Name: _____ Section: _____

Experiment 20 – DNA Extraction from an Onion

In this experiment, you will extract deoxyribonucleic acid (DNA) from the cells of an onion and will detect the presence of DNA in your sample using a qualitative test.

Discussion

DNA, a polymer found in all living cells, contains all of the genetic information needed for controlling cellular growth and development. Errors in DNA are the basis for thousands of genetic diseases.

In complex cells, such as those in multicellular animals and plants, DNA is found within a membrane-bound nucleus. To isolate DNA, the cells and associated membranes must be ruptured. A crude preparation of DNA can then be isolated from the dozens of other cellular components.

First, cell membranes are broken by grinding them with sand in a buffered detergent solution. The detergent weakens the cellular membranes and deactivates enzymes, called nucleases, that would destroy the DNA. DNA is more soluble and more stable under slightly basic conditions, so the detergent solution is buffered to pH 8.

To precipitate the DNA from the cellular solution, ethyl alcohol can be added to the extract. The DNA prepared in this manner will not be pure because cells contain ribonucleic acid (RNA) as well as thousands of different proteins that will precipitate under these conditions as well. Several additional purification steps would be required if DNA needed to be separated from these other substances.

To verify that DNA is present in the precipitate, it can be mixed with diphenylamine, which reacts with the deoxyribose portion of DNA producing a blue color. The intensity of the blue color is proportional to the amount of DNA in the sample.

In this experiment, you will extract DNA from onion cells using these principles and will verify the presence of DNA using the diphenylamine test.

Procedure

As you complete this experiment, record your observations on the Data and Observations sheet.

1. Obtain a chilled mortar and pestle.

2. Obtain approximately 5 grams of onion. Cut the onion into small pieces with a knife and place these into the cold mortar.

3. To the mortar, add an amount of sand similar in size to your portion of onion.

4. Obtain 10 mL of chilled buffered detergent solution and pour this into the mortar with the onion and sand.

5. Grind the onion-sand mixture for at least 5 minutes and record your observations.

6. Obtain a funnel, a ring support, a support stand, a piece of cheesecloth, and a 30-mL beaker. Assemble the filter assembly shown in figure 1. Fold the cheesecloth to make four layers large enough to cover the funnel and line the funnel with the folded cheesecloth.

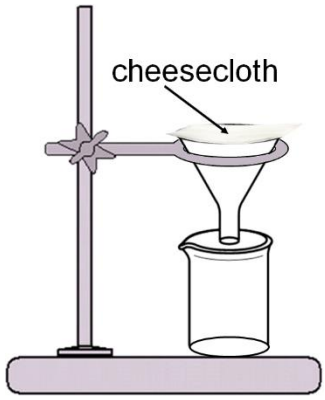

Figure 1: Filtration assembly

7. Pour the slurry of ground onion and sand onto the cheesecloth. Scrape any solid pieces out of the mortar. Lift up the cheesecloth by the edges, twist the cloth to enclose the solid pieces of onion, and press it against the wall of the funnel to remove as much liquid as possible. Record your observations.

8. Obtain two test tubes sized to fit your laboratory centrifuge. Label the tubes with your initials. Transfer the onion filtrate to one of the tubes. The second test tube is the balance tube. Fill the balance tube with water to a level that is equal to the level of the filtrate in the first tube.

9. Place the two tubes into opposite positions in a centrifuge. NEVER operate the centrifuge unless there are pairs of equally-filled test tubes on opposite sides to maintain balance. Close the cover and be sure that your hands and clothing are clear from spinning parts. Turn on the centrifuge for 5 minutes. While your sample is being centrifuged, rinse your beaker with deionized water and allow it to drain.

10. After 5 min, turn off the centrifuge and allow it to come to a stop. Record your observations of the supernatant (liquid portion) and the precipitate. Decant the supernatant back into the 30-mL beaker.

11. Dispose of the precipitate, the cheesecloth and the residual onion solids in the trash.

12. Into a second 30-mL beaker, pour enough cold ethyl alcohol to equal the volume of liquid in the beaker containing the DNA solution.

13. Tilt the DNA beaker slightly. Carefully pour the cold ethyl alcohol down the side of the beaker as shown in Figure 2. The ethyl alcohol should form a layer on top of the filtrate.

Figure 2: Precipitation of DNA

14. Examine the interface between the layers of ethyl alcohol and filtrate. Record your observations. The DNA should appear as fibers at the ethyl alcohol/filtrate interface while the gelatinous material consists mostly of proteins and RNA. Wait 2-3 minutes while you continue to record your observations. Then, carefully pierce the interface with a glass rod and rotate (but do NOT stir) the rod at the interface of the two layers to wind the DNA fibers around the rod. Remove any excess liquid by pressing the rod against the inner wall of the beaker.

15. Place your DNA onto a watch glass and record your observations about the appearance of the DNA fibers.

16. Pour the contents of the small beaker into the appropriate waste container.

17. **CAUTION:** ***The diphenylamine reagent is toxic and contains sulfuric acid and acetic acid, which are corrosive. It must be used in the fume hood.*** Prepare a boiling water bath for use in the fume hood. Add about 125 mL of water to a 250-mL beaker and place it on top of a hot plate. Use a ring support to prevent the beaker from tipping over (Figure 3) and bring the water to a boil.

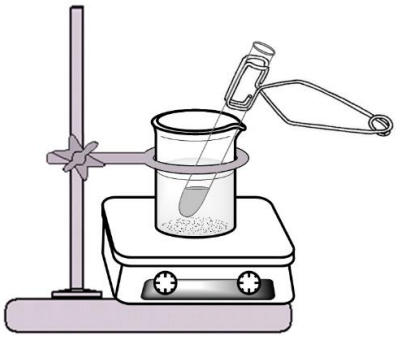

Figure 3: Boiling water bath set-up.

18. Transfer 2 mL of diphenylamine reagent to a small test tube. Add a small piece of your DNA sample. Mix well. Heat in a boiling water bath for 10 min. Record your observations.

19. Dispose of your test solution and any unused DNA as directed by the instructor. Be sure to wash your hands thoroughly with soap before leaving the laboratory.

Name: _____ Section: _____

Post-Lab Questions

1. Suggest several ways that the procedure might be changed to increase the amount of DNA extracted from the onion cells.

2. Suppose a student added ethyl alcohol directly to the onion cell filtrate without first centrifuging the sample. What do you think the student would observe? Would the student be successful in extracting the DNA? Explain.

3. Would you expect similar results if you were to use other cells, such as beef liver or yeast as sources of DNA? Explain your reasoning.

4. Consider a single-celled organism, such as a bacterium, whose DNA is not enclosed within a membrane bound nucleus.

   a) Would you predict that it would be easier or harder to extract the DNA from a bacterial cell compared to extracting DNA from onion cells? Briefly explain.

   b) Would you predict that a single-celled bacterial organism would have as much DNA as a cell from a more complex organism, such as an onion? Explain.

Name: _____ Section: _____

Data and Observations

Record your observations from…

Step 5:

Step 7:

Step 10:

Step 14:

Step 15:

Step 18: