

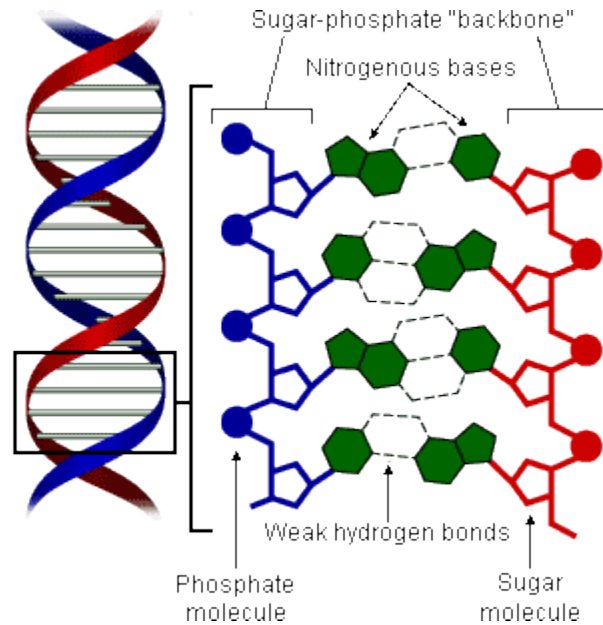
DNA Extraction

A DNA extraction activity

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Adapted from the activity *In Your Kitchen*, written and developed by the J. Craig Venter Institute.

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



evolution.berkeley.edu/evosite/history/dna2.shtml

Today, you will perform a DNA extraction to remove the DNA from cells.

NAME THREE SITUATIONS THAT WOULD REQUIRE DNA REMOVING FROM THE CELL:

In order to get to the DNA in eukaryotic cells, we must get into the nucleus of the cell. The cell membrane, a phospho-lipid bilayer, surrounds both the cell and the nucleus. Plant cells also contain a rigid structure called a cell wall, located just outside of the membrane. In addition to these layers, cells also contain other organelles made of proteins and carbohydrates.

How can we separate the NUCLEIC ACID from the lipids, proteins, and other components found in the cell?

THE EXPERIMENT

Scientists use controls to check the results of their experiments. You and your partner will use a positive and negative control for this experiment.

What **ABIOTIC** factors can you think of that do NOT contain DNA?

What **BIOTIC** factors can you think of that do contain DNA?

MATERIALS

Extraction buffer
Isopropyl alcohol
Micropipette
Zip-lock bag

Distilled Water
Positive control
Scissors
4 large test tubes

Fruit
Marker
Test tube rack
Microcentrifuge tubes

The following steps are your protocol for extracting DNA from cells. Work with your partner and follow the directions carefully – be sure to check off each step as you complete it.

1. There are four (4) test tubes in your tube rack: Label one tube **positive** and one tube as **negative**.

Positive control: _____
We know this contains DNA.

Negative control: _____
We know this does not contain DNA.

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. **What is the extraction buffer made of?**
5. Vortex the tube for five seconds to mix. **Compare the test tubes to each other. Did their appearance change? If so, how?**

Note: It is important to be gentle in this step of the DNA extraction to allow you to obtain DNA. Though it is a relatively sturdy molecule, DNA's tremendous length makes it prone to breaking once away from its protective environment. If the DNA is broken or sheared in too many places, it will not spool.

- 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 which contained DNA.

What happened to the positive control test tube when you added the isopropyl alcohol?

What happened to the negative control test tube when you added the isopropyl alcohol?

Now, you have your positive and negative controls. It is time for your experimental samples.

- 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.

Experimental Sample 1: _____

Experimental Sample 2: _____

Will one of the experimental samples contain DNA? Will one type of fruit have better DNA extraction than the other? Create a HYPOTHESIS (be specific):

- 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 gently use your fingers to mash the experimental samples into a paste.

What has happened to your experimental samples?

Why did you add Extraction Buffer?

- 12. Cut a small hole at the corner of the zip-lock bag. Slowly drip 2,000 μL of the liquid from the bag into the experimental test tubes. Do this again for your second sample.
- 13. Throw the bags and the rest of the mixture away.
- 14. Vortex the tube for five seconds to mix. **Compare the test tubes to each other. Did their appearance change? If so, how?**

Note: It is important to be gentle in this last step of the DNA extraction to allow you to obtain DNA. Though it is a relatively sturdy molecule, DNA's tremendous length makes it prone to breaking once away from its protective environment. If the DNA is broken or sheared in too many places, it will not spool.

- 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? What do you see? Compare these to your negative and positive controls. Write down your observations:

- 17. Use the sticks at your station to spool the DNA out of the large experimental test tubes. Place the stick into the tube and twirl it between your thumb and forefinger to twist the DNA onto the stick.
- 18. Put the DNA samples into the small microcentrifuge tubes. Label the tubes.
- 19. Add 500 mL of alcohol to your small tubes with the DNA.
These samples are now ready for further testing, if needed.

DATA ANALYSIS

Analyze the results of your experiment. Compare the three tubes to each other. Which tubes, if any, had DNA (circle your answers)?

Positive control?	YES	NO
Negative control?	YES	NO
Experimental sample 1?	YES	NO
Experimental sample 2?	YES	NO

CONCLUSION

Interpret the results of your experiment. Write a conclusion that answers your hypothesis: