

13-2 Manipulating DNA

The Tools of Molecular Biology

How do scientists make changes to DNA?

Scientists use their knowledge of the structure of DNA and its chemical properties to study and change DNA molecules.

Scientists use different techniques to:

- extract DNA from cells
- cut DNA into smaller pieces
- identify the sequence of bases in a DNA molecule
- make unlimited copies of DNA

In **genetic engineering**, biologists make changes in the DNA code of a living organism.

DNA Extraction

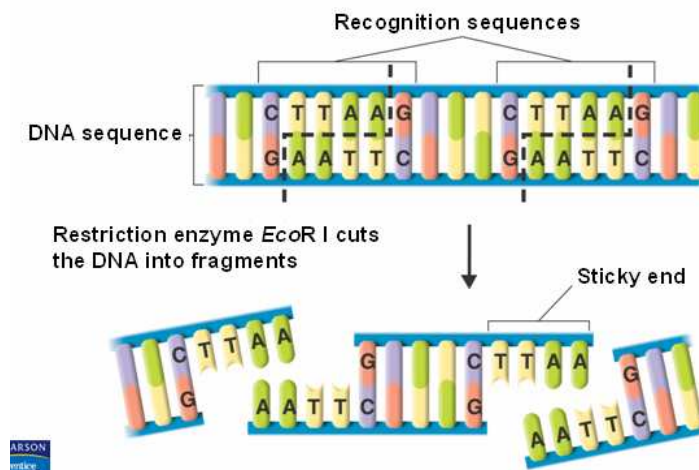
DNA can be extracted from most cells by a simple chemical procedure.

The cells are opened and the DNA is separated from the other cell parts.

Cutting DNA

Most DNA molecules are too large to be analyzed, so biologists cut them into smaller fragments using restriction enzymes.

Each **restriction enzyme** cuts DNA at a specific sequence of nucleotides. It will cut a DNA sequence only if it matches the sequence *precisely*.



Separating DNA

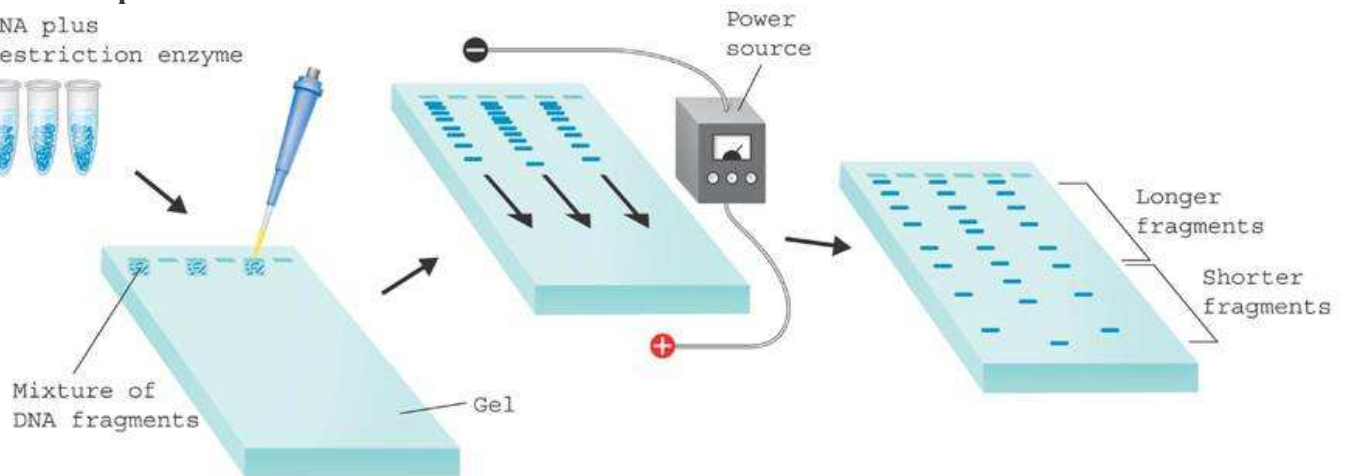
In **gel electrophoresis**, DNA fragments are placed at one end of a porous gel, and an electric voltage is applied to the gel.

When the power is turned on, the negatively-charged DNA molecules move toward the positive end of the gel. Gel electrophoresis can be used to compare the genomes of different organisms or different individuals.

