DNA Extraction Lab Basic Level Teacher Version
Credits: Austin CC BioTechEd project; Donald Bell, OCCC project.

DNA Extraction Lab

You can review lots of biology or teach lots of science concepts with this laboratory and make it unforgettable for your students, especially if you use the extension. Be sure to read the other versions of the extraction lab to see which one fits your class best before proceding. This is a great review of cell structure and biochemistry.

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 1 ml)
- 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.

Before starting, review the parts of the cell with your students: ask them "where is the DNA" "how do you get into the cell" - "what parts do you have to go through to get to nucleus" "what chemicals will you be dealing with from inside the cell?" Put cell parts that come up in discussion on a list on the board.

Back in the 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.*) ***Here you can talk about solutions - water the solvent, salt and soap the solutes - why is hot water a better solvent? keep listing terms on board.***
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.)* ***Why wheat germ and not all the grain - why is DNA concentrated there? parts of plant cell and plant seed can be reviewed here. Germ is embryo - seed full of food - carbohydrate - one of 4 biological molecules in cell, DNA another of biological molecules.***
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.* ***Why does water make germ swell - review diffusion and osmosis here.***
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.)* ***Proteins - another of the 4 biological molecules in the cell.***
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. ***(Here a demonstration of oil and water plus added soap to show emulsifying action of soap that breaks up lipid-rich cell membrane)***
6. Allow the solution to settle for about 2 minutes (or centrifuge for 30 seconds). In this time period
   Here go over all the parts of the cell - where is the DNA, what parts inside the cell before you reach nucleus. Summarize all the steps of the experiment that lead to extracting DNA exclusive of other cell parts and molecules - action of water, salt, soap, papain, mechanical pressure.
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. ***Note threadlike appearance - stringy - in cell, needs packaging into chromosomes.***
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.

If you are going to use the DNA in restriction analysis, better to store in refrigerator than freezer b/c freeze/thaw cycle of freezer and ice crystal formation breaks DNA. If using

with restriction enzymes, need to remove alcohol - air dry the prep or dry on paper towel or spool it out and add buffer.

Experiment Tricks of the Trade

Raw wheat germ

1. *can be purchased at a health food store or some large supermarket (toasted wheat germ does not work) How much?*

1 small plastic cup or beaker

- *3 oz. bathroom cups. Paper cups tend to soften due to the hot water and detergent which remove the wax coating.*

1 small (100 or 150 mL) beaker or cup

1. *to make initial mix of detergent, salt and water*

Non-iodized table salt

a. *sea salt or pickling salt; if none is available, the iodine shouldn't interfere with the reaction*

Hot but not boiling distilled water or tap water

- *approximately 20 ml per student*
- *50-6˚0 C–do not use water hotter than indicated as it will damage the DNA.*
- *Test your tap water—it may be hot enough right from the tap.*
- *The water will become cooler during the extraction procedure, but this does not matter.*

Dishwashing detergent

- *The following liquid soap products have been tested and work well: Lemon Fresh Joy, Woolite, Ivory, Shaper, Arm & Hammer, Herbal Essence shower gel by Clariol, Tide, Dish Drops, Kool Wash, Cheer, Sunlight Dish Soap, Dawn, Delicate, All,* and *Ultra Dawn.*
- *Liquid products that do not work well are Life Tree, Shout, Shaklee, Sunlight Dishwasher, and LOC.*

1. *Powdered detergents do not work in this protocol.*

About 1 mL meat tenderizer containing papain - 6 % solution

1. *Papain is the enzyme that will break down the structural proteins and other enzyme. Check the ingredient list. Not all meat tenderizers contain papain.*

Glass stirring rod or other stirrer

1. *you may substitute anything that can be used to stir- spoons, skewers, popsicle sticks, etc.*

Eyedropper or pasteur pipette and bulb

10 mL ice cold ethanol in a test tube on ice.

- *The colder the alcohol, the more likely the DNA will precipitate. You can add salt to the ice to make it even colder or keep it in the freezer until right before you use it.*
- *It is better to use 95% ethanol or Everclear grain alcohol but 70% ethanol or 70-90% isopropyl alcohol will work too.*

Large paper clip hook

- *used to spool the DNA*
- *A glass stirring rod is usually recommended because the charge on the glass attracts the DNA but it tends to slide off as it is pulled out of the alcohol.*
- *A glass pasteur pipette with the end curved into a hook over a bunsen burner may work even better.*
- *Paper clips are easily available but a thinner wire, if it is available, is easier to bend (small paper clips are too short).*

- *The hook must be fairly narrow if you want students to store their DNA in a microcentrifuge tube (see lab extensions)*

Sealable container (optional) such as a tube, vial or jar to store DNA.

