Supplemental Materials

for

The Use of Open-Ended Problem-Based Learning Scenarios in an Interdisciplinary Biotechnology Class: Evaluation of a Problem-Based Learning Course Across Three Years

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Module #1 – Choosing the Best GMO Detection Method

**Background:**

As part of The Clean Water Act of 1972 and its subsequent amendments, individual states are required to identify whether surface water systems are polluted with bacteria, and if so, to develop treatment action plans. Current efforts to develop methods to track the source of bacteria, as a field of study, are called both Bacterial Source Tracking (BST) and Microbe Source Tracking (MST). Most BST/MST methods are based upon developing a phenotype or genotype profile of the population of bacteria found at various point sources of pollution, then comparing a sample’s profile to profiles of known bacterial contaminants. While the current methods are sufficient to answer many questions, none have 100% predictive value. Efforts continue in this field because of the dire need to improve the tools available to aid in control of bacterial pollution.

An alternative to BST/MST methods is to add a genetically modified organism (GMO) to a suspected pollution site (e.g. a septic tank). The idea is that this GMO would be similar to many bacterial pathogens but unique because of its engineered genetic tag. Its movement and survival in the environment could be tracked, and if it was detected in a polluted stream, it could unambiguously identify the spiked site as a source of pollution.

**Problem Scenario:**

You are a member of a product development team that has started a biotech company (you choose the company's name). Your team has designed an *E. coli* bacterial strain that is
genetically engineered to contain on the bacterial chromosome a jellyfish gene that encodes for the protein called Green Fluorescent Protein (encoded by the *gfp* gene). There are variants of this *gfp* gene that encode Gfp proteins having different excitation and emission wavelengths, half-life, and which are controlled by various promoters (promoter is the regulatory region of a gene that controls expression of the gene). Your intention is to use the GMO as a tracer organism for spiking suspected pollution sources.

Once the GMO tracer has been designed, you need to identify a detection and quantification method that will be sufficient to determine whether the tracer GMO is present in a nearby surface water system, and if so, how many GMO cells are present. The introduced gene will change both the organism’s genotype and (under many circumstances) phenotype, hence your detection method could target the presence of the *gfp* gene DNA sequence, or of the fluorescent Gfp protein the *gfp* gene encodes. There are at least four methods that might work: (1) Samples could be plated on agar and allowed to grown up into visible colonies; (2) Samples could be subjected to PCR (polymerase chain reaction); (3) Samples could be subjected to FISH (fluorescent in situ hybridization); (4) or samples could be passed through a fluorescence activated cell sorter (FACS) could be used. For the PCR method, there are also multiple means of determining if amplified product has been made (i.e. visualization on a gel or one of multiple hybridization-based methods).

**Task:**
Your task is to assess and compare these methods as candidates for detection and quantification of your GMO in stream water and select a method that best meets the needs of
this project. Consider the capabilities, strengths and weaknesses of the test detection and quantification methods with respect to the characteristics of the organism you are trying to detect and the way you are planning to use the organism. Among other selection criteria, you should include sensitivity and specificity of detection.

- Recognize that many methods, including all those that are DNA-based, will not necessarily differentiate between living and dead bacteria.
- Furthermore, living bacteria can exist in a culturable form or a viable but nonculturable form (VBNC). Recognize that phenotypic-based methods presume the protein will be present.

**Resources / Readings:**

**Books:**

**Web sites:**

Wikipedia. Note: Wikipedia is a good source of information but should not be used as a primary reference.

Molecular Biology Basics: [http://plato.acadiau.ca/courses/biol/Microbiology/Basics.htm](http://plato.acadiau.ca/courses/biol/Microbiology/Basics.htm)

**PCR:** [http://www.escience.ws/b572/L3/L3.htm](http://www.escience.ws/b572/L3/L3.htm);


[animation]

**Waste water treatment plant (WWTP) / foam formation:**

**Action Plan:**

Your team should present a final written report targeted for your supervisors, who are administrators who know little about biology. The report should describe the purpose of your investigation, the desired attributes of an optimal detection and quantification method, the methods you evaluated, and the method you recommend to use based on your selection criteria. If your recommended method still falls short in some areas, you should discuss that as well.

