Course Name: Human Cytogenetics

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Unit-6 : Recombinant DNA Technology

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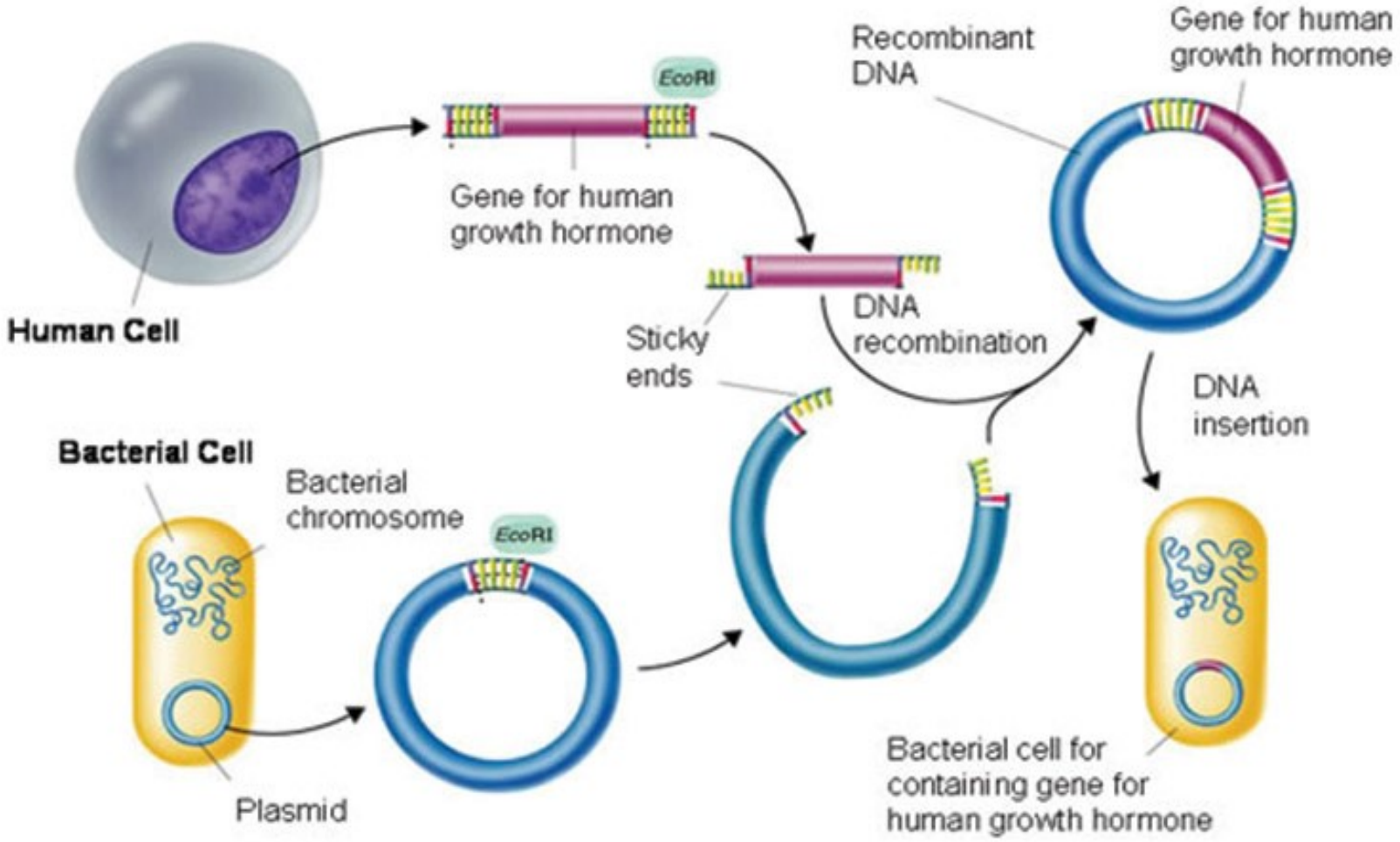
Recombinant DNA Technology

- A technique mainly used to change the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology.
- There are multiple steps, tools and other specific procedure followed in the recombinant DNA technology, which is used for producing artificial DNA to generate the desired product.

