

DNA Extraction Lab Basic Level Student Version

Credits: Austin CC BioTechEd project; Donald Bell, OCCC project.

DNA Extraction Lab

A complete copy of DNA is found in every cell in any organism. In order to release the DNA to analyze it, scientists must break open the cells and remove structural proteins and enzymes that interfere with the DNA structure. This simplified procedure releases a great deal of DNA so that you can see it. It allows observation of DNA's physical and chemical properties. It does not, however, purify the sample enough for the strict standards of a research or forensics lab.

To investigate DNA, you must know the following:

- DNA is found in the cell of every living thing
- Proteins and enzymes may obscure seeing the DNA, so it must be removed.
- You must break thru a cell membrane or cell wall to release DNA.
- Among eukaryotes DNA is contained in the membrane wrapped nucleus.
- DNA in prokaryotes is floating free in the cytoplasm.

You will have these materials to use:

- Raw wheat germ (premeasured)
- Non-iodized table salt (premeasured)
- Dishwashing detergent (premeasured)
- 6% papain solution (meat tenderizer) (premeasured)
- 10 ml ice-cold ethanol in a test tube (keep it on ice)
- Warm tap water (not boiling)
- Ice
- 2 small plastic cups
- 1 plastic cup to hold ice
- 1 small plastic cup for disposal of materials
- 1 dropper (for stirrer and to dispense solution)

When you have collected these materials, wait for instruction from your instructor.

Laboratory

1. In one of the small cups, mix about 50 ml of hot distilled water (or tap water) with the vial of dishwashing soap and salt. Stir easy to limit the bubbles. *(Soap and salt are used to disrupt the cell walls and membranes to release the DNA. Heat also helps “lyse” the cells and speeds up the reaction. The salt will also help later to precipitate the DNA so that it becomes visible and can be separated.)*
2. Place the raw wheat germ in the second small plastic cup. *(Wheat germ is the embryo of a kernel of wheat – purchased at the grocery, it is usually toasted which destroys the DNA, this wheat germ is raw.)*
3. Add enough of the soap and salt solution to the wheat germ to fill it about 1/3 full. *The wheat germ will absorb the water and swell so you may need to add more soap solution so there is clear liquid on top for step 7. If you add too much solution, the DNA will be diluted and you won't see as much in the last step.*
4. Add the vial of meat tenderizer solution that contains the papain. *(Meat tenderizers work by breaking down proteins to make the meat softer. There are proteins associated with DNA that will make it harder to spool and less likely to clump together and precipitate unless they are removed. Papain can also help break down DNAase, an enzyme that breaks down DNA.)*
5. To give the soap and salt time to work, stir the solution **slowly** for 5 minutes using the blunt end of the pipette. Stirring helps the reactions but don't stir too fast or you will get bubbles from the soap that traps the DNA.
6. Allow the solution to settle for about 2 minutes (or centrifuge for 30 seconds).
7. Use the pipette to withdraw 1 dropper full (about 1 ml) of the clearer fluid near the top of the solution.
8. Slowly add the fluid to the test tube containing 10 ml of ice-cold ethanol. DNA is soluble in water, but not in ethanol. The colder the ethanol, the less soluble the DNA is. The DNA may not appear immediately but will slowly appear over the course of about 3 minutes.
9. Use the pointed end of the pipette to try and spool the DNA. Stir the solution slowly with the rod trying to wrap the DNA around it enough that it won't slide off when you pull it out of the solution.

Questions:

1. What is the purpose of each of the following components in this protocol?

Dishwashing liquid

salt

meat tenderizer (papain)

ethanol

2. We can't really see a DNA molecule under the microscope unless it is tightly coiled into a chromosome. Why can you see the DNA after you put it into the ethanol?

3. If you were able to spool the DNA you could see that it is stringy and has the consistency of thick syrup or mucus. Based on what you know about the molecule, why do you think it has this consistency?

Your questions: Think about how you can use this technique to find out if you can extract DNA from other formerly living materials: fruits, vegetables, meats, etc. You could search the Internet to get a protocol to extract DNA from a banana for example, or just try it out. Your teacher may have additional suggestions on how to use your lab skills.

Explorations in DNA Extraction

Credits: Oklahoma Community College

Anything that is or was living has to have DNA in it. Hence, you should be able to extract DNA from a great variety of sources. There are a few basic steps to the process:

1. You need a source of DNA. Your sample could include split peas or dry lima beans, frozen spinach, chicken liver, onions, raw wheat germ, fruit, yeast, calf thymus, frozen worms, etc. Start with a measured amount (a one inch chunk, or 1 teaspoon, or 10 g—the amount isn't as important as the fact that you know exactly how much you used). Then if you don't see DNA at the end, use more of the sample and less water when you repeat.
2. Add water—about twice as much as your sample volume—to let the DNA go into solution. Many protocols call for hot water.
3. Add a large pinch of salt—about $\frac{1}{4}$ teaspoon or 1.5g
4. Add detergent (preferably clear) dishwashing liquid. You will need an amount about equal to the amount of water you started with. So if you used about 50 ml of water, add about 50ml of dishwashing liquid.
5. Mix by stirring or use a blender. At this point you want to have a thin opaque solution (you can't see through it). Allow 5-10 minutes for the salt and detergent to work to break down the cells' wall and membrane. Putting the sample container into a hot water bath may help at this point. Some protocols recommend heating to about 50°C at this point.
6. Filter to remove cell debris using cheesecloth or a coffee filter. You can also spin your sample in a centrifuge if it is available. You want to discard the solid material and keep the filtrate or supernatant from centrifugation. Put the filtrate/supernatant into a test tube.
7. Add a pinch of enzymes that break down proteins. Sources of these proteins include Adolph's meat tenderizer (a pinch is about 5g) or 6% papain (1ml) or contact lens cleaner with papain (1-2ml) or juice of fresh (not canned) papaya or fresh (not canned) pineapple. These enzymes will break down proteins clinging to the DNA and the proteins that can break down the DNA itself. Stir gently.
8. Tilt your test tube and slowly and gently drizzle **ice cold alcohol** (70% ethanol or isopropyl alcohol) down the side of the test tube. Use about the same amount of alcohol as filtrate. Let the alcohol form a layer on top of your liquid. **DO NOT MIX.** Handle it carefully. Let sit until you see DNA.

Now you should see DNA rising from the liquid on the bottom into the alcohol layer at the interface. It has been described as slimy or stringy or fluffy like mucus or snot.

Troubleshooting: If you don't see DNA, maybe your sample had a lot of water so try increasing the amount of sample you use. Make sure your blended sample is opaque.

1. Why can't you use cooked food for your sample of DNA?
2. What part of the cell would the detergent work on?
3. Why is keeping a record of what you did important?

If you do further experiments that compare results, you want to design a controlled experiment. You would use precise measurements and keep a detailed record of your procedures and results.

- You would use graduated cylinders to record volumes and a balance to record weights
- You would record how much time you allowed for each step for mixing and blending and incubation
- You would want to “control other variables.” That is, do every step exactly the same except for the one variable you are testing. For example, you might compare papaya juice versus meat tenderizer as the enzyme source. In this case you would keep all amounts and time identical and only change the enzyme source.
- You would record each step of your procedure fully and carefully so that someone else could read your record and exactly duplicate your experiment. Duplication of results is crucial to scientific investigations.

	Trial 1	Trial 2	Trial 3	Trial 4
Sample amount and source				
Water/buffer volume*				
Salt volume				
Detergent volume and source				
Mix time				
Filter/spin time				
Enzyme amount and source				
Alcohol amount and source				
DNA result				

For further research:

- Experiment with other DNA sources. Which source gives the most DNA? How can you compare?
- Experiment with different soaps and detergents: powdered v. liquid, shampoo?
- Experiment with leaving out or changing steps. See if that makes a difference in your DNA yield.
- *Some protocols use a buffer to maintain a proper pH, although this protocol did not. Here is a simple buffer recipe: 50g sugar, 3g Epsom salts, one buffered aspirin, add water to 500 mL. See if using a buffer instead of water in step 2 improves your results.
- When you change various parts of the protocols, be sure to write about how these changes affected your results and why you think the change happened.

Inquiry DNA Extraction Lab

Credits: Austin CC BioTechEd project; Donald Bell, OCCC project.

Title: DNA Extraction Extension

Purpose or Hypothesis

The purpose is to create a method of extracting DNA from plant or animal tissues, and to review cellular structure. We will also discuss how DNA from various organisms appears.

Things you need to remember:

DNA is found in (almost) every cell of every living thing

Proteins that help DNA maintain its form may obscure seeing the DNA so they must be removed.

There are also proteins in the cytoplasm that act to cut DNA

Among eukaryotes DNA is contained in the membrane wrapped nucleus

The cell membrane is made of lipids with embedded proteins.

Materials available

Plant tissue samples (from home, not cooked or processed)

Animal tissue samples (provided)

Mortar and pestle

Alcohol (cold) either isopropyl or ethyl

Warm water (50-60°C)

Test tubes

Plastic cups

Paper towels

Papain (meat tenderizer)

Plastic pipettes

Procedure

Develop the procedure based on what you think needs to be done to get to the DNA in the cells. Remember to record every step, especially those from the experiments that don't work.

Results and Data

Record the outcomes of your experiments. How did each procedure work? Did it work?

Conclusions

1. Did the experiment work the first time you attempted it?
2. If it did not work, what did you think was the problem, and what modifications did you make before it worked?
3. List the materials you used for each trial.
4. What was the purpose of the papain?
5. What was the purpose of the soap?
6. How did the alcohol help you to see the DNA?

Teachers: Consider using this as an authentic assessment of previous versions of DNA extraction.