Introduction to Recombinant DNA
A Genetics Teaching Plan—Final Lesson Sequence

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Objectives
Grade twelve biology students learned principles of biotechnology and DNA analysis using baker’s yeast and E. coli as model organisms. Activities were designed to carry the students through learning about model organisms, to extracting, analyzing and sequencing their DNA. The lessons were delivered in the context of several lectures coupled with lab activities, and an emphasis was placed on Science, Technology, Society and the Environment from the Ontario science curriculum.

In between the lectures and lab activities, students’ learning was reinforced through further reading and questions. This way, they were hearing and reading the concepts behind the labs, as well as doing both the labs and related questions.

The molecular genetics unit of 12U biology covers DNA isolation, restriction endonucleases, plasmids, and gel electrophoresis. In addition to learning real-life applications of these tools, students gained practical experience and laboratory skills.

Learning Outcomes
1. Students learned and refined techniques and skills related to:
   - microscopy
   - DNA isolation
   - gel electrophoresis
   - PCR
2. Students’ scientific literacy improved due to use of news articles and lectures relating the concept taught to real life
3. Students learned about the future of biotech and possible jobs in related fields

Addressing Misconceptions
Before the lessons began, Kate introduced the students to three science news articles to address ideas surrounding biotechnology. It was clear that students struggled to understand the articles, but they became much clearer at the end of the unit.

By the end of the lesson sequence, students had learned much more about various tools and techniques used in a biotech setting. The same news articles were then redistributed, and students were asked to suggest various ways the scientists involved in the studies could have gone about their research. For example, in sequencing Neanderthal genes, PCR may have been used, which students were now able to recognize. They analyzed these articles as an assignment, with a class average of 76% as a result.
Day one

Lecture 1: What is a model organism? (Monday)

Introductory materials for this lesson were taken from articles on model organisms, *E. coli*, and yeast found at
http://en.wikipedia.org/wiki/Model_organism
http://en.wikipedia.org/wiki/Escherichia_coli
http://en.wikipedia.org/wiki/Saccharomyces_cerevisiae

It was stressed to the students that wikipedia is a useful starting point for finding scientific information. In the third class, Corey led a discussion regarding the different types of scientific literature ranging from primary reports through reviews, to aggregator sources such as wikipedia.

In this class, Corey introduced himself to the students. As an introductory activity, he had the students write and share a brief description of DNA, so that they could examine their prior knowledge, and so that Corey could see what they knew at the beginning of the unit.

Corey then lectured on model organisms, while students took notes. He emphasized the qualities of good model organisms, why they are useful, and why different organisms are better for different investigations. He also noted that measures are taken by molecular biologists to ensure that biological agents do not end up in the environment.

Activity 1: Viewing Cells
For the second half of class, in groups, students worked to prepare and examine slides of bacteria and yeast. This served to review microscopy techniques (http://www.biologycorner.com/bio1/microscope.html), and to familiarize students with the model organism cells that they would be working with.

Day two

After Corey's first visit, Kate reviewed note-taking techniques with her students, as many of them had not experienced a university-style lecture before. She then set up stations around the room with the articles on model organisms, *E. coli*, yeast and recombinant DNA for students to take notes on.
Day three

Lecture 2: Recombinant DNA (Wednesday)

After Kate reviewed the principles and background information with the students regarding model organisms and recombinant DNA (http://en.wikipedia.org/wiki/Recombinant_DNA), Corey returned two for a lesson about plasmids, ligation, cloning, and gel electrophoresis (http://en.wikipedia.org/wiki/Gel_electrophoresis).

He walked students through the properties of DNA and the reactants that allow DNA to be extracted from cells. The class then followed the protocol below to extract plasmid DNA from *E. coli*, with students participating in the micropipetting and microcentrifuging. He also stressed that the components of kits represents years of research, and discussed the biochemistry that occurs at each step of the protocol.

Activity 2: Extracting plasmid DNA from *E. coli*: takes 1 day
20min plasmid DNA prep

1. Add 600 μl of bacterial culture to a 1.5 ml microcentrifuge tube.
2. Centrifuge 1.5 ml of bacterial culture for 30 seconds and Discard the supernatant.
3. Add 600 μl of water to the bacterial cell pellet and resuspend completely.
4. Add 100 μl of 7X Lysis Buffer (Blue) and mix by inverting the tube 4-6 times.
5. Proceed to step 3 immediately (solution should change from opaque to clear blue).
6. Add 350 μl of cold Neutralization Buffer (Yellow) and mix thoroughly (sample will turn yellow and a yellowish precipitate will form) Invert the sample an additional 2-3 times.
7. Centrifuge at 11,000 x g for 2 minutes.
8. Transfer the supernatant (~900 μl) into Spin column.
9. Place the column into a Collection Tube and centrifuge for 15 seconds.
10. Discard the flow-through and place the column back into the same Collection Tube.
11. Add 200 μl of Endo-Wash Buffer to the column. Centrifuge for 15 seconds.
12. Add 400 μl of Zyppy™ Wash Buffer2 to the column. Centrifuge for 30 seconds.
13. Transfer the column into a clean 1.5 ml microcentrifuge tube then add 30 μ1 of Elution Buffer3 directly to the column matrix and let stand for one minute at room temperature.
14. Centrifuge for 15 seconds to elute the plasmid DNA.

Once the DNA was extracted, the class helped to load the precast gel and run it. While it was running, Corey discussed, again, the properties of DNA that allow an electrophoresis to work.

Once the gel had been run, the DNA was observed DNA using a UV box. A brief description of DNA intercalating dyes and UV damage followed.
Day four

For further understanding of the previous day's activities, the students read and made notes on plasmids and restriction endonucleases. They also completed homework questions to make sure they were clear on the concepts covered so far.

