# DNA Background

Since DNA is an essential molecule to all living things (with the exception of some viruses), it is not surprising that elaborate mechanisms have evolved to protect it. To extract DNA successfully, it is helpful to understand these protective mechanisms.

The simplest organisms, **prokaryotes**, which include bacteria, do not have the protection of a membrane-bound nucleus. Rather, the DNA clings to an in-folding of the inner cell membrane and is protected from invading viral DNA by restriction enzymes that cut foreign DNA into small pieces. Methyl groups that are attached to its DNA protect the host cell from its own defenses. Methyl groups prevent restriction enzymes from cutting DNA.

As organisms become more complex, so do the mechanisms that protect their DNA. *Eukaryotic* DNA is contained within a membrane-bound nucleus. Plants have additional protection from a cell wall. All eukaryotes have DNAse enzymes in the cytoplasm that cut DNA. To produce spoolable DNA in the laboratory, it is necessary to denature the DNAses before interrupting the nuclear membrane, often by using heat or pH changes. DNA is a relatively sturdy molecule but its tremendous length makes it prone to breaking once it is away from its protective environment. If the DNA is broken or sheared in too many places, it won't spool. It is important to be gentle in the last steps of DNA extraction and to avoid violent shaking or vortexing that will shear the DNA.

**Historical Milestones**

**1869**
Johann Friedrich Miescher identifies a weakly acidic substance of unknown function in the nuclei of human white blood cells. This substance will later be called deoxyribonucleic acid, or DNA.

**1912**
Physicist Sir William Henry Bragg and his son, Sir William Lawrence Bragg, discover that they can deduce the atomic structure of crystals from their X-ray diffraction patterns. This scientific tool will be key in helping Watson and Crick determine DNA's structure.

**1924**
Microscope studies using stains for DNA and protein show that both substances are present in chromosomes.

**1928**
Franklin Griffith, a British medical officer, discovers that genetic information can be transferred from heat-killed bacterial cells to live ones. This phenomenon, called transformation, provides the first evidence that the genetic material is a heat-stable chemical.

**1944**

Oswald Avery, and his colleagues Maclyn McCarty and Colin MacLeod, identify Griffith's transforming agent as DNA. However, their discovery is greeted with skepticism, in part because many scientists still believe that DNA is too simple a molecule to be the genetic material.

**1949**

Erwin Chargaff, a biochemist, reports that DNA composition is species specific; that is, the amount of DNA and its nitrogenous bases varies from one species to another. In addition, Chargaff finds that the amount of adenine equals the amount of thymine, and the amount of guanine equals the amount of cytosine in DNA from every species.

**1953**

James Watson and Francis Crick discover the molecular structure of DNA.

**1962**

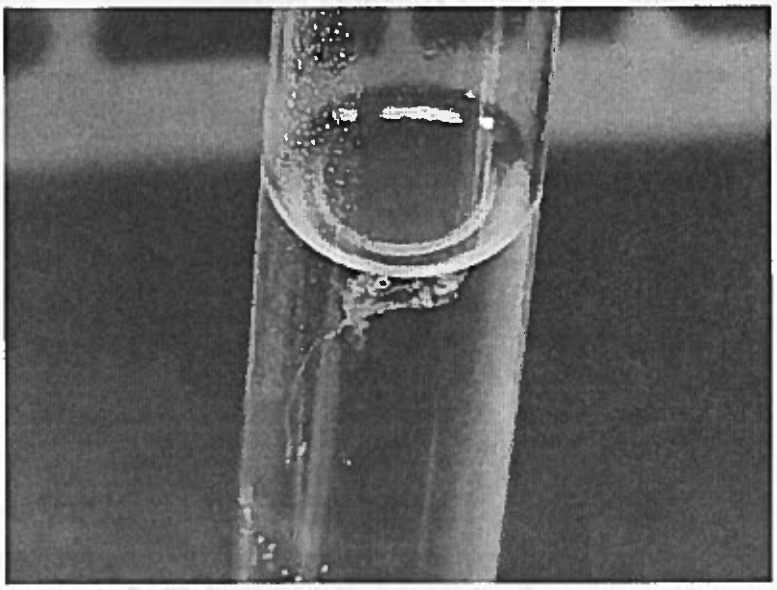
Francis Crick, James Watson, and Maurice Wilkins receive the Nobel Prize for determining the molecular structure of DNA.

*(Historic milestones link: Access Excellence, www.gene.com/gene/research/biotechnology/significant-milestones.jsp)*

## An Introduction to the DNA Extraction Lab

### Some basic, but cool, chemistry…

DNA is the largest known molecule. A single unbroken strand can contain millions of atoms. When DNA is released from a cell it typically breaks up into tiny strand fragments. These tiny fragments have a slightly negative electric charge. Salt ions, common in many solutions, are attracted to the negative charges on the DNA fragments and prevent them from adhering to one another. By controlling the salt concentration of the solution containing the DNA fragments, DNA can remain fragmented or become very "sticky" and form large globs of molecular material.



### Releasing the DNA…

The first step in obtaining DNA from any organism, be it a plant, animal, fungi, archae or bacterium, is to release the DNA from a cell. Detergents and soaps break down cell membranes, releasing the DNA, and they also break up proteins that may harm the DNA. Protein enzymes, or proteases, like those in contact lens solution or in "Ultra" forms of laundry detergent, can be used to further destroy proteins.

**DNA on a stick...**

Once the DNA fragments are released into solution, the DNA can be spooled together by using ice-cold isopropyl alcohol. Alcohol allows DNA fragments to stick together, or precipitate, producing a blob of DNA that you can examine. When a small layer of alcohol is added to the top of a solution containing cellular fragments and DNA, it will form an interface where the DNA will precipitate, allowing it to be captured, or spooled, onto a wooden stick or glass rod. Although this method is effective, the DNA produced is by no means pure; other materials such as protein and cell fragments are carried along.

