Genetic engineering

Modern genetic technology can be used to modify the genomes of living organisms. This process is also known as "genetic engineering." Genes of one species can be modified, or genes can be transplanted from one species to another. Genetic engineering is made possible by recombinant DNA technology.

General terms

- **Biotechnology**: use of microbes to make a protein product
- **Recombinant DNA technology**: – Insertion or modification of genes to produce desired proteins.
- **Genetic engineering**: manipulation of genes/insert DNA into cells.
- **Gene Cloning**: isolating genes from one organism, manipulating purified DNA in vitro, and transferring to another organism.

Genetic Engineering: can alter the DNA code of living organisms by:

1. Selective Breeding 2. Recombinant DNA **3.** PCR

4. Gel Electrophoresis 5. Transgenic Organisms

1. Selective Breeding:

- Selective breeding involves selecting parents that have characteristics of interest in the hope that their offspring inherit those desirable characteristics.
- Breed only those plants or animals with desirable traits
- People have been using selective breeding for 1000's of years with farm crops and domesticated animals.
- Example of selective breeding: cows that produce lots of milk, chickens that produce large eggs and wheat plants that produce lots of grain.

2. Recombinant DNA:

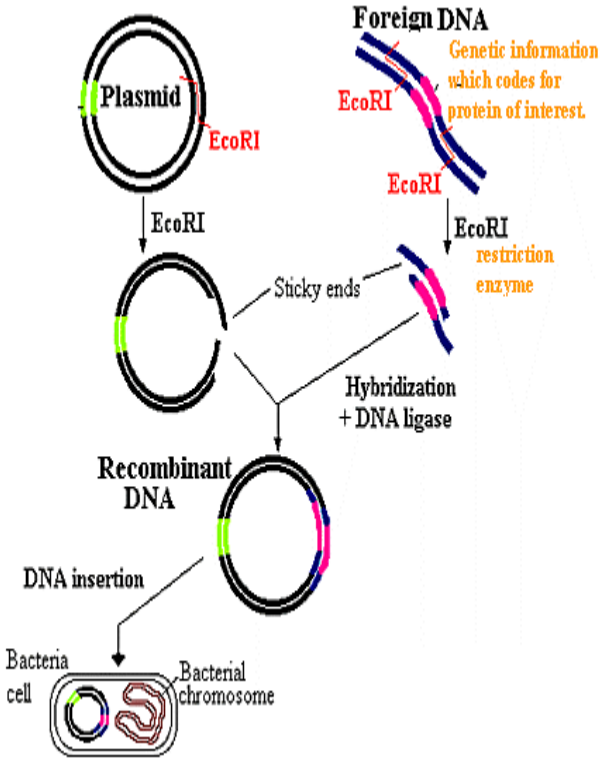
- The ability to combine the DNA of one organism with the DNA of another organism.
- Recombinant DNA technology was first used in the 1970's with bacteria

Recombinant Bacteria:

1. Remove bacterial DNA (plasmid).
2. Cut the DNA from another organism with "restriction enzymes".
3. Combine the cut pieces of DNA

together with another enzyme and insert them into bacteria.

4. Reproduce the recombinant bacteria.
5. The foreign genes will be expressed in the bacteria.



Restriction enzymes:

Recognize a specific sequence of bases and cut the DNA backbone

Enzymes are named from the organism they are isolated from

- EcoR1 (GAATTC)
- Sau3A (GATC)
- Generates "sticky ends"

Benefits of Recombinant Bacteria:

1. Bacteria can make human insulin or human growth hormone.
2. Bacteria can be engineered to "eat" oil spills.

3. Transgenic organisms

Transgenic organisms contain foreign DNA that has been introduced using biotechnology. Foreign DNA (the transgene) is defined as DNA from another species, or else recombinant DNA from the same species that has been manipulated in the laboratory then reintroduced.
Organisms that have altered genomes are known as transgenic. Most transgenic organisms are generated in the laboratory for research purposes. For example, "knock-out" mice are transgenic mice that have a particular gene of interest disabled.