Day five

Lecture 3: DNA Transformation (Friday)

After a review of the previous lesson and activities, Corey delivered a lecture on DNA transformation, and how it relates to gene therapy as a tie in to Science, Technology, Society and the Environment. He stressed that the principles for *E. coli* transformation are the same as those for human gene therapy.

Activity 3: Transforming plasmids into yeast: takes 2 days
To perform the yeast transformation, students plated cells as a class on Friday using transformation instructions from: [http://home.cc.umanitoba.ca/~gietz/Quick.html](http://home.cc.umanitoba.ca/~gietz/Quick.html)
The colonies had grown up by Monday for the subsequent activities. Because of time constraints, the students transformed a set of plasmids into *E. coli* in class, Corey's lab transformed the same plasmids into yeast, and both sets of transformations were analyzed by the students in class.

Day six

Lecture 4: PCR analysis of transformants (Monday)

After the yeast colonies had grown, a lesson was given on analyzing transformants and how this relates to forensic analysis as a tie in to Science, Technology, Society and the Environment.

Students were introduced to the Polymerase Chain Reaction using information from both the textbook and [http://en.wikipedia.org/wiki/PCR](http://en.wikipedia.org/wiki/PCR)

Activity 4: PCR
Students performed PCR on their yeast clones, *E. coli* clones and pure plasmid DNA (the same plasmid DNA they isolated on Day 2 to perform the transformations). They were involved in micropipetting and loading the chambers in the PCR machine. A significant portion of the time was used to discuss positive and negative controls and experimental design.

Protocol: Colony PCR

Overview
Colony PCR can be used to identify colonies containing a particular genetic manipulation (in our case they contain a new plasmid)
Protocol

An average-size yeast colony (0.5-2mm) is touched with a sterile pipette tip.

Rinse the cells off the tip with 10 uL water pipetting up and down and boil.

Use 2 uL boiled yeast cells for 25 uL PCR reaction.

We use a 2X master mix for PCR, add cells and primers (2ul each)

Cycle
1. 95 deg 5min
2. 95 deg 30 sec
3. 55 deg 30 sec
4. 72 deg 30 sec
5. go to step 2 34X
5. 4 deg C forever

The PCR machine was left at the school with Kate, with the resulting DNA to be used on Corey's next visit.

Day seven

Students read and answered questions surrounding PCR and what is needed to perform a successful PCR. Kate also showed YouTube videos on the subject:

http://www.youtube.com/watch?v=_YgXcJ4n-kQ An animation with explanation
http://www.youtube.com/watch?v=x5yPkxCLads&feature=related A humourous song about the uses of PCR

Day eight

Lecture 5: DNA Sequencing (Wednesday)
Corey delivered a lecture on traditional and next-generation DNA sequencing and how it will affect health care, as a tie in to Science, Technology, Society and the Environment. In addition, we discussed different types of jobs available in the Biotech/Pharmaceutical sector.

Activity 5: Electrophoresis
Students ran a gel of the DNA from the previous PCR reaction. The results were photographed by Corey and later emailed to Kate, who shared the results with the class.
Assessment and Evaluation
This was a very involved sequence of lessons; as such, a variety of strategies were used throughout in order to monitor student learning.

Methods of assessment

Diagnostic
*Use of news article to see what students already knew, and what terms they were familiar with

Formative
*Homework questions assigned day to day to review key concepts, techniques, and terms
*"Try This": each day at the beginning of class students were asked one question relating to the previous class. The questions were created by Kate and Corey. Students wrote their answers on a scrap piece of paper, and correct answers were entered into a draw held once a month. If it was found that many students were answering incorrectly, Kate and Corey went over the correct information.

Summative
*Article analysis assignment: students were asked to choose one of the introductory news articles, and provide an explanation of three biotech tools or techniques the scientists involved may have used in their study. In this way, the students were applying the knowledge they had gained, and seeing how it applies to current scientific studies.

*Short answer and multiple choice questions on the molecular genetics unit test relating to concepts and techniques we covered were included. As well, a new news article was on the test, and students were asked, again, to explain what biotech tools and techniques the scientists may have used to reach their results.
Introduction to Recombinant DNA
A Genetics Teaching Plan—Supplementary Report

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Overall, the sequence of lessons designed by Corey and Kate was successful in teaching grade 12 students the principles of biotechnology. The reason it worked is that the concepts were illustrated with the use of lab activities. The students did not just learn about concepts such as PCR and gel electrophoresis, they were able to perform them in a classroom.

At the beginning of the lesson sequence, students had a solid idea of the properties of DNA, thanks to their previous molecular genetics lessons. They did not know, however, how scientists use these properties to manipulate DNA. They were intrigued by the news articles we pre-read as a class, but could not explain them. This changed dramatically when they were assigned the same news articles for analysis after the biotech unit, with a class average of 76% on the assignment.

Some difficulties we faced were due to timing. It was difficult to get so many points across, as well as do a related lab activity, in a 75-minute period. The spacing of Corey’s visits helped this; he was able to introduce a concept and do a related lab, and in the day between each visit, Kate could go over any points that students were having trouble with, review concepts, and work on questions with them.

Another challenge, which was important for the students to face, was the overall reaction to having a university professor in the classroom. The students were used to more structured lessons, coming from a teacher they already knew; it took them a day or so to begin taking notes effectively while Corey was talking. By the end of the lesson sequence, they were more comfortable asking questions and participating; they also knew to take notes when he was explaining things. This, in addition to the laboratory skills they learned, will serve them well in university.

At the end of the lesson sequence, Kate and Corey surveyed the students for feedback. It was overwhelmingly positive, with any suggestions stemming from the challenges mentioned above. They remarked on the fast pace, and the difficulties in note taking, for example; but they were confident they had learned the concepts well.