CHAPTER 2

Case It! - Case-Study Learning Integrating Molecular Biology Computer Simulations and Internet Conferencing

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Introduction

Case It! is an NSF-supported project initiated by participants in the BioQUEST Curriculum Consortium. The overall goal is to develop a framework for collaborative case-based learning in molecular biology using interactive computer simulations, and to have students from around the world participate in web-based "poster sessions" via Internet conferencing. An automated system allows students to create posters by entering text and uploading graphics to a server located at UW-River Falls. The system is being integrated with an electronic bulletin board so that collaborative teams of students can discuss their results with students at other institutions. This chapter describes how the Case It! computer simulations can be used to enhance case-based learning. Contact mark.s.bergland@uwrf.edu for information concerning the web page-building/conferencing system, and to learn how you and your students can participate in the project. For further reading, including an analysis of class-testing, see Bergland et al. (1999b) and Klyczek and Bergland (1996).

Materials

Software modules described in this manual can be downloaded from the Case It! web site at no cost to educators (http://www.uwrf.edu/caseit/caseit.html), and are also available as part of the BioQUEST Library CD-ROM (Bergland et al. 1999a). If you download the software, please notify mark.s.bergland@uwrf.edu to that effect so that you can be apprised of future developments regarding the Case It! project.

Results of class-testing are also available at this web site, along with procedures used at Case It! workshops conducted at the University of Nebraska-Lincoln (ABLE) and the University of Liverpool (CUBE99) during the summer of 1999.

Notes for the Instructor

Purpose of Case It! Investigator

Case It! Investigator is a customizable software tool designed to help students gather background information on cases. It includes information from the "Student Outline" below (stored as a series of linked internal html files), and also enables users to link to remote Internet sites containing information relevant to the cases. When remote links are clicked, *Investigator* automatically opens the user's default web browser to these sites, and keeps track of sites visited.

Case It! Investigator also enables you to start any software application on your hard drive via a pop-up menu, including DNA Electrophoresis (described below). Complete instructions for customizing *Investigator* to fit your needs can be accessed from the Table of Contents screen of the program.

Purpose of DNA Electrophoresis

DNA Electrophoresis is an open-ended simulation which cuts any DNA sequence with any combination of restriction enzymes and runs realistic gels and Southern blots of the resulting DNA fragments. Eight wells are available for loading, with up to 40 fragments per well visible on the screen. The simulation cannot separate fragments larger than 38 kb on a 1.2% agarose gel, or larger than 94.2 kb on a 0.4% agarose gel. This is not a difficulty for any of the sample cases included with the Resource Manual, as fragment sizes are smaller than these maxima. The size of any large fragment can be shown, however, using the Gel menu which is accessible from the Data Screen or Lab Bench. The program will also generate "mapping fragments" which can be dragged by the user to solve restriction mapping problems.

NOTE: If the 'show genomic smear' option is selected in the 'Genomic' menu, a smear will cover the actual DNA file fragments whenever a DNA file ending with the suffix '.gen' is loaded and run. A Southern blot can then be run to reveal the fragments of interest. The genomic smear does not affect migration values and fragment sizes shown in the 40 boxes to the right of the gel; these values are generated from digestion of the DNA file sequences.

Suggestions for Class Use

The example cases described here were developed for use in introductory undergraduate biology classes to help students deal with concepts and issues in molecular biology, but they can be adapted to a variety of educational settings. Some of the approaches that can be employed when using these examples in classes are described below.

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 DNA 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 DNA 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 gel results, based on the background information about the molecular biology in the case, before running the gels.
- Start with the case analysis and gel results, and carry out their own research to obtain the information necessary to interpret the case.

In addition to using these cases and sequences, the module allows instructors to develop their own cases using DNA sequences obtained from GenBank or elsewhere (See "Building your own case study"). Sequences, restriction enzyme sites, and probes 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.

Student Outline

Instructions for using *Case It! Investigator*: After starting *Case It! Investigator* the "Table of Contents" screen will appear. Click the blue question mark on this screen to activate instructions which will appear as the cursor is rolled over various regions of the screen. Click the question mark a second time to deactivate the instructions.

Any words in blue are hot links either to internal pages or Internet sites. To navigate backwards and forwards, click either the blue words in the outline at upper-left, or the arrow buttons on the button bar. Web sites visited via buttons on the button bar will be listed in the window at upper-right; you can revisit these sites by clicking any of the listings in this window. Clicking the “Print/Save” button will automatically bring up the current *Investigator* html page in your web browser, from which it can be saved or printed using the File menu of your browser. If you get an error message when you click the Print/Save button, open the file of interest directly in your

browser, then print or save it (the files are located in the "html files" folder inside the "Case It Investigator" folder).

Instructions for using *DNA Electrophoresis*: The best way to learn how to use DNA Electrophoresis is to go through the *Case It! Tutorial* program, which will lead you step-by-step through a sample case. Another way is to use the instructions in the Help window which appear on every screen. For your convenience, an overview of these instructions is also printed below.

There are four main screens in the DNA Electrophoresis simulation: the DATA SCREEN, GENBANK SCREEN, PHOTO SCREEN, and LAB BENCH.
Use the **DATA SCREEN** to quickly digest DNA, load it into wells, and run gels. Southern blots can then be run after selecting an appropriate probe:

1. Use the DNA menu above to select a DNA sample.
2. Use the SITE menu to select an enzyme.
3. Use the DIGEST menu to digest the DNA.
4. Click a well button, then click the 'load' button.
5. Repeat steps 1-4 to digest and load additional samples.
6. Use the RUN menu to run the gel.
7. Select a probe file using the SITE menu.
8. Use the RUN menu to run a Southern blot.
9. To copy and paste from Well Data window to Gel Label:
   Select text you wish to copy in the Well Data window.
   Click the COPY button in the Well Data window.
   Click PASTE to paste copied text into the Gel Label.
10. Use the EDIT menu to clear and reset all parameters.

Gels can also be set up and run on the **LAB BENCH** using simulated laboratory equipment:

1. Use the TOOLS menu to assemble a gel box (or to get a preassembled gel box).
2. Use the DNA menu to select up to two DNA samples.
3. Use the SITE menu to select up to four enzymes.
4. Add enzymes to the DNA tubes using the micropipette.
5. Use the TOOLS menu to get a Heat Block.
6. Drag the DNA tubes into the Heat Block.
7. Click the Heat Block switch (top view) to digest the DNA.
8. Drag the DNA tubes from the Heat Block.
9. Use the micropipette to load wells in the gel box.
10. Use the TOOLS menu to get a Power Source.
11. Run the gel by turning on the Power Source.
12. Use the SITE menu to select a probe.
13. Use the RUN menu to run a Southern blot.
14. Use the EDIT menu to clear and reset all parameters before running another gel.

If you are developing your own case, the **GENBANK SCREEN** enables you to access the GenBank web site and filter files so that they are ready for analysis:

1. Use the GENBANK menu to open your web browser to the GenBank web site automatically.
2. Save any part of the GenBank sequence as a text-only file.
3. Use the FILE menu to open the saved GenBank sequence.
4. Use the FILTER menu to remove everything but the DNA sequence from the GenBank file.

5. Digest the DNA sequence using either the Data Screen or the Lab Bench.

The **PHOTO SCREEN** is used to compare photos taken of current gels and also to view photos taken of previous gels which have been saved as PICT or JPEG files. Labels are editable, and information can be copied and pasted from the Well Data window to any photo label.

1. Use the SAVE buttons to save photo 1 or photo 2 as PICT or JPEG files.
2. Use the REPLACE buttons to replace the photos on this screen with previously saved gel photos.
3. Use the ROTATE buttons to rotate horizontal photos into a vertical configuration (PC version only).

Sample cases

The DNA sequences for the cases described here are located in the Case It! examples folder. The necessary enzymes and probes for a particular case will be located in the same folder as the DNA sequences.

A. Human genetic diseases

Genetic diseases are caused by alterations 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 restriction enzyme digestion and Southern blotting. The following examples illustrate different types of DNA alterations associated with human genetic diseases.

Case 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.

The case: 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

Digest each of these DNA samples with MstII. Then use the probe corresponding to the region of the hemoglobin gene mutated in sickle cell anemia to determine the genotype of each individual.
[Note: There are three scenarios (A, B, and C) for this case, each with a potentially different outcome.]

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

Case 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. 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. Huntington's disease is considered a dominant disorder, since one copy of the amplified gene appears to be sufficient to cause disease symptoms.

The case: 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) Uncle (unaffected)
Father (affected) John (brother)
Aunt (affected)

Digest the DNA samples with EcoRI, and the 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.
[Note: There are two different scenarios (A and B) for this case, each with a potentially different outcome.]

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

Case 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.

The case: 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) 8-year-old son (possibly affected)
oldest son (unaffected) 7-year-old son (possibly affected)
daughter
[Note: There are three different scenarios (A, B and C) for this case, each with a potentially different outcome.]

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.)

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

Case 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 β-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 Glu to Gly mutation and the codon 717 Val to Ile mutation. The 693 mutation results in the loss of a MboII site, while the 717 mutation results in the gain of a BclI site.

The case: 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 wild type APP
Control 693 mutation
Control 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**.]

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

Case 5. 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 points 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 allele-specific oligonucleotide (ASO) probes to detect the presence of specific 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.

The Case: 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.

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 digested DNA is run on a gel and hybridized 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. Control DNA samples, known to have a specific mutation, are included.

Probes: 185delAG (AG deletion in exon 2)
4184delTCAA (TCAA deletion in exon 11)
5382insC (C insertion in exon 13)
Control BRCA1 (conserved region of BRCA1; this probe should bind to all DNA samples)

DNA samples: Elizabeth
Mother
Unrelated woman
Control 185delAG
Control 4184delTCAA
Control 5382insC
Control wild type BRCA1 (no mutations)

To analyze the DNA, load each DNA sample into the gel and run the gel. Then run a Southern blot with the various probes to see whether any of these mutations are present. The probe stringency must be set to 100% match.

Note: There are four different scenarios (A, B, C, and D) for this case, each with potentially different results

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

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 allele-specific oligonucleotide (ASO) probes.

Rapid screening for cystic fibrosis is also done using RFLP markers linked to the CF gene on chromosome 7. 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 ASO 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 below involves the use of RFLP markers linked to the CF gene, while **Case B** uses detection of the F408 deletion mutation within the CF gene using ASO probes.

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. A small portion of chromosome 7 near the CF gene, a locus called Mp6d.9, is amplified by PCR. Digestion 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.]

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

Case B: (Contributed by 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 detect if they are carriers and 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	Control (F508 mutant sequence)
Fetus	Control (wild type sequence)
Jim	

Procedure: Load each DNA sample into a well, and run the gel for 60 minutes. Next, perform a Southern Blot on the same gel using the wild type probe. Re-load and run the gel using the same DNA sequences as above and run a second Southern Blot on the same gel using the CF mutant probe. By comparing the Southern Blots of Jim, Lynda, and the fetus to those of the two controls, determine whether these DNA samples are homozygous positive for CF, homozygous negative for CF, or heterozygous carriers for CF.

- What conclusions can you draw from the results obtained?
- If you were put in Jim and Lynda's situation, would you choose to be tested to see if you were a carrier for CF? Would you choose to have your unborn child tested?
- Should there be mandatory genetic testing to determine who are carriers of CF?
- What options are available to the parents?
- If gene therapy was available to your unborn affected child, would you use it?

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 from 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 RFLP analysis of PCR-amplified DNA from the blood sample; the mutation causes the loss of a BsaI site. The RFLP test also allows prenatal testing.

The case: 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 are tested to confirm their carrier status and predict the risk of PKU in subsequent offspring.

PCR is used to amplify a portion of the phenylalanine hydroxylase gene from a blood sample. The amplified DNA is then digested with BsaI to detect the RFLP associated with PKU.

DNA samples:	Peter	Control wild type (no mutation)
	Pam	Control PKU mutation
	Infant	

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

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.

The Case: 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

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

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

9. Tay-Sachs Disease

(Contributed by Douglas Johnson, University of Wisconsin-River Falls)

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, that 18% have a G to C transversion causing an intron 12 spice-junction error and that 3% have an exon 7 missense mutation which substitutes a serine for glycine at position 269 and is associated with adult TSD.

The Case: When Megan and Greg announced their plans to get married, Megan's mother, Rachel, finally explained 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 told Greg this news, he questioned his parents and learned that they had chosen not to be tested because of fear of stigmatization and discrimination. Greg and Megan are now faced with some difficult decisions of their own.

NOTE: A complete analysis of DNA sequences associated with this case involves PCR techniques that have yet to be added to the Case It software. See the Case It web site below for information regarding this case, and also to download DNA sequences and software updates as they become available.

