This year my students did various biotechnology labs that helped them understand the concepts of diffusion through a membrane, polarity of molecules, DNA structure, and bacterial transformation. I used labs available in the internet, and kits purchased through Edvotek. I am providing the links for each.

**DNA isolation from strawberries, a common lab in bio classrooms, provided the foundation for the other 2 labs: electrophoresis of dyes, and transformation with green fluoroprotein. It helped the students understand that DNA is in the nucleus, and that for it to come out of the nucleus, chemicals to break the membrane must be used.**


**DNA Isolation from Strawberries**
*Developed by Diane Sweeney*

**Teacher Background**
This is a simple, effective protocol for spooling DNA. Ripe strawberries are an excellent source for extracting DNA because they are easy to pulverize and contain enzymes called pectinases and cellulases that help to break down cell walls. And most important, strawberries have eight copies of each chromosome (they are octoploid), so there is a lot of DNA to isolate.

The purpose of each ingredient in the procedure is as follows:

**Shampoo or dishwasher soap** helps to dissolve the cell membrane, which is a lipid bilayer.
**Sodium chloride** helps to remove proteins that are bound to the DNA. It also helps to keep the proteins dissolved in the aqueous layer so they don’t precipitate in the alcohol along with the DNA.
**Ethanol or isopropyl alcohol** causes the DNA to precipitate. When DNA comes out of solution it tends to clump together, which makes it visible. The long strands of DNA will wrap around the stirrer or transfer pipet when it is swirled at the interface between the two layers.

**Notes on Materials and Recipes**
- Use Ziploc TM freezer bags rather than sandwich bags, as they are thicker.
- Fresh or frozen strawberries can be used. Be sure to thaw the frozen berries at room temperature. Bananas or kiwi fruit can also be used but yield less DNA.
- Use non-iodized table salt or laboratory-grade sodium chloride.
- 95% ethanol or 91 or 100% isopropyl alcohol can be used to precipitate the DNA. Isopropyl alcohol can be purchased from a pharmacy. Whichever you use, make sure
it is ice cold by placing in an ice-water bath or in the freezer.

**DNA Extraction Buffer**

- 100 ml (3/8 cup) shampoo (without conditioner) or 50 ml dishwasher detergent
- 15 grams sodium chloride (2 teaspoons)
- water to 1 liter

The students prepared the gels and loaded the dyes and observed the direction that the dyes moved. The students became familiar with electrophoresis by doing the virtual electrophoresis lab at the following website using the school’s mobile labs.

*The GENETICS Project Department of Genome Sciences University of Washington*

[http://chroma.mbt.washington.edu/outreach/genetics](http://chroma.mbt.washington.edu/outreach/genetics)

2

**DNA Isolation from Strawberries**

**Student Directions**

**Materials per student group**

- 1-3 strawberries (about the volume of a golf ball). Frozen strawberries should be thawed at room temperature.
- 10 ml DNA Extraction Buffer (soapy salty water)
- about 20 ml ice cold 91% or 100% isopropyl alcohol
- 1 Ziploc TM bag
- 1 clear test tube
- 1 funnel lined with a moistened paper towel
- 1 coffee stirrer or transfer pipet

**Directions**

1. Remove the green sepals from the strawberries.
2. Place strawberries into a Ziploc TM bag and seal shut.
3. Squish for a few minutes to completely squash the fruit.
4. Add 10 ml DNA Extraction Buffer (soapy salty water) and squish for a few more minutes. Try not to make a lot of soap bubbles.
5. Filter through a moistened paper towel set in a funnel, and collect the liquid in a clear tube. *Do not* squeeze the paper towel. Collect about 3 ml liquid.
6. Add 2 volumes ice cold isopropyl alcohol to the strawberry liquid in the tube. Pour the isopropyl alcohol carefully down the side of the tube so that it forms a separate layer on top of the strawberry liquid.
7. Watch for about a minute. What do you see? You should see a white fluffy cloud at the interface between the two liquids. That’s DNA!
8. Spin and stir the coffee stirrer or transfer pipet in the tangle of DNA, wrapping the DNA around the stirrer.
9. Pull out the stirrer and transfer the DNA to a piece of saran wrap or clean tube. The fibers are thousands and millions of DNA strands.
10. To view in a microscope, put the glob on a clean slide and gently tease/stretch
apart using 2 toothpicks or dissecting pins. The fibers will be easier to see in the teased-apart area.

11. Rinse your funnel. Put the Ziploc TM bag and paper towel in the garbage.

2. Another biotech lab was the bacterial transformation with Green fluoroprotein, usually done in AP Bio labs. I used a kit purchased through Edvotek http://www.edvotek.com/223.html

To facilitate understanding, the students read the lab material attached, and answered the questions, before and after the lab., and visited the interactive website: http://www.hhmi.org/coolscience/resources/SPT--FullRecord.php?ResourceId=39

Bacterial Transformation with protein fluorescent green gene (DNA)

DNA is used to code for proteins, to identify individuals (like when solving a crime), or to do genetic engineering by inserting foreign DNA into an organism. There are three strategies for getting DNA into bacteria. Bacteria can insert DNA into each other by CONJUGATION; viruses can insert DNA into bacteria by TRANSDUCTION; or we can insert DNA into bacteria using chemicals or electricity, which is called TRANSFORMATION. When we 'poke holes' in the bacteria using chemicals, we allow the DNA to flow into the bacteria- this is called BACTERIAL TRANSFORMATION. We can use bacteria as little 'factories' to make more DNA, as they replicate, or to make protein, by transforming them with genes for proteins we want to make (like insulin). The DNA we insert is shaped in a little circle, called a plasmid. We can put one, two, or more genes in a single plasmid. One of the genes in the plasmid codes for the ampicillin resistance protein, and thus will allow bacteria with the plasmid DNA to grow in the presence of ampicillin. A plasmid is a small circle of DNA. Ampicillin is an antibiotic; antibiotics prevent bacteria from growing. Ampicillin specifically prevents bacteria from making cell walls. Thus, ampicillin will not kill bacteria (that already have a cell wall), but will prevent bacteria from reproducing (because they can't make new cell walls).

