Do Vampires Walk Among Us?
A Genetic Testing Scenario

Student Manual
Key Concepts

- Genetic testing for disease
- Genotype and phenotype
- Patterns of inheritance
- DNA sequencing
- Restriction Fragment Length Polymorphisms (RFLPs)

Prerequisite Understanding

- DNA replication
- Protein synthesis - transcription, translation
- PCR
- Electrophoresis
There are such beings as vampires, some of us have evidence that they exist. Even had we not the proof of our own unhappy experience, the teachings and the records of the past give proof enough for sane peoples.

Bram Stoker (Dracula)

In a small town, there is one family whose members keep to themselves. They have pale skin and dark circles under their eyes. They avoid sunlight and occasionally appear to lose control, acting out in frightening ways. Other people in the town come to fear them, conjuring fanciful explanations to explain what they can’t understand. Perhaps they are witches. Perhaps they are vampires who venture out only during the night preying on the weak and defenseless. Maybe that explains why some children die in their sleep, why a corpse was found lying in an alley. Maybe these people who don’t look and act like anyone else in town are to blame...

Vampire myths and legends abound in the history and folklore of many cultures. Some historians of folklore and legend have suggested that vampire myths may have originated from a community’s inability to comprehend different or strange appearances and behaviors of some people. Vampires of legend avoided sunlight, preferring to venture out by night. They had pale skin and were even rumored to sleep by day in coffins. Vampires were prone to erratic or violent behavior and were accused of sucking the blood from victims. These unusual behaviors and appearances may have been the symptoms of disease. It is important to remember that until recently, the causes of most diseases were unknown. Even relatively straightforward causes, such as viral and bacterial pathogens, were completely mysterious and incomprehensible to people who lived hundreds of years ago. Some historians suggest that rabies, tuberculosis, and porphyria, as well as many mental illnesses may have been confused with occult and supernatural phenomena attributable to witches, vampires, werewolves, or evil spirits.

What causes disease?

Fortunately, we now understand enough about what causes disease that we no longer have to come up with fanciful, and terrifying, explanations for the unusual appearances, behaviors, and experiences of some people. Common causes of disease:

- Pathogens such as viruses, bacteria, and fungi
- Genetic disorders such as hemophilia and cystic fibrosis
- Environmental effects of toxins, poisons and excess exposure to sunlight or certain nutrients
- Nutrient deficiencies such as rickets and scurvy

When it comes to disease, understanding the relationship between cause and effect can be very complicated. Some diseases, such as some skin cancers and porphyria, appear to be the result of complex interactions between a genetic predisposition and an environmental factor such as exposure to sunlight. Given how complex some disease etiologies (causes) are, we should extend some empathy to our ancestors for conjuring demons, spirits and even vampires in their attempts to understand scary and, at the time, inexplicable phenomena. More importantly, we should extend respect and acceptance to people whose symptoms and behaviors might be difficult to understand.

Classical Vampire Mythology

- Vampires crave the blood of the living to quell their hunger, hunting between dusk and dawn.
- They retreat to a coffin during the daylight hours.
- Vampires are able to shape-shift, taking on the form of a bat, wolf, rat, spider or a cloud of mist.
- Though they are “dead,” they appear quite healthy.
- Vampires have no reflection in a mirror because they do not possess a soul.
- They possess super-human strength, hearing, and seductiveness.
- They are not normally able to move about freely in the daylight and will suffer burns and depleted energy if exposed to intense light for any length of time.
- Garlic, holy water and crucifixes can repel a vampire but they will not kill him.
- The only sure ways to “kill” a vampire are: to drive a wooden stake completely through its heart, decapitate it, or keep it away from its coffin at dawn and allow it to “burn up” in the sunlight.
Porphyria - The Vampire's Disease?
The porphyrias are a group of disorders that result in a buildup of chemicals, called porphyrins (Figure 1), in the body. Porphyrins are normally made during the production of heme, the iron-containing component of hemoglobin, which is the large molecule responsible for carrying oxygen (and carbon dioxide) in red blood cells (Figure 2). Heme is produced in maturing red blood cells in a multi-step process.

Patients with porphyria lack a functional enzyme needed to convert the porphyrins in the next step of the reaction. Because the enzyme is not synthesized or is not functional, the next step of the heme production pathway is not catalyzed. The result is that abnormal amounts of porphyrins build up in the body. The cause is usually an inherited mutation in a gene that codes for the protein used to make one of the enzymes in the heme pathway.

The Porphyrias - A group of diseases with diverse symptoms
Some porphyrias affect the nervous system whereas some affect the skin. Still others affect both systems. Table 1 lists some symptoms that are associated with various forms of porphyria.

<table>
<thead>
<tr>
<th>Symptoms of Porphyria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Abnormally rapid heartbeat</td>
</tr>
<tr>
<td>Blistering skin lesions</td>
</tr>
<tr>
<td>Dementia</td>
</tr>
<tr>
<td>Depression</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
</tr>
<tr>
<td>Hypertrichosis (werewolf syndrome)</td>
</tr>
<tr>
<td>Psychosis (hallucinations, disorientation, paranoia)</td>
</tr>
<tr>
<td>Seizures</td>
</tr>
<tr>
<td>Trouble swallowing</td>
</tr>
</tbody>
</table>

Table 1

Genetics of Porphyria
Recall the relationship between genes and enzymes.

- Enzymes are made of polypeptides, the results of transcription and translation of a gene.
- The DNA sequence of a gene specifies the amino acids to be included in the polypeptide.
- The DNA is read in groups of three nucleotides, or codons.
- A genetic mutation alters the DNA sequence and can subsequently alter the sequence of amino acids inserted into the polypeptide.

Thus, genetic mutations may result in an enzyme that has little or no ability to catalyze chemical reactions. Because each of us inherits two gene copies - one from each parent - there are three scenarios possible at each locus.

- Two copies of the normal gene (homozygous normal)
- Two copies of the mutated gene (homozygous mutant)
- One copy of each (heterozygous)

Those who have two copies of the normal gene are free of disease associated with that locus. Those with two copies of the mutated form will not be able to make a functional enzyme. Heterozygous individuals will produce enzyme as coded by the normal gene. If one normal copy of the gene allows enough enzyme to be made, then this person is free of the disease associated with that locus. This scenario describes a recessive trait. But if having only one copy of the normal gene results in insufficient enzyme production, then this trait is dominant.

... in the relentless and meaningless manner one searches for something in a nightmare, coming on doors that won’t open or drawers that won’t shut, struggling over and over against the same meaningless thing, not knowing why the effort seems so desperate ...

Anne Rice (Interview With The Vampire)
In the porphyrias, a nonfunctional enzyme can result in an interruption of the heme production pathway and a buildup of the porphyrin intermediate. One enzyme in the heme synthesis pathway is UROD (uroporphyrinogen decarboxylase). A defect in the UROD enzyme can lead to either of two forms of porphyria.

- **Porphyria Cutanea Tarda (PCT)**
  - A individual heterozygous for a mutation in the UROD gene has the genotype for porphyria.
  - One mutant gene copy may or may not result in development of the disease. This is known as latency.
  - The porphyria disease phenotype (symptoms) is only present when the demand for heme production rises.
  - Demand for heme production increases upon exposure to an environmental factor, or trigger. Triggers include:
    - Drugs
    - Dieting or fasting
    - Smoking
    - Infection
    - Surgery
    - Stress
    - Alcohol use
    - Menstrual hormones
    - Sun exposure
    - Excess iron

- **Hepatoerythropoietic Porphyria (HEP)**
  - Individuals are homozygous for a mutation in the same UROD gene.
  - Symptoms are more constant, severe and can be fatal early in life.

With genetic diseases, understanding the relationship between genotype and phenotype involves identification of the locus or loci involved (genotyping) as well as determining whether or not the patient is experiencing disease symptoms (phenotype). Some patients seek medical help because they are experiencing symptoms. Other patients may seek genetic testing even in the absence of symptoms if they have a relative who has been diagnosed with the disease.

**Porphyria Cutanea Tarda**

One of the more common porphyrias is porphyria cutanea tarda (PCT). PCT is associated with mutations in the gene for the UROD enzyme. Along with being one of the most common forms of porphyria, PCT may also have the most complex etiology. This form of porphyria tends to occur only when other disease processes or sources of physical/emotional stress are present. For example, exposure to sunlight is the most consistent trigger for the primary symptom - skin blistering (Figure 3). Other symptoms of PCT include blistering skin lesions, hyperpigmentation, hypopigmentation, and hypertrichosis (excessive hair growth or werewolf syndrome). It is easy to see that some of these symptoms, such as skin blistering, would be frightening to those who knew a porphyria sufferer. Also, given that these severe symptoms tend to appear and disappear repeatedly, one can understand how someone might have appeared to be immortal, especially during a time when most people did not recover from severe disease.