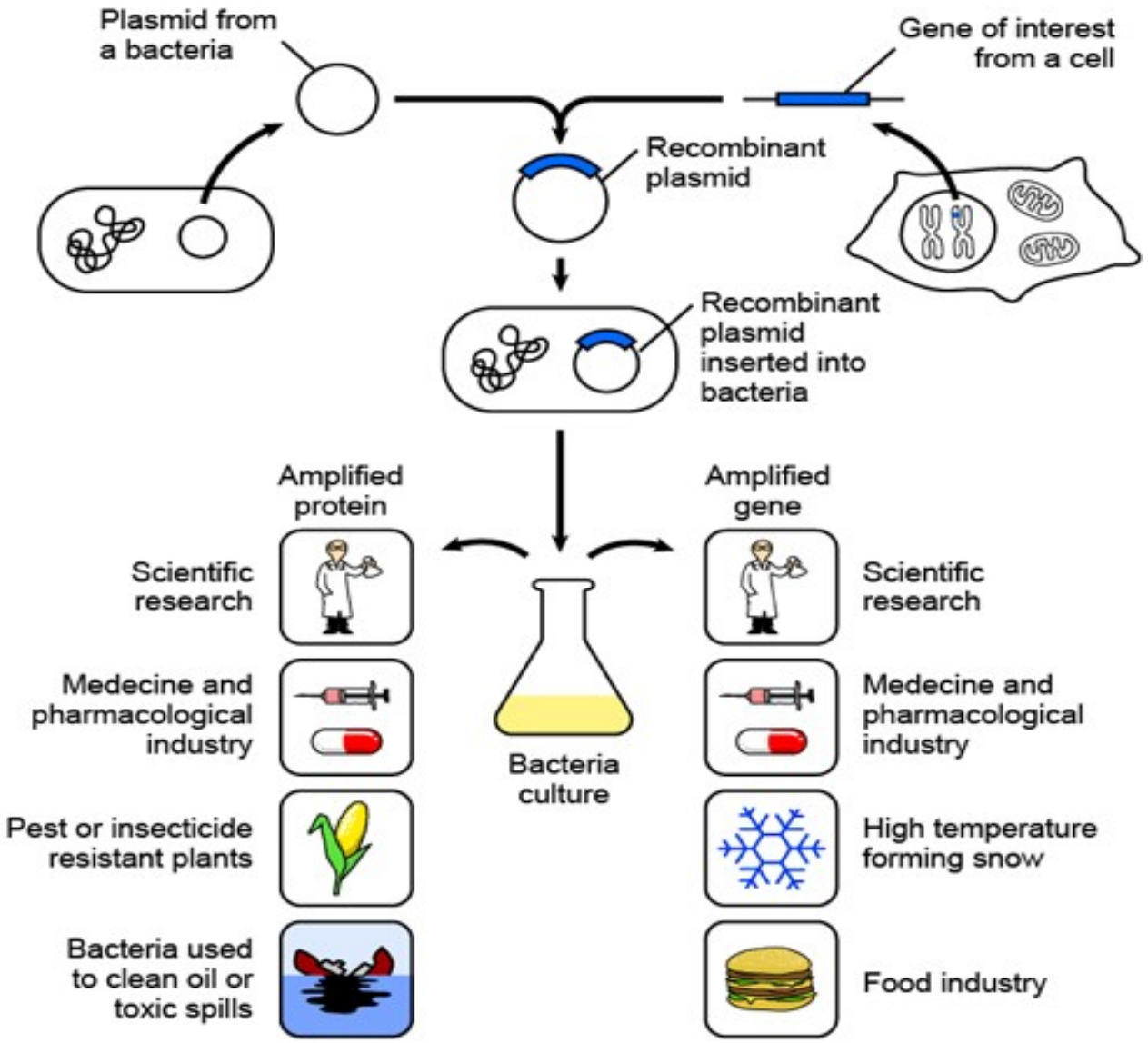
- Recombinant DNA technology was first applied to protein production in mammalian cells in the early 1980s. This approach was facilitated by the development of transfection methods for the efficient delivery of plasmid DNA into cultivated mammalian cells. Chinese hamster ovary (CHO) cells were the first mammalian host to be used for gene transfer, but many other cultured cell lines also proved to be acceptable hosts. The first recombinant proteins generated in CHO cells included β-interferon and tissue-type plasminogen activator (tPA). The latter became the first FDA-approved recombinant therapeutic protein (Activase) from mammalian cells in 1986.