To get the DNA into the bacteria, we have to poke holes in them with the chemical calcium chloride (CaCl₂). CaCl₂ will dissociate into Ca²⁺ and 2 Cl⁻, and the positive charge of the Ca²⁺ cancels the negative charge of the DNA, allowing it to cross the cell wall and cell membrane. The holes poked to allow the DNA in leaves the bacteria leaky. If we don't keep them on ice, they'll lose all the cell material and die. When doing the bacteria transformation experiment, each group should get one LB agar plate and one LB agar + ampicillin plate, and write the group name/pd on each plate. Plates should be always labeled on the bottom, the lids can get mixed up accidentally, so if the bottoms are labeled, the label will stay with the bacteria (which are growing in the bottom). The LB agar plate will show the existence of viable bacteria cells. If nothing grows on the LB agar plate then the bacteria are dead and we cannot expect transformation or growth on the LB agar + ampicillin. The tubes will be put tubes directly from ice into 42°C water bath.
Heating the bacteria helps the holes seal shut. It's like giving the bacteria a fever, so they start to heal themselves. It's called heat shock. The DNA tubes will be placed directly from water bath onto ice, and then LB broth added to the DNA tube and incubated again. The LB (Luria-Bertani) broth is both food and water for the bacteria. It will help make the bacteria healthy after poking holes in them, shoving DNA into them, and giving them a 'fever' to help them heal. The plates will be placed in a 37°C incubator for 24 hours. These bacteria are E. coli, which grow in human intestine. Because they grow in humans, they will grow best at human body temperature (37°C). Ampicillin is an antibiotic (described above). We use it in some of the plates to see if the plasmid DNA is there (bacteria will only grow on ampicillin if they have the ampicillin resistance gene). We leave it out of some of the plates to make sure the bacteria can grow if there is no ampicillin present (control.)

After the plates have been incubated, any of three things will be seen: - little white dots, called colonies (each colony starts as a single bacterium because they reproduce asexually, so each colony is like a house with a family of related bacteria) - a big smear, called a 'bacterial lawn' (this is like a city of bacterial houses, where there are so many colonies that we can't tell them apart any more) - nothing, where no bacteria are growing (ampicillin may kill all of the bacteria or we may not have spread the bacteria around the plate correctly,

DNA+ allows the bacteria to GROW and GLOW. DNA - allows the bacteria to GROW. The bacteria should form a lawn on the LB agar plate. On the plate with ampicillin, the bacteria shouldn’t grow or glow (you would see nothing) because they wouldn't have either the ampicillin resistance gene or the Green Fluorescent Protein (GFP) gene.

Name:

Date:

Period:

**Bacterial Transformation with PFG (Green fluorescent protein)**

**Some questions to get you thinking about today’s lab:**

1. What can we use DNA for?
2. Why would we want to put DNA into bacteria?
3. What is a plasmid?
4. What is ampicillin?
5. Why do you think we put the tubes on ice?

6. Where is the best place to label your plates? What is the control you are conducting?

7. What do you think heating the tubes does?

8. What is the LB broth for?

9. Why are the plates kept at 37°C?

10. What is ampicillin and why do you think we used it in some of the plates?

11. What do you expect to grow on each of the plates?

<table>
<thead>
<tr>
<th></th>
<th>LB agar</th>
<th>LB agar + ampicillin (stripe)</th>
<th>Do you expect to see any difference in bacterial growth on the two plates?</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria + DNA</td>
<td>Would expect see colonies, could count the number of colonies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12. What do you think would have grown on these plates if no DNA had been added to these bacteria?

<table>
<thead>
<tr>
<th></th>
<th>LB agar</th>
<th>LB agar + ampicillin (stripe)</th>
<th>Do you expect to see any difference in bacterial growth on the two plates?</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria (without DNA)</td>
<td>Would see bacterial lawns</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Day 2:**

1. What do you see on your plates?

2. Now look at your plates with UV light. What do you see?
3. Fill in the table with your data. What do you see on each type of plate?

<table>
<thead>
<tr>
<th>PFG (DNA)</th>
<th>LB agar</th>
<th>LB agar + ampicillin (3 black stripes)</th>
<th>Based on the phenotype, what is the DNA?</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFG/+DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFG/-DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. What does each DNA type (+DNA/-DNA) allow the bacteria to do?

5. What would the bacteria do on each type of plate (LB agar and LB agar + ampicillin) if you added no DNA?
With your group, brainstorm these AFTER LAB questions

1. Where do the bacteria grow best?

2. If the bacteria can grow on LB agar so well, why didn't they grow on LB agar with ampicillin?

3. If ampicillin is an antibiotic, why doesn't it completely stop the bacteria from growing?

4. Do both DNA types have the ampicillin resistance gene?

5. What would happen if no DNA is added?

6. What are the + DNA bacteria able to do? How is this different from the –DNA bacteria?

7. How is this kind of GENETIC ENGINEERING experiment helpful?