![Figure 3](Source: Porphyria South Africa website)

**The complex relationship between genetic traits and disease**

* (genotype and phenotype)

In some cases, the relationship between a genotype and phenotype is relatively straightforward. For example: eye color is, in part, determined by genes that direct the body to deposit a brown pigment, called melanin, in the iris of the eye. Someone with brown eyes has either one or two alleles at the brown-blue locus for depositing melanin in the iris, while someone with blue eyes is homozygous at the brown-blue locus for the allele that does not deposit melanin in the iris.

In another example: a patient with only one copy of the gene for Huntington’s Chorea (a progressive neurological disease that begins to show symptoms in adulthood), will develop the disease, as it is autosomal dominant.

However, in other cases, inheriting a genetic trait merely increases the likelihood of developing a disease. For example, patients with one copy of the mutated BRCA1 and BRCA2 genes have an increased risk of developing breast cancer compared to someone who has the normal form of the gene. In women with a BRCA gene mutation, other factors including alcohol consumption and smoking, sedentary lifestyle, and hormone treatments (including birth control pills) increase the likelihood of breast cancer developing.
**Diagnosing Porphyria**

Diagnosing porphyria can be difficult, especially when symptoms are intermittent, as in PCT. The following is a summary of the many steps usually taken before porphyria can be diagnosed.

- **Symptoms and patient history**
  - The patient will be asked to describe symptoms such as neurologic episodes, gastrointestinal pain, mood swings or skin lesions.
  - The clinician will note the timeline of symptoms including any patterns such as symptoms coinciding with stress, taking medications, concurrent illness, menstrual cycles or sun exposure.
  - The clinician will also ask if any family members have similar symptoms.

Early on in the diagnostic process, the clinician is unlikely to suspect porphyria because it is such a rare disease. Therefore, the clinician is likely to prescribe medications to alleviate symptoms or refer to a specialist.

- **Analysis of porphyrin levels.** Porphyria involves mutations in genes coding for enzymes in the heme production pathway. This causes an enzyme deficiency that results in the accumulation of abnormal amounts of porphyrin in the plasma and/or urine. Tests determine the amount of these substances present in patients suspected of having porphyria. Elevated levels of porphyrin can indicate that a patient has porphyria. However, elevated porphyrin levels can also be seen in patients who do not have porphyria. For example, because heme is produced in red blood cells and the liver is involved in breaking down old red blood cells, liver disease can cause porphyrin levels to rise.

- **Genetic testing is completed:**
  - If high urine or plasma porphyrin levels are found.
  - If a patient suspected of having porphyria is asymptomatic for an extended period of time.
  - If patient has a relative who has been diagnosed with porphyria.

When genetic testing is warranted, several methods are available. Here we will focus our discussion on two types of testing most likely to be used in diagnosing porphyria, DNA sequencing and RFLPs.

**DNA sequencing**

In order to confirm a porphyria diagnosis, genetic testing must be completed. One way in which this may be done is by DNA sequencing. In this process, the exact order of nucleotides in a segment of DNA is determined. Recall that there are many forms of porphyria, each being caused by a mutation in a different gene in the heme synthesis pathway. Thus, the symptoms, patient disease history, and initial laboratory tests (such as blood or urine porphyrin levels) will help to determine which gene in the pathway should be sequenced. Once determined, the patient's DNA sequence can then be compared to the normal DNA sequence. In the porphyrias, there are no mutations common to all patients, so the entire gene must be sequenced in each new family. If a mutation in the DNA sequence of a porphyria-causing gene is found, the diagnosis of a porphyria is confirmed. Once a mutation has been identified, DNA analysis (such as RFLP) can then be performed on other family members to determine if they have inherited that porphyria.

**Famous People Thought to Have Had Porphyria**

- Vincent van Gogh
- A large number of the British Royal family bloodline including:
  - James V of Scotland
  - Queen Mary I of Scotland
  - King George III
  - Queen Anne
  - King James I
  - Frederic the Great of Germany
  - Prince William of Gloucester

**Cultural References to Porphyria**

- Porphyria has been mentioned in the following TV shows:
  - House
  - Scrubs
  - Grey's Anatomy
  - CSI
Using RFLPs to Test for Porphyria
Once a diagnosis of porphyria has been confirmed using DNA sequencing, asymptomatic relatives (or ones who have symptoms but have yet to achieve a diagnosis) can opt to have their DNA tested to determine whether or not they have the same mutant form of the gene. This allows for identification of individuals with porphyria so they can then be counseled about appropriate disease management to avoid symptoms or to minimize disease complications.

