

Fruit DNA Extraction

PURPOSE

In forensic science, DNA is usually extracted from human cells to identify unknown parentage, crime scene suspects, missing victims and to investigate genetic diseases. Since 1985, DNA profiling has been used in legal cases around the world to link suspects to the scene of a crime. In this exercise, your class will be able to extract DNA from fruit to see what it looks and feels like. This process is similar to what a scientist must do before they can use the information contained in DNA to solve crimes. The exercise takes approximately **30 minutes**. Students can work in pairs or small groups as needed.



Why 90% alcohol?

DNA is insoluble in alcohol so the higher the alcohol content, the more DNA you will precipitate. You can find 90% alcohol at any drug store. However, if you cannot find 90%, the experiment will work with 70% alcohol as well.

MATERIALS

- Ethanol or Isopropyl (rubbing) alcohol (90% or greater)
- Liquid dish soap (for example: Ajax)
- Table salt
- Powdered meat tenderizer
- Coffee filters or cheesecloth
- Distilled water
- Cups of crushed ice or ice water
- Fruit: strawberries, kiwi, or bananas
- Ziploc *freezer* bags
- Small paper or plastic cups
- Test tubes or centrifuge tubes
- Plastic transfer pipettes or eyedroppers (optional)
- Paper clips

TEACHER PREPARATION

Prepare DNA extraction solution: Mix 100 mL liquid dish soap, 15 grams table salt, and distilled water to make a final volume of 1000 mL. This will be enough for about 40 individual experiments.

Aliquot ethanol or isopropyl alcohol: Aliquot into clear culture tubes or centrifuge tubes (15 ml or smaller). Fill tubes about half way. Keep these tubes covered and *on ice* until ready for use. You will need one tube for every student or pair of students.

Place at each student station: ziploc bag, piece of fruit, coffee filter, small cup, paper clip, ice cup containing tube of cold alcohol, plastic pipette (optional).

Photocopy worksheets: Photocopy one copy of the “DNA Extraction Analysis” worksheets for each student.

Fact Files

Ploid What?

We use fruit for this DNA extraction exercise because they are often polyploid, meaning they have more than 2 copies of every chromosome in each cell. Strawberries are actually octoploid: they have eight set of chromosomes in every cell. Humans are diploid, receiving one set from the mother and one from the father.

Go Online!

For: National DNA Day on April 25

Visit:

<http://www.genome.gov/Education/>

LESSON PLAN

Engagement

Inform your students that they are going to be extracting, or isolating, DNA from fruit. Ask your students what they know about DNA. What does it do? What color is it? Where is it found? How do scientists isolate DNA from different samples such as fruit? What steps do they think are involved?

Exploration

1. Have the students place a strawberry, piece of banana, or thick kiwi slice into the sealable freezer bag. Mash well for 2 minutes.
2. Pour 2-3 tablespoons of detergent solution into the bag. Add two or three shakes of powdered meat tenderizer. Seal the bag and mash together for 1 minute.
3. Set up the filtration apparatus: Place a coffee filter or triple layered cheesecloth over a small paper or plastic cup. Make sure the cheesecloth or filter creates a pocket deep enough to hold the mixture, and covers over the top sides of the cups. Secure with a rubber band if needed.
4. Open up the bag and slowly pour the mixture into the filter. **Do not overflow.**
5. When most of the mixture has filtered through, discard the cheesecloth or filter. If the mixture is very thick, more detergent can be added or the mixture can be gently stirred to aid in filtration.
6. *Slowly* pour or pipette some of the strained fruit mixture over the tube of cold alcohol until it is about 2/3 full. Let it run slowly down the sides of the test tube, do not pour it in all at once. The mixture will sink to the bottom of the tube and the alcohol will sit on top. Allow the test tube to sit undisturbed on the counter or on ice for about 5 minutes.
7. Observe occasionally and look for the DNA precipitating into the alcohol. This tends to occur at the boundary between the two layers. The DNA will appear as clear to whitish stringy clumps in the alcohol, and may even collect and float to the top of the tube.
8. Dip a straightened paper clip into the test tube and twirl it around gently to collect some DNA. If the DNA is gently stirred at the interface between the ethanol and detergent solution, it may continue to form fibers.

The Three Steps of DNA Extraction

- D**etergent – breaks open the cells
- EN**zymes – protect the DNA
- A**lcohol – Precipitates the DNA

Fact Files

Did you know?

If you unwound the DNA from just one of your cells, it would be about *six* feet long! If you unwound the DNA in your entire body, it would stretch to the sun and back about **400** times!

Go Online!



For: Paternity and Crime Scene Investigation Interactive
Visit:
http://www.biotechnologyonline.gov.au/popups/int_dnaprofilng.cfm

Explanation

Talk with the students briefly about the various steps involved in isolating DNA from the fruit. Why are these different steps necessary? Explain that DNA extraction or isolation is a necessary first step for many scientific processes, including DNA profiling, genetic cloning and paternity testing. Talk briefly about the process of DNA profiling: the DNA is isolated, cut with restriction enzymes and then separated using agarose gel electrophoresis.

Assessment

Have students complete the “DNA Extraction Analysis” worksheet on the following pages.

DNA EXTRACTION ANALYSIS

1. What was the purpose of the dish soap and salt mixture?
2. What was the purpose of the meat tenderizer?
3. Why was it necessary to “mush” the fruit?
4. What happened when the cold ethanol was added?
5. Would it make a difference if *warm* ethanol was added?
6. What are the steps required to isolate the DNA from the rest of the fruit?
7. What can we do with DNA once we have extracted it?

DNA EXTRACTION ANALYSIS

1. What was the purpose of the dish soap and salt solution?

The soap and salt both help to break open or lyse the cells. The soap/detergent destroys the cellular and nuclear membranes releasing the DNA. It does this by dissolving lipids and proteins that hold the membranes together. The salt will also help later to precipitate the DNA.

2. What was the purpose of the meat tenderizer?

Meat tenderizer contains a mixture of enzymes called bromelain. These enzymes help to break down proteins associated with DNA. These proteins will make the DNA harder to precipitate unless they are removed. Bromelain can also help break down DNAases, enzymes that breaks down DNA.

3. Why was it necessary to “mush” the fruit?

Pressing the fruit along with the detergent helps destroy the cell membranes while physically breaking apart the cell walls.

4. What happened when the cold ethanol was added?

DNA is soluble in water but insoluble in alcohol. DNA will precipitate out into the alcohol, while the other components of the mixture remain in solution in the watery (aqueous) layer.

5. Would it make a difference if warm ethanol was added?

Yes, the colder the alcohol, the more DNA you will precipitate. Here, solubility decreases along with temperature.

6. What are the steps required to isolate the DNA from the rest of the fruit?

The first step is to break apart the cells by physical (pressing) and chemical (detergent) means. This process breaks down the cell walls, membranes and nuclear membranes. The addition of meat tenderizer helps protect the DNA from proteins that can destroy it or hinder precipitation. Next, a filter is used to separate out some of the cell parts we don't need. Finally, DNA is precipitated out by the addition of cold alcohol.

7. What can we do with DNA once we have extracted it?

DNA can be used for DNA profiling to solve a crime, identify parentage, and check for genetic defects. We could look at the fruit DNA to see if it has been genetically modified, by comparing it to known modified and unmodified samples. DNA can also be used for transformation, to insert it into a foreign organism to give that organism a specific trait.