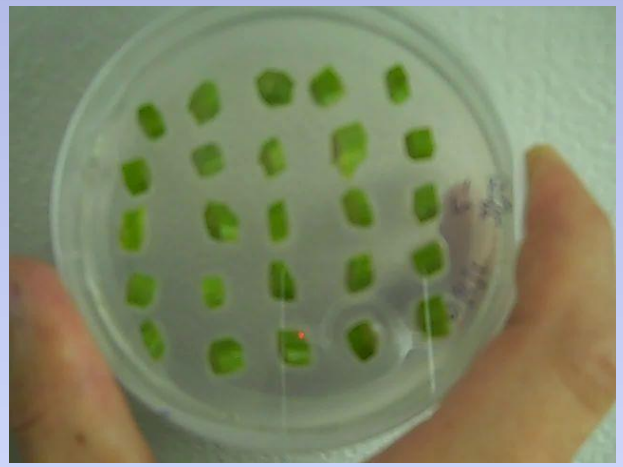
Kenan Fellowship

Using Biotechnology to Understand Lignin Production



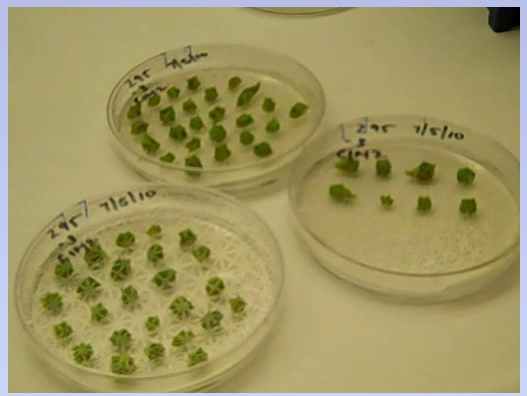
What is the Kenan Fellowship?

The mission of the Kenan Fellows Program is to enhance curriculum relevance for the benefit of all students; engage teachers, business, and universities through unique professional collaboration; and promote growth opportunities for teachers and the teaching profession.

Summer of 2010

- 1 week spent in workshops with other Kenan fellows

- 5 weeks spent at the Forest Biotechnology building learning all about the Lignin project and developing lessons for my students.



Lessons for Biology Students

- Goal – Give the students an opportunity to engage in hands on activities related to biotechnology

- 3 Lessons were developed:
  - Introduction to Lignin Biosynthesis
  - Genetic Transformation
  - DNA Fingerprinting

Lesson Model

- Exploration – engage students "hook"
- Activity – hands on, manipulating materials
- Content wrap-up - notes
- Guided Practice – allow them to make mistakes and discuss the material
- Assessment – formal assessment of knowledge

Lesson 1 - Intro to Lignin Biosynthesis

- Goals
  - Introduce students to steps involved in genetically transforming an organism and get them to think critically by organizing the steps.
  - Expose students to the steps involved in the lignin project from how a specific lignin gene is identified, to how it is altered and inserted into the black cottonwood tree.

Exploration – Answer the following questions:

1. What are some products that come from trees?
   There are a ton. Here are just a few.
   - **paper products – grocery bags, tissues, toilet paper, books, magazines**
   - **energy – burning wood**
   - **food – cereals, pet food**

   A huge list of products can be found at: http://www.idahoforests.org/wood_you.htm

2. How many trees are cut down per year worldwide?
   3-6 billion trees per year about one-third of this is used to make paper

Background Information

Lignin is a substance made by plants that makes up a portion of the cell wall of plant cells. Lignin provides the plant protection against pathogens (disease-causing agents). There are several different types of lignin. In the case of Populuas trichocarpa (black cottonwood) there are 2 main types of lignin that are found in the cell walls, G lignin and S lignin. G lignin is much harder to process than G lignin. The goal of the research being done at NC State is to understand how lignin is formed in plants. Once the scientists understand how lignin is formed, they can come up with strategies to improve the production of lignin which will in turn help with the processing of the trees. This will make the processing of wood for such things as energy, materials and food easier and less costly.

There are many steps to the Lignin Biosynthesis Project. Listed below are some of the main steps involved. These steps are out of order. Cut the steps out and place them in the correct order on a separate sheet of paper. Do not tape or glue them down until you know you have the correct order.

Student Prior knowledge:

1. Lignin is an important component in the cell wall of plants. What is another component of the cell wall that we discussed during other units?
   Cellulose

2. What is the purpose of the cell wall?
   To provide support and structure for the cell.

3. Lignin is a very large molecule. What is another word for a large molecule?
   Polymer

4. What are the individual units called of large molecules?
   Monomer

5. What does biosynthesis mean?
   Bio – **Life**
   Synthesis – **to make or produce**
   Biosynthesis – **the production of compounds by a living organism**

Steps of the Lignin Biosynthesis Project: **(Correct Order)**

- Identify the sequences of DNA (genes) in the black cottonwood that controls lignin production.
- Remove one of the identified lignin genes and change the gene slightly. The goal being to see what happens to the tree in the end if we change the DNA.
- Insert the lignin gene into a circular piece of DNA (plasmid) from a bacteria cell. The plasmid also contains a gene for antibiotic resistance. This modified DNA is called a construct or a vector.
- Insert the plasmid with the lignin gene into Escherichia coli (E.coli) bacteria.
- The E. coli bacteria reproduce in a petri dish that contains an antibiotic. This is to screen the bacteria to make sure they have the construct inside of them. Those that survive have the construct containing both the lignin gene and the antibiotic resistance gene.
- Run a PCR (Poymerase Chain Reaction) on the DNA inside the E.coli bacteria. This step amplifies (makes more) DNA so it can be analyzed.
- Gel electrophoresis is run on the amplified DNA to make sure the lignin gene is part of the construct. A marker (or known sample) is also run in the gel to identify that the construct is correct based on size.
- Place Agrobacterium tumefasciens (bacteria that is good at infecting plant tissue) into a solution containing the construct with the lignin gene. The Agrobacterium will take up the construct. This is done the same way as for the E. coli.
- Cut small pieces of the stem of a newly forming tree into 1/4" pieces and dip these into a solution of Agrobacterium that contains the plasmid/vector/construct.
- Place the small pieces of the tree stem onto a petri dish. The agrobacteria should "infect" the plant tissue with the modified lignin gene.
- Harvest the trees and analyze the changes to the lignin in the tree.

Follow-up Questions:

1. What are some benefits of the Lignin Biosynthesis project?
   To improve plant productivity
   Reduce the cost, energy and time involved in manufacturing trees

2. What are some risks or concerns with the Lignin Biosynthesis project?

   Risks of having genetically modified organisms in the environment with non-genetically modified organisms need to be analyzed.

3. One of the steps uses gel electrophoresis to make something called a DNA fingerprint? This is also a technique used on crime shows. What do you think a DNA fingerprint is and how can it be used?

   A DNA fingerprint is a unique set of bands made by DNA that can be used for comparison of individuals.

   It can be used to compare DNA from different species, identify people, and determine paternity. In the case of the lignin project it is used to identify if the gene was inserted correctly into the construct.

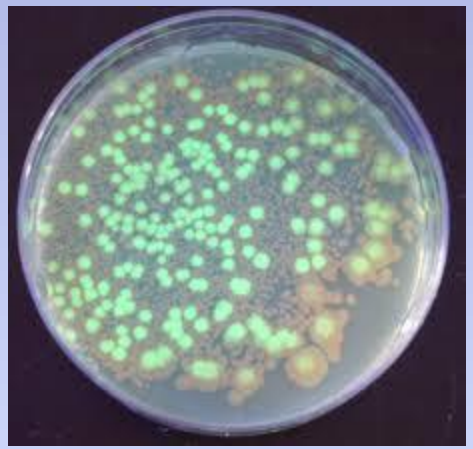
4. Another step of the project is inserting DNA from one organism into another. In this case altered DNA from a tree is put into a bacteria cell so the bacteria can carry a foreign gene. This is called genetic transformation. How do you think we can put DNA from one organism into another? Isn't the DNA of all organisms different?

