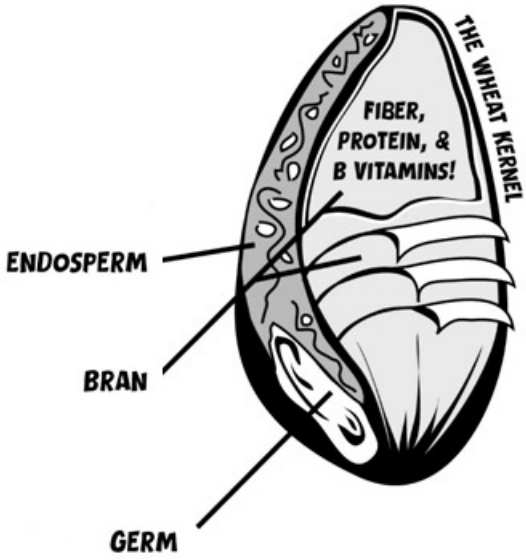
Name _____ Date _____ Period _____

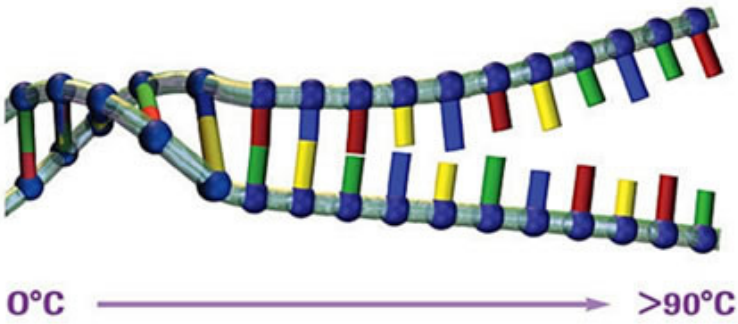
Lab: Wheat Germ DNA Extraction

BIOLOGY

BACKGROUND: Wheat germ is the ***embryo*** (sprouting) section of the wheat kernel; the remainder being the ***endosperm*** (storage). The germ is extremely rich in vitamins and nutrients, and for the purposes of this experiment, an excellent source of ***DNA***. The steps in this procedure can teach us a great deal about the properties of cells, cell membranes, and of ***deoxyribonucleic acid*** (DNA) itself.



Detergents ***solubilize*** and break down the lipids and proteins that form the primary cell membrane and disrupt the bonds that hold the membrane together. The cell contents, including the nucleus, and thus released for further treatment. Heat is applied to assist in softening the cell membranes and enhancing the action of the detergent. The heat also ***denatures*** enzymes that might otherwise damage the DNA. You will also be cautioned to keep the temperature below 60° C– *because higher temperatures denature the DNA and make spooling impossible*. Eventually, even and 55° C, the DNA would break down. For this reason, the incubation period is limited to 15 to 20 minutes, and the mixture must be cooled quickly once the incubation is complete.



The sodium bicarbonate solution is added to maintain a near-neutral pH– at which the DNA is most stable and at which the enzyme present in the meat tenderizer is most effective. The meat tenderizer contains the ***proteolytic*** (protein breaking) enzyme ***papain***– *naturally present in papaya, pineapple, and other fruits.* The papain completes the breakdown of the nuclear membrane, at which point the DNA is freely in solution.

The final step requires the cold alcohol. The solubilized DNA contacts the alcohol where the two liquid layers meet. The alcohol dehydrates and precipitates the DNA, as DNA is insoluble in the alcohol (especially cold alcohol). If the procedure is carried out properly, fine, long strands of DNA will form at the interface– and can be readily spooled onto a paper clip.

PURPOSE: The purpose of this lab is to extract the nucleic acid DNA from wheat germ, by carefully following the procedures as listed below. In this investigation, you will develop not only good scientific laboratory skills, but also discover many important properties of the cell, the cell membrane and DNA itself.

MATERIALS NEEDED:

- Raw (untoasted) wheat germ, 2g
- Tap water
- Liquid detergent (Palmolive, Dawn, Woolite)
- Thermometer
- Meat tenderizer (Adolph's unseasoned original), 2g
- Large test tubes (25 x 200ml)
- Alcohol, 95% (ethyl or isopropyl), 20 ml
- Graduated cylinder, 50 ml
- Water bath at 55° C
- Ice bath
- Sodium bicarbonate, $NaHCO_3$ (baking soda)
- Serological pipet, 10 ml
- Paper clip (giant sized)
- fine sand paper

PROCEDURE:

1. Measure 45 ml of tap water into a large test tube and place it in a warm water bath. Allow it a few minutes to warm. The optimal temperature for the procedure is 55℃– ***do not allow the temperature to exceed 60℃.***

2. Sprinkle the wheat germ into the test tube and gently stir in 3 ml of detergent. Allow this mixture to incubate in the 55℃ water bath for 5 minutes.

