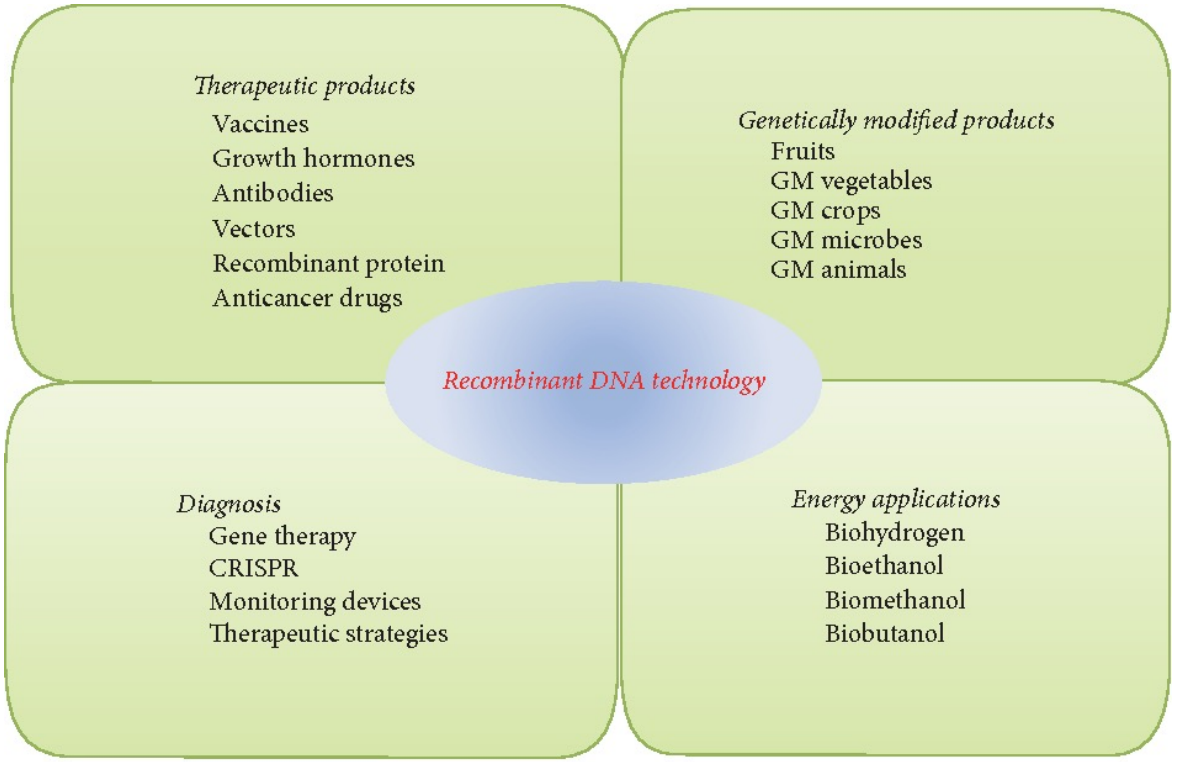

FIGURE 1: Illustration of various applications of recombinant DNA technology.

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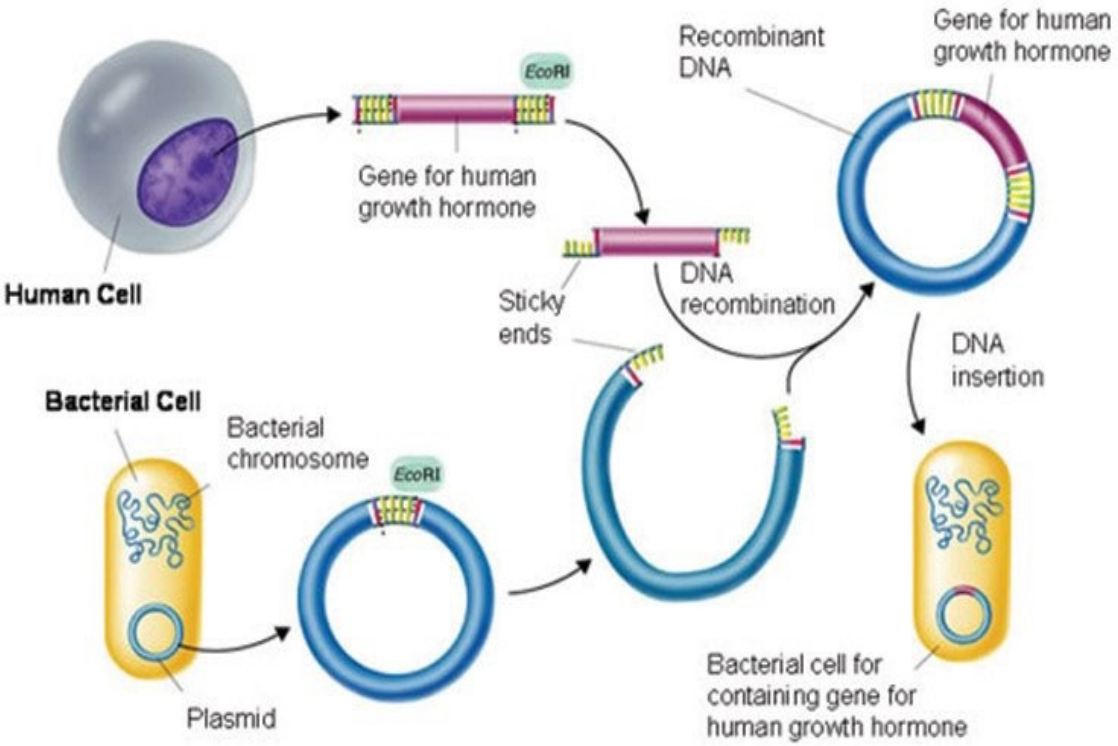
Processes of Recombinant DNA Technology

Isolation of Genetic Material

- We already know that the genetic material of all living organisms is 'nucleic acid'. In most organisms, it is DNA, whereas in some it is RNA. The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.
- However, in a normal cell, the DNA not only exists within the cell membrane, but is also present along with other macromolecules such as RNA, polysaccharides, proteins, and lipids. So, how do we break open the cell and obtain DNA that is free from other macromolecules?



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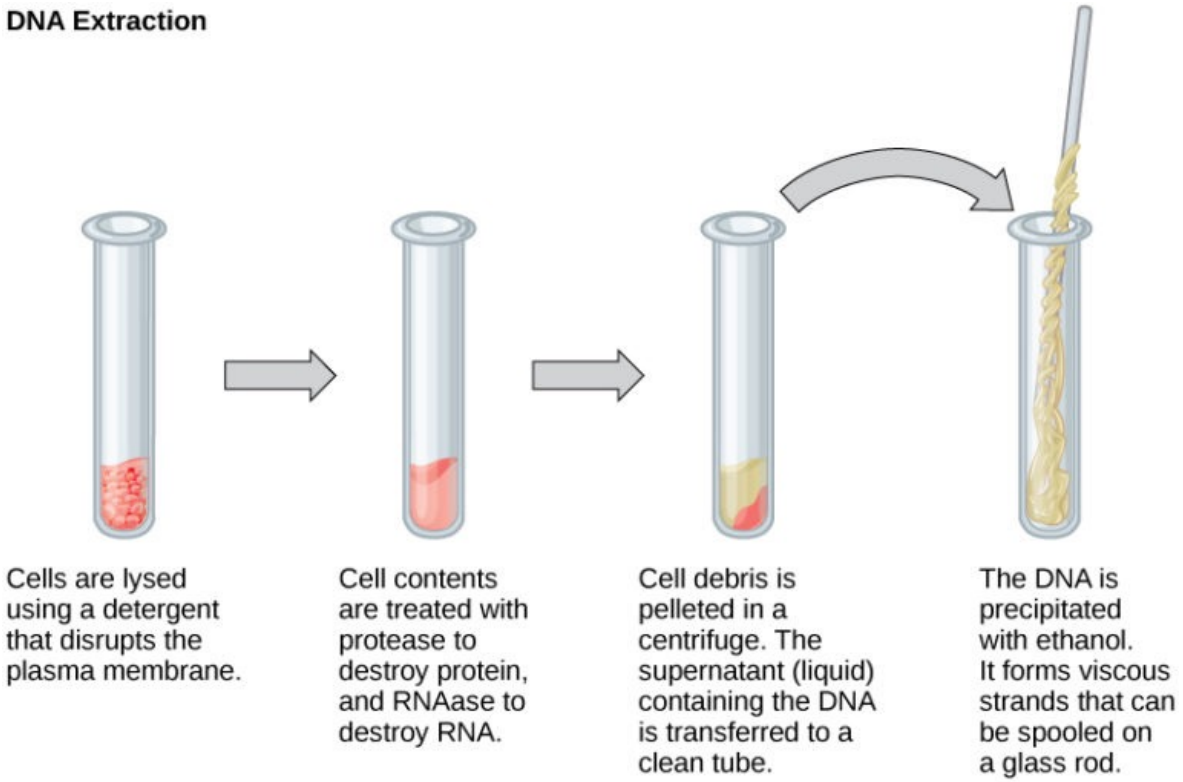
Processes of Recombinant DNA Technology

We can use the following enzymes for specific purposes:

- **Lysozyme** – to break bacterial cell wall.
- **Cellulase** – to break plant cell wall.
- **Chitinase** – to break fungal cell wall.
- **Ribonuclease** – removes RNA.
- **Protease** – removes proteins (such as histones that are associated with DNA).

Other macromolecules are removable with other enzymes or treatments. Ultimately, the addition of ethanol causes the DNA to precipitate out as fine threads. This is then spooled out to give purified DNA.



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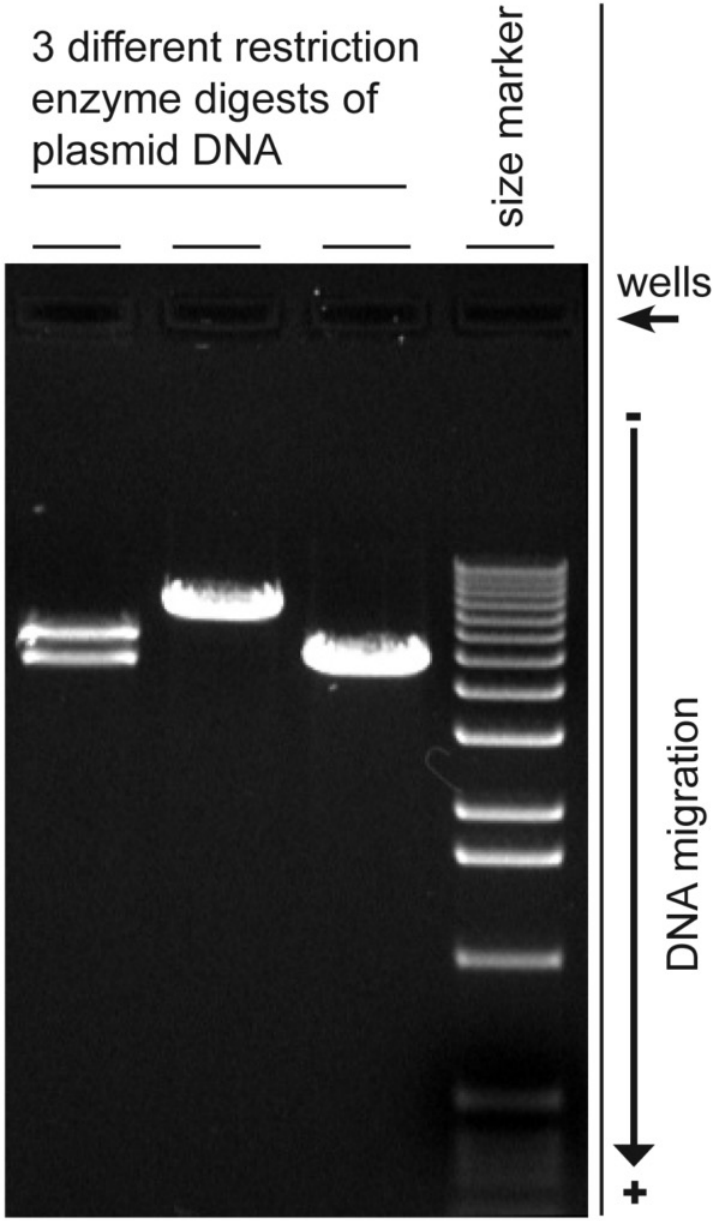
Processes of Recombinant DNA Technology

Restriction Enzyme Digestion

- Restriction enzymes act as molecular scissors that cut DNA at specific locations. These reactions are called ‘restriction enzyme digestions’. They involve the incubation of the purified DNA with the selected restriction enzyme, at conditions optimal for that specific enzyme.
- The technique – ‘Agarose Gel Electrophoresis’ reveals the progress of the restriction enzyme digestion. This technique involves running out the DNA on an agarose gel. On the application of current, the negatively charged DNA travels to the positive electrode and is separated out based on size. This allows us to separate and cut out the digested DNA fragments. The vector DNA is also processed using the same procedure.



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Processes of Recombinant DNA Technology

Amplification Using PCR

- Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – DNA polymerase. It helps to amplify a single copy or a few copies of DNA into thousands to millions of copies. PCR reactions are run on 'thermal cyclers' using the following components:
- **Template** – DNA to be amplified
- **Primers** – small, chemically synthesized oligonucleotides that are complementary to a region of the DNA.
- **Enzyme** – DNA polymerase
- **Nucleotides** – needed to extend the primers by the enzyme.

The cut fragments of DNA can be amplified using PCR and then ligated with the cut vector as explained in upcoming slides.

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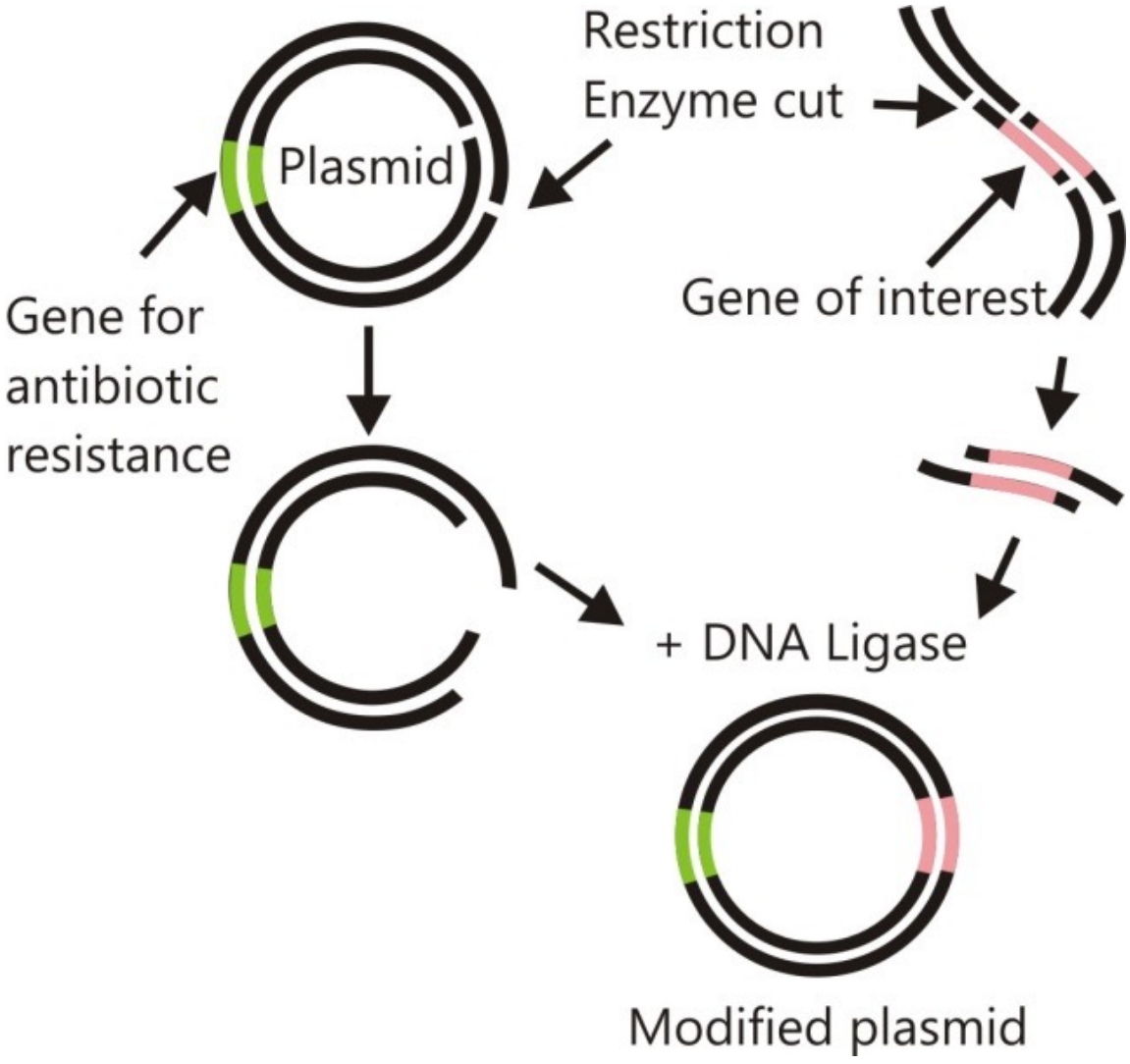
Processes of Recombinant DNA Technology

Ligation of DNA Molecules

- The purified DNA and the vector of interest are cut with the same restriction enzyme. This gives us the cut fragment of DNA and the cut vector, that is now open. The process of joining these two pieces together using the enzyme 'DNA ligase' is 'ligation'. The resulting DNA is '**recombinant DNA**'.



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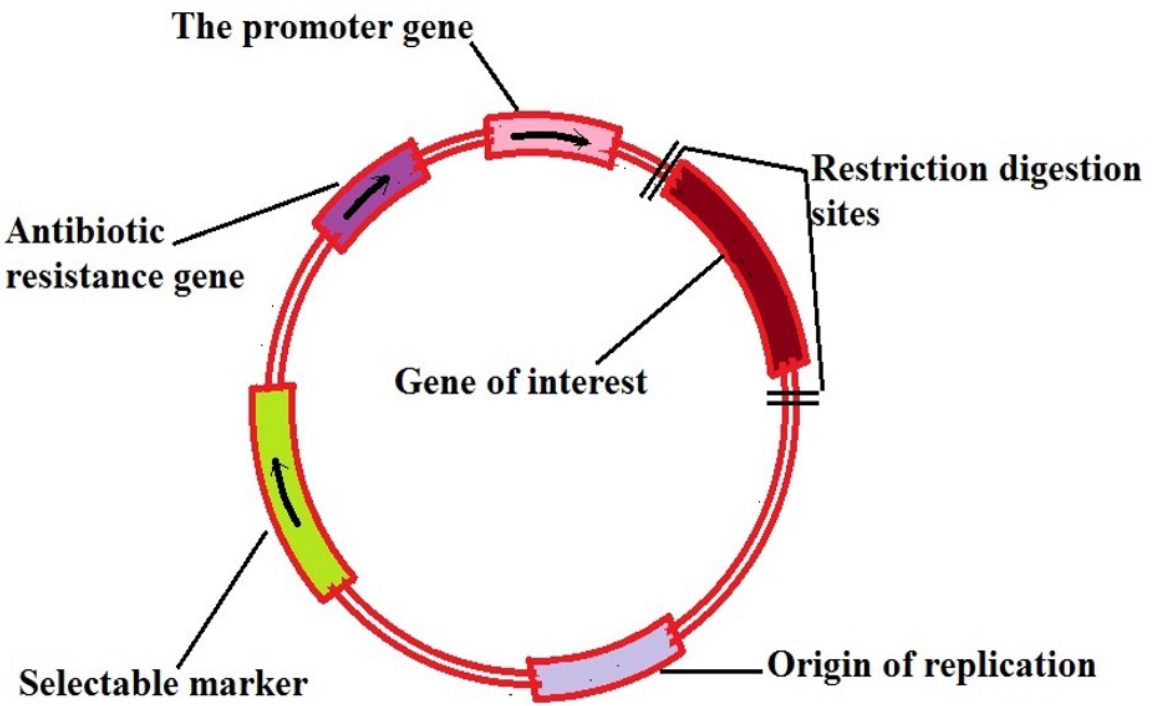
Processes of Recombinant DNA Technology

=Insertion of Recombinant DNA Into Host

- In this step, the recombinant DNA is introduced into a recipient host cell. This process is **'Transformation'.** Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them **'competent'** to accept new DNA. (The topic – Tools of Biotechnology explains a few ways to make cells competent).
- During transformation, if a recombinant DNA bearing a gene for ampicillin resistance is transferred into recipient *E. coli* cells, then the *E. coli* cells also become ampicillin-resistant. This aspect is useful in differentiating transformed cells from non-transformed cells.
- For example, if we spread the transformed cells on agar plates containing ampicillin, only the transformed, ampicillin-resistant cells will grow while the untransformed cells will die. Therefore, in this case, the ampicillin resistance gene acts as the 'selectable marker'.



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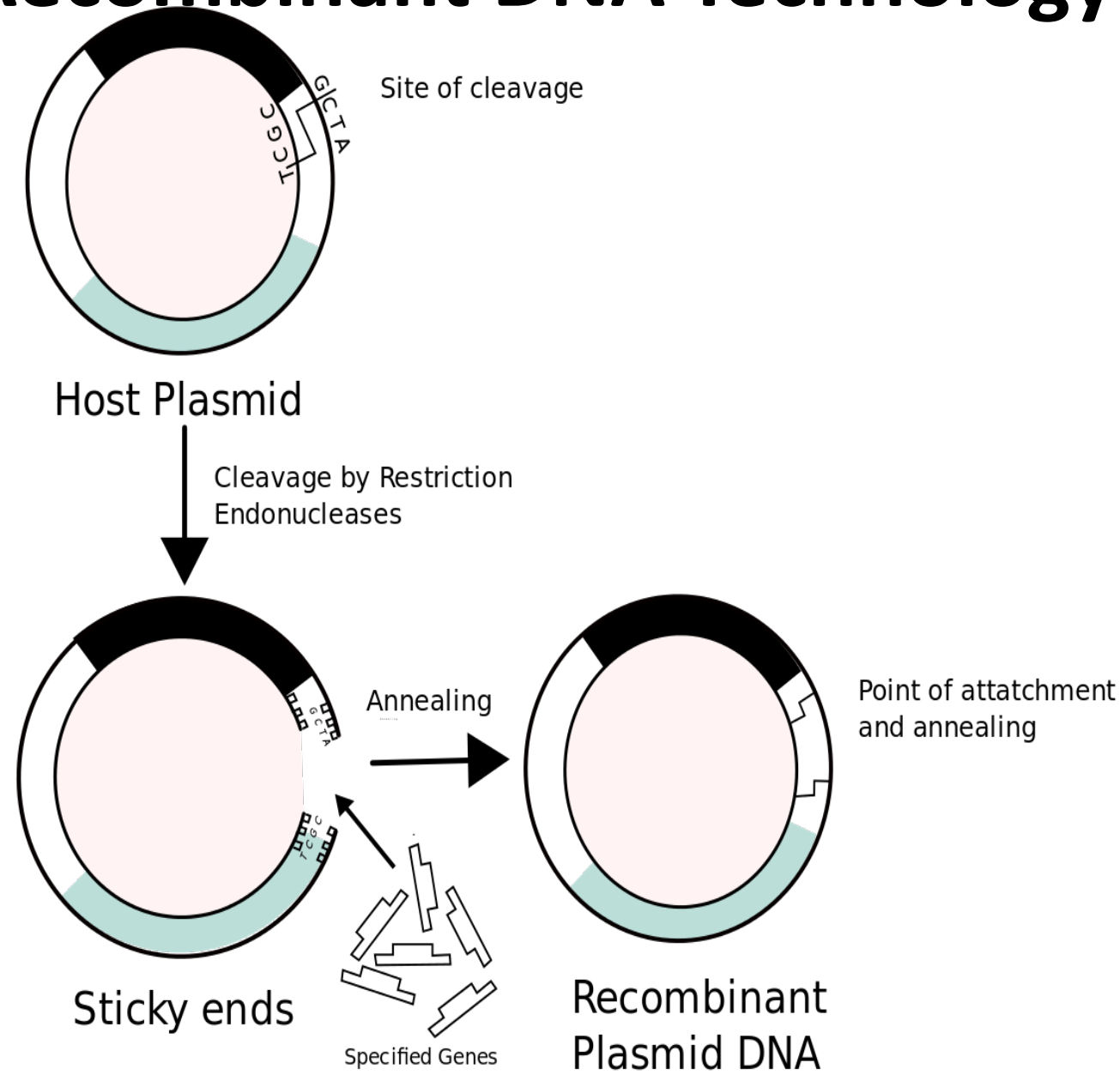
Processes of Recombinant DNA Technology

Obtaining Foreign Gene Product

- The recombinant DNA multiplies in the host and is expressed as a protein, under optimal conditions. This is now a **recombinant protein**. Small volumes of cell cultures will not yield a large amount of recombinant protein. Therefore, large-scale production is necessary to generate products that benefit humans. For this purpose, vessels called **bioreactors** are used.
- Bioreactors are large containers with a continuous culture system, where the fresh medium is added from one side and used medium is taken out from another side. Bioreactors can process about 100-1000 litres of cell cultures. A bioreactor provides optimum conditions (temperature, oxygen, pH, vitamins etc.) to biologically convert raw materials into specific proteins, enzymes etc.



