# Performing Electrophoresis

HOW CLOSELY RELATED are modern humans and Neanderthals? Where did your ancient ancestors originate? These questions can be answered, to some degree of certainty, with modern DNA analysis techniques. An important step in the process is to separate DNA into smaller portions so it can be more easily analyzed. The process used for the separation is called gel electrophoresis.

## Objective:

Describe how electrophoresis is used to separate and analyze DNA.

## Key Terms:

agrose
EDTA
electrophoresis
gel box
histone
mtDNA
PCR
probes
restriction digest
RFLP
SDS
SNP
Southern blot
STR
TBE
VNTR

## Understanding Electrophoresis

The first step in any DNA analysis is to obtain the DNA. It may be crime scene evidence, a medical test, or something drilled from the tooth of a Neanderthal skeleton. The DNA must then be processed to reduce the large chromosomes to more manageable pieces. The smaller portions that result are sorted and analyzed for the desired information.

### DNA EXTRACTION

To collect DNA, you must break up cells that contain the DNA you want to analyze. You must also separate the DNA from other biochemical components of the cell. The cells can be

physically broken by grinding or running them through a blender. Very small cells can be broken by freezing or by ultrasonic vibrations.

## EDTA

Ethylenediaminetetraacetic acid (EDTA) is added to the resulting cell solution. **EDTA** is a chelating agent that removes metallic ions (e.g., $Mg^{2+}$ and $Ca^{2+}$) from the solution, which prevents enzymes from degrading the DNA. You may notice EDTA on an ingredients list for many foods. It is used to slow many enzyme reactions.

## SDS

Sodium dodecyl sulfate (**SDS**) is a detergent used to remove the lipids from the cell membrane. SDS is sometimes called sodium lauryl sulfate and is a common component of household detergents and shampoo.

### *Histone Proteins*

The DNA in cells is tightly coiled around spherical proteins that need to be removed to uncoil the DNA. **Histone** proteins are the globular proteins to which DNA is attached, causing it to coil up. They can be removed by adding protein-digesting enzymes.

### *Alcohol*

The membrane lipids and digested proteins are attracted to water, unlike the DNA. You can now separate the DNA by adding alcohol. The DNA dissolves in the alcohol while the other chemicals stay in the water.

## EXTRACTING RNA

RNA extraction is more difficult. All living systems have many active RNA-digesting enzymes that must be controlled. Otherwise, your RNA sample will be degraded quickly. These enzymes must be chemically denatured. The extraction is done using a phenol-chloroform mixture. The pH is adjusted to 4 to allow the RNA to be extracted and the DNA to stay in solution. DNA can be extracted or purified with this method at a higher pH. However, the chemicals required are quite hazardous. As a result, they must be handled by trained personnel.

## ELECTROPHORESIS

**Electrophoresis** is the use of an electrical field across a gel matrix to separate charged molecules on the basis of how quickly they move through the gel. It is frequently used in the study of DNA to analyze segments of different sizes and different nucleotide sequence patterns.

Electrophoresis uses a gel box attached to a direct current electrical source. The **gel box** is a container with a tray for an agrose gel and embedded wires for the electric connection. **Agrose** is a polysaccharide made up of many galactose units. The gel is placed into the box and is flooded with a buffered solution that will conduct electricity. The usual buffer is Tris/Boric acid/EDTA (**TBE**). The agrose gel has small wells, and the DNA samples are placed into the wells using a micropipette. Then the box is covered, the wires are connected, and the current is started. The wells are located at the negative end of the box. DNA has a negative charge due to the phosphate groups on the outer rails of the helix. The negative charge causes the DNA to be attracted to the positive terminal. DNA fragments of different lengths have different masses and move at different rates. The smaller fragments move faster. The gel is run until the fragments have been spread out. They are then stained, and the position of the fragments is compared to a size marker standard.

