Using *Case It!* materials in classes

Example of class implementation: HIV case with introduction to bioinformatics

Overview: This document provides more detail on classroom implementation of infectious disease cases using the Anna scenario from the HIV cases as an example. These cases have been used in Introductory Biology courses for majors and non-majors at the University of Wisconsin-River Falls, as well as in upper level Immunology and Virology courses. Each team of students chooses or is assigned one of the HIV cases to analyze. There are 12 different HIV case scenarios, some set in the U.S. and some in Africa. All cases include video scenarios, and in all but one of the cases these are based on real people living with HIV/AIDS.

Students review the background information for HIV/AIDS, read the case scenario, and use the *Case It* software to run ELISAs and Western blots on samples representing individuals described in the case scenarios. They export their results as image files and incorporate them into their report, where they interpret the results and answer questions. The same online role playing strategy described for the genetic disease cases also can be used for the HIV cases, i.e. results are posted on web pages, and interpretations of results are written as statements to the person or family in the case. Then students read other teams' 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.

Each HIV case also includes an optional viral load test, which is simulated as a 96-well PCR showing the fluorescence intensity results (rather than qPCR with Ct values). The purpose of this extension is to illustrate how the amount of virus in a person can be monitored.

Each case also includes a sequence analysis component, as an introduction to bioinformatics concepts such as sequence alignment and tree building. HIV sequences representing people in the case scenario, or in some cases representing samples not directly related to the case scenario, are analyze using MEGA software which is activated from within the Case it simulation. This activity can be used as a starting point for more open-ended bioinformatics investigations, such as the HIV problem space on the BEDROCK web site (http://bioquest.org/bedrock/problem_spaces/hiv/).

Biology content: This particular exercise helps students understand the nature of infectious agents, HIV transmission, virus structure and multiplication, the immune response to viruses, and techniques used to detect antigens and antibodies (e.g. ELISA, Western blot). In addition, students are introduced to phylogenetic trees and representations of nucleotide sequence comparisons. Students also must grapple with the social and ethics aspects of HIV/AIDS as they present their analyses.

Class time required: One to three lab sessions are required, depending on how much work students do outside of class time, whether the online role playing activity is included, and the extent of the bioinformatics analysis. We have used one two-hour lab period to demonstrate the software and allow students to run and analyze their case scenarios, a second lab period for the sequence analysis and bioinformatics extension. If an open-ended bioinformatics project is included, this can require an additional lab period, although students can also complete this assignment outside of class time. The role playing exercise worked particularly well when our students were interacting with students from other institutions, especially if these are located in another geographic area with a different experience with HIV/AIDS (such as the University of Zimbabwe).

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 and build trees, 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 ELISA, Western blot, PCR, sequence alignment and tree building. An instructor's manual with keys to cases and additional background information is available by emailing mark.s.bergland@uwrf.edu

- Determine teams and how cases will be assigned. Two students per team works well in our experience. For the online role playing component, we have both members of the team conduct the analysis and set up the web page, but then have team members send discussion messages independently when role-playing. Students prefer this method, and that way we know who is contributing to the discussions and can assign credit accordingly.

- Set up the web page system for sharing results and discussion, if using. Otherwise, determine the format for the students product (lab report, oral presentation, etc.)

  An example of how the group web pages could be set up for a class is provided at http://caseitconferencing.wikispaces.com. Wikispaces (http://www.wikispaces.com) offers free full-service wikis for education, but there are other options. Any system allowing students to upload images and text could be used, providing it also allows for communication via discussion boards.

  Wikispaces allows you to upload a spreadsheet with usernames, passwords and email addresses to quickly set up accounts for the class and send students login information automatically. 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.

- If using the online discussions, determine discussion assignments, i.e. which other web pages students will view, asking questions of students who constructed those pages. We have found that it is better to make assignments rather than let students choose which cases to view, so that each team receives roughly the same 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 to view web pages for Teams 3 and 4, etc.