3. After 5 minutes, gently stir in 2 grams of meat tenderizer and 5 ml of the 1 M sodium bicarbonate solution. Incubate this mixture at 55℃ for an additional 15 to 20 minutes.

4. Transfer the test tube containing the wheat germ mixture to an ice bath for a few minutes to ***quickly*** cool it to room temperature. Stir gently during this period.

5. Using the serological pipet, carefully layer the ice-cold alcohol over the wheat germ solution in the test tube. Allow the alcohol to flow from the pipet with the pipet tip held against the inside surface of the test tube just above the liquid level.

6. There will be a visible interface between the alcohol layer and the wheat germ mixture layer. A fibrous white precipitate should be evident at the interface. This is DNA. Use the paper clip hook, immersed to the depth of the interface, to spool up the DNA fibers. Use a slow, twirling motion to avoid breaking and separating the strands.

7. Store the DNA in a test tube with a 4% salt solution as directed by your teacher.

8. Carefully dispose of the remaining mixture as directed by your teacher, and clean all glassware and beakers that you used in the lab.

QUESTIONS:

1. What was the purpose of using a detergent to prepare our DNA sample?

2. What does the term ***denature*** mean? What affect does increased temperature have on this process?

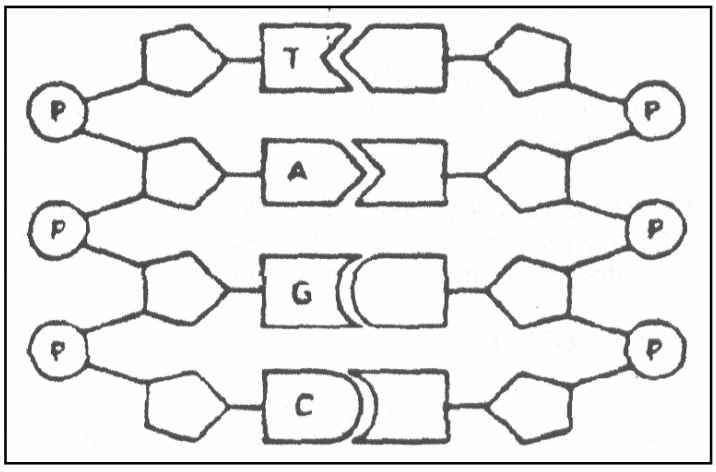
3. What is a ***proteolytic enzyme***, and how was it used in this laboratory experiment?

4. What was the reason you added sodium bicarbonate to your mixture?

5. Why was cold alcohol used to precipitate the DNA?

6. If you did not get good results in this lab, what do you think you did wrong? (If you were successful, do you think there was anything you could have done to improve your results?)

7. Below is a diagram showing a small section of the DNA molecule. Redraw this diagram and label each part of the helix, filling in the base code letters that correspond to the correct nucleotide. Be sure to label: ***phosphate unit, deoxyribose, hydrogen bond, guanine, cytosine, thymine, and andenine.***



8. Describe the general structure of the DNA molecule?

9. DNA is a polymer formed from units called nucleotides. These nucleotides are composed of three basic parts. What are they?

10. DNA contains four nitrogenous bases. Two of the nitrogenous bases belong to a group of compounds known as ***purines***. The remaining two are known as ***pyrimidines***. Identify the bases that belong to each group and give the letters that are used to represent each of them in the DNA molecule.

11. The DNA molecule is often described as looking like a 'twisted ladder'. What parts of the nucleotide building blocks form the sides (or backbone) of the ladder and what part forms the 'rungs' of the ladder?

12. What holds the nitrogenous bases together?

CONCLUSION:

1. What is the major significance of having 2 strands in the DNA molecule?

2. DNA is said to control all of the cells structure and function. How does it accomplish this?

3. What is the biggest thing you learned from this lab?

4. Give 3 examples of how DNA is used by scientists today.

5. How did the contribution of Erwin Chargaff eventually enable Watson and Crick to determine the structure of the DNA molecule?