3.4 GENETIC ENGINEERING & BIOTECHNOLOGY

PCR (Polymerase Chain Reaction)

- PCR copies and **amplifies** minute quantities of nucleic acids.
- In the polymerase chain reaction, DNA copies itself continuously to produce copies of the original DNA molecule (millions of identical copies).
- The whole procedure takes place in only a few hours.
- PCR is useful when large amounts of DNA are needed for experimental use, but only very small quantities are found in the real samples.
- DNA from very small samples of semen, blood, or any other tissue or even from long-dead specimens can be amplified using PCR.
- To speed up PCR, high temperatures are needed, so a special type of heat-stable DNA polymerase is used (**Taq DNA polymerase**).
- DNA is heated to 95 ^{0}C to separate the two strands. The temperature is then reduced to 53 ^{0}C, which allows primers to bind to both strands. Finally, the temperature is increased to 73 ^{0}C, which encourages Taq DNA polymerase to replicate both strands.
- The specific enzyme is obtained from a bacterium (*Thermus aquaticus*) that lives in hot springs hence its enzymes are active even at high temperatures.

DNA is copied in small tubes called **eppendorfs**. By the end of PCR there should be more than a hundred million copies of a gene in a 0.2 ml eppendorf**.**

The identification/ presence of DNA is achieved by Gel Electrophoresis.

GEL ELECTROPHORESIS

- A molecule with a net charge will move in an electric field. This phenomenon is called electrophoresis.
- Therefore, Gel Electrophoresis is a method of separating mixtures of proteins and other macromolecules such as DNA and RNA, that are charged.
- The mixture is placed on a thin sheet of gel which acts as a molecular sieve.
- An electric field is applied to the chamber containing the gel by attaching electrodes to both ends. Depending on whether the particles (macromolecules) are positively or negatively charged, they move towards the one or the other electrode.
- The rate of movement depends on the **size** and **charge** of the molecules (small and highly charged molecules move faster along the gel than larger and less charged molecules).

Gel Electrophoresis of DNA is used in DNA Profiling (ταυτοποίηση DNA)

DNA PROFILING

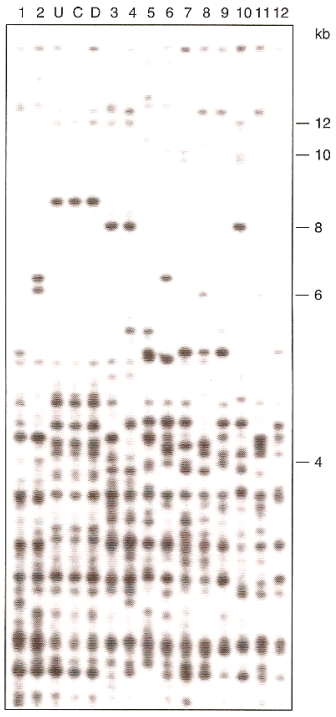
- Humans and other organisms have short base sequences (3-5 bases) that are repeated many times, called **"satellite DNA" or "short tandem repeats (STR).** This satellite DNA varies greatly between different individuals in the number of repeats.
- If the satellite DNA is copied using the PCR and then cut up into short fragments using restriction enzymes, the lengths of the fragments will vary greatly between the individuals.
- Gel Electrophoresis can be used to separate these fragmented pieces of DNA according to their size and charge.
- The pattern of bands on the Gel is very unlikely to be the same for any two individuals. This technique is called **DNA Profiling** or **DNA Fingerprinting**

Applications:

1) Criminal (murder or rape) Investigations
2) Research into population variation and tracking individuals in populations such as migrating whales
3) Paternity tests
4) Identification of people died a long time ago (mummies)

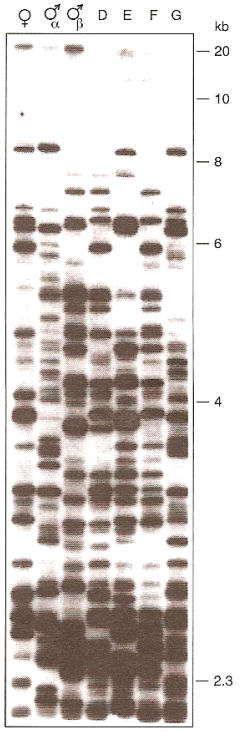
Description of two applications:

Testing whether samples of DNA show differences using DNA profiling



The results of DNA profiling of Dolly the sheep are shown above.
U = the udder cells from the donor sheep
C = cells in the culture derived from the udder cells
D = blood cells from Dolly the sheep
1 – 12 = results from 12 other sheep for comparison.
The results confirm that Dolly is a clone of the donor udder cells.

