

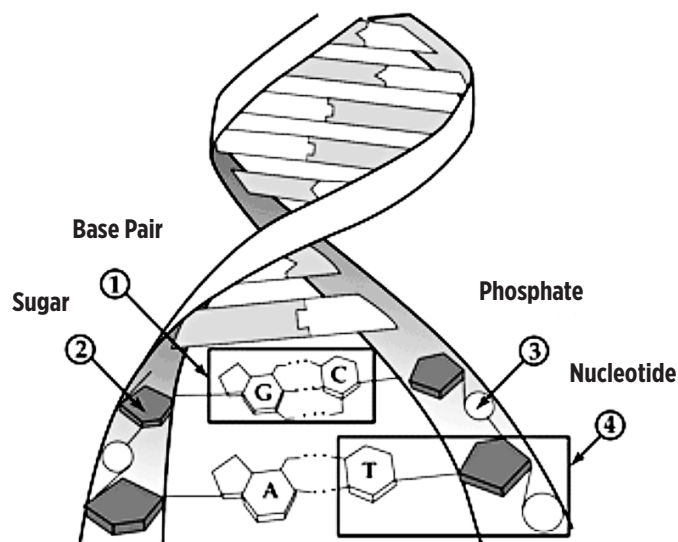
DNA Extraction

Extracting DNA from Fruit

Name:

All living things are made of cells. Most cells contain Deoxyribonucleic Acid, or DNA, which is the blueprint of all living things. In prokaryotes (single celled organisms such as bacteria), the DNA is floating freely in the cytoplasm of the cell. In eukaryotes (multi-celled organisms), the DNA is enclosed within a nucleus. DNA contains the genetic code needed to synthesize proteins.

Amazingly, the structure of DNA is exactly the same across all organisms. DNA has a very distinct shape called the double helix, which looks very similar to a spiral staircase. This structure is held together by parallel backbones made of alternating phosphate and sugar groups. Attached to each sugar-phosphate group is the storage unit called the nitrogenous base. Together, this entire unit is called a nucleotide. The nitrogenous base on each nucleotide has a complimentary base that, when paired, is referred to as a base pair.



Today, you will perform a DNA extraction to determine whether or not the fruit of a plant is biotic (i.e., whether it is living or produced by a living entity). In order to get to the DNA in eukaryotic cells, we must first get into the nucleus of the cell. In animal cells, the DNA is contained within two phospholipid bilayers, the nuclear envelope and the cellular membrane. Plant cells also contain a rigid structure called a cell wall, located just outside of the cellular membrane. In addition to these layers, both plant and animal cells contain organelles made of proteins and carbohydrates.

State the general hypothesis that you will test in this activity

MATERIALS

- Extraction buffer
- Isopropyl alcohol
- Micropipette and tips
- Zip-lock bag (x2)
- Distilled Water
- Positive control
- Scissors
- 4 large test tubes
- Fruit
- Microcentrifuge tubes

PART I — Prepare the controls

1. Locate the four (4) test tubes in your tube rack. Label one tube positive and one tube negative. In the chart below, identify which of your materials is the positive control and which is the negative control. Also, indicate whether these controls are biotic or abiotic.

	ID	Biotic or Abiotic?	DNA?
Positive Control			
Negative Control			

2. Put 2,000 μL of distilled water into your negative control test tube.
3. Put 2,000 μL of the positive control into your positive control test tube.
4. Add 1,000 μL of extraction buffer to each of the test tubes.

QUICK CHECK:

 What is the extraction buffer made of?

5. Vortex the tube for five seconds to mix. Compare the test tubes to each other.
6. Add 2,000 μL of rubbing alcohol slowly down the side of each tube to form a layer that floats on top of each sample.
DO NOT MIX, VORTEX, OR SHAKE.
7. If there is DNA in the test tube, it should form gray clumps with air bubbles. Look closely at each test tube to see if any contained DNA.

PART II — Test the fruit for DNA

8. Label one test tube E1 (Experimental 1). Label one test tube E2 (Experimental 2).
9. Choose two experimental samples from the fruits near your station. Record the name of the fruit below and circle whether you predict that fruit is biotic or abiotic.

Experimental Sample 1: _____
Biotic / Abiotic

Experimental Sample 2: _____
Biotic / Abiotic

 How will you test these hypotheses?

- 10. Place a piece of your experimental sample into a zip-lock bag. Do this for your second sample also.
- 11. Add 7,000 μL of water and 3,000 μL of extraction buffer to the zip-lock bags. Close the bags and use your fingers to gently mash the experimental samples into a paste.
- 12. Get a new tip for your micropipette and using scissors cut off about 1 cm of the tip. Transfer approximately 2,000 μL of the mixture into the appropriate test tube.
- 13. Throw away the bags and the rest of the mixture.
- 14. Vortex the two experimental sample tubes for five seconds to mix.
- 15. Add 2,000 μL of rubbing alcohol slowly down the side of each tube to form a layer that floats on top of each sample.
DO NOT MIX, VORTEX, OR SHAKE.
- 16. Take a look at your experimental samples, and record your observations in the table below.

PART III — Data analysis

Analyze the results of your test by filling in the table below.

Sample	ID	DNA?	Biotic or Abiotic?
Positive Control	Juice	Y	Biotic
Negative Control	Water	N	Abiotic
Experimental 1			
Experimental 2			

PART IV: Conclusion

Use the results of your data analysis to make a conclusion about your hypotheses: How did you determine whether or not the experimental samples were biotic or abiotic? Do your results support or reject your hypotheses?