One way to test a patient’s DNA is to subject it to RFLP analysis, which relies on the use of restriction enzymes. Thus, it is not always necessary to sequence the DNA of the relatives of the original patient. If the particular mutation causing the disease happens to coincide with a restriction enzyme cleavage site (Figure 5), it is possible to use RFLP analysis to determine if relatives of the patient have the same disease-causing mutation. Figure 6 outlines the steps required to complete an RFLP analysis. It is important to understand that the procedures performed in this laboratory are only a portion of the complete genetic analysis.

Cutting DNA with Restriction Enzymes
Bacterial cells can selectively protect their own DNA while enzymatically destroying the DNA of foreign intruders. The enzymes involved in restricting infection of bacteria are appropriately called restriction enzymes. By using these enzymes (several hundred have been identified) in a laboratory setting, we can cut (a.k.a. cleave or digest) DNA with such exactness that individual genes can be isolated. Additionally, DNA sequences can be checked for the gain or loss of restriction sites, which are short sequences of nucleotides where the DNA is cut by a restriction enzyme. The DNA fragments that result are then separated by size using gel electrophoresis. This produces different patterns that allow researchers to distinguish genotypes based on the presence or absence of a particular restriction site (RFLPs).

**Figure 5** Gain of Restriction Site.
Restriction enzyme recognition sites can be gained or lost through mutation. In this simplified hypothetical DNA sequence, a single base mutation in the wild type DNA results in the gain of a ScaI restriction site.

**Figure 6** RFLP Analysis.

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**1. Tissue or blood**

**2. DNA extracted and copied by PCR so enough is available for testing.**

**3. DNA cut with restriction enzyme**

**4. DNA separated into bands gel electrophoresis**
Laboratory Procedures

Purpose

The purpose of this lab is to introduce you to an application of DNA technology - genetic testing for disease. The class will be divided into groups and each group will receive eight DNA samples to run on an agarose gel. Before receiving your samples, read the introductory material about porphyria, DNA sequencing, genetic testing for disease, and restriction fragment length polymorphisms.

Scenario

Your class has received samples of PCR amplified and restriction enzyme digested DNA from patients involved in a research study of the genetics of porphyria cutanea tarda (PCT). One goal of this study is to evaluate the effectiveness of RFLP analysis in diagnosing PCT in asymptomatic and symptomatic patients.

The research study includes:

- Four patients with confirmed PCT (called probands). Each has undergone thorough diagnostic testing as outlined in the Introduction. The UROD gene in these four patients has been sequenced and it has been determined that each patient has a different mutation. In addition, each mutation involves different restriction sites. Sites may be lost or created as a result of the mutation.

- Two asymptomatic relatives of each proband from whom the UROD gene has been PCR amplified and subjected to the same restriction enzymes as their proband relative.

- Two unrelated, symptomatic patients from the same community. For these two patients, the results of preliminary screening are suggestive of PCT. The UROD gene from these two people has also been PCR amplified and subjected to enzyme digestion in order to determine if any of the four UROD mutations previously identified in this community (probands) can also be found in these unrelated patients.

Procedure

Your team has received a set of DNA samples from one proband, two relatives, and two unrelated patients (Vladimir and Isabella). The other teams in your class have received sets of samples from other probands and their relatives plus the DNA from Vladimir and Isabella (the same two unrelated patients). For each sample set, the DNA has been digested with enzymes that correspond to a restriction site known to be affected by that proband’s mutation (site is gained or lost). Different teams have DNA that has been digested with different enzymes.

You will also receive a 100bp DNA ladder, a reference DNA control (wild type allele = normal UROD gene) and one sample of uncut (undigested) UROD DNA.

Your job is to run a gel and analyze the results. To do this effectively, you will need to compile results from the entire class.