Case It! home page: http://www.uwrf.edu/caseit/caseit.html

B. 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. 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) suspect 1 (ex-husband)
blood spot 2 (from glove) suspect 2 (acquaintance)
victim's blood

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

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 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-line 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. The alleles are detected by Southern blotting and RFLP analysis. It is possible to determine whether two individuals are closely related by comparing how frequently their alleles match.
NOTE: A complete analysis of DNA sequences associated with this case involves PCR techniques that have yet to be added to the Case It software. See the Case It web site below for information regarding this case, and also to download DNA sequences and software updates as they become available.
Case It! home page: http://www.uwrf.edu/caseit/caseit.html

C. 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:

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?
   You also can modify the probe search properties (in the Site menu): the default setting is the first 10 bases of the probe have to match for a fragment to be highlighted (regardless of stringency setting); decreasing this requirement may generate more data by highlighting more fragments.
   Use the "calculate and display probe match percentages" under the Run menu to obtain quantitative data on the extent of similarity between the probe and DNA files.

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).

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

D. 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)

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 genes 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?

E. Build your own case

Develop a case study, research problem, etc. that can be addressed using restriction enzyme digestion and/or Southern blotting. The general steps involved include:

a. 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 some notation containing the key words; one of these files was the complete human hemoglobin gene.

b. 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 literature review. Often the GenBank files will include information about the location of key mutations. Save sequence files generated as text-only files.

c. Determine what restriction enzymes, if any, are needed, and generate the enzyme site files (again, saved as text-only files).

d. For Southern blotting, determine what portion of the sequence to use as a probe, e.g. near polymorphisms affecting restriction enzyme sites. 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. Remember to 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.

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Appendix: Key to cases

This section of the manual explains how each case was developed and describes the expected outcome. For some cases, multiple scenarios with different outcomes have been included. Instructors can rename and reorganize sequence files to change the outcome of a case.

The key below is available online, and can be assessed automatically from *Case It! Investigator* (contact mark.s.bergland@uwrf.edu for the free password that will permit *Investigator* to access these web pages).

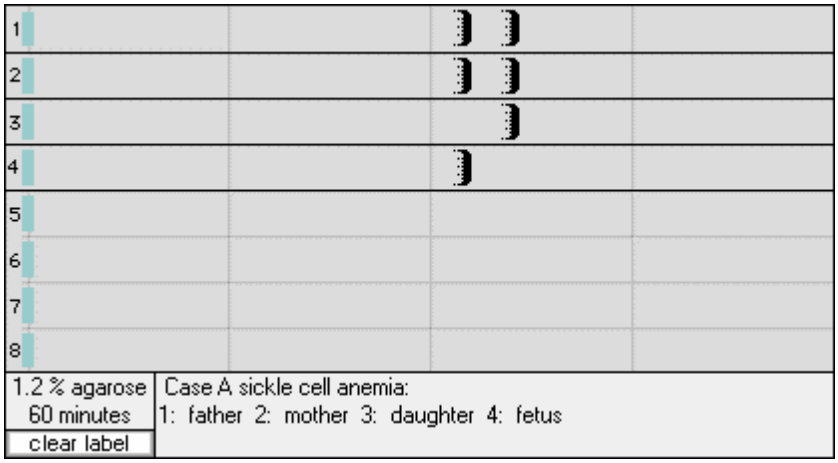
IMPORTANT NOTE: To create the heterozygous DNA files included with this program, the mutated, filtered sequence was pasted at the end of the wild type, filtered sequence, separated by a hard return. The homozygous files include two copies of the wild type or the mutated sequences. This is to represent the two alleles found in each body cell. The Case It! program tallies the numbers of cuts on this representative pair of genes.
Like the software itself, this is a beta version of the manual. More information will be added - keep checking the ftp site for updated versions. Suggestions and other comments are welcome.

A. Human genetic diseases
1. Sickle cell anemia

The DNA sequence files are the human beta globin gene, on chromosome 11, locus HUMHBB from GenBank, 73,308 bp. To create the sickle cell mutation, nucleotide 62206 was changed from A to T. The probe corresponds to nucleotides 61023 to 61210 and hybridizes to the fragment which will be larger if the MstII site is eliminated by the mutation at position 62206. The enzyme MstII recognizes the site CATNAGG, where N can be either A, C, T, or G. The Case It! program searches sequentially for each of these 4 forms of the restriction enzyme, and cuts the sequences accordingly.

Mutation/technique illustrated: Point mutation generating loss of restriction site detected by RFLP; mutated fragment is larger.
Based on Fig. 27-9 in Watson et al., *Recombinant DNA*, 2nd ed., Scientific American Books, 1992.
There are three cases, each with a different outcome and therefore different issues are raised (see gel photo):

Case A. father - heterozygous (carrier)
mother - heterozygous (carrier)
daughter - homozygous wild type
fetus - homozygous sickle (affected)



Issues: Fetus will be born with sickle cell anemia. Should the pregnancy be terminated? Should the parents have additional children knowing they are carriers?

Case B. father - heterozygous (carrier)
mother - homozygous wild type
daughter - heterozygous (carrier)
fetus - homozygous wild type

Issues: Fetus is OK, but daughter is a carrier. Should she be counseled about whether to have children?
Father is a carrier. What if the parents want to have additional children? Although they cannot have a homozygous sickle child, should they risk passing along the sickle gene?

Case C. father - homozygous wild type
mother - heterozygous (carrier)
daughter - homozygous wild type
fetus - heterozygous (carrier)

Issues: Would the parents be justified in terminating the pregnancy because the child would be a carrier? Should the parents have any more children and risk passing along the sickle gene again?

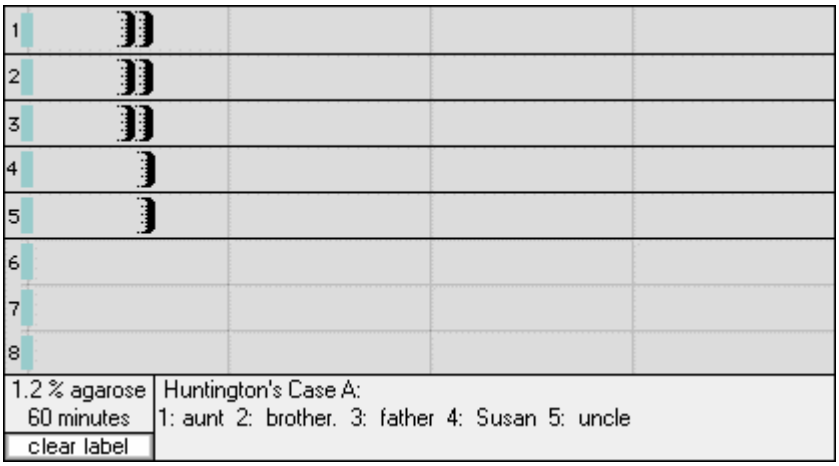
Case 2. Huntington's chorea

The DNA sequence files are from the Huntington's disease gene sequence, GenBank accession #L12392, locus HUMHDA, 10,348 bp. To create the Huntington's mutation, 200 additional CAG repeats were inserted at nucleotide position 363 (the wild type sequences contains 24 repeats). The probe corresponds to nucleotides 4390 to 5160.
Mutation/technique illustrated: Triplet nucleotide amplification detected by Southern blotting and RFLP; mutated fragment is larger.

