

# Biotechnology: Recombinant DNA and Gene Amplification

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**Biotechnology is the use of biological organisms or processes to develop products or improve the quality of life. One common technology studied is Recombinant DNA technology. DNA of two organisms is combined to improve the characteristics and properties of an organism. Modification of the DNA also is done to develop new pharmaceutical therapies and diagnostic tests. In this article, different techniques in biotechnology are discussed, namely, recombinant DNA technology, Polymerase Chain Reaction, Gel Electrophoresis, and CRISPR Cas9.**



## Introduction

Biotechnology is an interdisciplinary field of study defined as **a combination of biological sciences and engineering technologies**. It is characterized by the use of biological systems found within organisms or the use of actual living organisms to make technological advances and produce biological products. The two main branches of biotechnology are agricultural and medical biotechnology. Subbranches include bioinformatics, blue biotechnology, industrial biotechnology, and red or bio-pharmaceutical biotechnology.

Some examples of products manufactured using biotechnology are DNA vaccines, RNA-based therapeutics, synthetic humanized insulin, and other drugs produced through genetic engineering.

# Recombinant DNA

Recombinant DNA (rDNA) technology involves **combining DNA fragments from two sources**. Molecules of DNA from two different sources or species are inserted into a host organism. The resulting new genetic combination is of value to science, medicine, agriculture, and industry.

The resulting DNA is called recombinant DNA. Naturally, DNA recombination that involves the crossing over of homologous chromosomes occurs during meiosis (prophase I) in sex cell production (see image). In the context of biotechnology, recombinant DNA is the artificial or uncommon union of DNA fragments from two different sources of genetic material. Some scientists also use the term **chimeric DNA** for this “unnatural” combination of genes. The experimental manipulation of genetic material to produce rDNA is what is now called recombinant DNA technology.

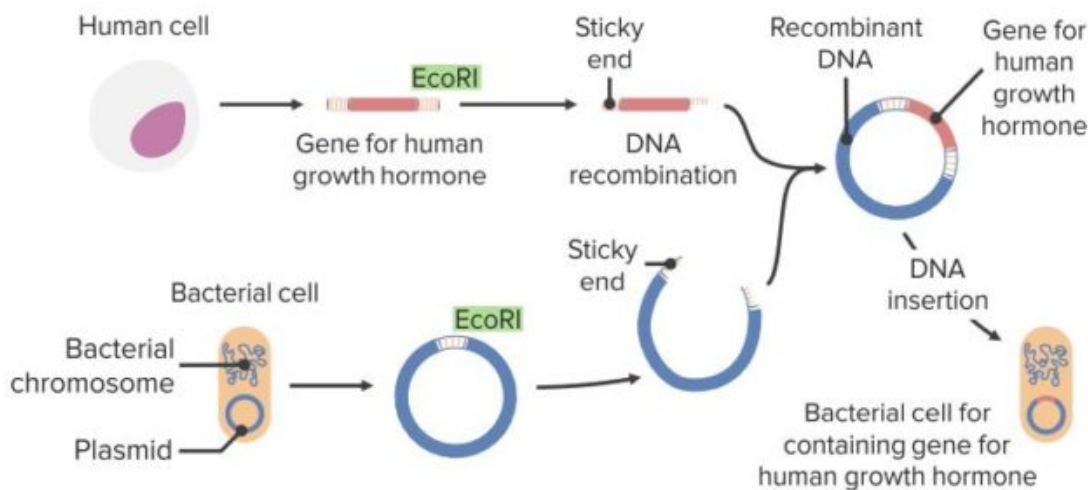


Image: A single DNA molecule created from the DNA of two different organisms. By: Lecturio.

## The preparation of rDNA involves four main steps:

1. DNA isolation
2. DNA splicing
3. DNA joining
4. rDNA amplification

Before preparing rDNA, it is important to identify and isolate the correct gene of interest; this depends on the desired results. One of the main goals of rDNA technology is to **develop species of organisms with characteristics or properties that are not naturally found in these species**.

For example, a bacterial species that does not naturally glow can be inserted with a specific gene from bioluminescent algae to produce a bacterial strain that can produce the same bioluminescent protein as the algae. The organism that serves as the source of the desired DNA fragment is called the **donor**, while the organism whose DNA is modified is called the **vector**.

**The first step in the preparation of rDNA is to isolate both the vector and donor DNA.** For prokaryotic applications, **the most commonly used vector is a circular bacterial DNA plasmid**. To isolate the plasmid from the rest of the DNA genome, differential centrifugation is performed via the addition of cesium chloride and ethidium bromide. The two DNA fragments selectively bind to the ethidium bromide, producing a

cesium chloride gradient after centrifugation. The plasmid DNA, which sinks to the bottom, is collected for the next step.