As a minimum, your report should be presented using the section headings shown below. You may add additional sections or sub-headings and include other information as you deem necessary.

- Cover Page, showing names of all team members
- Introduction
- Method Comparisons
- Conclusions and Recommendations
- References
Grading:

85% Report grading is based upon: a) rationale used in decision making, including selection criteria used, b) choice and use of information sources, and c) clarity of writing, including explanation of rationale and selection criteria used in decision making.

10% Peer evaluations

5% Class participation (in-class, on-line discussion board)

Discussion Questions:

1) What additional information (about the methods) would have allowed you to make a more definitive choice?

2) There isn’t consensus among scientists on which of these four methods is best. Why do you think this is true?

3) When would someone not need a MST method that has a 100% predictive value (see background paragraph)?

4) What are the three most common reasons why the public expressed concern over a GMO release? What are the three most scientifically-based, valid reasons for concern over a GMO release? Why are these lists different?

5) How would your method ranking have changed if the problem scenario goal was changed from detecting a released GMO to detecting the presence of pathogenic bacteria in food products?

NOTE: Every student should email the course instructors any questions they would like answers to or problems they are having in developing their solution strategy, and how they
would like the instructors to help (e.g., cover certain material in the next class, suggest a resource to obtain certain information).
Appendix 2

MODULE #2 – Genetic Testing

Background

“Rabbi, who sinned, this man or his parents, causing him to be born blind?” (John 9:2).

Scientific advancements in the past 20 years would now allow us to answer this 2000 year-old question.

There is a genetic component to almost every disease, and in some cases a single gene determines whether the gene carrier will contract a disease. Usually, the genetic locus responsible for a “genetic disease” is an altered (or mutated) form of a gene that is found in all individuals. For example, in Huntington’s disease the length of a tri-nucleotide repeat (sequence “CAG”) located at the HTT gene determines disease status. If there are fewer than 35 contiguous copies of this repeat at the gene, the individual is unaffected; if there are more than 40 copies, the individual is affected. For cystic fibrosis, many types of DNA changes to the CFTR gene can result in disease, but about 70% of mutations in CF patients result from a three-base deletion, resulting in loss of one amino acid from the wild-type protein (this mutation is called ΔF508 because the amino acid lost/deleted [Δ] is normally a phenylalanine [abbreviated “F”] located at the 508th amino acid position on the protein). In both of these examples, having the mutated gene results in an almost 100% chance of acquiring the disease (in genetic terms, they have a high penetrance).

Traditionally, diagnostic tests were developed after therapeutic strategies. After all, why develop a diagnostic test if a cure for the disease didn’t exist? In some cases, a diagnostic test
led to discrimination. For example, between 1970 and 1972 sickle cell testing was mandatory in 12 states. At the time, the disease could not be prevented or treated, so there was no clear benefit to being tested (i.e. what life decisions would be affected by knowing one was sickle-cell positive)? The discrimination that too often resulted from a positive test result was prevented by passage of the National Sickle Cell Anemia Control Act in 1972. Today, the traditional order of therapeutic strategy \( \rightarrow \) diagnostic test is usually reversed. This is because sequencing of the human genome combined with advancements in molecular biology techniques (e.g., sequencing, PCR, microarrays) has made it fairly easy to identify the DNA sequence responsible for a genetic disease and to develop a diagnostic test for the presence of that sequence.

Today, there are approximately 900 genetic tests available to identify genetic disorders. Their uses include: confirm a diagnosis based on disease symptoms; test for a genetic disease before symptoms appear; predict the likelihood of someone contracting a disease; screen embryos for disease, preimplantation genetic diagnosis (PGD); and identify carriers for a disease.

