Massachusetts Institute of Technology
Department of Biology

DNA Spooling Experiment

adapted from http://www.fernbank.edu/museum/genomic/wheatgermprotocol.pdf

By Brenda Minesinger
and revised by Mandana Sassanfar

Overview: In this laboratory, you will learn how to extract total genomic DNA from fruits or vegetables using readily available house hold and kitchen ingredients.

(Note: This exercise is suitable for any high school level biology class. It is an easy, fast and cheap hands–on activity that is none less very informative and useful)

Objectives: Before doing this laboratory you should try to learn more about:

- DNA: Its structure and function in cells
- chromatin
- Its relevance in human health, and forensics
- Why one would want to extract genomic DNA from cells.
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After doing this laboratory you should be able to discuss:

- The purpose of each step in the isolation of genomic DNA
- The function of each ingredient used in the DNA extraction protocol
- Variation that may be required in the protocol to isolate DNA from other organisms
- How you could assess the size of a genome
- What you would do with the DNA you have just extracted

Exercise:

Ingredients and material: What You'll Need …

- Water (cool or lukewarm)
- Baking Soda
- Wheat Germ (must be raw, not toasted; can purchase at health food store or larger supermarket)
- Hand Soap (antibacterial OK)
- Meat Tenderizer (unseasoned works best; seasoned or smoked can interfere with results)
- Rubbing Alcohol (isopropyl alcohol)
- Teaspoon
- Tablespoon
- 50 ml test tube, spice jar or small clear container with lid

Protocol: What to Do …

1. Use 50 ml conical tube to measure 25 mls of water. If you do not have a conical tube, use a measuring cup and pour the water into a small capped container. If you do not have a metric measuring cup, simply use 2 tablespoons of water.
2. Add 1 teaspoon of wheat germ. Swirl to mix thoroughly.
3. Add 1 pump of hand soap (about ¼ teaspoon).
4. Close lid. Invert tube or container slowly about 45 times to mix. Do not shake as too many bubbles will form.
5. Add 1 teaspoon of baking soda.
6. Add 1 teaspoon of meat tenderizer.
7. Close lid or cover. Invert tube slowly about 30 times to mix.
8. **Open lid or cover**. Let tube or container sit for 3 minutes while excess wheat germ settles to the bottom.
9. Pour off the top liquid layer of the wheat germ mixture into a clean tube or container. If using a tube, this should be about 15 to 20 mls of liquid; this is your **supernatant**. Put aside the first tube or container with your solid mixture — you won't need it anymore.
10. Slowly add an equal amount (15 to 20 mls) of rubbing alcohol down the side of your tube containing your **supernatant**. Try not to mix the two layers! If you do not have a tube, pour an equal amount of alcohol (or 1 tablespoon) into your container. Measurements do not have to be exact for the experiment to work.
11. Once the alcohol is added, you should see cloudy, stringy material begin to rise to the top. This is DNA!
12. Use a toothpick or wooden coffee stirrer to "spool" the DNA from in between the water and alcohol layers.

If you want to keep the DNA for further experiments:

13. Dunk several time the spooled DNA into a small tube containing clean alcohol and let air dry.
14. At this point you can store the DNA in sterile water in the freezer.

Notes:

- If you do not open the lid while the wheat germ is settling, carbon dioxide will build up in the tube and can cause some solution to be sprayed when you open the tube to remove the DNA.
- Try to add approximately the same amount of alcohol but more is better than less. Do not mix the layers or it will be difficult to separate the DNA.
- This DNA might need to be cleaned up further before if can be digested by enzymes into smaller pieces.

The reactions: How it Works …

- When you add the soap, the mixture should get very thick. The soap is destroying cell membranes, allowing the cell contents to spill out (**cell lysis**). We often think that the cytoplasm of cells is quite watery. In fact, the cellular milieu is quite thick composed of hundreds of thousands of proteins, nucleic acid molecules and other cell components.

- DNA is only soluble at a pH near physiological levels. The baking soda serves as a buffering system that raises the pH and releases the DNA from bound proteins.

- The meat tenderizer is an enzyme (proteinase) that removes proteins bound to the DNA and destroys enzymes (endonucleases) which would chew up the DNA.

- Since the supernatant is thick from the cellular contents, carefully pouring the alcohol on top of the supernatant leaves two distinct layers. DNA is soluble in water, but not in alcohol; thus, the DNA present at the water-alcohol interface precipitates out of solution, allowing it to be seen.