Reference: MacDonald, M. and Ambrose, C.M., Cell 72 (6), 971-983, 1993.

There are two cases, each with a different outcome:

Case A. Susan - homozygous wild type (unaffected)
father - heterozygous
aunt - heterozygous
uncle - homozygous wild type
John (brother) - heterozygous (affected)



Case B. Susan - heterozygous (affected)
father - heterozygous
aunt - heterozygous
uncle - homozygous wild type
John (brother) - homozygous wild type (unaffected)

Issues: Should someone with a family history of HD be required to undergo testing? Should they have children if they test positive, or if they have not been tested? Should someone as young as John be tested? Should the results of such tests be made available to insurance companies? to potential employers? to potential mates?

Case 3. Duchenne's muscular dystrophy

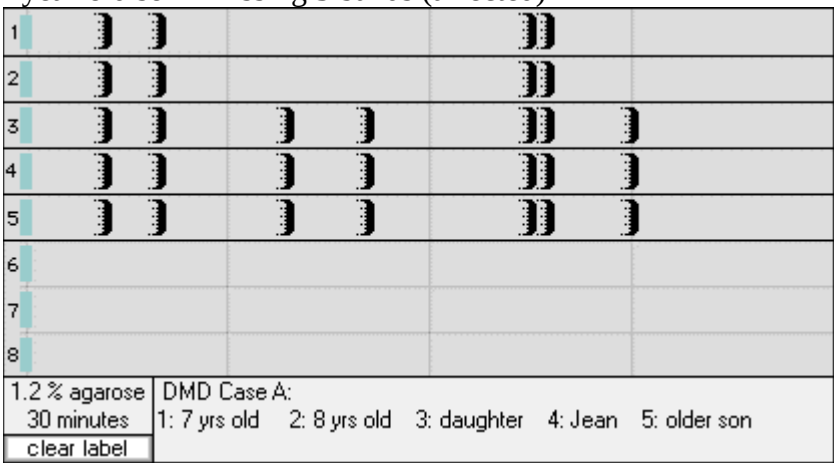
These sequences are somewhat contrived in order to demonstrate the loss of exons associated with DMD. The DNA sequence files are from the human dystrophin gene sequence, exons 1, 2, 7-9, 44, 48, 60 and 61, GenBank accession #L01538, U60822, M23261, M86844, M86885.

Note that the human dystrophin gene contains over 70 exons, not all of which have been sequenced. The separate GenBank containing the indicated exons (as well as some associated intron sequences) were joined together, with the individual sequences separate by HindIII sites. To simulate the "cocktail" of probes that would be used to hybridize to each exon, 22 bp from exon 61 is inserted at the beginning of each exon in the cDNA. After digestion, electrophoresis and Southern blotting each exon fragment is highlighted. The mutated files were created by deleted 3 (exons 7-9) or 4 (exons 1-9, 44) exon sequences.

Mutation/technique illustrated: Exon deletion detected by Southern blotting and RFLP; mutation results in missing fragments on Southern results.
References: Watson et al., *Recombinant DNA*, 2nd ed., Scientific American Books, 1992, Fig. 27-8; Hoffman,E.P., et al. Science 238 (4825), 347-350, 1987.

There are three cases, each with a different outcome:

Case A. Jean (mother) - all bands (exons) present
Oldest son - same as mother, all exons present
Daughter - same as mother, all exons present
8-year-old son - missing 3 bands (affected)
7-year-old son - missing 3 bands (affected)



Since DMD is X-linked, Jean is probably heterozygous. However, that can't be determined from the Southern blot since as long as she has one wild-type sequence all exons will be present and missing exons generally can't be observed. Similarly, it can't be determined whether the daughter is homozygous or heterozygous. The oldest son inherited the wild type X chromosome, while the younger sons inherited the chromosome bearing the mutation.

Case B. Jean (mother) - all bands (exons) present
Oldest son - same as mother, all exons present
Daughter - same as mother, all exons present
8-year-old son - missing 3 bands (affected)
7-year old son - missing 4 bands

These results are similar to case A, except that the youngest son appears to have an extra deletion (or a longer deletion). Explanations for their difference include: a) A spontaneous additional deletion in the youngest son's DMD gene, occurring in the egg cell or early zygote; b) Jean is not the biological mother of both boys.

Case C. Jean (mother) - all bands (exons) present
Oldest son - same as mother, all exons present
Daughter - same as mother, all exons present
8-year-old son - missing 3 bands (affected)
7-year old son - same as mother, all exons present

In this case, the youngest son appears to be genotypically normal, although he was beginning to show some symptoms. Explanations include: a) The youngest son has different deletions or some other mutation in the dystrophin gene that are not picked up by this test; b) The youngest some is "mimicking" his brothers symptoms, either consciously or subconsciously; c) The parents are imagining or exaggerating physical characteristics in the youngest son which are similar to DMD symptoms in the 8-year-old.

Issues: If the mother is a carrier, is she justified in having additional children? Who should have access to the test information?

Case 4. Alzheimer disease

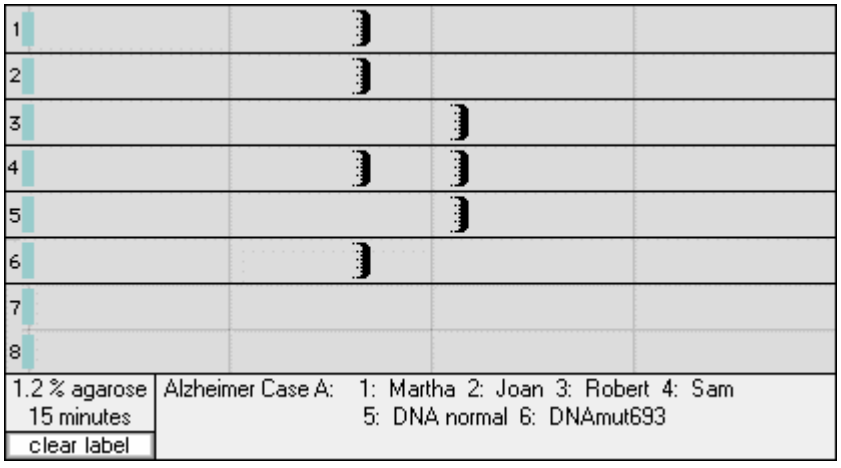
The DNA sequence files are from the human APP gene, GenBank accession number A33293, 2265 bp. Mutated sequence files were created by introducing point mutations at codons 693 (G to C) and 717 (G to A).