Most diseases/traits are not caused by a single, simple DNA change having a high penetrance. In many cases, a genotype is found to have a less than 100% correlation with a certain phenotype. For example, having a mutation in either the BRCA1 or BRCA2 gene means your chance of acquiring breast cancer is \( \sim 80\% \) (approximately 5% of breast cancers are attributed to heredity) and your chance of acquiring ovarian cancer is \( \sim 20\% \). In other types of disease/traits/cancers, the correlation between genotype and phenotype is much lower. This
makes finding the genetic link more difficult; it also makes knowing how to use the information less clear.

In the past (pre-2000), investigators would focus on a given disease/trait (i.e. phenotype) and work backwards to identify the responsible gene. This would often be done by identifying proteins involved in the wild type phenotype. The sequence of the gene(s) encoding the protein(s) would be obtained and then compared between normal and affected individuals. If using an animal model, the gene from a healthy animal may be intentionally mutated and introduced into the animal to see if it induced the trait. Once the responsible gene was identified, it would be further studied. Today, this approach is still used, but additional tools are available, based upon knowing the sequence of the entire animal’s genome, including that of humans.

The human genome contains approximately 3.3 billion base pairs (per haploid). Within these 3.3 billion base pairs, there are approximately 10 million locations in which humans differ from one another. That is, of the 3.3 billion base pairs that comprise the haploid genome, all humans are identical at 3.29 billion of those base pairs. Of those 10 million remaining locations, the difference between people resides in being able to find more than one of the four different bases (e.g., A, T, C or G). Expressed another way, there are 10 million single-nucleotide polymorphisms (SNPs) in the human genome.

Once the number and location of SNPs was determined, the strategy for identify links between genes and diseases changed. Now, instead of looking for which of 3.3 billion base pairs may
be responsible for causing a disease, researchers need focus only on those 10 million SNPs. Furthermore, SNPs can be used for more than identifying what DNA responsible for causing a disease; it could be correlated with any phenotype that has a genetic component. For example, pharmacogenetics involves determining how response to a given drug is affected by an individual's genotype; the goal is to be able to individualize drug treatments based on the patient’s genotype (e.g., a liver enzyme cytochrome P450 breaks down certain drugs, and alterations in the gene encoding P450 affects how readily drugs are broken down; knowing this information affects optimal drug dosage). And since SNPs are the only part of a person's genotype that is relevant, it’s accurate to say that drug treatments will be tailored to a person's SNP profile. Within 15 years, it is likely that patients will be able to take their personalized SNPchip with them when they visit their physician or pharmacist.

The ability to determine the genetic profile of both the born and unborn raises many ethical issues. Who should be tested and are there any controls on which genetic tests should be available? Who has access to test results? How can those results be used? Who decides answers to these questions?

**Problem Scenario and task:**

Consider the scenario from the three perspectives given below.

**Scenario 1)** You are the VP of Human Resources for Wal-Mart and have been asked to: a) investigate the genetic screening test offered by a company called 23andMe to determine its reliability, accuracy, and potential use in hiring/firing/health insurance decisions, and b)
determine whether it is legal to use the test results in making hiring/firing/health insurance
decisions. *23andMe* advertised itself as a company with a “goal of democratizing genetic
information by giving our customers access to even more of their SNP data through our next
generation custom content, all at a lower price.”

Task #1: Submit a position paper for the task above. Your audience is the CEO of Wal-Mart
(interestingly enough, a biology minor in college). Include rationale for decisions made (with
citations).

Include in your report a description of how you might use results from this assay in
employee assessment. Indicate one specific SNPs/trait that *23andMe* screens for that you feel
would be relevant in making hiring decisions, another that is relevant for considering health
care intervention for an existing employee, and one that, if used, could open the company to a
law suit (and indicate why).

Scenario 2) Your sister needs a job and has applied to work at a new Wal-Mart store. They
have asked her to volunteer to be genetically screened by *23andMe*, free of charge, as part of
their job interview process. They haven’t indicated it is required, but she assumes that if she
doesn’t agree, it will hurt her employment chances. She has exhausted all other legal source of
revenue and this seems to be the only job opportunity in her community. She has come to you
for advice.

Task #2: What advice would you give her (give your rationale behind each suggestion)?
Provide her a list of at least three pros and cons for being tested, with a counter argument for
each item on the list. If you recommend she agree to be tested only on certain conditions, list those conditions (e.g., access to test results, specific traits to not be screened, etc.).

Scenario 3) You are a member of the NC Senate, which is currently considering a bill that would require genetic screening be performed on all a) prison inmates, b) people requesting welfare (including pregnant women without medical insurance who are receiving health care from a government-sponsored entity), c) and all newborns. Bill advocates indicate that this bill will help the state assess current and future health needs; bill proponents object on the grounds of violation of privacy rights.

Task #3: Submit a position paper to indicate whether this bill should be approved or not. Give three arguments-counter arguments for each position for each class of individuals to be screened.

Indicate for which SNPs, if any, you would approve the bill in its current form?

Indicate for which SNPs, if any, you would approve the bill in a modified form (indicating the modification)?

Indicate for which SNPs would you oppose the bill in any form?

List two examples of currently mandated health screens required of some segment of the population.

Resources / Readings:
Web sites:
www.23andMe.com (“Genetics 101” link)
  - http://spittoon.23andme.com/. SNPwatch. SNPwatch gives you the latest news about research linking various traits and conditions to individual genetic variations
  - https://www.23andme.com/gen101/. A tutorial on genetics and SNPs.

Genetic Nondiscrimination Information Act (GINA).
  - See also http://blog.wired.com/wiredscience/2008/05/genetic-protect.html

Genetic testing overview.
http://www.genome.gov/19516567

References


Action Plan:
The three tasks are given above; each requires a written report. For all three, your audience is scientifically-literate. Include the rationale behind each decision/recommendation. Specific SNP examples can only be used once. Suggested section headings for your reports are shown below.
  - Cover Page, showing names of all team members
• Background
• Task statement
• Conclusions and Recommendations
• References

**Grading:**

85% Report grading is based upon: a) rationale used in decision making, including selection criteria used, b) specific SNPs used to support a position, and c) clarity of writing.

10% Peer evaluations

5% Class participation (in-class, on-line discussion board)

**Discussion Questions:**

1) Who should be able to decide whether a pregnant mother undergoes preimplantation genetic diagnosis (PGD) to determine if her fetus carries the marker for an early age lethal trait (e.g., Infantile Tay-Sachs)? For a trait that will require life-long hospitalization? For a trait that can be “cured” by proper nutrition of the mother during pregnancy? Under what circumstances would you expand your list?

2) Assume the following: a) the test for Huntington’s disease has a false-positive rate of 1 in 1000; b) the incident of Huntington’s disease in the high-risk group is 1 in 100, and in the low risk group is 1 in 10,000. The test is performed on someone in the low risk group and the result is positive. The patient asks the physician what is their chance of having Huntington’s disease, and the response is “Well, the test is wrong in only 1 out of a 1000 times, so you have a 99.9%
chance of having Huntington’s disease.” Is the physician correct? What are the false-positive and false-negative rates for the BRCA1 and BRCA2 test?

3) When should the government require, or pay, for genetic tests (i.e. how does one balance the public welfare issue against the personal privacy issue)? When does the balance lean towards public welfare? towards privacy?

4) If paid for by a government entity (e.g., Medicare), should the information be made available to government agencies (e.g., state health department? defense department)? insurance agencies (w/ or w/out their paying for this information)?

5) In Vermont, euthanasia is legal in cases of terminal illness. Would obtaining a positive result for the test for early Alzheimer’s qualify? For Huntington’s disease? What positive genetic test result, if any, would qualify? NOTE: this is not a debate of the legality of Vermont’s law.

6) Should employers have the right to require employees to be genetically tested for predisposition to traits that directly impact their jobs? How about companies testing employees who have filed compensation claims for specific syndromes/disease that may have a genetic component? Give a brief description of the lawsuit against Burlington Northern Sante Fe Railroad (BNSF) about genetic testing related to carpal tunnel syndrome.

