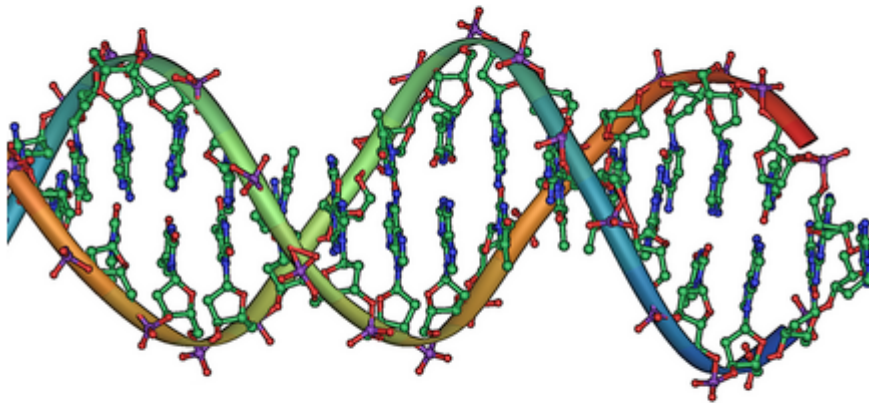


# Biotechnology: DNA Libraries, Analysis and Applications

[See online here](#)

**Recombinant DNA technology can be used for a variety of applications. It is therefore important to develop libraries for the genomes of organisms so that it will be easier for scientists to isolate desired genes of interest. This article discusses DNA libraries and how they are developed, and the applications of biotechnology in forensics, medicine, and agriculture. Blotting techniques and how they apply in the forensic investigation are also examined.**



## DNA Library

A **DNA** library is a **collection of DNA fragments** that were **cloned in vectors so that scientists can identify and isolate desired fragments** for **genetic studies**. Two kinds of DNA libraries are constructed.

Some genomic DNA libraries contain **a representation of an organism's entire genome**. The number of clones produced in a genomic library depends on the size of the genome being studied and the size of genes that a cloning vector system can tolerate. Application of genomic libraries includes complete genome sequencing for organisms, the

source of genomes in the production of transgenic animals, and studies on the function of regulatory sequences and [genetic mutation in cancer cells](#).

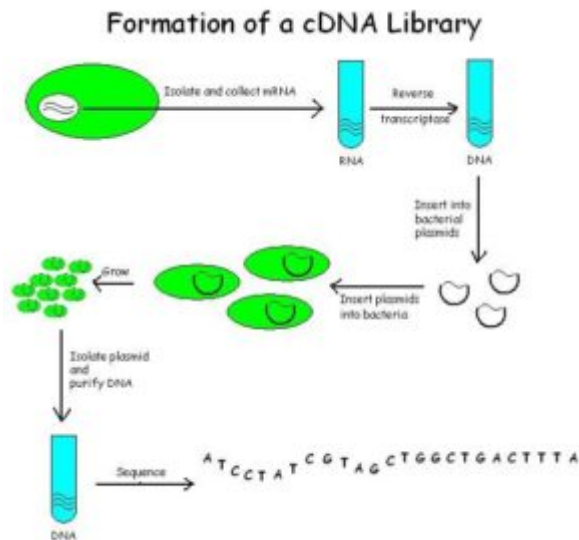


Image: "Formation of a cDNA Library" by PhD Dre. License: [CC BY-SA 3.0](#)

Another type of library is the cDNA library. This is a collection of cloned complementary DNA fragments inserted into a host cell, which combines with some portions of the transcriptome of the organism to be stored as a library. It is produced from fully transcribed [mRNA](#) found in the nucleus and thus **only contains copies for coding sequences**.

Using cDNA libraries, bacterial cells can express eukaryotic genes. Applications of cDNA libraries include the cloning of full-length cDNA molecules in vitro, the discovery of novel genes, studies on alternative gene splicing in different cells and tissues, and studies on the variety of mRNAs expressed by different cells and tissues.

## Probes and Blotting Methods

DNA libraries make it easier to access a gene of interest. However, because of the variety of DNA fragments included in certain libraries, several techniques have been developed to easily isolate and identify genes of interest from the libraries.

### Southern blot method

One of the most common gene identification techniques is the **Southern blot method**. It combines **electrophoresis and probe hybridization**. The gel containing the separated DNA fragments is transferred or blotted to a nitrocellulose paper to produce a nucleic acid "print."

