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Chapter 3

Case It! Case Study Learning via Simulations of Molecular Biology Techniques

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Professor Karen Klyczek is Chair of the Biology Department at UW-River Falls. She was the PI for two NSF Teacher Enhancement Awards to provide biotechnology in-service training for secondary life science and agriculture education teachers. She has presented at numerous workshops and conferences dealing with biotechnology education, and has received NIH funding for the study of gene regulation in tumor cells. In 2000, she was named the University Distinguished Teacher at UW-River Falls, and is a past recipient of the Outstanding Teaching award for the College of Arts and Sciences, Science Division.

Developed by Karen Klyczek, Kim Mogen, Douglas Johnson, and Mark Bergland, University of Wisconsin-River Falls, with contributions from UWRF Biology students and a network of beta-testers in the U.S. and Canada. Contact mark.s.bergland@uwrf.edu for additional information. This project was supported, in part, by the National Science Foundation. Opinions expressed are those of the authors and not necessarily those of the Foundation. Copyright 2004

Contents

Introduction

Case It! is NSF-supported project initiated by participants in the BioQUEST Curriculum Consortium. The 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; no knowledge of html is required. The system is integrated with an electronic bulletin board so that collaborative teams of students can discuss their results with students at other institutions.

Software modules (both Macintosh and Windows versions) can be downloaded from the Case It web site at no cost to educators (http://www.uwrf.edu/caseit/caseit.html). Results of class testing are also available at this web site. Contact Mark Bergland (*mark.s.bergland@uwrf.edu)* for additional information concerning the project, and to learn how you and your students can participate.

This manuscript is an abbreviated version of the Resource Manual, which can be downloaded from the Case It! Site at http://www.uwrf.edu/caseit/caseit.html. In this paper, we describe a number of cases developed using DNA sequences obtained from GenBank via the Internet.

Case It! Investigator

Case It! Investigator is a software tool that students can use to gather background information on cases using internal links within *Investigator* itself, and external links to Internet sites. In essence, *Investigator* is an interactive version of the Resource Manual with hyperlinks to various sections of the manual, viewed as html pages (a password is required for access to the Key to Cases section, however. Instructors can obtain this password by emailing *mark.s.bergland@uwrf.edu)*. In addition, Investigator includes pop-up menus that enable users to access important Internet sites providing background information, especially those relating to human genetic diseases.

Case It! software

Case It! is an open-ended simulation which includes restriction digestion and mapping, polymerase chain reaction (PCR), DNA electrophoresis, Southern blotting and dot blotting analysis. The restriction digestion simulation will cut any DNA sequence with any combination of restriction enzymes and runs realistic gels and Southern blots of the resulting DNA fragments. The software download includes cases and DNA sequences for breast cancer, Alzheimer's disease, sickle-cell disease, Huntington's disease, PKU, Tay-Sach's disease, cystic fibrosis, DMD, and fragile X, as well as forensics and phylogenetic cases. Students can also design their own cases.

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 Gel/Southern Blot or Lab Bench screen.

PCR simulation will generate the DNA fragments that would be amplified using the primer sequences supplied. These fragments can be run in the gel electrophoresis simulation to analyze the sizes of the fragments or to carry out Southern blotting, or they can be used in the dot blot simulation. The dot blot simulation will determine whether there is a match (hybridization) between the DNA applied to a dot and the DNA added to the hybridization solution (represented by the corresponding "square" surrounding the dot). The DNA loaded in the square would be labeled and would hybridize to the dot DNA if there is a sequence match. Hybridization is detected by the dot turning dark because of the labeled DNA sticking to it. The relationship between a probe and a DNA sample may be the reverse of what is used for Southern blotting, in that unlabeled probe is often applied to the dot, while the sample DNA (e.g. from a patient in a human genetic disease case) is labeled (usually by PCR amplification using labeled primers) and allowed to hybridize to the probe.