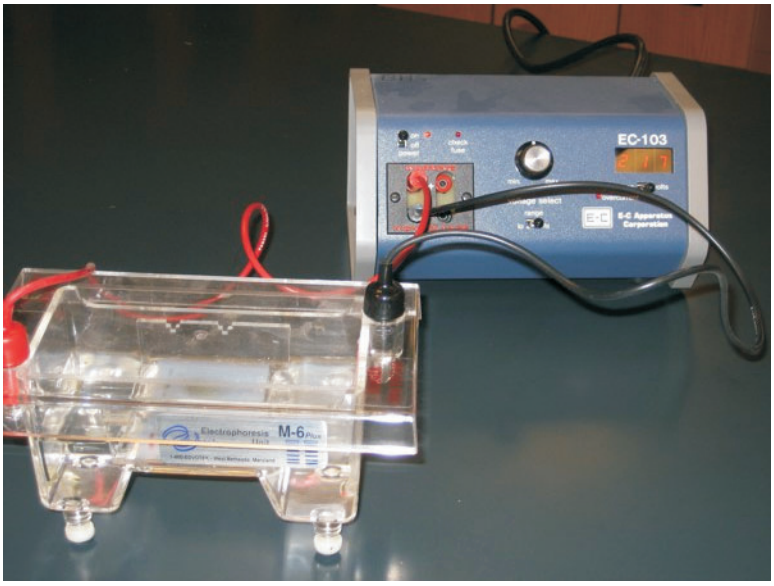


**FIGURE 1. Electrophoresis mini gel box and power source.**

## IMPORTANCE OF ELECTROPHORESIS RESULTS

### *Criminal Forensics*

DNA analysis using electrophoresis is used extensively in criminal forensic laboratories. DNA can be used to identify unknown victims. It is often used to identify individuals who left DNA at the scene of a crime. You are constantly losing cells into the environment. As a result, skin cells, hair, blood, saliva, or semen can be collected from many crime scenes. The identification of the person who left the DNA puts him or her at the scene of the crime. The circumstances of the type of cells left might further implicate the individual. For instance, the DNA identification of semen is powerful evidence in cases of sexual assault.

### *Identification*

DNA is useful in identifying victims who have been burned or decayed. The DNA identification supplements other methods (e.g., dental records and fingerprints). The military keeps DNA records for all members to aid in positive identification. Also, DNA collected from personal belongings and family members was used to identify human remains after the World Trade Center attack.

# FURTHER EXPLORATION...

### ONLINE CONNECTION: Ancient Human Migrations

You can investigate the Department of Energy's Human Genome Information Web site to learn about the science behind the DNA analysis that has been used to track ancient human migrations.

On the National Geographic Web site, you can see how the gathered information has been interpreted. Talk to your parents about the possibility of participating in adding to the database by submitting your DNA sample. For more information, visit the following links:

http://www.ornl.gov/hgmis/elsi/humanmigration.shtml

http://genographic.nationalgeographic.com/

## *Research*

A great deal of genetic research is conducted by examining family DNA when a child has a genetic disease. DNA analysis looks for markers in the family that are not common in the rest of the population. This type of searching has led to the specific identification of a number of genetic disorder genes. The search for disorder markers and similar research has led to the development of huge libraries of genetic sequences. These can be searched when researchers obtain new DNA samples. As a result, near and distant relationships will be revealed. This has enabled researchers to hypothesize ancient migration routes and other indicators about the past of ancient human history.

### DNA Libraries

DNA libraries are readily available. A business has developed to analyze anyone's DNA and to determine the probable history of your family origins. The dog genome has also been analyzed for disorder markers in our quest to understand many related disorders in humans. This has enabled a new test being sold to allow you to analyze the genetic heritage of your dog.

DNA libraries are also being developed for most agricultural plants and animals. Using electrophoresis, specific gene locations can be inferred. This is important for identifying the most desirable parents for selective breeding. The identification is particularly important in plants, because many crop plants have multiple sets of chromosomes rather than the two sets found in animals. Selecting the parents that will contribute the desired trait is less complicated if you are able to identify the desired gene with markers found with electrophoresis. Extensive mapping has been done on wheat, rice, corn, sugar cane, tomato rape seed, soybeans, and onion. Similar programs are used in working with traits in chickens, pigs, and cattle. Electrophoresis has also been used to accurately identify caught wild mackerel for proper labeling.

## ELECTROPHORESIS TECHNIQUES

Electrophoresis is the actual separation of molecules using an electrical current. The actions taken with the samples before electrophoresis are also an important part of the process. Some

modifications are accomplished by changing the size of the gels and the purity and concentration of the material used in the gel matrix.

## Restriction Digest

To analyze DNA using electrophoresis, you first have to cut it into smaller pieces. The pieces will not be meaningful if they are random, so a restriction digest is done. A **restriction digest** is the cutting of DNA at specific nucleotide sequences with restriction enzymes. With this technique, you will always get the same size pieces by cutting the same DNA with the same restriction enzyme.

## Agrose Gels

Most DNA electrophoresis has been done on agrose gels. These are the gels with the fluorescent bands seen on some crime dramas. This is the type of gel used in a beginning molecular biology lab. Huge gels requiring cooling systems were used in the early work on gene sequencing.

## Capillary Gel Electrophoresis

Capillary gel electrophoresis is a specialized process where a thin capillary tube of gel (instead of a slab of agrose) is used. It uses electronic detectors instead of staining techniques and gives greater resolution of molecular size for research purposes. Television crime labs often use this type of analysis, though very few crime labs are so well equipped.