Mutation/technique illustrated: Point mutation resulting in loss (693) or gain (717) of restriction site detected by Southern blotting and RFLP; 693 mutation results in a larger Mbo I fragment; 717 mutation results in a smaller Bcl I fragment.

Reference: Kamino, K. *et al.* Am. J. Hum. Genet. 51:998-1014, 1992.

There are two cases scenarios, each with a different outcome:

A. Martha (mother) - homozygous 693 mutation
Sam (son) - heterozygous 693 mutation (possible increased risk)
Joan (daughter) - homozygous 693 mutation (definite increased risk)
Robert (son) - homozygous wild type



B. Martha (mother) - homozygous 717 mutation
Sam (son) - homozygous wild type
Joan (daughter) - heterozygous 717 mutation (possible increased risk)
Robert (son) - homozygous 717 mutation (definite increased risk)

Issues: Does the lack of one of these mutations mean that the person cannot get Alzheimer disease? Does the presence of one of these mutations mean that the person will definitely contract AD? Should someone with a family history of AD be required to undergo testing? Should they have children if they test positive, or if they have not been tested? Should the results of such tests be made available to insurance companies? to potential employers? to potential mates?

Case 5. Breast Cancer

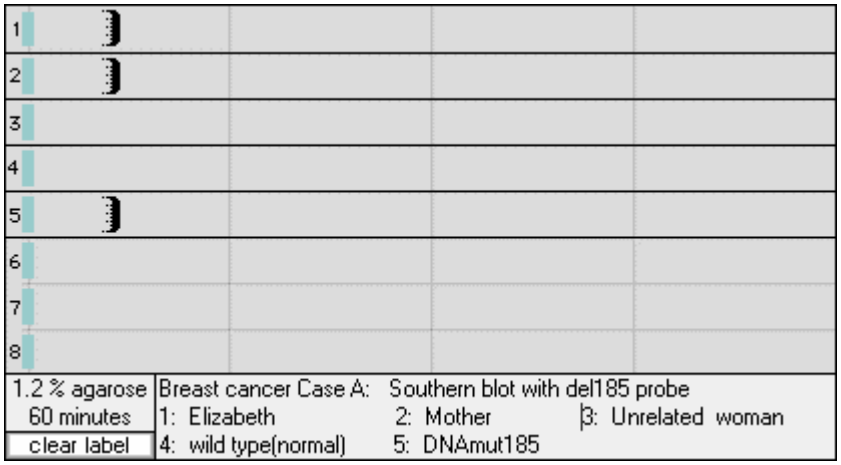
The DNA sequence files are from the human BRCA1 gene, GenBank accession number U14680, locus HSU14680, 5711 bp. Mutated sequence files were created by introducing the indicated nucleotide deletions or insertions into the text file prior to filtering. The probes are approximately 20 bp sequences spanning the mutation region and containing the mutated sequences. The wild type probe is from a BRCA1 region outside any of the mutated regions so it is unchanged in all sequence files.

Mutation/technique illustrated: Detection of a deletion mutation in the disease gene using ASO probes in Southern blots.

Reference: Friedman, L.S. *et al.*, Am. J. Hum. Genet. 57:1284-1297, 1995.

There are four case scenarios, each with different outcomes:

A. Elizabeth - positive for 185delAG mutation
Mother - positive for 185delAG mutation
Unrelated Woman - positive for 5382insC mutation



B. Elizabeth - no mutations detected
Mother - positive for 185delAG mutation
Unrelated Woman - positive for 4184delTCAA mutation

C. Elizabeth - positive for 185delAG mutation
Mother - no mutations detected
Unrelated Woman - positive for 5382insC mutation

D. Elizabeth - positve for 4184delTCAA mutation
Mother - positive for 4184delTCAA mutation
Unrelated Woman - positive for 185delAG mutation

Issues: If either Elizabeth or her mother test positive, is a prophylactic double mastectomy appropriate? Does the lack of any of these mutations mean that the women will not get breast cancer? Who should have access to these test results? Does the daughter have the right to know the results? She is only seven now, but what about when she is 16? Should this type of screening be mandatory?

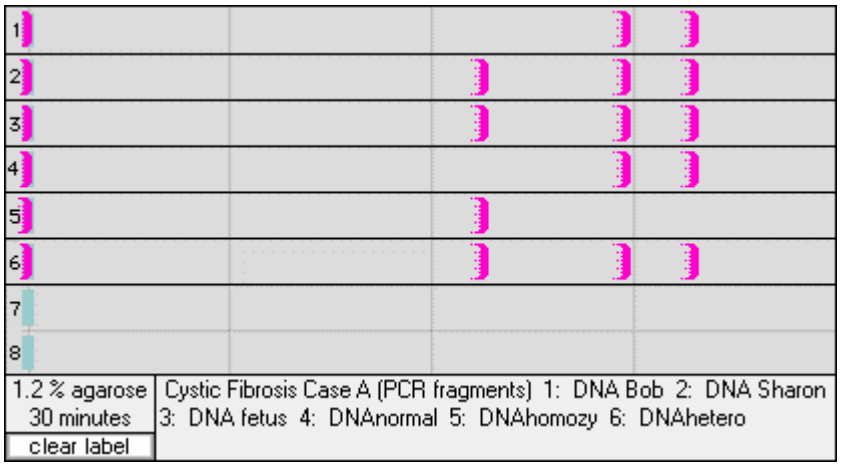
Case 6. Cystic fibrosis

Case A. The DNA sequence files are from the human MP6d9 gene, GenBank accession #M37523, 383 bp, which is linked to the cystic fibrosis gene on chromosome 7. This simulates a PCR-amplified region near the CF gene. The mutated sequence files were created by replacing the G to A base change at nucleotide 170 which causes the loss of an Msp I site. No Southern blotting is necessary to observe the mutation.

Mutation/technique illustrated: RFLP marker outside of the actual disease gene, but genetically linked to the disease gene.

Reference: Huth,A., et al., Nucleic Acids Res. 17 (17), 7118, 1989.