<table>
<thead>
<tr>
<th>Lane 1</th>
<th>Lane 2</th>
<th>Lane 3</th>
<th>Lane 4</th>
<th>Lane 5</th>
<th>Lane 6</th>
<th>Lane 7</th>
<th>Lane 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Ladder</td>
<td>Reference DNA</td>
<td>Proband</td>
<td>Symptomatic Non-relatives</td>
<td>Relative 1</td>
<td>Relative 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>1 Edward</td>
<td>Isabella</td>
<td>Vladimír</td>
<td>Alice</td>
<td>Esme</td>
<td>Uncut DNA</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>2 Lucy</td>
<td>Isabella</td>
<td>Vladimír</td>
<td>Jonathan</td>
<td>Mina</td>
<td>Uncut DNA</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>3 Lestat</td>
<td>Isabella</td>
<td>Vladimír</td>
<td>Claudia</td>
<td>Louis</td>
<td>Uncut DNA</td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>4 Stefan</td>
<td>Isabella</td>
<td>Vladimír</td>
<td>Damon</td>
<td>Elena</td>
<td>Uncut DNA</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Loading diagram.
Each team will have samples from an affected patient (proband) plus two of the person’s relatives. In addition, all teams will analyze samples from the same two unrelated patients.
Materials:

Per group:
- preamplified and prestained (MolecuLab 217) DNA samples from one proband
- two asymptomatic relatives of that proband
- two unrelated members of the community whose preliminary screening test results are suggestive of PCT
- one reference UROD DNA sample
- one uncut UROD DNA sample
- one DNA ladder

Per every group:
- electrophoresis chamber
- agarose (0.3g)
- gel tray
- staining trays (218)
- 8-well comb or larger
- vinyl gloves

Per class:
- power supplies
- methylene blue (218)
- Tris Borate EDTA (TBE) buffer

Protocol:

1A. Preparation of 1.0% agarose gel. (If pre-dissolved agarose is available go to step 1B).

a. Add 0.3 g of agarose powder to 30ml of 1X concentration TBE buffer in 100 ml flask or 50 ml glass screw cap tube. Stir well to suspend agarose.

b. Cap flask or tube loosely and dissolve agarose by heating in a microwave (60 - 90 seconds), in a boiling water bath (10 - 15 minutes), or directly on a hot plate at a moderate setting until all agarose is in solution (solution will be clear). In all cases, swirl the solution occasionally and do not allow it to boil over.

c. After dissolving the agarose, allow it to cool to about 65°C (or until it can be comfortably held by the bottom). Alternately, it can be placed in a 65°C water bath until ready to pour your gel.

1B. Predissolved Agarose. (If agarose solution has been prepared earlier and allowed to solidify.

a. Remelt agarose by heating in a microwave oven (30 - 60 seconds) or in a boiling water bath (10 - 15 minutes) until it becomes completely clear with no lumps.

b. After melting the agarose, allow it to cool to about 65°C (or until it can be comfortably held by the bottom). Alternately, it can be placed in a 65°C water bath until ready to pour your gel.

2. Casting the Gel

a. Slide the plastic gates on both ends of the gel tray into the up position and secure them in place by tightening the screws. Do not overtighten as this may cause leaking.

b. Place the gel tray on a flat surface and insert the comb (at least 8 wells) in the slots near the end of the tray. Make certain that the comb is even across the bed of the tray and that there is a small space between the bottom of the teeth and the tray bed.

c. Slowly pour the melted agarose into the tray to a depth of about 5 mm (30 ml). Allow the agarose gel to stand undisturbed until it has solidified for at least 20 - 30 minutes. The gel will become opaque as it solidifies.

d. After the agarose has gelled, loosen the screws slightly and move the gates to the down position. Place the tray onto the platform in the electrophoresis chamber so that the comb slots are closest to the black, or negative, electrode.

e. Fill the electrophoresis chamber with 1X TBE buffer to a level that just covers the surface of the gel.

f. Carefully remove the comb from the gel. Use both hands to grasp the ends of the comb and pull straight upward with a steady motion. Do not tear the agarose or the wells will be useless. Make sure the wells are completely submerged in buffer. If dimples appear around the wells add buffer until they disappear.

3. Load the DNA Samples - Table 3, page 6

Set the volume of a micropipetter to 10 µl. Load 10 µl of each DNA sample into its corresponding well in the agarose gel. Steady the pipet tip so that it is under buffer and over the well. Be careful not to puncture the bottom or sides of the well. The loading dye makes the sample denser than the buffer so that it sinks to the bottom of the well.

