Resource Manual for *Case It! Version 7*

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This manual includes case descriptions and instructions for case studies included with the Case It! v7 download. See the tutorials for more detailed instructions on use of the software simulation. Click here for a PDF version of this Resource Manual. For the Instructor's version, including additional background information and keys to cases, contact Mark Bergland at mark.s.bergland@uwrf.edu. Page updated 2/21/18.

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I. Overview of the Case It! project

Case It! is an NSF-supported project initiated in 1995 by participants in a BioQUEST Curriculum Consortium workshop. The overall goal is to develop a framework for collaborative case-based learning in molecular biology. The Case It software is an open-ended simulation which reads nucleotide or amino acid sequence files, and includes methods for analyzing DNA (restriction digestion and mapping, polymerase chain reaction (PCR), DNA electrophoresis, Southern blotting and dot blotting, microarray analysis) and proteins (protein electrophoresis, Western blotting, ELISA). Detailed tutorials that describe the various features and the steps for using them can be accessed from the Case It home page (http://www.caseitproject.org).

Version 6 of the software has several new features, including separate, movable windows and automated loading features for gels, blots, and ELISAs. In addition, bioinformatics capabilities (sequence alignment, tree building) have been added via integration with MEGA software. Several existing cases have bioinformatics extensions that utilize these new features. [**Important note**: Use the DNA sequences designated for bioinformatics for these extension activities. The DNA files provided for other parts of the case may not give meaningful results when used for sequence alignment and tree building.]

The Case It software can be downloaded from the Case It web site at no cost to educators. MEGA software must be downloaded and registered separately from the MEGA web site, http://www.megasoftware.net (also free of charge). Contact mark.s.bergland@uwrf.edu) for additional information concerning the project..

II. Suggestions for Class Use of Case It!

Most of the cases described here were developed for use in introductory undergraduate biology classes to help students address concepts and issues in molecular biology, but they can be adapted to a variety of educational settings. These cases guide students through the analysis steps and then provide focused questions to prompt interpretation and application of the results.

Each case description includes the case scenario and instructions for analyzing the case, as well as background information and discussion questions. The cases can be presented to students using this format, having them read the background information and perhaps do some additional research, then carry out the analysis, interpret the results and discuss the significance and the issues raised. Alternatively, instructors can edit the cases to add or omit information as appropriate for the backgrounds of students and the course objectives. Students may be required to:

- focus on the ethical and social issues raised by the analysis and the decision-making process involved.
- take on a particular role, e.g. genetic counselor or family member, and present the case interpretation from that perspective.
- develop hypotheses about the results, based on the background information about the molecular biology in the case, before running the analysis
- start with the case analysis and results, and carry out their own research to obtain information necessary to interpret the case.

In addition to using these cases and sequences, instructors may develop their own cases using DNA sequences obtained from GenBank or elsewhere (see "Building your own case study" below). Sequences, restriction enzyme sites, probes, primers and antibodies all are editable text files. Case development also can be assigned to students in more advanced biology courses. The student-designed cases then can be subjected to peer review via poster presentations, etc. and used by students in introductory courses.

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III. Cases

The DNA and protein sequences for the cases described here are located in the Cases folder that is downloaded with the Case It! Software simulation. The necessary enzymes, probes, antibodies, or proteins for a particular case will be located in the same folder as the DNA sequences. The keys to the cases are available online to instructors - contact Mark Bergland (mark.s.bergland@uwrf.edu) for access.

A. Human genetic diseases

Genetic diseases are caused by mutations, or changes in the DNA, which result in loss of function or altered function of a protein. These changes in the DNA can be detected, even in the absence of disease symptoms, by isolating DNA from the patient and using various molecular biology lab techniques to detect the mutation. The following cases illustrate different types of DNA mutations associated with human genetic diseases, as well as different lab techniques, including restriction enzyme digestion and restriction fragment length polymorphism (RFLP), Southern blot, polymerase chain reaction (PCR), and dot blot. Each case scenario indicates which technique to use, and the necessary sequences (DNA samples, primers, probes) are found in the case folder. Note that the techniques used here do not necessarily represent what would be done in a clinical laboratory - PCR and DNA sequencing are increasingly being used for diagnosis, but these cases are intended to illustrate the variety of techniques used to detect DNA differences. The Huntington's chorea case now includes PCR detection as well as the original Southern blot.

[A note about terminology: The term “normal” is generally used to refer to DNA samples or probes without the disease mutation, i.e. the normal or most common sequence for this DNA. No value judgment regarding individuals who have inherited the disease-associated mutations is intended or implied.]

1. Sickle cell anemia

Background: Sickle cell anemia is a disease of red blood cells. It is caused by a mutation in the hemoglobin gene. A single base change results in a single amino acid substitution. This mutation causes the hemoglobin to change its conformation to a more elongated form under certain conditions, distorting the red blood cells and impairing their ability to carry oxygen. Sickle cell anemia is considered a recessive trait, since both chromosomes have to carry the mutation in order for the full blown disease symptoms to appear.

The sickle cell mutation also eliminates a restriction enzyme site - the recognition site for the enzyme MstII. To detect the sickle cell mutation, a patient’s DNA is digested with MstII and a Southern blot is performed using a probe corresponding to this region of the hemoglobin gene. The presence or absence of the sickle cell mutation can be determined based on the size of the fragment identified by the probe.

Case A: Steve and Martha are expecting their second child. They know that sickle cell anemia runs in both of their families. They want to know whether this child could be affected. Neither they nor their 10-year-old daughter, Sarah, have shown any symptoms of the disease. They decide to have DNA tests to determine the status of the fetus, as well as to find out whether they in fact are carriers of the disease gene.

DNA samples: Steve (father)

Martha (mother)
Sarah (daughter)
Fetus
Control DNA, homozygous for sickle cell mutation
Control DNA, homozygous normal, without sickle cell mutation

Digest each of these DNA samples with MstII. Then run a Southern blot, using the probe corresponding to the region of the hemoglobin gene mutated in sickle cell anemia, to determine the genotype of each individual.

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing?

Case B: Mattie has just returned form the hospital after visiting KC, her favorite nephew. She and her family are already grieving the loss they know is coming. She has watched her only brother, Josiah, and his wife, Emma, deal with KC's illness over the years. She feels as helpless for them as she does for KC. Josiah shook her up when he blurted out, during a period of overwhelming stress, that if they had known ahead of time, perhaps they would have chosen a different route, and that she should get tested to avoid the same suffering. Mattie knew it was the stress talking, and that Josiah would not trade any of his moments with KC, but maybe he was right about her. Maybe she should go into parenting with her eyes open. Maybe she should find out if she could bear a child with sickle cell anemia.

DNA samples: Mattie (sister)
Josiah (brother)
KC (nephew)
Emma (wife)
Control DNA, homozygous for sickle cell mutation
Control DNA, homozygous normal, without sickle cell mutation

Digest each of these DNA samples with MstII. Then run a Southern blot, using the probe corresponding to the region of the hemoglobin gene mutated in sickle cell anemia, to determine the genotype of each individual.

1. What chance does Mattie have to bear a child with sickle cell anemia?
2. What other conclusions can you draw from the results?
3. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
4. What issues are raised by this type of testing?

Case C: Claudine and Andre Kasonga live in a small community in sub-Sahara Africa, surrounded by family and friends whose children frequently suffer from malaria or sickle cell anemia. They themselves have both had siblings succumb to each of these diseases. While they both appear to be fine, they are expecting their first child and wish to know how to prepare themselves. Should they move away from the malaria-carrying mosquitoes, or wouldn't it matter? They decide to get tested.

DNA samples: Claudine (mother)
Andre (father)
Fetus
Control DNA, homozygous for sickle cell mutation
Control DNA, homozygous normal, without sickle cell mutation

Digest each of these DNA samples with MstII. Then run a Southern blot, using the probe corresponding to the region of the hemoglobin gene mutated in sickle cell anemia, to determine the genotype of each individual.

1. What is the connection between the malaria-carrying parasite and sickle cell anemia?
2. Under what fetal genetic conditions would it make sense to move out of the area where malaria is endemic?
3. What conclusions can you draw from the results?
4. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
5. What options are available to the family?
6. What issues are raised by this type of testing?

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2. Huntington's chorea

Background: Huntington's chorea is a neurodegenerative disease characterized by motor, cognitive, and emotional symptoms. The age of onset for symptoms is generally 30-50 years. The genetic basis of the disease is an amplification in a gene with an (as yet) unknown function. A triplet (CAG) is repeated 20-50 times in asymptomatic individuals; having more than 50 repeats is associated with disease symptoms. Huntington's disease is considered a dominant disorder, since one copy of the amplified gene appears to be sufficient to cause disease symptoms. This amplification can be detected by restriction enzyme digestion and Southern blot analysis, since the size of the fragment bound by the probe is increased as a result of the amplification of the triplet repeat. Alternatively, PCR can be used to isolate the region containing the triplet repeats; the relative size of the repeat region can be determined by running the PCR products on a gel.

For **Southern blot**: Digest the DNA samples with EcoRI, and then perform a Southern blot with the Huntington's probe. By comparing the sizes of the fragments bound by the probe, determine the Huntington's gene status of Susan and her brother.

For **PCR**: Use the HD primers to perform PCR on the DNA samples. Run the PCR products on a gel (note that the DNA fragments are small, so shorter gel run times may be needed).

Case A: Susan is a 23-year-old whose father, age 55, and paternal aunt, age 61, have been diagnosed with Huntington's chorea. A paternal uncle, age 66, appears to be unaffected by the disease. Susan wants to know if she inherited the mutated gene from her father so that she can prepare for that future if necessary. She arranges to undergo DNA testing for Huntington's disease. Her 17-year old brother, John, also decides to be tested after talking with Susan.

DNA samples:	Susan (patient)
	Father (affected)
	Aunt (affected)
	Uncle (unaffected)
	John (brother)
	Control DNA with HD mutation
	Control DNA, normal (without HD mutation)

1. What conclusions can you draw from these results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. How would you counsel Susan and her brother based on the results of the test?
4. What issues are raised by this type of testing?

Bioinformatics:

1) Use BLAST to identify the gene associated with the larger gel fragments. Click on the large gel fragment from one of the samples that is positive for Huntington's disease. The sequence of that DNA should be visible in the lower window (if not, check the Sequence box above the window). The entire sequence, just a portion containing the repeat region, can be sent for BLAST analysis (the latter will work better if using fragments from the Southern blot). See the BLAST tutorial for more detailed instructions.

2) Compare the lengths of the repeated regions by aligning sequences. How many triplet repeats does each individual have? Select the PCR fragments from the Opened & Processed window or, if Southern blot was used, highlight the region containing the repeats from each sample and export for alignment (the Search feature can be used to find the CAG repeats in the original DNA file). Note that trees built using these sequences are not meaningful. Use the Alignment Viewer window in MEGA to locate the repeat regions.

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Case B: Josiah and Eldrea were worried about their 52-year-old father. He was starting to act sometimes like this older brother, their uncle Theo. Theo was 15 years older than their father and he had been recently diagnosed with Huntington disease. After speaking with the family physician they learned a diagnostic DNA test was available. They wanted to their father to have the test, and they decided they should take it themselves so that they can better prepare for their future.

DNA samples:	father
	uncle Theo
	Josiah
	Eldrea
	Control DNA with HD mutation
	Control DNA, normal (with no mutation)

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing?

Bioinformatics: See Case A

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Case C: Forty-four year old Jerry is haunted by the specter of Huntington disease. It took his grandmother, a favorite uncle, and now he sees signs of motor impairment in his 67-year-old mother, Sophie. He worries that he might have inherited the disease and wonders, too, if he may have passed it to any of his 3 children. After several late night family discussions, a date is set for them to provide samples for DNA testing. While he is certain he and his mother should be tested, he wonders if his children are making the right choice.

DNA samples:	Sophie (mother)
	Jerry (father)
	22-year-old son
	19-year-old daughter
	18-year-old son
	Control DNA with HD mutation
	Control DNA, normal (without HD mutation)

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing?

Bioinformatics: See Case A

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3. Duchenne's muscular dystrophy

Background: One form of inherited muscular dystrophy, Duchenne's, is X-linked and therefore affects primarily males. The symptoms of Duchenne's muscular dystrophy (DMD) include progressive and severe skeletal muscle weakness. A common mutation associated with DMD is a deletion of one or more exons in the dystrophin gene. These deletions can be detected by restriction enzyme digestion and Southern blotting using a combination of probes that will bind to multiple dystrophin exons.

Case A: Jean and Bill have three sons, ages 12, 8, and 7, and a daughter, age 6. The oldest son and daughter are healthy, but the two younger sons are exhibiting symptoms of muscle weakness consistent with early muscular dystrophy. Jean knows that she has a family history of muscular dystrophy, but she does not know whether she is a carrier of the disease gene. She seeks DNA testing to determine whether her younger sons may have inherited the form of the dystrophin gene associated with Duchenne's muscular dystrophy (DMD).

DNA samples:	Jean (mother)
	oldest son (unaffected)
	daughter
	8-year-old son (possibly affected)
	7-year-old son (possibly affected)

Digest each DNA sample with HindIII, then perform a Southern blot with the dystrophin gene probe (DMD probe). Based on the number and sizes of the fragments bound by the probe, determine the status of each of the individuals tested. (Hint: Some fragments are small, so you may need to use shorter run times to see them all.)

1. What conclusions can you draw from these results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What issues are raised by this type of testing

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Case B: Tabitha walked slowly to the kitchen table and sat down. She gazed out the window where her two daughters (aged 10 and 12) were playing in the sprinkler and chuckled at their antics. She thought of the son she and her husband lost nearly 2 years ago. He would have been 14 this year. And she just found out she was pregnant again - unplanned and unexpected. As she sipped her coffee she knew

she could not endure a pregnancy wondering if the baby would turn out healthy, nor could she bear to lose another child to muscular dystrophy. She called the clinic to set up an appointment for DNA testing. She knew now that she was the source of the mutated gene and she wondered if she had passed it to her new baby. She also wondered if either of her daughters were carrying it, and hoped fervently they were not.

DNA samples: Tabitha (mother)
fetus
10 yr old daughter
12 yr old daughter

Digest each DNA sample with HindIII, then perform a Southern blot with the dystrophin gene probe (DMD probe). Based on the number and sizes of the fragments bound by the probe, determine the status of each of the individuals tested. (Hint: Some fragments are small, so you may need to use shorter run times to see them all.)

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing?
5. Is the fetus healthy?
6. Is the fetus male or female?

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Case C: Scott and Mary met as teenagers at the local MD telethon. Each was there volunteering their time in support of brothers they watched slowly suffer from progressive muscle degeneration. Now, years later, they were married to each other and ready to start a family of their own. Mary’s pregnancy test came back positive and the news filled them with both joy and dread. What if their child had muscular dystrophy? Mary decides to go in for DNA testing to find out is she is a carrier, and if the baby is affected.

DNA samples: Mary (mother)
Scott (father)
fetus

Digest each DNA sample with HindIII, then perform a Southern blot with the dystrophin gene probe (DMD probe). Based on the number and sizes of the fragments bound by the probe, determine the status of each of the individuals tested. (Hint: Some fragments are small, so you may need to use shorter run times to see them all.

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing?
5. Is the fetus healthy?
6. Is the fetus male or female? How do you know?

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4. Alzheimer disease

Background: Alzheimer disease is by far the most common cause of dementia in aging persons. The disease symptoms are identical to other forms of senile dementia, and diagnosis had been possible only at autopsy by the detection of protein clusters called amyloid plaques in the cerebrum. The disease is multifactorial and inheritance patterns are complex. Some forms of familial Alzheimer disease appear to be inherited as autosomal dominant traits, while others are recessive. Spontaneous Alzheimer disease also can occur in the absence of inherited factors.

Mutations in at least four genes have been linked to Alzheimer disease. One of these is the amyloid precursor protein (APP) gene, which encodes the b-amyloid peptide found in the cerebral plaques of Alzheimer patients. The function of APP is not yet known, but certain APP point mutations are associated with inheritance of late-onset Alzheimer disease in some families. Two examples which can be detected by RFLP analysis are the codon 693 Glutamic acid to Glycine mutation and the codon 717 Valine to Isoleucine mutation. The 693 mutation results in the loss of a MboII site, while the 717 mutation results in the gain of a BclI site.

Case A: Martha, age 71, has been exhibiting increasingly severe symptoms of senile dementia and has been hospitalized for testing. She is in good health otherwise. Her three children - Sam (age 43), Joan (age 41) and Robert (age 38) - want to find out the cause of the dementia and determine the prognosis for Martha's future condition. They are also concerned that Martha may have a form of familial Alzheimer

disease and want to know if they are at risk. The physician decides initially to test Martha for two mutations, 693 Gly and 717 Ile, in the amyloid precursor protein (APP) gene which are associated with inherited Alzheimer disease.

DNA samples: Martha (mother)
Sam (son)
Joan (daughter)
Robert (son)
Control normal APP gene
Control with 693 mutation
Control with 717 mutation

To test for the 693 Gly mutation, digest the DNA with MboII and perform a Southern blot using the APP probe. To test for the 717 Ile mutation, digest the DNA with BclI and then use the APP probe. Compare the test samples to the control samples, and use the results to determine the genotype of each individual. [Note: Small fragments are generated with the MboII digestion - use 1.2% agarose and short run times.]

1. Does Martha have either of these two APP mutations?
2. Did any of Martha's children inherit an APP mutation?
3. What conclusions can you draw regarding Martha's diagnosis?
4. What can you tell Martha's children about their risk for Alzheimer disease
5. What issues are raised by this type of testing?

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Case B: Lisa, age 17, and her cousin Jen age 18, were half-listening to music in the den and half-listening to their mothers discuss Grandma Eloise and her older sister Florence. Lisa and Jen loved Eli and Flo dearly but even they could tell something wasn't quite right about their increasingly odd behavior. The teens moved into the kitchen to join the conversation. "Is Grandma's erratic behavior and forgetfulness Alzheimer's or just senile dementia commonly associated with old age?" They decide to talk to Eloise and Florence about DNA testing. The mothers also wonder about their risk for Alzheimer disease and decide to be tested.

DNA samples: Eloise (grandmother)
Florence (Eloise's older sister)
Lisa's mother
Jen's mother
Control with 693 mutation
Control with 717 mutation
Control normal APP gene

To test for the 693 Gly mutation, digest the DNA with MboII and perform a Southern blot using the APP probe. To test for the 717 Ile mutation, digest the DNA with BclI and then use the APP probe. Compare the test samples to the control samples, and use the results to determine the genotype of each individual. [Note: Small fragments are generated with the MboII digestion - use 1.2% agarose and short run times.]

1. What conclusions can you draw from the results?
2. What is the molecular basis of this disease, and why does this result in the observed gel patterns?
3. What options are available to the family?
4. What issues are raised by this type of testing

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6. Cystic Fibrosis

Background: Cystic fibrosis (CF) is generally considered the most common severe autosomal recessive disorder in the Caucasian population, with a disease frequency of 1 in 2,000 and a carrier frequency of 1 in 20. The major clinical symptoms include chronic pulmonary disease, pancreatic insufficiency, and an increase in sweat electrolyte concentrations. The cause of the disease appears to be a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a membrane protein involved in transporting ions across epithelial surfaces, such as the linings of the lungs and intestines. Several mutations have been identified as being associated with a non-functional CFTR protein. The most common mutation, accounting for about 50% of CF cases, is called delta F508; it is a three-base deletion resulting in the loss of a phenylalanine at position 508, in the ATP-binding portion of the protein. This mutation is detected by

sequence analysis of PCR-amplified DNA, or by hybridization with mutation-specific probes (the latter method is illustrated in Case B).

