

DNA Extraction-Liver and Banana

Introduction

The goal of this lab is to extract DNA from a tissue such that you can actually see the isolated DNA. There are three basic steps in DNA extraction. First, the cell must be broken open (lysed). Next, the nucleus must be broken open to release the DNA. Last, the DNA must be precipitated out of solution. The reagents necessary for the extraction procedure are **detergent, salt and alcohol**.

How can a cell be opened to extract DNA?

Both the cell and nuclear membranes are composed primarily of phospholipids and proteins. Phospholipids, like other lipids are “oily”, and as dishwashers know, detergents cut grease. Likewise, **detergent** will “cut” or emulsify the lipid-based membranes, enabling extraction of DNA.

Phospholipids have two parts, a polar (hydrophilic) end, and a non-polar (hydrophobic) end. In water, these molecules will spontaneously arrange themselves in a double layer with the polar ends facing out. Likewise, the cell membrane consists of a double layer of phospholipids, with the polar ends facing the external and internal environments of the cell, with the nonpolar fatty acids forming the inside of the membrane. Just as detergent gently dissolves fats in a frying pan, a little detergent gently dissolves cell membranes (Fig. 1).

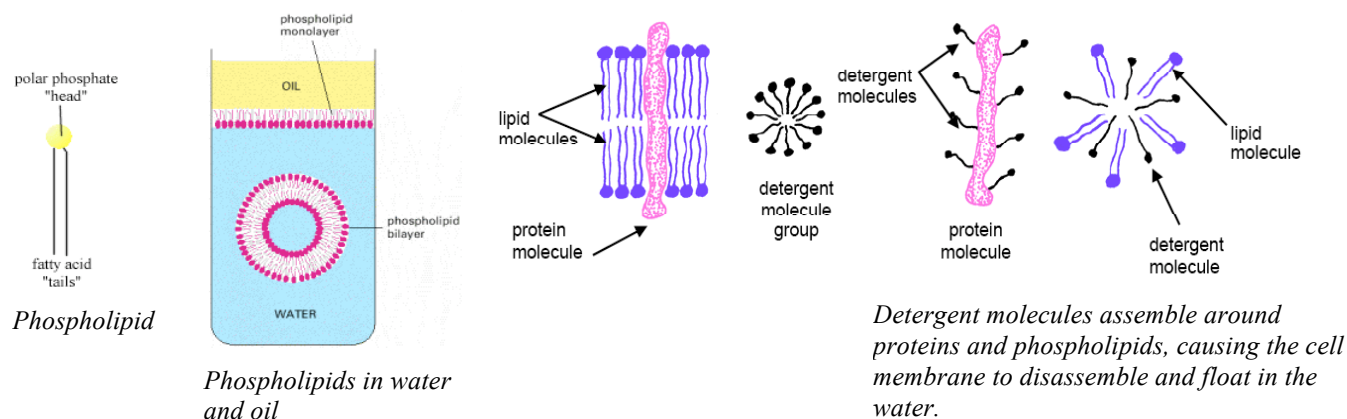


Figure 1. How detergent disrupts the cell membrane.

The detergent used in this protocol is sodium dodecylsulfate (SDS). SDS is also called sodium lauryl sulfate, and is a detergent that is found in dish detergent and shampoo.

As the cell membranes dissolve, the DNA is released from the cell into the surrounding liquid. DNA is soluble in water, but not in alcohol. In **alcohol**, DNA uncoils and precipitates. When alcohol is added to a solution of water and DNA, the DNA precipitates from the water and becomes visible in a boundary layer between the water and alcohol.

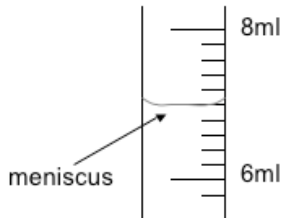
Salt provides DNA with a favorable environment for precipitation in alcohol by contributing positively charged atoms that neutralize the normal negative charge of the DNA. This allows the DNA to clump together.

Notes on measurement:

Accurate measurement is essential to successful implementation of molecular biology techniques. Techniques such as PCR often fail in the hands of undergraduates because of inaccurate pipetting. These techniques are extremely sensitive to having exactly the right amount of each enzyme and chemical.