**Student spoolers...**

Following an introduction to DNA, students will have an opportunity to extract DNA from some interesting samples: salmon sperm and some common vegetables or fruits. Some samples will be prepared ahead of time (positive controls), but students will have to prepare other samples for themselves. The students will then compare their DNA extractions to positive samples. The steps are not complicated but do require that students work carefully to optimize the yield of DNA product that they are attempting to extract. The experiment will be a bit messy but very rewarding if students are able to make their own extraction.

# Extracting DNA From Fruit

**Pre Lab: You were to have read about the extracting DNA article**
Cells are the functional units of living things. They reproduce, in part, by making and passing deoxyribonucleic acid (DNA) from the parent cell to the offspring cell during cell division . All DNA in every living thing on earth is made up of the same chemical bases, **adenine, thymine, guanine, and cytosine.** The order of the bases determines the proteins the cell makes and the functions the cell performs which will be different for us as it would be different for a bear or a mosquito.

In this activity, students extract DNA (and also some RNA) from 30g-40g of bananas, strawberries and apples and will compare and contrast how much DNA they will produce with each sample by comparing data with their classmates.

- **DNA is a component of living and once-living things.**
- **DNA can be extracted and observed.**
- **Every individual has the same DNA in all of their cells**

## Key Terms

**DNA:** Deoxyribonucleic acid, which is the hereditary material in cells that contains the instructions for producing proteins in the cell and enabling it to function, and repair and reproduce.
**Extraction Solution:** A solution that breaks down lipids
**Filtrate:** The material collected after a solution or mixture passes through a filter
**Precipitate:** Solid material that comes out of solution as a result of a chemical or physical change

## Materials:

- *Extracting DNA from Fruit* Student Handout
- DNA article from UMBI
- 1 sample of fruit with a mass around 30-40g
- ¼ cup or 50ml of extraction solution –(*distilled water, Pantene shampoo, salt*) placing the plastic baggie
- 15 ml 91% isopropyl (i.e., rubbing alcohol) in 25 ml or 50 ml sealed test tube; chill the alcohol in ice
- 1 set of measuring spoons and a measuring cup with 1/4-cup markings
- 1 paper coffee filter and rubber band or tape
- 2 glass stirring rods
- 2 250 ml beaker
- 1 plastic pipette
- test tube holder
- Bag of ice
- Plastic baggies
- stop watch
- test Tube

## Procedure

1. **In your group choose who is going to be the *Director, Spokesperson, Techi, and Reader***
   Start by reading the article on **DNA Extraction.**
2. Reader: start by reading through the entire procedure
3. Take a piece of fruit, (strawberry or banana) into a plastic baggie
4. Mash the fruit with your hands for 30-60 seconds making sure the it is completely pulverized.

5. Put 1/4 cup of *Extraction solution*. Agitate the bag for 10 minutes.
**Extraction Solution: (distilled water, *Pantene* shampoo, salt)**
to the plastic bag with the fruit.

**Debrief:**
**Salt** *in the water helps the DNA precipitate (solidify and appear) when alcohol is added.*
**What is thePantene for***? Hint: it breaks down oils and grease, what is made of oils/lipids in the cell?*
**What is EDTA**? Pantene contains EDTA a preservative it prevents DNA from breaking down and DNase (enzyme) from forming
**Why does the fruit have to be pulverized?**

6. Insert a filter into a clean glass beaker 250 ml glass beaker **IT IS EXTREMELY IMPORTANT** that the filter does not touch the bottom of the cup. If necessary, use a rubber band to secure all sides of the filter around the glass beaker.

7. Pour the mixture from step 3 into the filter. After 3-5 minutes, some liquid, called the filtrate, should have collected in the bottom of the cup. Gently stir with a glass stirring rod the mixture in the filter and let it sit for another minute so that all the filtrate will filter down into the glass. Be extremely careful not to break the filter, or you will have to start your collection over.

8. Remove the filter and throw away in the garbage. Be sure not to get any of the foam in the beaker.
*Cold water helps keep the DNA intact during the extraction process. How? Cooling slows down enzymatic reactions. This protects DNA from enzymes that can destroy it.*

8. Get a test tube of 15 ml cold alcohol and place in a cold water bath using the second beaker and ice

**Debrief:**
**Why must the alcohol be kept cold?**
*Keeping the alcohol cold and ice-cold water will increase your yield of DNA. The cold water protects the DNA by slowing down enzymes that can break it apart. The cold alcohol helps the DNA precipitate (solidify and appear) more quickly.)*
**Why is it important not to get any foam into the bottom filtrate material?**

9. Use a pipette or eyedropper to collect your fruit filtrate. Add it to the alcohol in the test tube. Slowly running the DNA **filtrate** down the side of the test tube

9. Place the test tube with the alcohol and filtrate in a beaker ice bath for 3-5 minutes. Let it sit undisturbed. The white material coming out of solution as a **precipitate** is DNA.

10. Dip the glass rod into the tube, slowly rotating it to spool out the DNA.
Look closely. The DNA may be lingering between the two layers of alcohol and pea soup. Try to help the DNA rise to the top, alcohol layer. Dip a glass rod stick into the fruit mixture and slowly pull upward into the alcohol layer. Also, look very closely at the alcohol layer for tiny bubbles. Even if your yield of DNA is low, clumps of DNA may be loosely attached to the bubbles.

11. Now look at your *student version* **DNA Extraction Lab** and finish answering the questions and the writing up the lab. **Make sure to answer the Debrief Questions in the lab.**