Classroom activity:

1. We recommend that the instructor first demonstrate *Case It v6.06* 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 Anna HIV case can be presented:

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

Some general discussion questions that can be posed to encourage to students to consider the biology and social issues for each case include:
- How did this person become infected with HIV?
- Have others also been infected?
- Who else should be tested?
- How reliable are the tests?
- How often should someone be tested?
- Why do people engage in risky behaviors?
- What impact is the infection having on this person's life?
- How can this person prevent further transmission of the virus?
- What other ethical decisions does this person face?
- How common is this case?
- Are there cultural differences regarding attitudes about HIV and prevention?

Show the Anna video, linked from the Case It web site (www.caseitproject.org > Cases > HIV/AIDS > U.S. cases > Anna). Here is the text version: "Anna is a 27-year-old woman from Guatemala, who is living with her boyfriend and is pregnant with her first child. A blood test during her second trimester revealed that she was HIV positive. Anna is surprised, because her first trimester test was negative, and she did not have sexual contact with anyone other than her boyfriend. She is very concerned about the fate of her child, who may contract the virus from Anna."

Run the analysis: Use the ELISA simulation to test the following blood samples for antibodies that can bind to HIV proteins:

    Anna, first trimester
    Anna, second trimester
    Anna's baby, 6 weeks after birth
    Anna's boyfriend
    Positive and negative controls

The procedure to run an ELISA in the Case It simulation, using the rapid autoloading procedure, is as follows:
(Note: For a real-time demonstration, see video tutorials at www.caseitproject.org):

a. Open the HIV protein file and all of the antibody files (representing blood samples that are being tested for the presence of antibodies). These files will appear in the "Opened & Processed" window.

b. Select all of the antibody samples and click the Quick Load/Run button. Select ELISA > Option 1: Load same protein, different antibodies. The 96-well plate will appear, with protein and antibody samples loaded into the wells.

c. Click the Run button, which will run the ELISA and display the color results. These results can be saves as a .jpg file. Absorbance values for the color are obtained by clicking Options > Data > ELISA > Absorbance.
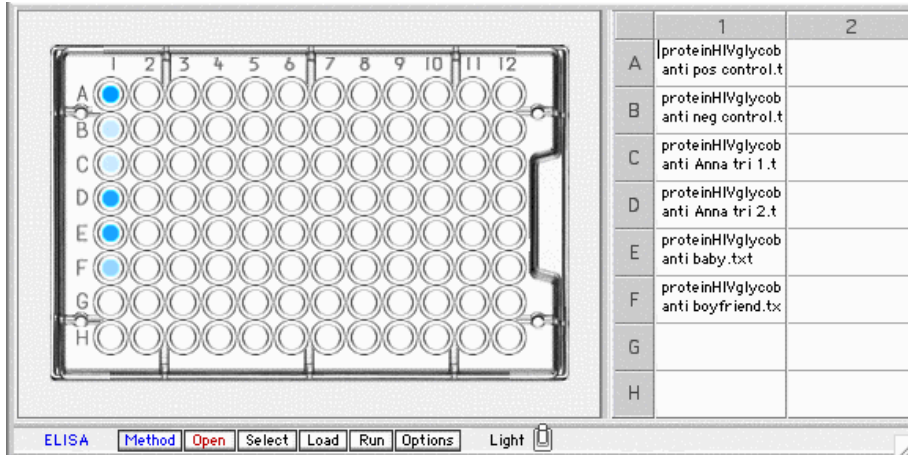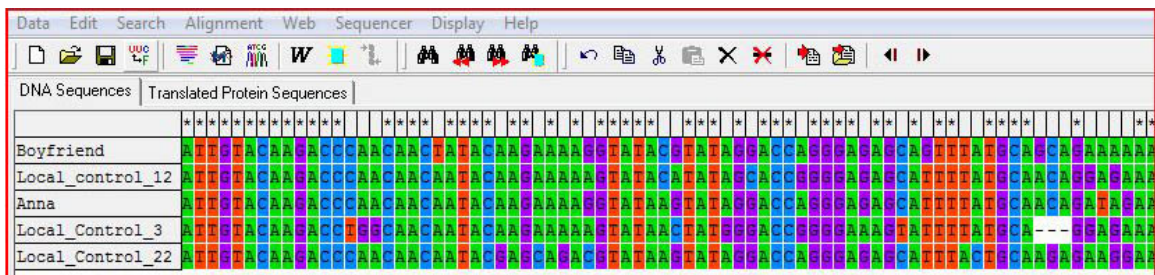

d. Discuss the results, including which samples appear to be positive (darker color than the negative control). Note that the ELISA test has a false positive rate of approximately 1/1000, and a Western blot is needed to confirm a positive result.

e. To run the Western blot, the same protein and antibody samples are used. Click on the HIV protein in the Opened & Processed window, then highlight all of the antibody samples. Click the Quick Load/Run button and select Western blot > Option 1: Load Same protein, different antibodies (the third item under Option 1 will load and run the Western blot in one step).
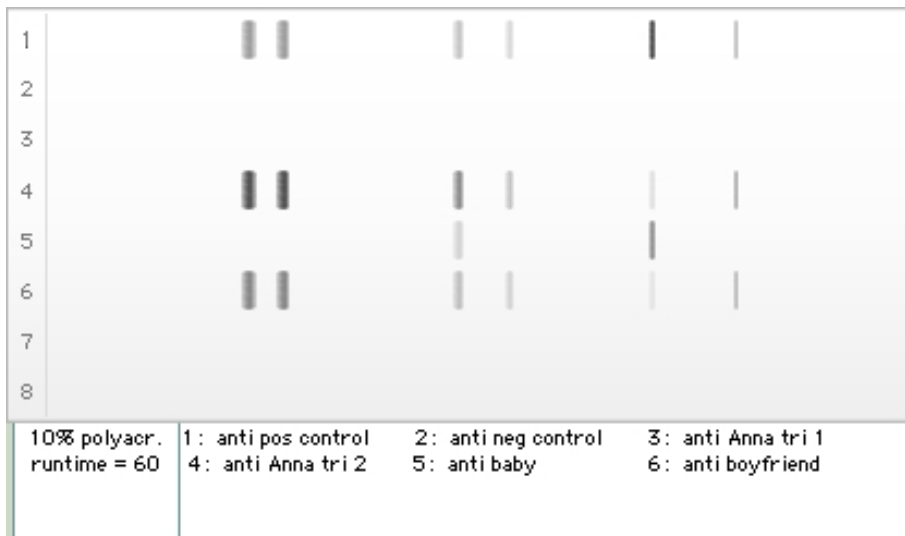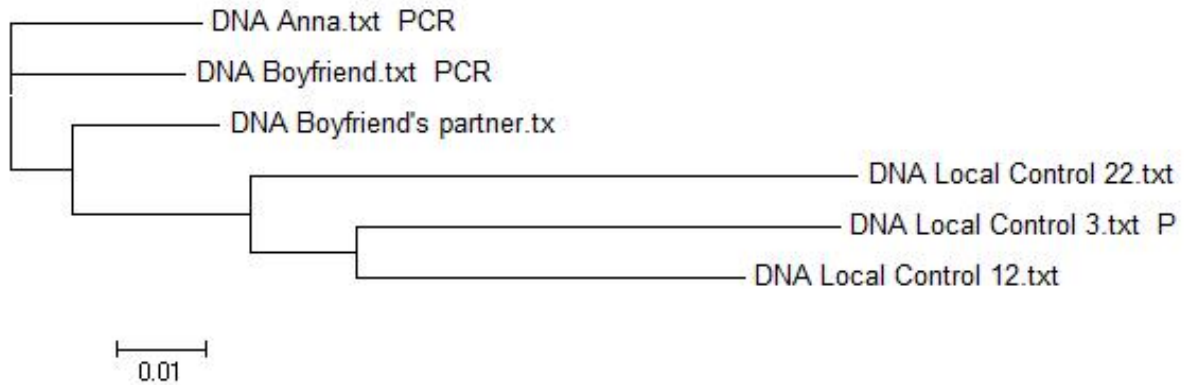

f. The following questions can be used to discuss these results (see the online Case descriptions and Instructors Manual for additional guides to interpret the results):

1) What is the status of each person tested?
2) How would you explain these results to Anna?

