

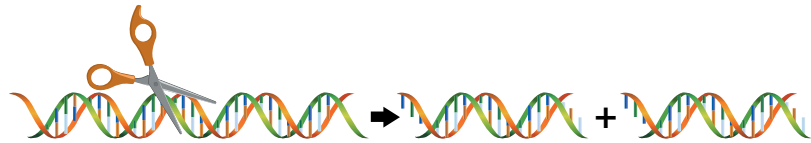
Chapter Four: **The Technology**



Biotechnology scientists depend on a wide variety of constantly evolving laboratory techniques and tools. This section focuses on some of these platform technologies. To understand the industry, it's helpful to have some basic knowledge of what goes on in the lab.

Transformed Cells

Transformation of bacterial cells for the production of recombinant proteins usually involves *E. coli*. Transformation of animal cells, called transfection, usually involves a cell line derived from Chinese hamster ovary (CHO) cells. CHO cells were introduced in the 1960s and remain the most commonly used mammalian host cells for industrial production of recombinant protein therapeutics.



Restriction enzymes are proteins that function as molecular scissors and cut DNA.

Restriction Enzymes

Biotechnology employs a process called **genetic engineering**, which combines DNA sequences in order to produce recombinant proteins as potential therapeutics. The process utilizes restriction enzymes.

BIOFACT



Scientists have identified more than 3,800 restriction enzymes, and more than 600 are commercially available for purchase from scientific supply companies.

Scientists discovered **restriction enzymes** (endonucleases) in bacteria in the 1970s. They found that these enzymes cut up viral DNA into small, nonfunctional pieces, thereby protecting the bacterium from an invading virus.

There are hundreds of restriction enzymes—each of them recognizing a specific sequence of DNA called a restriction site. For example, EcoR1, a restriction enzyme found in *E. coli*, recognizes and cuts at the six-base sequence GAATTC. HaeIII, a restriction enzyme found in *Haemophilus-aegyptius*, recognizes and cuts at the four base sequence GGCC.

All DNA, regardless of where it comes from, is made up of the same four bases—As, Ts, Gs and Cs. HaeIII reads any DNA segment and cuts the DNA every time it encounters the sequence GGCC. All restriction enzymes are specific and reproducible, which are two key characteristics that allow researchers to utilize restriction enzymes to manipulate DNA.

The counterpart to cutting is called pasting. **DNA ligase** is a protein (enzyme) that seals two DNA segments together in a process called ligation. The ability to cut and paste DNA is the basis of genetic engineering.

BIOFACT



Daniel Nathans, Werner Arber and Hamilton Smith received the 1978 Nobel Prize in Physiology or Medicine for their discovery of restriction endonucleases. Their discovery led to the development of recombinant DNA technology.

Recombinant DNA

When segments of DNA are cut and pasted together, the new DNA is called **recombinant DNA**. Recombinant DNA can be inserted into cells to produce cells with new characteristics. This genetic altering can include a single-base (letter) change or multiple gene changes.

Recombinant DNA can be introduced into a host cell by a **vector**, which is used to physically carry DNA into a host cell. A host cell can be bacterial, yeast, plant, insect or mammalian.

Common bacterial vectors include plasmids and phages. A plasmid is a circular unit of DNA that can be engineered to carry a gene of interest. A phage is a genetically engineered virus that injects DNA into bacteria. Cells that contain recombinant DNA are referred to as genetically modified, **transgenic** or transformed cells. The process is called **transformation**.

BIOFACT



The FDA approved human insulin in 1982—the first medicine made via recombinant DNA technology.

Recombinant Proteins

Recombinant DNA can be used to produce **recombinant proteins**. The host cells use the new DNA information and their own cellular machinery to produce the protein encoded by the recombinant DNA.

When recombinant proteins are produced for use as human therapeutics, host cells must be grown in large quantities so that enough recombinant protein is produced to meet demand. The recombinant protein is isolated, purified and analyzed for activity and quality before it goes to market.

Producing a protein with the proper order of amino acids isn't always the whole story. Sometimes further processing is required to yield an active or fully functioning protein. Many human proteins are **glycosylated**, meaning, they have a particular pattern of sugar molecules linked to them. If a protein is translated but not correctly glycosylated, it may not function properly.

Adding a phosphate group—a process known as **phosphorylation**—can act as an on-switch, allowing the proteins to become active proteins. Other biochemical functional groups may be added to the protein to allow it a larger range of function.

Recombinant proteins for therapeutic use include vaccines, **hormones**, monoclonal antibodies and hematopoietic growth factors for the treatment of cancer, AIDS, aller-

gies, asthma and many other conditions. The number of recombinant proteins has increased greatly in recent years as the technology used for their production and purification has advanced.