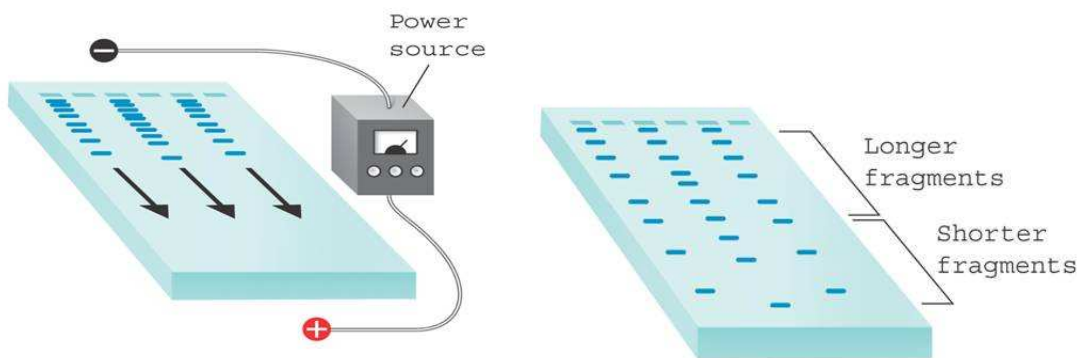
It can also be used to locate and identify one particular gene in an individual's genome.

Gel Electrophoresis

DNA plus
restriction enzyme



An electric voltage is applied to the gel. This moves the DNA fragments across the gel. The smaller the DNA fragment, the faster and farther it will move across the gel.



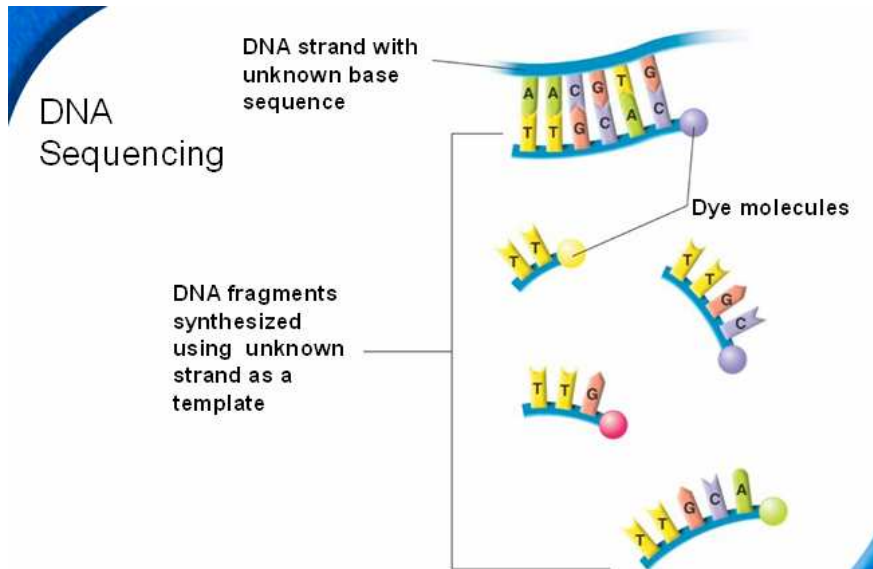
Based on size, the DNA fragments make a pattern of bands on the gel. These bands can then be compared with other samples of DNA.

Using the DNA Sequence

Knowing the sequence of an organism's DNA allows researchers to study specific genes, to compare them with the genes of other organisms, and to try to discover the functions of different genes and gene combinations.

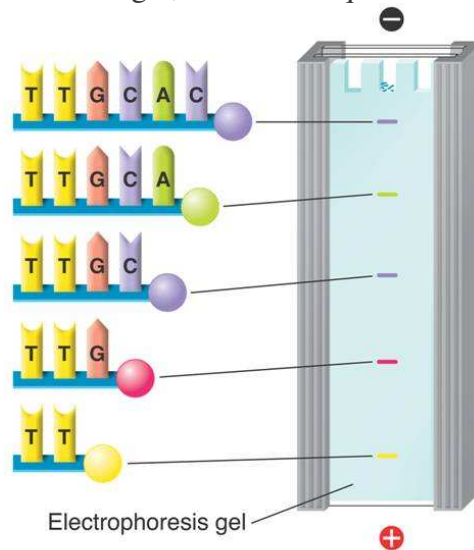
Reading the Sequence

In DNA sequencing, a complementary DNA strand is made using a small proportion of fluorescently labeled nucleotides.



Each time a labeled nucleotide is added, it stops the process of replication, producing a short color-coded DNA fragment.

When the mixture of fragments is separated on a gel, the DNA sequence can be read.



Base sequence as “read” from the order of the dye bands on the gel from bottom to top: **T G C A C**

Cutting and Pasting

Short sequences of DNA can be assembled using DNA synthesizers.

“Synthetic” sequences can be joined to “natural” sequences using enzymes that splice DNA together.

These enzymes also make it possible to take a gene from one organism and attach it to the DNA of another organism.

Such DNA molecules are sometimes called **recombinant DNA**.

Making Copies

Polymerase chain reaction (PCR) is a technique that allows biologists to make copies of genes.

A biologist adds short pieces of DNA that are complementary to portions of the sequence.

DNA is heated to separate its two strands, then cooled to allow the primers to bind to single-stranded DNA.

DNA polymerase starts making copies of the region between the primers.