Results:
Brown family:
Sharon - heterozygous
Bob - homozygous wild type
fetus - heterozygous



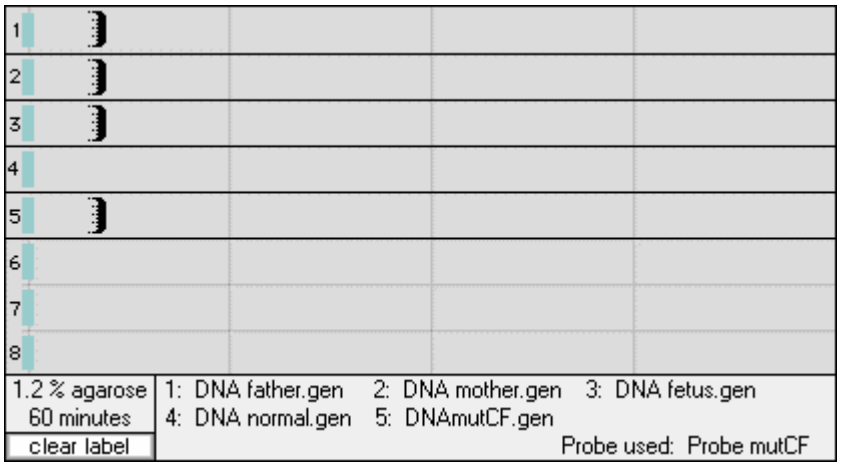
Jones family:
Jill - heterozygous
Mike - heterozygous
fetus - homozygous CF mutation

Smith family:
Carol - heterozygous
Ron - heterozygous
fetus - homozygous wild type

Issues: See Sickle Cell case.

Case B. The DNA sequences are from the human CFTR gene, GenBank accession #M28668, 6129 bp. The mutated sequence files were created by deleting nucleotides 1653-1655 (delTTC). The wild type probe is 25 bp spanning this region, while the CF probe contains the TTC deletion.

Mutation/technique illustrated: Detection of a deletion mutation in the disease gene using ASO probes in Southern blots.



Reference: Riordan et al., Science 245:1066-1073, 1989.

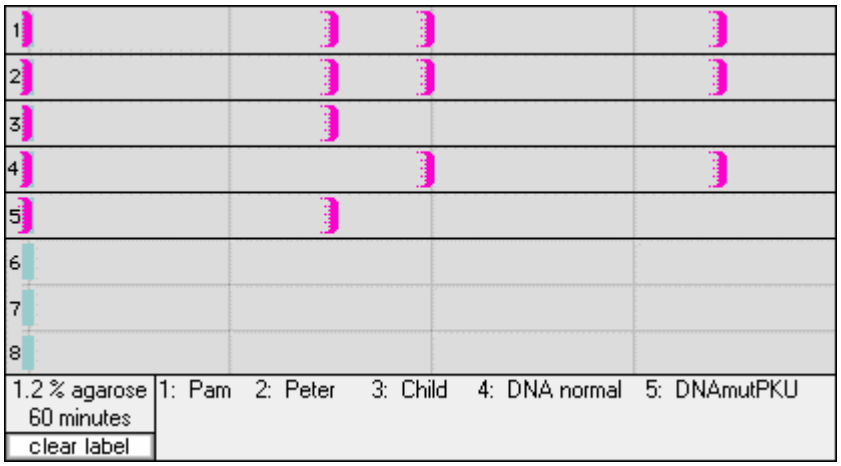
Results:

Jim - heterozygous
Lynda - heterozygous
fetus - homozygous CF mutation

Case 7. Phenylketonuria

DNA sequences are from the human phenylalanine hydroxylase (PAH) cDNA, GenBank accession #K03020, 2429 bp. The mutated file was created by introducing a base change (C to T) at the position corresponding to amino acid 408.

Mutation/technique illustrated: Point mutation resulting in loss of a restriction enzyme site, detected by PCR amplification of that region followed by restriction enzyme digestion; mutation results in one larger fragment instead of two smaller fragments.



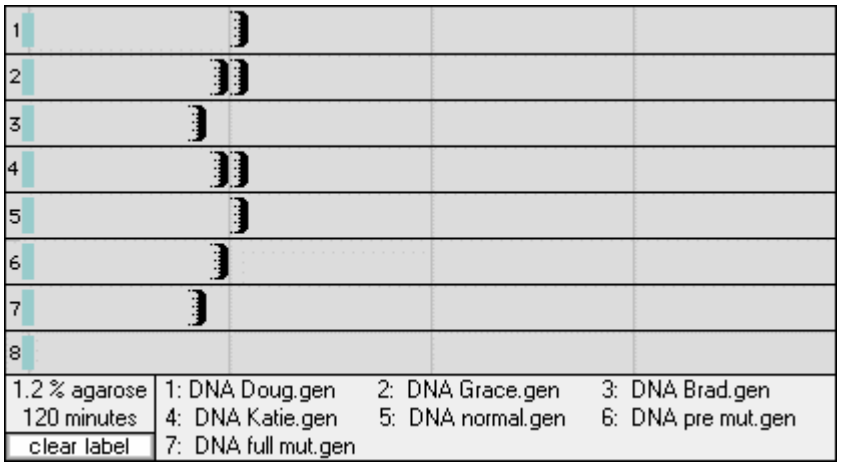
Reference: Zschocke, J. *et al.* Am. J. Hum. Genet. 57:1311-1317, 1995

Case 8. Fragile X Syndrome

The DNA files are from the human *fmr1* gene, GenBank accession #L29074 and L38501, 185,775 bp. The first 20,000 nucleotides were used for the files to reduce size and make them manageable. Mutated files were created by inserting 100 (premutation) or 200 (full mutation) cgg repeats into position 13833. EcoRI is used because it cuts outside of the repeat region.

Note that relatively large fragments are generated; using 0.4% agarose and/or longer run times helps separate the fragments better.

Mutation/technique illustrated: Similar to Huntington's disease (triplet nucleotide amplification detected by Southern blotting and RFLP; mutated fragment is larger) with the addition of an intermediate pre-mutation.



Results:

Doug - wild type

Grace - heterozygous pre-mutation

Brad - full mutation
Katie - heterozygous pre-mutation
fetus - wild type (homozygous if female)

Note: These results suggest that Brad's X chromosome exhibits expansion from pre-mutation to full mutation, if in fact Doug and Grace are his biological parents.