Testing parentage using DNA profiling



The DNA profiles of a family of dunnocks (*Prunella modularis*) are shown above. Dunnocks are small birds found in Europe, North Africa and Asia. The tracks from left to right are: the female, two resident males that might have been the father of the offspring and four offspring. The results show that the β male fathered three of the four offspring (D, E and F), despite being less dominant than the α male.

HUMAN GENOME PROJECT (HGP)

The Human Genome Project (1990 - 2000) was an international cooperative venture established to sequence the complete human genome.

- The human genome consists of ~ 23,000 genes
- The HGP aimed to find the location of all of these genes on the human chromosomes and the base sequence of all of the DNA that makes them up.
- Sequencing of the entire human genome will make it easier to study how genes control human development. It will allow easier identification of genetic diseases, production of new drugs based on DNA base sequences of genes or the structure of the proteins coded for these genes.
- More specifically, the HGP should lead to an understanding of many genetic diseases, the development of genome libraries to detect sufferers and carriers of genetic diseases. It may also lead to the production of pharmaceuticals based on DNA sequences.

→ By 2008 the genomes of over 1,000 humans had been sequenced

→ By 2012 the cost of sequencing dropped below 10,000 USD

→ By 2014 the genomes of hundreds of prokaryotes and more than one hundred eukaryotes had been sequenced

GENETIC MODIFICATION AND ITS USES

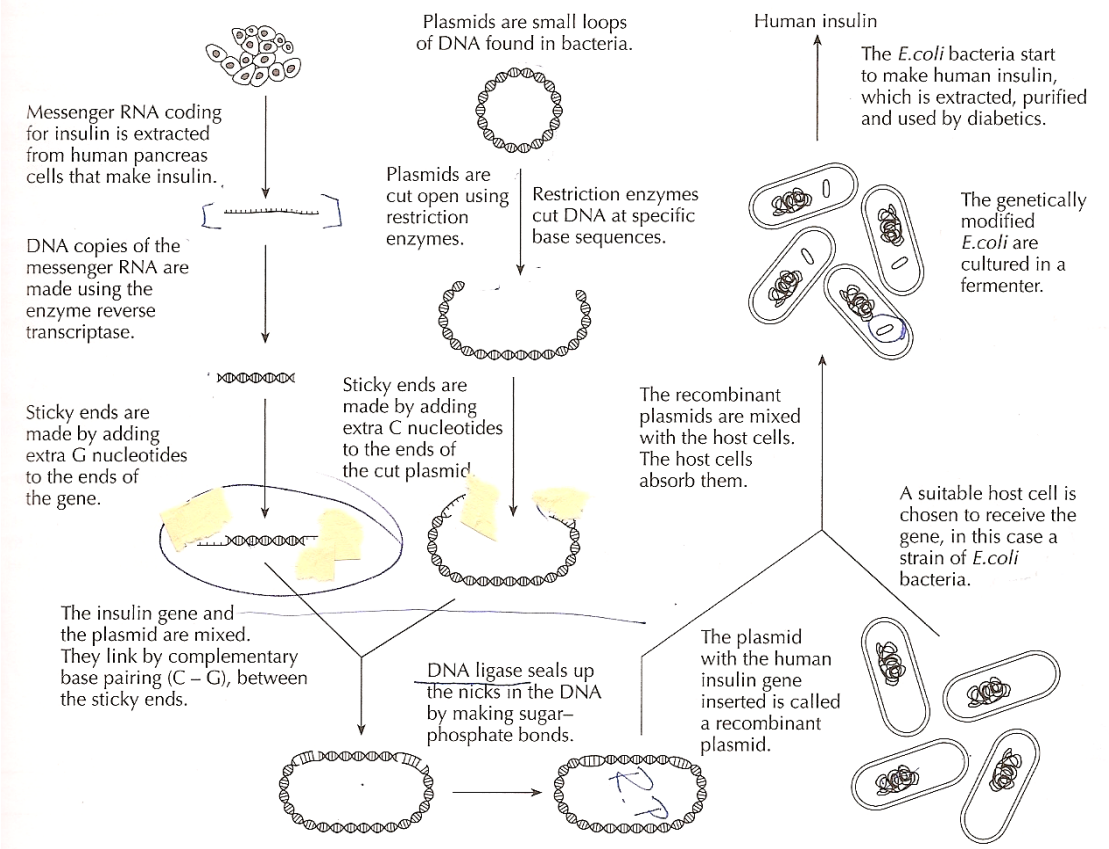
- The genetic code is **universal** allowing the genes to be able to be transferred from one organism to the other even if they are members of different species.
- A gene codes for the same polypeptide whether it belongs to a human cell, a bacterium or any other cell.
- Organisms that have had genes transferred to them are called genetically modified organisms (GMO) or transgenic organisms.
- The process of gene transfer is called genetic modification.