- *to save DNA for restriction analysis experiments (see lab extentions) or to teach students proper storage of DNA*

Paper towels or filter paper –for drying DNA.

1. *Paper towels can be considered sterile until touched by human hands. If you choose to have students dry the DNA it is not necessary to spool it.*

Laboratory Extensions: An Industry Perspective

This lab is very short and straightforward and so lends itself well to supplemental activities. Below are two approaches that introduce students to the working world of the laboratory as well as improve critical thinking skills.

1. Comparison to an S.O.P. (Standard Operating Procedure)

1. The students compare their extraction protocol to at least one professional protocol such as the "Extraction of DNA from Mouse Tail". Additional DNA extraction procedures can be found at company websites such at Stratagene (www.stratagene.com) or Ambion (www.ambion.com). Using the keywords "DNA extraction techniques" on a search engine such as google (www.google.com) will bring up other protocols.
2. Have the students determine the differences between their lab and the professional protocol. They can then fill out a table such as the one included for the mouse tail extraction to highlight these differences.
3. A worksheet for this activity is available through Austin Community College's Biotechnology Program (biotech@austin.cc.tx.us)

2. Developing an S.O.P.

This is an excellent lab to allow students to be researchers. The procedure is short and so can be repeated many times to test, the materials are inexpensive, and the original procedure is very vague.

1. Ask students to figure exact measurements (amounts of materials to add, temperatures, stirring time, etc.) to ensure the largest yield of DNA
2. Have students keep complete records of everything they change <u>as they change it</u> (make sure they don't do it and then try to remember what they did to write it down).
3. Figure a way to measure yield of DNA for comparison such as:
    1. allow the DNA to settle in the test tube or centrifuge for a brief time and measure the height
    2. dry the DNA on a paper towel and use surface area or weight (if you have a analytical balance)
    3. ask a scientist to measure it for you in their lab
    4. use the "characterizing DNA extension below

4. Have students rewrite the protocol and get another group to perform it to ensure it is repeatable.

3. An Industry Case Study

See activity #2 for more information.

The Problem: You are working at a company that makes DNA for sale to other scientists and research institutions. Since your company wants to stay in business, the DNA it sells must be of good quality and each extraction must give the highest possible yields. The basic protocol has been established for extracting DNA from wheat germ.

The Brainstorming Session: The lab supervisor (teacher) calls a meeting of all the biotechnicians to brainstorm ways to improve yield by modifying the protocol.

1. Write down biotechnicians' suggestions for changing the protocol such as changing the amounts and brands of wheat germ, meat tenderizer, detergent and water, type and temperature of water, and percent and temperature of ethanol. Other considerations may be stir mechanics (i.e. vigorous versus easy stirring or shaking versus stirring)
2. Each group of biotechnicians chooses a parameter (or two) to explore and designs experiments to test the parameter changes. They hand these in to the lab supervisor for approval.

The Research and Modification: The groups perform their experiments and write up their results and conclusions. These are presented to the entire company (class) about whether their modifications should be included in the company S.O.P.

4. Characterizing the DNA

Depending on the tools and time available, students can characterize their DNA.

1. Dry DNA on paper towel and weigh to determine yield. (It helps if they weigh the starting material first.)
2. Record color of product
3. Determine volume of the DNA obtained
4. Read O.D.260 in a spectrophotometer and determine yield using the equation

$$\frac{\text{1 O.D.}}{\text{50 µg/ml}} = \frac{\text{expt'l value}}{\text{? µg/ml}}$$

   This method requires a spectrophotometer that measures ultraviolet light absorption and expensive quartz cuvettes that allow UV light to pass through.

1. Help the students determine the quality control tests that measure the final specifications of their extracted DNA
2. Keep the DNA in buffer for use in another lab such as restriction enzyme analysis

5. Restriction Analysis

DNA from the extraction can be used in a restriction analysis exercise along with plasmid or lambda DNA.

- The DNA should be stored in alcohol for short periods or a buffer containing EDTA (TE, TBE, or TAE) for longer periods. The EDTA binds metal ions such as calcium and magnesium which enzymes require to break down the DNA.

The amount of DNA can be very important to the outcome of the lab.

1. You need about 1 µg of DNA to be able to see it on a gel. If you go much beyond this the DNA will make a streak and not distinct bands.
2. One unit of a restriction enzyme is defined as enough to digest 1 µg of DNA in 1 hour. If you have large quantities of DNA it will not be digested properly.
1. You can use the characterization of DNA activity described in #2 to find out how much DNA you have or do a trial and error method by using several different dilutions of the stored DNA. Since every group probably started with different amount of DNA you should have a variety of results that show what happens when incorrect amounts are used. A researcher who deals with DNA would be able to quickly quantify the DNA and help you figure a dilution to bring it to a good concentration.