The nitrocellulose containing the bound DNA is then exposed to a hybridization probe containing a single DNA fragment with a specific sequence that can determine the **presence of the target DNA**. It employs a radioactively labeled atom or molecule tags using fluorescent or chromogenic dyes.

### Northern blot and western blot methods

Other blotting techniques are the **northern blot and the western blot methods**. The northern blot method is used to detect isolated **mRNA** in a sample, while the western blot

is used to detect **proteins** using antibodies. Probes for northern blotting are composed of nucleic acids with sequences complementary to all or part of the RNA of interest.

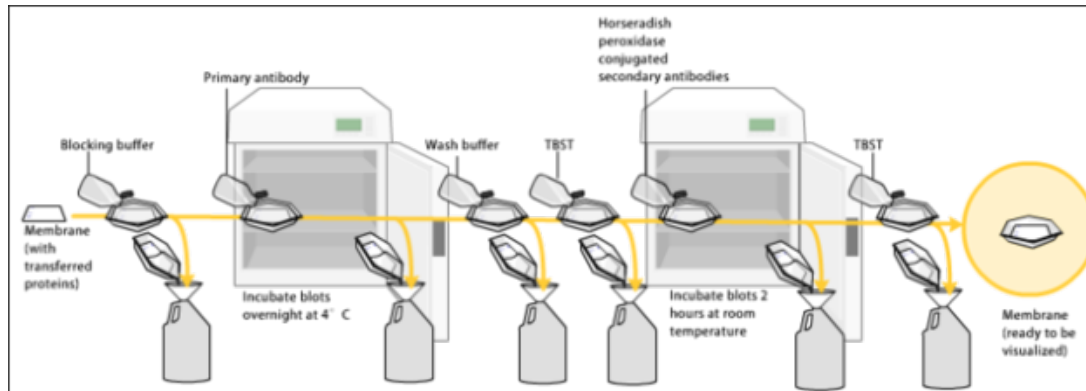


Image: "Western Blot binding" by Bensaccount. License: [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/)

## DNA Fingerprinting

Just like an individual's fingerprint, the DNA of an individual is said to be unique. This means that no two individuals have the same genetic code. This is why DNA fingerprinting is commonly used in forensic investigation to identify suspects who have left behind fluids that can be sources of DNA.

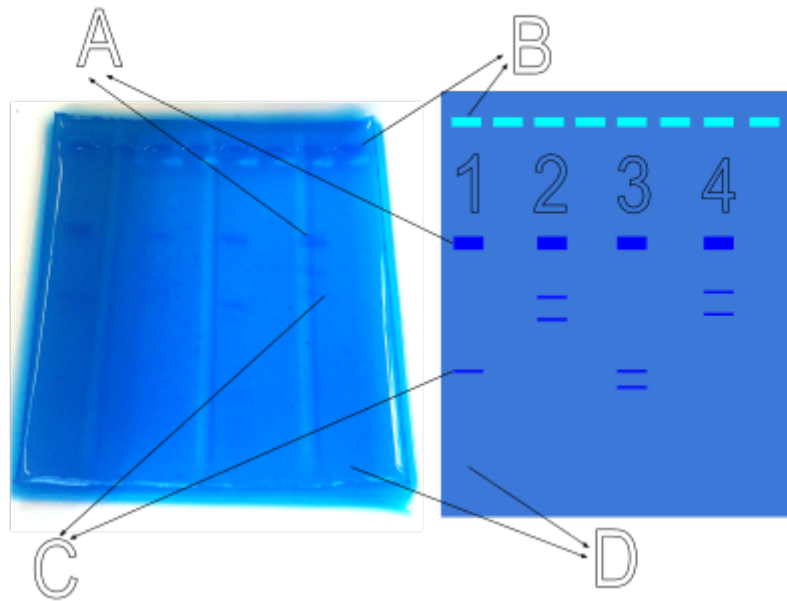
## Restriction Fragment Length Polymorphism (RFLP)

One of the most common DNA fingerprinting technique is the **restriction fragment length polymorphism (RFLP)**. This technique uses special enzymes to cut segments of a sample where DNA can be extracted. It involves **looking for repetitive DNA base sequences in individual DNA using specific restriction endonucleases**. It specifically looks for differences in the homologous DNA sequences.

