

Using *Case It!* materials in classes

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Contents:

Overview of Case It! project	1
Suggestions for class use	1
Example: Huntington's disease case with online role playing	3
Example: Colon cancer case with emphasis on central dogma	8

Overview of the Case It! project

Case It! is an NSF-supported project to develop a framework for collaborative case-based learning in molecular biology using interactive computer simulations. The *Case It* software is an open-ended simulation that reads nucleotide or amino acid sequence files, and includes methods for analyzing DNA (restriction digestion and mapping, polymerase chain reaction (PCR), DNA electrophoresis, Southern blotting and dot blotting, microarray analysis) and proteins (protein electrophoresis, Western blotting, ELISA). Detailed tutorials that describe the various features and the steps for using them can be accessed from the Case It home page (<http://www.caseitproject.org>). The download includes the simulation as well as all of the sequences necessary to run the cases described on the web site. The case descriptions can be viewed from the *Cases* menu of the web site and downloaded as a pdf file. An instructor's manual that includes the keys to the cases and additional background information is available at <http://www2.uwrf.edu/caseit/InstructorsManualv6.htm>.

Version 6 of the software has several new features, including separate, movable windows and automated loading features for gels, blots, and ELISAs. In addition, bioinformatics capabilities (sequence alignment, tree building) have been added via integration with MEGA software. Several cases have bioinformatics extensions that utilize these new features. The Case It software can be downloaded from the Case It web site (<http://www.caseitproject.org/download-2/>) at no cost to educators. MEGA software must be downloaded and registered separately from the MEGA web site, <http://www.megasoftware.net> (also free of charge). Contact mark.s.berglund@uwrf.edu for additional information concerning the project.

Suggestions for Class Use of Case It!

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

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

- Focus on the ethical and social issues raised by the analysis and the decision-making process involved
- Take on a particular role, e.g. genetic counselor or family member, and present the case interpretation from that perspective (see below for more details)

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

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

Presentation of case results

Students can present their case analyses in a variety of formats, including

- Conventional lab reports, where students insert images from the simulation, write an interpretation, answer the questions, and/or extend the analysis with additional research
- Presentation to the class, as individuals or groups, displaying the images from the software interpretations and background information
- Posters with images and text, which can be printed and used in a poster session where students interact in person to ask questions about the cases
- Web pages or other online presentations

Role-playing and online communication

With the human genetic disease, cancer, and infectious disease cases, we have had particular success when students interact online, taking on different roles as they analyze and discuss the case results. Students work in teams to analyze the samples in the case, using the Case It simulation, and present their results in a web-based format. We previously used a custom system that generated web pages with associated discussion boards, but a wiki system provides the same features (for example, see <http://caseitconferencing.wikispaces.org>). Any online system that allows students to upload images and text and communicate interactively could be used to accomplish these steps.

1. Playing the role of a lab technician, students use the Case It simulation to analyze the DNA or protein samples. Images from the simulation are saved and posted to a web page, along with background information and an interpretation of the results.
2. Students play the role of a genetics or health counselor as they compose a statement to the patient or family.
3. While interacting online, students switch between the roles when they ask and answer questions. For example, when students visit other web pages they play the role of patients/family members in the case, whereas when responding to questions about their own web pages they play the role of genetic/health counselors. It is possible to do something similar in person rather than online, but in our experience students prefer the anonymity of online communications because they can be more open in their answers and it is easier to assume roles.

Example of class implementation: Huntington's disease case with online role playing

Overview: This document provides more detail on classroom implementation of genetic disease cases using *Case It*, as described in the accompanying essay for the Huntington's disease case. These cases have been used in Introductory Biology courses for non-majors at UW-River Falls, as part of a genetics unit. Each team of students chooses or is assigned one of the genetic disease cases. There are 8 different diseases, each with three case scenarios, for 24 cases total. Students review the background information for their disease, read the case scenario, and use the *Case It* software to run PCR and gel electrophoresis on samples representing the individuals described in the case scenario. Students export their results as image files and upload them to a web page along with a summary of the background information, interpretation of the results, and statement to the person or family in the case that. Students read other team's web pages and use a discussion board to pose questions to the authors in the role of the people in the case. They respond to questions about their own web page in the role of a health care professional.

