Appendix 2: Examples of Interdisciplinary Chemistry-Biology Laboratory Experiments

INTERDISCIPLINARY LAB # 2: SYNTHESIS AND PROPERTIES OF SOAP

Relevance to Your Life

Big Question: Why is soap considered a surfactant?

Biological Relevance: Soap comprises of two distinct ends: the *hydrocarbon end*, which *is lipothilic* and *non-polar*, and a *hydrophilic end*, which is *polar*. The non-polar end is capable of dissolving non-polar molecules, whereas the polar end of the soap is capable of dissolving polar molecules.

Chemical Relevance: The efficiency of soap is affected by various factors such as pH, the composition of the solvent (e.g., the elements/ions present in the solvent), and temperature. Temperature is a physical property of matter that is vital not only in the soap efficiency, but also in the formation of soap. High temperature in soap making increases the rate of collision of the reactant molecules thus increasing the reaction rate, hence high product yield. Moreover, low temperature increases the rate of separation of soap from the solvent.

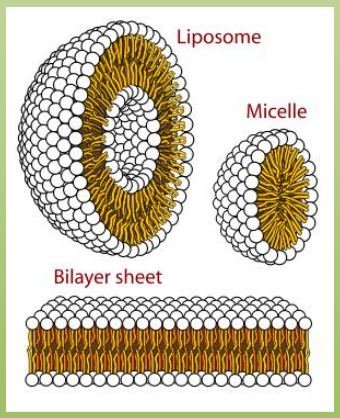
Real-World Relevance: Why does soap do a better job in cleaning greasy surfaces (e.g., dishes, clothes, etc) than plain water?

Objectives:

- Describe a procedure for making soap.
- Explain with the use of equations how soap is formed.
- Devise an experimental procedure to test the chemical properties of soap.
- Explain why soap has the ability to clean greasy surfaces or stains compared to plain water.
- Explain why soap lathers well with soft water, but forms scum with hard-water or acid rain water.

Biology Connection

Biological significance of fatty acids



Soap qualifies as a surfactant due to its molecular structure. The non-polar tail is capable of dissolving non-polar molecules (such as oils and grease) and the polar head is capable of dissolving polar molecules (such as water). Multiple soap molecules associate into droplets called “micelles” when the non-polar tails dissolve the non-polar molecules in the center and the hydrophilic heads coat the outward surface where they can interact with water molecules. Biological membranes form using the same principle. Cellular membranes and liposomes are composed of a lipid bilayer. The non-polar tails of the lipid molecules face the inner membrane space where water is absent. The polar heads line the inner and outer surfaces where they can interact with water molecules on both sides of the membrane.