Examples include: gene transfer from cattle to chickens for making growth hormone and gene transfer to bacteria for making human insulin, salt tolerance in tomato plants, delayed ripening in tomatoes, herbicide resistance in crop plants, factor IX (human blood clotting) in sheep milk.

Example of a techniques used for gene transfer:

- The use of *E.coli* in gene technology is well documented.
- Most of its DNA is in one circular chromosome but it also has **plasmids** (small circles of DNA helix).
- These plasmids can be removed and cleaved (cut) by **restriction enzymes** (endonucleases) at target sequences.
- DNA fragments of another organism can also be cleaved by the same restriction enzyme and these pieces can be added to the open plasmid and join up together by **ligase**.
- The recombinant (ανασυνδυασμένα) plasmids formed can be inserted into new host cells, can be cloned and produce the required protein.

Diagram:



BENEFITS & RISKS OF GENETIC MODIFICATIONS

There are some genetic modification applications such as insulin production which has beneficial effect on humans. However, there are examples which can be beneficial but also harmful at the same time.

Example:
Maize crops (*Zea mays*) are often seriously damaged by some insects. A gene from a bacterium (*Bacillus thuringiensis*), which codes for the protein Bt toxin is transferred to maize. The production of this toxin kills the insects feeding on the maize.

A) BENEFITS of the Bt maize

1) Less pest damage and therefore higher crop yields
2) Less land needed for crop production
3) Less use of insecticides

B) RISKS of the Bt maize

1) Humans or animals that feed on genetically modified maize might be harmed by the bacterial DNA in it or by the Bt toxin itself
2) Insects that are not pests could be killed. The caterpillars of the Monarch butterfly (*Danaus plexippus*) is has been greatly affected.
3) Populations of wild plants might be changed due to cross-pollination that will spread the Bt gene
4) Interruption of food chains