## Polyacrylamide Gels

Polyacrylamide gels are sometimes used for DNA and RNA separations and are commonly used for protein separations. The polyacrylamide gels are usually purchased precast due to the toxic nature of the acrylamide used to make them. In the polymerized form, the gels are no longer toxic. The polyacrylamide gels are run vertically as opposed to the horizontal agrose gels. When running proteins, SDS is added to denature or unfold the proteins so they run based on overall size.

## Polymerase Chain Reaction

Early use of DNA analysis required large samples. In forensic work and many research situations, large samples are often hard to obtain. The development of another process made this unnecessary. A Polymerase Chain Reaction (**PCR**) is a process to copy and greatly increase the amount of DNA. This has allowed for the forensic analysis of minute samples from crime scenes to Neanderthal DNA from teeth.

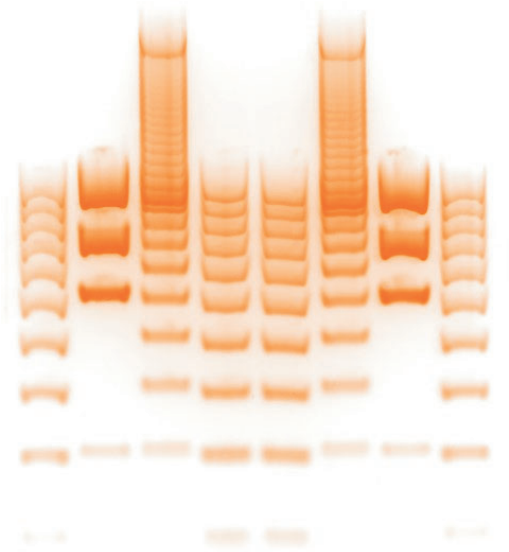

**FIGURE 2. Stained electrophoresis gel. Outer lanes are size markers.**

# INTERPRETING ELECTROPHORESIS RESULTS

## Restriction Digest

The result of a restriction digest is a mix of DNA segments of different lengths called RFLP (pronounced "riff lip"). A **RFLP** is a restriction fragment-length polymorphism caused by differences in genetic sequence between DNA from different sources cut by restriction enzymes. Restriction enzymes cut the DNA to be analyzed in specific locations based on the specific DNA nucleotide sequence. There are more than 900 different restriction enzymes. Cutting at different sequences even while using only a few enzymes can give many different RFLP patterns for the same DNA. If two similar DNA sequences from different organisms are cut with the same enzyme, the small differences in sequence will give different patterns.

**FIGURE 3. One of the steps of a restriction digest is keeping the DNA sample on ice to prevent degradation.**

## Stain

The pattern is visualized by placing the digested DNA into wells in an agrose gel and separating the RFLP by electrophoresis. The DNA is colorless, so the bands of same-sized fragments must be stained. Safe, inexpensive staining can be done in beginning labs with methylene blue stain. However, it is slow, has low resolution, and will not show bands with only a little DNA. Ethidium bromide is the preferred method because it is faster and has far better resolution. It readily binds with DNA and, therefore, has potential biological hazards. As a result, it should only be handled by trained personnel. The stained gels are placed on a light box, visible for methylene blue and UV for ethidium bromide (another potential hazard). Since the gels will dry out and the position of the bands might then change, the gel is usually photographed. Analysis of the fragment distribution is conducted by comparing the band position to bands (with known base pair length) that were run at the same time. A comparison of relative positions can be used to calculate the base pair length of the newly cut fragments.

## Blotting

An additional method for analysis involves transferring the DNA bands to a nylon or nitrocellulose for a procedure called a Southern blot. A **Southern blot** is the transfer of DNA to the membrane by absorbing water through the gel. Once the DNA is chemically locked onto the membrane, it can be treated with probes to show further differences in the DNA. The **probes** are short sequences of DNA that will hybridize or link up to complimentary sequences on the DNA bands. The bands that the probes attach to are visualized by additional chemicals that are linked to the probe. Some are radioactive and are visualized by placing the membrane on X-ray film and leaving it for a time until spots develop. Those bands that

develop spots had the sequence being tested. Fluorescent chemicals can also be used. After each test, the hybridized DNA is rinsed off and another probe can be used.