Biology content: Inheritance patterns, DNA structure, nature of mutations, techniques used to detect mutations (PCR, restriction enzyme digestion, Southern blot, Dot blot depending on the case; the original Huntington's disease case uses Southern blotting, but a PCR-based version is also included).

Class time required: 1-3 lab sessions, depending on how much of the work students would do outside of class time. We have used one 2-hr lab period to introduce the software and for students to run and analyze their cases. A second lab period is used for the students to prepare their web pages, and a third to give them time for discussions. Students can download the simulation and access the web pages and discussion boards, so part of the activity can be assigned to complete outside of class. However, we have found that there is more and richer discussion when class time is devoted to this activity.

Preparation:

- Download *Case It* software and determine how it will be distributed to the students. It can be installed on the server for a computer lab, copied onto flash drives or to a class web site for downloading, or students can download it themselves. The folder extracted from the downloaded zipped file includes the *Case It* executable file, which requires no further installation and no administrative access on lab computers.
- In order to perform sequence alignment of the PCR products, to demonstrate the variable number of triplet nucleotide repeats in the Huntington's gene, it will be necessary to install MEGA software. MEGA is downloaded <http://www.megasoftware.net>. See the tutorial on the *Case It* web site for instructions to download and install MEGA. Not all of the genetic disease cases have a sequence analysis component, so it might not be necessary for all students to have MEGA installed, and you may opt to use this feature as a demonstration.
- Become familiar with the software features that will be used in class. There are tutorials available on the *Case It!* web site, including video screencast tutorials for PCR and gel electrophoresis. An instructor's manual with keys to cases and additional background information is available by sending an email to mark.s.bergland@uwrf.edu.
- Determine teams (2-3 students) and how cases will be assigned.
- Set up the web page system for sharing results and discussion. We originally used a custom web editor and discussion board (referred to as the "*Case It* Launchpad"), but are switching to a wiki system. Wikispaces (<http://www.wikispaces.com>) offers free full-service wikis for education, but there are many other options. Any system that allows students to upload images and text and

communicate could be used. See <http://caseitconferencing.wikispaces.com> for an example of how this could be set up for a class. You can upload a spreadsheet with usernames, passwords and email address to quickly set up accounts for the class and send students log in information automatically. Contact mark.s.bergland@uwrf.edu if you need assistance setting up this type of system.

[Wikispaces also recently added a “Projects” feature that allows you to set up teams that will each have a page to edit. Each page includes a discussion board. Access to pages can be restricted to teams while they are building them, then released to the class for review and discussion.]

- Determine discussion assignments, i.e. which other web page each student will view and ask questions. We have found that it is better to assign these rather than let students choose which cases to view, so that each team receives an equal number of questions. One way to do this is to number the teams, and assign Team 1 to view web pages for Teams 2 and 3; Team 2 views web pages for Teams 2 and 4, etc.

Classroom activity:

1. The instructor demonstrates the simulation software using an example case. Alternatively, students can be directed to view the tutorials on the Case It web site. However, a demonstration allows the instructor to model data analysis and interpretation. As an example, here is how the Huntington’s disease case can be presented:

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

Case A scenario: 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.

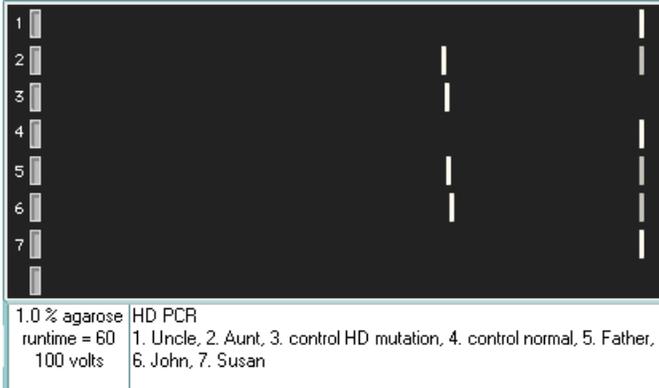
Run the analysis: Use the HD primers to run PCR on the following DNA samples:

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

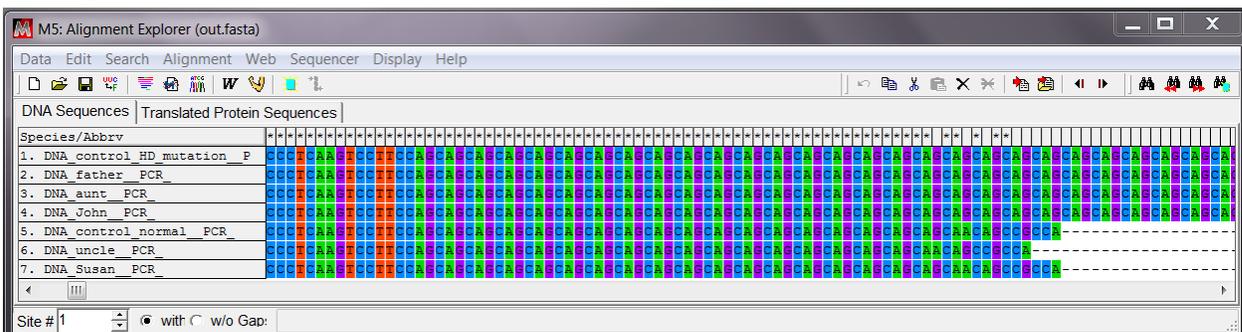
The procedure to run PCR in the Case It simulation includes (see detailed tutorials at www.caseitproject.org):

- a. Open the DNA sample files and the HD primers file. The files will appear in the “Opened & Processed” window.

- b. Select all of the DNA samples and click the Run button. New files corresponding to the PCR products for each sample will appear in the “Opened & Processed” window.
- c. Select all of the PCR projects and use the Quick Load/Run button to load them into the gel and run the gel. Staining the gel using the UV photograph option yields the following result:



- d. Discussion questions can include:
 - 1) What conclusions can you draw from these results? What is the status of each individual? Since the mutation is dominant, heterozygous individuals will be affected.
 - Susan - homozygous normal (unaffected)
 - father - heterozygous (affected)
 - aunt - heterozygous (affected)
 - uncle - homozygous normal (unaffected)
 - John (brother) - heterozygous (affected)
 - 2) What is the molecular basis of this disease, and why does this result in the observed gel patterns? The mutation is amplification of a triplet repeat (CAG). The PCR products from the samples containing the mutation are larger in because there are more repeats. The fragment sizes vary because each individual has a different number of repeats. This can be demonstrated in *Case It* by aligning the sequences of the PCR products.
 - a) Clicking on a band in the gel will display the sequence of that DNA in the lower window. **Choose the largest band (farthest to left) in each lane.**
 - b) From the Analyze menu, select Export sequence to add it to the Export window.
 - c) Once all of the sequences have been exported, use the Analyze menu to align the sequences. This will activate MEGA to and display the alignment:



Rubric for evaluating web posters

Molecular Biology of the disease: Quality of Information 1 2 3 4 (5 pts.)

Major inaccuracies	Some inaccuracies	Correct basic information	Correct info and some in-depth investigation is apparent
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Symptoms and treatments: Quality of Information 1 2 3 4 (5 pts.)

Major inaccuracies	Some inaccuracies	Correct basic information	Correct info and some in-depth investigation is apparent
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Laboratory procedure: Quality of Information 1 2 3 4 (5 pts.)

Major inaccuracies	Some inaccuracies	Correct basic information	Correct info and some in-depth investigation is apparent
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Results: Accuracy of gel photos 1 2 3 4 (5 pts.)

Gel blot is missing	Gel blot is wrong	Gel blot is slightly wrong Not all blots are present or labeled	Gel blot labeled correctly and done correctly
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Interpretation of gels 1 2 3 4 5 (5pts.)

No interpretation	Some Inaccurate interpretation	Slight misconception of meaning of gel blot, e.g., confusing terms; over interpreting results	some interpretation	Understanding and interpretation is clear
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Statement to Family 1 2 3 4 5 (5 pts.)

No diagnosis or counsel given	gives poor/vague counsel	Correct diagnosis but offers no counsel for family	Correct diagnosis & presents treatment or resources to family	Mentions treatment and resources to family
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Ethical issues 1 2 3 (3 pts.)

No ethical issues mentioned	Issues mentioned, but not relevant to case or do not show much thought	Identifies pertinent ethical issues, evidence of thought
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References 1 2 3 (2 pts.)

No references included	References included, but incomplete and/or not cited in page	Complete references included and cited in page
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Grammar, spelling 1 2 3 (2 pts.)

Many organizational, spelling or grammar problems	Some problems	Good organization of information, few spelling or grammar errors
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Individual contribution to project 1 2 3 (3 pts.)

Did not fulfill obligations to project	Completed some obligations	Fulfilled all obligations to project
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Example of class implementation: Colon cancer case, focus on the central dogma

Overview: This document provides more detail on classroom implementation of the case on inherited susceptibility to colon cancer. This case is based on “Colon Cancer: A Case of Genetic Bad Luck?” by Anne M. Casper, from the collection of the National Center for Case Study Teaching in the Sciences (http://sciencecases.lib.buffalo.edu/cs/collection/detail.asp?case_id=266&id=266). The case scenario is about a man diagnosed with colon cancer at a relatively young age, which, along with his family history, suggests that his cancer is a result of inherited susceptibility. The *Case It* version of this case can extend the information in the NCCSTS case, adding molecular biology techniques to determine the nature of his mutation, testing family members to see if they inherited the same mutation. The mutation described in this case is in the adenomatous polyposis coli (*APC*) gene. We have presented this in workshops as an investigation of the central dogma of molecular biology, investigating mechanisms by which a change in DNA nucleotide sequence can affect the function of a protein.

Students review the background information for colon cancer and read the case scenario. They use the *Case It* software to run a diagnostic Western blot to determine the size of the APC protein in the tumor tissue compared to normal tissue. The sequence of the cDNA from the patient’s *APC* is aligned with the normal sequence to identify the location of the mutation. The amino acid sequence translated from the mutated and normal DNA sequences are aligned to determine the effect of the mutation on protein sequence. Along the way, they are encouraged to think about the possible mechanisms by which an abnormal protein can be generated, and which mechanisms are supported or eliminated by the lab results.

This case also includes DNA sequences for testing other family members using a dot blot. Students make recommendations for the family members based on their test results. They can export images of their results and include them in a report or presentation.

Biology content: This exercise helps students understand DNA and protein structure and function, the process of translation, and the effect of DNA mutations. The nature of inheritance patterns and the concept of inherited susceptibility vs. disease are emphasized.

Class time required: One to two lab sessions are required, depending on how much work students do outside of class time.

Preparation:

- Download *Case It* software from <http://www.caseitproject.org> and determine how it will be distributed to the students. It can be installed on the server for a computer lab, copied onto flash drives or to a class web site for downloading, or students can download it themselves. The folder extracted from the downloaded zipped file includes the *Case It v6.06* executable file, which requires no further installation and no administrative access on lab computers when run from a flash drive.
- In order to perform sequence alignments directly from *Case It*, it will be necessary to install MEGA software (1). MEGA is downloaded from <http://www.megasoftware.net>. See the tutorial on the *Case It* web site for instructions to download and install MEGA. Note that *Case It v6.06* works with either MEGA 4 or MEGA 5.
- Become familiar with the software features that will be used in class. There are tutorials available on the *Case It!* web site, including video screencast tutorials for Western blot, PCR, and sequence alignment. An instructor’s manual with keys to cases and additional background information is available by emailing mark.s.berglund@uwrf.edu

- Determine how groups will be organized to work on the case. Two students per team works well in our experience.
- Determine the format for the students' products (lab report, oral presentation, etc.)

Classroom activity:

2. We recommend that the instructor lead students through the case, demonstrating the use of *Case It v6.06*. Alternatively, students can be directed to view the tutorials on the Case It web site prior to class time. The questions can be used for class discussion, or answered in a report.
2. Provide an overview of colon cancer (from www.caseitproject.org > Cases > Colon Cancer): Colorectal cancer is one of the leading causes of cancer death in the United States. Each year, about 150,000 Americans are diagnosed with colorectal cancer, and more than 50,000 die from the disease. Colorectal cancer usually begins with a polyp, which can be detected through the use of several tests: double contrast barium enema, flexible sigmoidoscopy, colonoscopy, or CT colonography (virtual colonoscopy). Early detection and removal of polyps can prevent the development of colon cancer.

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

3. Review the case scenario, linked from the Case It web site (www.caseitproject.org > Cases > Colon Cancer). Sam called his younger sister Jane to tell her he had just been diagnosed with colon cancer. Although Sam was only 40, he had suffered with diarrhea and found bright red blood in his stool, so his physician recommended a colonoscopy. Because their father had died at the age of 45 from colon cancer, Sam's doctor recommended that he get additional genetic testing to see if the cause was hereditary. Sam encouraged Jane who was 35 to get a colonoscopy and planned to also call the twins, Mark and Caroline, age 33, to encourage them to get screened for colon cancer as well. Sam also recommended that if he had the mutated APC gene that his siblings also undergo genetic testing.
4. Run the western blot analysis: Proteins from Sam's tumor tissue were analyzed by Western blot, using antibodies specific for the APC protein. What do the results of this test indicate about the nature of his mutation?

Protein samples:

Sam's tumor tissue

Normal colon tissue

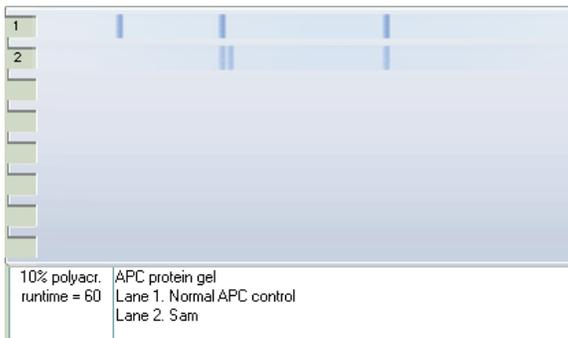
Antibody: antibody specific for APC protein

The procedure to run a Western blot in the Case It simulation, using the rapid autoloading procedure, follows:

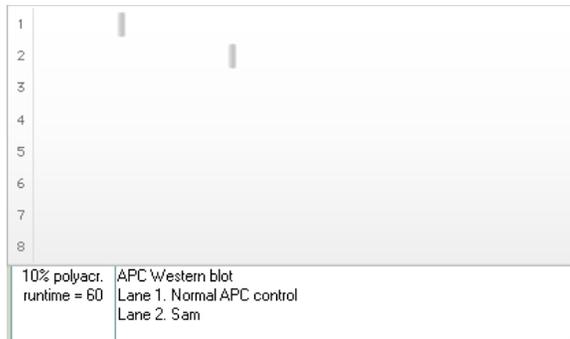
(Note: For a real-time demonstration, see video tutorials at www.caseitproject.org):

- e. Click the Protein button on the button bar and open both protein files, Sam and APC normal control, in the Cases > Cancer > Colon cancer > Case A > Western blot folder. The two protein samples should appear in the Opened & Processed window.

- f. Open the antibody file. The name of the antibody (anti APC) should appear in the Opened & Processed window as well as in the upper left panel of the main data screen.
- g. To run the Western blot, click once on “anti APC” in the Opened & Processed window to make sure it is the active antibody file. Then highlight both of the protein samples by clicking on the first one and then holding down the Shift key while clicking on the second one. The Western blot can be run in one step, but running the protein gel first allows a more thorough explanation of how a Western blot is performed. To run the protein gel, Click the Quick Load/Run button at the bottom of the Opened & Processed window and select Protein gel > Load selected proteins & run gel > Stain proteins blue. The default setting is to run the samples on a 10% polyacrylamide gel for 60 minutes.



- 1) Do you see any differences between the two samples?
 - The band patterns are different. Proteins ran left to right, largest to smallest. The largest band in the control sample is missing in Sam’s sample, and instead Sam has an additional, smaller band.
- 2) How do you know which proteins are the APC gene product?
 - If you know the expected size of the APC protein, and include a size marker on the gel, then you can identify a band at the correct size. (Note that the Case It software will allow you to click on a band and see the size of the protein in kD.) Since other, unrelated proteins may happen to be the same size as APC, the Western blot using antibodies specific for APC is more definitive.
- h. To run the Western blot after running the protein gel, **associate the anti-APC antibody with each lane of the protein gel by clicking the white numbers next to the boxes representing the gel lanes (in the upper middle part of the screen)**. Click the Quick Load/Run button and select Western blot > Option 2 > Run Western blot after loading samples. (The third item under Option 2 will load and run the Western blot in one step.) The APC antibody will be associated with each lane of the gel. The image below shows the result of blotting the proteins from the gel onto a membrane, and letting labeled antibodies bind to their target protein.

