DNA Strawberry Extraction Background

DNA

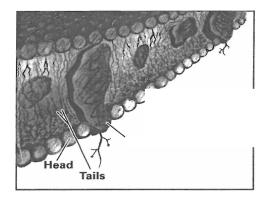
Deoxyribonucleic acid (DNA) can be considered the hereditary "code of life" because it possesses the information that determines an organism's traits and is transmitted from one generation to the next. DNA can be compared to a recipe or a list of instructions about how to create and maintain a specific living thing. The DNA in an individual's cells contains unique genetic instructions about how to make and operate that individual.

DNA can be removed from organisms through a common and useful scientific procedure called DNA extraction. In order to understand this process, it is useful first to identify the basic structures that hold DNA molecules within living things.

Cells Contain DNA

DNA is located inside the cells of all species. However, different organisms are made up of different types of cells. Members of the Animal, Plant, Protist (algae, amoebas, paramecia, etc.), and Fungi (mushrooms, yeasts, molds, etc.) kingdoms are comprised of eukaryotic cells. This means that these cells have a true nucleus, a membrane bound organelle within which the DNA is contained. The nucleus of eukaryotic cells is the "control center" that directs all cellular activities. Members of the kingdom Monera (bacteria and cyanobacteria) are comprised of prokaryotic cells that do not have nuclei. In these cells, DNA exists as a long loop coiled loosely within the cytoplasm of the cell.

The nucleus of eukaryotic cells is surrounded by a nuclear membrane (also called a nuclear envelope) and the entire cell is bound by a cell membrane (also called a plasma membrane). These barriers are both made up of two layers of fatty, oily compounds called lipids. The most abundant types of membrane lipids are phospholipids. These molecules have hydrophilic ("water-loving") heads linked by a phosphate group to two hydrophobic water-hating") tails. The formation and stability of cell membranes is based on the orientation of phospholipid molecules in an aqueous (watery) environment. In such surroundings, phospholipids form a barrier of two rows with their hydrophobic tails facing each other (away from water) and their hydrophilic heads pointed outward (in contact with the aqueous environment). This two-layered structure is known as a *phospholipid bilayer* (see Figure below). Protein and carbohydrate molecules are also imbedded within the phospholipid bilayer of cell membranes to transport particular molecules into and out of the cell, and to conduct cellular messages.



All eukaryotic cells have a nuclear membrane that encircles the nucleus, as well as a cell membrane that encases the entire cell. However, plant cells (and some bacterial, fungal, and protist cells) have an additional barrier surrounding the cell membrane called a cell wall. Animal cells do not have cell walls. Plant cell walls are made of cellulose, which is a sturdy polysaccharide material comprised of glucose units. Cellulose gives plants their rigidity and provides a tough barrier that enables plant cells to hold a great deal of fluid without bursting.

Packaging and Structure of DNA

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 Xshaped) 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.

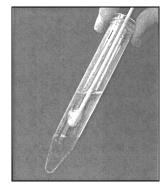
All living things are dependent on DNA, and the structure of DNA is consistent among all species. However, the particular sequence of nitrogenous bases within DNA molecules differs between organisms to create explicit "blueprints" that specify individual living things. This sequence of base pairs is 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 wound onto a wooden stick in a process known as "spooling" the DNA.



Strawberry cells are excellent sources of DNA for extraction and visualization. They are multicellular and octoploid. This means they have eight copies of their seven chromosomes in each of their many cells. Therefore, just one berry will yield enough DNA to be easily seen and spooled. Strawberries are also a soft fruit, which makes them easy to mash. Mashing the berries breaks down the strawberry tissue, releases the individual cells, separates the seeds from the cells, and breaks the cell walls. In addition, ripe strawberries produce pectinase and cellulase—enzymes that contribute to the breakdown of cell walls.