Transgenic animals:

1. Mice – used to study human immune system

2. Chickens – more resistant to infections
3. Cows – increase milk supply and leaner meat
4. Goats, sheep and pigs – produce human proteins in their milk

4. Polymerase Chain Reaction (PCR):

The polymerase chain reaction (PCR) is a technique to amplify a piece of DNA very rapidly outside of a cell. It is one of the most powerful technologies in molecular biology. Using PCR, specific sequences within a DNA or cDNA template can be copied, or "amplified", many thousand- to a million-fold using sequence specific oligonucleotides, heat stable DNA polymerase, and thermal cycling.

Basic concept of PCR:

One DNA molecule is used to produce two copies, then four, then eight and so forth. This continuous doubling is accomplished by specific enzyme that able to string together individual DNA building block to form long molecular strand.

Steps in PCR: A cycle of PCR consists of three steps:

1. **DNA denaturation at 95 degrees C.**

High temperature incubation is used to "melt" double-stranded DNA into single strands and loosen secondary structure in single-stranded DNA. The highest temperature that the DNA polymerase can withstand is typically used (usually 95°C). The denaturation time can be increased if template GC content is high.

2. **Primer annealing at 50-60 degrees C.**

During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers (5°C below the Tm of the primer). (Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA).

3. **DNA polymerization by a thermostable DNA polymerase at 72 degrees C**

At 70-72°C, the activity of the DNA polymerase is optimal, and primer extension occurs at rates of up to 100 bases per second. When an amplicon in real-time PCR is small, this step is often combined with the annealing step using 60°C as the temperature.

Applications of PCR:

1. Forensic medicine.
2. Preimplantation Genetic Diagnosis (PGD).
3. Archeology.

4. Paternity testing.
5. Gene manipulation and expression studies
6. Comparison studies of genomics.
7. Comparison studies with gene cloning
8. Detect pathogens
9. Diagnose genetic disease

4. Gel Electrophoresis:

It is a technique used to separate DNA fragments according to their size. This technology allows scientists to identify someone's DNA.

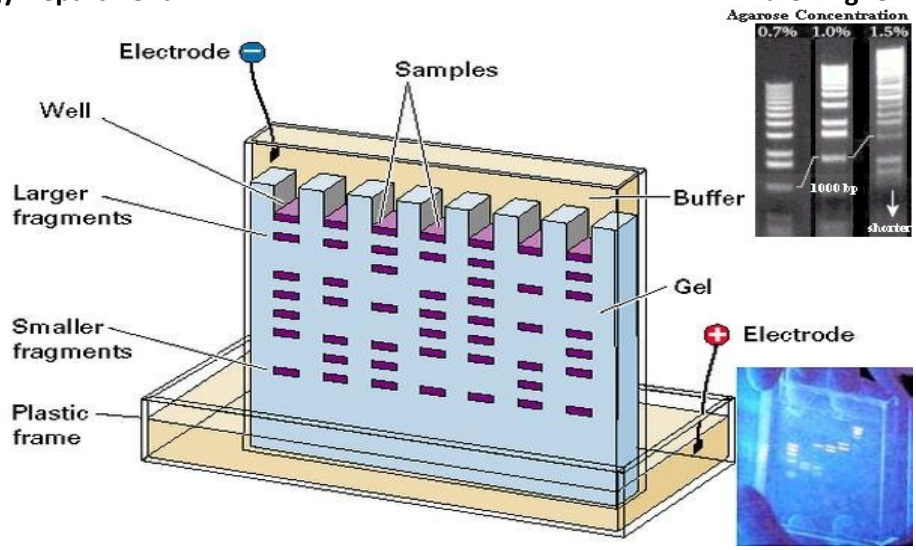
- DNA samples are loaded into wells (indentations) at one end of a gel, and an electric current is applied to pull them through the gel.

- DNA fragments are negatively charged, so they move towards the positive electrode. Because all DNA fragments have the same amount of charge per mass, small fragments move through the gel faster than large ones.

- When a gel is stained with a DNA-binding dye, the DNA fragments can be seen as **bands**, each representing a group of same-sized DNA fragments.

Steps Involved in Gel Electrophoresis:

1. "Cut" DNA sample with restriction enzymes.
2. Run the DNA fragments through a gel.
3. Bands will form in the gel.
4. Everyone's DNA bands are unique and can be used to identify a person.
5. DNA bands are like "genetic fingerprints".



Why is genetic engineering important?

1. Purify protein

– Insulin

– Growth factor

– Interferon

2. Generate more copies of a particular gene: “amplify DNA”

3. Research gene function and regulation

GOOD LUCK