Explorer's Guide

Fruitful DNA Extraction

See and touch the hereditary molecules

Do you think you have very much in common with a kiwi fruit? Believe it or not, a kiwi's genetic material is very similar to your own! See and touch the genetic material that you'll extract from the cells of a kiwi fruit.

Things You Will Need

- 1 500-mL or larger beaker, or 1 250-mL or larger flask
- 2 200-mL or larger graduated beakers
- 1 100-mL graduated cylinder
- 1 100-mL beaker
- a kiwi fruit
- knife and fork for cutting and mashing
- thermometer
- funnel
- paper coffee filter to fit in the funnel
- 2 saucepans for water baths
- ice
- cold water
- hot water
- 50 mL chilled ethanol
- 2 g table salt
- 10 mL detergent
- coffee stirrers
- waxed paper or paper plate
- access to a balance
- hot plate (optional)
- clock or watch
- 1 test tube for each group member
- rack for holding test tubes
- 1 medicine dropper
- hook made from thin wire (optional)

To Do and Notice

Extracting the DNA

❶ Prepare an ice water bath by putting ice and water into a saucepan or similar container to a depth of 5 to 8 cm. Put about 50 mL of chilled ethanol into the 100-mL beaker and place the beaker in the ice bath.

❷ Prepare the DNA extraction solution: Dissolve 2 g of salt in 90 mL of water in a 200-mL or larger beaker. Then add 10 mL of detergent and stir (gently!) with a stirrer.

❸ Peel the kiwi fruit over waxed paper or a paper plate and cut it into chunks.

❹ Use a balance to measure 30 g of kiwi chunks, then thoroughly mash that amount with a fork.

❺ Place the mashed kiwi in a 200-mL or larger beaker.

❻ Pour the DNA extraction solution (detergent and salt solution from step 2) over the fruit until the total volume of fruit and liquid is about twice the volume of the mashed fruit alone.

❼ Prepare a hot water bath by putting hot water into a saucepan about 5 to 8 cm deep. Check the temperature of the hot water bath with the thermometer. Add colder or hotter water to get the water to 60°C. If you have a hot plate, put the water bath on it to help it reach and maintain a 60° temperature. Check the temperature periodically and adjust as needed.

❽ Place the beaker with fruit and extraction solution into the hot water bath. Note the time.

❾ Let the fruit and extraction solution mixture incubate in the hot water bath for 10 to 15 minutes. Stir the solution occasionally to distribute the heat. The temperature of the water bath must be monitored and maintained between 50°C and 60°C during this incubation period.

❿ After 10 or 15 minutes of incubation, transfer the beaker containing the fruit and extraction solution mixture to the ice bath. Allow it to stay there for 5 minutes, stirring occasionally as it cools.

⓫ While the extraction mixture is cooling, set up the filtration system. Place a funnel over a clean 500-mL or larger beaker or 250-mL flask, and insert a coffee filter into the funnel.

⓬ Pour the cooled extraction mixture into the filter-lined funnel. Allow the liquid to filter for about 5 minutes.

⓭ Thoroughly swirl the filtrate (the fluid that drains through the filter).

⓮ Pour about 5 mL of the filtrate into a test tube.

Precipitating the DNA

❶ Gently layer about 10 mL of cold ethanol (as cold as possible) on top of the filtrate. You can add the ethanol with a dropper or gently pour it down the side of the test tube while you hold it at an angle.

❷ Place the test tube in a test tube rack. Observe what's happening in the test tube at the area where the ethanol and filtrate layers meet. Record your observations.

❸ Let the solution sit for 2 minutes without disturbing it. A white precipitate will form in the alcohol layer. This is the DNA, and it will appear as a slimy, white mucus.

❹ If you like, you may collect the DNA with a wire hook or medicine dropper at the ethanol/filtrate interface. It's safe to touch, so go ahead and explore!

Interpreting Your Observations

What do you think is the purpose of each step in the extraction and precipitation of the DNA? The DNA here isn't pure; what other types of molecules might be present?

Facilitator's Guide

Fruitful DNA Extraction

Materials

for the whole group

- 70% or higher percentage ethanol, chilled overnight in a freezer
- light-colored detergent, such as dishwashing liquid
- balance

for each small group

- 1 500-mL or larger beaker, or 250-mL or larger flask
- 2 200-mL or larger graduated beakers
- 1 100-mL graduated cylinder
- 1 100-mL beaker
- a kiwi fruit
- knife and fork for cutting and mashing
- thermometer
- funnel
- basket-style paper coffee filter to fit in the funnel
- ice
- cold water
- hot tap water (60°C)
- 2 3-qt saucepans for water baths
- coffee stirrers
- 10 mL detergent
- 50 mL chilled ethanol
- 2 g table salt
- waxed paper or paper plate
- 1 test tube for each group member
- test tube rack or similar device
- 1 medicine dropper
- hook made from thin wire (optional)
- hot plate (optional)

Management

- Amount of time for the activity: 45 minutes
- Preparation time: 30 minutes
- Group size: 1–5

Preparation and Setup

Activity Overview

Extract DNA from kiwi fruit using simple household chemicals.

Concepts

› DNA is the genetic material in organisms.

› The sequence of DNA subunits determines an organism's traits.

› We can extract DNA from tissue using a very simple procedure.

Preparation

❶ Obtain the materials. Nearly pure ethanol (99–100%) works best. Less-pure ethanol can be purchased at some grocery stores, but be aware that it contains acetone as a denaturant and is extremely toxic. If neither of these forms of ethanol is unavailable, Bacardi 151 Rum (75.5% ethanol) works well as a substitute. If you choose to use this as your source of ethanol, be sure to conceal its identity.