There are five main screens in the DNA Electrophoresis simulation:

1) Use the **Digest DNA/PCR Screen** to quickly digest DNA and load it into wells, or to generate PCR fragments and load them into gels, if appropriate.
2) Use the **Gel/Southern Blot Screen** to run Southern blots. This screen shows the gel, along wityh migration or size values for DNA fragments in each lane.
3) Use the **Dot Bot Screen** to determine whether there is a sequence match between DNA samples and probes.

4) The **Genbank Screen** enables you to access the GenBank web site and filter files so that they are ready for analysis:
5) Gels can also be set up and run on the **Lab Bench Screen** using simulated laboratory equipment.

In addition, the Gel/Southern Blot and Dot Blot screens have **PHOTO SCREENS** for documenting results:

- The **Gel Photo Screen** is used to compare photos taken of current gels and 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.
- The **Dot Blot Photo Screen** can be used to record dot blot results.

Suggestions for Class Use of Case It!

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 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" on page 15). 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

Example cases

The DNA sequences for the cases 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. (Provided here are just a few of the many cases included in the Case It! Software modules. Keys to the cases are found in the "Instructor's Notes" section of this manuscript.)

A. Human genetic diseases

Genetic diseases are caused by alterations in the DNA that 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.

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 because 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.

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)

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.

a. What conclusions can you draw from these results?
b. What is the molecular basis of this disease, and why does this result in the observed gel patterns?

c. How would you counsel Susan and her brother based on the results of the test?
d. What issues are raised by this type of testing?

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 that 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.]

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

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 contributes 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. DNA samples known to have specific mutation also are included. If a mutation is detected, use the probe corresponding to the normal sequence for that mutation site to determine whether the individual is homozygous or heterozygous for the mutation.

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

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

B. Forensics

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)
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.)

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

C. Phylogenetic studies

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?

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

D. Build your own case

Develop a case study, research problem, etc. that can be addressed using restriction enzyme digestion, PCR, gel electrophoresis, Southern blotting and/or dot 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 notations 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 a literature review. Often the GenBank files will include information about the location of key mutations. Save sequence files generated as text-only files.

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

d. 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.

e. 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. Remember to save the probe as a text-only file.

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

Instructor's Notes

Keys to cases

This section how each case presented here was developed and describes the expected outcome. Note that instructors can rename and reorganize sequence files to change the outcome of a case as desired.

IMPORTANT NOTE: To create the heterozygous DNA files included with this program, the mutated, filtered sequence was pasted at the end of the normal, filtered sequence, separated by a hard return. The homozygous files include two copies of the normal 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. In reality, many copies of each gene would be present on a gel because of the number of cells involved when preparing DNA samples, so many more cuts would be made in an actual digest.

Human genetic diseases

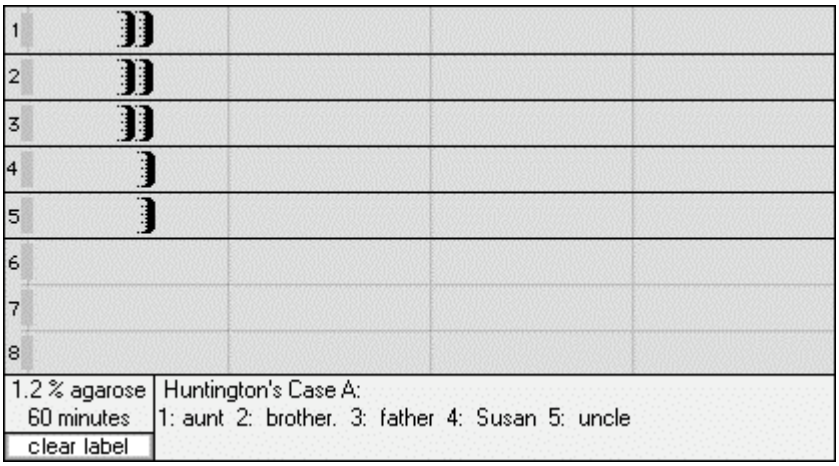
Huntington's chorea

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.