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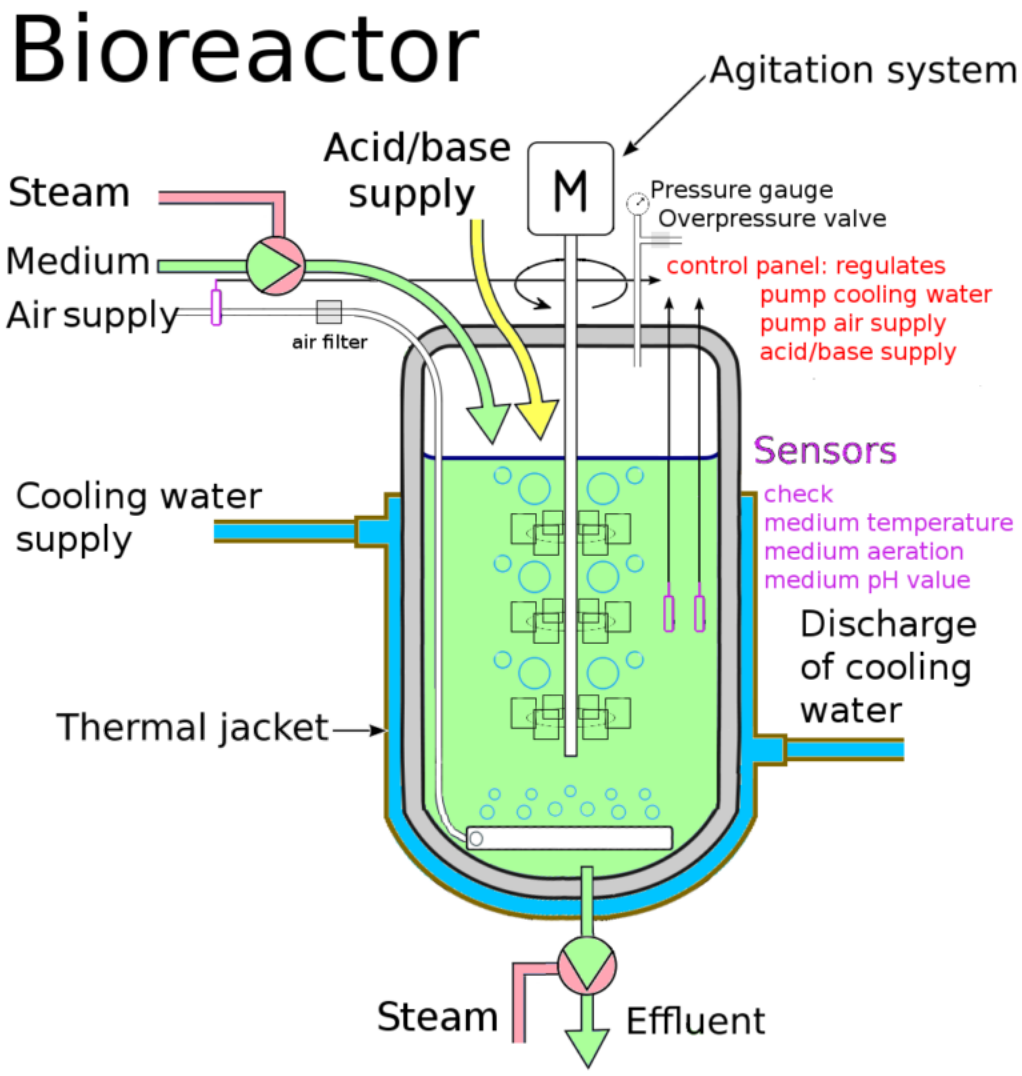
Biotechnology: Principles and Processes

‘Stirred-tank bioreactor’ is the most common type of bioreactor. It is usually cylindrical and has the following parts:

- Agitator system – to stir the contents evenly
- Oxygen delivery system – to introduce air into the system
- Foam control system
- Temperature control system
- pH control system
- Sampling ports – to take out small amounts of culture



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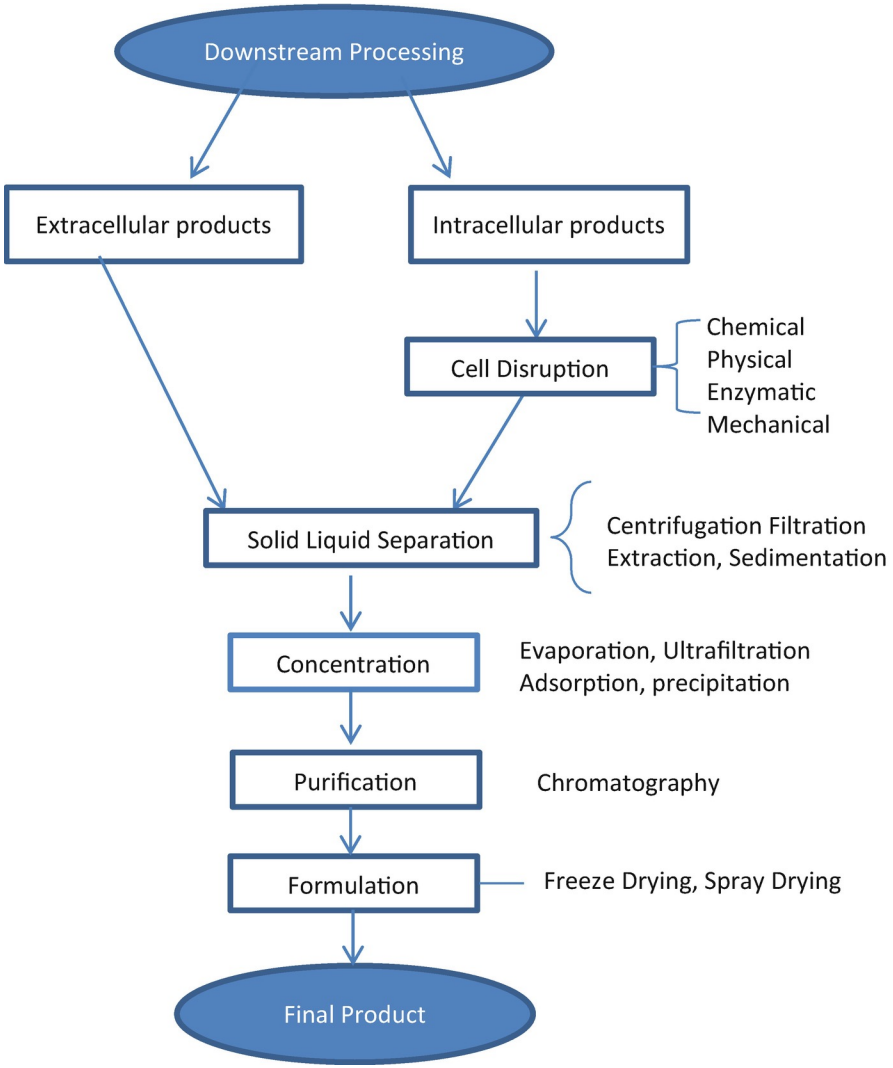
Biotechnology: Principles and Processes

Downstream Processing

- Before the protein is marketed as a final product, it is subjected to downstream processing which includes:
- Separation and purification.
- Formulation with suitable preservatives.
- Clinical trials to test the efficacy and safety of the product.
- Quality control tests.

Note: These processes differ from product to product.



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Tools of Biotechnology

Principles Of Biotechnology

- The field of biotechnology has evolved tremendously over the years. It has gone from the traditional view of using microbes to generating products for human use, to the modern view of using genes for developing vaccines. Therefore, the European Federation of Biotechnology (EFB) has come up with a new definition that involves both the traditional and the modern view of biotechnology. It states that –
- 'Biotechnology is the integration of natural science and organisms, cells, parts thereof, and molecular analogs for products and services'. The two core techniques responsible for the birth of modern biotechnology are:
- Genetic Engineering: It involves techniques that change the chemistry of the genetic material such that when they are introduced into the host, the host phenotype changes.
- Maintenance of a contamination-free environment to enable the growth of only the desired microbe in large quantities during the manufacture of products like vaccines, antibiotics etc.

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Genetic Engineering

- Traditional hybridization methods in plant and animal breeding introduce desirable as well as undesirable genes. The techniques of genetic engineering such as recombinant DNA technology, gene cloning etc. overcome this problem, allowing us to introduce only desired genes. Genetically modifying an organism has the following basic steps:
- Identification of DNA containing desired genes.
- Introduction of desired DNA into the host organism.
- Maintenance of the introduced DNA in the host genome and transfer of the DNA to its progeny.

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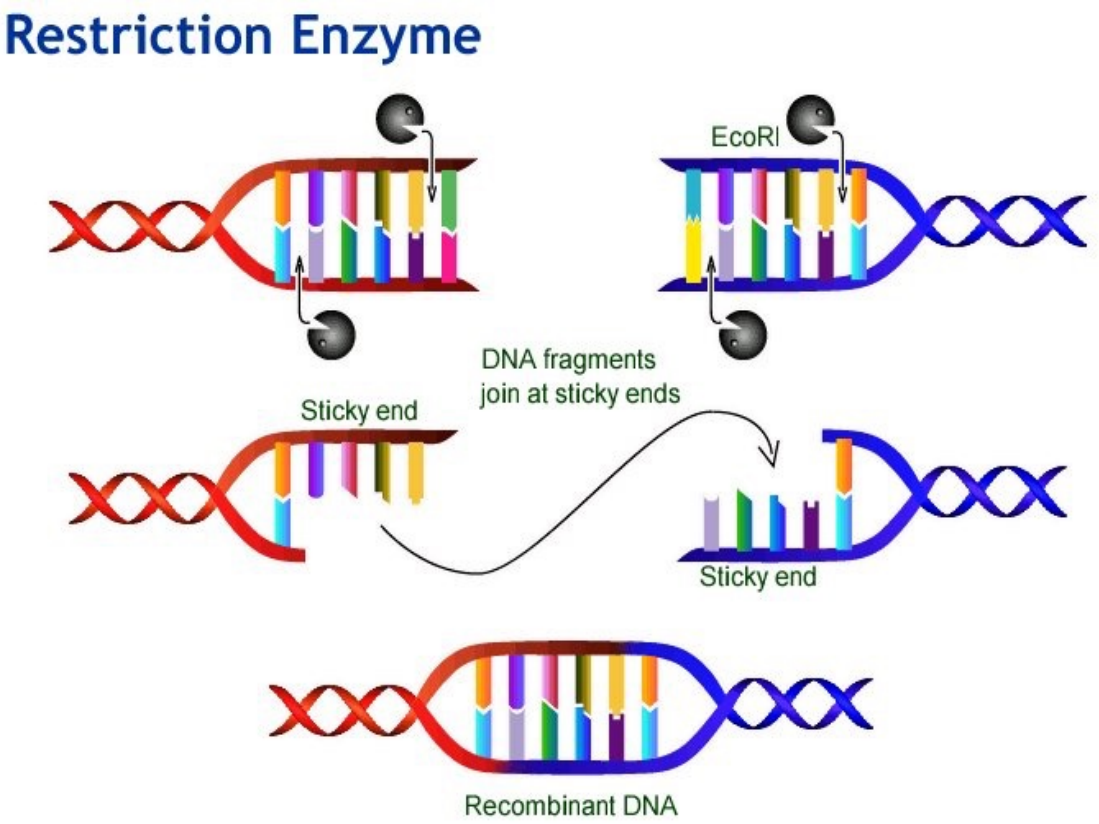
Tools of Biotechnology

Tools Of Biotechnology

- Genetic engineering or recombinant DNA technology is possible only if we have some important tools. The following are some important tools of biotechnology.
- **1) Restriction Enzymes**
- Restriction enzymes, also known as 'restriction endonucleases' are molecular scissors that can cut DNA at specific locations. These are part of a larger class of enzymes called 'Nucleases'. Nucleases are of two kinds:
- Exonucleases – Enzymes that remove nucleotides from the ends of DNA.
- Endonucleases – Enzymes that make cuts at specific positions in the DNA.



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'*Hind II*' – the first restriction endonuclease to be isolated, identifies a specific six base pair sequence and always cuts the DNA at a particular point. This specific sequence is the '**Recognition sequence**'. Today, there are more than 900 restriction enzymes, isolated from about 230 bacterial strains. Each of these enzymes recognizes different recognition sequences. There is a naming convention to name restriction enzymes. Let's learn this using **EcoRI** as an example:

- **Eco** – indicates that this enzyme was isolated from *Escherichia coli.*
- **R** – refers to the name of the strain.
- **I** – is the Roman numeral that indicates the order of isolation of this restriction enzyme from the strain of bacteria.

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Mode of Action of Restriction Enzymes

- Endonucleases perform their function in the following manner:
- Inspect the length of DNA for the specific recognition sequence.
- Bind the DNA at the recognition sequence.
- Cut the two strands of DNA at specific points in their sugar-phosphate backbone.
- Restriction enzymes recognize specific **palindromic nucleotide sequences** in the DNA. What is a palindrome? In terms of words, a palindrome is a group of letters that produce the same word when reading forward or backwards. An example is 'RADAR'. In terms of DNA, a palindrome is a sequence of base pairs that reads the same on both DNA strands when reading in the same orientation.

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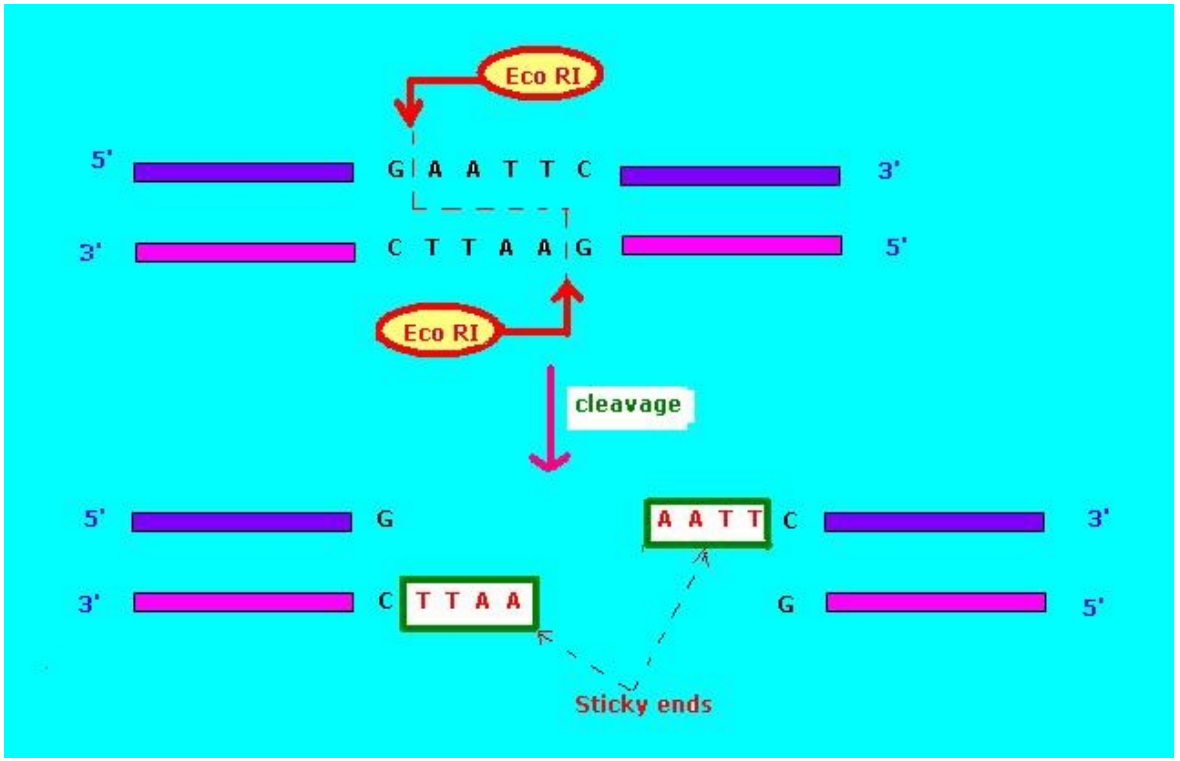
For example –

- 5′ —— GAATTC —— 3′
  3′ —— CTTAAG —— 5′

On reading both the strands shown above in the 5′ to 3′ direction, they give the same sequence. This is true even when they are both read in the 3′ to 5′ direction.

Restriction enzymes cut the DNA strand a little away from the center of the palindromic site, but between the same two bases on both strands. This gives rise to single-stranded, overhanging stretches on each strand called ‘sticky ends’. They are called ‘sticky’ because they can bind to their complementary cut counterparts.

Using restriction enzymes we can generate recombinant DNA that contains DNA from different sources. Cutting these DNA sources with the same restriction enzyme gives fragments with the same kind of ‘sticky ends’, that can then be joined using DNA ligases.



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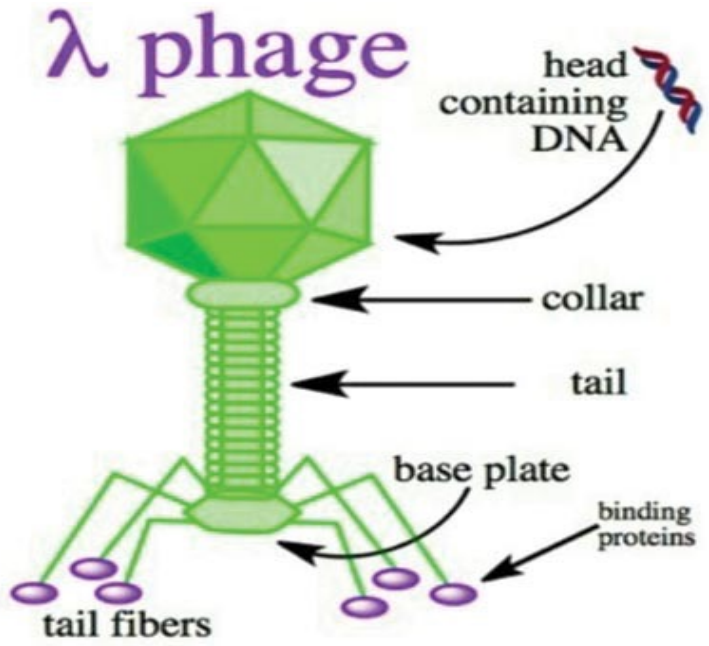

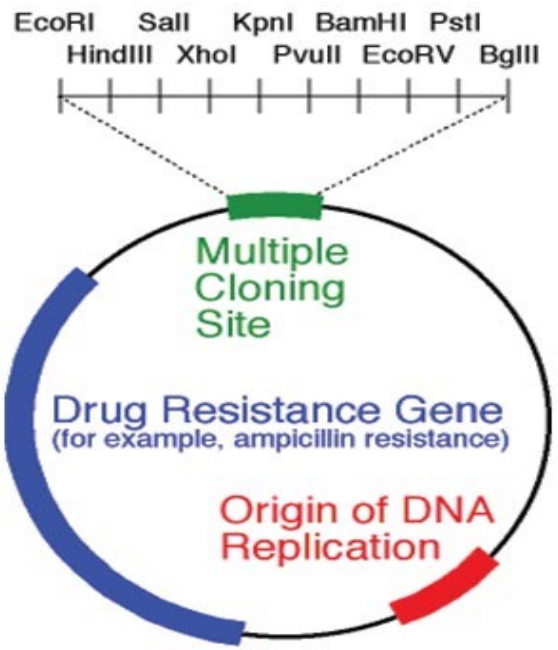

2) Cloning Vectors

Just the way a mosquito acts as a 'vector' to transfer the malarial parasite into the human body, we need vectors to transfer the cut DNA into a host organism. Vectors are one of the important tools of biotechnology.

Plasmids make good vectors because they can replicate in bacterial cells, independent of the control of the chromosomal DNA. The vectors in use currently are engineered such that they help in easy linking of foreign DNA and allow selection of recombinants over non-recombinants.

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A vector needs the following features to enable cloning:

(i) Origin Of Replication (ori)

This is the sequence from where replication begins. Linking a piece of DNA to this sequence causes it to replicate in the host cell. This sequence also controls the copy number of the linked DNA. Therefore, the target DNA needs to be cloned into a vector whose '**ori'** supports high copy number, in order to recover large amounts of the DNA.

(ii) Selectable Marker

The vector also needs to have a selectable marker which allows the selection of recombinants over non-recombinants. In terms of *E. coli,* some useful selectable markers are genes that provide resistance to antibiotics like ampicillin, kanamycin, chloramphenicol etc. Since the normal *E. coli* cells do not carry these resistance genes, it becomes easy to select the recombinants.

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(iii) **Cloning Sites**

- In order to attach the foreign DNA to a vector, the vector should have a recognition site for a specific restriction enzyme. Multiple recognition sites will result in multiple DNA fragments, complicating the process of cloning. A vector has more than one antibiotic resistance gene. The foreign DNA is ligated into a restriction site in one of the antibiotic resistance genes.
- For example, let's say an *E. coli* cloning vector has genes for ampicillin and tetracycline resistance. On ligating the foreign DNA into a recognition site within the tetracycline resistance gene, the plasmid loses its tetracycline resistance. But, it can still be selected for non-recombinants by plating on ampicillin-containing medium.
- Now, by transferring the ones that grow in the ampicillin medium to a medium with tetracycline, we can dissect out recombinants from non-recombinants. The recombinants will grow in ampicillin but not in tetracycline medium; while non-recombinants will grow in both mediums.

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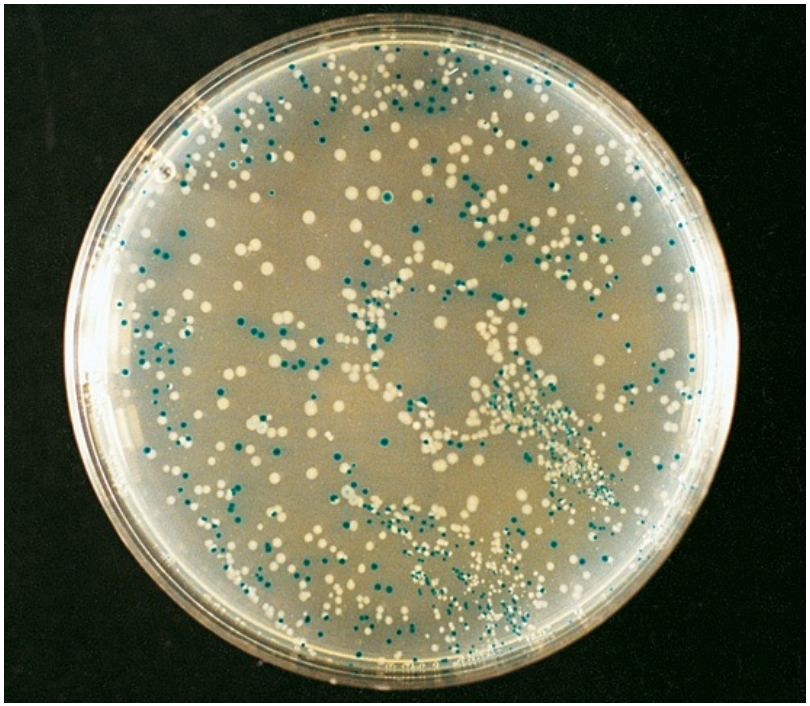
Currently, alternative markers are available that can differentiate recombinants from non-recombinants based on their ability to produce color. This involves insertion of the foreign DNA in the DNA sequence of an enzyme like β-galactosidase, which inactivates the enzyme. This is **'insertional inactivation'.** On reaction with a substrate, the recombinants do not produce color whereas non-recombinants produce color.



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(iv) Vectors to Clone Genes in Plants and Animals

- Long before us, bacteria and viruses knew how to transfer genes into plants and animals. For example, *Agrobacterium tumifaciens,* a pathogen on dicot plants, transfers 'T-DNA' that transforms normal plants cells into tumours. These tumours then produce chemicals that the pathogen requires. With better understanding, we have now converted these pathogens into useful vectors to deliver genes of interest to the plants or animals.
- Now, the tumour-inducing (Ti) plasmid of *Agrobacterium tumifaciens* has been modified into a cloning vector. This vector is no longer harmful but is useful in delivering genes of interest to plants. Retroviruses transform normal cells into cancerous cells in animals. These have also been modified so that they are no longer harmful and can deliver genes to animals.

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Tools of Biotechnology

3) Competent Host

- Host cells are bacterial cells which take up the recombinant DNA. Since DNA is hydrophilic, it cannot pass through the cell membrane of bacteria easily. Therefore, the bacterial cells have to be made 'competent' to take up the DNA.
- Some procedures that make the cells competent are treatments with a specific concentration of divalent cation like calcium. This makes it easy for the DNA to enter the cell wall through pores. Incubation of cells with the recombinant DNA on the ice, followed by heat shock at 42°C and another incubation on ice, enables the cells to take up the DNA.
- There are several other methods to introduce foreign DNA into host cells. The **'microinjection'** method involves injecting the recombinant DNA directly into the nucleus of an animal cell. The **'bolistics'** or **'gene gun'** method bombards plant cells with high-velocity microparticles of gold or tungsten coated with DNA. The last method uses 'disarmed pathogen vectors' (discussed above) to transfer the recombinant DNA into the infected host cells.

Ref: https://www.toppr.com/guides/biology/biotechnology-principles-and-processes/

Biotechnology Applications In Medicine

Recombinant DNA (rDNA) technology is very important in healthcare because it allows for the mass production of safe and more effective medicines. It also prevents undesirable immune responses which are common with medical products from non-human sources.

Currently, about 30 recombinant therapeutics have been approved for human use worldwide. Let's take a look at some of the applications.

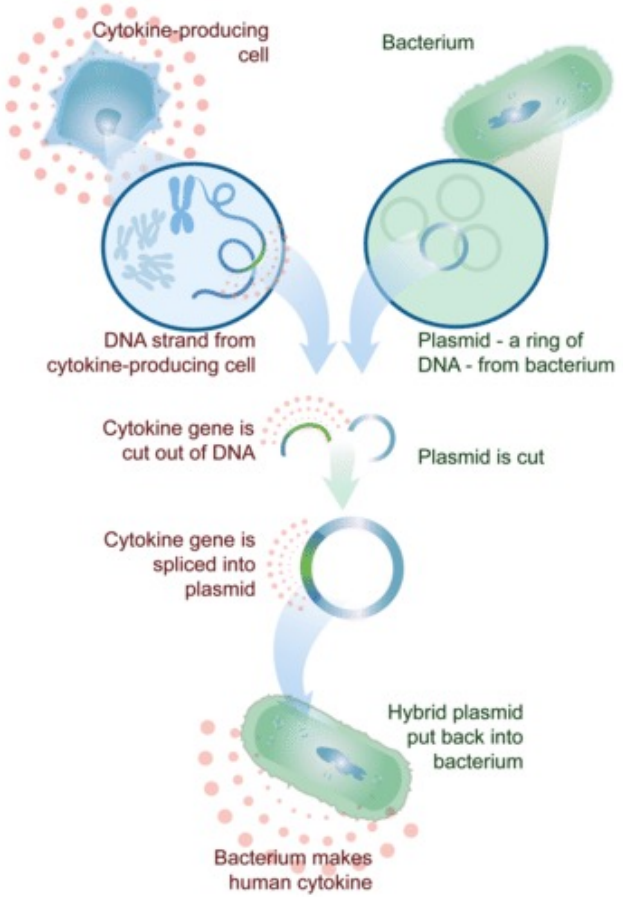

Figure shows how biotechnology can be used to transform bacteria so they are able to make human proteins is done to produce a **cytokine**, which is a small protein that helps fight infections.

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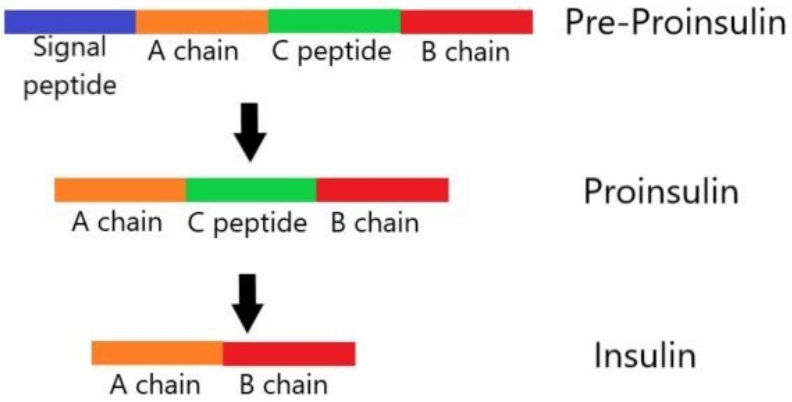
Biotechnology Applications In Medicine



Genetically Engineered Insulin

- Earlier, diabetes was treated using insulin from the pancreas of slaughtered pigs and cattle. Do you think this insulin causes any side-effects in humans? Yes! Insulin from animal sources induces allergies and other unwanted immune reactions in humans. This is why there was a need to isolate human insulin. Is there a way to do this? What if we can use bacteria to produce human insulin? Not only can we grow bacteria in large amounts, but we can also mass-produce human insulin!
- Insulin consists of two short, polypeptide chains – chain A and B, linked via disulfide bridges. Insulin is produced as a 'prohormone' in mammals (including humans). This prohormone has an extra peptide, the **C peptide,** which needs to be removed to give rise to mature insulin.

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Biotechnology Applications In Medicine

The major challenge while generating human insulin is to assemble insulin into its mature form. An American company called ‘Eli Lilly’ overcame this hurdle in 1983. They prepared two DNA sequences that correspond to the A and B chains of human insulin. They then incorporated these sequences into plasmids of *E.* Coli to generate insulin chains. Further, they produced the chains separately, extracted and combined them by creating disulfide bonds to give rise to human insulin.

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Biotechnology Applications In Medicine

Gene Therapy

- If a child is born with a genetic defect, is there a way to correct that defect? Yes, there is, with gene therapy! Gene therapy is a biotechnology application involving a collection of methods that can correct a gene defect in a child or an embryo. It involves inserting a normal gene into the person's cells or tissues to compensate for the non-functional gene. Let's understand how this works.
- In 1990, the first clinical gene therapy was applied to treat a 4-year old girl with a deficiency in the enzyme adenosine deaminase (ADA). This disorder is due to the lack of the gene for ADA, which is an enzyme important for the function of the immune system. Bone marrow transplantation helps cure this disorder in some cases. Enzyme replacement therapy, which involves injecting the patient with functional ADA, is also effective in some cases. However, both these procedures are not completely curative.

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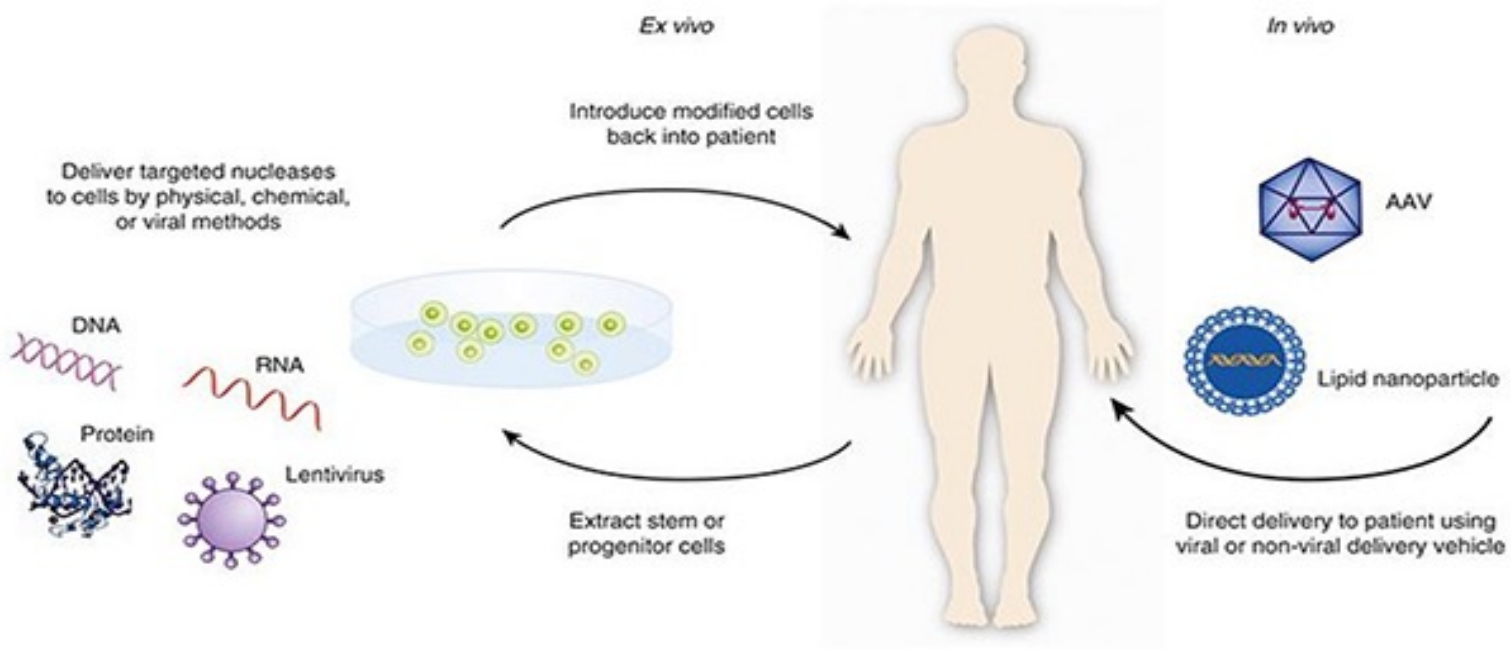
Biotechnology Applications In Medicine



In gene therapy, blood lymphocytes of the patient are grown in a culture outside the body. Subsequently, a functional ADA cDNA is incorporated into these lymphocytes and re-introduced into the patient. This alleviates the symptoms of the disorder. However, the patient requires periodic infusions of these genetically-engineered lymphocytes, since these cells are not immortal. A permanent cure for this could be to introduce the gene producing ADA from marrow cells into cells at early embryonic stages of life.

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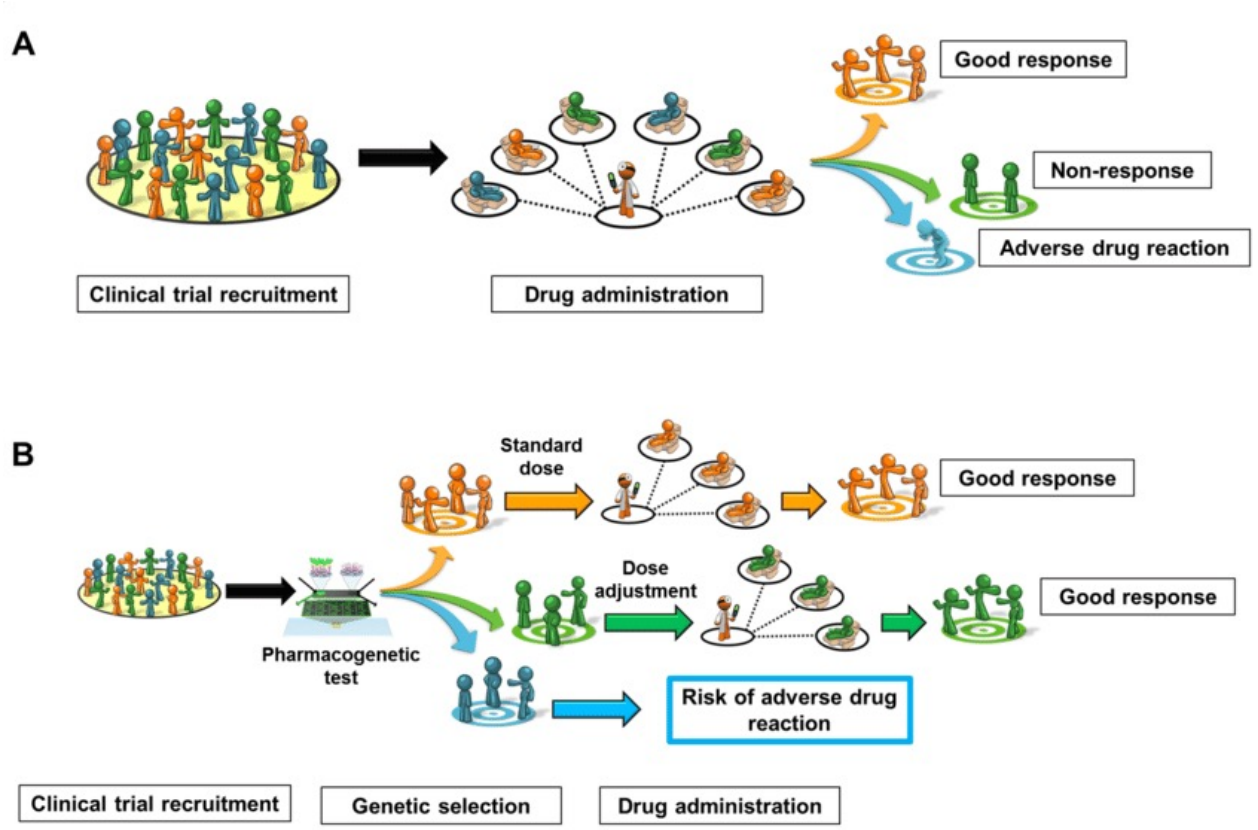
Biotechnology Applications In Medicine



Pharmacogenomics

We know that, thanks to our DNA, each of us is a little bit different. Some of those differences are obvious, like eye and hair color. Others are not so obvious, like how our bodies react to medication. Researchers are beginning to look at how to tailor medical treatments to our genetic profiles, in a relatively new field called **pharmacogenomics**. Some of the biggest breakthroughs have been in cancer treatment. For additional information on this "personalized medicine," listen to this.

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Biotechnology Applications In Medicine

Synthetic Biology

Imagine living cells acting as memory devices, biofuels brewing from yeast, or a light receptor taken from algae that makes photographs on a plate of bacteria. The new field of **synthetic biology** is making biology easier to engineer so that new functions can be derived from living systems. Find out the tools that synthetic biologists are using and the exciting things they are building at www.kqed.org/quest/television...thetic-biology.

Molecular Diagnosis

We all know that early diagnosis of a disease is important to effectively treat the disease. Early detection is not possible using conventional methods like serum and urine analysis. Let's look at some biotechnology applications that help in early diagnosis of diseases.

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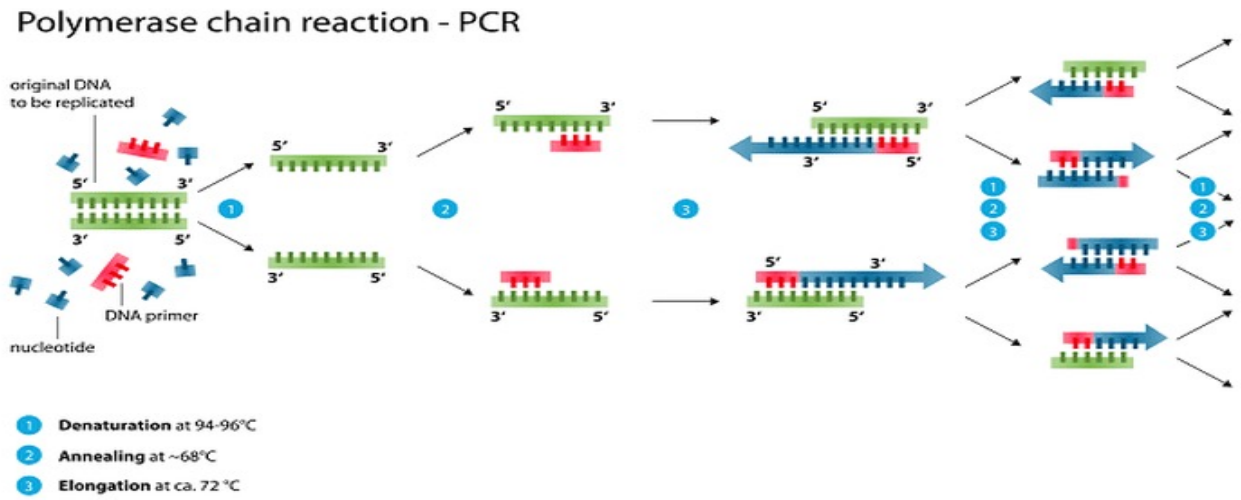
Biotechnology Applications In Medicine: Molecular Diagnosis



- Polymerase Chain Reaction (PCR)
- Normally, we can detect a pathogen (bacteria, virus etc.) only when the disease symptoms start to appear. However, by this time, the pathogen concentration in the body is very high! Is there a way to detect pathogens at initial stages of the disease when their concentrations are low?
- Yes, using a technique called PCR. PCR involves amplification of the nucleic acid in the pathogen allowing us to detect the pathogen at very low concentration. Today, we use PCR routinely to detect HIV in suspected AIDS patients and to detect gene mutations in suspected cancer patients.

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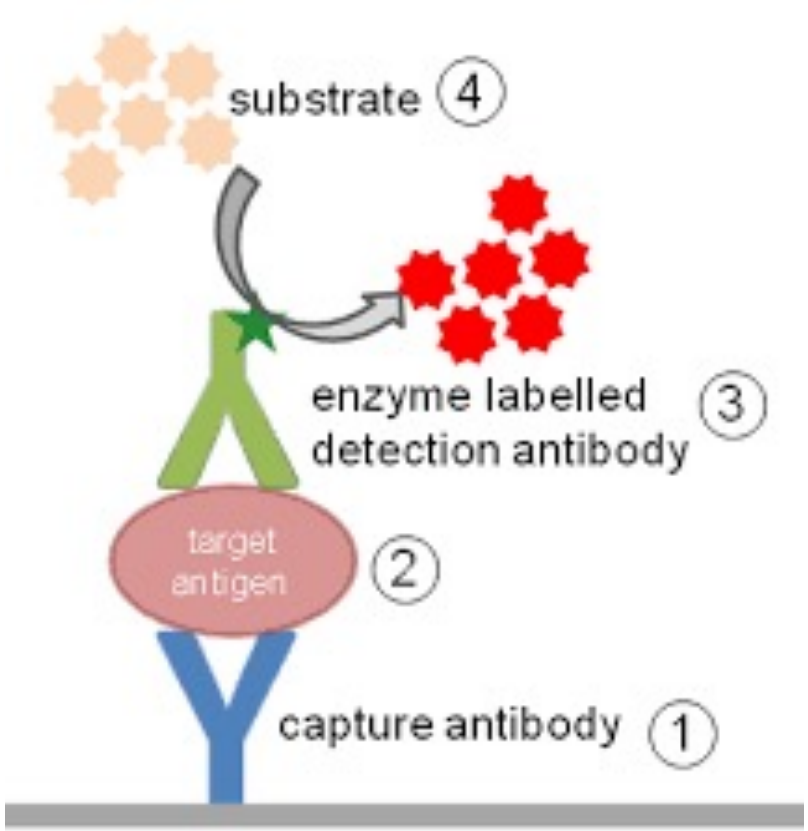
Biotechnology Applications In Medicine: Molecular Diagnosis

Enzyme-Linked Immunosorbent Assay (ELISA)

- The basic principle of ELISA is antigen-antibody reactions. ELISA can diagnose infections by detecting the presence of antigens (proteins of the pathogen) in the patient serum or by detecting the antibodies produced against the pathogen.



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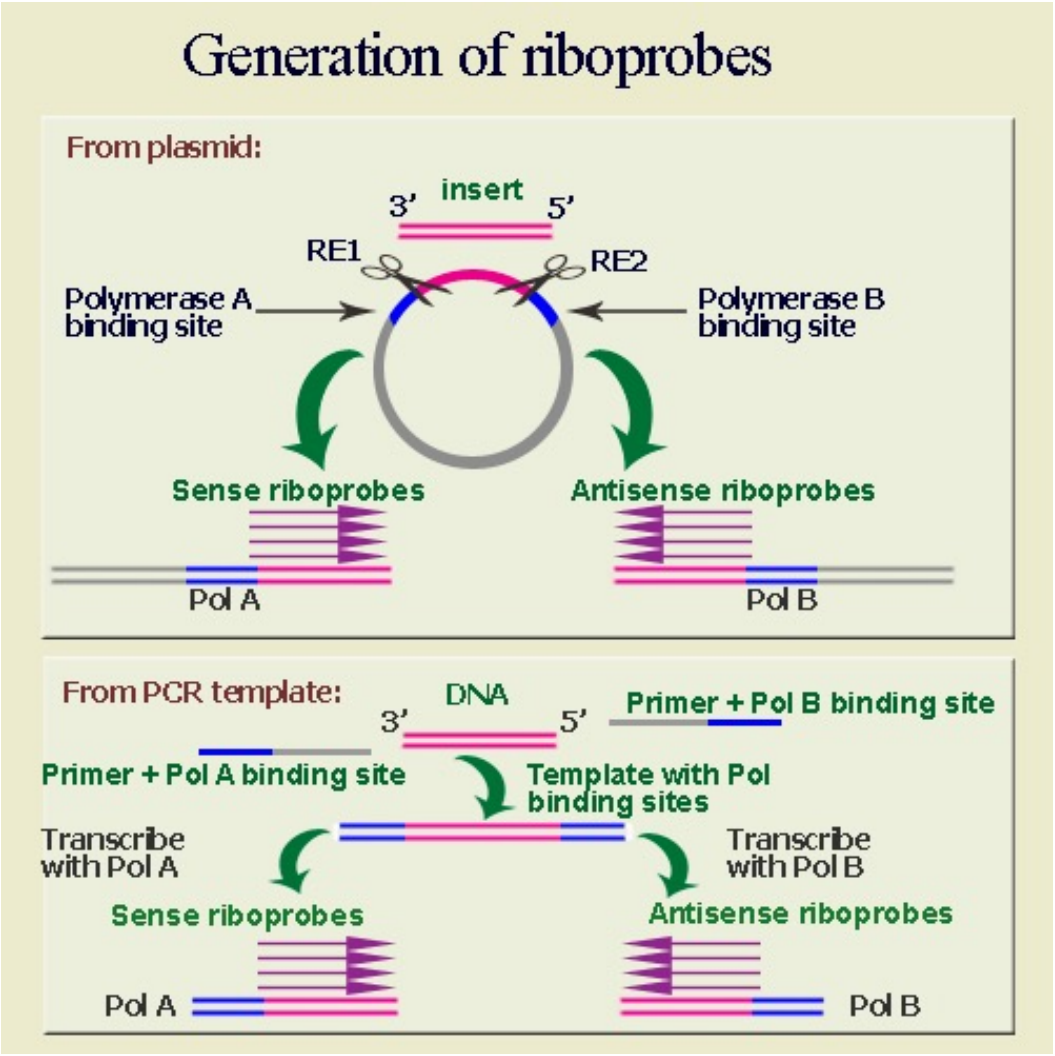
Biotechnology Applications In Medicine: Molecular Diagnosis

In Situ Hybridisation

- This technique involves tagging a single-stranded DNA or RNA with a radioactive molecule (probe). This then hybridizes with its complementary DNA in a clone of cells. On detection using autoradiography, the clone with the mutated gene will not appear on the photographic film because the probe is not complementary to the mutated gene.



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Biotechnology Applications In Agriculture

Application of Biotechnology in Agriculture involves scientific techniques such as Genetically Modified Organisms, Bt Cotton, Pest Resistant Plants. It helps in modifying plants, animals, and microorganisms and improve their agricultural productivity. Techniques like vaccines, tissue culture, genetic engineering are also used.

Ref: https://www.toppr.com/guides/biology/biotehnology-and-its-applications/application-of-biotechnology-in-agriculture/

Biotechnology Applications In Agriculture

Genetically Modified Organisms

- You must have heard the term ‘GMO’ used by people around you or in the news every now and then. What does this mean? GMO stands for ‘Genetically Modified Organisms’. GMOs are plants, animals, bacteria or fungi whose genes have been modified by genetic manipulation.

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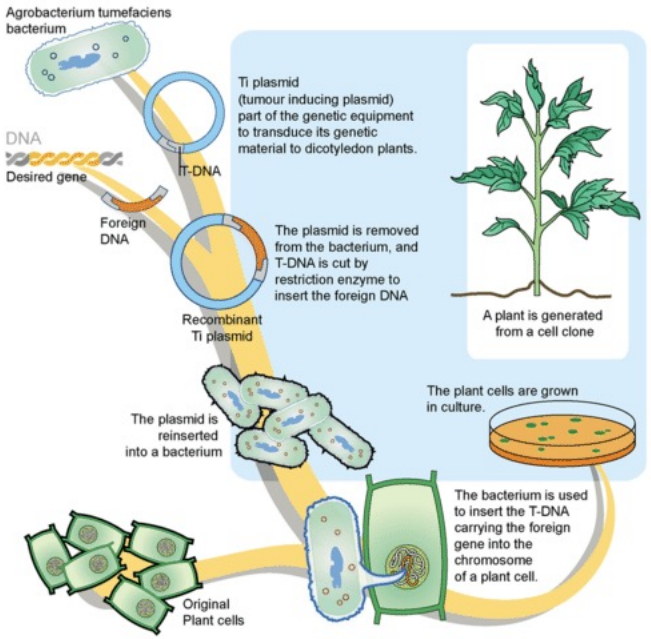
Biotechnology Applications In Agriculture

Genetically modified crops or GM crops are used in the following ways:

- They are more tolerant to stresses such as drought, cold, heat etc.
- They are pest-resistant and therefore less dependent on chemical pesticides.
- Genetically Modified crops help to reduce post-harvest losses.
- They help to increase the mineral usage by plants, thereby preventing early exhaustion of soil fertility.
- Genetically modified crops have enhanced nutritional value. Example – Vitamin A enriched rice.

Genetic modifications also help to create tailor-made plants to provide alternative resources to industries, such as fuels, starches, and pharmaceuticals. Let's look at some examples of GM crops and how they are useful.



Creating a Transgenic Crop. A transgenic crop is genetically modified to be more useful to humans. The bacterium transfers the T-DNA (from the Ti plasmid) fragment with the desired gene into the host plant's nuclear genome.

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Biotechnology Applications In Agriculture

Creating a Transgenic Crop.

A transgenic crop is genetically modified to be more useful to humans. The bacterium transfers the T-DNA (from the Ti plasmid) fragment with the desired gene into the host plant's nuclear genome.

- Transgenic crops have been created with a variety of different traits, such as yielding more food, tasting better, surviving drought, and resisting insect pests. Scientists have even created a transgenic purple tomato that contains a cancer-fighting compound and others that have high levels of antioxidants (see **Figure** below). Seehttp://extension.oregonstate.edu/...tomato-debuts-'indigo-rose' for more information. To learn how scientists have used biotechnology to create plants that can grow in salty soil, watch the video "Salt of the Earth - Engineering Salt-tolerant Plants" at this link:http://www.sosq.vcu.edu/videos.aspx.

Transgenic Purple Tomato. A purple tomato is genetically modified to contain a cancer-fighting compound. A gene for the compound was transferred into normal red tomatoes.

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Biotechnology Applications In Agriculture

Bt Cotton

- This is a genetically modified version of cotton. 'Bt' stands for the microbe *Bacillus thuringiensis.* This microbe produces an insecticidal protein or toxin that kills other insects such as tobacco budworm, flies, mosquitoes, beetles etc. Why is this protein not toxic to the *Bacillus* itself?
- This is because it stays inactive (as protoxin) in the *Bacillus.* It gets activated only once it comes in contact with the alkaline pH in the insect gut when the insect ingests it. The activated toxin then binds to the surface of epithelial cells and creates pores in it. This causes the cells to swell and lyse, eventually leading to the death of the insect.

Scientists isolated the Bt toxin genes from *Bacillus thuringiensis* and incorporated it into various crop plants such as cotton. This variety is 'Bt cotton'. Since most Bt toxins are insect-group specific, the choice of genes to be incorporated depends on the crop and the targeted pest. A gene named *cry* codes for the toxin protein and there a number of these genes. For example, the genes *cryIAc* and *cryIIAb* encode toxins that control cotton bollworms whereas the gene *cryIAb* controls the insect 'corn borer'.

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Biotechnology Applications In Agriculture

- Pest Resistant Plants
- Several nematodes live as parasites on multiple hosts like plants, animals, and even human beings. A specific nematode '*Meloidegyne incognitia*' infects the roots of tobacco plants and causes a great decrease in yield. To prevent this infestation, a novel strategy was adopted which is based on the process of **RNA interference (RNAi)**.
- RNAi is a method of cellular defence in all eukaryotes. It involves the silencing of a specific mRNA by a complementary double-stranded (ds) RNA that binds and inhibits the translation of this mRNA. The complementary RNA can come from an infection by viruses that have RNA genomes or genetic elements called 'transposons'.

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Biotechnology Applications In Agriculture

- Scientists took advantage of this process and introduced nematode-specific genes into host plants using *Agrobacterium* vectors. The introduced DNA produces both sense and anti-sense strands in the host cells. These complementary strands then produce dsRNA and initiate RNAi and thus silence the specific RNA of the nematode. Consequently, the parasite cannot survive in the host that expresses this RNA, leading to resistance against that parasite.

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Transgenic Animals and Ethical Issues

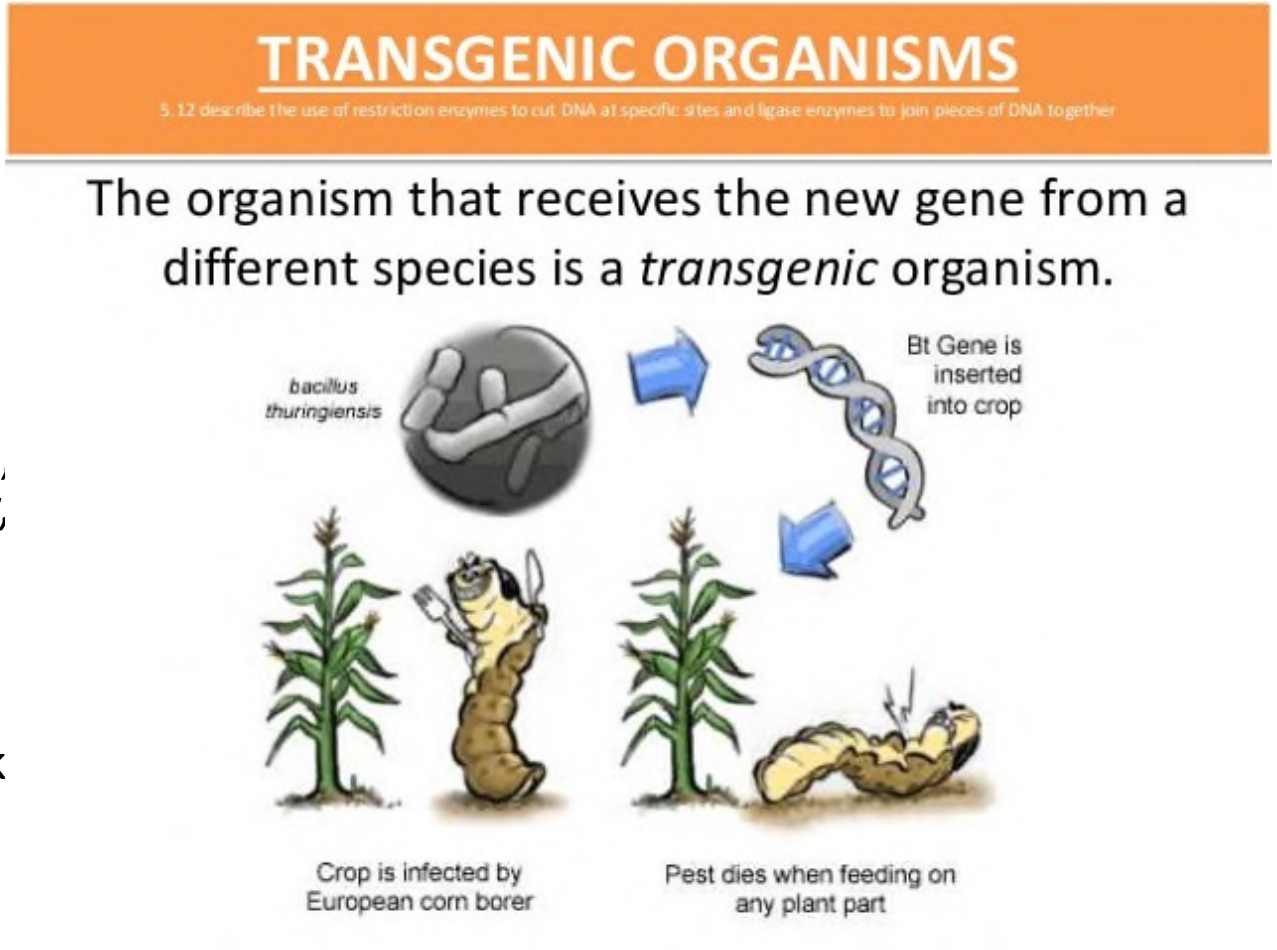
- You may have heard of scientists using mice or rats to do their research. Are these the same mice or rats we find around us commonly? No! Scientists use genetically modified mice or rats for their research. These are ‘Transgenic animals’. Let’s learn more about these animals.

Ref: https://www.toppr.com/guides/biology/biotechnology-and-its-applications/transgenic-animals-and-ethical-issues/

Transgenic Animals and Ethical Issues

Transgenic Animals

- These are animals that have had their DNA manipulated to express an additional (foreign) gene. Although more than 95% of transgenic animals are mice; there also exist transgenic rats, sheep, rabbits, pigs, cow, and fish. What is the need to generate these animals and how do we benefit from these modifications? Let's look at some common reasons.