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

2. After the above demonstration (with students following along on their computers), students read the background information and scenario for their own case. Additional time may be provided for students to conduct additional research into HIV/AIDS testing, treatment, etc.

3. Teams of students use the simulation to run samples for their case. Results are saved as .jpg images. (Note that the .jpg suffix must be part of the filename if the image is to save properly.

4. For the bioinformatics extension, the instructor can again demonstrate the procedure using the Anna case. Here is the scenario for this part of the case: "Did Anna's boyfriend infect her with HIV? Compare Anna's and her boyfriend's HIV sequence with local controls – does tree suggest that their sequences are more similar than random samples? Can you tell from this who gave it to whom? Anna's boyfriend acknowledges that he had another partner. Does adding this sequence help you determine the sources of Anna's infection?"

    a. Use the Clear menu to clear the ELISA and Western samples.

    b. Open the DNA samples from the bioinformatics folder. These samples will appear in the Opened & Processed window. For the Anna case, the samples are

        Anna
        Anna's boyfriend
        Sexual partner of Anna's boyfriend
        Three local controls (unrelated individuals in the same geographic region)

    c. The DNA samples contain entire HIV genome sequences (almost 10 kb), so PCR is necessary to amplify a small segment of the *env* gene to facilitate multiple sequence alignment. Open the PCR primers, then highlight all of the DNA samples. Click on the Quick Load/Run button and select PCR > Run PCR. New files representing the PCR products will appear in the Opened & Processed window. These could be run on a gel at this point to demonstrate how they would be analyzed that way, or you can go directly to sequence alignment.

    d. Select all of the PCR products, and click the Analyze button on the bottom of the Opened & Processed window and select Align/Tree > From O&P window > Show alignment and tree. This will activate MEGA software (if it has been installed in the Case It folder) and the multiple sequence alignment and tree will appear in separate windows. The default settings in MEGA (DNA distance calculations, neighbor joining tree) are used.

DNA Anna.txt PCR
DNA Boyfriend.txt PCR
DNA Boyfriend's partner.tx
DNA Local Control 22.txt
DNA Local Control 3.txt P
DNA Local Control 12.txt

0.01

   e. Discuss the results, including the general interpretation of trees and how they present sequence relationships, and specifically how this information might be used to determine how Anna may have been infected with HIV. (Refer to the Instructors manual for more information about how to interpret these results.)

5. If the online role playing will be used, students will prepare web pages with the results and interpretations for their case. Students are assigned to view another team's web pages, read the information, and post questions in the role of one of the people in the case. They should identify their role when asking questions. We recommend that this be assigned as an individual activity rather than having the team pose questions, as it is easier to give credit and students become more involved personally in the interactions.

   Authors of web pages respond to questions in the role of a health care professional. This can be an individual or team effort (i.e. team members consult before posting their answers). We tell students that they can consult with team members, but that they should give their own responses to questions, as a "second opinion". That way they can put their own interpretation on a response, rather than rely on a team member to frame a joint response.

6. An alternative or additional activity is to extend the bioinformatics analysis to more open-ended projects, where students ask their own questions and choose their own sequences to analyze. One effective strategy is to use a problem space approach, where relevant sequences are gathered for a particular type of problem or question, but students still have to select specific sequences to analyze to address their question.

   We have used the BEDROCK HIV problem space at http://bioquest.org/bedrock/problem_spaces/hiv/. The HIV problem space is based on a paper that describes how researchers followed disease progression in several HIV-infected individuals. The data include CD4 T cell counts at different clinical visits over several months, as well as the sequences of viruses isolated from the patients at each time point. Students were asked to pose questions about the expected degree of sequence variation in virus populations isolated from patients whose CD4 counts decreased quickly ("rapid progressors" to AIDS), compared to patients whose CD4 counts remained stable for the duration of the study ("non-progressors"). They tested their hypotheses by choosing sequences from the problem space and generating trees using Case It and MEGA software.

   Students prepared web pages of their results (see http://caseitconferencing.wikispaces.com for example web pages). We then used a variation on the role playing activity, where students viewed each others web pages in the role of a peer reviewer, asking questions for clarification and follow up.

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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    (*Assessment of learning gains*)

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