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 the results of such tests be made available to insurance companies? to potential employers? to potential mates?
Should someone as young as John be tested?

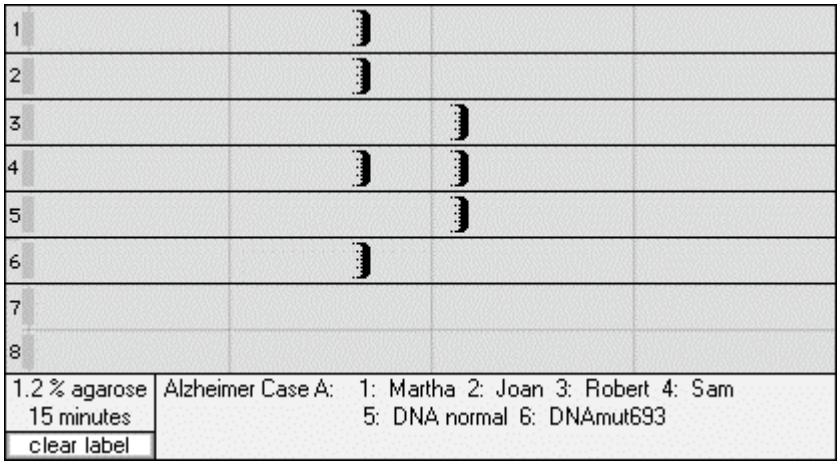


Case A.
Susan - homozygous normal (unaffected)
father - heterozygous
aunt - heterozygous
uncle - homozygous normal
John (brother) - heterozygous (affected)

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.



Case 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 normal

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?

Breast Cancer

The DNA sequence files are from the human BRCA1 gene, GenBank accession numbers U14680, locus HSU14680, Mutated sequence files were created by introducing the indicated nucleotide deletions or insertions into the text file prior to filtering. The probes are approx. 20 bp sequences spanning the mutation region and containing the mutated sequences. The control probe is from a BRCA1 region outside any of the mutated regions so it is unchanged in all sequence files. This probe serves as a positive control to confirm that BRCA1 DNA has been loaded onto the dot blot.

Note to instructors: DNA files containing PCR-amplified DNA fragments from each sample are also included, since this is how the case was constructed in Case It! v.3. This case also could be analyzed using Southern blots rather than dot blots, as in Case It! v. 3, but this requires running separate blots for each probe.

Mutation/technique illustrated: Detection of a deletion mutation in the disease gene using mutation-specific probes in dot blots.

References: Friedman, L.S. *et al*., Am. J. Hum. Genet. 57:1284-1297, 1995 and Smith, T.M. *et al.,* Genome Res. 6 (11):1029-1049, 1996

Case A. Elizabeth - pos. for 185delAG mutation (heterozygous)
Mother - pos. for 185delAG mutation (heterozygous)

Breast Cancer Case A

10 positive results

1	7	13	19	1	DNA 185 mutation (PCR) Probe 185 mutation	7	DNA 185 mutation (PCR) Probe 4184 mutation	13	DNA 185 mutation (PCR) Probe 5382 mutation	19	DNA 185 mutation (PCR) Probe normal 185
2	8	14	20	2	DNA 4184 mutation (PCR) Probe 185 mutation	8	DNA 4184 mutation (PCR) Probe 4184 mutation	14	DNA 4184 mutation (PCR) Probe 5382 mutation	20	DNA 4184 mutation (PCR) Probe normal 185
3	9	15	21	3	DNA 5382 mutation (PCR) Probe 185 mutation	9	DNA 5382 mutation (PCR) Probe 4184 mutation	15	DNA 5382 mutation (PCR) Probe 5382 mutation	21	DNA 5382 mutation (PCR) Probe normal 185
4	10	16	22	4	DNA BRCA1 normal (PCR) Probe 185 mutation	10	DNA BRCA1 normal (PCR) Probe 4184 mutation	16	DNA BRCA1 normal (PCR) Probe 5382 mutation	22	DNA BRCA1 normal (PCR) Probe normal 185
5	11	17	23	5	DNA Elizabeth (PCR) Probe 185 mutation	11	DNA Elizabeth (PCR) Probe 4184 mutation	17	DNA Elizabeth (PCR) Probe 5382 mutation	23	DNA Elizabeth (PCR) Probe normal 185
6	12	18	24	6	DNA Mother (PCR) Probe 185 mutation	12	DNA Mother (PCR) Probe 4184 mutation	18	DNA Mother (PCR) Probe 5382 mutation	24	DNA Mother (PCR) Probe normal 185

