BIOLOGY 11

DNA Extraction Lab

OBJECTIVE: To isolate genomic DNA from an onion

MATERIALS:

onion (frozen 24 hours)	stand
mortar & pestle	ring clamp
25 ml graduated cylinder	250 ml beaker
spatula (long)	18 mm x 150 mm test tube
50 ml centrifuge tube w/cap	10 ml grad. cylinder
funnel	long stirring rod
filter paper	timer
test tube holder	grease pencil

ice cold ethanol w/iced 10 ml grad. cylinder

detergent/salt/enzyme solution w/ dispensing beakers

water bath @ 60°C

ice bath with test tube racks

PROCEDURE:

1. Grind an onion section well in mortar and pestle to rupture the cells. This releases the cell contents (proteins, DNA, RNA, lipids, ribosomes and various small molecules).

2. Slowly mix in approximately 20-25 ml of the detergent/salt/ enzyme solution, grinding well. The solution will degrade the proteins, emulsify the fat and create a polar environment to dissolve the DNA.

3. Transfer the mixture to the 50 ml centrifuge tube, using the spatula to transfer all of the ground onion.

4. Cap the tube and incubate in a 60°C water bath for **exactly** 15 minutes. **NOTE:** Timing and temperature are very important. Higher temperatures or longer incubation will denature the DNA.

5. Remove the tube from the water bath and immediately cool in an ice bath for 5 minutes.

6. Remove the tube from the ice bath and filter the mixture into a 250 ml beaker.

PROCEDURE (CON'D):

7. Place 6 ml of the filtrate into the test tube.

8. **Slowly and carefully** add 9 ml ice cold ethanol down the side of the test tube and let sit for 5 minutes. DNA is insoluble in ethanol and will precipitate out in a layer between the filtrate and the ethanol.

9. SHOW YOUR TEACHER!

10. Very slowly and carefully extend a glass stirring rod into the test tube past the layer of DNA. **DO NOT STIR** but gently roll the stirring rod between your fingers. The DNA fibers will wrap around the rod (a technique called "spooling").

11. Clean everything up!