Rapid screening for cystic fibrosis is also done using RFLP markers linked to the CF gene on chromosome 7 (illustrated in Case A). Several RFLP analyses can be performed relatively quickly on PCR-amplified DNA from a blood sample or chorionic villus sample. Then, if a positive result is obtained with an RFLP marker, sequence analysis or mutation-specific probe hybridization can be done to confirm the CFTR mutation. An example of a linked RFLP marker is in the locus Mp6d.9, in which a point mutation linked to CF results in the loss of an MspI site.

Case A: As Sharon Brown browsed the local newspaper, she noticed the story about the five-year old boy with cystic fibrosis who lives on the next block. The article was mainly a human interest story about how the family was coping. There also was some background information about the disease and its inheritance patterns, including the statistics indicating that approximately 1 in 18 people in this part of Minnesota carried a cystic fibrosis mutation.

Sharon is two months pregnant. She realizes that she and her husband, Bob, should have been tested for the cystic fibrosis (CF) mutation since they each have some family history of the disease, but they really hadn't expected to have a child so soon. She discusses this with her physician during her check-up the next day, and together they decide to test Sharon and Bob for a mutation in linked to the CF gene. They also decide to test the developing fetus. Two other families in the same town who also are in the first trimester of a pregnancy, Jill and Mike Jones and Carol and Ron Smith, also decide to be tested after reading the article.

Blood is drawn from the parents, and a chorionic villus sample is taken from each fetus. DNA is isolated from the samples, and a small portion of chromosome 7 near the CF gene, a locus called Mp6d.9, is amplified by PCR. (Use the PCR function on the Data Screen, rather than 96-well PCR.) Control DNA samples with and without the CF mutation are also included. Digestion of the PCR fragments with the enzyme MspI is used to detect the RFLP linked to the mutated CF gene, which results in the loss of a MspI site. [Note: Small fragments are generated, so use shorter run times to see all of the fragments.]

a. What conclusions can you draw from the gel results?
b. What options are available to the parents?
c. What issues are raised by this type of testing?

Case B: (Contributed Stephanie Dahlby, Dan Tally, and Janelle Veerkamp, Biol 305 Students, Spring 1997, UW-River Falls)

Lynda and Jim are expecting their first child. Recently, however, they learn that Lynda's aunt died of CF and Jim's uncle died of CF. They are worried that they might be carriers for the disease and pass cystic fibrosis on to their unborn child. They learn about a procedure which can determine whether they are carriers. They also learn about a procedure called amniocentesis which can detect if their unborn child has CF or is a carrier. However, amniocentesis is a very risky procedure. Jim and Lynda ultimately decide that they first want to be tested to see if they are carriers for the disease. If they learn that they both are carriers, they would like to go through with the amniocentesis to see if their child is affected.

DNA Samples:
- Lynda
- Fetus
- Jim
- Control DNA with F508 mutation
- Control normal DNA, without mutation

Procedure: Run PCR on each of the DNA samples using the CF primers (NOTE: Use the PCR function on the Data Screen rather than the 96-well PCR). Then, using the dot blot screen, load the probes into the dots. Load the DNA samples into the corresponding wells. By comparing the dot blot patterns of Jim, Lynda, and the fetus to those of the two controls, determine whether these DNA samples are homozygous positive for the CF mutation, homozygous negative for CF, or heterozygous carriers for CF.

1. What conclusions can you draw from the gel results?
2. What options are available to the parents?
3. Should large-scale screening for CF carriers be done?
4. How has the prognosis for children with CF changed and how might it change in the future?
5. What other issues are raised by this type of testing?

Case C: The pre-marriage counseling session Carl and Maggie are having with Pastor Frank is not going at all as they had expected it to. After some of the anticipated discussion of relationship issues, the conversation turns to family planning. When both Carl and Maggie say they want to have children, Pastor Frank, instead of giving advise on how to properly rear children, begins to talk about genetic testing for Cystic Fibrosis! It turns out that Pastor Frank and his wife had two children affected with CF who died in their early teens. Because of the relatively high frequency of CF carriers and his opposition to abortion, Pastor Frank believes that all couples should be tested for the CF gene

before getting married. Carl and Maggie are not sure they share Pastor Frank's beliefs but decide to go along with being tested.

DNA Samples: Carl
Maggie
Control DNA with F508 mutation
Control normal DNA without mutation

Procedure: Run PCR on each of the DNA samples using the CF primers. (NOTE: Use the PCR function on the Data Screen rather than the 96-well PCR.) Then, using the dot blot screen, load the probes into the spots. Load the DNA samples into the corresponding wells. By comparing the dot blot patterns of Carl and Maggie to those of the two controls, determine whether these DNA samples are homozygous positive for the CF mutation, homozygous negative for CF, or heterozygous carriers for CF.

1. What conclusions can you draw from the gel results?
2. What options are available to the parents?
3. Should large-scale screening for CF carriers be done?
4. How has the prognosis for children with CF changed and how might it change in the future?
5. What other issues are raised by this type of testing?

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7. Phenylketonuria (PKU)

(Contributed by Duane Zimmerman, Biol 451 Student, UWRF, Fall 1996)

Background: PKU is a genetic metabolic disease caused by a mutation in the phenylalanine hydroxylase enzyme. In the most common form of PKU, a C to T point mutation causes an arginine to be replaced by tryptophan at amino acid position 408, resulting in an inactive enzyme and incomplete metabolism of phenylalanine-containing compounds such as proteins. The resulting buildup of phenylalanine can cause mental retardation, eczema, loss of skin pigmentation, and other disorders. If detected early, the disease is treatable by excluding foods high in phenylalanine form the diet.

PKU is typically tested by measuring the blood level of phenylalanine in a blood sample taken at birth. The molecular test would be valuable as a follow-up to confirm the cause of high phenylalanine levels and to be better able to predict treatment outcomes. The mutation can be detected by dot blot analysis of PCR-amplified DNA from the blood sample. Probes that will bind either the mutated or non-mutated sequence are used in the dot blot to determine which form of the gene is present.

To analyze these cases, use PCR with the PKU primers to amplify a portion of the phenylalanine hydroxylase gene from blood DNA samples. (NOTE: Use the PCR function on the Data Screen, rather than the 96-well PCR.) Then, using the dot blot, load the probes into the spots and add the PCR-amplified DNA samples into the corresponding wells.

Case A: Peter and Pam just had their first child. The PKU blood test performed at birth indicated a high level of phenylalanine in the blood. The physician suggests a follow-up DNA test immediately to confirm the PKU diagnosis and to determine the most appropriate treatment. She also suggests that Peter and Pam be tested to confirm their carrier status and predict the risk of PKU in subsequent offspring.

DNA samples: Peter
Pam
Infant
Control DNA containing the PKU mutation
Control normal DNA without mutation

Questions

1. What conclusions can you draw from the results of the DNA test?
2. What is the molecular basis for the test, and how does this explain the test results?
3. What issues does this type of testing raise?

Case B: Angie watched her little brother, Alan, grow up with PKU. She knows how wonderful it is that the dietary treatment that he has undertaken since being diagnosed by neonatal screening has prevented development of the worst PKU symptoms. But she has also seen that his life has not been an easy one. It is never easy being different and Alan's strict dietary regimen has significantly affected his social interactions at school. Angie has always said that if she ever decides to have a child, she will be tested before she gets pregnant to see if she carries the PKU gene. This makes her current situation, an unplanned pregnancy by a man who was out of her life before either of them even knew, especially difficult. There are so many unknowns. Does she want to continue the pregnancy under these circumstances, even if she

isn't a carrier? If she is a carrier and the fetus is unaffected, is this her best chance to have child unaffected by PKU? Angie decides that the starting point for her difficult decisions must be to find out if she is a carrier for PKU.

DNA Samples:	Angie
	Control DNA containing the PKU mutation
	Control normal DNA without mutation

1. What is the chance that Angie is a carrier?
2. What conclusions can you draw from the results of the DNA analysis?
3. How would you counsel Angie based on the results of her test?
4. Is the role of a genetic counselor different in a case in which an unaffected fetus may be aborted?

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Case C: When Richard and Kathy's first child, Robert, was tentatively diagnosed with Phenylketonuria on the basis of neonatal screening for high levels of phenylalanine, they were relieved to learn that appropriate dietary restrictions are an effective treatment for PKU. After some experience with maintaining the strict diet and the constant medical monitoring, they make some decisions about family planning. They know they still want to have a large family, but feel that they cannot handle the rigors of more children with PKU. When Kathy becomes pregnant again, they seek genetic testing to confirm the diagnosis and test the fetus for PKU.

DNA Samples:	Robert
	Richard
	Kathy
	Fetus
	Control DNA containing the PKU mutation
	Control normal DNA without mutation

1. What conclusions can you draw from the results of the DNA analysis?
2. How would you counsel Richard and Kathy based on the results of their tests?
3. Is termination of the pregnancy appropriate in the case of a treatable disease?

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8. Fragile X Syndrome

(Contributed by Gretchen Hessler, Melissa LeFebvre, and Jenni Swanson, Biol 305 Students, Spring 1997, UWRF)

Background: Fragile X syndrome is the leading cause of inherited mental retardation. The mutated gene that causes the disorder is called *fmr1* and is located on the long arm of the X-chromosome. It is currently unclear whether this trait is dominant or recessive, because both types of expression have been demonstrated.

The mutation involves exaggerated repetition of the CGG triplet in a portion of the *fmr1* gene near the 5' end. Those with a functional gene have 6 to 50 CGG repeats, whereas those with a full mutation have 200 or more such repeats. Between 50 and 200 repeats of the codon constitute a premutation. An individual with a premutation is considered a carrier, but does not display any symptoms of fragile X. A premutation may undergo additional repetition to generate a full mutation.

The *fmr1* gene was discovered in 1991, and therefore DNA testing for the disorder is relatively new. In the past, those with this disorder were often diagnosed as being learning disabled, autistic, or hyperactive. With the advent of DNA testing, accuracy of diagnosis has increased tremendously.

Case A: Doug and Grace are expecting their third child. They have recently learned of fragile X syndrome and strongly suspect that their son, Brad, might have this disorder. For this reason, they would like their family to undergo genetic testing. Their daughter, Katie, shows no symptoms of fragile X. They also decide at this time to test the fetus for the same disorder.

DNA Samples:	Doug
	Grace
	Brad
	Katie
	Fetus
	Control normal DNA
	Control DNA with premutation

Control DNA with full mutation

Digest each of these DNA samples with EcoR1. Then perform a Southern blot using the probe corresponding to the region of the *fmr1* mutation to determine the genotype of each individual.

1. What conclusions can you draw from these results?
2. What options are available to the parents?
3. What issues may be raised by the results of the testing?

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Case B: Melissa has always found dealing with her brother, David, very difficult. His developmental disability and behavior problems were an embarrassment to her as she was growing up. When she married Paul and moved away, she was delighted finally to be free of her "problem brother." However, her freedom for her "problem" is to be very short lived. She and Paul want to start their family as soon as possible and Melissa gets pregnant soon after their marriage. When Melissa calls home to tell her family the good news, her mother, Emma, bursts into tears. Melissa listens in shock as her mother tells her that their family physician has learned of new research on a genetic condition called Fragile X Syndrome and has suggested that this might be the cause of David's problems. Emma and David have already made an appointment for genetic testing and Melissa quickly decides that she and her fetus should also be tested.

DNA Samples: David
Emma
Melissa
Fetus
Control normal DNA
Control DNA with premutation
Control DNA with full mutation

Digest each of these DNA samples with EcoR1. Then use perform a Southern blot using the probe corresponding to the region of the *fmr1* mutation to determine the genotype of each individual.

1. What is the chance that Melissa is a carrier?
2. What conclusions can you draw from the results of the DNA analysis?
3. How would you counsel Melissa based on the results of her test?
4. What issues may be raised by the results of the testing?

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Case C: As Janet, Beth and Alison sit in the reception area of the genetics clinic, they discuss their anxiety about the upcoming test results and their anger at their mother. Ever since Uncle Al, their mother's brother, was diagnosed with Fragile X Syndrome, their family has discussed genetic testing for the disorder. Their mother has steadfastly refused to consider being tested and even her unexpected pregnancy at age 42 has had no effect on her decision. The daughters have decided that they will be tested because they want to know their status and also because they intend to use any positive results as leverage to convince their mother to change her mind about testing for her and the fetus.

DNA Samples: Janet
Beth
Alison
Control normal DNA
Control DNA with premutation
Control DNA with full mutation

Digest each of these DNA samples with EcoR1. Then perform a Southern blot using the probe corresponding to the region of the *fmr1* mutation to determine the genotype of each individual.

1. What conclusions can you draw from the results of the DNA analysis?
2. How would you counsel Janet, Beth and Alison based on the results of their tests?
3. What issues may be raised by the results of the testing?
4. Does their mother have a right not to know the results?
5. What other genetic counseling issues are there for their mother?

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9. Tay-Sachs Disease

Background: Tay-Sachs Disease (TSD) is an autosomal recessive inborn error of metabolism whose basic biochemical defect is a deficiency of a lysosomal enzyme known as hexosaminidase A (*hex A*), which normally catalyzes a step in the degradation of a membrane glycolipid called ganglioside GM2. In the absence of *hex A* activity, GM2 accumulates in central nervous system cells, eventually compromising their function. In the classical form of TSD, infantile TSD, clinical symptoms typically appear at three to six months of age and progress rapidly to blindness, deafness, uncontrollable seizures and death before age five years. The disease occurs with increased frequency in the Ashkenazi Jewish population, with frequencies of heterozygotes ranging from 1 in 25 to 1 in 45. An adult form of TSD, resulting from a partial deficiency of *hex A* activity, is associated with an age of onset in the twenties or thirties and is characterized by an unsteady gait followed by progressive central nervous system deterioration. There are no effective therapies currently available for either form of TSD. Characterization of the enzyme defect in infantile TSD in the 1960's resulted in development of a test for hex A activity that allowed for identification of heterozygotes and prenatal diagnosis of affected fetuses through amniocentesis. The availability of these tests combined with the relatively high frequency of heterozygotes in a well defined population led to TSD carrier screening programs being instituted in most major cities in the United States. The Tay-Sachs gene has now been identified on chromosome 15 and three mutations that result in TSD have been characterized, allowing for more accurate diagnosis. Studies of TSD carriers have shown that 78% have a four-nucleotide insertion mutation in exon 11.

To analyze these cases, use PCR with the TSD primers to amplify a portion of the hexosaminidase A (*hex A*) gene from blood DNA samples. (NOTE: Use the PCR function on the Data Screen, rather than the 96-well PCR.) Then, using the dot blot, load the probes into the spots and add the PCR-amplified DNA samples into the corresponding wells.

Case A: When Megan and Greg announce their plans to get married, Megan's mother, Rachel, finally explains why Megan never got the baby brother or sister that she always asked for when she was younger. Shortly after Megan was born, her parents learned that a Tay-Sachs Disease carrier screening program was being organized in their area. Since they were planning to have more children, they decided to be tested. The news they received was not what they had hoped for; they both tested positive for carrier status. Because they did not want to risk having a child with TSD and their religious beliefs did not permit aborting an affected fetus, they chose not to have any more children. When Megan tells Greg this news, he questions his parents and learns that they had chosen not to be tested because of fear of stigmatization and discrimination. Greg and Megan decide that they must be tested before they get married.

DNA samples:
- Megan
- Greg
- Control DNA, with the TSD mutation
- Control normal DNA without the TSD mutation

1. What is the chance that Megan carries the Tay-Sachs gene, based on her parents' test results?
2. What conclusions can you draw from the results of the DNA analysis?
3. How would you counsel Megan and Greg based on the results of their tests?
4. What issues are raised by large-scale genetic screening?

Case B: Lisa had always wondered about the results of her first Tay-Sachs Disease carrier test. She had been tested at age 18 when large-scale screening was done in her hometown of Minneapolis. The test used then measured levels of the Tay-Sachs enzyme and Lisa's test results were in a range that made the diagnosis uncertain. Even repeat testing could not resolve the question. Since she was not planning to have children right away, Lisa had put her concern aside and gone on with her life. With a busy career and an active social life, she had never married. Now, at age 40, she suddenly found herself with an unplanned pregnancy and facing some difficult decisions. Although the thought of being a single parent is daunting, Lisa decides that she wants to have a child. The father is not interested in being involved in the child's future and also refuses to undergo genetic testing. Lisa decides to have DNA testing done to resolve her carrier status and to determine the genotype of the fetus.

DNA samples:
- Lisa
- Fetus
- Control DNA, with the TSD mutation
- Control normal DNA without the TSD mutation

1. What conclusions can you draw from the results of the DNA analysis?
2. How would you counsel Lisa based on the results of her tests?
3. What issues are raised by this case?

Case C: When Lisa (see Case B) tells her younger sister, Rose, about her decision to be tested for Tay-Sachs, Rose informs Lisa that she and

her husband, Frank, are also expecting a child. Rose is now concerned about her own Tay-Sachs status and decides that she and Frank should be tested. They also decide to test their unborn child, while she is in the early stages of the pregnancy.

DNA samples:	Rose
	Frank
	Fetus
	Control DNA, containing the TSD mutation
	Control normal DNA without the TSD mutation

1. What conclusions can you draw from the results of the DNA analysis?
2. How would you counsel Rose based on the results of her tests?
3. What issues are raised by this case?

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B. Cancer

1. Breast Cancer Susceptibility

Background: Breast cancer is the most common malignancy among women. Current estimates are that one in eight women born in 1990 will contract breast cancer by age 85. Many factors contribute to breast cancer risk. Inheritance of breast cancer susceptibility genes contribute to approximately 5-10% of all breast cancers. The breast/ovarian cancer susceptibility gene BRCA1 has been identified on chromosome 17. Women who inherit certain BRCA1 mutations have an 80% risk of breast cancer.

BRCA1 appears to encode a tumor suppressor protein. Mutations that affect the function of this protein cause increased rates of cell division and a predisposition towards the development of malignancy. Several BRCA1 mutations, including point mutations, deletions, and insertions, have been identified that may contribute to loss of tumor suppressor function. These mutations can be identified by amplifying portions of the BRCA1 gene by PCR and then using RFLP analysis, direct sequencing, or hybridization with specific probes to detect the presence of mutations. Large scale screening trials are underway to gain more information about the nature of the mutations responsible for increased cancer risk. One deletion mutation in exon 2, 185delAG, is highly prevalent among women of Eastern European Jewish descent, and screening efforts have targeted this population of women for further study.

For the screening, a small amount of blood is drawn. DNA is isolated from the blood, and part of the BRCA1 gene is amplified by PCR. The amplified DNA is run on a dot blot with specific probes corresponding to mutations known to be linked to increased breast cancer susceptibility. The probe will only bind to the DNA if that mutation is present. Probes corresponding to the normal sequence for that mutation site will help you determine whether the individual is homozygous or heterozygous for the mutation. Control DNA samples known to have specific mutation also are included. To analyze these cases, use the PCR function on the Data Screen, rather than the 96-well PCR, to generate DNA samples for the dot blot. Load probes in the spots and load the DNA samples into the corresponding wells.

Case A: While Elizabeth is reading the morning newspaper, she notices an ad for a free genetic screening for breast cancer at the clinic next week. The ad specifically invites women of Ashkenazi Jewish ancestry to participate. According to the newspaper ad, subjects will be tested to see whether they have mutations in the BRCA1 gene which would predispose them to breast cancer. Elizabeth, age 27, had heard about the discovery of the gene and about the mutation linked to Jewish women. Her paternal grandmother had been diagnosed with breast cancer at age 51 and died two years later, and Elizabeth worried that she had inherited the disease. She also worried about her mother, age 52 and apparently cancer-free so far, and her 7-year old daughter. Her daughter is not allowed to participate in the screening, but Elizabeth convinces her mother to go with her to get tested.