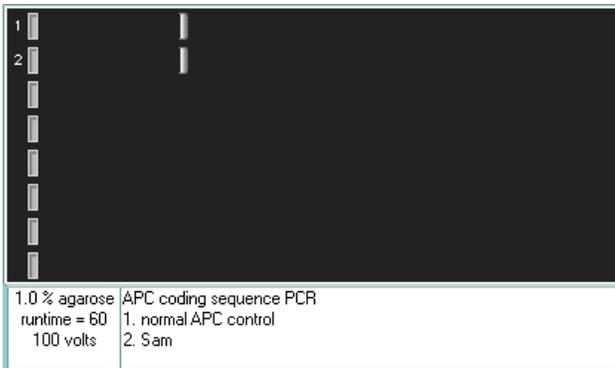


- 1) What is the difference between the two samples?
 - Sam's APC protein ran farther, so it is smaller

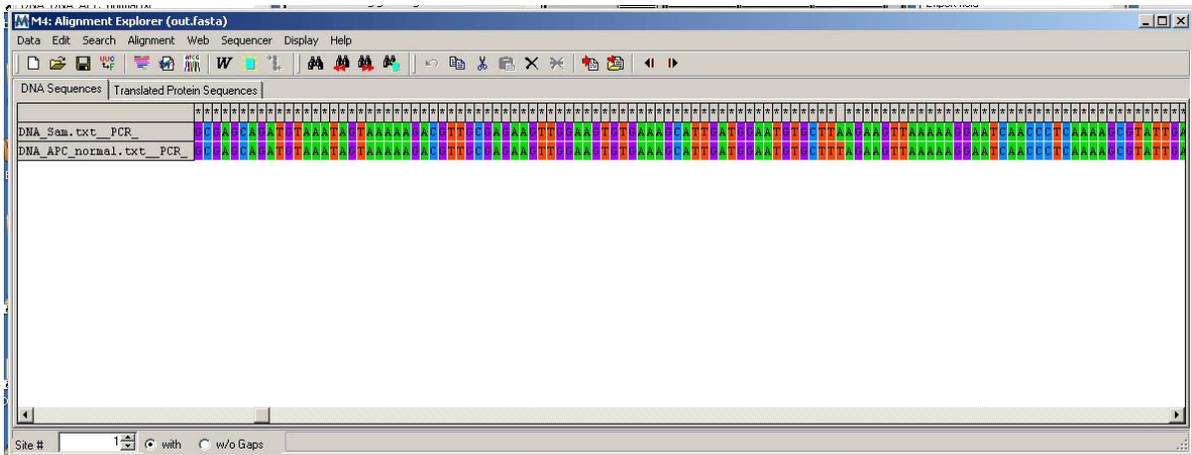
- 2) What are the possible mechanisms that could cause this difference?
 - Protein level: degradation or other processing that removes part of the protein
 - RNA level: differential RNA splicing resulting in an altered protein size
 - DNA level: deletion in the gene that removes part of the coding sequence, nonsense mutation resulting in a stop codon

- 3) How could you determine which is the actual mechanism in this case?

