DNA Extraction and Gel Comparison of Cut DNA—Lab Experiment

Introduction

The primary purpose of this lab is to extract DNA from the cells of a plant to help you understand a common step in many microbiology and genetics protocols. To extract the DNA from plant tissue, you must first break open the cell wall and cell membranes using a mechanical action and using a soapy detergent that can break up the lipid membranes.

After the cells have been torn apart, the proteins and cellular components must be stripped away from the DNA so they do not denature or degrade the molecule. To remove these components, you will add salt, which will bind to the debris and will cause it to clump or fall to the bottom of a tube. Straining the mixture removes the salty clumps and leaves a supernatant (liquid runoff) of macromolecules.

After the cell components have been removed, the DNA can be coaxed away from the macromolecular soup by using an alcohol that attracts the charged portion of the molecule. The procedure of extracting DNA is similar for any cellular tissue from any living organism.

After extracting the DNA of an organism, you can thenn cut the DNA with restriction enzymes to create fragments of differing lengths. For the second part of this procedure, you will run out the DNA of three different chestnut trees on a gel matrix to look for similarities and differences between related organisms: (a) the American chestnut, (b) the Chinese chestnut, and (c) a hybrid offspring of those two parent species. The instructions for the hybrid DNA analysis are included in the Edvotek kit that your teacher will give you.

Materials

- One Ziploc® (or similar) plastic bag
- One strawberry (or half if they are big)
- One 15-milliliter (ml) disposable graduated tube with a cap
- One 50-ml disposable tube with a cap
- One 15-centimeter x 15-centimeter square of four-ply cheesecloth
- A container with 5 ml of ice-cold 95 percent ethanol
- A container with 10 ml of extraction buffer
- One toothpick-diameter wooden stick that is long enough to reach the bottom of the 15-ml tube
- One 1-ml disposable pipet
- One 1-ml microcentrifuge tube (optional)
- One rubber band (optional)

Procedure

1. Place one strawberry in the Ziploc® plastic bag, and press out the air as you seal the bag. Mash the strawberry until the tissue is smooth and creamy.

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2. Add 10 ml of DNA extraction buffer to your plastic bag, reseal it after removing the air, and mix it again for 1–2 minutes.

3. Pour the mixture through four-ply cheesecloth that has been draped over a 50-ml test tube (you may want to ask you neighbor to hold the cheesecloth during this step or may use a rubber band to hold it in place). Do not squeeze the contents of the cheesecloth; you want to collect only the liquid that drips through on its own.

4. Pour off 2 ml of the liquid from the 50-ml tube into a 15-ml tube.

5. Tilt the 15-ml tube at an angle, and slowly pipet 5 ml of ice-cold ethanol onto the top layer of the mixture. Do not mix the layers or shake or swirl the tube. Watch for a transparent, mucus-textured, bubble-filled substance to form at the interface of the two layers. This substance is the DNA from your strawberry.

6. Gently insert the wooden stick into the 15-ml tube until it is below the first layer. Twirl the stick to spool long threads of DNA onto the stick.

7. Gently remove the stick containing the DNA, and scrape your DNA into a 1-ml microcentrifuge tube to keep.

Conclusion

Answer the following questions after you have finished the DNA extraction lab.

- Describe how each of the following cell components was removed.

  Proteins: _____

  Nucleus: _____

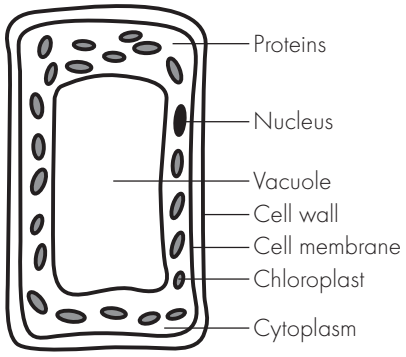
  Vocuole: _____

  Cell wall: _____

  Cell membrane: _____

  Chloroplast: _____

  Cytoplasm: _____



- How would the DNA extracted from bacteria, fungi, or an animal cell differ from the DNA extracted from a plant cell?

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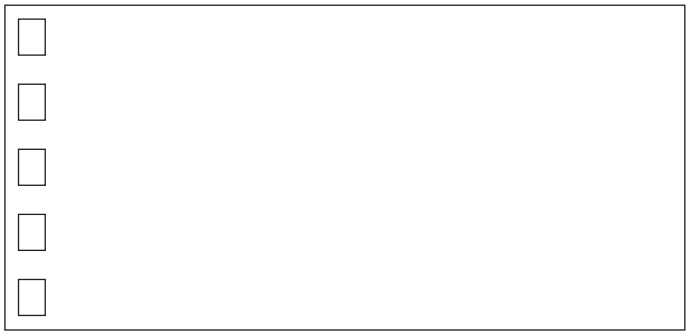
Answer the following questions after you have finished the Edvotek No. 114-PLT hybrid DNA analysis lab:

- Sketch the gel and the bands found in each lane. Label the lanes for each species.



- Using your gel results, explain the genetic relationships among the three species.

- Was the hybrid more genetically similar to the American chestnut or the Chinese chestnut? Support your answer with scientific data.

- How does the DNA analysis relate to the physical and chemical traits in the hybrid organism?

- Predict what the gel would look like if you were to perform a DNA fingerprint analysis using your DNA and DNA from your biological parents.