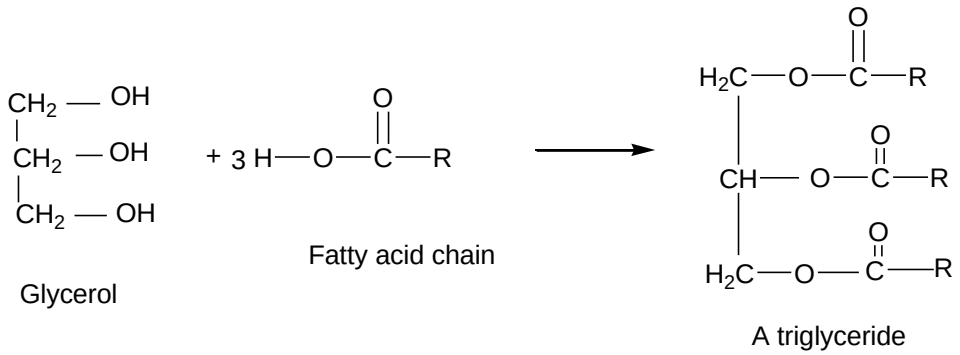
History of Soap

The discovery of soap dates back to about 6000 years ago. Around 2800 B.C.E, the ancient Babylon excavations uncovered cylinders with inscriptions for making soap.[1] In 1500 B.C.E, records from ancient Egypt described how animal and vegetable oils were combined with alkaline salts to make soap. According to a Roman legend, *"soap* got its name from *Mount Sapo*, where animals were sacrificed. Rain washed the fat from the sacrificed animals along with alkaline wooden ashes from the sacrificial fires into the Tiber River, where people found the mixture helpful in cleaning clothes. This procedure for making soap remained unchanged for centuries, with American colonists collecting and cooking down animal tallow (rendered fat) and then mixing it with an **alkali potash** solution obtained from the accumulated hardwood ashes of their winter fires. Similarly, Europeans made *castile soap using olive oil*. Since the mid-nineteenth century, the process became commercialized and soap became widely available at the local markets."[1] To date, most people use similar methods to make home-made soaps.

The Chemistry of Soap

Soap making involves the **hydrolysis** of a triglyceride (fat or oil) using an alkaline solution usually **lye,** chemical name **sodium hydroxide**. Triglycerides are typically triesters consisting of 3 long-chain aliphatic carboxylic acid chains appended to a single glycerol molecule (see Equation 1). This process of making soap is known as **saponification.** The common procedure involves heating animal fat or vegetable oil in lye (sodium hydroxide), therefore hydrolyzing it into *carboxylate salts* (from the combination of carboxylic acid chains with the cations of the hydroxide compound) and *glycerol*.

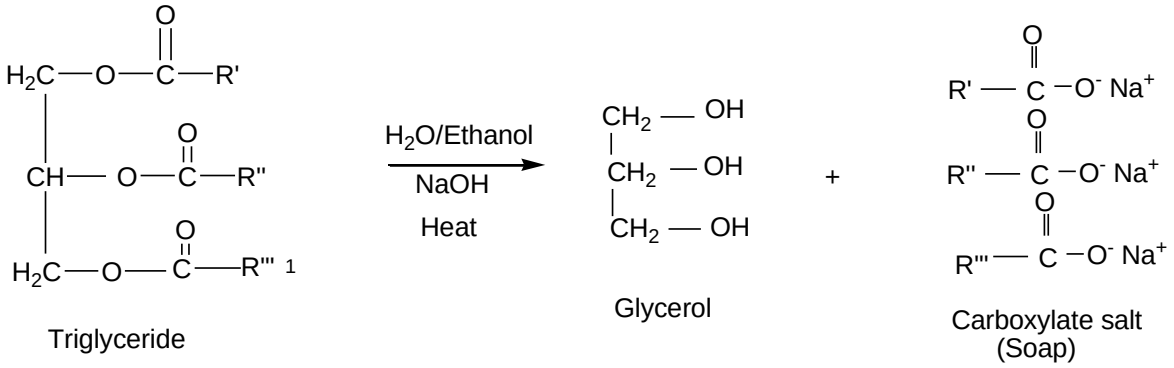
Equation 1:



Eqn 1: Reaction between glycerol and fatty acids to form a triglyceride

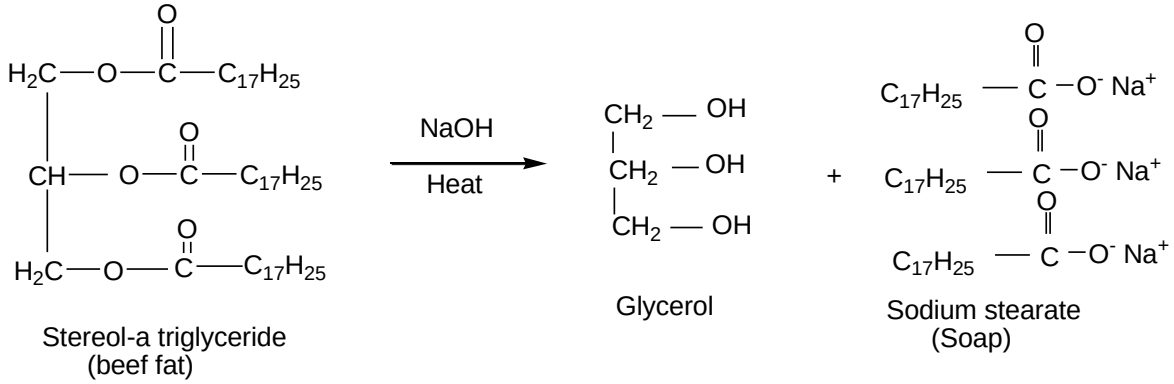
Equation 2 shows the general reaction between triglycerides and sodium hydroxide, while Equation 3 shows an example of a specific reaction between beef fat and sodium hydroxide to form soap.[1, 2] Notice that from these reactions, I mole of triglyceride requires 3 moles of NaOH to produce I mole of glycerol and 3 molecules of soap. Soap has unique properties that make it an excellent **surfactant** ("surface active" compound) or cleaning reagent. In this lab, you will: (1) synthesis soap, and (ii) study the physical and chemical properties of your soap.

