Lesson N°08

Twentieth century: gene therapy and molecular cloning

I. Gene therapy

- **Gene therapy** involves introducing genetic material into cells to treat a disease. It was initially conceived as a therapeutic approach for monogenic diseases (i.e. linked to the dysfunction of a single gene), delivering to cells a "healthy" gene capable of replacing the "sick" gene.
- The aim of gene therapy is to provide a sick individual with a gene in good working order, in order to :

a) Bypass the blockage of a metabolic pathway (e.g. lactose intolerance, etc.)

b) Alleviate the consequences of a genetic disease (e.g. hyper-fragility of red blood cell walls following microspherocytosis, etc.)

c) Supply the diffusible substance whose absence is at the root of symptoms

d) Fight cancer by releasing tumor cell-destroying factors in situ

II. Molecular cloning

Molecular cloning involves producing recombinant DNA molecules and using them to transform a host organism, in which they are replicated.

- **Principe**

A molecular cloning reaction generally involves the following two elements:

- The DNA fragment of interest to be replicated;
- A plasmid vector containing all the components required for replication in the host organism.

The DNA of interest (a gene, regulatory elements or operon, for example) is prepared for cloning in a variety of ways: it can be excised from the source DNA using restriction enzymes, amplified by PCR (polymerase chain reaction) or assembled from individual oligonucleotides.

In parallel, a linearized plasmid vector is prepared by restriction enzymes or PCR. A plasmid is a small fragment of circular DNA replicated within the host and independent of the host chromosome or genome.

The DNA of interest is inserted into the plasmid vector via phosphodiester bonds to form a new recombinant plasmid that will be replicated by the host.

During the cloning process, the ends of the DNA of interest and the vector must be modified to make them compatible, so that they can be linked by a DNA ligase, a recombinase or an in vivo DNA repair mechanism. These steps generally involve enzymes such as nucleases, phosphatases, kinases and/or ligases. Numerous methods and, more recently, cloning kits have been developed to simplify and standardize these processes.