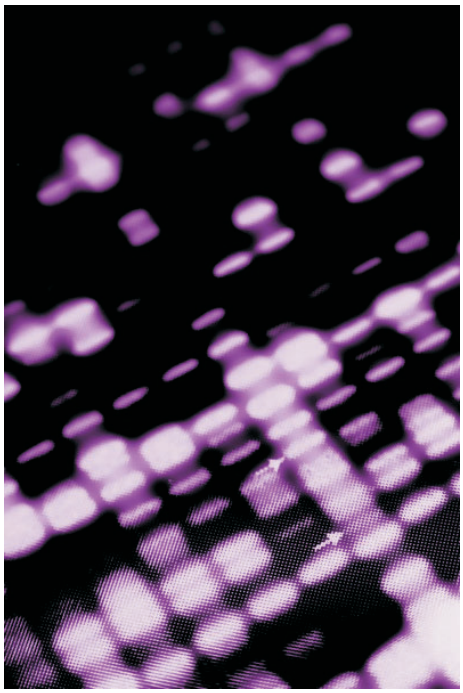

**FIGURE 4. Southern blot results for analysis.**

## *Extending the Technology*

The RFLP/probe method was the original DNA analysis used in forensic cases and was very time consuming. Faster and more precise methods have been developed. For instance, a variable nucleotide tandem repeat (**VNTR**) is a short sequence of nucleotides repeated at a specific chromosome location. The number of repeats is variable. The knowledge of these repeats can facilitate the use of RFLPs and Southern blots. The probes you use will match the targeted VNTRs. You can use PCR to amplify those areas of a sequence where the VNTR is located rather than having to digest and blot the whole genome.

### Short Tandem Repeats

Short tandem repeats (STRs) are VNTRs of short length. An **STR** is a repeated short sequence of DNA. The ones used for forensic identification are usually tetra- or penta-nucleotide sequences. Shorter and longer sequences have PCR problems. Longer sequences also degrade more easily. Of the more than 10,000 STRs identified, the U.S. law enforcement agencies have identified 13 used in their CODIS database, which is the reference used for forensic identification. When these 13 have been probed, the chance that two individuals will have the same pattern of STRs is 1 in 1 billion.

### Single Nucleotide Polymorphisms

SNPs are single nucleotide polymorphisms (usually pronounced "snips"). A **SNP** is a sequence of DNA with a single nucleotide difference. It may be in a non-coding area or in a coding gene; it causes a detectable difference in a protein. The study of SNPs is important in genetic analysis of evolutionary relationships and genetic disorders. Numerous new technologies can detect SNPs. They can be analyzed by RFLP and probes, but it is time consuming and labor intensive. Therefore, newer highly technological methods are typically used.

### Mitochondrial DNA

In cases where nuclear DNA is not available, identification or analysis is still possible. This may be the situation in forensic cold cases where blood, hair, or semen samples were not stored properly and DNA has degraded. It may also be used for very old DNA (e.g., in testing Neanderthals). Mitochondrial DNA (**mtDNA**) is the small, circular DNA in the mitochondria. Because it is circular, it is less susceptible to degradation. In addition, there are many copies in each cell. However, it has a different hereditary pattern than nuclear DNA. The DNA is passed from mother to offspring, so it is not usable to determine paternity or paternal heredity. It is easily amplified with PCR and analyzed by RFLP, Southern blot, and probes.

## Summary:

The first step in any DNA analysis is to obtain the DNA. The DNA must then be processed to make the large chromosomes manageable. The smaller portions that result are sorted and analyzed for the information you are seeking.

Electrophoresis is the use of an electrical field across a gel matrix to separate charged molecules on the basis of how quickly they move through the gel. DNA analysis using electrophoresis is used extensively in criminal forensic laboratories and to identify unknown victims. It is also used in research on genetic diseases and human evolutionary history.

A restriction digest is the cutting of DNA at specific nucleotide sequences with restriction enzymes. The digest is then separated by electrophoresis and is analyzed through the use of stains, Southern blots, and probes. The identification of highly specific variable areas of DNA such as VNTR, STR, and SNP have made analysis faster and more refined.

## Checking Your Knowledge:

1. What is DNA separated from in a DNA extraction?
2. What is electrophoresis?
3. What does a restriction digest yield?
4. How is a Southern blot used?
5. How many STRs are used in CODIS?

## Expanding Your Knowledge:

On television crime dramas, DNA evidence is routine and results are returned quickly. Contact local law enforcement to determine how often DNA is usually collected and how long it takes to obtain the results.

## Web Links:

**DNA Extraction**
http://learn.genetics.utah.edu/content/labs/extraction/howto

**Animation and Virtual Lab on Electrophoresis and Analysis**
http://learn.genetics.utah.edu/content/labs/gel/

**How DNA Evidence Works**
http://science.howstuffworks.com/genetic-science/dna-evidence.htm

**Agricultural Career Profiles**
http://www.mycaert.com/career-profiles