Equation 2:



Eqn 2: General model showing the hydrolysis of triglycerides with sodium hydroxide

Equation 3:



Eqn 3: A specific model of hydrolysis of beef fat with sodium hydroxide[1]

Questions to ponder:

- *What are the differences between cooking fat and cooking oil?*
- *Do you think different fats or oils will produce the same type of soap in terms of color, smell, and texture?* (Share your ideas with your group members, then with the rest of the class).

A. Synthesis of Soap

Experimental Procedure

Obtain triglyceride (cooking fat/oil) from your TA, and place about 6 mL of the cooking oil or 6g of cooking fat into a 120 mL Erlenmeyer flask. Dissolve 5 g of NaOH into 40 mL of 50/50 water- 95% ethanol **(CAUTION: NaOH is very corrosive and may result into severe burns. If the chemical comes into contact with your skin, wash the affected area with copious amount of water).** Add the

NaOH solution into the 120 mL flask containing the oil, swirl the solution gently. Clamp the flask and submerge it in a 250 mL beaker of boiling water. Heat the solution for 45 minutes. **(CAUTION: Be careful with the hot NaOH. Do not look into the mouth of the flask. If splattering of the mixture occurs, remove or lower the heat).** While allowing for the stated reaction time, prepare a salt solution of 25 g of NaCl in 150 mL of distilled water in a 250 mL beaker. Place the beaker in an ice-water mixture to cool the salt solution. **(Also, brainstorm about the questions on section B, and prepare solutions for part B (II) as you wait for your soap to form)**. Pour the soap solution into the cooled salt solution after the reaction time is complete (i.e., after 45 minutes) and stir for several minutes. Filter the precipitated soap through **vacuum filtration** (as directed by the TA). Wash the solid (soap) in the funnel at least **thrice** with a10 mL ice-cold water each time. Weigh 3 g of your soap and preserve for part B. Place the remaining soap into a paper cup to harden.

Discussion questions

1. Why is it important to cool the salt solution before adding the soap solution?
2. What is the smell of your soap (record the smell in the **data sheet A**)? How does the soap smell differ from the smell of the triglyceride used?
3. What is the color of your soap (record your observation in the **data sheet A**)? Does the color of your soap differ from those of your classmates? If so, why?
4. What texture is your soap? Does you soap have same texture as those of your peers in other groups? If so, why?

B. Investigating the Chemical Properties of Soap

In part A, you have synthesized soap and studied some physical properties such as the soap color, soap smell, and soap texture. In this section, you will investigate the chemical properties of soap. You will also compare the chemical properties of your soap with the detergent provided by your instructor. ***How is this possible? Think about how you can design an experiment to study the chemical properties of soap and detergent, and share your ideas with your group partners, then with the rest of the class. How do your ideas compare or differ from the other groups' ideas.***

Experimental Procedure

Label two clean dry 150 mL beakers; one 'soap' and the other 'detergent'. Dissolve the 3 g of soap preserved in part A in 100 mL of boiling water in the beaker labeled 'soap' and 3 g of detergent provided in 100 mL of boiling water in the beaker labeled 'detergent'. Preserve these solutions for the subsequent sections. ***You are required to share your ideas with your group members on how you can test for the chemical properties of soap and detergent using the reagents/materials provided.***

I. The pH of soap versus detergent

In your group, discuss how you can determine the pH of your soap and the detergent using a pH paper. **Make sure you get approval of your test method from the TA before carrying out the test.**

What is the pH value for your soap and the detergent? Specify if acidic, neutral, or basic and record your observations and inferences on the **data sheet B** provided.

II. Testing for water hardness using soap and detergent

Using the reagents provided, devise a procedure with your group members to determine the behavior of your soap and the detergent provided with the reagents below **(make sure your TA approves your method before proceeding with the test)**. Record your observations in **data sheet B** and account for the observed phenomena as much as possible.

- 3 mL distilled water
- 3 mL distilled water + 5 drops 3% calcium chloride solution
- 3 mL distilled water + 5 drops 3% magnesium chloride solution
- 3 mL distilled water + 5 drops 3% iron (III) chloride solution
- 3 mL distilled water + 5 drops 3% sodium chloride solution
- 3 mL distilled water + 5 drops 3% ammonium sulfate solution

III. Testing for the solubility of soap and detergent in acidic water

Earlier in the semester, you carried out solubility tests on some reagents. Using the knowledge acquired from that lab, how can you devise a procedure to test for the solubility of soap and detergent in 1 M HCl solution? **Present your ideas to the TA for approval before carrying out your test.** Record your observations in data sheet B and account for the observed phenomena (obtain 1M HCl solution for TA).

IV. Testing for the emulsifying power of soap, detergent, and distilled water on mineral oil

In this test, you will investigate the behavior (reaction) of each reagent (soap, detergent, and distilled water) with mineral oil. Devise a method to set-up the experiment and **get approval from your TA before proceeding with the test.** Record your observations in data sheet B and account for the observed phenomena.

References

1. http://www.chemistryexplained.com/Ru-Sp/Soap.html.
2. Dueno et al., *Journal of Chemical Education Vol 75(5) 1998.*
3. C. E. Harland, Ion exchange: Theory and Practice, The Royal Society of Chemistry, Cambridge, 1994.
4. Muraviev. D., Gorshkov, V., Warshawsky), Dekker, M. (2000). Ion exchange, New York.
5. http://chemistry.about.com/od/cleanerchemistry/a/how-soap-cleans.htm
6. David A. Katz (2000). The science of soaps and detergents.

Post-Lab Questions

1. Using the structure of the triglyceride **(Canola oil)**, deduce the structure of the soap **(show your reaction equations).**

2. What kind of organic compound is soap?

3. Explain the reason(s) behind the observed difference in ***texture*** between the Crisco fat and the cooking oils.

4. Based on your findings, which reagent (soap or detergent) lathered well with hard water? Why?

5. Using equations summarize your observations on **part B (III).**

6. Which solutions in **part B (III)** formed scum, and which ones formed suds? Account for the observed phenomena.

Lab 7i

Polymerase Chain Reaction

Relevance to your life

Big Question: Why is the Polymerase Chain Reaction (PCR) such an important component of modern biology and increasingly to many other disciplines?

Biological Relevance: PCR is based on our knowledge of DNA and DNA replication. Without this understanding, development of PCR would not have been possible. As long as a little DNA is known at both ends, unknown DNA in between can be identified and acquired.

Chemical Relevance: Underlying the Polymerase Chain Reaction are two important chemical bonds; DNA is held together by hydrogen bonds between the two strands and held together between the nucleotides by a phosphodiester bond. Understanding this chemistry made PCR possible.

Real-World Relevance: The power of PCR lies within its simplicity and its ability to amplify and find the needle in the haystack. It has been used to detect virus infections, bacterial contamination of food, and perpetrator evidence at crime scenes. In this experiment we use PCR to determine if your corn chips were made from genetically modified crops.

Objectives

- Learn and master PCR technology.
- Utilize the biology and chemistry that underlie DNA replication to design an experiment.
- Determine if your corn chips are contaminated with genetically modified corn.

