DNA SPOOLING
ISOLATION OF DNA FROM ONION

INTRODUCTION
This laboratory protocol will demonstrate several basic steps required for isolation of chromosomal DNA from cells. To extract the chromosomal DNA, both the cell membrane and the nuclear membrane must be lysed, or broken open. This is accomplished by disrupting the membranes with a solution of detergent and salt, creating a cell homogenate. Once the DNA is released from the nucleus, it must be protected from nucleases, enzymes which will degrade the DNA. Keeping the cell homogenate cold and various chemical components of the homogenization medium help restrict the action of these nucleases. The final step of this protocol involves precipitation of DNA from the homogenate. When the homogenization medium is added in the first step, the positive ions of the salts (sodium chloride and sodium citrate) bind to the negatively charged DNA backbone, creating a DNA molecule with a neutral charge. When a cold polar solvent, like ethanol, is added to the DNA solution, the DNA is precipitated out of solution, leaving other cell components behind (proteins, lipids, polysaccharides, general cell debris).

DNA can be isolated and precipitated using tissue from an onion. The cells are disrupted by manually chopping the onion into small bits, adding homogenization medium to the onion bits (a solution of dish detergent and salts), and then grinding this mixture in a blender. The pureed onion is filtered with cheesecloth to remove any solid material, resulting in a clear cell homogenate. Ice cold ethanol is added to this homogenate to precipitate the DNA. The DNA can then be isolated by slowly stirring a glass stirring rod in the homogenate, literally spooling the DNA onto the glass rod.

PURPOSE
The goal of this exercise is to demonstrate the isolation of DNA from living tissues. The students will be able to:
- a. isolate DNA from onion tissue.
- b. make observations regarding the results of the isolation.
- c. develop a hypothesis from their observations regarding the nature of the molecule.
- d. design an investigation to test their hypothesis.

MATERIALS
- medium size onions
- homogenization medium
- latex gloves
- 95-100% ethanol, ice cold
- cheesecloth, cut in 6-8” lengths
- clean 50 mL glass beakers

- cutting boards and knives
- blender
- 250 mL beakers (2 per group)
- glass stirring rods
- ice buckets/styrofoam containers and ice

1 Adapted from “DNA Spooling: Isolation of DNA from Onion”
http://eagle.clarion.edu/~faculty/biscuits/OnionSpooling.html (8 May 2002)
SAFETY PROCEDURES & PRECAUTIONS

1. Caution should be taken when the students are chopping the onion and using the blender. Also, goggles are recommended when chopping the onion.
2. Gloves should be worn at all times to prevent contamination of the onion sample with nucleases that naturally occur on the skin. Care should be taken not to touch the glassware or utensils with bare hands as contamination can occur here as well.

PROCEDURES

1. Prepare the homogenization solution ahead of time (no more than 24 hrs before). The ethanol should be chilled overnight in a freezer, with the cap loosened.

   **Homogenization Medium (1 L)**
   …to 800 mL of distilled water add:
   
   - 30 mL liquid dish soap (ex. Joy)
   - 8 g NaCl
   - 4 g sodium citrate
   - 3 g EDTA
   
   Bring volume up to 1 L with more distilled water.

   Each group will require approximately 100 mL of homogenization medium.

2. Gloves should be worn at all times when handling the onions. Do not touch the insides of the glassware with bare hands.

3. Remove the inside of the onion and chop approximately 50 g of tissue. Place the chopped onion in a 250 mL beaker.

4. Add enough homogenizing medium to cover the onion tissue.

5. Cool the onion solution by placing it on ice for 5-10 min. (An ice water bath will cool the solution more quickly.)

6. To break open the cells, place the chilled onion solution into a blender and mix for 45 sec.

7. Filter any solid material out of the onion solution by pouring it through 2 – 3 double layers of cheesecloth into a clean beaker that is set in an ice water bath. Discard the cheese cloth. The solution in the beaker is the cell homogenate. It is very important to keep this cold during the final steps of the procedure.

8. To precipitate the DNA from the cell homogenate, ice cold ethanol should be added to the homogenate in a ratio of 2:1. For example, if you have 10 mL homogenate, you would need to add 20 mL of cold ethanol to effectively precipitate the DNA. Estimate the amount of chilled homogenate in the beaker. (Use of graduated cylinders can
improve the accuracy of this measurement.) Then determine the appropriate volume of chilled ethanol required for DNA precipitation.

9. **Slowly** layer the appropriate amount of chilled ethanol on top of the homogenate. A layering effect can be achieved by tilting the beaker containing the cell homogenate and then slowly pouring the ethanol down the side of the beaker. White strands of precipitated DNA may appear.

10. Using a glass stirring rod, wind the DNA around the rod by slowly spinning the glass rod while stirring the mixture in one direction. Strands of precipitated DNA should begin to appear at the interface of the cell homogenate/ethanol mixture. Continue stirring and collecting precipitated DNA for a few minutes.

11. Once the DNA is collected, gently scrape it into a 1.5 mL micro centrifuge tube that contains 1 mL of water or low salt buffer (e.g. TE). If stored in a refrigerator, the DNA sample can last for several months and be used for other experiments.
DNA SPOOLING

Analysis of Results

Questions:
1. Why do you think it is important to wear gloves while doing this procedure?

2. Why is it important to keep the cell homogenate and the solutions cold?

3. The homogenizing solution contains high concentrations of liquid detergent and salt. What does the detergent do to the cells? Why is it important to have a lot of salt in the solution?

4. What is the importance of the blender step?

5. What parts of the cell do you think are filtered out by the cheese cloth?

6. What do you know about the DNA molecule that makes it possible to spool it out of the homogenate? Why don’t other molecules in the homogenate do this?

7. Can you think of a way to prove that the molecule you have isolated is DNA? Design an experiment to show this.
TEACHER NOTES

1. It should be possible to complete this procedure in one 50 minute period, provided that required solutions are made and chilled prior to class.

2. If the DNA isolation goes well, it is possible to store and use this DNA for subsequent experiments. The DNA can be resuspended in water, but for long-term storage a low salt buffer like TE is recommended.

3. Chromosomal DNA isolated with this protocol can be subsequently used to demonstrate agarose gel electrophoresis and function of restriction enzymes as well as numerous other nucleic acid techniques.