Strawberry DNA Extraction

Overview

DNA is the "Code of Life" .The DNA of eukaryotic cells is about 100,000 times as long as the cells themselves. However, it only takes up about 10% of the cells' volume. This is because DNA is highly convoluted (folded) and packaged as structures called chromosomes within cell nuclei. A chromosome is a bundle of tightly wound DNA coated with protein molecules. An organism's chromosomes bunch together within the nucleus like a ball of cotton, but during cell division (mitosis) they become individually distinct (human mitotic chromosomes are X-shaped) and can be observed as such with microscopes. DNA is not visible to the eye unless it is amassed in large quantity by extraction from a considerable number of cells. When chromosomal DNA is unfolded and the proteins coating it removed, the structure of DNA is exposed as a twisted ladder called a double helix. The sides of the ladder form the DNA backbone with alternating sugar and phosphate molecules linked by covalent bonds. The rungs of the ladder are comprised of pairs of nitrogenous bases [adenine (A) with thymine (T) and cytosine (C) with guanine (G)] joined by hydrogen bonds. Although the structure of DNA is well known and clearly defined, even the most powerful microscopes cannot visualize the DNA double helix of chromosomes.

All living things are dependent on DNA, and the structure of DNA is consistent among all species. However, the particular sequence of nitrogenous bases within DNA molecules differs between organisms to create explicit "blueprints" that specify individual living things. This sequence of base pairs is what makes an organism an oak tree instead of a blue jay, a male instead of a female, and so forth.

DNA Extraction From Plant Cells

The DNA of a plant cell is located within the cell's nucleus. The nucleus is surrounded by a nuclear membrane and the entire cell is encased in both a cell membrane and a cell wall. These barriers protect and separate the cell and its organelles from the surrounding environment. Therefore, in order to extract DNA from plant cells, the cell walls, cell membranes and nuclear membranes must first be broken. The process of breaking open a cell is called cell lysis. Physical actions such as mashing, blending, or crushing the cells cause their cell walls to burst. The cell membranes and nuclear membranes may then be disrupted with a detergent-based extraction buffer. Just as a dishwashing detergent dissolves fats (lipids) to cleanse a frying pan, a detergent buffer dissolves the phospholipid bilayer of cell membranes. It separates the proteins from the phospholipids and forms water-soluble complexes with them. Once the cell wall and cell membranes are degraded the cell contents flow out, creating a soup of DNA, cell wall fragments, dissolved membranes, cellular proteins, and other contents. This "soup" is called the lysate or cell extract.

DNA molecules are then isolated away from the cell debris in the lysate. For this purpose, the detergent-based extraction buffer also includes salt. The salt causes some of the cellular debris in the soup to precipitate out of solution while the DNA remains dissolved. This means that the cell debris become suspended particles that can be seen. The cell extract is then filtered through layers of cheesecloth. The cheesecloth traps the precipitated cell debris while the soluble DNA passes through. DNA is soluble in the aqueous cellular environment and in the presence of the extraction buffer, but is insoluble in alcohol (such as ethanol and isopropanol). Applying a layer of ethanol on top of the filtered lysate causes the DNA to precipitate out of the solution, forming a translucent cloud of fine, stringy fibers at the point where the alcohol and cell extract meet. Cold ethanol works best to precipitate DNA to the fullest. DNA extracted from multiple cells

is visible by eye and can be wound onto a wooden stick in a process known as "spooling" the DNA.

Importance of DNA Extraction

DNA extraction is a fundamental procedure in scientific laboratories around the world. By extracting DNA, scientists can learn how DNA encodes the instructions for all life processes. DNA extraction is important to the study of heredity and to the treatment of many diseases through the creation of gene therapy DNA molecules. Extracted DNA can also be used to create DNA fingerprints to help diagnose genetic diseases, solve criminal cases, identify victims of disaster and war, and establish paternity or maternity. Scientists can genetically engineer changes in DNA to create robust, disease-resistant genetically modified plants and animals. DNA extraction is also necessary in order to sequence the DNA code (order of base pairs) of different organisms (as in the Human Genome Project) and compare different species.

What does DNA look like? What will we see?

The structure of DNA is like a twisted ladder, forming what is called a double helix. The sides of the ladder are sugar-phosphate groups joined by covalent bonds and the rungs are nitrogenous bases joined by hydrogen bonds. However, in order to package DNA within the nucleus of eukaryotic cells, DNA is wound around protein molecules and tightly folded into chromosomes. Can we see DNA? Yes and no. Chromosomes have been studied using microscopes, but the double helix of unraveled chromosomes is so thin that even the most powerful microscopes cannot detect it. How will we see the DNA we extract? Chromosomal DNA from a single cell is not visible by eye, when DNA is extracted from multiple cells, the amassed quantity is visible and looks like strands of mucous-like, translucent cotton.

Objectives

Understand how cell barriers are broken and how to extract DNA from strawberry cells.

Material

This kit accommodates up to 32 students working in pairs.

17 50-mL tubes with lids and bases

33 15-mL tubes with lids

16 resealable plastic bags

16 wooden sticks

16 funnels

1 pack of cheesecloth

16 transfer pipets

1 bottle of ethanol, 95% (100 mL)

1 bottle of liquid detergent

10 g salt (sodium chloride, NaCl)

Procedure

1. Obtain one fresh or one frozen and thawed strawberry. If you are using a fresh strawberry, remove the green sepals (tops) from the berry.
2. Place the strawberry in a resealable plastic bag.
3. Close the bag slowly, pushing all of the air out of the bag as you seal it.
4. Being careful not to break the bag, thoroughly mash the strawberry with your hands for two minutes.
5. Pour the 10-mL aliquot of extraction buffer into the bag with the mashed strawberry. Reseal the bag.
6. Mash the strawberry for one additional minute.
7. Place a funnel into a 50-mL centrifuge tube. Fold the cheesecloth in half along the longer side and place it in the funnel to create a filter. The cheesecloth will overlap the edge of the funnel.

8. Pour the strawberry mixture into the funnel, filtering the contents through the cheesecloth and into the 50-mL centrifuge tube.
9. Carefully pour 2 mL of the filtered contents from the 50-mL tube into a clean 15-mL tube. Use the lines on the side of the 15-mL tube to help measure the amount added.
10. Hold the 15-mL tube at an angle. Using a transfer pipet, carefully add 5 mL of cold 95% ethanol by running it down the inside of the tube. Add the 95% ethanol until the total volume is 7 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers. Caution: Do not mix the strawberry extract and the ethanol!
11. Watch closely as translucent strands of DNA begin to clump together where the ethanol layer meets the strawberry extract layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.
12. Slowly and carefully rotate the wooden stick in the ethanol directly above the extract layer to wind (or "spool") the DNA. Remove the wooden stick from the tube and observe the DNA (see Figure 4: DNA Extraction on the next page).

Resource: Strawberry DNA Extraction.2004. World-class Support for science & math. Carolina, USA