Chemistry Connection

The Polymerase Chain Reaction, while using biological molecules, is essentially a chemical reaction involving DNA. It exploits two main bonds in DNA; hydrogen bonds between DNA strands and the phosphodiester bond between nucleotides.

Hydrogen Bonds: At temperatures below 80C double-stranded DNA is stable. Above 80C the first A-T hydrogen bonds begin to break and finally as the temperature increases, the G-C hydrogen bonds between strands begin to break. By 95C, all hydrogen bonds are dissolved and DNA falls apart into single strands. The reverse is also true; as temperature decreases, hydrogen bonds between base pairs begin to form between correctly paired strands.

Phosphodiester Bonds: The covalent bond formed between nucleotides by DNA polymerase is a phosphodiester bond. DNA polymerase catalyzes the joining of the 5'phosphate of a new nucleotide to the 3'OH at the end of the DNA strand (or primer). This reaction is a condensation reaction with H_20 formed as a byproduct.

PCR Reaction: The reaction itself is a repeating series of steps that take place in a computer-controlled incubator (thermocycler); dissolve hydrogen bonds, anneal DNA primers, extend and make new DNA. It is all chemistry.

Introduction

For thousands of years, farmers have selectively bred crops such as corn and soy beans to produce desirable qualities like higher yields, greater insect resistance, better nutrition and/or being more resistant to drought. However, this kind of selective breeding takes time and crop character manipulation can sometimes be unpredictable, as increasing one quality sometimes has the unintended consequences of decreasing another.

Advances in molecular biology have sped up the pace of these changes by allowing the insertion of genes or fragments of genes from other organisms that have already evolved their genes to handle these problems. This recombinant DNA revolution has produced improved crops with increased resistance to insects and therefore allows farmers to use fewer pesticides. Other changes to crops have provided crops with higher nutritional value and the ability to grow in areas where the conditions were not sufficient previously.

Genetically Modified Foods

A plant with DNA from another organism is described in biology as a transgene (the inserted DNA) or a transgenic plant/animal. You may also have heard it called a genetically modified organism or GMO plant/animal. While both describe the same thing, one is a biological term and the other is a political term used by those against creating transgenic organisms. GMO opponents worry that moving genes between organisms or even adding new genes may cause environmental hazards, allergic reactions in humans or even super weeds if the gene gets passed to weeds. Genetically modified (GM) foods do not have to be labeled in the USA. Even if a GMO food contains 5% GMO crops, the food can be labeled GMO free. In Europe and other countries labeling is required.

One popular type of GMO corn has a gene from the soil bacteria *Bacillus thuringiensis* (BT) inserted into it. BT has been used for years by organic gardeners to kill caterpillars. Caterpillars that ingest this organism receive a toxin called delta endotoxin that specifically kills corn borers. By putting the gene for the endotoxin in the corn, farmers no longer have to spray the plants. The corn grows, expresses the toxin and if a caterpillar eats it, eventually dies.

The transcription promoter from cauliflower mosaic virus regulates the expression of the endotoxin gene so that it will be transcribed in all plant tissues. To terminate transcription, the NOS *Agrobacterium* gene is added to the end of the endotoxin gene. The result is a promoter from one organism, the endotoxin gene from BT, and the termination sequence from a third gene. This new combined gene is then transformed (we will look at this process later in the course) into a single corn cell which can then be grown into a corn plant. The final step is to cross this modified corn strain with the latest high-yielding corn strain. This process may take several years to produce a pure line, but in the end it is much faster than breeding and adds new possibilities to a plant-breeders arsenal.

PCR Technique

We will be using the Polymerase Chain Reaction (figure 1) in today's lab to detect the presence of transgenes in tortilla chips. An excellent video of the technique can be found at http://youtu.be/eEcy9k_KsDI.

