Diagnosing Genetic Disease Through Electrophoresis
HASPI Medical Biology Lab 18b

Background/Introduction

DNA, Genes, and Chromosomes

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person’s body has the same DNA. Most DNA is located in the cell nucleus, but a small amount of DNA can also be found in the mitochondria.

The information in DNA is stored as a code made up of four bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99% of those bases are the same in all people. The order, or sequence, of those bases determines which protein it has the instructions to create. A specific sequence of DNA to make a specific protein is called a gene. Genes are wound up and organized into chromosomes.

Gene Interaction and Genetic Variation

Even though all humans have the same genes, there are variations of these genes called alleles. For example, we all have the gene for eye color, but there are different alleles for brown eyes, blue eyes, or green eyes. We inherit one of these alleles from our mother and one allele from our father for each gene. These alleles then interact in a variety of ways to create a variety of genetic variation. The most common gene interactions include:

<table>
<thead>
<tr>
<th>Dominant/Recessive</th>
<th>Definition</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant/Recessive</td>
<td>One allele “dominates” the other, which is the recessive allele. Individuals who inherit two dominant alleles (AA) are homozygous dominant. If they inherit two recessive alleles (aa) they are homozygous recessive. If they inherit a dominant and a recessive allele (Aa) they are heterozygous.</td>
<td>Brown eyes are dominant to blue eyes.</td>
</tr>
<tr>
<td>Incomplete Dominance</td>
<td>The dominant allele does not completely dominate the recessive allele. In heterozygous (Aa) individuals, an in-between phenotype appears.</td>
<td>Breeding a plant with red flowers and white flowers produces pink flowers</td>
</tr>
<tr>
<td>Co-dominance</td>
<td>There may be more than one dominant allele that competes with the other. Both dominant allele traits appear.</td>
<td>A spotted cow will have a dominant brown allele and a dominant white allele.</td>
</tr>
<tr>
<td>Multiple Alleles</td>
<td>There are more than two alleles that contribute to the gene.</td>
<td>Human blood types have 3 alleles (I^A, I^B, and i).</td>
</tr>
<tr>
<td>Epistasis</td>
<td>There are two or more genes that interact with one another to produce a trait.</td>
<td>Human skin color is created through an interaction of several genes.</td>
</tr>
</tbody>
</table>

During meiosis, traits can be shuffled even further. Chromosomes can exchange part of their information in a process known as crossing-over. Crossing-over creates more genetic variation in the sperm or egg that is produced.
Genetic Mutations

When a DNA has been damaged or reproduced incorrectly, a genetic mutation has occurred. Sometimes these mutations are caught by the cell’s checking mechanisms (think of it like spell check), but sometimes these mistakes get through and are replicated in other cells. Mutations can occur randomly during meiosis and chromosome separation, or in nucleotides during replication. A gene that has the incorrect order of bases has the wrong directions to create a protein. For example, the protein that gives color to our skin is called melanin. Individuals who are albino have a mistake in the gene that makes this protein. The following diagrams demonstrate mistakes that can happen at the chromosome level or at the nucleotide level.

Errors in Chromosomes

In addition to these mutations, it is also possible for chromosomes to not separate correctly during meiosis. This is called nondisjunction. Nondisjunction of chromosome 21 is common and can result in a child with 3 copies of chromosome 21. This condition is more commonly known as Down syndrome.

Mutations During Replication

Mutations can be completely random by internal factors, called endogenous factors, or can be caused by external environmental factors, called exogenous factors. Endogenous factors may include errors in DNA replication, toxic by-products of metabolism, or a hereditary predisposition to mutations. Exogenous factors may include radiation, harmful chemicals, or sunlight exposure.
Electrophoresis
In order to diagnose a genetic disease, the presence of the incorrect gene or wrong protein must be identified. One method that can be used to do this is called electrophoresis. **Electrophoresis** is a technique that uses electricity to separate pieces of DNA by size through a gel. DNA is negatively charged so it moves through the gel to the positive pole. Larger pieces move slower than smaller pieces, which causes them to separate.

**Detailed Steps**
1. Wells, or holes, in the gel are created in order to have a space for the DNA to be placed.
2. A micropipette is used to place small amounts of DNA into the wells.
3. A DNA sample with standard-sized pieces of DNA for comparison, a mutated gene for comparison, and the patient sample are loaded into different wells.
4. An electrical power source is used to run an electrical current through the gel.
5. The DNA moves from negative to positive over time.
6. The smallest pieces of DNA move the farthest and the larger pieces of DNA move the shortest distance. The distance each section of DNA moves can be measured and the number of bases in the DNA can be calculated.

Restriction Enzymes
In order to identify and separate out a specific gene through electrophoresis, the gene has to be separated, or "cut," out of the DNA. **Restriction enzymes** are enzymes found in bacteria that are capable of "cutting" DNA into smaller fragments. Each restriction enzyme is named for the bacteria from which it was taken. There are several types of restriction enzymes that cut DNA at the same place every time. For example, the bacteria *Haemophilus aegypticus* produces a restriction enzyme called **HaeIII** that cuts DNA everywhere it sees the following sequence of nucleotides - “GGCC.” Some other restriction enzymes include:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Bacteria Source</th>
<th>Cuts at:</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td><em>Escherichia coli</em></td>
<td>GAATTC</td>
</tr>
<tr>
<td>BamHI</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>GGATCC</td>
</tr>
<tr>
<td>HindIII</td>
<td><em>Haemophilus influenzae</em></td>
<td>AAGCTT</td>
</tr>
</tbody>
</table>

[Image links]

Electrophoresis, HASPI Medical Biology Lab 18b & 18c
Electrophoresis is one of the most used tools in molecular biology research. Every human has a unique DNA electrophoresis “fingerprint,” and DNA for an individual can be collected from a single cell. This means DNA could be found in saliva, blood, skin, hair, etc. For this reason, DNA electrophoresis can be used to analyze evidence in criminal cases, solve paternity issues, and diagnose genetic diseases. Electrophoresis can also be used to separate proteins and amino acids.

Review Questions – answer questions on a separate sheet of paper

1. What is DNA? Where is it found in a cell?
2. What are the four bases of DNA?
3. Explain why the sequence of DNA bases is just like a sentence.
4. What is a genetic mutation, and at what point(s) can it occur?
5. What is the difference between exogenous and endogenous factors involved in causing genetic mutations? Give an example of each.
6. What is electrophoresis? Summarize the steps of electrophoresis.
7. What is a restriction enzyme? Where do they come from?
8. Look at the electrophoresis sample below. Which DNA samples are the most similar (A-F)? Hypothesize what these similarities mean.