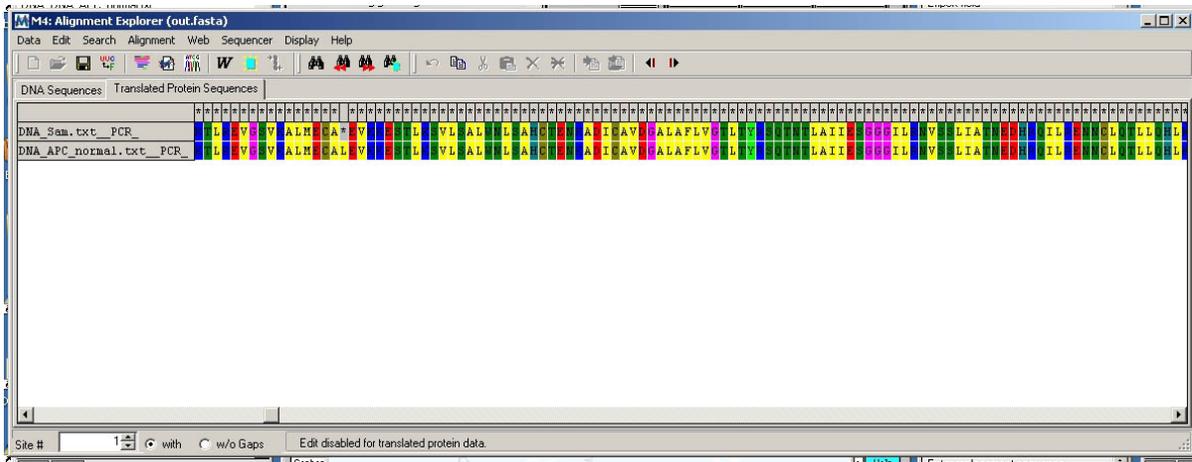
5. DNA and protein sequence analysis. To determine whether there is a difference in the DNA encoding APC, use PCR to amplify the coding sequence of the APC gene. This would be accomplished by isolating RNA from the tissue samples and using RT-PCR to generate cDNA from the RNA. The cDNA will include untranslated sequences both 5' and 3' of the translated sequence. The coding sequence can be amplified by PCR, using primers that bind to nucleotides at the beginning and end of the translated sequence.
 - a. To carry out PCR, you will need to open the DNA and primer files. First clear the program of the files used for the Western blot, using the Clear Menu > Clear everything > Yes. Then click on the DNA button and go to "Cases > Cancer > Colon cancer > Case A > PCR and alignment" to open the two DNA files, for Sam's DNA and a normal APC control.
 - b. Open the primers file from the same folder. Highlight both DNA samples using the shift-click method described in step 4.c. Click the Quick Load/Run button and select PCR > Run PCR. New files representing the PCR products will appear in the Opened & Processed window.
 - c. Run the PCR products on a gel to see if there are any differences between the two samples. Shift-click to highlight both PCR products. Then click on the Quick Load/Run button and select DNA gel > Load wells & run gel > Show light fragments on dark background (UV photo). The resulting image represent a photo of a gel stained with fluorescent dye (e.g. Ethidium bromide) taken on an ultraviolet light box.



- 1) Are there any differences between the samples that can be observed on the gel?
 - No, the bands are the same size
 - 2) Does this result rule out any of the possible mechanisms discussed in 4.d. above?
 - It rules out a large deletion in the DNA causing the smaller protein
- d. The sequence of the coding sequence of Sam's APC gene can be compared to the normal sequence by aligning them in MEGA. To do this, shift-click to highlight both PCR products. The click on the Analyze button at the bottom of the Opened & Processed window. Select Align/Tree > From O&P window > Show alignment only. This will activate MEGA software (if it has been installed in the Case It folder) and the multiple sequence alignment will appear in a separate window.



- 1) Is there a difference between the two sequences?
 - There is a single base change at nucleotide **1730**, from T to A
 - 2) If so, how might this difference cause a change in the protein size when translated?
 - That depends on the reading frame; it will either change the amino acid translated at that position or it may results in a stop codon at that position.
- e. The amino acid sequence translated from this nucleotide sequence can be viewed by clicking on the Translated Protein Sequence tab just about the alignment.