The starting material for PCR, the 'target sequence,' is a gene or segment of DNA. In a matter of hours, this target sequence can be amplified (copied) a million fold. The complementary strands of a double-stranded molecule of DNA are separated by heating. The heat breaks the hydrogen bonds holding the two DNA strands together. When the temperature is decreased, the primers (two small synthetic pieces of DNA each complementary to a specific sequence) will bind to the complementary sequence in the target. DNA polymerases start at each primer and extend the strand by making a complementary copy of the opposite strand. Within a short time, exact replicas of the target sequence have been produced without knowing anything about the DNA between the primers. In subsequent cycles (heating/cooling), double-stranded molecules of both the original DNA and the copies are separated; primers bind again to complementary sequences and the polymerase replicate. At the end of many cycles, the DNA pool is greatly enriched in the small pieces of DNA that contain the target sequences. This amplified genetic information is then available for further analysis.

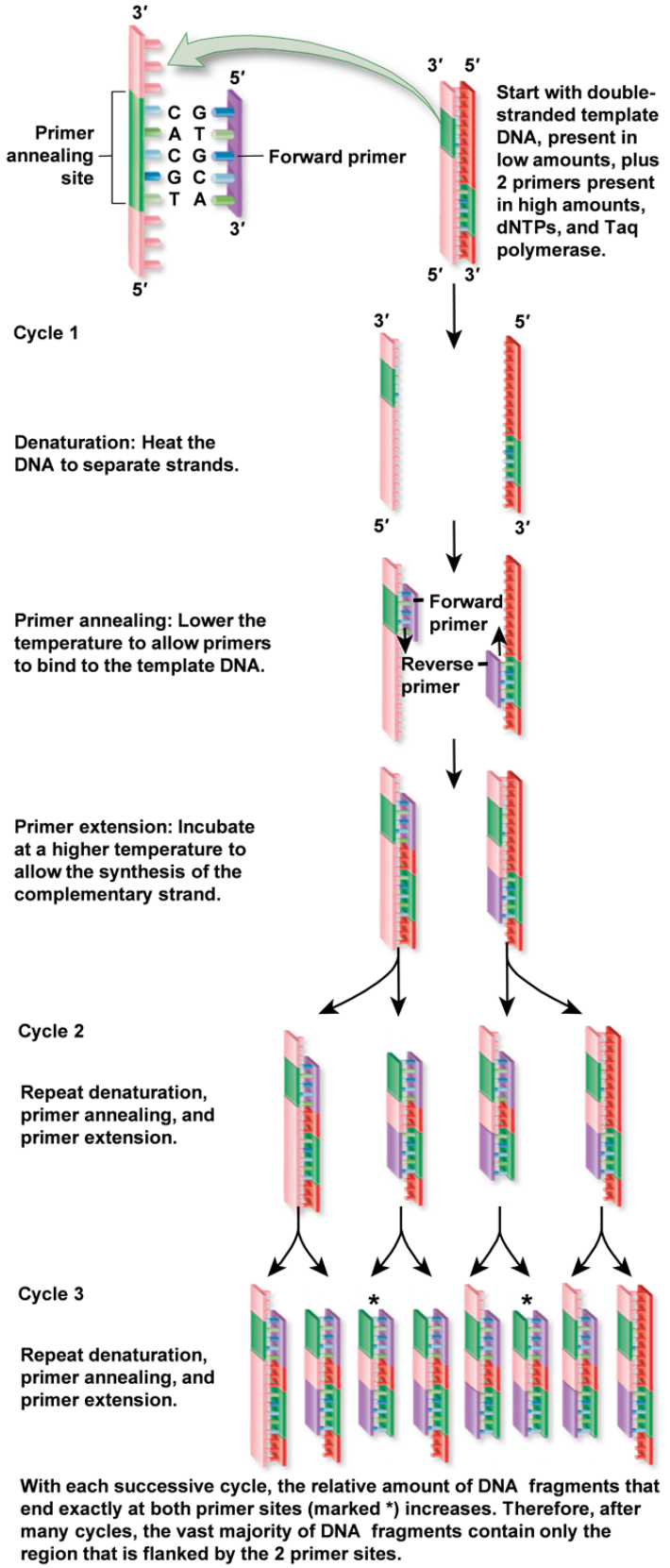


Figure 1

Equipment Used in this Class

Micropipette

A micropipette is an expensive and delicate piece of equipment used to measure very small amounts of liquid. There are several different sizes of micropipette. The range of the pipette is always printed on the top end. Regardless of the size, they all work in the same way. Watch the http://youtu.be/4AZHoi3hdWM to familiarize yourself with the use of a micropipette.

