Lab 13 Microbial Genetics and Recombinant DNA

Experiment 1: DNA Extraction

Extraction of DNA from bacteria is a key starting step for many procedures in recombinant DNA technology. A bacterial culture is grown in liquid growth media (from 3 mL for small laboratory preps to over 300L for industrial applications) then concentrated by spinning the growth in a centrifuge to collect the cells in a pellet. The liquid media is removed and the cells are lysed in a solution containing a detergent (in the lab this is usually sodium dodecyl sulfate, or SDS). This lysis releases all DNA, RNA, and proteins from the cells into the lysis solution. DNA is soluble in water but not in alcohol; therefore, alcohol is added to the solution to force the DNA out of solution. This process is called precipitation. Salt aids in precipitation by neutralizing the negatively charged phosphate backbone of DNA and allowing the DNA to stick to itself. In this experiment, you will use strawberries as a substitute for bacteria to extract DNA.

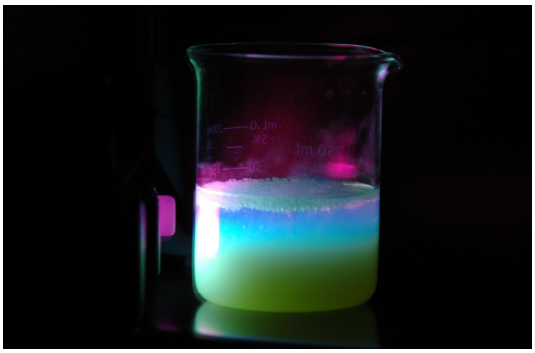

***Figure 6:* DNA extraction. The color has been enhanced by dying the fruit with a substance that glows under black light.**

Materials

**DNA Extraction solution

(2) 100 mL Beaker

10 mL Graduated cylinder

Measuring spoon

Funnel

(1) 50 mL Conical tube

Cheesecloth

Drinking glass or bowl

Plastic bag

Rubber band

1 Wooden stir stick

*Scissors

*Fresh soft fruit (such as strawberry, grapes, banana, etc.)

*Water

***Ice cold Ethanol

*You must provide

**Sodium chloride, detergent, and water

***For ice cold ethanol, store it in the freezer for 60 minutes prior to use

Procedure:

1. Pour at least 5 mL of ethanol into a graduated cylinder. Transfer the alcohol into a 100 mL beaker, and place in the back of your freezer for approximately 60 minutes.
2. Put pieces of soft fruit (approximate size of five grapes) into a plastic zipper bag and mash with your fist.

3. Using a 100 mL beaker, measure out 10 mL of the DNA extraction solution and pour it into the bag with the fruit it in. Seal the bag completely.
4. Mix well by kneading the bag for 2 minutes.
5. Create a filter by placing the center of the cheesecloth over the mouth of the standing test tube, pushing it into the tube about 2 inches, and securing the cheesecloth with a rubber band around the top of the test tube.
6. Carefully cut a hole in the bottom corner of the bag and filter your extraction by pouring it into the cheesecloth (the filtered solution in the standing test tube is what you keep).
7. Take your ethanol out of the freezer. Hold the test tube at a 45° angle, and use your funnel to slowly pour 5 mL of ice-cold ethanol into the test tube.
8. DNA will precipitate (come out of solution) after the ethanol has been added to the solution. Let the test tube sit for 2 - 5 minutes. You should begin to see air bubbles form at the boundary line between the ethanol and the filtered fruit solution. After enough bubbles form you will see the DNA float to the top of the ethanol.
9. Gently insert the stir stick into the test tube and slowly raise and lower the tip several times to spool and collect the DNA. If there is an insufficient amount of DNA available, it may not float to the top of the solution in a form that can be easily spooled or removed from the tube. However, the DNA will still be visible as white/clear clusters by gently stirring the solution and pushing the clusters around the top.