Recombinant DNA Technology

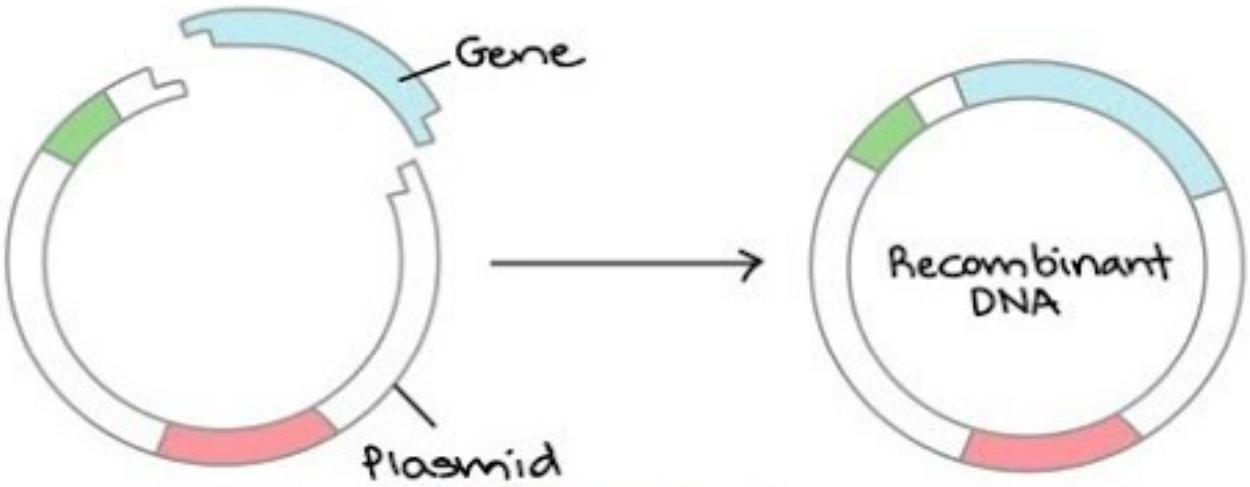
What is Recombinant DNA Technology?

- The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to as Recombinant DNA Technology.
- Recombinant DNA technology is popularly known as genetic engineering.
- The recombinant DNA technology emerged with the discovery of restriction enzymes in the year 1968 by Swiss microbiologist Werner Arber,
- Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed.
- Thus the recombinant DNA has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring.


Plasmid from a bacteria
Gene of interest from a cell
Recombinant plasmid
Recombinant plasmid inserted into bacteria
Amplified protein
Amplified gene
Scientific research
Medecine and pharmacological industry
Pest or insecticide resistant plants
Bacteria used to clean oil or toxic spills
Bacteria culture
Scientific research
Medecine and pharmacological industry
High temperature forming snow
Food industry

Tools Of Recombinant DNA Technology

- The enzymes which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases.
- The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.



Tools Of Recombinant DNA Technology

- The vectors – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have a very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.
- Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.
- There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

Process of Recombinant DNA Technology

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

➢ **Step-1.**

Isolation of Genetic Material

- The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.
- Since DNA exists within the cell membrane along with other macromolecules such as RNA, polysaccharides, proteins, and lipids, it must be separated and purified which involves enzymes such as lysozymes, cellulase, chitinase, ribonuclease, proteases etc.
- 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.