4. Gel Electrophoresis

a. Put the cover tightly on the electrophoresis chamber and, with the power supply off, connect the electrical leads to the power supply. The leads should be connected anode (+) to anode (red to red) and cathode (-) to cathode (black to black).

b. Turn the power supply on and set it to 120 volts. Confirm that current is flowing through the chamber by looking for small bubbles along the electrodes. If none are observed, turn off the power supply and recheck the connections.

c. You will begin to see the loading dyes move toward the anode (+) side of the gel as three bands (MolecuLab 217) or two bands (MolecuLab 218). Stop electrophoresis after the dark blue dye runs 3/4 of the way down.

d. Turn off the power supply and remove its plug from the outlet. Grasp the plastic connectors (not the wires) and remove the leads from the power supply.
e. Carefully remove the gel tray from the electrophoresis chamber. Raise the gates and finger-tighten the screws.

f. **MolecuLab 217: Go to step 6.**
The DNA has been pre-stained with FOTO/Vision™ stain and requires no additional staining or destaining.

5. **MolecuLab 218: Methylene Blue Staining of DNA (Wear vinyl or latex gloves when performing this procedure)**
   a. Place gel in gel tray into plastic staining tray. Cover with methylene blue solution (~150 ml) and stain for at least 30 minutes or until the bands appear.
   b. Using a funnel, drain the stain into the methylene blue storage bottle.
   c. Rinse the gel with tap water for several seconds and then fill the staining tray with water. Allow the gel to destain with occasional rocking for at least 30 minutes. Pour the water down the drain.

6. **Viewing RFLP Products in Stained Gel**
   a. Dry the bottom of the gel tray with a paper towel. Place the tray with the gel on the surface of a FOTODYNE FOTO/Phoresis UV (FOTO/Vision™) or White Light (methylene blue) transilluminator. Close the cover if using the UV transilluminator.

   b. Turn on the transilluminator. With the UV transilluminator and FOTO/Vision you should be able to see blue-green bands in the gel. With the white light transilluminator and methylene blue you should see dark blue bands in a lighter blue background of the gel.

7. **Photodocumentation of RFLP Products**
   Using a Polaroid, digital or CCD camera, take a picture of your gel.

   The following chart is designed to help you organize your group's results and the results of other groups.

In the chart below, use a “+” symbol to indicate that the patient has a pattern matching Proband. Make a note of any other observations made of collected data.

<table>
<thead>
<tr>
<th>Group 1 / Proband 1</th>
<th>Edward</th>
<th>Isabella</th>
<th>Vladimir</th>
<th>Alice</th>
<th>Esme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 / Proband 2</td>
<td>Lucy</td>
<td>Isabella</td>
<td>Vladimir</td>
<td>Jonathan</td>
<td>Mina</td>
</tr>
<tr>
<td>Group 3 / Proband 3</td>
<td>Lestat</td>
<td>Isabella</td>
<td>Vladimir</td>
<td>Claudia</td>
<td>Louis</td>
</tr>
<tr>
<td>Group 4 / Proband 4</td>
<td>Stefan</td>
<td>Isabella</td>
<td>Vladimir</td>
<td>Damon</td>
<td>Elena</td>
</tr>
</tbody>
</table>
Glossary of terms

Acute - Disease symptoms having sudden onset and lasting a short duration.

Allele - One of several alternative forms of a gene or DNA sequence occupying a given locus on a chromosome. Alleles are inherited separately from each parent and can be recessive or dominant.

DNA Sequencing - The process of determining the exact order of base pairs in a segment of DNA.

Etiology - The study of the causes or origins of disease.

Gene - A locus on a chromosome that encodes a specific protein or several related proteins.

Genetic Marker - A DNA sequence with a known physical location on a chromosome. Genetic markers can help link an inherited disease with the responsible gene. The genetic marker itself may be a part of a gene or may be located near the gene of interest, allowing researchers to pinpoint the locus of the gene of interest.

Genotype - The genetic composition of an organism, the actual DNA sequence.

Heterozygous - The presence in an individual of different alleles at corresponding homologous chromosome loci.

Homozygous - The presence in an individual of identical alleles at corresponding homologous chromosome loci.

Latency - The period of a disease where the individual experiences no symptoms.

Locus - A specific, physical position on a chromosome.

Mendelian Inheritance - Following the principles of heredity of sexually reproducing organisms formulated by Gregor Mendel.

Law of Segregation - Certain paired characteristics, one from each parent, do not blend or alter each other in the offspring, thus accounting for contrasting traits in successive generations.

Law of Independent Combination - The genes determining such pairs of traits combine in the offspring according to the statistics of chance.

Law of Dominance - If one of a pair of genes is dominant and the other recessive, the appearance of the trait depends on the combination of the genes in the pair. If there is a dominant gene in the pair, the dominant trait will appear. This is called a dominant inheritance pattern. The recessive trait may appear in the offspring only if both genes of its pair are recessive. This is called a recessive inheritance pattern.

Neuropathy - An abnormal and usually degenerative state of the nervous system or nerves where the electrical nerve signals are not functioning normally causing a large range of symptoms.

PCR (Polymerase Chain Reaction) - A technique that uses repeated cycles of denaturation, primer annealing, and extension with DNA polymerase to amplify a target DNA sequence by several orders of magnitude.

Phenotype - The physical characteristics or behavior of a cell or an organism.

Polypeptide - A protein or molecular chain of amino acids; formed by translation of a gene.

Porphyrrins - Pigment proteins found in both animal and plant life. They have a heterocyclic structure with metals and are constituents of hemoglobin, chlorophyll, and cytochromes.

Prevalence - The percentage of a population that is affected with a particular disease at a given time.

Proband - An individual affected with a disorder who is the first subject in a study (as of a genetic character in a family lineage).

RFLP (Restriction Fragment Length Polymorphism) - Variation in the length of a restriction fragment produced by a specific restriction enzyme acting on DNA from different individuals that usually results from a genetic mutation and may be used as a genetic marker.
REFERENCES


http://www.springerlink.com/content/t28l86p12j1jw786/


FURTHER READING

For detailed information about each gene associated with porphyria, including gene maps, mutations, and patterns of inheritance:

Heme
http://www.umass.edu/molvis/tutorials/hemoglobin/heme.htm

Genetics of Disease

Heme synthesis pathway (contains copyrighted animation)
http://rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/heme.htm#animat

Porphyria, Congenital Erythropoietic (autosomal recessive)

General porphyria and genetics (testing)

http://jcp.bmj.com/content/54/7/500.full.pdf

Porphyria Laboratory
The University of Texas Medical Branch, Galveston TX
http://pmch.utmb.edu/Files/Porphyria_Testing_packet.pdf

INTERNET RESOURCES

Web resources used included the following:
http://www.mayoclinic.com/health/porphyria/DS00955
http://neuromuscular.wustl.edu/nother/porph.htm
http://www.porphriafoundation.com/testing-for-porphyria
http://www.mayoclinic.com/health/brcagene-test

The following sites relate to the Dracula myth and some associated controversy:
http://metabolic-disorders.suite101.com/article.cfm/porphyria-the-disease-that-created-dracula
http://www.straightdope.com/columns/read/1321/did-vampires-suffer-from-the-disease-porphyria-or-not
Discussion Questions

1. Mount a print of the gel (or draw your results) in the space provided and label each lane with the name of the sample loaded.

2. Based on your results, which study subjects, if any, have the allele for porphyria cutanea tarda?

3. Explain why a patient could be positive for the PCT allele yet have no symptoms.

4. Using the materials provided as well as other resources (such as the websites listed in the References section) provide at least two reasons why a patient who tests negative for a PCT allele might have symptoms consistent with PCT.

1.

2.
5. Explain why we include the Reference DNA (a digested copy of the wild type or normal) allele in each gel.

6. Explain why we include an undigested copy of the UROD gene in each gel.

7. Using the internet resources listed in the References section (plus others if you like) critique the use of RFLP analysis in one of the following applications:

   1. Identifying remains, such as after a plane crash

   2. Testing genetic diseases for which restriction sites are known. Examples include:

      3. Paternity testing

8a. Evaluate the advantages and disadvantages of using RFLPs (in place of DNA sequencing) to test for PCT?

8b. Which technique do you think is more effective at testing for PCT? Defend your answer with your own opinion based on your evaluation.

9. There are two patients with PCT living in Seattle. A third patient lives in Phoenix. One patient in Seattle is showing symptoms of PCT, but the other is not. The patient in Phoenix is showing severe symptoms. Hypothesize why these patients are having or not having symptoms of PCT.

10. The RFLP pattern seen for Damon resembles neither the RFLP for the proband (Stefan) nor the RFLP for the normal UROD gene. State a hypothesis that may explain this result and propose an experiment to test your hypothesis.