DNA samples:	Elizabeth
	Mother
	185delAG (DNA containing this mutation)
	4184delTCAA (DNA containing this mutation)
	5382insC (DNA containing this mutation)
	Normal BRCA1 (no mutations)

Probes:	185delAG (AG deletion in exon 2)
	Normal 185 (no mutation at this site)
	4184delTCAA (TCAA deletion in exon 11)
	Normal 4184
	5382insC (C insertion in exon 13)

Normal 5382

Primers: Forward and reverse PCR primers for the BRCA1 gene

Questions

1. What conclusions can you draw from the results of the DNA analysis?
2. How would you counsel Elizabeth and her mother based on the results of the test?
3. Who should have access to the test results?
4. What other issues does this type of testing raise, and how should these issues be addressed?

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Case B: The time passes slowly as Deborah waits for Aunt Millie to come out of surgery. It had come as no surprise when Aunt Millie was diagnosed with breast cancer. After all, for as long as Deborah could remember, her mother had talked about how breast cancer "ran in the family." Deborah has already read the literature the doctor gave them about genetic testing for breast cancer susceptibility genes. It is one thing to know that several women in her mother's family had developed breast cancer; it is quite another to learn that Aunt Millie has tested positive for such a gene and therefore, Deborah and her mother are at higher risk. Her mother has made it clear that she has no intention of being tested but as Deborah sits in the surgery waiting room, she comes to the inevitable conclusion that she has to find out if she carries the gene.

DNA Samples: Aunt Millie
Deborah
185delAG (DNA containing this mutation)
4184delTCAA (DNA containing this mutation)
5382insC (DNA containing this mutation)
Normal BRCA1 (no mutations)

Probes: 185delAG (AG deletion in exon 2)
Normal 185 (no mutation at this site)
4184delTCAA (TCAA deletion in exon 11)
Normal 4184
5382insC (C insertion in exon 13)
Normal 5382

Primers: Forward and reverse PCR primers for the BRCA1 gene

Questions

1. What is the chance that Deborah carries the same breast cancer gene as Aunt Millie?
2. What conclusions can you draw from the results of the DNA analysis?
3. How would you counsel Deborah based on the results of the test?
4. What are the implications for Deborah's mother if Deborah's test is positive?
5. Does Deborah's mother have a right not to know her status?

Case C: Cindy took the news very hard when her mother was diagnosed with breast cancer. The surgery and chemotherapy her mother has gone through have taken a significant toll on the whole family. Discovering that the breast cancer is related to the presence of a breast cancer susceptibility gene has only added to the concern. Cindy's sister, Ellen, decided to have genetic testing done to determine if she carried the gene, but Cindy chose not to go with her for testing. The news that Ellen tested positive was devastating to Cindy and it has been even more difficult to accept Ellen's subsequent decision to have a prophylactic double mastectomy. These events have caused Cindy to reevaluate her decision not to be tested; she almost feels an obligation to go through this experience for her sister. She schedules the appointment for testing, still undecided how she will react if the test is positive

DNA samples: Mother
Ellen
Cindy
185delAG (DNA containing this mutation)
4184delTCAA (DNA containing this mutation)
5382insC (DNA containing this mutation)
Normal BRCA1 (no mutations)

Probes:	185delAG (AG deletion in exon 2)
	Normal 185 (no mutation at this site)
	4184delTCAA (TCAA deletion in exon 11)
	Normal 4184
	5382insC (C insertion in exon 13)
	Normal 5382

Primers: Forward and reverse PCR primers for the BRCA1 gene

Questions

1. What is the chance that Cindy carries the same breast cancer gene as her mother?
2. Does Ellen's positive test affect that chance?
3. What conclusions can you draw from the results of the DNA analysis?
4. Was Ellen's decision to have a prophylactic double mastectomy appropriate?
5. How would you counsel Cindy based on the results of her test?

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2. Colon cancer

(Adapted from a case developed by by Anne M. Casper, Department of Molecular Genetics and Microbiology, Duke University. Copyright held by the National Center for Case Study Teaching in Science, University at Buffalo, State University of New York. Used with permission.)

Background: Colorectal cancer is one of the leading causes of cancer death in the United States. Each year, about 150,000 Americans are diagnosed with colorectal cancer, and more than 50,000 die from the disease. Colorectal cancer usually begins with a polyp, which can be detected through the use of several tests: double contrast barium enema, flexible sigmoidoscopy, colonoscopy, or CT colonography (virtual colonoscopy). Early detection and and removal of polyps can prevent the development of colon cancer.

About 5% of people who develop colorectal cancer have an inherited genetic susceptibility to the disease. The two most common inherited syndromes linked with colorectal cancers are familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). Inherited mutations in a tumor suppressor gene called adenomatous polyposis coli (APC) are responsible for familial adenomatous polyposis (FAP). The APC gene regulates the formation of polyps in the colon.

Case A: Sam called his younger sister Jane to tell her he had just been diagnosed with colon cancer. Although Sam was only 40, he had suffered with diarrhea and found bright red blood in his stool, so his physician recommended a colonoscopy. Because their father had died at the age of 45 from colon cancer, Sam's doctor recommended that he get additional genetic testing to see if the cause was hereditary. Sam encouraged Jane who was 35 to get a colonoscopy and planned to also call the twins, Mark and Caroline, age 33, to encourage them to get screened for colon cancer as well. Sam also recommended that if he had the mutated APC gene that his siblings also undergo genetic testing.

Proteins from Sam's tumor tissue were analyzed by Western blot, using antibodies specific for the APC protein. What do the results of this test indicate about the nature of his mutation?

Protein samples:	Sam's tumor tissue
	Normal colon tissue
Antibody:	APC

To determine the genetic status of each family member, DNA is isolated from a blood sample, and a DNA dot blot is performed using probes corresponding to the mutated and normal sequence in the APC gene.

DNA samples:	control normal APC
	control mutated APC
	Sam
	Jane
	Mark
	Caroline

Probes: normal APC
mutated APC

Bioinformatics: PCR is used to amplify cDNA containing the protein coding region of the APC gene from tumor cell RNA. Use sequence alignment to compare Sam's APC sequence to the normal gene sequence. Are there any difference? If so, where? In the MEGA sequence alignment window, change the nucleotide sequence to the amino acid sequence by clicking on the "Translated protein sequence" tab. How does Sam's translated protein sequence compare to the normal sequence?

Questions:

1. What conclusions can you draw from the results of the DNA analysis?
2. What do you notice when you compare Sam’s APC protein translation to the normal APC protein?
3. How would you counsel Jane and Sam based on the results of the test? What would you advise Mark and Caroline?
4. If someone is predisposed to getting cancer, does that mean that he or she will definitely get cancer someday?
5. In a small number of patients whose families appear to have all the classical characteristics of FAP, a mutation cannot be found in the APC cDNA. What are two possible reasons for why mutations may not be found in some patients whose families appear to have FAP?
6. How might environmental factors such as diet influence the development of colon cancer?
7. Are there risks associated with screening such as colonoscopy? How do these risks compare with the risk of contracting colon cancer?

C. Infectious Diseases

Infectious diseases are caused by bacteria, viruses, or other pathogenic agents. Diagnosis may involve detecting the presence of proteins or nucleic acid from the suspected pathogen using ELISA or PCR, respectively. In some cases, the patient's blood will be tested for the presence of antibodies specific for a pathogen, as an indication that the person was previously infected with that agent.

1. HIV/AIDS

Background: Human immunodeficiency virus (HIV) causes the disease Acquired Immunodeficiency Syndrome (AIDS). AIDS is characterized by the inability to mount an effective immune response to bacteria and other pathogens, resulting in a variety of life-threatening infections. The virus is spread when bodily fluids, such as blood and semen, from an infected person directly enter the bloodstream or tissue fluids of an uninfected person. For example, unprotected sexual intercourse and sharing needles during injected drug use can spread the virus. Once in the body, HIV infects and destroys certain white blood cells (called CD4 cells) and impairs the immune system. It may take years after the initial HIV infection for the symptoms of AIDS to appear. HIV infection is routinely detected indirectly, via tests which measure whether a person's blood contains antibodies against HIV; if so, they must have been previously infected with the virus. PCR can also be used to directly measure the amount of HIV in a person's blood or lymph nodes. For additional information about HIV/AIDS-related disease, detection methods, treatment, and prevention, visit the CDC's web site, www.cdc.gov/hiv .

These case scenarios are based on real people infected with HIV. Each case includes a video, accessed from the Case It web site, showing that person discussing their experience with the disease. As you study the cases, here are some general questions you might consider:

- How did this person become infected with HIV?
- Have others also been infected? Who else should be tested?
- How reliable are the tests?
- How often should someone be tested?
- Why do people engage in risky behaviors?
- What impact is the infection having on this person's life?
- How can this person prevent further transmission of the virus?
- What other ethical decisions does this person face?
- How common is this case?
- Are there cultural differences regarding attitudes about HIV and prevention?

Video introduction to HIV (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Analyzing an HIV/AIDS case using the Case It! simulation software includes the following components. For details about how to use the software, refer to the tutorials linked from the Case It! Home page (left panel) and also the Help window in the software.

1. Run an **ELISA** to test blood samples for the presence of antibodies to HIV. The ELISA involves adding antibody samples to wells of a 96-well plate that have been coated with HIV proteins. Samples produce color in the wells if they contain antibodies that can bind to the HIV proteins. An ELISA test is generally considered positive if the color production (absorbance) for a sample is at least twice that of the negative control. Choose the "use antibodies as samples" option when setting up the ELISA.

2. Since the ELISA has a 1/1000 rate of false positive results, positive ELISA results are confirmed by **Western blot**. For the Western blot, HIV proteins are separated by size using polyacrylamide gel electrophoresis. Then blood samples are tested to see whether they bind specific proteins in the gel. The HIV proteins visible in the gel (from largest to smallest, running left to right), include gp160*, gp120, p55, p41, p31 and p24. The positive control antibody will bind to all of the proteins. To be considered HIV positive, a sample must bind to two of these three proteins: gp160/gp120*, gp41, and p24. Any other binding pattern is considered "indeterminate". A result can only be called negative if there is no binding to any of the HIV proteins. (*gp160 is a precursor that includes both gp120 and gp41, before they are cleaved into separate envelope proteins; antibodies that bind gp120 are likely to also bind gp160.)

   Use the ELISA and Western blot results to determine the HIV status of each person in the case. You should be able to explain the results and make recommendations for treatments and for preventing further spread of the virus.

3. The **viral load** test detects virus genetic material in blood or lymph node samples, using a PCR method performed in a 96-well plate. This test is used to monitor the progress of the disease and to determine the effectiveness of drug treatments. The DNA samples represent RNA isolated from blood that has been copied to DNA using reverse transcriptase. PCR primers specific for HIV sequences will amplify HIV DNA and produce fluorescence. The amount of fluorescence correlates with the number of copies of HIV present in the original sample. The data are recorded as “viral load values”, i.e. how many copies of the virus were detected. To run the viral load test, Click the Method button on the 96-well plate and choose PCR (or use the auto-loading feature on the "open & processed" window).

4. The **bioinformatics** extension for each case is a new feature of version 6.0. Case It! software has been integrated with MEGA 4 software, which performs multiple sequence alignments and builds phylogenetic trees. Each case includes a scenario where building a tree provides additional information that addresses questions about the case. The DNA samples are complete HIV genomes, and PCR must be performed to amplify a portion of the *Env* gene to use for alignment and tree building. **Important note**: use the DNA sequences provided in the **Bioinformatics folder** for each case, rather than the sequences in the Viral load folder.

U.S. HIV cases

Case A. **Anna** is a 27-year-old woman from Guatemala, who is living with her boyfriend and is pregnant with her first child. A blood test during her second trimester revealed that she was HIV positive. Anna is surprised, because her first trimester test was negative, and she did not have sexual contact with anyone other than her boyfriend. She is very concerned about the fate of her child, who may contract the virus from Anna.

Video version of Anna case (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
- Anna, first trimester
- Anna, second trimester
- Anna's baby, 6 weeks after birth
- Anna's boyfriend
- Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Anna?
2. How would explain the difference between Anna's first and second trimester results?
3. What recommendations would you give Anna as she cares for herself and her baby
4. How did Anna get infected? How did her boyfriend get infected?
5. Why did Anna think it was OK to have unprotected sex with her boyfriend?
6. What does an indeterminate test mean for the baby? Should the baby be tested again?

Viral load: After her baby is born, Anna begins taking antiretroviral medications. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: Did Anna’s boyfriend infect her with HIV? Compare Anna’s and her boyfriend’s HIV sequence with local controls - does tree suggest that their sequences are more similar than random samples? Can you tell from this who gave it to whom? Anna’s boyfriend acknowledges that he had another partner. Does adding this sequence help you determine the sources of Anna’s infection?

Case B. **Katrice** grew up in rural Alabama, where there was not much discussion about HIV and AIDS. At seventeen, she had sex with a popular boy, who she later learned was very promiscuous and a drug user. She tested HIV positive during a routine blood test several months later. Katrice went untreated for four years, living in denial about her HIV infection. She became involved with another man and had a daughter. She finally sought medical treatment when she thought her daughter might have been exposed to the virus.

Video version of Katrice case Part 1 and Part 2 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Katrice
Latranya's father
Latranya, 3 months old
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Katrice?
2. What recommendations would you give Katrice as she cares for herself and her baby?
3. How did Katrice get infected?
4. Why did Katrice wait so long to get treatment?
5. Should Katrice have gotten pregnant? There is no indication that she sought treatment for HIV while pregnant; what was the risk for her baby?
6. Should Latranya be tested again?

Viral load: On the advice of her physician, Katrice begins taking antiretroviral medications. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: HIV has been spreading rapidly in the southern U.S. How does the rate of HIV mutation here compare to the viruses spreading in other parts of the world? Compare several virus sequences from southern Alabama to sequences isolated in Zimbabwe and Thailand. Does there appear to be a difference in the rate of mutation, based on the variation between these viruses? What factors could contribute to different mutation rates?

Case C. **Laverne** is a 31-year-old African-American woman who is pregnant and HIV positive. When she found out she was pregnant, she and her partner, Henry, decided that they could not terminate the pregnancy. She already has named her baby Marcus, and she is trying to do everything possible to prevent him from becoming infected with the virus. She is taking medications and eating healthy foods. She will undergo a Caesarian section, and Marcus will take medications for his first six weeks until he is tested for HIV.

Video version of Laverne case (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Laverne
Henry
Marcus, 6 weeks old
Marcus, 3 months old
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Laverne?
2. What recommendations would you give Laverne as she cares for herself and her baby?
3. How did Laverne get infected?
4. How did Henry get infected?
5. Should Laverne have gotten pregnant? What she right to keep the baby rather than terminate the pregnancy? What was the risk to Marcus?
6. Why did Marcus test positive and then negative?
7. Should Marcus be tested again?

Viral load: After her baby is born, Laverne continues taking antiretroviral medications. A viral load test is performed one, three, and six

months after later. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: Although Laverne has been diligent about taking her the drugs and they seemed to be working to reduce her load, her most recent tests show that her viral load is on the rise. Some strains of HIV isolated in that part of the U.S. have been shown to develop unusually high resistance to the drug treatments. Compare Laverne's HIV sequence to several isolated in the region, some of which have shown resistance. Does she appear to be infected with a drug-resistant virus? What other options does she have for treatment?

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Case D. **Doug** grew up in southern California, an all-American boy who surfed and played volleyball. When he was 20 years old and a junior in college, he revealed that he was gay and moved to San Francisco. He had a hard time adjusting, and he felt that, in order to fit in, he had to engage in the same risky behaviors as everyone else. Even though he knew the risks, he had unprotected sex. Four days before his 24th birthday, he tested positive for HIV.

Video version of Doug case Part 1 and Part 2 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Doug
Doug's partner the night he believes he was infected (partner 1)
An earlier partner of Doug's (partner 2)
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Doug?
2. What recommendations would you give Doug?
3. How did Doug get infected?
4. Can you tell from the results which partner infected Doug?
5. Why was Doug willing to take risks that affected his health?

Viral load: Doug and his partner begin antiretroviral drug treatments. A viral load test is performed one, three, and six months after they begin this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatments?

Bioinformatics: Which of Doug's partners infected him with HIV? Compare the three sequences to each other and to random controls in the San Francisco area. Can you tell from these data who infected whom? What additional information would you need to make a more definitive determination?

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Case E. **Lisa** grew up in a wealthy neighborhood, and the kids she grew up with didn't think they needed to worry about HIV and AIDS. However, she believes she was infected with while on an island vacation during her college years. Her father, a physician, helped her find the best medical care, and she immediately began taking medications which seemed to keep her healthy. A few years later, she married David and wanted to start a family. They decided to have unprotected sex during times when her viral load was low. She continued to take medications during her pregnancy, and had C-section deliveries to reduce the risk of passing the virus to her baby. Lisa and David now have three children.

Video version of Lisa case Part 1 and Part 2 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Lisa
David
3-year old child
6 1/2-year old child
9-year old child
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Lisa?

2. What recommendations would you give Lisa as she cares for herself and her family?
3. How did Lisa get infected?
4. How do you think David and Lisa felt when he received his ELISA result?
5. Is it worth the risk to have unprotected sex in order to have children?
6. What does the indeterminate result mean for their 9-year old child?
7. Do the negative results for the other children mean that they don't ever have to worry about becoming HIV positive?

Viral load: In order to become pregnant, David and Lisa chose to have sexual intercourse during times when her viral load was low. Analyze the DNA samples provided by PCR. Based on the PCR results, when do you think they should have tried to conceive?

Bioinformatics: The boy from whom Lisa thinks she contracted was from New York City, where they had an outbreak of an especially pathogenic HIV strain. He quickly progressed to AIDS and died shortly after Lisa found out she was infected. Does it appear that Lisa was also infected with a similar strain? Compare her sequence to the boy's and to other high- and low-pathogenic strains. How does her access to medical affect her situation? What are the implications for her family?

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Case F. Jennifer is a white female who grew up, in her words, "with old-fashioned parents and old-fashioned values." She got good grades throughout high school and did not date until her late teens. But after she graduated from high school and went away to college, she was eager to change her lifestyle. She started to party a lot and dated several different people. However, after a drunken sexual encounter with a man she met at a party that left them both feeling horrible the next morning, she decided to take better care of herself and stopped having sex. Several months later she underwent a series of routing medical tests, including a blood test for HIV. The HIV test came back positive. Shocked, Jennifer decided to be tested again at a different clinic. She contacted two of the boys with whom she had sexual contact (including Jeff, the boy from the party), and suggested they also get tested.

Video version of Jennifer case Part 1 and Part 2 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Jennifer
Jeff
Paul
Positive and negative controls

Questions:

1. What is the HIV status of each person tested?
2. Do the results provide any information about how Jennifer may have been infected?
3. What recommendations would you give to Jennifer as she deals with her HIV diagnosis?
4. How do you think Jennifer's story would influence other college-age students?

Viral load: Jennifer begins antiretroviral drug treatments. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatments?

Bioinformatics: Jennifer's HIV infection was detected early, and since she started drug treatments right away her virus levels have stayed low. However, her physician is concerned by recent reports that people who start drug treatment early are more prone to develop drug-resistant viruses. Compare viruses isolate early in her infection to viruses isolated four years later. An increase in virus diversity may make drug-resistant mutations more likely. Can you tell from these results whether Jennifer is more at risk for drug resistance now?

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Case G. In the early 1990's **Steve** was an avid long distance runner and the picture of health. But he suddenly started getting a lot of colds and unusual infections. Eventually a blood test determined that he was HIV positive. By the time he was tested he was already exhibiting full-blown AIDS and his prognosis was poor. He started antiretroviral drug treatments, but they did not seem to help. When a new class of drugs, protease inhibitors, was approved, Steve changed his medications and immediately began to show improvement. Amazingly, his immune system seemed to return to normal and he regained much of the weight he lost. He began running again and finished a marathon. Two of his running partners were inspired by his situation and decided to get tested. They both had reason to believe they have been exposed to HIV, but were reluctant to get tested because they were afraid that a positive test would be a "death sentence".

Video version of Steve case Part 1 and Part 2 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Steve
Runner 1
Runner 2
Positive and negative controls

Questions:

1. What is the HIV status of each person tested?
2. What recommendations would you give each of them based on their results?

Viral load: Viral load tests were run three, six, and nine months after Steve started each of his drug treatments. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatments?

Bioinformatics: The drug treatment that seems to be effective for Steve has shown a tendency to develop resistant strains of the virus. Steve is concerned, since he experienced problems with his original drug regimen and does not want the treatments to fail again. Compare viruses isolated from Steve with strains that have shown resistance to these drugs. What can you tell Steve about the chance that he will develop drug resistance?

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Case H. Marie thought she had found her perfect match when she started dating Rick, a divorced man who seemed to live a healthy lifestyle and treated her well. They traveled together frequently and became very close. But suddenly, Rick's behavior changed and he became unpredictable, His mood swings eventually caused them to split up. Several months later, Marie learned through a mutual friend that Rick was dying of AIDS. Marie was shocked, and she immediately got tested for HIV. She also began a program of exercise and nutritional supplements to try to improve her chances of staying healthy.

Video version of Marie case Part 1, Part 2 and Part 3 (from "AIDS: A Changing Epidemic", copyright 2002 Discovery Education - used with permission)

Blood samples for ELISA and Western blot:
Marie
Rick
Positive and negative controls

Questions:

1. What is the HIV status of each person tested?
2. What recommendations would you give to Marie as she deals with her HIV diagnosis?
3. What role do you think Marie's exercise and nutrition program will play in her health?
4. What would happen if Marie stopped taking the antiviral drugs

Viral load: Marie begins antiretroviral drug treatments. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatments?

Bioinformatics: Although the drug treatments have lowered Marie's viral load, the side effects of the drugs are greatly reducing her quality of life. Her most recent liver function test show that her current drug regimen is damaging her liver, in spite of her efforts to stay healthy. Her physician recommends an new, experimental drug that blocks virus entry into cells. This drug has only been tested on certain strains of HIV. Compare Marie's sequence to strains that have been shown to respond to this drug treatment. Would you recommend that she switch to the new drug treatment?

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African HIV cases

Video introduction to African cases (from "The Faces of AIDS", Media for Development International - used with permission)

Case I. Nicole is a 25-year old woman living in Cameroon. She has been diagnosed with AIDS, and is staying in a hospital because she is unable to care for herself. Her father has refused to help her because he believes she contracted the disease by "sleeping around". Her father also forbids her brothers and sisters from seeing her. She believes she may have been infected by a boy she was seeing for a while,

but she has not heard from him in months.

Video version of Nicole case (from "The Faces of AIDS", Media for Development International - used with permission)

Blood samples for ELISA and Western blot:
Nicole
Nicole's former boyfriend
Positive and negative controls

Questions:

1. What is the status of each person tested?
2. How would you explain these results to Nicole?
3. What options does Nicole have?
4. What do you think about Nicole's father's decision not to help her?
5. How important is it to get medications to people like Nicole?
6. Why did Nicole's father abandon her?
7. What resources are available for someone in Nicole's situation
8. How did Nicole get infected?

Viral load: Because her father will not support her financially, she is given only supportive care at the hospital. As part of a UNAIDS study, blood samples are taken every few months and sent to a hospital in the capital city, Yaounde, so a viral load test can be performed. Based on the results of testing Nicole's samples 3, 6, and 12 months after her diagnosis, what is her prognosis?

Bioinformatics: Nicole's disease is progressing rapidly, and the physicians in Yaounde suspect that she may be infected with a highly pathogenic strain of the virus that has been detected in other patients tested at the hospital. Compare Nicole's virus sequence to highly pathogenic and low-pathogenic strains. Can you tell from these results if she was infected with a highly-pathogenic strain? Does it matter in Nicole's situation? What should the scientists involved in the study do with this information?

Case J. Auxilia, who has just been diagnosed with AIDS, lives with her five children on a small plot of land in Zimbabwe. Her husband died several years ago. Auxilia is worried about who will look after her children if she dies. She knows that there is a lot of stigma associated with AIDS and that people are afraid to interact with her.

Video version of Auxilia case (from "The Faces of AIDS", Media for Development International - used with permission

Blood samples for ELISA and Western blot:
Auxilia
Auxilia's oldest child
Auxilia's youngest child
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Auxilia?
2. What recommendations would you give Auxilia as she cares for herself and her baby?
3. What can Auxilia tell people to help them understand that they should not be afraid of her?
4. How did Auxilia get infected?
5. How can she get help for her children if she gets sicker?
6. What treatment options does she have?
7. What does an indeterminate result mean?

Viral load: Auxilia is selected to enroll in a program that allows her to receive antiretroviral medications. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: Zimbabwe is experiencing an increase in the spread of HIV. How does the rate of HIV mutation here compare to the viruses spreading in other parts of the world? Compare several virus sequences from Zimbabwe to sequences isolated in the U.S. and Thailand. Do you see a difference in the rate of mutation? What factors could contribute to different mutation rates?

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Case K. Marie lives on a small plot of land in Cameroon with her five children. Her husband died two years ago, and Marie has just been diagnosed with AIDS. Before she was diagnosed, she did not believe that the disease existed in her country. Fortunately, Marie's brother is very supportive and willing to look after her and her children. He is not afraid of catching the disease from her.

Video version of Marie (African) case (from "The Faces of AIDS", Media for Development International - used with permission

Blood samples for ELISA and Western blot:

Marie
Marie's brother
Marie's husband
Marie's oldest child
Marie's youngest child
Positive and negative controls

Questions:

1. What is the status of each person tested?
2. How would you explain these results to Marie?
3. What recommendations would you give Marie as she cares for herself and her baby?
4. What precautions does her brother need to take to keep from getting infected?
5. How did Marie get infected?
6. How did Marie's beliefs about AIDS not being in Cameroon affect her risk of being infected?
7. What precautions does her brother need to take to keep from being infected?
8. What resources are available to help Marie care for her children?
9. Are Marie's children at risk for HIV?

Viral load: Marie is selected to enroll in a program that allows her to receive antiretroviral medications. A viral load test is performed one, three, and six months after she begins this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: Some strains of HIV isolated in that part of the Cameroon have been shown to develop unusually high resistance to the drug treatments. Compare Marie's HIV sequence to several isolated in the region, some of which have shown resistance. Does she appear to be infected with a drug-resistant virus? What other options does she have for treatment?

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Case L. Tendayi and her husband, **Farayi**, a married couple in Zimbabwe, learned that they were both HIV positive when their baby died two years ago. They are supporting each other and planning to stay together; neither blames the other for what happened. They are focused on finding a way to live with AIDS and to educate others about it.

Video version of Tendayi case (from "The Faces of AIDS", Media for Development International - used with permission

Blood samples for ELISA and Western blot:

Tendayi
Farayi
Their 3-year old child
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Tendayi?
2. What recommendations would you give Tendayi and Farayi as they care for themselves and their remaining child?
3. What can they do to help others understand the disease?
4. What would happen to them if the medications were no longer available?

Viral load: Tendayi and Farayi are selected to enroll in a program that allows them to receive antiretroviral medications. A viral load test is performed one and six months after they begin this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: How did Tendayi and Farayi become infected? Both have acknowledge having other sexual partners. Compare each of the virus sequences to viruses isolated from their known partners and from random local controls. Can you determine how they became infected from these data? Should this information be share with Tendayi and Farayi, even though they have said that they do not want to swell on the past?

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Case M. **Safari** grew up in a rural village in Kenya and owns a small plot of land. After he marries, he decides that the land won't support his family, so he goes to the city to find work. Eventually, he finds a job and is able to send money home to his wife. He is only able to visit his home once in a while, and he spends most of his time in the city. Lonely, he turns to other women for companionship. His wife, meanwhile, is left to take care of the house and the land by herself. She becomes pregnant and gives birth to a child, and Safari continues to work in the city. Safari becomes chronically sick and starts to miss a work frequently. Safari's doctor eventually tests him for HIV, and he is positive. Are his wife and child also infected?

Video version of Safari case Part 1, Part 2, Part 3 and Part 4 (from "AIDS - Life at Stake", Media for Development International - used with permission)

Blood samples for ELISA and Western blot:

Safari
Safari's wife
Their baby
Positive and negative controls

Questions:

1. What is the status of each person tested? How would you explain these results to Safari and to his wife?
2. What recommendations would you give to the couple as they care for Safari?
3. How did Safari get infected?
4. What does an indeterminate result mean for his wife?
5. How common is this story?
6. If Safari had been aware of the risks of HIV infection, would his behavior been different?
7. Is their baby at risk for HIV infection

Viral load: Safari is selected to enroll in a program that allows him to receive antiretroviral medications. A viral load test is performed one and six months after they begin this drug treatment. After running the PCR analysis on these samples, what would you conclude about the effectiveness of the treatment?

Bioinformatics: Although the drug treatments have lowered Safari’s viral load, the side effects of the drugs are greatly reducing his quality of life and he has still been unable to return to work. His physician recommends an new, experimental drug that blocks virus entry into cells. The clinical trial will include African as well as U.S. subjects. This drug has only been tested on certain strains of HIV. Compare Safari’s sequence to strains that have been shown to respond to this drug treatment. Would you recommend that he switch to the new drug treatment?

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2. Human Influenza

Background: Influenza virus causes respiratory infections ("the flu") that can be quite severe. About 36,000 people die from flu complications every year in the U.S. The symptoms of the flu include fever, headache, sore throat, cough, and muscle aches. These symptoms are difficult to distinguish from other respiratory infections. A more definitive diagnosis can be made by testing respiratory fluids, often obtained via a throat or nasopharyngeal swab, for the presence of influenza proteins (using ELISA or similar techniques) or nucleic acid (using RT-PCR) (Since the virus genetic material is RNA, reverse transcription (RT) is used to copy the RNA into cDNA for the PCR test). Since it takes a few weeks for antibodies to influenza to be detected in a patient's blood, testing the patient's blood for influenza antibodies is usually used to confirm an infection after the illness has subsided, e.g. to monitor the extent of an epidemic. Influenza virus is highly contagious and is easily spread via respiratory droplets. There are drugs available to treat influenza virus infections, but they are generally only used when an infected person is at risk for serious complications, or to control an epidemic. The influenza vaccine ("flu shot") can protect individuals from getting infected, and it is highly recommended for individuals most at risk for the serious complications from flu, such as people older than 65, children under 2 years old, and anyone with chronic heart or lung conditions. The vaccine usually contains three different strains of influenza, and the antibodies generated to the vaccine can protect against these strains and related strains. Influenza virus has a high mutation rate and there are new strains of the virus appearing every year. There are two main strains of influenza, A and B, that cause the annual outbreaks of flu. Strain A viruses are further distinguished based on their surface proteins, abbreviated H and N. The strain names also include the location and year where they were isolate. For example, strain A/Fujian/02 (H3N2) was isolated in the

Fujian province in China in 2002 and its surface proteins are designated H3N2.

Note: An ELISA test is considered positive if the color production (absorbance) for a sample is at least twice the negative control value. PCR results are recorded as viral load values, i.e. how many copies of the virus were detected. Use the 96-well PCR feature to analyze these cases.

Case A. This fall, for the first time in several years, Sheila did not get a flu shot. She has been very busy, especially since she started babysitting her grandchildren (ages 1 and 3) on weekdays. She also does not like needles and shots, so it was easy for her to come up with excuses not to go get the shot. Sheila is 67 years old, but she has been in good health and does not have any chronic health conditions. Two days ago, she came down with a fever (102 degrees F), sore throat, and a bad cough. She has been taking ibuprofen, but it does not seem to be helping. Sheila feels just awful, but she drags herself to the clinic. The physician is concerned that the fever has not subsided, and because Sheila's age places her at some risk for serious complications from influenza, she decides to test Sheila for influenza and takes a throat swab sample. Although Sheila's grandchildren have not been with her the past two days, they did stay at her house the day before she got sick. The physician suggests that both children be tested, even though they have not yet shown any symptoms.

To analyze this case, first run an ELISA on the throat swab samples from the following sources to test for the presence of influenza virus proteins, using antibodies specific for influenza A and B viruses. Optionally, you can perform a Western blot on each sample to confirm the ELISA results. Then perform a PCR test on the cDNA isolated from the swabs, using primers specific for influenza A and B, to see if influenza virus genetic material can be detected. Use the 96-well PCR format for this test.

Protein and DNA samples:	Negative control (no influenza virus)
	Positive control for influenza A
	Positive control for influenza B
	Sheila
	1-year old
	3-year old

Questions:

1. Do Sheila's symptoms appear to be caused by infection with influenza virus?
2. Are either of the grandchildren infected?
3. What should the physician recommend for treatment for these patients?
4. What should Sheila do to avoid spreading her illness to others?

Bioinformatics: Influenza B viruses generally respond well to the antiviral drugs oseltamivir (Tamiflu) and zanamivir (Relenza), which inhibit neuraminidase activity. However, influenza B viruses have been isolated this season that show some resistance to oseltamivir. Compare the neuraminidase sequence isolated from Sheila's virus to these resistant virus isolates as well as to some drug-sensitive viruses. Does it look like oseltamivir will be an effective treatment for Sheila? What other options does she have? **[Note**: Use the sequences in the Bioinformatics folder, rather than the PCR folder, for this analysis.]

Case B. Shannon, a college junior, was really looking forward to playing in her first big basketball tournament. The whole team had flow to Hawaii for one week during the semester break to play against teams from all over the country. Unfortunately, Shannon's team was not in top form. Three team members had to stay home due to illness. They had headaches, fever, and muscle aches that prevented them from getting out of bed, let alone play basketball. Four other teammates had similar symptoms the week before but had recovered enough to join the team on the trip, although they were somewhat out of practice. On the morning of their first game, Shannon woke up in the hotel room she was sharing with three other teammates feeling terrible. She was distressed to realize she was experiencing the same symptoms as the sick team members, and she did not want to tell her coach because she did not want to miss the game. But soon after getting up she knew she was too sick to play and reluctantly told her coach. The coach was anxious to find out what was sweeping through her team, so she brought Shannon to a clinic in Honolulu. The physician there took a throat swab and tested for influenza virus. She also recommended that the other team members rooming with Shannon be tested. In addition, she tested blood from the team members who had recovered from a similar illness, to see if they had antibodies against the same virus.

To analyze this case, run an ELISA on the throat swab samples from the following sources to test for the presence of influenza virus proteins, using antibodies specific for influenza A and B viruses. Then perform a PCR test on the cDNA isolated from the swabs, using primers specific for influenza A and B, to see if influenza virus genetic material can be detected. Use the 96-well PCR format for this test.

Protein and DNA samples:	Negative control (no influenza virus)
	Positive control for influenza A
	Positive control for influenza B
	Shannon

Roommate 1
Roommate 2
Roommate 3

Test the blood samples from the four players who recovered from the illness using an ELISA to see if they have antibodies to influenza A or B proteins.

Antibody samples:	Negative control
	Positive control for Influenza A
	Positive control for Influenza B
	Recovered roommate 1
	Recovered roommate 1
	Recovered roommate 1
	Recovered roommate 1

Questions:

1. Is Shannon infected with Influenza virus?
2. What are Shannon's prospects for playing in any of the games this week?
3. What should the physician recommend for Shannon?
4. Are any of her roommates infected?
5. Does is appear that the recovered players had the same virus infection?
6. What should be done to prevent the rest of the team from getting sick?

Bioinformatics: A novel variant of H1N1 influenza, with a possible origin in swine, has been reported in Mexico and part of the U.S. So far, it has not been detected in Hawaii. But since the antibody testing does not determine which type A influenza Shannon and her roommates have contracted, hemagglutinin sequences from their virus isolates are compared to the novel H1N1 virus, as well as to seasonal H1N1 viruses that have been circulating in Honolulu the last two years. Do Shannon and her roommate appear to be infected with seasonal flu or with the novel swine flu? Does it matter in terms of their treatment? **[Note**: Use the sequences in the Bioinformatics folder, rather than the PCR folder, for this analysis.]

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3. Avian influenza

Background: Certain strains of influenza virus preferentially infect birds rather than humans. These viruses occur naturally in wild bird populations, particularly waterfowl, and can be spread to domestic birds. Like human influenza, avian influenza is highly contagious. The virus is shed in the saliva, nasal secretions, and feces of infected birds. Wild birds generally do not get sick from the infection, but some forms are highly pathogenic for domestic poultry and can be over 90% fatal. In 1997, one type of avian influenza strain A with surface proteins designated H5N1 (see the description of human influenza virus above) was found to be able to infect humans who came into contact with infected birds. Humans infected with avian influenza have a high mortality rate, but the virus does not seem to be able to spread from person to person. If the virus mutates such that it can spread more easily between people, it has the potential to cause a global pandemic, because it is different enough from the human influenza viruses that few people will have developed immunity from previous exposures.

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Case C. An outbreak of avian influenza recently swept through several farms in a rural area outside Ho Chi Minh City, Viet Nam. The government has ordered that all farms in the area destroy their chickens to prevent the virus from spreading any further. World Health Organization (WHO) officials are collecting samples and testing them for influenza. The Vanh family's farm is located just outside this area and they are hoping that they do not have to destroy their flock. But a few of their chickens have recently died, so the WHO workers take samples for testing. Their daughter, age 8, has a respiratory infection that the family worries may be related to the avian flu. WHO officials arrange for her to be tested for influenza, and recommend that other family members be tested even though they are not showing symptoms. Throat swab samples are taken from the father, mother, and two children (the sick daughter and her brother).

To analyze this case, test the five samples of respiratory fluids from three of the dead chickens on the Vanh farm, and from the four family members, by ELISA for the presence of influenza H5N1 proteins. Then perform a PCR test on the cDNA isolated from the samples to see if influenza virus genetic material can be detected.

Questions:

1. Did the Vanh's chickens die from influenza infection?
2. Should the rest of their chickens be destroyed? What are the alternatives?

3. Is the Vanh's daughter infected with avian flu? What are the options for treating her illness?
4. Do any other family members appear to be infected?
5. What can be done to reduce the risk of avian influenza infection?

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Case D. Turkey was one of the first European countries to report avian flu H5N1 infections in domestic poultry. Dozens of birds have died from the infection, and thousands more have been destroyed in order to stopped the spread of the virus. Several people in the same regions have come down with serious respiratory infections, and two people have died. Two of the patients are sisters in a family whose chickens tested positive for H5N1 influenza. Local health official want to test the two girls to see if their illness is caused by influenza H5N1 infection. The also want to test the rest of the family members, so throat swabs samples are taken from the mother, father and brother in addition the two girls. The family is adamant that only the older sister had direct contact with sick chickens so they do not understand why both girls would be ill if this virus was causing the symptoms.

To analyze this case, run an ELISA to test the throat swab samples from each of the family members for the presence of influenza H5N1 proteins. Then test cDNA isolated from each sample for influenza genetic material using PCR.

Questions:

1. Are the two sisters infected with avian influenza?
2. Do any of the other family members appear to be infected?
3. What are the implications if the second sister was infected without having had direct contact with infected chickens? How else might she have become infected?
4. Is it possible to determine whether the two children were infected from the same source?
5. What is the risk to the other family members?
6. What can be done to reduce the further spread of the virus?

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4. Severe Acute Respiratory Syndrome (SARS)

Background: SARS is caused by infection with a coronavirus called SARS-associated coronavirus (SARS-CoV). The disease was first reported in Asia in 2003, and SARS-CoV was unrecognized prior to this outbreak. It infected over 8,000 people and killed over 700. There have been no new cases since April 2004. SARS-CoV is spread person-to-person via respiratory droplets produced when a person coughs or sneezes. Symptoms include high fever, headache, and body aches, with a rapid progression to pneumonia. Laboratory diagnosis is made using RT-PCR to detect the presence of SARS-CoV genetic material in respiratory samples, or the testing the blood for antibodies to SARS-CoV by ELISA. Antibodies may not be detectable until a week or longer after infection, so RT-PCR is preferred early in an infection. (Since the virus genetic material is RNA, reverse transcription (RT) is used to copy the RNA into cDNA for the PCR test). There is no specific treatment for SARS, nor is there a vaccine for SARS-CoV.

Note: An ELISA test is considered positive if the color production (absorbance) for a sample is at least twice the negative control value. PCR results are recorded as viral load values, i.e. how many copies of the virus were detected.

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Case A. Dr. Smith, a physician at a hospital in Hamilton, Ontario observed four cases of what appeared to be a viral pneumonia within the last week. The symptoms resemble those associated with SARS. The patients all had high fevers and difficulty breathing, and their x-rays showed signs of pneumonia. Bacterial cultures were negative. None of the patients reported traveling out of the country recently. The first patient who was admitted seems to be recovering, but the second patient has taken a turn for the worse, and may not last the night. Dr. Smith remembers very well the SARS outbreak in Toronto, just one hour north of Hamilton. Most of the more than 300 people infected were exposed to the virus while in a Toronto hospital, as a patient or visitor. Some of the casualties were health care workers who contracted the virus while treating patients. He and the rest of the clinical staff have been taking every possible precaution, and the patients are in an isolation ward in the hospital. Dr. Smith is anxiously awaiting the results of the laboratory tests.

To analyze this case, two types of tests are necessary. Run an ELISA test on blood samples to test for the presence of virus proteins. Also, run RT-PCR on the samples to try to detect the virus genetic material; DNA from these samples is used in the PCR test. Samples include:

Protein and DNA samples:	Positive control for SARS-CoV
	Negative control
	Patient 1 (57-year-old male)
	Patient 2 (51-year old female, wife of patient 1)
	Patient 3 (43-year-old female)
	Patient 4 (69-year old male)

Questions:

1. Are any of the patients infected with the SARS coronavirus?
2. If so, what should be done to treat them?
3. What are the risks of spreading the virus in a hospital setting?
4. Where/how might these patients have been exposed to the virus?
5. If any of the patients are negative for SARS CoV, what else could be causing their symptoms?
6. How risky is it for the health care workers who treat SARS patients?

Case B. Shi Jiao-hui has lived in the New York City almost all of this life. His parents moved there when he was two years old. He is a U.S. citizen and considers himself a New Yorker, but the rest of his extended family still lives in Guangdong province in China. Finally, at age 30, he and his wife, Ming, and their son and daughter traveled to China to visit his relatives and see his homeland. Their 3-week trip took them through Hong Kong and several cities in China, and they spent the last week with relatives near the city of Guangzhou. Unfortunately, Ming was very sick most of that week. She had a high fever and difficulty breathing. She was taken to a hospital in Guangzhou where she was treated for pneumonia. She recovered enough to fly back home and is doing fine now. However, Jiao felt ill soon after returning home and now has the same symptoms as Ming. When he goes to the clinic the physician is alarmed by the description of the symptoms and the fact that the family had recently visited the area where SARS was first reported. He recommends that Jiao be tested for SARS coronavirus infection and collects a throat swab. He also recommends that the two children be brought in for testing. In the meantime, Jiao is hospitalized and placed in an isolation ward.

To analyze this case, run an ELISA on the proteins in throat swab samples from Jiao and the two children, as well as a PCR test on cDNA isolated from the sample. Also, test Ming's blood for antibodies to SARS-CoV proteins.

Protein and DNA samples:	Positive control for SARS-CoV
	Negative control
	Jiao
	Son
	daughter
Antibody sample:	Ming

Questions:

1. Is Jiao infected with SARS-CoV?
2. Do either of the children appear to be infected?
3. What should the recommended treatment be?
4. Was the physician right to place Jiao in isolation?
5. Was Ming infected with SARS-CoV while in China? If so, why didn't the doctors there tell her?
6. What should be done to minimize the risk that Jiao will infect anyone else?

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5. West Nile virus

Background: West Nile virus (WNV) was first reported in the U.S. in 1997. It is spread by mosquitoes that bite an infected animal (usually a bird), and then bite another animal, transmitting the virus. In addition to birds, the virus can be spread to humans and other mammals including dogs, cats, and horses. It can also be transmitted via blood transfusion or organ transplant from an infected person. Many humans experience no symptoms, but about 20% will contract "West Nile fever", with fever, headache, body aches, nausea, and rash that can last for weeks. In a few cases (less than1%), the illness will become more serious, leading to permanent neurological effects such as muscle weakness, vision loss, and coma. There is no specific treatment for WNV illness. Diagnosis of WNV infection is accomplished by detecting virus proteins (by ELISA) or genetic material (by PCR) in blood or cerebrospinal fluid samples. (Since the virus genetic material is RNA, reverse transcription (RT) is used to copy the RNA into cDNA for the PCR test). Antibodies to WNV can also be detected in the patient's blood by ELISA, but these may not be detectable until later in the infection process.

Note: An ELISA test is considered positive if the color production (absorbance) for a sample is at least twice the negative control value. PCR results are recorded as viral load values, i.e. how many copies of the virus were detected.

Case A. The annual blood drive in Mitchell, SD is usually held during September each year. However, this year there was an outbreak of West Nile infections in mid-August. No one died, but at least 30 people were diagnosed with West Nile fever, and five cases were severe

enough to require hospitalization. It is likely that many more people were infected with WNV but did not have symptoms, so all of the donated blood will be screened for antibodies to WNV. Any samples testing positive for antibodies will be tested for WNV genetic material by PCR. The presence of WNV cDNA would indicate an active infection, otherwise the individual has probably recovered from the infection.

To analyze this case, run an ELISA on the set of 10 donated blood samples provided, testing them for antibodies to WNV proteins. If any of the samples test positive for antibodies, test those samples for WNV genetic material by PCR.

Questions:

1. How many blood samples, if any, tested positive for WNV antibodies?
2. Did any of the samples test positive for WNV genetic material?
3. What is the significance of finding WNV antibodies or genetic material in blood?
4. Is the number of positives what you would expect, based on the number of cases of West Nile fever?
5. Should the individuals who donated the blood that tested positive be notified?
6. What can be done to reduce the number of WNV infections next season?

Case B. Rachel has lived in Anaheim, CA, all of her life, and had never noticed anything like this. For the past several weeks she had been finding dead birds, mostly crows and a few sparrows, in her yard. She has also seen them in the neighbor's yards when she walked her dog. She estimates that she has seen at least 35 dead birds. Rachel finally contacts the California Department of Health, which collects some of the birds for West Nile virus testing. Lab technicians take samples from brain tissue and test them for the presence of WNV proteins by ELISA. Although no humans have reported symptoms of West Nile disease, health officials decide to test individuals for the presence of antibodies to WNV in their blood, to determine the extent of human infections. Rachel, her husband, and two children are tested.

To analyze this case, run an ELISA on the six samples of brain tissue from dead birds (four crows and two sparrows) for the presence of WNV protein. Also, test the blood samples from Rachel's family members for antibodies to WNV proteins.

Questions

1. Did the birds die from WNV infection?
2. Were any members of Rachel's family infected with WNV?
3. Are there any consequences if someone is infected with WNV but exhibits no symptoms?
4. Should more people in the area be tested?
5. What can the residents do to protect themselves from WNV infection?
6. What impact will that many dead birds have on the local crow population?
7. What is the potential environmental impact reducing crow numbers?

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6. Ebola

Background: Ebola virus causes a hemorrhagic fever that is often fatal. The disease was first reported in 1976 in the Democratic Republic of the Congo (formerly Zaire), and outbreaks have appeared sporadically since then. The disease progresses quickly upon infection, usually in 2-10 days. The symptoms begin with fever, headache, and muscle aches, followed by diarrhea and vomiting. In severe cases, internal and external bleeding occur. The virus is spread from person to person via contact with infected blood or secretions. The source of the initial infection in an outbreak is not known, but it is hypothesized to be transmitted from an infected animal. The infection can be diagnosed within a few days of the onset of symptoms, using an ELISA to test for Ebola virus proteins or RT-PCR to test for virus genetic material. (Since the virus genetic material is RNA, reverse transcriptase (RT) is used to copy the RNA into cDNA for the PCR test). After recovery, patients can be tested for antibodies to Ebola virus proteins in their blood to confirm infection. There is no treatment for Ebola hemorrhagic fever other than supportive care. The death rate from infection is typically 70-80%. There is no vaccine available for Ebola virus.

Note: An ELISA test is considered positive if the color production (absorbance) for a sample is at least twice the negative control value. PCR results are recorded as viral load values, i.e. how many copies of the virus were detected.

Case A. An outbreak of a disease that resembles Ebola hemorrhagic fever has been reported in a village outside of Kinshasa, the capital of the Democratic Republic of Congo. Local health officials are concerned about the outbreak spreading to such a large population. Health workers are sent to the village to determine whether the disease is caused by Ebola virus. Most of the victims so far belong to one family. Blood samples are collected from the family members showing symptoms of hemorrhagic fever and tested for Ebola virus proteins and genetic material.

To analyze this case, run an ELISA on the proteins in throat blood samples from the various family members, as well as a PCR test on cDNA isolated from the samples.

Protein and DNA samples: Negative control
Positive control for Ebola
Mother
Father
Son
Daughter

Questions:

1. Are these family members infected with Ebola virus?
2. What should be done for these individuals if they are infected?
3. What precautions should be taken when handling these blood samples?
4. What should be done to prevent the virus from spreading to the city of Kinshasa?

Case B. One of the animals suspected of being a reservoir for Ebola virus is the fruit bat. After an outbreak of Ebola hemorrhagic fever in Gabon, scientists captured dozens of bats in the forests near the village and tested their blood for antibodies to Ebola virus. The presence of Ebola antibodies would indicate that the bats had been infected with Ebola at some time and survived. None of the bats were showing symptoms of hemorrhagic fever at the time they were captured.

To analyze this case, run an ELISA on the 10 bat blood samples provided, testing them for antibodies to Ebola proteins. If any of the samples test positive for antibodies, test those samples for Ebola cDNA by PCR.

Questions:

1. How many bats, if any, tested positive for Ebola antibodies?
2. Did any samples test positive for Ebola genetic material? What does a positive result mean?
3. Does a positive result mean that bats started the Ebola outbreak in Gabon?
4. If bats test positive for Ebola, should efforts be made to eliminate bats in areas near human populations?

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7. Salmonella

Background: Salmonella is a group of bacteria that can cause digestive illness in humans. They pass from the feces of people or animals to other people or other animals. There are many different kinds of Salmonella bacteria. Salmonella serotype Typhimurium and Salmonella serotype Enteritidis are the most common in the United States. Salmonella bacteria were discovered about 100 years ago by an American scientist named Salmon, for whom they are named.

Salmonellosis is an infection with Salmonella bacteria. Most persons infected with Salmonella develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the Salmonella infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness.

Source: Center for Disease Control web site, http://www.cdc.gov/nczved/dfbmd/disease_listing/salmonellosis_gi.html

Case A. Sarah, a 6-year-old girl, was admitted to the hospital with a 4-day history of fever, vomiting, abdominal pain, and diarrhea. Lab tests indicated that her white blood cell count is elevated and that she has mild liver dysfunction. A stool culture taken on admission yielded a Gram negative rod that appeared to be Salmonella. Blood, nasopharyngeal, and urine cultures were negative. Sarah had no history of overseas travel, and there was no indication that she had ingested any suspect foods. No other family members were ill. Sarah has a pet turtle, a red-eared slider, for which she is the sole caregiver. A water specimen from the turtle's tank also yielded a culture of Salmonella. To determine whether the bacteria isolated from the turtle's tank and from Sarah were the same, genomic DNA was isolated from each of the bacteria samples.

To analyze this case, digest each of the DNA samples with XbaI. Then digest each of the original DNA samples (not the XbaI-digested samples) with BlnI. Run the digested samples on a gel, using a 0.5% agarose gel. (Note that this would normally be done using pulse-field gel electrophoresis, due to the large sizes of the DNA fragments, but here the fragments will run correctly using the standard agarose gel procedure). Do the band patterns for the DNA isolated from Sarah and from the turtle match?

Multiplex PCR can be used to determine which strain of Salmonella Sarah has. To perform this procedure, use the *Salmonella* primers to run PCR on the DNA isolated from the turtle's tank, the DNA isolated from Sarah, and from control DNA samples isolated from *S. typhimurium*

and *S. paratyphi.* The primers file contains two sets of primers, one which identifies all strains of *Salmonella* and amplifies a 204 bp DNA fragment; the other is specific for *S. typhimurium* and amplifies a 402 bp fragment. Use a 1.0% agarose gel to run the PCR products.

Bioinformatics: What gene is amplified by the primers? Use BLAST to identify the gene associated with the gel fragments. Click on the gel fragment from one of the samples. The sequence of that DNA should be visible in the lower window (if not, check the Sequence box above the window). The entire sequence, just a portion containing the repeat region, can be sent for BLAST analysis (the latter will work better if using fragments from the Southern blot). See the BLAST tutorial for more detailed instructions.

Additional sequences of similar genes from other species of *Salmonella* and also from *E. coli* in the Bioinformatics folder included with this case. Open these sequences and build a tree from the Opened & Processed window. How similar are the *S. typhimurium* and *S. paratyphi* strains, compared to the other bacterial strains included?

Questions

1. Which strain of Salmonella is Sarah infected with?
2. Do the results indicate that Sara contracted the Salmonella infection by handling the turtle?
3. How should Sarah be treated so that she can recover from the infection?
4. What is the level of risk associated with reptile pets?
5. What other pets are associated with possible bacterial infections?

References

Nagano, N. *et al.* Jpn. J. Infect. Dis. 59, 132-134, 2006
Alvarsez, J. *et al.*, J. Clin. Microbiol. 42, 1734-1738, 2004

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8. Sexually transmitted infections

(developed by C. Dinitra White, North Carolina A & T State University, as part of the 2006 BioQUEST summer workshop at Beloit College.)

Background: Sexually transmitted diseases are a major public health problem in the U.S. Several organisms, including bacteria and viruses, can be transmitted through sexual contact. *Neisseria gonorrheae* (which causes gonorrhea) and *Chlamydia trachomatis* can cause urethritis inflammation of the urethra) in males and females, and pelvic inflammatory disease (PID) in women. PID is infection of the uterus and fallopian tubes that can lead to chronic pelvic pain, infertility, ectopic pregnancy, abscess formation, and internal bleeding. Organisms that cause genital ulcer disease (GUD) include the bacteria *Haemophilus ducreyi* (which causes chancroid), *Treponema pallidum* (syphilis), and herpes simplex virus type 2 (genital herpes). GUD results in painful genital lesions in both males and females. These organisms can be detected by culture methods, antibody-based tests such as ELISA, and nucleic acid amplification tests such as PCR. Multiplex PCR can test for several organisms simultaneously. Treatment is primarily antibiotics (or anti-viral drugs for Herpes simplex virus).

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Case A. The Soldier's Unexpected Gift

Robert Jr. is a 22 year old soldier who very recently returned to his hometown in the Midwestern region of the United States. Lucky for Robert, his assignment in Asia ended just in time to return to Old School University to complete a degree in Science and Mathematics Education. To celebrate his return, Robert's girlfriend Jenna, a foreign exchange student, gathered lots of food, alcohol, and party favors for a night of celebration with friends. Several days later, the director of Old School University's student health department issued an alert to the university president and the local department of health to report a dramatic increase in the number of genital ulcer disease (GUD) cases on campus. There were lots of rumors about an outbreak of HIV or gonorrhea on campus, however no official warnings were released by the university. Three days after the party, Robert became very worried upon noticing an unusual, inflamed 'bump' on his penis during a shower. However, he resisted a trip to student health… he hoped it would simply go away. Two days later, the bump began bleeding. Immediately, Robert called Jenna and urged her to go with him to the student health facility to be tested for what he thought may be HIV. But rather than test them for HIV, the student health nurse took an endocervical swab sample from Jenna and a urethral swab sample from Robert. DNA was extracted from the swab samples and multiplex PCR was performed to test for five different sexually-transmitted diseases.

Options For Case Analysis Using Multiplex PCR

Option A: Perform multiplex PCR on the DNA samples listed below, using multiple primer sets (in a single combined file) that recognize five sexually transmitted organisms. Use the standard PCR protocol and gel electrophoresis for this option.

Option B: Perform 96-well PCR on the DNA samples listed below, testing each sample for the five sexually transmitted organisms. Each sample should be tested separately for each the five sexually transmitted organisms (the Option B folder includes five primer sets, in five separate files).

DNA samples:
- Robert
- Jenna
- Another male student at the same college (Student 1)
- Another female student at the same college (Student 2)
- Negative Control
- Positive control, containing the following:
  Haemophilus ducreyi (chancroid)
  Herpes simplex virus type 2 (herpes)
  Neisseria gonorrheae (gonorrhea)
  Chlamydia trachomatis serovar D (Chlamydia)
  Treponema pallidum (syphilis)

If the target DNA is present, the primers will amplify the following sizes of DNA fragments (in kilobases):
Haemophilus ducreyi, 1.24
Herpes simplex, 0.34
Neisseria gonorrheae, 0.55
Chlamydia trachomatis, 1.93
Treponema pallidum, 0.40

Questions:

1. What organism is most likely causing Robert's genital ulcer?
2. Was his girlfriend Jenna also infected?
3. Were the other college students tested infected with the same organisms?
4. Do these results give you any information about how the diseases are being spread?
5. What would you recommend as a treatment for Robert?
6. How would you discuss with Robert the importance of preventing the spread of these infections?

References

BioQUEST 2006 summer faculty workshop resources, http://www.bioquest.org/summer2006/resources.php
Centers for Disease Control and Prevention, Sexually transmitted disease information, http://www.cdc.gov/std/default.htm

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9. Methicillin-resistant *Staphylococcus aureus* (MRSA)

(contributed by Rafael Tosado, Interamerican University of Puerto Rico, Metropolitan campus)

Background: Approximately one out of 20 hospitalized patients acquires an infection during their hospital stay; such infections are known as nosocomial infections. Oftentimes, the microorganisms that cause such infections are transmitted accidentally by personnel working at the health care facility who have not followed Safety Universal Precautions such as changing their gloves often and washing their hands carefully. The most common nosocomial pathogens include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Enterococcus spp, Clostridium difficile,* and *Staphylococcus aureus.*

Antibiotic-resistant microbes may develop due to genetic mutations or genetic transformations. In the latter case, an antibiotic-sensitive bacterium acquires DNA containing genes that confer antibiotic resistance either from another bacterium (horizontal transfer) or as a result of transfection from a bacterial-infecting virus (a bacteriophage or phage). Spontaneous genetic mutations may also happen in antibiotic-sensitive bacteria rendering them resistant to certain drugs that normally prevent them from growing. The drug-resistant bacteria will then be able to grow and replicate even in the presence of the antibiotic, giving rise to the spread of antibiotic-resistant strains.

The selective drug pressure induced by antibiotic abuse or misuse usually causes the genetic mutations associated with antibiotic resistance. Examples of such misuse include: using antibiotics without medical prescription; not finishing the full doses of a prescribed antibiotic; or not complying with the instructions given by the physician or pharmacist.

Staphylococcus aureus is one of the most problematic nosocomial pathogens because certain strains of the bacterium are resistant to multiple antibiotics. One such strain is Methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant not only to Methicillin but also to penicillin and many other penicillin derivatives known as Beta lactams *S. aureus is* commonly found on the human skin in areas of the body such as the underarms, the groin, and the perianal area, but the bacterium tends to concentrate in higher amounts in the nostrils. It can cause infections of the respiratory tract and bones and joints, endocarditis, septicemia, and toxic shock syndrome. However, the most common infection caused by MRSA is the necrotizing infection of soft tissues that may lead to amputations.

There are also antibiotic-resistant strains of bacteria that can cause infections outside of hospitals and healthcare facilities. These

community-associated (CA) resistant strains can be transmitted easily from one person to another and are often present in crowded places such as schools, childcare centers, eldercare nursing facilities, jails, and gyms. A person carrying CA-MRSA may not develop an infection right away, but any trauma of the skin such as a cut or bruise could allow the bacteria to penetrate the skin and establish an infection. A bacterial-culture medium named Chromagar can be used to discriminate between MRSA and antibiotic-sensitive strains of *S. aureus*. However, the only way to differentiate between hospital-acquired (HA) and community-associated (CA) MRSA is by molecular diagnostic procedures that rely on DNA analysis. CA-MRSA strains often harbor a prophage encoding the *pvl* toxin gene that is not found in HA-MRSA (see diagram).

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Case A.

Nelson was a very talented boy who grew up in the mountains of Utuado, Puerto Rico. At twelve years of age, Nelson sang at the *Fiestas Patronales* where he shared the stage with the famous Puerto Rican singer Ednita Nazario, who had just returned to the island after her Broadway debut in Paul Simon's *The Cave Man*. Ednita was ecstatic with Nelson's talent and said to him "keep up the good work, because you are Broadway material". Her words still resounded in Nelson's mind when at age 22, he received a call confirming an audition to enter the American Music and Dramatic Academy (AMDA) in New York City.

Just two months from graduating with a double major in Dramatic Arts and Music from the University of Puerto Rico, he was confident that the AMDA was going to be his way to finally make it to Broadway. There was only one detail that he thought could become an obstacle to his triumph, and that was his nose. Nelson had inherited the distinctive big nose of the Castillo family and he was terrified when he learned that the nose and ears are the two parts of the human body that continue to grow as we get older. It was then that he decided to undergo cosmetic surgery to reduce and re-shape his nose before going to New York.

"What's wrong with our nose?" his sister Daliana asked. "It's just like Barbra Streisand's and it certainly was never an obstacle to her success in showbiz." But Nelson had made up his mind. He gathered all of his savings and convinced his sister to put down her signature for a $15,000 loan. With that amount of money, he would be able to schedule an abdominal-sculpting surgery as well. The abs-sculpting surgery went perfectly well and three weeks later, Nelson was ready for the nose job.

A week after the second surgery, Nelson's nose and face were quite swollen, bruised, and aching, and his body temperature was 99.5°F. "There must be something wrong," said Daliana, who was taking care of her brother during his convalescence. "You were not this sick a week after the abs surgery." Nelson reminded Daliana that the surgeon warned him about the swelling and pain because some bone had been shaved to fix his nose. The surgeon also mentioned that fever was one of the potential side effects of beta-lactam antibiotics and he was on amoxicillin to prevent Staph infections. "I know you very well, Nelson," Daliana said. "You are willing to risk your health in order to get what you want. I'm taking you to the emergency room even if I have to carry you." Reluctantly, Nelson agreed to go.

The physician at the ER confirmed Daliana's fears. The swelling and fever where not just normal post-surgical side effects, and the bruises where actually necrotic lesions. Nelson was suffering an infection, probably a Staph infection. As they waited for the lab results, Nelson did not understand how he could develop an infection since he never missed a single dose of his antibiotic and he had been very careful in complying with the surgeon's post-surgical instructions. Daliana, on the other hand, was looking into the possibility of filing a lawsuit against the hospital and the surgeon on the basis that her brother acquired a nosocomial infection during the surgery.

DNA has been isolated from the following bacteria samples:
- culture of bacteria from Nelson's lesions
- an antibiotic-sensitive *S. aureus*
- a hospital-acquired methicillin-resistant *S. aureus*
- a community-acquired methicillin-resistant *S. aureus*

PCR primers are available for the following (individually or all together in one primers file):
- *glp*, a housekeeping gene found in all strains of *S. aureus* (fragment size = 843 bp)
- *pvl*, a toxin gene from a prophage common in community-acquired MRSA in this region (fragment size = 433 bp)
- *mec*, part of the methicillin-resistance cassette found in most samples of MRSA (fragment size = 393 bp)

Use these primers to run PCR and gel electrophoresis on the DNA samples, to see if the corresponding sequences are detected. What do the results tell you about Nelson's infection?

Bioinformatics: Compare the *glp* and/or *mec* PCR products using the sequence alignment/tree building feature. (You can obtain the sequence for each band on a gel by clicking twice on the band. Send these sequences to the Export window for alignment and tree building). Do the tree results support your initial conclusions about the source of Nelson's infection?

Questions:

1. Is Nelson suffering a Staphylococcal infection? If so, with what strain of *S. aureus* is Nelson infected?
2. Does Daliana have grounds to file a lawsuit against the hospital? According to the DNA analysis, do you think that Nelson developed a nosocomial infection or could he have picked up the bacteria before going to the hospital?

3. What treatment options are there for Nelson?
4. Explain the difference between HA-MRSA and CA-MRSA at the molecular level.
5. How does antibiotic resistance happen in bacteria?
6. How could Nelson get a Staph infection while taking antibiotics as prescribed by his doctor?
7. How is MRSA diagnosed? Compare and contrast the culture-based methods vs. the molecular methods of diagnosis in terms of turn-around time and specificity.
8. What do you think of Nelson's decision to undergo cosmetic surgery?
9. Is the risk of developing an infection greater for cosmetic surgery than for other kinds of surgeries?
10. What are Safety Universal Precautions? How could antibiotic-resistant bacteria be prevented?

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10. *Vibrio*

(contributed by Arlin Toro, Interamerican University of Puerto Rico, San German campus)

Background: Bacteria belonging to the *Vibrio* group are commonly found in brackish and marine environments worldwide. *Vibrio cholerae*, *Vibrio vulnificus* and *Vibrio parahaemolyticus* are the most important human pathogens within this family. Some of the members of this group can cause diseases like gastroenteritis, open wound infections and septicemia. These diseases are related to the ingestion of contaminated water and seafood as well as to the exposure to contaminated water. There are several factors linked to severity of the symptoms. People with diabetes, liver diseases and with impaired immune system are more likely to suffer a more severe disease. There are several clinical cases of *Vibrio vulnificus* reporting gastroenteritis, open wound infections and septicemia. Infections with this bacterium may lead to death. *Vibrio parahaemolyticus* is involved in most of the gastroenteritis is related to the ingestion of raw oysters and seafood. Nevertheless, *Vibrio cholerae* has a greater pandemic potential and is responsible for several pandemics throughout the entire world. As of today, thousands of cases and deaths were reported every year. This bacterium is the causative agent of cholera. The disease is characterized by severe watery diarrhea, vomiting and prostration. Severe dehydration may occur due to the fact that the patient will experience 20 -30 bowel movements within a day. The diarrhea will be watery and profuse and may lead to death within a few hours if patient does not receive appropriate rehydration. There are many serogroups of V. cholera, of which 01 and 0139 were related to pandemic episodes. Other serogroups such as non-01 and non-0139 were related to less virulent outbreaks. *V. cholerae* 01, *Vibrio vulnificus* and *Vibrio parahaemolyticus* harbor many genes that encode different virulent factors and the expression of these genes accounts for the severity of the disease caused by this strain. The genes coding for toxins as well as other several virulence factors within *Vibrio cholerae* 01, *Vibrio vulnificus* and *Vibrio parahaemolyticus* can be targeted using molecular tests such as PCR which is critical to differentiate pathogenic from other variants.

For each case, DNA is isolated from cultured bacteria, and multiplex PCR using primers specific for the three *Vibrio* species is performed to see which organism is causing the infection. The individual primers as well as control DNA samples for the three species are provided. The following PCR fragments sizes are obtained: *V. cholerae*, 162 bp; *V. parahaemolyticus*, 367 bp; *V. vulnificus*, 205 bp.

Bioinformatics: BLAST the sequence of the PCR product to determine which toxin gene is being detected and which strain is implicated in the infection.

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Case A. A hurricane struck the coastal area of the island. The residents were evacuated ahead of time, but many of them came back trying to rescue their belongings right after the hurricane passed. The houses were still flooded with one foot of water. Despite the efforts of the authorities, some of the residents managed to walk inside the houses and spent hours trying to recover what is left. One day later, two elderly ladies, neighbors Emily and Luisa, reported to the emergency room with fever, severe vomiting and abdominal pain. Their pulse was weak and their blood pressure was low. The skin was dry and lacked turgor. Both women presented painful leg cramps. The profuse diarrhea had dehydrated both of them in such a severe way that they were experiencing cardiac and respiratory problems. Although it was obvious to the doctors that they were dealing with a gastrointestinal disease, the absence of blood, based on the color of the diarrhea, was the key for the treatment as well as for the laboratory tests that had to be done to confirm the cause of the disease. Later that day two other males and one female from the same neighborhood arrived at the hospital with similar but less severe symptoms.

Stool samples from all patients were collected and sent to the laboratory for further microbiological, serological and molecular analysis of the suspected microorganism. *Vibrio* was indicated by selective cultures. DNA was extracted from these cultures to perform PCR analysis of the potential toxin genes.

Questions

- Why is the color of the stool important in the presumptive diagnosis?
- How do you think the dehydration may affect the laboratory analysis such as CBC or urinalysis?
- How are the leg cramps are related to the cholera disease?
- Which culture methods should be used in order to isolate the bacteria from the stool sample?
- What kinds of information do the cultural methods provide?

• What did you need to know in order to determine the *Vibrio* serogroup?
• Using the information that is available with cultural and serogroup methods, were you are able to discriminate between the strains infecting all of the patients?
• Did the patients get infected by same strain? How do you know?
• What are the advantages and disadvantages of the molecular methods and the bioinformatics analysis?
• What are the advantages and disadvantages of using cultural methods?

Case B. It is midsummer, and the Ortiz family is enjoying an extended vacation. However, the family became concerned because of the sudden appearance of abdominal pain, nausea, vomiting and diarrhea in two of their family members, brothers Marcos and Vincent. They were wondering about the possible cause, as they were all eating the same foods during their vacations. Suddenly, one of them remembered that the day before they bought raw oysters from a street vendor. Almost all of them consumed the oysters, but, why were only two sick? After several hours, the brothers were becoming more ill so they decided that was time to visit the emergency room. At the hospital they were interviewed about the general health status of the sick men. Both are diabetic, but in general they do not have any other health problems. Both were treated with antibiotics and rehydrated. Blood samples and stool samples were sent to laboratory for further analysis. After treatment, both men recovered and were questioned further about the food that they consumed within the last few days. The doctors paid attention to the fact that they had consumed raw oysters and order an analysis for the detection of suspected bacteria. Selective culture methods indicated possible infection with *Vibrio*. DNA was extracted from these cultures to perform PCR analysis of the toxin genes.

Questions
• Which is the toxin that pathogenic strains of *Vibrio parahaemolyticus* harbor?
• Compare this toxin with the cholera toxin. Consider clinical, molecular and physiological factors.
• Why were only two of the family members were ill if everyone ate raw oysters?
• Does the fact that this occurred in summer have something to do with this outbreak?
• What are the advantages and disadvantages of the molecular methods and the bioinformatics analysis?
• What are the advantages and disadvantages of using cultural methods?

Case C. The annual family picnic gathered a lot of family members at the beach. Many generations enjoyed the beautiful day. However, the grandmother did not feel well that day. She is diabetic and she had an open wound in the leg that had not improved. Finally, she decided that maybe the salty water will helped her in the healing process so she entered into the water for a while. Two days passed and she became even more ill. The wound was worse and she could not stand. The family doctor hospitalized her and ordered cytological and microbiological examination of the wound. The Doctor discussed the results with the family and they were startled by the fact that the cause appeared to be a marine bacterium that must have been acquired while she enjoyed the beach. DNA was isolated from the cultured bacteria to identify which species of bacteria was the culprit.

Questions

• If all the family were at the beach, why did only the grandmother become ill?
• Why do she and many people think that salty water will improve the condition of the wound?
• Which are the differences between the different biotypes within *Vibrio vulnificus*?
• Do other *Vibrios* have different biotypes? If so, what are the differences between them?

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11. Malaria

(contributed by Rafael Tosado, Interamerican University of Puerto Rico, Metropolitan campus, and Bjorn Wolter, Michigan State University)

Background:

What is malaria? Malaria is a blood-borne pathogenic disease caused by protozoans of the genus *Plasmodium* spp., which is an internal parasite that preys on human red blood cells. It is common in tropical and subtropical regions and transmitted to humans via infected bites from *Anopheles* spp. mosquitoes. Approximately 500 million infections occur each year, and more than one million die from the parasite, many of whom are young children.

What causes malaria? Malaria is thought to have originated in tropical African and spread with ancestral hominids as they migrating across the globe, affecting populations from the Mediterranean to eastern China and all parts in between. Before the advent of modern science the causes of most pathogenic diseases were unknown, even if the symptoms were common. Malaria was often observed in individuals who lived and worked in or near marshy, estuarine habitats where mosquitoes breed; however, people did not associate the disease with insect bites, rather connecting it with the bad fumes that often arise from swamps as pockets of methane created by rotting vegetation. The name "malaria" is in fact derived from the Latin words for "bad (mala)" and "air (aria)" referring to these fumes, which ancients thought caused the

disease.

There are four species of malaria-causing Plasmodium: *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*. Each of these species causes an individual variation of malaria.

	P. malariae	***P. vivax***	***P. ovale***	***P. falciparum***
Disease variant	"Benign malaria"	"Tertiary malaria"	"Tertiary malaria"	"Cerebral malaria"
Endemic area	Southern Europe, Middle East, North Africa	Asia, Africa, & South America	West Africa & southeast Asia	Sub-Saharan Africa
Malignancy level	Low	Medium	Medium	Extreme
Mortality rate	None	Low	Low	High
Fever cycle	Every 4 days "quartan fever"	Every 3 days "tertiary fever"	Every 3 days "tertiary fever"	No cycle
Treatment	?	Chloroquine	Chloroquine or Primaquine	Artemisinin
Recurrence	Infrequent	Possible	Possible	Often deadly

What are the symptoms of malaria? Malaria is frequently mistaken for other, more pedestrian disorders, especially in areas where it is not endemic such as the United States and many European nations. Many of the symptoms are similar to those of the common flu and include: fever, chills, headache, general weakness, nausea, vomiting, muscle pain, cough, and/or diarrhea. Malaria is diagnosed by microscopic examination of blood, looking for the *Plasmodium* organisms in blood cells. Molecular biology techniques, such as PCR, and be used to make a more definitive diagnosis. Nested PCR may be used in a laboratory diagnosis. First a general primer set that should react with all *Plasmodium* species is used, which will amplify an approximately 1100 bp fragment. Then this product is used as a target for a second round of PCR with species-specific primers. The products expected with the specific primers are 205 bp for *P. falciparum*, 120 bp for *P. vivax*, 800 bp for *P. ovale*, and 144 bp for *P. malariae*.

What drugs are used to treat malaria? As with most diseases, the sooner malaria is treated, the better. The Centers for Disease Control recommend that treatment begins no later than 24 hours after the first symptoms occur. There are a variety of drugs available to treat malaria, the most common of which are synthetic, but chemically based on quinine, which is derived from the bark of the Cinchona tree (and commonly found in tonic water). This group of drugs includes: quinine, chloroquine, mefloquine, and primaquine. Other drugs used in the treatment of malaria are a combination of atovaquone and proguanil, a combination of sulfadoxine and pyrimethamine, the antibiotic doxycycline, and Artemisinin. Artemisinin is a lactone product of the Sweet Wormwood plant and has been used by Chinese physicians since 340 A.D.

Where may one contract malaria? One of the four species of Plasmodium that causes malaria can be found in most tropical and subtropical countries throughout the Old and New Worlds. Note that some areas that have historically been endemic areas (such as southern Europe) now pose very low infection risks due to eradication programs.

Case A. Sergio Román was glad to be surrounded by his family during the holidays. This would be the first Christmas without his older sister, Milagros, who died after contracting malaria during a missionary visit to Columbia. She was diagnosed when she returned to Puerto Rico with a high fever that came and went every few days. Milagros took the drugs they prescribed and seemed to recover, but a month later the fevers returned, and this time she did not recover. Sergio was frustrated because he did not understand why the drugs did not work for Milagros. Other members of the mission group, including Sergio's younger sister, Rosario, had also become infected but recovered fully after the drug treatments. Both were treated with the same dosage of chloroquine. It did not seem fair that a disease that was supposedly eradicated from Puerto Rico many years ago should take such a toll on Sergio's family.

Milagros and her family had agreed to allow her blood samples to be used for research into drug resistant malaria. To determine which species of *Plasmodium* infected the two sisters, perform a nested PCR by first amplifying DNA isolated from their blood with a general *Plasmodium* primer set. Then use a second round of PCR, amplifying the first PCR product using species-specific primers.

Bioinformatics: SIngle nucleotide polymorphisms (SNPs) in the multidrug resistance gene *pfmdr-1* have been associated with resistance to the drug treatments used for Milagros. Test *P. falciparum* DNA samples from Milagros and Rosario by using PCR with primers that will amplify regions containing SNPs. Compare the sequences of the PCR products by exporting the sequences of corresponding bands and submitting them for sequence alignment.

Questions:

1. Which species of *Plasmodium* infected the two sisters?

2. Does the *Plasmodium* DNA isolated from Milagros appear to contain any SNPs that might be associated with drug resistance?
3. Is there a different treatment that may have worked better?
4. How would you explain why Rosario was able to recover from malaria but Milagros was not?

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Case B. Jennie could not wait to tell her best friend the news. “You’re kidding me! Your dad actually bought you that ‘round-the-world’ ticket you’ve been asking for every birthday since you turned 18?” Synove looked at her best friend incredulously and continued, “I just can’t believe it!”

“Yup.” Jennie answered, looking a little smug. But then her face brightened as she smiled, telling Synove, “But, he said I couldn’t go alone. So he bought TWO tickets and told me to pick a friend to go with me! That’s you!”

“No way!” Synove shouted. “Where are we going to go?”

“Well, I thought we’d start off heading to Europe first just to get acclimated to traveling on our own. Maybe start in Paris and bike to southern Spain. Then cross the Straits of Gibraltar to Morocco and head down west Africa. Then we hop a flight Victoria Falls in Zimbabwe; safari in Botswana and Namibia; Cape Town; and up the east coast for a break in Dar es Salaam. From there we can fly to India and bum around a bit before heading to Thailand and Cambodia. Then we visit my extended family in Taiwan before catching a flight to Japan and from there back to Seattle. What do you think?” Jennie asked.

“Wow. Sounds great! Do we have to get any shots before we go?”

“I’m pretty sure, but I’ll ask my mom tonight.” Jennie said.

“Of course! A doctor would know…”

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“Well girls, you’re going to need lots of shots before you go on this world tour of yours." said Jennie's mother. You’ll need Yellow Fever, Hepatitis A and B, Typhoid, Rabies, and Meningococcal. Jennie I know you’ve had it, but Synove, do you know if you were vaccinated for Polio as a child? If not, you’ll need that. For India, Thailand, and Cambodia you’ll probably need to get Japanese encephalitis too if you’re going to be in rural areas. You should probably get Dengue Fever immunizations too, just to be on the safe side. Good thing you’ve got about eight months before you leave. All these shots are going to take a while.”

“Mom, what about malaria? Do we need to worry about that?”

“Absolutely. I can prescribe a prophylaxis for the both of you to take. Be sure you take it every week at the same time, ok?”

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About a week after returning from their trip, Jennie began to feel ill. She was feverish, felt weak, had a headache, and just ached all over. Both she and Synove thought it was just the flu. But one afternoon, Synove couldn’t wake Jennie from a nap she was taking between classes and called an ambulance. Based on Jennie's recent travels and her symptoms, the physician at the hospital orders a blood analysis, which revealed evidence of *Plasmodium*.

To confirm that Jennie is infected with Plasmodium, perform a nested PCR by first amplifying DNA isolated from her blood with a general *Plasmodium* primer set. Then use a second round of PCR, amplifying the first PCR product using species-specific primers.

Questions:

1. Which species of *Plasmodium* infected Jennie?
2. What treatment(s) would you recommend based on the diagnosis?
3. What other advice would you have for Jennie? What is her prognosis?
4. Is there anything the girls could have done to prevent infection?

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D. Microarrays

Background: Microarrays perform the simultaneous detection of thousands of DNA sequences. Single-stranded DNA probes are spotted onto a small chip, and a labeled DNA sample is added. Matching sequences will hybridize, and the label (e.g. fluorescence) will be detected on the spots corresponding to probes that match the sample’s sequences. The Case It software displays a representative 64-spot section from a microarray chip, so that students can view the fluorescence data used to analyze the samples.

The Case It software simulates two types of microarrays, **SNP microarrays** detect single nucleotide polymorphisms in DNA samples. SNP chips contain pairs of probes containing a single nucleotide difference between them. DNA samples are tested for their ability to bind to one or both probes, as measured by the amount of fluorescence detected on each probe spot. The genotype of each sample is determined by the

relative amount of fluorescence bound to each probe in a SNP pair. Genotypes may be homozygous for one or the other SNP, or they may be heterozygous. SNPs are associated with a variety of conditions, including disease susceptibility or resistance, and can be used for diagnosis or for research into understanding disease mechanisms. The SNPs in these cases are identified by the dbSNP ID names, beginning with the designation “rs” followed by a number.

Expression microarrays measure levels of gene expression in cells and tissues under specific conditions, based on the amount of RNA transcribed from the genes. RNA isolated from the samples is copied into cDNA, incorporating a fluorescent label. DNA from control is typically labeled with a green dye, while DNA from experimental samples is labeled red. A control and an experimental sample are mixed and added to a chip containing probes corresponding to genes of interest. The relative amount of green-labeled DNA that binds to each probe, compared to the amount of red-labeled DNA that binds, is an indication of whether the expression of that gene increased or decreased (or did not change) under the experimental conditions, relative to the control. A computer program displays the relative red and green fluorescence on each spot on the chip, where equal amounts of green and red are displayed as yellow.

Expression microarrays typically include probes for genes that should not change expression regardless of conditions, as controls for variability. These control genes include beta-actin, GAPDH, GUS, RPLPO, and TFRC.

More background information about microarrays is also available on the NCBI site, http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html.

1. SNP Microarrays

SNP microarray quick start instructions

Video tutorial for SNP microarrays

a. Prostate cancer

Greg just celebrated his 50th birthday. He does not feel “old” and thought he was in very good health. However, his physician recommended that, because of his age, he undergo screening for various diseases that are more common in men over 50. One of these is the PSA (prostate-specific antigen) test, used to detect potential prostate diseases. Although Greg has not had any of the symptoms associated with prostate disease, he has his blood tested for PSA. His result of 8 ng/ml is outside of the normal range of 0-4 ng/ml. Greg’s physician suggests that they perform a needle biopsy to look for abnormal cells in Greg’s prostate that might indicate inflammation or even cancer. Greg does not want any unnecessary tests, especially any involving needles, and he discusses his concerns with his physician. Greg’s physician just read an article describing men with higher than normal PSA levels in the absence of any prostate disease, and suggests the possibility that higher PSA blood levels may be normal for Greg due to his genetics. The physician arranges for Greg’s DNA to be tested for the SNPs associated with high PSA levels.

According to this study, two SNPs (rs10788160 near the FGFR gene, and rs17632542 in the KLK3 gene) were associated with higher blood PSA levels in the absence of prostate cancer. One SNP (rs10993994 in the MSMB gene) was linked to higher PSA levels but also to higher risk for prostate cancer.

To analyze this case, open the file SNP PSA levels.csv and run the microarray. Compare Greg’s DNA with the sample from a patient with genetically high PSA levels and the sample from someone with normal PSA levels to determine whether Greg’s DNA contains SNP alleles that would explain his higher blood PSA levels.

Questions:

- Does Greg’s DNA contain the SNP alleles associated with high PSA levels?
- Should Greg get the needle biopsy? Why or why not?
- How do these results affect the interpretation of Greg’s PSA test results?
- What role might these gene products play in prostate function?
- Should routine PSA testing continue to be recommended if It is not completely accurate?

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b. Cardiac disease

Jonathan is a 44-year old male who is in good health, although he is about 30 pounds overweight. It has been a difficult year for Johnathan. His marriage ended, and after a bitter custody fight he has full custody of his two daughters, ages 7 and 9. Soon after, he lost his full time job. He quickly found work again in a position that pays a lower salary. but he feels lucky, though, to have found a job that provides health insurance. He blames the stress of the past year for the fact that he has gained weight. It has been hard to find time to exercise with his new

job and taking care of his daughters. So he was relieved when his recent physical exam indicated that his overall health was reasonably good. His cholesterol levels were slightly elevated since his last exam, but his blood pressure was in the normal range.

Jonathan has been concerned about heart disease ever since a former classmate, who had seemed in perfect health, died of a sudden heart attack while running a 10K race. Jonathan does not know very much about his own family history of heart disease since his father died in a car accident when Jonathan was very young, and he has few other male relatives. When he mentioned his concerns to his physician, he tells Jonathan that there is a genetic test that can determine whether his DNA contains certain SNPs associated with sudden cardiac disease. Having one of these SNPs may be associated with a 2-5 times greater chance of sudden myocardial infarction (heart attack). Having more than one of the associated SNPs can increase the risk significantly. Since this clinic is involved in researching the role of these SNPs in sudden cardiac disease (SCD), Jonathan can get his DNA tested for no cost. He decides to go ahead with the test and submits a blood sample.

To analyze this case, open the SNP Cardiac disease.csv file and run the SNP microarray. For comparison, this file also contains samples from a man who had a sudden myocardial infarction (SCD) at age 40 with no prior symptoms, and from a man with is in his 70's and has had no heart problems nor does he have any family history of heart disease. Determine the genotype for these SNPs associated with increased risk for sudden cardiac disease:

SNP	Risk allele
rs4687718	A
rs4665058	A
rs3918242	T
rs11970286	T

Questions:

- Does Jonathan's DNA contain any of the SNPs associated with risk for sudden cardiac disease? How would you explain the results of the test to him?
- What would you recommend to Jonathan in terms of managing his health and reducing his risk of cardiac disease?
- Should Jonathan be concerned about the results of his DNA testing being used in a research study, or being shared with anyone else?
- Are there SNPs other than those mentioned in the tables whose genotype pattern indicate they might be associated with increased risk for cardiac disease?
- Are these SNPs located in or near genes? If so, what are the functions of these genes in cardiac function?

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c. Pharmacogenomics

Each year, thousands of people die from drug complications, and millions have adverse side effects. There is significant genetic variability in how patients will respond to certain drugs. For example, Warfarin is an oral anticoagulant drug that is widely used to prevent and treat thromboembolic disease in patients with deep-vein thrombosis, pulmonary embolism, mechanical heart valves, and atrial fibrillation. It inhibits the vitamin K epoxide reductase complex subunit 1 (VKORC1), which results in decreased formation of vitamin K-dependent clotting factors and provides the therapeutic effect of anticoagulation. It is associated with a substantial risk of major bleeding, which can be fatal, and patients taking Warfarin must be monitored closely. SNPs have been identified in genes encoding enzymes that metabolize Warfarin, that are associated with increased risk for bleeding. Additional SNPs in the VKORC1 gene are associated with resistance to Warfarin, rendering it ineffective at protecting against blood clots.

Sally is a 55-year-old woman with a recent history of atrial fibrillation who requires long-term anticoagulation therapy. She and her daughter are quite concerned about the potential for bleeding and ask the pharmacist about their concerns. The pharmacist suggests that Sally undergo genetic testing to avoid this adverse event. Sally's DNA is tested for SNPs associated with higher risk of excessive bleeding due to Warfarin. To analyze this case, open the file "SNP pharmacogenomics warfarin.csv" and run the SNP microarray. A DNA sample from an individual known to have a SNP associated with risk for Warfarin-induced bleeding is included, as well as DNA from someone who has a SNP associated with resistance to Warfarin.

Questions:

- Based on the results of these tests, should Sally be prescribed Warfarin to treat her atrial fibrillation?
- What other recommendations do you have for Sally to help her manage her health?

- What are the functions of the SNP-associated genes and how are they involved in modulating the effect of Warfarin?

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d. Resistance to HIV infection

Researchers have been studying a population of Kenyan sex workers who appear to be resistant to HIV infection despite repeated exposure to the virus. Identifying the mechanism for HIV resistance could lead to treatments that would protect individuals at risk for HIV exposure. To determine whether there are genetic factors that contribute to this resistance, the researchers obtained blood samples from HIV-resistant sex workers as well as from individuals who became infected with HIV upon exposure, and isolated DNA from these samples. A SNP microarray was performed to identify any SNPs consistently associated with HIV resistance.

To analyze these samples, open the file “SNP HIV resistance.csv” and run the microarray. Compare the resistant and susceptible samples against each other to identify SNPs genotypes that correlate with resistance.

Questions:

- Are there SNPs that appear distinguish the resistant vs. susceptible individuals?
- Do any of these SNPs appear to be in or near genes that you would expect to be involved in HIV resistance? What is the gene function?
- How might a change in one nucleotide affect the ability of a cell to be infected by HIV?

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2. Expression microarrays

Expression microarray quick start instructions

Video tutorial for expression microarrays

a. Breast cancer

Sarah was devastated when she received a diagnosis of breast cancer. It did not seem to run in her family, so she assumed she did not have to worry about it. She is grateful for the support of her friends, especially Molly, who is a clinical lab pathologist. Molly is helping her think about the difficult decisions about how aggressive her treatment should be, in terms of surgery, chemotherapy, etc. She explained that the oncologist recommended running a lab test that uses a microarray to measure the expression of specific genes. The pattern of gene expression can predict how quickly the tumor cells will grow and whether they will respond to treatments. Sarah is meeting with the oncologist to review the results, and she has asked Molly to go with her.

Researchers have identified genes whose increased expression is associated with increased proliferation, or rapid growth in breast cancer tumors: Ki-67, STK15, Survivin, Cyclin B1, MYLB2. Other genes are associated with increased tumor cell invasion if their expression is increased: Stomelysin 3 and Cathepsin L2. Some breast cancers cells respond to the hormone estrogen by proliferating, which provides a possible mechanism for treatment since estrogen receptors can be blocked. Increases in the expression of estrogen receptor genes might indicate these cells are responsive to the hormone.

To analyze this case, open the file "Expr breast cancer.csv" and run the microarray. Compare the levels of gene expression, indicated by fluorescence intensity, in the sample from Sarah's normal breast epithelium to a sample of the tumor tissue.

Questions:

- What genes are elevated in the tumor tissue? What genes are decreased?
- What are the function of the genes with the most significant difference in expression between normal and tumor tissue?
- Does that pattern of gene expression provide any information about Sarah's prognosis?

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b. Melanoma

Background: Melanoma accounts for about 3% of human cancers, and it is one of the most lethal. If caught early, it can be treated successfully with surgical excision. However, once it has spread it does not respond well to chemotherapy or other treatments. The cure rate is less than 5% and average survival times are 6-9 months from diagnosis. Melanoma arises from melanocytes, the pigment-producing cells

in skin. These cells also produce benign skin growths such as moles (nevi). One of the challenges in detecting melanomas is to determine when an apparently benign nevi may have developed into a cancerous growth. Patients are advised to seek medical advice if a mole changes shape, size, or color. If the physician thinks that further evaluation is necessary, a surgical biopsy is performed and a pathologist will determine microscopically whether there are abnormal cells, with the potential to invade other tissues, spreading from the tissue. Diagnosis via standard histological criteria can be very difficult with these samples, and cases of invasive melanoma can be missed. Recently, molecular methods have been developed to support the microscopic pathology. Genes that are expressed at increased levels in invasive melanoma tissue relative to benign nevi can be used to identify tumor cells. Furthermore, identification of these genes provides a possible strategy for developing therapies that target invasive melanoma cells. Genes associated with increased expression in melanoma cells include OPN (a secreted phosphoprotein involved in cell adhesion and migration), PHACTR1 (phosphatase inhibitor), STAT1 (signal transduction and transcription activator), and FABP7 (fatty acid binding protein).

Scenario: Catherine usually did not spend much time looking closely at her skin. At 23, she was not yet worried about the signs of aging. Growing up on the gulf coast of Texas, she had spent most of her summers on the beach without worrying very much about excessive sun exposure. Her college roommate and best friend, who was from Minnesota, constantly commented on how attractive Catherine's tanned skin was, and what a "healthy glow" she had. So when her physician questioned her about a large mole on her shoulder, she could not really tell her whether it had changed in appearance recently. She had many moles and did not pay much attention to them. But once it had been pointed out to her, and after doing some internet searching on how to identify potential skin cancers, she became anxious enough to seek the advice of a dermatologist. After examining the mole, the dermatologist recommended a biopsy, since it appeared to be abnormal. The pathologist report came back as "uninterpretable", because there was some trauma to the tissue during the excision and processing that made it difficult to characterize the cells. A second biopsy with wider margins was planned, but in the meantime the original tissue sample was submitted for an experimental evaluation that involved extracting RNA for purposes of gene expression studies that might provide more information about whether the tissue contained cancerous cells. A biopsy of a mole with a normal benign appearance was taken for comparison.

To analyze this case, open the file "Expr melanoma.csv" and run the microarray. Compare the levels of gene expression, indicated by fluorescence intensity, in the sample from Catherine's questionable tissue (possible melanoma) to that in the normal, benign mole.

Questions:

- Is the expression of any of the gene associated with invasive melanoma elevated in the questionable mole sample relative to the benign mole?
- Are there any other differences in gene expression between the two samples? If so, what are the functions of the genes showing differential expression?
- What would you recommend to Catherine based on the results of this analysis?
- What else could Catherine do to reduce her risk for melanoma?

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c. Herpes simplex virus multiplication

Microarrays can be used to determine a time course of virus gene expression during the infection of cells in culture. Virus multiplication cycles include attachment to the cell and penetration of the cell membrane, replication of the virus genome synthesis of virus proteins, and assembly and release of new virus particles. Virus gene expression is regulated temporally, with some genes expressed early in the multiplication process, while other genes are not expressed until later in the process. In this experiment, HSV-1 was used to infect HeLa cells. Samples of infected cells were taken at 3 and 8 hours, and total RNA was extracted. The RNA was copied into cDNA, incorporating Cy5 (red) fluorescent dye into the cDNA. RNA was also extracted from the uninfected HeLa cells, and cDNA was copied incorporating Cy3 (green) dye.

To analyze the results of this experiment, open the file "Expr HSV 3hr.csv" and run the microarray. Compare the levels of gene expression, indicated by fluorescence intensity, in the sample from uninfected and HSV-infected cells. Note which genes appear to be elevated in the virus-infected cells at that time point, relative to uninfected cells. Then repeat this procedure with the file “Expr HSV 8hr.csv”. Genes that were not elevated at 3 hr but appear at 8 hr would be considered "late genes". See http://darwin.bio.uci.edu/~faculty/wagner/hsv9fnew.html for background information about HSV microarray experiments and to identify the virus genes (HSV gene names include the letter U followed by a number). Probe sequences can also be submitted to BLAST analysis to gain more information about the genes.

Questions:

- Which HSV genes would be designated “early” genes based on these results? What are their functions in virus multiplication?
- Which HSV genes would be designated “late” genes based on these results? What are their functions in virus multiplication?
- Which cellular genes show increased expression in HSV-infected cells relative to uninfected cells? What role would these genes have in virus multiplication?

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I. Honey bee cases

Case A: Pesticides and viruses (contributed by Brad and Kim Mogen, University of Wisconsin-River Falls)

Background: Honey bees are commonly exposed to pesticides as they forage for pollen and nectar. Some pesticides are known to affect the central nervous system of bees and thus impact their behavior. Sub-lethal exposures of some pesticides are considered possible contributing factors to Colony Collapse Disorder (CCD).

Scenario: Dr. Muskiver was curious if pesticide exposure was linked to virus infection, another possible contributing factor to CCD. To test this question, Dr. Muskiver set up test colonies, and fed the honey bees either with untreated pollen or pollen treated with sub-lethal doses of pesticides. She then tested the bees for the presence of several viruses using multiplex PCR on cDNA isolated from the bees.

DNA samples:

Negative control - bee sample with no viruses present
Positive control - bee sample containing all four viruses
Hive 1 - exposed to pesticides
Hive 2 - exposed to pesticides
Hive 3 - no pesticides exposure
Hive 4 - no pesticide exposure

Procedure: To analyze this case, open the DNA sequences and multiplex primer, and run multiplex PCR. Then load and run the gel. Click on fragments and BLAST the associated sequence to verify that the fragments are correctly associated with the viruses.

Questions

- Do the control samples produce the results you expected?
- What are the results for each experimental hive, in terms of viruses that are detected?
- Is there any correlation between pesticide exposure and viruses detected?
- How would you explain these results to Dr. Muskiver?
- What changes would you make to the experiment design if this is repeated?
- What would you suggest that these researchers do next?
- What are some other tests that could be done to address this question?

Case B: Mites and virus diversity

Background: Recent declines in honey bee populations have given rise to the syndrome named Colony Collapse Disorder (CCD). Several potential stressors have been identified. It has recently been reported that V. destructor transmits certain strains of DWV more effectively, and that long-term mite infection reduces virus diversity and leads to the prevalence of more pathogenic viruses.

Scenario: A team of research scientists, funded by the North American Honey Bee Council, decide to survey colonies from around North America for two of the notable stressors - Deformed Wing Virus (DWV), a virus that causes wing deformation, and Varroa destructor, a parasitic mite that feeds on the bee. The scientists are interested in testing the relationship between DWV strains and the Varroa mite in North America.

Bees tested from:

- Central Ontario - low mite levels
- Northwestern Washington - low mite levels
- Southeast Florida - high mite levels
- Oahu, Hawaii - high mite levels
- Northern Arizona - moderate mite levels
- Southern British Columbia - moderate mite levels

Procedure

The file contains a total of18 sequences, 6 from Florida, 6 from Ontario, and 6 from Washington (Arizona and Hawaii sequences are not

included in the file). The tree can be built three ways. If using MEGA software, a single menu command will open MEGA and build the tree via the Analyze button of the Opened & Processed window. If using MABL or MAFFT, then the sequences need to be transferred to the Export field of the Sequence Analysis window (using the Analyze button). The Analyze button can then be used again to opent the MABL or MAFFT web site, after which the contents of the Export field (copied to the clipboard automatically) can be pasted into the input fields of either web site. Instructions for using the web sites are included in the menus of the Analyze button.

Questions

•If three different methods are used to build the tree (MABL, MAFFT, MEGA), why are the three trees different? Or are they?

•The hypothesis is that long term mite infection reduces viral diversity. Is that hypothesis supported by the data?

•What else do we need to know before concluding that relatively high mite infections are associated with relatively low viral diversity?

Case C: Comparing relative amounts of viral DNA in honey bee hives in relation to mite loads

Background: Quantitative PCR (qPCR) is a method for determining both relative and absolute quantities of DNA in samples. In this procedure, DNA amplification is monitored over time, as the DNA doubles each cycle. The point at which the amount of DNA present (measured as a fluorescence value) crosses a predetermined threshold is called the 'Ct' value (see qPCR tutorial for a more detailed explanation of Ct and Delta Ct).

Scenario: A local beekeeper is experiencing declines in honey bee production from his hives and has asked biology instructors if their classes can study the problem. This was a 'single-blind' study, so the instructors knew which hives had high, moderate and low mite infestations (loads) and good, moderate and poor overwintering success, but the student researchers did not. The students were told that 4 of the hives had high mite loads, 4 had low mite loads, and 2 had moderate mite loads, but they were not told which hives fell into each of these categories.

Procedure: The students determined relative amounts of DWV and BQCV viral levels for the 10 hives using the reverse-transcriptase qPCR procedure (via amplification of cDNA). Resulting data is a file ("qPCR honeybee") containing fluorescence levels resulting from amplification of cDNA for DWV and BQCV over time. The students were then asked to analyze the data to see if any trends were present. They were also asked to search the literature for known causes of DWV and BQCV, that might offer an explanation for any trends that they found.

Hint: With the Case It software, qPCR can be run for subsets of the data by selecting wells (by clicking or dragging) so that they turn purple in color, and then qPCR can be run for "purple wells only" (or "orange wells only"). This makes it easier to examine the data for any trends that might be present.

Questions:

•Are there relationships among mite infestation levels and viral levels in Mr. Smith's honey bee hives?

•If there are relationships, are they dependent on the threshold level set before the qPCR procedure is run?

•From the literature, what hypotheses have been advanced about how DWV and BQCV are transmitted? Did the class data support or not support these hypoetheses?

•What additional information would you need to know before drawing conclusions from the results of this study? How could the experimental design be improved?

•What other viruses and environmental factors have been implicated in honey bee declines in the U.S.? How important are honey bees, both ecologically and economically? Are honey bees used commerically in the U.S. native to this country? What is their impact on native bee populations?

"Case" D. Determination of absolute quantities of DNA from samples using standard curves

Although not set up as a case, standard curve data is included in the Cases ->qPCR folder, to demonstrate how standard curves are used to quantify the amount of DNA present in samples. An outlier is deliberately included as it was present in the original data set. See the qPCR tutorial (in video, pdf or Powerpoint format) for more detail on how to generate and use a standard curve. Note that this data set was not generated from honey bee viruses, but rather is a generic data set included here because it is part of the qPCR tutorial.

F. Plants

1. Bt Corn

(contributed by Eric Ribbens, Western Illinois University Biology Department)

Background: A significant proportion of the corn grown in the U.S. has been genetically modified to contain the crystal toxin gene from *Bacillus thuringiensis* (Bt corn). The protein translated from this gene is toxic to caterpillars and other larval stage insects. When these insects eat leaf tissue containing the toxin protein, it causes the lining of their digestive tract to deteriorate, eventually killing the insect. There are several versions of the Bt protein. Any that are in the human food supply are tested by the FDA for safety. However, several years ago corn used for human food was contaminated with a Bt protein that had not been approved for human consumption (known as Starlink). This strain of the protein, Cry9C, showed allergen potential in preliminary tests and was being analyzed further. Although no one was apparently harmed by consuming this corn, this incident resulted in more thorough testing of corn prior to its use in human food. See http://www.organicconsumers.org/ge/starlinkdisrupt.cfm for more background information.

Case A: As a lab technician for a genetic testing company, your job is to determine whether corn samples contain any genetically engineered corn. A corn farmer, Mr. Keller, has hired your company to test his corn. Mr. Keller does not grown any genetically modified corn, but a local elevator has rejected his corn saying that is contaminated with Bt corn. There are three batches of corn to test, each with a different number of samples. Choose one batch to test and use PCR with primers that will amplify any *Cry* genes present. The primers also contain a primer set that will amplify a portion of the 16SrRNA from corn as a positive control. Run the PCR products on a gel. Report your results to Mr. Keller.

Questions:

1. Did any of the samples contain the the Bt gene?
2. How many samples should be tested in order to get an accurate result?
3. How could Mr. Keller's corn be contaminated if he did not plant Bt corn?
4. Is the corn that tested positive for Bt safe for human consumption?

Bioinformatics: Which version of the Cry gene is present in the corn samples you tested? Use BLAST to identify the sequence of the PCR product. Click on the gel fragment from one of the samples that is positive for the Bt cry gene. The sequence of that DNA should be visible in the lower window (if not, check the Sequence box above the window). The entire sequence, just a portion containing the repeat region, can be sent for BLAST analysis. See the BLAST tutorial for more detailed instructions. Should this corn be allowed to enter the human food supply?

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2. *Cannabis* - hemp vs. drug

(developed by Eric Ribbens, Western Illinois University Biology Department, and Ethel Stanley, BioQUEST, at the 2008 SCOPE workshop, http://bioquest.org/scope/march_2008.php)

Background: Arizona has legalized growing and selling hemp. Hemp is a subspecies of *Cannabis sativa*, but instead of selecting for high THC levels (the chemical associated with marijuana drug use) hemp growers have chosen varieties that produce long strands of fiber. Hemp is easier to grow than cotton in some areas, and is becoming more popular as a fiber source. However, because the THC-containing varieties of *Cannabis* are illegal to grow or sell in Arizona, the Drug Enforcement Agency (DEA) has been vigilant about making sure that no one takes advantage of the new law.

Case A. George

The Arizona state DEA has arrested George Malkay. George has a record of drug abuse, but claims he is innocent. He grows hemp, which is legal. However, the DEA claims they found 6 bags of dried leaves in his warehouse, bags they think contain high-potency marijuana that, according to an informant, came from Guatemala. George's lawyer has hired your genetic testing company to test his samples. You have researched *Cannabis*, and found that several regions with small tandem repeats (STRs) have been identified in the genome. While you cannot directly measure whether a plant is hemp or drug by analyzing these STRs, it is quite possible to make inferences about geographic differences.

You have been given 6 samples to test, and have isolated DNA from these samples. Use primers that amplify regions of *Cannabis* DNA with small tandem repeats (STRs) to carry out PCR tests on the samples and run a gel with the PCR products. The length of the STRs, as indicated by the band patterns on the gels, identify related varieties. Note that there are eight different primer sets that amplify different STR regions that you can use for individual PCR tests, or a file containing all of the primers that you can use for multiplex PCR. Explain the results of your testing to George's lawyer.

Case B. Bob

Bob Propson is a farmer who has become convinced hemp is worth growing. The DEA, in a routine inspection, collects six samples from his farm. Your genetic testing company has been contracted to test these sample, and it is your job to carry out the tests. You have researched *Cannabis*, and found that several regions with small tandem repeats (STRs) have been identified in the genome. While you cannot directly measure whether a plant is hemp or drug by analyzing these STRs, it is quite possible to make inferences about geographic differences.

You have isolated DNA from these samples. Use primers that amplify regions of *Cannabis* DNA with small tandem repeats (STRs) to carry out PCR tests on the samples and run a gel with the PCR products. The length of the STRs, as indicated by the band patterns on the gels, identify related varieties. Are all of Bob's samples from Arizona?

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G. Forensics

1. Murder case

A woman has been brutally stabbed to death outside of her home. Two suspects have been arrested – 1) her ex-husband, whom the deceased woman claimed had been stalking her in the two months prior to her death, and 2) an acquaintance of her ex-husband who had been living in the ex-husband's house for about six months and who could not provide an alibi for the time of the murder. Blood samples are taken from the crime seen – one spot found near the victim's body and one taken from a glove found near the crime scene.

DNA is isolated from these blood spots, as well as from blood samples taken from the victim and the two suspects. Each DNA sample is subjected to PCR analysis, amplifying a polymorphic region of chromosome 1. (NOTE: Use the PCR function of the Data Screen, rather than 96-well PCR.) Digesting this amplified DNA with HindIII will yield distinctive banding patterns that should help identify the source of the blood spots from the crime scene.

DNA samples:	blood spot 1 (from sidewalk)
	blood spot 2 (from glove)
	victim's blood
	suspect 1 (ex-husband)
	suspect 2 (acquaintance)

(Note: There are three versions of this scenario, Case A, B, and C, each with a different outcome.)

Questions:

1. What conclusions can you draw from these results?
2. Do you think these data are sufficient to convict someone?
3. What additional issues are raised by this type of testing?

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2. Thomas Jefferson / Sally Hemings case

Background

There has long been controversy regarding whether Thomas Jefferson fathered any children with Sally Hemings, one of his slaves. Jefferson was accused of fathering two of Hemings' sons: Thomas Woodson, who was born in 1802 shortly after Jefferson and Hemings returned from an extended stay in France, and Eston Hemings Jefferson (born 1808), who bore a striking resemblance to Jefferson and took his name as an adult. No known documentation either directly supports or refutes these claims. Recently, researchers in the United Kingdom have attempted to address these questions scientifically by analyzing DNA from the Y chromosome of male descendants of Jefferson's uncle, Jefferson's sister, and Hemings. Thomas Jefferson himself had no undisputed, surviving sons.

Most of the Y chromosome passes unchanged from father to son, except for occasional mutations. Several Y chromosome genetic markers, some of which are genes while others are non-coding, can be used for this analysis since they can be inherited in one of two allelic forms (called bi-allelic). The alleles are detected by dot blot analysis using probes that will bind to one allele or the other. It is possible to determine whether two individuals are closely related by comparing how frequently their alleles match. Another type of marker that can be used in this analysis is microsatellite short tandem repeats (microsatellite STRs). These are regions where short (2-3 base pairs) sequences are repeated. The number of repeats is inherited like an allele, and can be determined by the size of the band detected on a gel after PCR amplification of that region.

The Case

DNA was isolated from the following individuals (numbers correspond to reference numbers used in the study):

H21 – Eston Hemings Jefferson's great-great grandson

W55, W70 – Thomas Woodson's great-great grandson

J41, J47, J49 – Descendants of Field Jefferson, Thomas Jefferson's uncle

C27, C31 – Descendants of John Carr, Thomas Jefferson's nephew

To determine which allele each individual has at each of the Y chromosome bi-allelic markers, use the appropriate primers to amplify the

DNA by PCR (use the PCR function on the Data Screen rather than 96-well PCR) and then perform a dot blot using the probes for that marker. Load the probes into the spots and the amplified DNA into the corresponding wells. To detect microsatellite STRs, use the appropriate primers to amplify the DNA and then run the PCR products on a gel to determine the relative sizes of the fragments.

Bi-allelic markers:	Microsatellie STRs:
YAP	19
sY81	389A
92R7	389D
SRY	392

Questions:

1. What can you conclude from the dot blot and gel results? Which individuals appear to be the most closely related?
2. Are these results consistent with Thomas Jefferson having fathered either Easton Hemings Jefferson or Thomas Woodson?
3. Are there other ways to interpret these results?

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H. Phylogenetic studies

1. Primate relationships

(suggested by Rick Berken, East High School, Green Bay, WI)

Compare hemoglobin genes from human, chimpanzee, and gorilla to determine how closely related these species are. Two types of analyses can be performed using restriction enzyme digestion and gel electrophoresis:

a. Digest each DNA sample with restriction enzyme(s) (choose one or a combination) and compare the fragment patterns generated. Are the patterns for one pair of species more similar than another pair (e.g. is gorilla more similar to chimp or to human)? How many different enzymes do you need to use in order to yield reliable data?

b. After digestion, perform a Southern blot with one of the hemoglobin probes from chimp. With the probe stringency (match) set at 100%, does the probe hybridize to DNA from either of the other species? If not, how much do you have to reduce the stringency before the probe hybridizes to the other samples?

Bioinformatics approach: Compare these sequences, plus additional primate hemoglobin sequences, using the sequence alignment and phylogenetic tree building feature. How do these results compare to your original analysis?

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2. Squirrel taxonomy

(contributed by Steven Rice, Wake Forest University, Winston-Salem, NC)

In this example you will compare mitochondrial cytochrome b sequences from various squirrel populations. Cytochrome b is an integral part of the mitochondrial electron transport system. One DNA sample is from *Sciurus aberti* aberti, the tassel-eared squirrel that resides in Arizona, extending to the southern rim of the Grand Canyon. DNA samples also are available for individuals from a different subspecies, *Sciurus aberti* ferreus, and also from another species in the genus, *Sciurus niger*. The former is an individual of the Kaibab squirrel that has been isolated on the north rim of the Grand Canyon. The latter is a fox squirrel that is common in the midwest.

Open each DNA sample, digest the DNA fragments with the AluI enzyme, load each into a different well and run the gel. Use a short run time (10 minutes).

1. Which of the types had similar restriction fragments?
2. How do these differences compare with what you would expect based on the taxonomic differences among the individuals?

Bioinformatics approach: Compare the squirrel sequences using the sequence alignment and phylogenetic tree building feature. How do these results compare with your original analysis? Re-construct the tree including the cytochrome c sequences from other organisms. Does this change the relative relationships between the squirrels?

3. Color vision in primates and other animals (research project)

Background

Color vision in humans and other primates, and in many other animals, is dependent upon amino acids present at three critical positions in the opsin gene (position 180 in exon 3 and positions 277 and 285 in exon 5). Differences of a single amino acid at these positions

determines wavelengths of maximum light absorption. Combinations of amino acids at these three locations is a predictor of whether vision is dichromatic (two-color) or trichromatic (three-color). For example, trichromatic vision is most common in Old World primates including humans, whereas color vision is highly variable in New World primates, with a mixture of dichromatic and trichromatic vision even within individuals of the same species. Because of the ecological and evolutionary significance of these variations among primates and other animals, the study of genetic sequences related to color vision has considerable potential for undergraduate research projects.

A good basic overview of this topic is the Monkey Opsin section of the Evo-Ed web site. A more in-depth overview of color vision in humans is Genetics of variation in human color vision and the retinal cone mosaic.

Other articles of interest include:

Highly polymorphic colour vision in a New World monkey with red facial skin, the bald uakari

Color Vision Polymorphism in Wild Capuchins and Spider Monkeys in Costa Rica

Elephants and Human Color-Blind Deuteranopes Have Identical Sets of Visual Pigments.

Many other research articles are available on this topic that provide background information and generate ideas for hypothesis testing.

Research project

Start by reviewing "Tutorial - searching sequences", which gives an overview of how sequence searching works in Case It. After reviewing the tutorial and background publications linked above, examine the following folders and see if you can propose a hypothesis and devise a research project to test this hypothesis. See the **tutorial on searching sequences** (in either PDF or Powerpoint) format for ideas and techniques for this project.

Folders available:

DNA for 180 277 285 locations
Primers for exons 3 and 5
New World monkeys
Old World monkeys
Vertebrate comparisons
Sample sequences

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I. Simulation of wet labs

These were developed to be used along with electrophoresis labs, to prepare students for the lab and/or to allow extensions of the lab activity. They also could be used in place of the lab if time or equipment is not available.

1. Digestion of Lambda DNA

(contributed by Brack Gillespie, Ashwaubenon High School, Ashwaubenon, WI and Rick Berken, East High School, Green Bay, WI)

This is a standard lab activity to illustrate the basic features of restriction enzyme digestions. DNA isolated from bacteriophage Lambda is digested with common restriction enzymes - EcoRI, BamHI, HindIII - to demonstrate that enzymes with different recognition sites will yield different band patterns on a gel.

Activity extensions:

- Use additional enzymes (provided for other case studies or generate your own enzyme files)
- Simultaneous digestions with two or more enzymes
- Mapping restriction sites along Lambda DNA (see part 2.a. below)

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2. Mapping of phage T7 DNA

(Contributed by Barbara Moffat, University of Waterloo, Ontario, Canada)

This activity has two parts: a) generating a restriction map of phage T7 DNA; and b) determining to which T7 gene(s) the probe binds.

a. Generating a T7 restriction map

Choose two of the enzymes in the T7 folder (NruI, BclI, StuI, BglII). Digest the T7 DNA with NruI, and also perform a double digest by cutting the DNA with NruI and then with one of the other enzymes. Based on the sizes of the fragments generated, determine the relative locations

of the enzyme sites along the T7 DNA. Use the map menu on the data screen to convert the gel fragments to mapping fragments to help you put the map together.

b. Determine which genes are located in the probe binding region

After digesting the T7 DNA and running it on the gel, open the T7 probe and perform a Southern blot. The probe should bind to one fragment. Using the T7 map you created, determine where in the T7 DNA the fragment bound by the probe is located. What T7 genes are located in this region? How would you find this information?

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3. Detection of Alu insertion in human DNA

This simulation is demonstrates the detection of the PV92 Alu insert by PCR amplification that is used in many biology classes. This lab allows students to isolate and analyze their own DNA, usually from cheek cells. The human genome contains a variety of transposable elements or "jumping genes" that increase genetic variability. The PV92 locus on chromosome 16 contains a Ya5 Alu insert in some individuals. Since the presence or absence of this insert is not associated with any known phenotype, it is suitable to use with students in a classroom setting. By using primers that bind just outside the insertion site, the presence of the Alu insert can be detected based on the size of the amplified DNA. More background information is available at the DNA Learning Center (http://www.geneticorigins.org/pv92/aluframeset.htm). There are three genotypes that can be detected: Alu insert on both chromosomes 16, i.e. homozygous for the insert (+/+), absence of the Alu insert on both chromosomes (homozygous -/-), and heterozygous (+/-).

DNA representing each of the three phenotypes is provided. Several primer sets that amplify part of the PV92 Alu locus are available through biology education companies. The primers provided here will amplify a fragment of 416 bp if the Alu insert is absent, and a fragment of 731 bp if the fragment is present.

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J. Build your own case

1. DNA-based cases

Develop a case study, research problem, etc. that can be addressed using restriction enzyme digestion, PCR, gel electrophoresis, Southern blotting, dot blotting, sequence alignment, or phylogenetic trees. The general steps involved include:

- Find the relevant DNA sequence(s). This can be done by searching the GenBank database using key words. For example, the human hemoglobin gene for the sickle cell anemia case study was obtained by using the key words “hemoglobin” and “sickle”. This search actually returned dozens of sequence files that had been submitted to GenBank with notations containing the key words; one of these files was the complete human hemoglobin gene.

a. Determine how the sequence should be modified, if at all, to fit the case. Do you need to use only a portion of the gene? Do you need to create a wild type and/or a mutated version? This is often the most difficult part of preparing the case and requires some prior knowledge about the system and/or a literature review. Often the GenBank files will include information about the location of key mutations. Save sequence files generated as text-only files.

b. If restriction enzymes are needed, generate the enzyme site files (again, saved as text-only files).

c. For PCR, generate primer files. Determine from the literature which region of the DNA to amplify and which forward and reverse primers sequences will be used Create a text file containing both primer sequences (first the forward, then the reverse sequence) separated by a carriage return. Primers should be written in the conventional format, i.e. 5’ to 3’; the reverse primer sequence will be complementary to that region of the target DNA sequence. For quantitative PCR in 96-well format, a viral load value can be added to the end of the sequence; this value will be reported if the Viral Load option is selected for 96-well PCR data.

d. For Southern blotting or dot blotting, determine what portion of the sequence to use as a probe, e.g. near polymorphisms affecting restriction enzyme sites (for Southern blotting) or spanning the region containing the mutation (for dot blotting) Again, this requires literature references. Generate the probe file by copying from the sequence file and pasting into a new file or by typing a new text file. Save the probe as a text-only file.

e. Filter all of the sequence files using the GenBank filter in the program and save the filtered files.

IMPORTANT NOTE: DNA sequence files should only contain the letters A, C, G, and T. Any other letters (other than N, indicating an unknown base) should be removed to avoid an error message.

2. Protein-based cases

The steps involved for generating protein cases are very similar to those for DNA-based cases. However, unlike probes, primers and restriction enzyme where the precise sequence used in a real lab setting can be obtained, proteins are usually detected by antibodies. Antibodies may bind conformational determinants, dependent on the shape of the protein, which the Case It! software cannot simulate. Therefore, antibody files are a short amino acid sequence from one or more proteins. It is sometimes possible to find antibody binding sites reported in the literature, but in some cases it is necessary to just use a short peptide unique to that protein. The contents of the antibody file do not appear in the upper left corner of the data screen.

The Case It! simulation will calculate the molecular weight in kD based on the amino acid sequence of the protein, and the proteins will migrate on the gel according to this calculated size. If proteins samples are glycoproteins, and carbohydrates contribute to the apparent size, the weight value of the carbohydrate must be added to the calculated size in order for the protein to run accurately on the gel. Indicate this value at the end of the protein sequence between + signs. There are additional codes that are placed in the protein and antibodies files to prescribe the strength of the reaction, in order to achieve some variability among results. Contact mark.s.bergland@uwrf.edu for more information if you are developing these types of cases.

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