Module A -- DNA isolation from fruit

DESCRIPTION

DNA will be isolated from food material and the final DNA product will be visible to students. Scientists today are able to study the genetic code – DNA - of most living things and then compare that code to a number of different databases to determine likely gene sequences and the types of proteins that may be made by the organism. Isolation of DNA is a necessary first step in analyzing DNA for use in DNA fingerprinting used in research, forensics, or paternity tests. In plant research, the information in DNA ultimately helps us to understand properties of a food crop such as its nutritional value, its ability to withstand the effects of invasion by other organisms and its response to changes in the growing environment.

Time Required: ~ 30 – 45 minutes

The activity can be performed by groups of students, or as a demonstration for the class, depending on class size and time available.

OBJECTIVES

This exercise is designed to:

- Demonstrate how DNA is extracted from cells
- Demonstrate the common occurrence and abundance of DNA in food

MATERIALS

- 10 ml of clear shampoo (use something like Suave daily clarifying shampoo – do not use baby shampoo – its too mild, or shampoos with conditioner included – the conditioner interferes with DNA precipitation)
- 1.5 g of table salt (about ¼ teaspoon)
- H_2O (use distilled water if available, although tap water will work too)
- 1 liter Zipper bag (one per student group)
- Freshly cut kiwi fruit and skinned (each fruit cut into 12 pieces) or one large strawberry, allocate ~ 30 g per student pair
- A large beaker (class)
- Hot water plate with beaker of water or saucepan set at a constant 60 ^{o}C (class)

- Cheese cloth or coffee filter (cut to fit over beaker)
- Tape or a rubber band
- Ice water bath or large cooler with ice bath (class)
- Ice cold 95% ethanol or 91% isopropanol (rubbing alcohol) (2 ml per student pair)
- 1 small test tube (1 per student group) (glass or clear plastic is best)
- 1 wood applicator (1 per student group)
- Large transfer pipettes

PROCEDURE (This procedure can be modified for amount depending on the size of a class.)

1. In a 100 ml graduated cylinder, mix 90 ml of water and 1.5 g of salt (sodium chloride) (about ¼ of a teaspoon). Cover the cylinder with plastic wrap and your hand and mix by inversion.

2. Add shampoo until solution volume is 100 ml. Stir slowly to avoid foaming of the shampoo or mix slowly by inversion.

3. Measure 20 ml of solution into each Ziploc bags (1 bag per student group)

4. Add kiwi/strawberry fruit into extraction solution in the zipper bag. Close bag, let as little air in as possible.

5. Mash the kiwi/strawberry thoroughly for 5 minutes, without breaking the bag.

6. Place the zipper bags with fruit and extraction solution into the 60^{o}C hot water bath for 10 minutes. Occasionally shake the bag to distribute heat.

7. Put the "mashed" bags of kiwi/strawberry fruit and solution into the ice bath for 1 minute. Remove and mush the kiwi/strawberry fruit more. Repeat this procedure 5 times.

8. Use tape or a rubber band to attached the cheese cloth/coffee filter over the beakers. Filter this mixture through the filter. Student groups can combine their solutions at this point. Let the solution drain 5 minutes.

9. Using the plastic transfer pipettes, distribute approximately 2 ml of the kiwi/strawberry fruit solution into each test tube, one for each group of students. Try to avoid creating foam when you do this step.

10. Carefully, without disrupting the test tube contents, GENTLY add approximately 2 ml of ice-cold ethanol or isopropanol to each tube. Hold the tube at about a 45-degree angle and let the drops of ethanol/isopropanol run slowly down the side of the test tube and rest on top of the kiwi/strawberry fruit mixture.

11. Let the solution sit for two minutes without disturbing it. The DNA will appear as transparent, slimy, mucous-like strands.

12. If desired, you can use a wooden stick or glass rod to spool the DNA. Place one end of the stick into the solution and slowly twirl. The DNA should adhere to the stick and you can lift it out of the solution.

ANALYSIS

What was the purpose of the shampoo? The plasma membrane around the outside of a cell, and the nuclear membrane, are made up of fatty components called lipids. The detergent in the shampoo destroys the cell and nuclear membranes to allow the DNA to get out. It does this by dissolving lipids and proteins that hold the membranes together.

What was the purpose of the salt? The salt enables the DNA strands to stick together or "precipitate". The positive charge of the sodium interacts with the negatively charged phosphate groups at the 5' ends of DNA strands to neutralize the molecule – this helps to make the DNA less soluble in the water/alcohol mixture.

Why was it necessary to mash the kiwi/strawberry fruit? Pressing the kiwi/strawberry fruit helps to physically break apart the cell walls that surround plant cells. This process of breaking up the cells is also aided by the detergent.

Why do you heat and cool the mixture? Heating helps with the cell lysis. Cooling protects the DNA from other cellular components. DNases or enzymes that destroy DNA are present in the cell's cytoplasm. The DNA's nuclear membrane is destroyed by the soap and the DNA is now susceptible to the DNases. These enzymes are temperature sensitive and cooling the solution slows down the process of degradation. DNases are in cells to protect the organisms from invading viruses and to help in normal cell functions.

What happens when the cold ethanol/isopropanol is added? DNA is not soluble in alcohol, the other components of the mixture stay dissolved in solution. The DNA will become apparent as white mucous-like strands that can be spooled with a glass pipette or rod.

Would it make a difference if it were warm ethanol/isopropanol that was added? The colder that this DNA solution is, the more likely it will precipitate or solidify. Cooling the ethanol increases the amount of DNA that is precipitated. The temperature has nothing to do with the DNases at this point; it has to do with precipitating or solidifying the DNA out of the solution.

Student Page

Overview

This activity will allow you to remove the DNA from cells of a plant. DNA is the chemical code that determines the activity within a cell, and ultimately determines the biology of the entire organism. It is found in virtually all living cells, including the cells in your food and your body.

Procedure

Extraction solution preparation:

1. In a 100 ml graduated cylinder, mix 90 ml of water and 1.5 g of salt (sodium chloride) (about ¼ of a teaspoon). Cover the cylinder with plastic wrap and your hand and mix by inversion.

2. Add shampoo until solution volume is 100 ml. Stir slowly to avoid foaming of the shampoo or mix slowly by inversion.

3. Measure 20 ml of solution into each Ziploc bags (1 per student group)

DNA isolation

4. Add kiwi/strawberry fruit into extraction solution in the zipper bag. Close bag, let as little air in as possible.

5. Use your fingers to mash the kiwi/strawberry on a tableop thoroughly for 5 minutes, without breaking the bag.

6. Place the zipper bags with fruit and extraction solution into the 60^{o}C hot water bath for 10 minutes. Occasionally mix the bag to distribute heat.

7. Put the "mashed" bags of kiwi/strawberry fruit and solution into the ice bath for 1 minute. Remove and mush the kiwi/strawberry fruit more. Repeat this procedure 3 to 5 times.

8. Use tape or a rubber band to attach the cheese cloth or coffee filter over the beaker(s). Pour this mixture through the filter and allow it to drain into the beaker. Student groups can combine their solutions for this step. Let the solution drain 5 minutes.

9. Using the plastic transfer pipettes, distribute approximately 2 ml of the kiwi/strawberry fruit solution into each test tube, one for each group of students. Try to avoid creating foam when you do this step.

Student page (continued)

10. Carefully, without disrupting the test tube contents, GENTLY add approximately 2 ml of ice-cold ethanol or isopropanol to each tube using a clean transfer pipette. Hold the tube at about a 45-degree angle and let the drops of ethanol/isopropanol run slowly down the side of the test tube and rest on top of the kiwi/strawberry fruit mixture.

11. Let the solution sit for two minutes without disturbing it. The DNA will appear as transparent, slimy, mucous-like strands.

12. If desired, you can use a wooden stick or glass rod to spool the DNA. Place one end of the stick into the solution and slowly twirl. The DNA should adhere to the stick and you can lift it out of the solution.

Questions

1. Propose ideas about the purpose of each chemical or substance you added to remove the DNA from the fruit. For example, what do you think the shampoo did?

2. Why do you think it might be important for scientists to be able to remove the DNA from an organism?

3. Why does the alcohol added in step 7 "float" on top of the water?

4. Why was it necessary to mash the fruit tissue?

5. Why do you suppose we cooled the sample on ice?

REFERENCES

1. www.accessexcellence.org
2. www.biotech.iastate.edu
3. www.biology.arizona.edu
4. http://www.uga.edu/discover/sbof/

The following website provides a protocol for extracting your own DNA!

http://www.nature.ca/genome/05/051/pdfs/DNAextract_e.pdf