➢ Step-2.

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 separating and cutting out the digested DNA fragments.
- The vector DNA is also processed using the same procedure.

➢ Step-3.

Amplification Using PCR

- Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – DNA polymerase in vitro.
- 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.

➢ Step-4.

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 result-ing DNA molecule is a hybrid of two DNA molecules – the interest molecule and the vector. In the ter-minology of genetics this intermixing of dif-ferent DNA strands is called recombination.
- Hence, this new hybrid DNA molecule is also called a recombinant DNA molecule and the technology is referred to as the **recom-binant DNA technology**.

➢ Step-5.

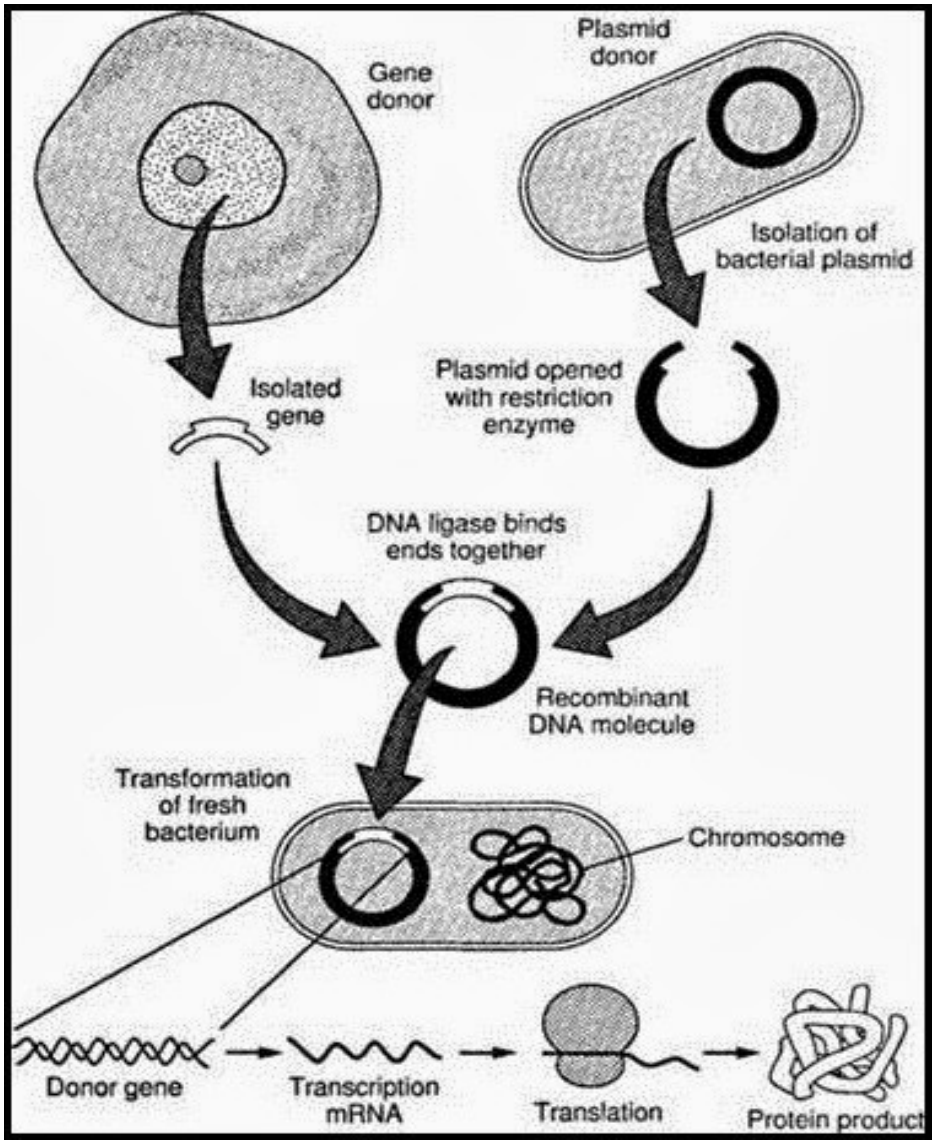
Insertion of Recombinant DNA Into Host

- In this step, the recombinant DNA is introduced into a recipient host cell mostly, a bacterial 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 processes used may be thermal shock, Ca^{++} ion treatment, electroporation etc.