7) What is a SNPchip, and how will it be used to advance patient treatment?

8) You want to determine if you have a genetic predisposition to breast cancer, and cost is not a factor. Would you choose gene sequencing or gene chip analysis? Why?

9) 23andMe indicates that their assay for Type II Diabetes gives results at 9 markers. What do they mean by “marker?”

10) Find one new SNP-trait link identified in 2009. Explain how the SNP is associated with a trait/disease.
11) What is the current status of the privacy law concerning genetic testing?

12) What does the Genetic Nondiscrimination Information Act still not protect against?

13) List two advantages and disadvantages of having genetic test results delivered directly to the patient/consumer.

**NOTE:** Every student should email the course instructors any questions they would like answers to or problems they are having in developing their solution strategy, and how they would like the instructors to help (e.g., cover certain material in the next class, suggest a resource to obtain certain information).
Appendix 3

Module #3 - Bioremediation of Oil Using a Suicidal GMO

Background

Oil serves as a primary energy source for humans, but it is also a major environmental pollutant. Oil sludge is present not just spill areas, but also in refineries and storage tanks. To clean up oil-contaminated areas, biological methods are often used, and such methods are often referred to as forms of “bioremediation.” One challenge in biodegradation of oil compounds is that oil binds to soil particles and is highly hydrophobic, while the microbes that may metabolize oil compounds tend to grow in aqueous conditions. Use of surfactants has helped to blend and improve contact between the two, and microbial biosurfactants (surfactants produced by microbes) are generally preferred over synthetic ones.

The ideal bacterium to remediate oil contaminated areas would be one that both degrades hydrocarbons and produces biosurfactants. Some organisms have been genetically modified to contain or enhance both of these desired features, and one (an obligate aerobe) has emerged as a likely candidate for field testing. The concern with releasing such an ideal oil-metabolizing organism is that it might escape the contaminated release area and enter the environment “at large,” where it could degrade other petroleum-based products and even invade oil reserves.

To minimize the potential for inadvertent GMO release, preliminary field tests of the GMO are being carried out in a storage tank. The microbe is added directly to a tank
containing oil sludge, and neither is removed from the tank during the testing period. However, even if the GMO succeeds in this testing phase, its full potential will not be realized unless the escape risk can be satisfactorily addressed.

One genetic engineering technique that could be considered for this application is the creation of an internal feedback mechanism whereby the cell could be configured such that it could not survive in the absence of oil. There are multiple examples of “suicidal” genetic systems in bacteria that have potential application in this scenario. Many of these systems exist in nature as part of a plasmid maintenance system.

**Goal:**

You are a university research team writing a proposal to EPA to allow testing of the oil-consuming, biosurfactants producing GMO. You will propose to test it under controlled conditions for future use in the field. The purpose of your research is to develop a molecular biology strategy to cause the oil-degrading, surfactant-producing bacteria to survive only when in the presence of oil. The strategy will be tested in aerated storage tank containments with the expectation that if the trials are successful, the microbe could ultimately be used at open bioremediation sites.

The research objectives are to:

a) Identify gene(s) to use to make the oil-eating, biosurfactants producing GMO suicidal in the absence of some threshold of oil concentration.
b) Determine where the gene(s) should be located inside the bacterial cell (i.e. if in the chromosome, indicate where), and suggest a method to place the gene(s) at this location. For purposes of this objective, assume the target organism has the same chromosome sequence as *E. coli* strain MG1655.

c) Design an aeration system for the storage tank test containment where oil sludge will be degraded. Show that the system can provide oxygen to the microbes at a rate that will allow the sludge-microbe mix to maintain a minimum DO of 2 mg/L.

d) Prepare a list of concerns that would/should likely be raised by the public with regard to this type of open-ended GMO release. Rank them in order of validity. Prepare counter arguments to each argument. Rank your counter-arguments in order of validity.
Appendix 4

Classroom Strategy and Problem Solving Skills Survey (CSPSSS)

Last 4 numbers of your SSN ___________

Instructions: During this course, your professors used specific instructional strategies to enhance your learning.