❷ Chill the ethanol or ethanol substitute overnight in a freezer.

Questions for Getting Started

› Have you heard of DNA before? What does DNA do in living organisms?

› Do you look like your parents or siblings? Why do you think that this is so?

TIPS!

- The activity can be streamlined by preparing the extraction solution and hot and cold water baths ahead of time.
- The activity can be completed over a 2-day period. When most of the liquid has been filtered, the filtrate can be covered and stored in the refrigerator for use the next day. Or the entire filtration procedure may be conducted in a refrigerator overnight.

After the Exploration

Expected Results

A slimy white material will precipitate at the interface of the ethanol and filtrate layers. This material consists of clumped-together DNA strands and some protein.

What's Going On?

The procedure used in this activity has the same essential elements as more advanced laboratory DNA extraction procedures: mechanical and thermal disruption of cells, liberation of the DNA, and precipitation of the DNA.

In this procedure, the kiwi cell walls are broken down by the mechanical mashing and then the heating, and the detergent dissolves the lipids in the cell membranes and nuclear envelope (just like the detergent dissolves grease on your dishes). No longer confined inside nuclear membranes, the DNA—highly soluble in water because the phosphate group of each nucleotide carries a negative charge—goes into solution. However, the positively charged sodium ions from the salt in the extraction solution are attracted to the negatively charged phosphate groups on the DNA backbone, effectively neutralizing the DNA's electric charge. This neutralization allows the DNA molecules to aggregate with one another. When the ethanol is added, the DNA clumps together and precipitates at the water/ethanol interface because the DNA is not soluble in ethanol.

Each glob of material in the precipitate will contain millions of DNA strands clumped together, along with some of the protein that is normally associated with DNA. (Since the DNA was not highly purified, some protein precipitates out with the DNA.)

It is possible to analyze the extracted DNA in a research laboratory to provide good evidence that it really is DNA.

Each type of molecule, because of its unique structure, has a characteristic pattern of absorption of the electromagnetic spectrum. This pattern can be determined by an instrument called a spectrophotometer, which shines light of specific wavelengths through substances and records the degree of absorbance for each wavelength. DNA exhibits maximal absorbance at approximately 260 nm, while a typical protein shows peak absorbance at 280 nm. This difference can be used to distinguish the two types of molecules.

Kiwi fruit DNA extracted by the procedure outlined here was removed from solution, dissolved in a buffer, and subjected to spectrophotometric analysis in order to obtain a crude idea of its constituents and purity. The material showed an absorbance peak at 264 nm, indicating that it likely contains DNA with some contaminating protein.

Discussion Questions

❶ We can't isolate and touch most of the other molecules that make up living things as easily as we can the DNA from kiwi fruit cells. Why do you think this is so?

❷ Have you heard of the Human Genome Project? What is it and why is it important?

❸ Some people are concerned that we may be able to manipulate the DNA of people and that it will change them into something that they are not. Can you give some examples of the types of human genes that might be changed? Do you think that scientists should proceed with this type of research? Why or why not?

Going Further: Ideas for Inquiry

› Try to extract DNA from other fruits or vegetables using this procedure.

› Research and try other types of DNA extraction procedures. Compare the yield of another procedure with the yield from the one described here.

› Calculate how many times to the moon and back a human's DNA would reach if it was removed from each cell and each strand laid end-to-end. Here is the information you need: Each cell nucleus in a human holds about 2 meters of DNA and a typical adult human is composed of 60 trillion cells. The distance from the earth to the moon is 380,000 kilometers.

The Basics and Beyond

Background

Deoxyribonucleic acid (DNA) is the genetic material present in all organisms, from bacteria to humans. A single subunit of DNA is called a nucleotide and consists of a nitrogen-containing base, a sugar, and a phosphate group. Hundreds of thousands of nucleotides are hooked together to form a chain, and two chains are paired together and twisted into a double helix to form the finished DNA molecule (see Figure 1). In organisms with nucleated cells such as humans, DNA is coupled with protein in structures called chromosomes that are contained within a membrane-bound nucleus inside a cell.

Very pure DNA can be easily extracted from cells in a research laboratory, and somewhat less-pure DNA can be extracted with some simple techniques easily performed at home or in the classroom.

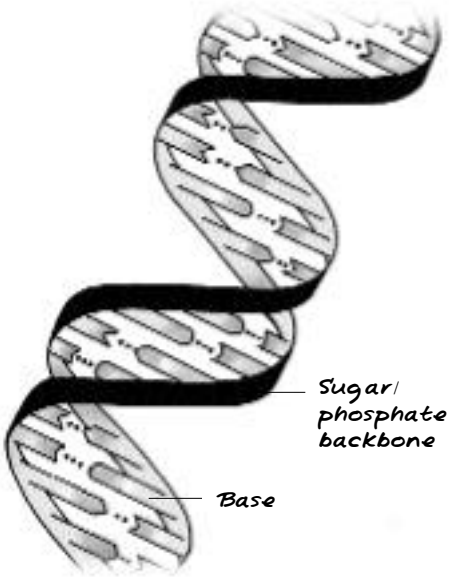

Figure 1: DNA double helix

Tidbits

› There are about 3×10^9 nucleotide base pairs in the human genome (the complete set of genes in one cell). If you took all of the DNA from a single human cell and laid the strands end to end, it would be about 2 meters long!

› All of that DNA is folded and packed into the nucleus of a human cell. The diameter of the nucleus is about 0.005 mm or $^1/_{500}$ the width of a dime.

› There are 6 billion bits of information coded by DNA in each of our nucleated cells (a bit is a measure of information). Each human cell contains twenty-one times the information that is found in the *Encyclopædia Britannica,* which is thought to have about 280 million letters.

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