Graduated cylinder:

For measuring larger volumes of liquid (2-1000ml), the graduated cylinder is the best tool. When using the graduated cylinder, a meniscus forms because the water molecules are more attracted to the glass than to each other. To measure the volume of liquid in a graduated cylinder, read the level at the bottom of the meniscus. In the image below, the cylinder contains 7ml of liquid.



Pipettor use:

Look on the disk at the top of each pipettor to see the maximum volume that the pipettor will hold. If it says 1000 μ l, we call this a 1000 μ l pipettor. We also have 200 μ l, 100 μ l and 10 μ l pipettors. 10 μ l isn't very much liquid.

All of the pipettors should have 3-4 digits that change as you rotate the disk on the top of the pipettor. These digits tell you the volume that you will be picking up. But the digits have different meanings on the different-sized pipettors, so it can be a bit confusing. When some of the 1000 μ l pipettor read 100, this means you will pick up 1000 μ l, and when it reads 020, you will pick up 200 μ l. The 1000 μ l pipettor does not go below this volume. However, on the 100 μ l and 200 μ l pipettors, 100 means that you will pick up 100 μ l. On the 10 μ l pipettors, 100 means 10 μ l. Is that confusing or what? Always check the top of the pipettor to see the maximum volume. Use this information to help you interpret the digits on the side.

Some pipetting rules:

Never rotate volume adjuster beyond the upper or lower range.

Never use without a disposable tip in place.

Never lay pipettor down with filled tip-fluid could run back into the piston.

Never let plunger snap back after withdrawing or ejecting fluid. This could damage the piston.

Use a fresh tip for every sample and every solution to avoid contamination.

Always use the smallest-volume pipettor that will hold the desired volume.

When picking up sample, depress plunger to first stop, place tip into tube, suck up liquid slowly. Always watch the liquid as you suck it into the pipettor. Make sure you don't pick up air bubbles. Check to see that the volume looks correct

When ejecting sample, touch tip to side of tube or place tip into liquid already in tube, push plunger down slowly to second stop. Eject tip into trash so you don't accidentally reuse it

Materials: (you'll need to share some items)

Coffee filter or piece cheese cloth

Glass rod

Piece of fruit (tomato or banana, etc)

ziploc bags

50 ml conical bottomed Falcon tube

10% SDS (20g SDS in 200ml) or dish soap

0.9% NaCl (9g in 1L)

95% ethanol ice cold (stays in freezer or on ice till you are ready for it)

10ml graduated cylinder

pipetter (1000ml)

Procedure (work in pairs - one student can do tomato and the other banana)

- 1 Place a small piece of tomato or banana tissue (about 1cm x 2 cm) into a **ziploc bag and smash** with fist.
- 2 **Add 10 ml of NaCl solution and 2 ml SDS** to the tissue. Continue breaking down the tissue.
- 3 **Strain** the cell suspension through several layers of **cheesecloth or 1 layer of coffee filter** into a falcon tube to remove any unpulverized tissue.
- 4 **Gently** drip in **15ml ice cold ethanol** to the falcon tube containing the cell solution. **The ethanol should form a layer** on top of the extract. Below, **draw** what you see—be sure to include the “interface”.
- 5 At the “interface” between the solutions, a white, mucus-like substance will appear. You can see the air bubbles attached to it. This is DNA with its attached proteins. After the DNA has formed, **use a stirring rod to slowly twirl the strands of extracted DNA.**
6. When you are finished, wash all your dirty dishes and return them to where you got them. You can throw away the plastic bag.

Questions: (If you can't answer these, ask your neighbors or TA) **Name:** _____

- 1 What makes up the cell membranes, and what are the chemical properties?
- 2 How many membranes does a eukaryotic cell have? Where are they? (You don't really need a number here, just list a few)
- 3 What happened to the cells when you added SDS? Why?
4. Draw and label your Falcon tube with the "interface"
5. Why does the DNA end up at the boundary layer after the addition of ethanol?
6. How could you prove that the substance you extracted is DNA? Brainstorm with your lab partner and other lab members. Describe one or more possible experiments