Issues:

If any of the women test positive for a mutation, is a prophylactic double mastectomy appropriate?

Does the lack of any of these mutations mean that the women will not get breast cancer?

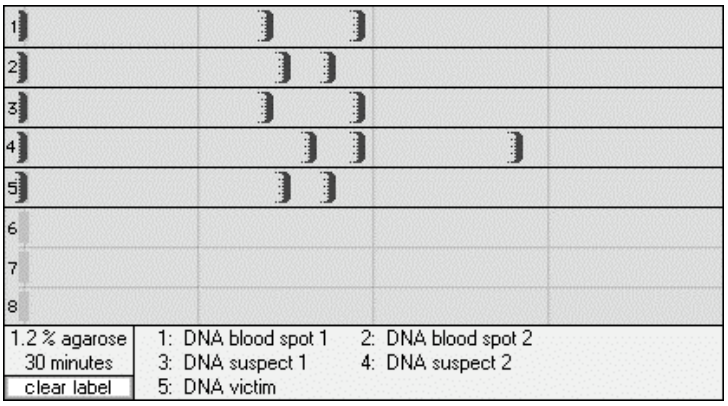
Who should have access to these test results?

Should this type of screening be mandatory?

Forensics

Murder Case

Note that there are three scenarios (A, B, and C) each with a different outcome. Scenario A is shown below:



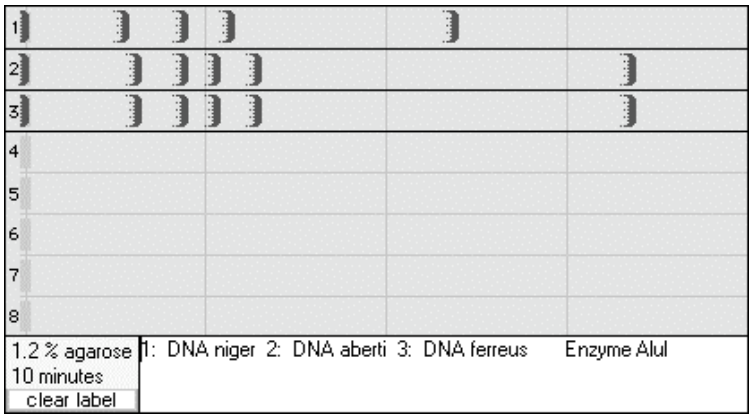
Scenario A.
Blood spot 1 = ex-husband's blood
Blood spot 2 = victim's blood

Phylogenetic studies

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 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)

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" feature 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)

Web resources

http://www.uwrf.edu/caseit/caseit.html
Case It home page – updates on latest versions, class testing, tutorials, and more.

http://www.ncbi.nlm.nih.gov/
National Center for Biotechnology Information (NCBI) - part of the National Institutes of Health; access to several molecular biology databases including GenBank, Online Mendelian Inheritance in Man, Medline, and Chromosome Maps

http://www.bioquest.org
The BioQUEST Curriculum Consortium

See the Case It! Investigator software for additional links to relevant web sites.