

Lab #2: DNA Extraction

In this lab, we will extract DNA from **two sources** using **two** different methods

1- Duck muscle tissue extraction – using QIAGEN’s DNeasy Tissue Kit

2- Tomato and banana tissue extraction – using common household products

The QIAGEN protocol will extract DNA from muscle tissue. This lab component uses commercially available kit-based technology from QIAGEN that frequently is used in many genetics labs around the world, including several labs at UAF. In practice, geneticists might use this protocol or a variety of other methods to extract DNA. No single method is best for all applications. In addition to quantity and quality of DNA, the time and cost invested in each method and the end use of the DNA also must be considered. Will the DNA simply be used once, or will it be archived and analyzed repeatedly? Different methods offer different advantages and disadvantages. In the second lab component, DNA will be isolated from tomato and banana cells by lysing (breaking) the cellular membrane and separating the DNA from cellular components through ethanol precipitation. The purpose of this lab component is to show you what large quantities of DNA look like (basically a lot of slimy mucus---compare to it what you like!) and familiarize you with DNA precipitation. This part of the lab exercise is meant to be informative and fun.

1- QIAGEN’s DNeasy Tissue Kit

In this lab component we will use a commercial kit from QIAGEN, Inc. The DNeasy Tissue Kit allows you to follow simple steps to produce a ready-to-use DNA sample in about three hours. First, cellular components are lysed and histones and other proteins associated with the DNA are digested with an enzyme called proteinase K. This step requires an incubation period of approx. 1.5 hours. The DNA is next bound to a mini-column holding a silica-gel membrane that selectively binds DNA. The cellular debris is washed off using a series of buffers containing salt and ethanol and the centrifuge. Lastly, the DNA sample is eluted, or concentrated with water. You should wear gloves for this lab to minimize the possibility of cross-contamination with somebody else’s tissue sample.

Materials

- ✓ Gloves
- ✓ Tissue sample
- ✓ Forceps/scissors
- ✓ Pipettes and tips
- ✓ 1.5 mL microcentrifuge tube (2)
- ✓ Buffer ATL
- ✓ Proteinase K
- ✓ Buffer AL (contains chaotropic salt, an irritant)
- ✓ Ethanol (96%-100%)
- ✓ DNeasy mini-column

- ✓ Buffer AW1 (also contains chaotropic salt)
- ✓ Buffer AW2 (contains sodium azide)
- ✓ 2 mL collection tube
- ✓ Microcentrifuges
- ✓ 70°C heat block

Methods for QIAGEN DNA extraction

Lyse

1. Obtain tissue sample about $\frac{1}{4}$ the size of a pea and place it in a 1.5 mL microcentrifuge tube.
2. Add 180 uL Buffer ATL.
3. Add 20 uL Proteinase K and incubate at 55°C in a heat block until tissue is lysed. Shake occasionally, ~every 20-30 minutes. The lysate will appear as a cloudy material in the microcentrifuge tube. The incubation will take ~1.5 hours.
- 4. Proceed to the tomato/banana DNA extraction.**
5. After 1.5 hours, add 200 uL Buffer AL to sample, mix by hand, and incubate at 70°C for 10 min in a heat block.

Bind DNA

6. Add 200 uL ethanol to sample, mix thoroughly by hand.
7. Place a DNeasy mini-column in a 2 mL collection tube. Pipet the mixture from the microcentrifuge tube into the mini-column so that the mixture touches the filter but the pipet tip does not. Centrifuge for 1 min. at maximum speed.

Wash

8. Place mini-column in a new 2 mL collection tube. Discard flow-through. Add 500 uL Buffer AW1. Centrifuge for 1 min. at maximum speed.
9. Place mini-column in a new 2 mL collection tube. Discard flow-through. Add 500 uL Buffer AW2. Centrifuge for 3 min. at maximum speed. The filter should be spun dry so there is no ethanol. Remove the DNeasy mini-column carefully so that the column does not contact the flow-through.

Elute

10. Place the mini-column in a new 1.5 mL microcentrifuge tube. Pipet 200 uL distilled H₂O directly onto the mini-column membrane. Incubate at room temperature for 5 min then centrifuge for 1 min at 9,000 rpm (start on low and slowly bring up) to elute. At this point, the DNA has been pulled through the filter and is in solution with the H₂O. Discard the filter, keep the flow through this time.
11. **Label your DNA sample as indicated.** The DNA samples will be collected and frozen until you use them for the PCR lab next week.
12. Take notes of your sample ID, the species you extracted DNA from, and all other information that will be relevant when we analyze our sequences.

2- Tomato and Banana DNA extraction (using common household products)

The goal of this part of 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.

- ❖ **SDS (Sodium dodecyl sulfate)** - a biological detergent that breaks down cell membranes by dissolving the lipids and proteins of the cell and disrupting the bonds that hold the membrane together. SDS is also called sodium lauryl sulfate, and is a detergent that is found in dish detergent and shampoo.
- ❖ **Sodium chloride (salt)** - shields the negative phosphate ends of DNA and allows for precipitation in ethanol.
- ❖ **Ethanol** - used to separate the DNA from other cellular components (lipids and proteins). DNA is insoluble in ethanol, while lipids and proteins are not. This property leads to easy separation of DNA.

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.

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.

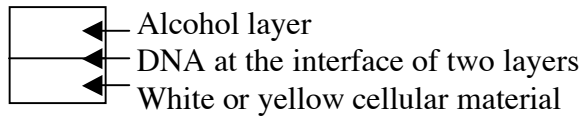
Supplies

- ✓ Coffee filter or a piece of cheesecloth
- ✓ Glass rod
- ✓ Piece of fruit (tomato or banana)
- ✓ Ziploc bags
- ✓ 50 ml conical bottomed Falcon tube
- ✓ 10% SDS (20g SDS in 200 ml)
- ✓ 0.9% NaCl (9g in 1L)
- ✓ 95% ethanol ice cold (stays in freezer or on ice till you are ready for it)
- ✓ 10 ml graduated cylinder

✓ pipetter (1000 ml)

Procedure (work in groups of 2-3 people/group)

1. Place a small piece of tomato/banana into 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 the cheesecloth or filter paper into a falcon tube to remove any pulverized tissue.
4. Slowly add 15 ml of cold 95% ethanol down the side of the falcon tube with the squirt bottle. Look for the precipitate. The ethanol should form a layer on top of the extract. DNA should be visible as a clear, mucus-like substance at the interface of the alcohol layer.



5. Let mixture stand for a minute. After DNA has formed, use a stirring rod to slowly twirl the strands of extracted DNA.
6. Draw what you see in your lab notebook, and write down if you extracted DNA from tomato or banana.