	Name	Low Volume	High Volume
Small	P-20	0.5µL	20µL
Medium	P-200	20µL	200µL
Large	P-1000	200µL	1000µL

Setting the micropipette - On your micropipette you will see a window with numbers in it. This tells you how many microliters the micropipette will withdraw from your liquid. The black wheel changes the setting. Always make sure that you do not set the micropipette any higher or lower than the range of the micropipette shown above. If you do, it may jam or break.

Putting on a tip - You always need to use a tip when you are using the micropipette. You should use a new tip each time you change solutions (to avoid contamination). Hold the box of tips on the desk (so it doesn't flip over) and slide the micropipette into a tip. Withdraw micropipette and tip from the box.

Use of the plunger - First, look at the plunger part of the micropipette. When you push the plunger down there are two different stops. The first stop is for drawing up liquid. If you keep pushing down you will notice that it gets a bit harder to push and you come to the second stop. This second stop is for pushing out the liquid.

Using the micropipette - Push the plunger down to the first stop and put the tip into the liquid. You don't need to stick the tip very far into the liquid. Release the plunger while the tip is in the liquid. Release it slowly and smoothly so it doesn't just pop up. Take the tip out of the liquid and push the plunger down all the way to the second stop to push out all the liquid into your new tube. Remove the tip by pushing down on the ejector button by your thumb and place the used tip in the trash.

NOTE: You can review the proper use of the micropipette http://bit.ly/qJzfEb.

Thermocycler

The PCR machine or thermocycler is a heating and cooling block. The block heats the samples to 94C to make the DNA single stranded, then cools to 59C to allow primers to bind to DNA and then finally to 72C so the polymerase will be able to make the complementary DNA strand using the primers as starting points. We will run the thermocycler for 40 rounds of heating and cooling, at that point there should be sufficient DNA to observe in next week's lab. The process takes about 4-5 hrs. so your TA will put the finished PCR tubes in the freezer until next lab.

PCR, Lab Session 7 - Day of Class

Experiment

Part A – Instructions, 15 minutes (00:15)

PCR utilizes enzymes needed for DNA replication to make millions of copies of specific fragments of DNA. To PCR amplify a fragment of DNA you need to know a little of the sequence of the DNA you are interested in amplifying. Two small (20-25 bp (bp=base paris) single strand DNA strands called primers were purchased which are complementary to the two ends of the BT toxin DNA sequence; this is all that is needed. For BT, one primer is 5'-ACCATCAACAGCCGCTACAACGACC-3' (the other is not listed but is required). The size of the resulting double-stranded PCR product is 225 bp in length.

As a positive control, we will be using another plant gene called PSII. This gene is found in all plants since it is a major constituent of photosystem II and is needed for photosynthesis.

The question you will be trying to answer today and during next week's lab is: "Do any of the Tortilla chip brands contain transgene DNA (GMO)"?
Even after storage, grinding, harsh (lye, strong base) treatments to remove the corn kernel cover, pressing into tortillas and frying of chips, there are still DNA fragments large enough to be detected.

Part B – Isolation of DNA, 30 minutes (00:45)

Your table will be given the choice of four bags of corn chips labeled A, B, C or D. **PICK two bags** to check for the presence of GMO. You will only need one chip from each bag. Make sure you mark down and identify (keep good notes) which bag you took the chip from.

1. Measure out 1 gram of chip from one bag (a chip weighs about 2 grams). Break off pieces until you get the correct weight. Place the pieces in a mortar. **Repeat the same procedure for the second bag and the GMO free corn meal sample.**

2. Grind the chip/corn meal up with the pestle until it forms a fine powder.

3. Add 5 ml of distilled water to the mortar. Grind powder and water with a pestle for at least 2 min to form the chip slurry.

4. Add 5 ml of water again and grind further until smooth enough to pipet.

5. Prepare three tubes with an appropriate label corresponding to your samples. The label should include your initials (or group number), day and chip bag.