**The second step involves cutting, or splicing, the DNA fragment by using restriction enzymes.** Restriction enzymes are responsible for cutting the bacterial DNA and, in the process, deactivating it. They **cut at specific locations in the DNA strand to selectively obtain the desired DNA fragment.** These enzymes can recognize the specific DNA sequence where the cutting will occur.

These sequences **occur as palindromes**; that is, the two strands of DNA have the same sequence but are structured in opposite directions. The restriction enzyme recognizes this sequence and cuts the fragments by opening up the circular DNA. To be able to successfully produce rDNA, the donor DNA strand should also be cut by the same restriction enzymes. This is done to ensure that the sticky or cohesive ends (points where the cut was made) of the donor DNA are compatible with the sticky ends of the vector DNA.

**The third step is the joining of the two DNA fragments: the isolated donor fragment and the remaining vector fragment.** The **two DNA fragments that were cut using the same restriction enzymes will be combined.** Since the sticky ends of both fragments are complementary, the two DNA fragments can be combined to form the new rDNA. To further strengthen the connection, **DNA ligase is used** to create phosphodiester linkages between the fragments.

**The last step involves DNA amplification.** The resulting rDNA is inserted into a bacterial cell via a process called plasmid or vector transformation. The **recombinant plasmid will then be replicated through natural DNA-replication processes.** When it is replicated, the donor DNA is also replicated and multiple copies of it are produced. Continued cell division leads to millions of cells containing the desired DNA fragment (amplification).

## Gel Electrophoresis

In the laboratory, large DNA fragments are difficult to analyze because of the amount of

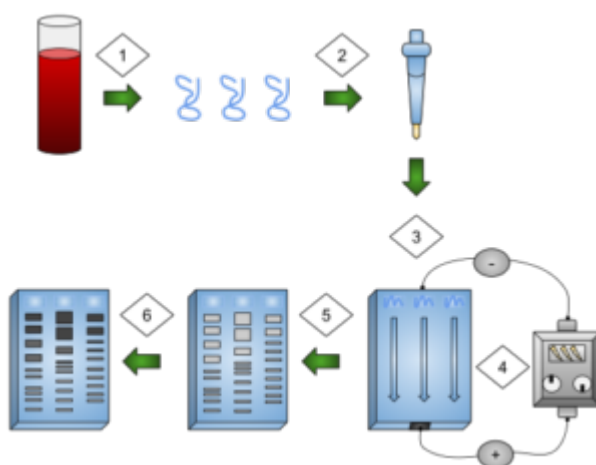


Image: Gel electrophoresis in DNA fingerprinting.  
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nucleotide bases contained within them. The use of restriction enzymes enables the fragmentation of a DNA sequence into fragments of varying lengths or sizes. To be able to obtain a single desired fragment, a separation technique must be employed.

In molecular biology, these **DNA fragments are separated by gel electrophoresis** (see image). In this technique, mixtures of DNA fragments are placed on the top lanes of the gels. Electricity is then applied to separate the fragments according to their size and charge. Smaller DNA fragments will naturally move faster than larger ones. After the separation, the DNA fragments can be visualized by adding fluorescent dyes that bind to the DNA.

## Bacterial Transformation

Bacterial transformation is a process of horizontal gene transfer that **occurs when bacteria take up foreign DNA fragments from the environment** (see image). These DNA fragments enter the cell and can be integrated into the bacterial chromosome by nonreciprocal recombination. When recombination is successful, a stable transformation occurs and the inserted DNA fragments may be expressed by the organism. Otherwise, the DNA fragment is degraded.

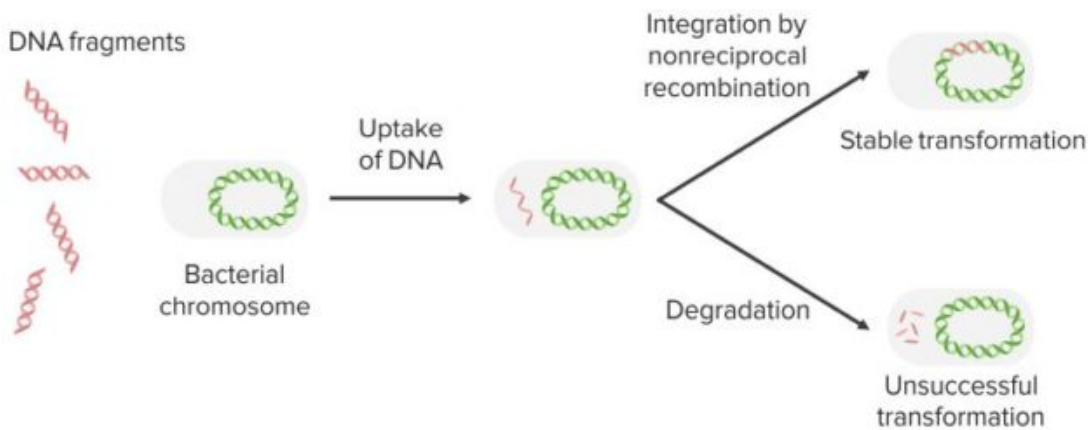


Image: Bacterial transformation—the integration of foreign DNA from the environment into the bacterial genome. By: Lecturio.