CLONING

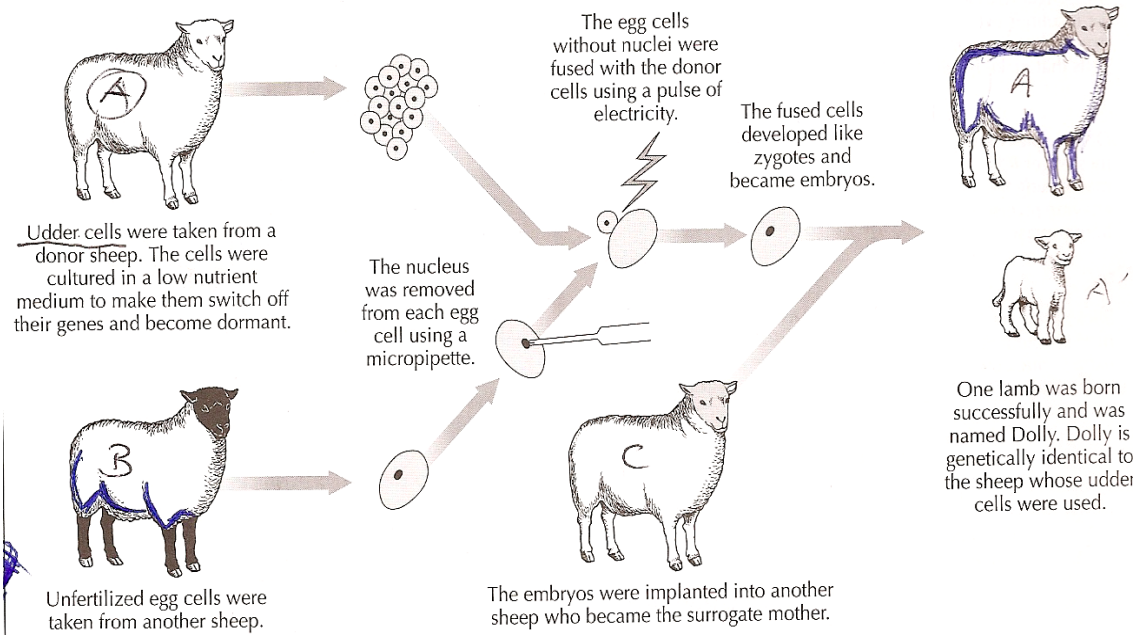
Cloning is the procedure by which identical copies of genes, cells or organisms are produced. Cloning is very useful if an organism has a desirable combination of characteristics, and more organisms with the same characteristics are wanted.

CLONE

A group of genetically identical organisms (eg. identical genotype) or a group of cells artificially derived from a single parent cell.

CLONING USING THE DIFFERENTIATED CELLS

The first successful cloning of an adult with known characteristics produced **Dolly the sheep**. The method is called somatic-cell nuclear transfer, in which the nucleus is removed from an egg cell and replaced by a nucleus from a differentiated somatic (body) cell.



ETHICAL ISSUES OF CLONING IN HUMANS

Research has shown that cloning of humans is possible. However, there are many ethical issues and human cloning has been banned in many countries.

Therapeutic cloning is the creation of an embryo to supply embryonic stem cells for medical use.

Arguments <u>for</u> cloning in humans

- It is not a new phenomenon since it happens naturally when identical twins are formed.
- Cloning of embryos would make screening of embryos for genetic disease easier.
- Infertile couples might have more chance of success with IVF if their embryos were cloned.
- Through cloning, it will be possible to manufacture cells or replace tissues damaged by illness (e.g muscular dystrophy) by embryos.

Arguments against cloning in humans

- Groups of genetically identical people might suffer psychological problems of identity or individuality.
- Cloning using differentiated cells would often cause suffering because it carries a high risk of fetal abnormalities and a high rate of miscarriage.
- DNA taken from differentiated cells has already begun ageing and humans cloned from it might grow old faster than usual.
- Others could see this as leading to the selection of those “fit to be cloned” and visions of “eugenics and a super race”….
- The embryo would be allowed to develop only to a stage where stem cells could be separated and cultured from it. Although the embryo would consist of a few hundred cells there would be many ethical issues raised by this technique.

Investigating factors affecting rooting in stem cutting

Stem cuttings are short lengths of stem that are used to clone plants artificially.

If roots develop from the stem, the cutting is an independent new plant.

The cutting can be placed in water or other solid media to develop roots.

Independent variables: Leaves left on the cutting, use of a hormone rooting powder, temperature where the cuttings are kept, plastic bag covering the cutting (to increase humidity and decrease transpiration)

Dependent variable: number of roots formed

Controlled variables: same plant species, number of repeats/trials

! An experiment could be designed to check one factor that affects the rooting of stem-cuttings ☺

The clones of Polo – Adolfo Cambiaso Argentina:

https://www.youtube.com/watch?v=6NhDY3IDp00