**RFLP markers are highly locus-specific, which is why they can be used to detect genetic diseases.** An RFLP probe is a labeled DNA sequence that can hybridize with 1 or more fragments of the DNA sample. When they are separated using electrophoresis technique, different viewing techniques may be used, including UV light or autoradiography, in the case of use of radioactive markers. When a mutation occurs, there are changes in the profile of the normal and mutated DNA fragments obtained after electrophoresis.

## DNA Profiling

### RFLP (Restriction Fragment Length Polymorphism) Analysis



**Image:** "Seen here is a diagram of agarose gel for DNA Profiling: RFLP (restriction fragment length polymorphism) Analysis. 1. Known DNA 2. DNA of suspect 1 3. DNA of suspect 2 4. DNA from the crime scene A. DNA fragments: larger fragments B. Wells: where people load DNA samples C. DNA fragments: smaller fragments that are further separated D. Agarose gel: electric field" by Phoenix\_src. License: [CC BY-SA 4.0](https://creativecommons.org/licenses/by-sa/4.0/)

## Short Tandem Repeats Polymorphisms (STR)

Another technique used in DNA fingerprinting is a **short tandem repeat (STR) polymorphisms**. STR are short DNA sequences that are repeated a dozen or more times in a head-to tail-manner. This technique does not involve using restriction enzymes like the ones used in RFLPs. **It uses probes that attach to a specific region in the DNA.** The polymerase **chain reaction is then done to determine the length of the short tandem repeats.**

STR regions are variable or polymorphic, which is why they can be used to discriminate 1 DNA profile from another; 1 individual can have 5 repeats of a specific sequence while another may have 7. **The number of repeats can be used to profile individuals.**

## Biotechnology in Medicine

### Production of Insulin

Biotechnology has a variety of applications in the field of medicine. One common example of this is in the production of **insulin**. **Human insulin is important for the control of blood sugar levels.** Patients with **diabetes mellitus** cannot produce their own insulin and so have difficulty regulating their sugar levels. **Genetic engineering has paved the way for the production of synthetic insulin.**

Part of this process involves extracting the gene responsible for the production of insulin and inserting it into a plasmid vector to form recombinant DNA with a human insulin-producing gene. The recombinant DNA is then introduced to a bacterial cell. When the gene to produce insulin is expressed by the recombinant bacterium, insulin will be

extracted, purified, and bottled. This will then be injected into patients as supplements. The same procedure is used in the production of human growth hormone protropin.

## Production of vaccines

Another application of recombinant DNA technology is in the production of [vaccines](#). Recombivax HB, for example, is an approved **hepatitis B vaccine developed using rDNA technology**:

1. The vaccine was produced by isolating the HB antigen-producing gene from the HB virus.
2. The gene was then inserted into the plasmid DNA of a bacterium to form the rDNA.
3. The rDNA was then introduced to a yeast cell which multiplies and when fermented, produces the HB antigens.
4. The HB antigens were then extracted, purified, and bottled, and injected as a vaccine.