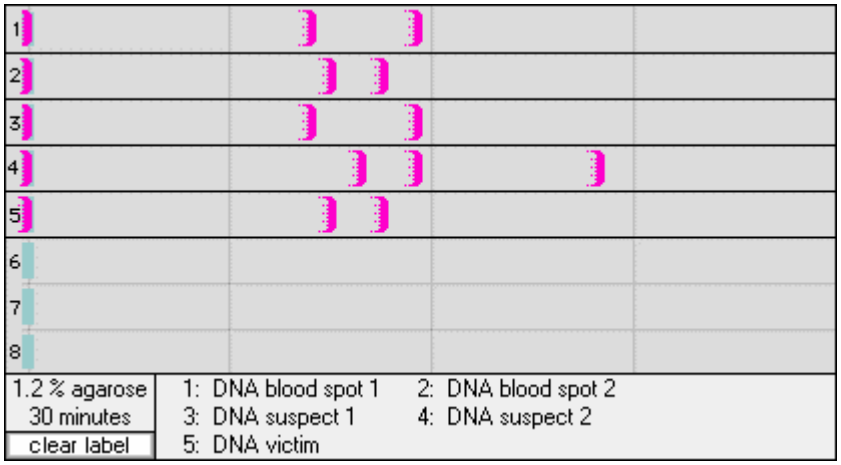
References: American College of Medical Genetics, http://www.faseb.org/genetics/acmg/pol-16.htm
Verkerk,A.J.M.H., et al., Cell 65 (5), 905-914, 1991.

B. Forensics

1. Murder Case

Note that there are three scenarios (A, B, and C) each with a different outcome:

a. Blood spot 1 = ex-husband's blood
Blood spot 2 = victim's blood



b. Blood spot 1 = acquaintance's blood
Blood spot 2 = victim's blood

c. Blood spot 1 = victim's blood
Blood spot 2 = no match with either suspect

C. Phylogenetic studies

1. Primate relationships - human, chimp, gorilla

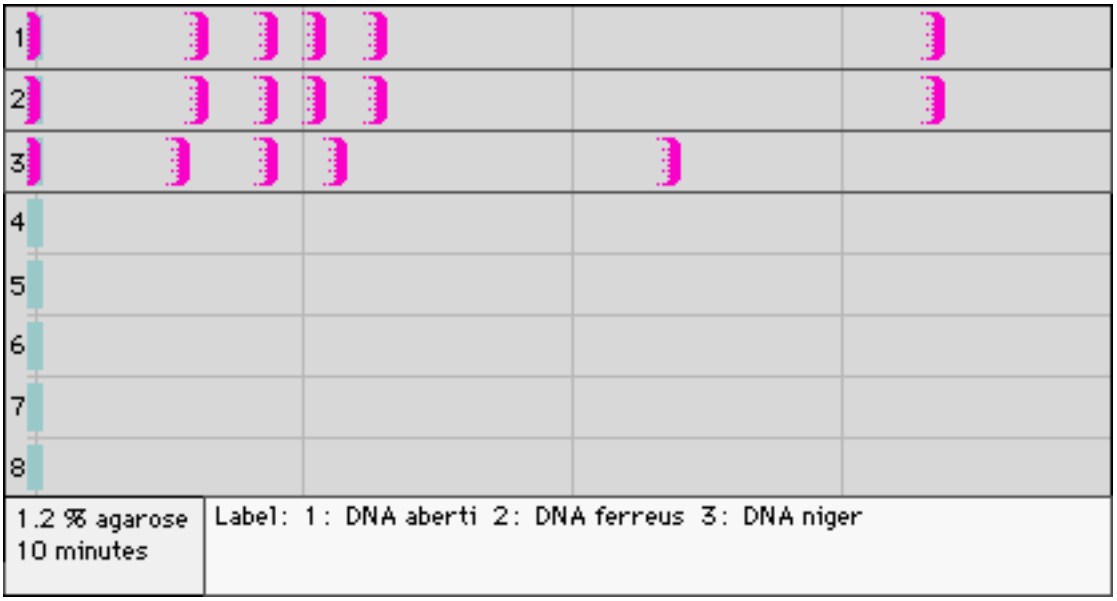
The DNA sequence files are from the human, chimpanzee (*Pan troglodytes*), and gorilla (*Gorilla gorilla*) beta globin genes, GenBank locus HUMHBB (human), CHPHBBPCH (chimp), and GORHBBPG (gorilla). The chimp and gorilla sequences are 7,025 and 7,055 bp, respectively.

This case is the most "open-ended" in that students chose which enzyme or combination of enzymes and probes to use and it encourages follow-up exploration. The general consensus is

that humans and chimpanzees are more closely related to each other than either is to gorillas. However, depending on which enzyme(s) are used for restriction digestion, other relationships may be demonstrated.
Reference: Miyamoto, M.M., J.L. Slighton, and M. Goodman. Science 238:369-372 (1987)

2. Squirrel taxonomy

DNA sequence files are from the mitochondrial cytochrome b gene of *Sciurus aberti aberti* (GenBank accession #10163), *Sciurus aberti ferreus* (#10171), and *Sciurus niger* (#10180). Using the enzyme Alu I, the two *Sciurus aberti* subspecies will yield identical patterns, while the *S. niger* DNA will generate a different pattern.



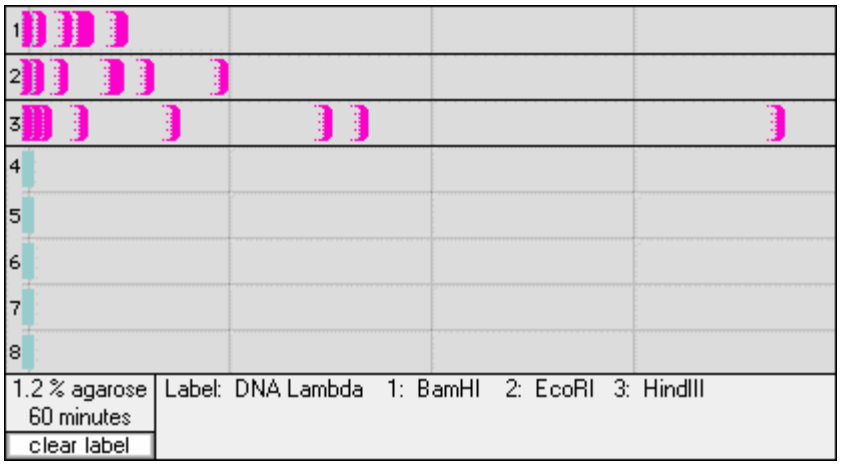
Like the primate example above, this case could be expanded to include other enzymes. Additional extensions could include generating probes from one of the sequences and using the "calculate and display probe match percentages" featured under the Run menu to obtain more quantitative data on the extent of similarity between the sequences.