*For #1-12, circle the number that reflects the extent to which you agree that each activity below assisted your learning during this course. If you wish, provide comments on the reverse side of this survey.*

<table>
<thead>
<tr>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly Agree</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

1. The use of problems
2. Working in groups
3. Communicating about environmental biotechnology with your group members
4. Peers as teachers
5. Working individually on assignments
6. Class discussions led by the professor
7. Class discussions led by classmates
8. Lectures by the professor
9. The coursepack of readings
10. The use of electronic resources, primarily the Internet, to find information
11. Library resources, other than electronic ones
12. The use of computers as an investigative tool
For #13-20, indicate the extent to which you agree that the activities during this course helped you improve your skills in the following areas:

|   |                                                                 |   | 1 | 2 | 3 | 4 | 5 |
|---|----------------------------------------------------------------|---|---|---|---|---|---|---|
| 13| Communicating literature and/or research results               |   | 1 | 2 | 3 | 4 | 5 |
| 14| Participating in discussions                                   |   | 1 | 2 | 3 | 4 | 5 |
| 15| Writing about environmental biotechnology                      |   | 1 | 2 | 3 | 4 | 5 |
| 16| Working collaboratively with classmates                        |   | 1 | 2 | 3 | 4 | 5 |
| 17| Finding relevant information                                   |   | 1 | 2 | 3 | 4 | 5 |
| 18| Analyzing and synthesizing information                         |   | 1 | 2 | 3 | 4 | 5 |
| 19| Using computers for information retrieval and data analysis    |   | 1 | 2 | 3 | 4 | 5 |
| 20| Thinking critically about environmental biotechnology issues   |   | 1 | 2 | 3 | 4 | 5 |
Appendix 5

Student Content Knowledge Survey (SCKS)

Last 4 numbers of your SSN __________

Instructions: Having almost completed this course, circle the number that most closely corresponds to how you feel about each item below.

Specifically, indicate the extent to which you believe that you are currently able to accomplish the following objectives of this course:

<table>
<thead>
<tr>
<th>Not Knowledgeable</th>
<th>Not Very Knowledgeable</th>
<th>Somewhat Knowledgeable</th>
<th>Very Knowledgeable</th>
<th>Extremely Knowledgeable</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
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</table>

1. Name and describe the principles behind a variety of biotechnology methods
2. List examples of molecular biology applications
3. List examples of molecular biology applications specific to environmental engineering
4. Name some potential future applications of the methods
5. Explain the basics of bioprocess engineering
6. Describe the ethical issues and arguments associated with genetic engineering
7. Describe the advantages and disadvantages of biotechnology methods relative to conventional methods
Appendix 6
Faculty Perceptions about PBL in Class (FPC)

Name: __________________________  Gender: _________   Ethnicity: __________
Department: ___________________   Current Position: ________________________

Years of Teaching Experience:
A. K-12: ___________
B. College Level: ___________
C. Graduate Level: ___________
D. Other: _________________

Please explain your previous experience with PBL (e.g., training, workshop, reading, etc.).

At this point in time, what concerns do you have regarding implementation of PBL in your course?

Please answer the following questions:

1 – Very Well    2 - Fairly Well    3 - Not Sure    4 - Not Too Well    5 - Not Well

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<tbody>
<tr>
<td>1</td>
<td>At this point in time, how well do you think that you understand the principles of problem-based learning (PBL)?</td>
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<td>2</td>
<td>At this point in time, how well do you think that you will be able to develop a PBL model that helps you satisfy your course objectives?</td>
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<td>3</td>
<td>At this point in time, how well do you think that PBL will allow the students to learn the course content?</td>
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<td>4</td>
<td>At this point in time, how well do you think that PBL will enhance your students' critical thinking skills?</td>
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<tr>
<td>5</td>
<td>At this point in time, how well do you think that you will be able to develop a PBL model that can be used at other Institutions of Higher Education?</td>
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