6. Pipette 50ul of each ground slurry to the small plastic tubes containing 500 μl of InstaGene, Recap tube (check with the instructor to make sure you use the correct size tube). Note: the tips used to transfer the 50 ul are very small and easily clogged with particles of corn chips. Look at the fluid in the tip to make sure fluid comes to the 50 ul line.

7. Shake or flick the tubes and place in a 95 C water bath/heat block for 5 minutes. The InstaGene mixture contains a resin that removes the Calcium, Magnesium and other ions from the solution (like a water softener) and the heat helps to liberate the DNA.

8. Place the tubes in a centrifuge and centrifuge for 5 minutes at the set speed. This will pellet the resin and any large fragments of chips.

The supernatant now contains the DNA. Be careful not to disrupt the pellet which will contaminate your DNA!

Part C – Experimental Design, 30 minutes (01:15)

The DNA you have made has 20,000 potential genes. PCR allows you to make millions of copies of one of those genes specified by the primer set. You are essentially looking for a needle in a haystack. But things can go wrong.

1. You could have contaminated your sample with GMO DNA from another sample.
2. You might not have properly heated or spun your DNA preparation, thus no DNA in your solution.
3. You could have made good DNA but left out some component of the PCR reaction, thus no product.
4. Or there could be a technical problem with the PCR machine, thus no product.

Here is where experimental design becomes important. The experiment you design should take into account that you have a maximum of 7 tubes to set up (this is due to the limitations in next week's lab). Some tubes will be used to test for the unknown chip DNA samples but others should be used as controls to make sure the various parts of the PCR are working properly.

A. You have available in the lab two sets of primers; one for the BT transgene and the other for PSII (photosystem II used in photosynthesis).
B. You have your unknown DNAs.
C. You have your DNA from a known GMO free source (corn meal).
D. You have available DNA from a known GMO source.

1. State the hypothesis that you will be testing.

2. Using your isolated DNA samples and the four primers (A, B, C, D), design an experiment to test your hypothesis while controlling for problems 1-4 above. This design, when done correctly, will tell you what to put into each of the 7 PCR tubes. Use the table below to help in your design process.

PCR design table

Tube	Type of DNA	Primer Mix	Purpose	Result [to be filled in next week]
1				
2				
3				
4				
5				
6				
7				

Part D – Group Discussion, 15 minutes (01:30)

Your TA will pull you all together and discuss the experimental design you derived. Be prepared to share with the class what you decided. After class consensus is reached for each of the seven tubes, you will set up your 7 tubes for the PCR test. For your lab report you will be asked to indicate why PCR was done on each of these samples so keep good notes.

Part E – PCR Set Up, 30 minutes (02:00)

1. It is now time to pipette samples and reagents into each of the 7 tubes. Following the experimental design above, place 20 ul of the correct DNA into each tube. **USE a NEW tip each time! Be careful not to disturb the pellet when you take the DNA solution. Only put your tip into the tube far enough to get the fluid, not the resin pellet.**

2. With new pipet tips, add 20 ul of the appropriate primer mix to each tube. The **primer mix** contains the forward primer, reverse primer, deoxynucleotides (dATP, dCTP, dGTP, dTTP) and the heat stable DNA polymerase enzymes needed for PCR.

3. When all tubes are completed, cap each tube and mix each of the tubes by flicking with your finger, then tap the bottom on the table to get the fluid to the bottom of each tube.

4. Give your tubes to the TA who will help you load the tubes into the PCR machine.

Part F – Regroup, 30 minutes (02:30)

Your TA will pull the class back together to discuss the following questions.

1. What is happening at the **chemical level** during PCR cycles?

2. What will we do next time with our PCR fragments?

3. Is GMO food of any concern?

Clean up your work area:

1. Before leaving the lab make sure you table is clean. You can discard the remainder of the DNA samples.

2. Throw away tips and any other garbage in the regular trash.

3. Scrub the mortars and pestles with soap and water, rinse with water and leave upside down to dry for the next class.