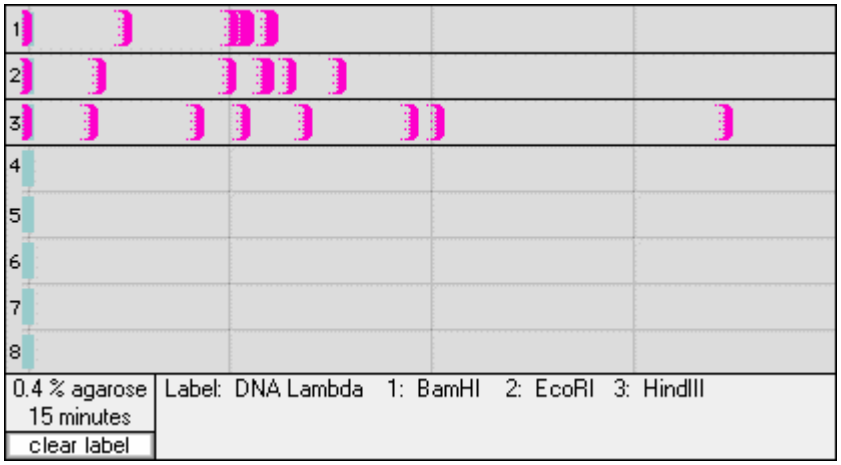
Reference: Wettstein,P.J. *et al*. Mol. Phylogenet. Evol. 4 (2), 150-162 (1995)

D. Simulation of wet labs

1. Digestion of bacteriophage lambda DNA

The DNA sequence file is from the Lambda genome, GenBank locus LAMCG, 48502 bp. Many users will be familiar with the fragment patterns for these three digestions (see reference). Note that even the smallest fragments, normally not visible on agarose gels, will be seen in the simulation. Both 1.2% and 0.4% digestions are shown below:

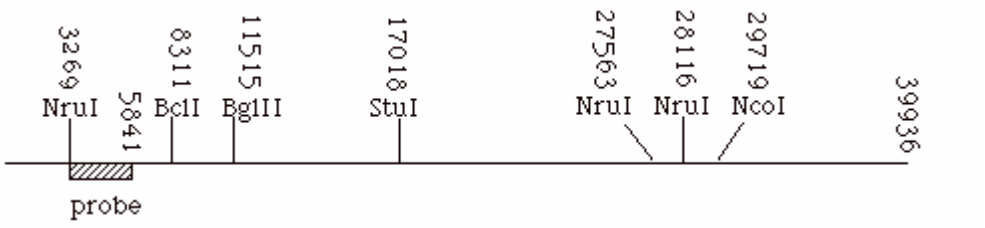




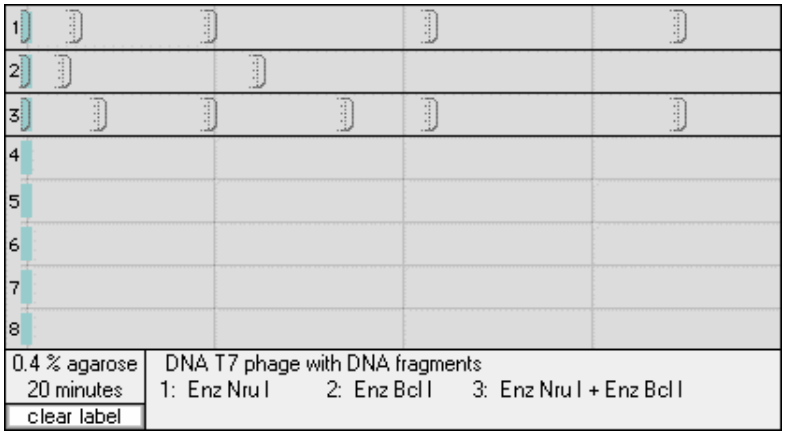
Reference: Micklos, D.A. and D.A. Freyer, *DNA Science*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990, p. 268.

2. Mapping of bacteriophage T7 DNA

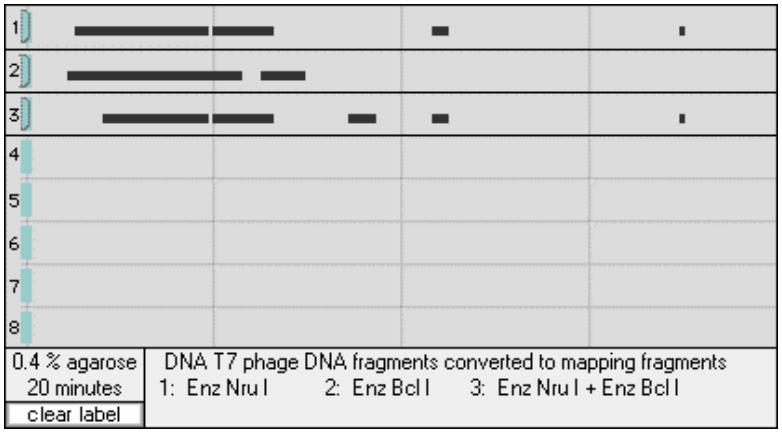
The DNA sequence files are from the bacteriophage T7 genome, GenBank locus T7CG, 39,937 bp. The restriction map of T7 is below:



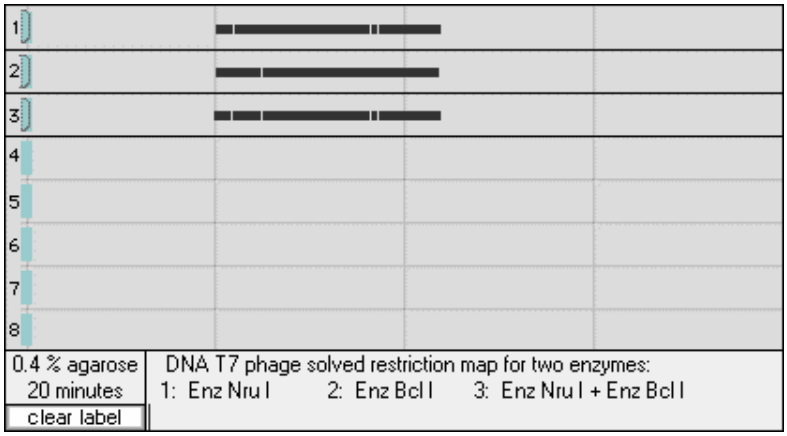
Example: To solve the restriction map for two enzymes (Nru and Bcl), first run a single digest using Nru, a single digest using Bcl, and a double digest using Nru and Bcl:



Next, use the Map menu of the Digest Screen to convert the above DNA fragments into "mapping fragments" proportional in width to the size of the fragments in base pairs:



Finally, drag the individual mapping fragments around within each lane until the map is solved, as shown below:



This procedure could be repeated to map the other enzyme sites for the T7 phage.
Reference: Dunn, J.J. and Studier, F.W. J. Mol. Biol. 166 (4), 477-535 (1983).