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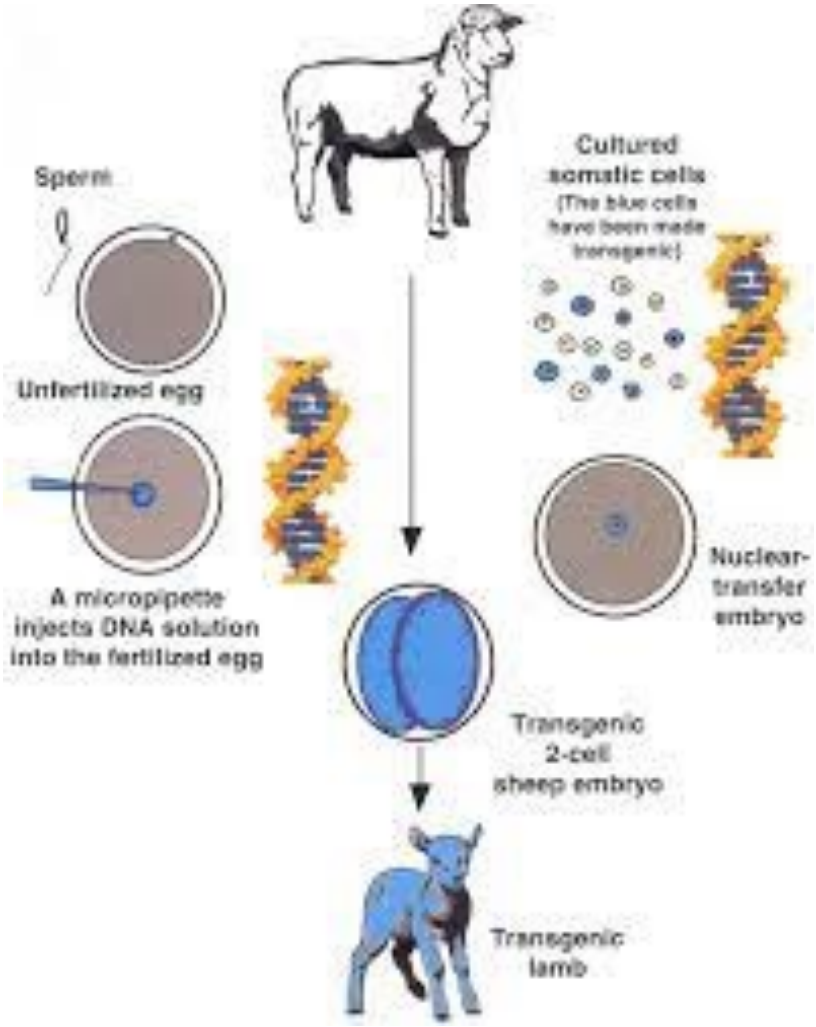
Transgenic Animals and Ethical Issues

Normal Physiology and Development

- Transgenic animals allow us to study how genes affect the normal functions of the body and its development. In addition, they also help us understand how genes are regulated in the body.
- For example, by introducing genes from other species, that alter the formation of a particular factor and by studying its biological effects, we can gain knowledge about the biological role of the factor in our body.



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Transgenic Animals and Ethical Issues

Study of Disease

- Transgenic animals also serve as models for human diseases and increase our understanding of how genes contribute to disease development. They also make it possible to investigate new treatment methods for diseases. Currently, transgenic models exist for many human diseases such as Alzheimer's, cancer, rheumatoid arthritis, cystic fibrosis etc.

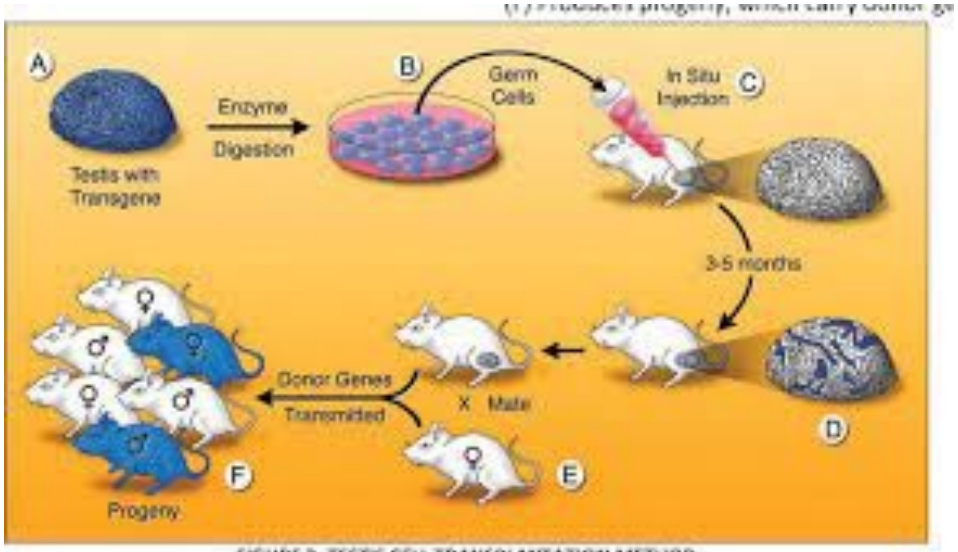

FIGURE 2: TESTIS CELL TRANSPLANTATION METHOD

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Transgenic Animals and Ethical Issues

Biological Products

- Treatment of certain human diseases requires medicines that contain biological products, which are usually expensive to make. Transgenic animals can be used to make these biological products too. Incorporation of genes of a particular product into transgenics will result in the production of that biological product.
- An example is human α-1-antitrypsin which is used to treat emphysema. The most noteworthy example is Rosie – the first transgenic cow, generated in 1997. The milk from this cow contained human α-lactalbumin and had a higher nutritional value for human babies, compared to natural cow milk.

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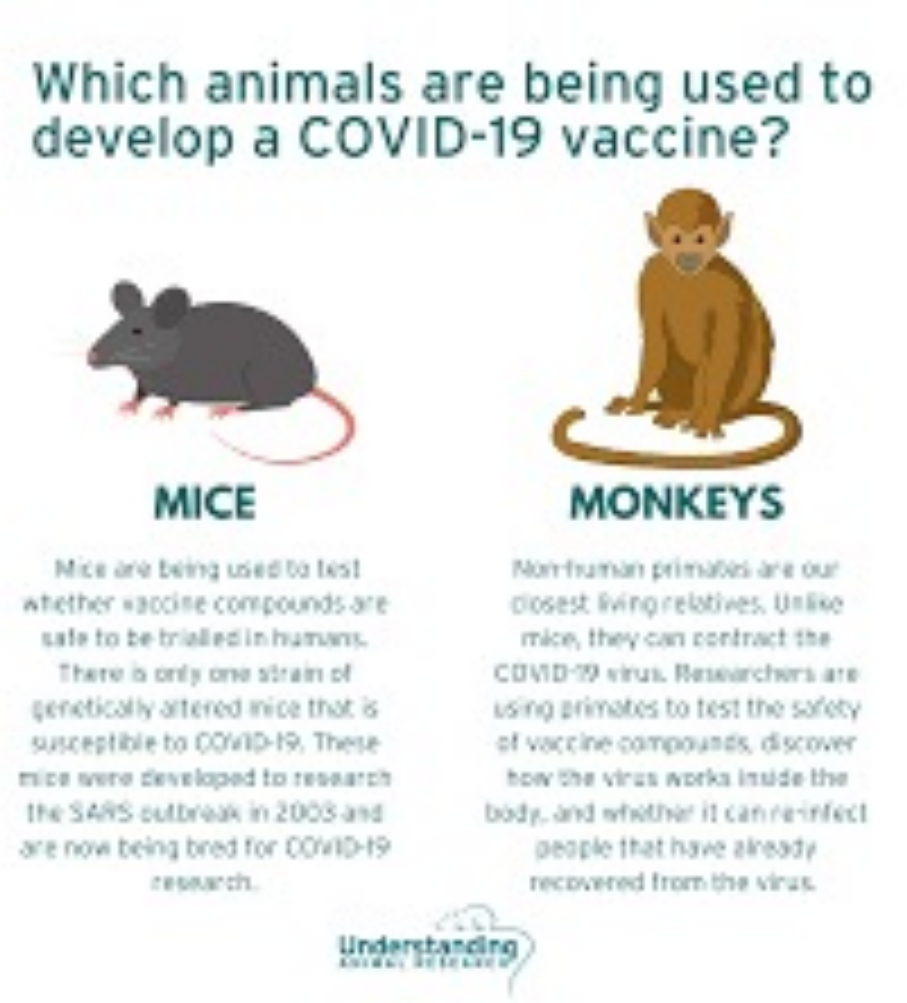
Transgenic Animals and Ethical Issues

Vaccine Safety

- Before using vaccines on humans, they are first tested for safety on transgenic mice. Transgenic mice are currently in use to test the safety of polio vaccines. If the test is successful and reliable, transgenic mice will replace the use of monkeys to test the safety of these vaccines.



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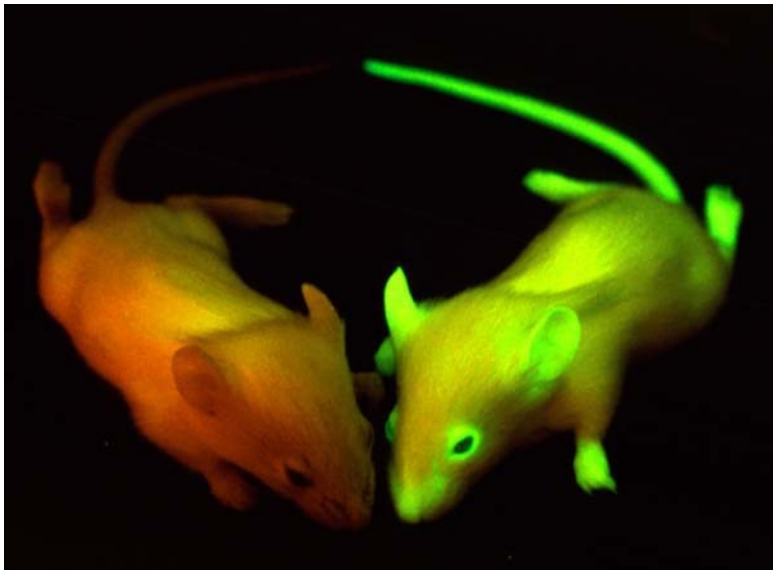
Transgenic Animals and Ethical Issues

Chemical Safety Testing

- The toxicity or safety of substances is also tested using transgenics. For this, transgenic animals are created with genes that make them more sensitive to the toxic substance under study. Subsequently, they are exposed to the toxic substance and the effects are studied. Using transgenics for toxicity testing gives results in a short time.



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Transgenic Animals and Ethical Issues

Ethical Issues

- What do you think will happen if the genetic manipulation of living organisms by humans goes unregulated?! It will not only lead to exploitation of the organisms but will also have harmful impacts on our ecosystem. It is wrong at the moral and biological level! This is why we need ethical standards to regulate the human manipulation of organisms.

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Transgenic Animals and Ethical Issues

GEAC

- In terms of genetically modified organisms (GMOs), the Indian Government has set up the **Genetic Engineering Approval Committee (GEAC)**. This organization makes decisions regarding the validity of research involving GMOs and addresses the safety of GMOs introduced for public use.

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Transgenic Animals and Ethical Issues

Patents And Rights

- Recently, certain companies were granted patents for products and technologies that involve genetic material or other resources developed and used by people of a specific region over many years. This has angered a lot of people.
- A recent example is Basmati rice, which was developed by Indian farmers over hundreds of years. An American company, in 1997, obtained the patent rights for Basmati rice from the US Patent and Trademark Office. This patent not only allows this company to sell new variations of Basmati rice and make a profit but also restricts other people from selling it.
- Similar attempts have been made to patent Indian herbal medicines too. Therefore, people and countries need to be vigilant and counter patent applications that exploit products and techniques native to their land.

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Transgenic Animals and Ethical Issues

Biopiracy

- Biopiracy refers to the use of bio-resources by multinational companies without proper authorization or compensation to the people or country concerned. Most developed, financially rich nations are poor in biodiversity or traditional know-how; while it is the other way around for developing nations. This leads to exploitation of the traditional knowledge to develop modern, commercial applications that save the makers time, money and effort.
- People are now more aware of the injustice and inadequacy in compensation and sharing of benefits between the developing and developed nations. As a result, several nations now have laws that prevent other nations from exploiting their bio-resources and traditional knowledge. The Indian Parliament recently cleared the second amendment of the Indian Patents Bill, that takes the issue of biopiracy into consideration.

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