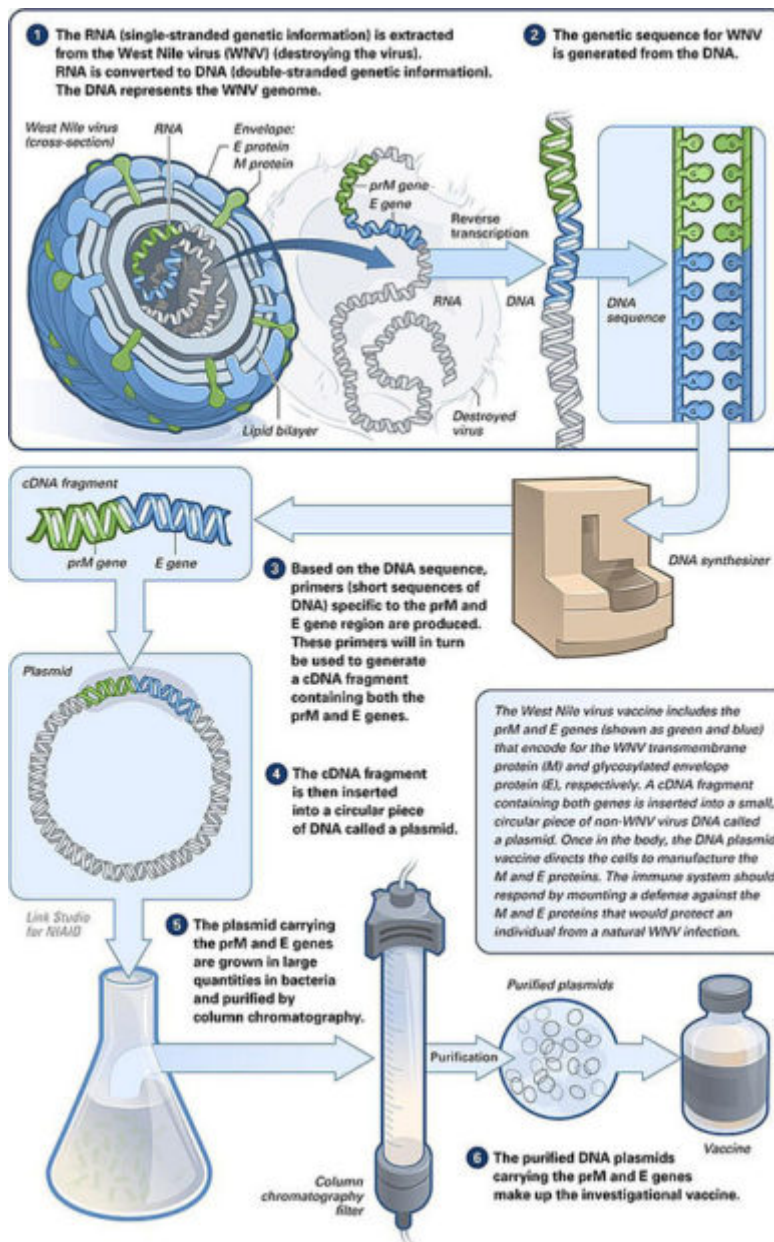


Image: "Making of a DNA vaccine" by Unknown. License: [Public domain](#)

## Gene therapy

Gene therapy is another application of biotechnology in medicine. This therapy involves injecting nucleic acid polymers into a patient's cell to **treat diseases**. A common approach in gene therapy is to **extract stem cells** from the patient first.

A working copy of the gene is then inserted into a virus. The virus will then insert the working gene into the extracted stem cells. When the stem cells are returned to the patient, the **patient will be able to express the working gene**. This can best be coupled through the CRISPR/Cas9 system, which allows for better targeting of genes.

## Biotechnology in Agriculture

### Pharmaceutical products

Biopharming is an interesting application of biotechnology in the field of agriculture. Through this technique, plant species can be used to produce pharmaceutical products. The same general process is used in the production of rDNA.

Plant cells are modified by inserting genes responsible for the production of certain pharmaceutical compounds. When the cells are grown, they will express the gene inserted and produce compounds they do not normally produce. The plant materials can later be collected and extracted to obtain the pharmaceutical compound of interest. The extracts are then processed to produce drugs to be sold in the market.

### Herbicide and pest resistant plants

Herbicide- and pest-resistant plants have also been developed using rDNA technology. A common example of this is *Bacillus thuringiensis* (Bt) corn. This bacteria naturally produce proteins that kill Lepidoptera larvae.

The **gene responsible for the production of this protein is inserted into corn cells** so that it is also able to produce the same protein, giving the corn protection from the corn borer larvae. The protein produced is **highly selective, as it kills only Lepidoptera larvae**. This means that BT corn is safe for human consumption.

## Transgenic Animals



Image: "GloFish fluorescent fish. Genetically modified" by www.glofish.com. License: [Attribution](#)

Transgenic animals are **animals that carry foreign genes inserted into their genome**. This is achieved via the same process as in common rDNA applications. Some complexity is involved because this **technique involves eukaryotic animals**.

One common example of a transgenic animal is the **GloFish**, which is a transgenic zebrafish. The gene inserted into the genome of this fish is responsible for the production of fluorescent-green proteins. Differences in how combinations occur during transformation result in a fish that can also produce fluorescent-yellow and -red proteins.

## Review Questions

The correct answers can be found below the references.

**1. What do you call the technique that uses restriction endonucleases to cut DNA samples at specific sites and when coupled with electrophoresis technique and autoradiography, can discriminate one individual from another?**

- A. Restriction fragment length polymorphisms
- B. Short tandem repeats polymorphisms
- C. Southern blot method
- D. Gene Therapy

**2. Which blotting method uses antibodies to detect proteins?**

- A. Northern blot
- B. Western blot
- C. Southern blot
- D. Eastern blot

## References

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**Correct answers:** 1A, 2B

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