## Molecular Cloning

Molecular cloning **follows the basic steps of rDNA technology (isolation, insertion, and multiplication)**. The first step is the isolation of the vector DNA. The next step is to use the correct restriction endonuclease to cut the DNA at a specific location. The foreign DNA is then inserted and connected using DNA ligase. The resulting transformation incorporates the modified DNA into the bacterial species. The species containing the modified gene is amplified via natural cell division and will exhibit different properties than the natural species.

## Host Vector System

To be able to produce multiple copies of a gene, the gene must be inserted into DNA segments that can spontaneously replicate. A DNA fragment that can replicate with a newly inserted DNA fragment is known as a vector or cloning vehicle. A good vector should have an origin of replication, one or more genetic markers for selection, and a cloning site where foreign DNA can be inserted.

**Replication of DNA can only be achieved inside a host that provides the proper enzymes and factors needed for replication.**

### Common hosts include:

- *Escherichia coli*
- Yeast cells
- Mammalian tissue culture cells
- Insect cells

### Common vectors include:

- Plasmids
- Bacterial and yeast artificial chromosomes

## Transforming Eukaryotic Cells

Unlike in the transformation of prokaryotic cells, transformation in eukaryotic cells is more challenging. A gene gun is commonly used. This instrument inserts DNA by bombarding electrically charged cells with gold or tungsten particles that are coated with DNA.

## CRISPR-Cas9 System

Clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 **enables the editing of DNA** by having the Cas9 act like a pair of molecular scissors to cut pieces of DNA from the disease-causing organism (see image). The organism later incorporates part of the invader's DNA into its own genome so that it can recognize the invader in the future.

In this technique, scientists create a guide RNA sequence that matches the DNA they want to modify. This sequence is then added to cells together with a protein called Cas9, an RNA-guided DNA endonuclease that interrogates and cleaves foreign DNA. The Cas9 protein cuts the DNA and inserts the guide RNA into the DNA sequence. Enzymes then repair the cuts and produce the completed modified DNA.

This technique has been successfully used to fix genetic defects such as **Duchenne muscular dystrophy and Huntington's disease in mice.**

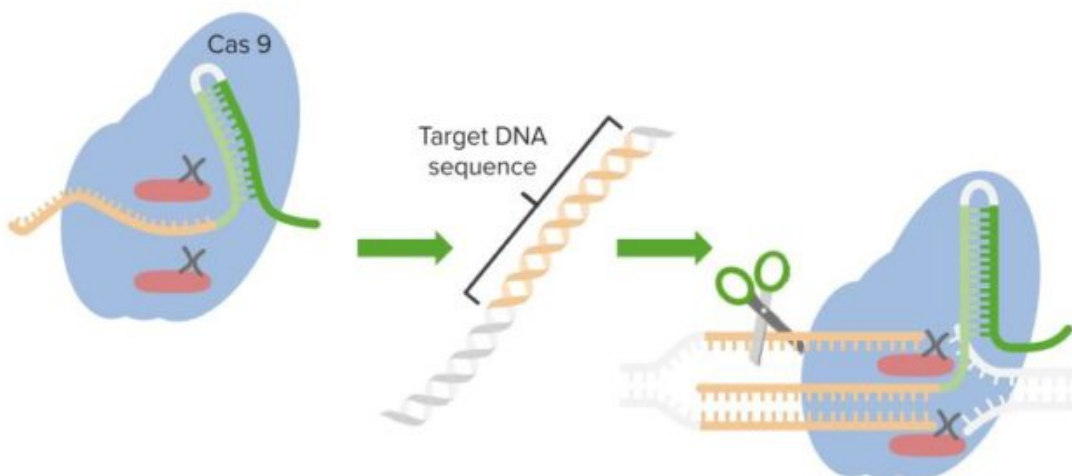


Image: The CRISPR-Cas9 system—upgraded splicing and editing of genomes. By: Lecturio.

# Polymerase Chain Reaction

Polymerase chain reaction is a technique used to produce multiple copies of DNA fragments. The process of DNA amplification is divided into three stages: the **denaturing stage, annealing stage, and extending stage**. In the denaturing stage, the DNA molecule is heated up to 95°C to separate the two strands. Primers then attach to each of the strands in the annealing stage. The extending stage then proceeds to add more nucleotides into the growing chain. The steps are repeated over and over to produce more copies of the DNA.

## References

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