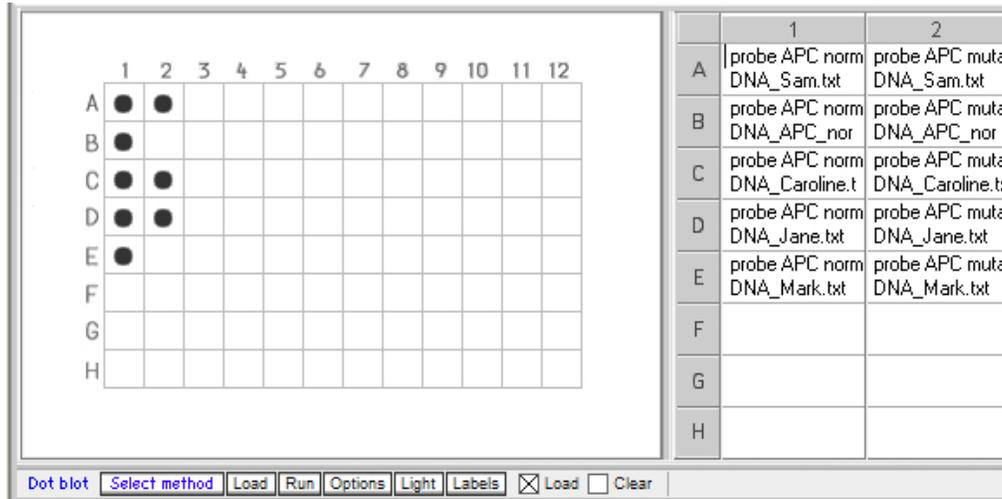


- 1) Is there a difference between the two amino acid sequences?
 - Yes, at amino acid **577**, there is no amino acid translated from Sam's sequence. This is due to a stop codon at that position as a result of the mutation (TTA to TAA)

- 2) If so, does it explain the protein size difference between Sam and the normal control?
 - Yes, since the translation would end at this point, resulting in a truncated protein. Note that MEGA does not end translation, but rather continues it after the skipped amino acid – but in a cell that would not happen and the protein would be smaller.

6. Dot blot testing of Sam and family members. In order to determine the APC genotype of Sam and his siblings, a dot blot test will be performed on their DNA samples. To run a dot blot, probes corresponding to the normal and mutated sequences are spotted separately on a membrane. Labeled DNA from the patient is added to the blot and allowed to bind to the probes. The probes spot will turn dark if the DNA sample binds to that probe, i.e. if the DNA contains the sequence corresponding to the probe. DNA samples to test include:
 - Sam
 - Jane, Sam's sister, age 35
 - Mark and Caroline, Sam's twin siblings, age 33
 - Normal APC control
 - a. To run the dot blot, first clear the DNA samples from the sequence alignment test, using the Clear menu > Clear everything > Yes. Then click on the DNA button to open DNA samples for the dot blot, from Cases > Cancer > Colon cancer > Dot blot. Shift click to open all of the DNA samples at once. These will appear in the Opened & Processed window.
 - b. Click on the Probe button and open the files for the mutated and normal APC probes.
 - c. To load the dot blot, first click once on "probe APC normal" in the Opened & Processed window to make that the active probe. Then shift-click to highlight all of the DNA samples. Click on the Quick Load/Run button and select Dot blot > Load samples. The wells A1 through E1 should turn orange, indicating they are loaded, and the corresponding wells should indicate which samples have been loaded in each well. Click on "probe APC mutation" to make that the active probe, then shift-click on all of the DNA samples again to highlight them. Repeat the steps described above to load these samples; they will appear in wells A-E in column 2.

- d. Click the Run button under the wells to run the dot blot.



- 1) What is the genotype of each person tested?
- 2) How would you counsel Jane, Sam, Mark and Caroline based on the results of the test? What would you advise Mark and Caroline?
- 3) If someone is predisposed to getting cancer, does that mean that he or she will definitely get cancer someday?
- 4) In a small number of patients whose families appear to have all the classical characteristics of FAP, a mutation cannot be found in the APC cDNA. What are two possible reasons for why mutations may not be found in some patients whose families appear to have FAP?
- 5) How might environmental factors such as diet influence the development of colon cancer?
- 6) Are there risks associated with screening such as colonoscopy? How do these risks compare with the risk of contracting colon cancer?
- 7) If Mark and Caroline are twins, how do you explain the fact that they have different DNA results?
- 8) If Sam is heterozygous for the APC mutation, why is only the mutated protein, and not the normal protein, detectable in the Western blot?

The NCCSTS case includes instructions for analyzing the DNA sequence using BLAST alignment and OrfFinder to demonstrate the effect of the mutation. In addition, it includes additional information about the function of the APC gene product and the functional effect of the mutation.

Additional case scenarios are available on the *Case It!* home page, <http://www.caseitproject.org>, from the Cases menu. The Instructors manual, with keys to cases and additional background information, is available by emailing mark.s.bergland@uwrf.edu.

References:

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(Credit for development of MEGA software, which is used in conjunction with Case It v6.06)
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