Summary of the Lessons 2, 3 and 4 (Autrice prof.ssa Elisabetta Schietroma)

TECHNIQUES USED TO AMPLIFY, SEPARATE AND ANALYZE DNA

1. Polymerase chain reaction (PCR)

https://www.youtube.com/watch?v=JRAA4C2OPwgù

A sample of DNA is **replicated** without the need to clone it in a living organism (bacterium). It is carried out in a PCR machine.

The PCR machine is loaded with: the DNA to be replicated; DNA nucleotides; DNA primers (**DNA primers** → short section of DNA to initiate replication) and a **thermostable DNA polymerase**.

Steps:

1. **DNA denaturation** → 95°C to separate the strands of DNA
2. **Annealing** → 50°C primers bind ss DNA to initiate the replication process
3. **Extension** → 72°C DNA polymerase uses the nucleotides to make complementary strands.
4. The cycle is repeated.

Each cycle (c) doubles the amount of the DNA as the molecule replicate.

After the PCR machine runs 30 cycles (c=30) the number of DNA copies produced (**T**) by Nmolecules of DNA is **N x 2^c**.

***Virtual lab*:**

Add the PCR Master Mix solution[1] to the positive and negative control and to sample of a DNA (in a tube) to replicate it.

Animations

1. Grow bacterial colonies on a solid medium culture dish
2. Pick up a single colony (by a wire loop) and drop it into a microcentrifuge tube
3 Add digestive enzymes buffer to the tube
4. Heat in a water bath (37°C)
5. Spin down cellular debris for removal from the sample
6. DNA is in the supernatant
7. Transfer the supernatant
8. PCR amplification
9. Gel electrophoresis

[1]The PCR Master Mix solution contains water; ions, a buffer (→pH is important for the enzyme); A,T,G and C; oligonucleotides, DNA primers that bind DNA to initiate the replication process and a **heat stable DNA polymerase.**

It is prudent to run a gel to confirm that a PCR reaction worked. The gel should contain the line for bacterial DNA, the lanes for the negative control (water) and the lanes for the positive control (a known DNA sequence).

2. Restriction endonuclease and gel electrophoresis

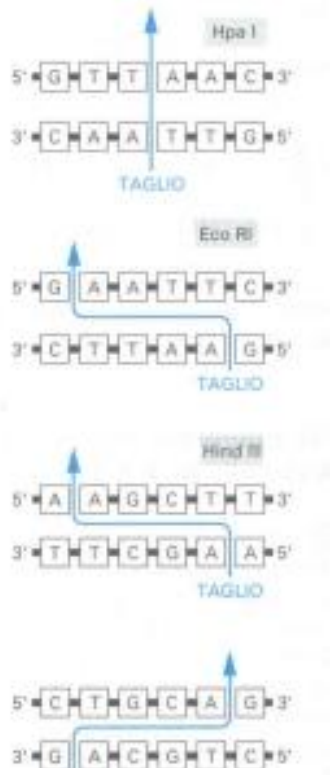
DNA separated by applying an **electric field** to move the negatively charged molecules through the pores of the gel (**agarose**). Shorter molecules move faster than longer and they migrate farther.

1. A sample of DNA is cut into fragments using a restriction endonuclease. A **restriction endonuclease** (eg. *EcoRI*) is an enzyme that cuts DNA at two different points in **a palindromic sequence** (eg.GAATTC; as result 7 fragments of DNA are produced if the enzyme cuts the DNA 6 times). This enzyme acts like chemical scissors.

2. The fragments of different lengths are separated by gel electrophoresis.

3. These pieces form bands which can be studied.

4. The different position of the bands depends on their molecular weight (or the number of the basis pairs) and is important to identify a sample of DNA (eg. gene to transfer).



Restriction endonucleases

5. The genetic fingerprint is used for example to identify people using their unique genetic code, to find out whether people are related to each other, or to help solve crimes.

Gel electrophoresis

https://www.youtube.com/watch?v=lgmq_HsuZIU

https://www.youtube.com/watch?v=6_4AY3lYRgo

https://www.youtube.com/watch?v=kjJ56z1HeAc