Cell Culture

Cell culture is the technique of growing cells in the laboratory under controlled conditions. Growing large quantities of transformed cells is a major step in the process of producing recombinant protein products. Both transformed bacterial cells and transformed animal cells are used in this process.

Simple proteins can be produced using recombinant DNA technology in bacterial cell cultures. Typically, more-complex proteins that are, for example, glycosylated are made in animal cell cultures.

During cell culture, cells are grown in petri dishes or flasks containing liquid media. Culture media provides all the nutrients necessary for cell growth. The cultures are grown in an incubator that maintains the appropriate temperature and environment, often requiring certain gas mixtures of oxygen and carbon dioxide. It is very important to maintain specific conditions for cultures, because variation in these conditions may affect the proteins expressed and, therefore, the product that is produced.

In the process of commercial production of proteins, cell cultures must be scaled up to produce enough protein to meet demand. Since only a limited number of cells can be grown in small petri dishes or flasks, the cell culture can be transferred to large containers called **bioreactors**, which are involved in the manufacturing process.



Research Tools

Biotechnology professionals depend on leading-edge laboratory equipment. Here are a few pieces used in genetic engineering:

Thermocycler

Polymerase chain reaction (PCR) is a technique that replicates DNA in a machine called a **thermocycler**. PCR is a series of cycles that takes a small amount of original DNA and exponentially copies, or amplifies, it. Each three-step cycle doubles the amount of DNA present. It's like a photocopier for DNA. A single piece of DNA can be turned into millions of copies of the same DNA piece.

PCR enables researchers to make enough DNA to work with in the lab. There are many applications for PCR, including producing enough of a DNA sequence or gene for use in creating recombinant proteins.

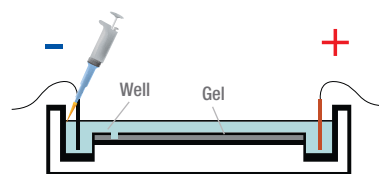
BIOFACT



Kary Banks Mullis developed the polymerase chain reaction in 1983 and received the 1993 Nobel Prize in chemistry for the invention.

Gel Electrophoresis

Gel electrophoresis is a technique commonly used in the laboratory for analyzing DNA fragments. Gel electrophoresis allows DNA fragments to be separated within a gel. A gel electrophoresis apparatus holds the gel and allows electricity to run through it. Each DNA fragment is negatively charged, causing it to migrate toward the positive pole of the gel apparatus. Larger fragments of DNA move more slowly than smaller fragments because they encounter resistance from the gel matrix.



Gel electrophoresis.

There are different types of electrophoresis. Each is based on the type of separating material used, gels being one type, and the types of molecules being separated. DNA, RNA and proteins can all be separated using electrophoresis in an appropriate apparatus.

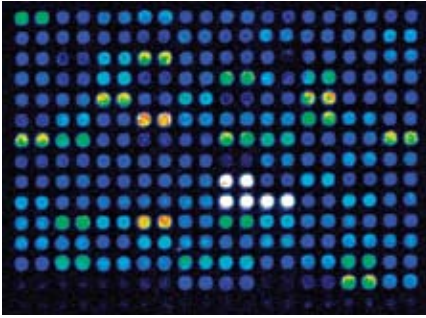
Gel electrophoresis has many applications in both clinical and research labs. One common use is for verifying PCR products—that is, checking to see whether the reaction generated the correct DNA fragment.

DNA Microarrays

A DNA microarray (also called a gene chip) is a small piece of glass or silicon divided into thousands of sections in a grid pattern. Each section has a single-stranded gene fragment corresponding to either a healthy or diseased gene.

DNA from an individual is separated into single strands and tagged with a fluorescent dye. The tagged DNA is washed over the microarray. The individual's DNA binds to any complementary DNA sequences on the slide, if they are present, to become double-stranded DNA.

With the aid of a computer, the double-stranded, fluorescently tagged DNA spots can be located and measured. The individual's DNA must match the attached gene fragment exactly to bind, thereby indicating the presence of healthy or diseased DNA.



Microarray.

Microarrays are powerful tools allowing researchers to analyze thousands of genes in one test. Microarrays are used for genetic testing, for comparison of genetic information from different individuals or species, and in the discovery of potential drug targets.

Microarrays can also be used for researching and identifying genes of interest to be used with recombinant DNA technologies. Other microarrays include protein, tissue, chemical compound and **antibody** microarrays—all of which allow researchers to analyze thousands of data points at one time.

BIOFACT



Microarrays are capable of generating so much data that special microarray databases are needed for storage, searches, analysis and interpretation. There are both public and private microarray databases.