   The sequence of DNA of organisms is different but all DNA is made up of the 4 same nucleotides (A, T, C, and G).

Lesson 2 - Create Glowing Bacteria through Genetic Transformation

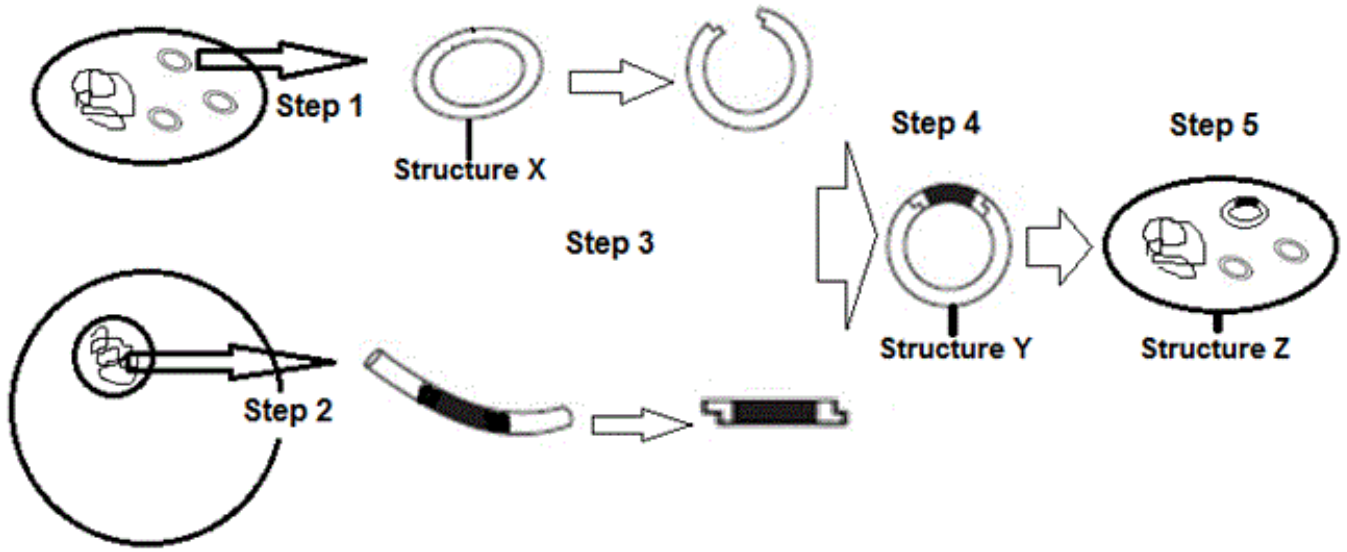
- Goal is for students to understand how transgenic organisms are created and how they are used in industry.

- The lab that we do is transforming bacteria with a pGlo jellyfish gene.



Genetic Transformation Guided Practice

Most genetic engineering experiments include four basic steps as shown in the figure below. In this example the gene responsible for producing human **INSULIN** is the gene of interest. Use the figure below to answer the questions.



1. What is structure X? _____
2. During which numbered step (#1-5) is a restriction enzyme used? _____
3. Which structure (X, Y or Z) shows **recombinant DNA**? _____
4. Which structure (X, Y or Z) shows a **transgenic organism**? _____
5. What is the benefit of going through the above process? In other words, what can the bacteria now do for humans? _____
6. What are two other examples of genetically modified organisms we discussed in class?

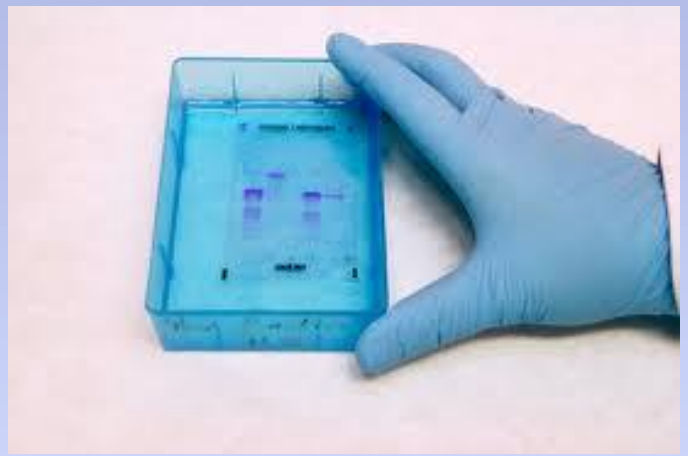
   _____
7. What is one problem or concern with making genetically modified organisms?

   _____

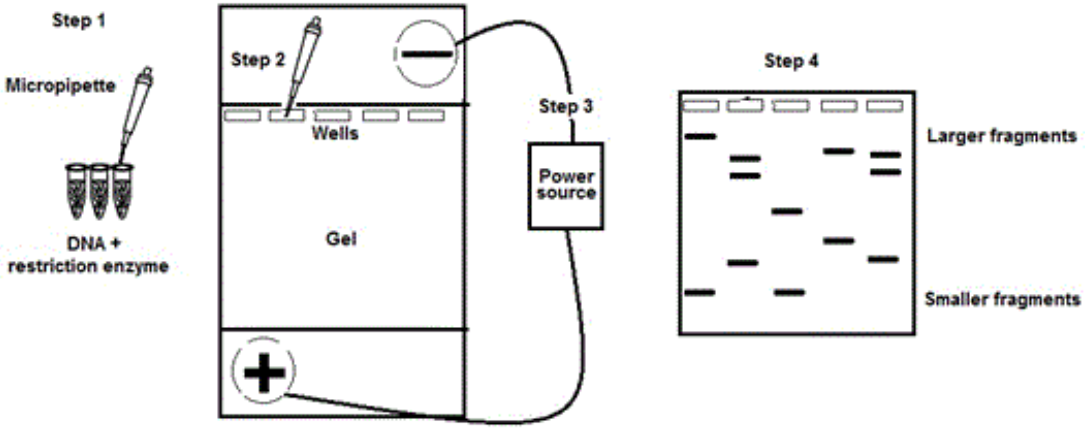
Lesson 3 – DNA Fingerprinting

- Goal is for students to understand that gel electrophoresis is a technique to separate molecules based on size and charge and how this is used to make a DNA fingerprint.

- Lab – Gel electorphoresis with food coloring



Notes: DNA Fingerprinting



Steps of DNA fingerprinting	Why is each step performed? Think back to the DNA fingerprinting lab you did.
1. DNA from blood or other tissues is placed into a tube. Restriction enzymes are also added to the tube.	
2. A gel electrophoresis chamber has been set up. There is a gel that has wells in it at the negative end of the chamber.	
3. Small amounts of DNA are placed inside each of the wells.	
4. A solution is poured into the chamber. The chamber is then hooked up to an electrical source.	
5. The DNA begins to move towards the positive side of the chamber.	
6. The DNA separates based on size.	
7. The gel is taken out of the chamber and stained.	

Ultimate goals

- Expose students to techniques used in biotechnology and give them hands on experiences.
- Show them what is going on right in their own town.
- Get the students to think critically and problem solve.
- Get the students excited about doing science.

What's Next?

- Summer 2011 – 1 week in professional development workshops and 5 weeks working in the Forest Biotechnology department at NC State.

- Develop 3-5 more lessons for my students
  - Tissue culture
  - Ecology lesson - Human impact on the environment and sustainability versus the fears of using GMOs
  - Link to DNA & Protein Synthesis unit and/or Biochemistry unit.