➢ Step- 6

Isolation of Recombinant Cells

- The transformation process generates a mixed population of transformed and non-trans- formed host cells.
- The selection process involves filtering the transformed host cells only.
- For isolation of recombinant cell from non-recombinant cell, marker gene of plasmid vector is employed.
- For examples, PBR322 plasmid vector contains different marker gene (Ampicillin resistant gene and Tetracycline resistant gene. When pst1 RE is used it knock out Ampicillin resistant gene from the plasmid, so that the recombinant cell become sensitive to Ampicillin.



Steps in Recombinant DNA Technology

Application of Recombinant DNA Technology

- Recombinant DNA is widely used in biotechnology, medicine, and research.
- The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences.
- Recombinant DNA is used to identify, map and sequence genes, and to determine their function.
- Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms.
- Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering.

- DNA technology is also used to detect the presence of HIV in a person.
- **Application of recombinant DNA technology in Agriculture** – For example, manufacture of Bt-Cotton to protect the plant against ball worms.
- **Application of medicines** – Insulin production by DNA recombinant technology is a classic example.
- **Gene Therapy** – It is used as an attempt to correct the gene defects which give rise to heredity diseases.
- **Clinical diagnosis** – ELISA is an example where the application of recombinant DNA is possible.

DNA Cloning

- A clone is a cluster of individual entities or cells that are descended from one progenitor.
- Clones are genetically identical as the cell simply replicates producing identical daughter cells every time.
- Scientists are able to generate multiple copies of a single fragment of DNA, a gene which can be used to create identical copies constituting a DNA clone.
- **DNA cloning** takes place through the insertion of DNA fragments into a tiny DNA molecule. This molecule is made to replicate within the living cell, for instance, a bacterium. The tiny replicating molecule is known as the carrier of the DNA vector.

- Yeast cells, viruses, Plasmids are the most commonly used vectors.
- Plasmids are the circular DNA molecules that are introduced from bacteria.
- They are not part of the main cellular genome. It carries genes, which provide the host cell with beneficial properties such as mating ability, drug resistance.
- They can be conveniently manipulated as they are small enough and they are capable of carrying extra DNA which is weaved into them.

Applications Of Gene Cloning

- Gene Cloning plays an important role in the medicinal field. It is used in the production of hormones, vitamins and antibiotics.
- Gene cloning finds its applications in the agricultural field. Nitrogen fixation is carried out by cyanobacteria wherein desired genes can be used to enhance the productivity of crop and improvement of health. This practice reduces the use of fertilizers hence chemical-free produce is generated
- It can be applied to the science of identifying and detecting a clone containing a particular gene which can be manipulated by growing in a controlled environment
- It is used in gene therapy where a faulty gene is replaced by insertion of a healthy gene. Medical ailments such as leukemia and sickle cell anaemia can be treated with this principle.

Limitations of Recombinant DNA technology

- Destruction of native species in the environment the genetically modified species are introduced in.
- Resilient plants can theoretically give rise to resilient weeds which can be difficult to control.
- Cross contamination and migration of proprietary DNA between organisms.
- Recombinant organisms contaminating the natural environment.
- The recombinant organisms are population of clones, vulnerable in exact same ways. A single disease or pest can wipe out the entire population quickly.
- Creation of superbug is hypothesized.

- Ethical concern about humans trying to play God and mess with the nature's way of selection. It is exaggerated by the fear of unknown of what all can be created using the technology and how is it going to impact the civilization.
- Such a system might lead to people having their genetic information stolen and used without permission.
- Many people worry about the safety of modifying food and medicines using recombinant DNA technology.

THANK YOU