Split Peas

How to Extract DNA From Anything Living

(http://gslc.genetics.utah.edu/basic/howto/index)

DNA (deoxyribonucleic acid) is a very long polymer made up of nucleotides. (deoxyribose sugar + organic base + phosphate). Single molecules of DNA are long and stringy. Each cell of your body contains 6 feet of DNA, but it is only one-millionth of an inch wide. To fit all this DNA into your cells, it needs to be packed efficiently. To solve this problem, DNA twists tightly and clumps together inside cells. Even when you extract DNA from cells, it still clumps together, though not as much as it would inside the cell.

The human body contains about 100 trillion cells, each of which contains 6 feet of DNA. If you do the math, you'll find that our bodies contain more than a billion miles of DNA. The DNA, which is acidic, is "packaged" in each cell by interactions with proteins, especially basic proteins called histones. Anything that interferes with that acid-base attraction (such as salt or certain detergents) will allow the DNA to uncoil and spring free. If cells and nuclei are broken open gently, it is possible to isolate the DNA in the form of long strands. Detergents disrupt membranes by dissolving their lipids. During today's laboratory, we will attempt to isolate DNA from dry green split peas.

Instructions for Procedure

1. Place in a blender jar:
   - 84 grams (100 ml or 1/2 cup) of green split peas or other DNA source
   - 200 ml (1 cup) of water
   - A pinch of salt (1 gram or 1/8 teaspoon).

2. Blend on high for 15 seconds.

3. Strain the mixture through a sieve into a measuring cup to remove the unblended peas.

4. Add 30 ml (2 tablespoons) of detergent. Swirl gently to mix. Let sit for 5 – 10 minutes.

5. Fill a test tube about 1/3 full with the split pea liquid.

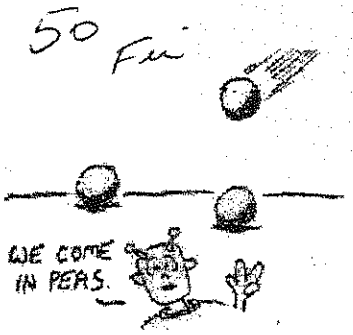
6. Wet a wooden stick by dipping it into the split pea liquid. Then dip the wet stick into meat tenderizer. Mix the meat tenderizer into the split pea liquid.

7. Tilt the test tube and slowly pour an equal amount of alcohol down the side of the tube so that it layers on top of the split pea liquid.

8. Stringy DNA should appear at the boundary between the split pea liquid and the alcohol.

9. Use a wooden stick to gently move the split pea liquid up into the alcohol so that more DNA will precipitate out; you can also let the tube sit for 30 minutes or more.

10. You can keep the DNA indefinitely in a sealed container with alcohol or dry it on paper. *(use a wooden stick or a pipette (eyedropper) to transfer the DNA)*



DNA Extraction Discussion Questions:

1. Are pea plants prokaryotic or eukaryotic?

2. Why do detergents disrupt cell and nuclear membranes?

3. Meat tenderizers break down certain proteins found in meat. This apparently makes the meat easier to chew and a bit more palatable. Knowing this, what is the role of the meat tenderizer in this lab?

4. In animal cells, what other organelle besides the nucleus contains DNA?

5. List 5 other things available to you that you might use for a DNA source.

6. List 5 things that would not be a DNA source.

7. Which "ingredient" do you think might have the most effect on the amount of DNA you extract? Describe an experiment to test your hypothesis.

8. DNA is "packaged" in the cell nucleus with what molecules? Why are they likely to be attracted to DNA?

9. How does pea DNA differ from human DNA?

REAGENTS

edvotek®
The Biotechnology Education Company®

1-800-EDVOTEK (1-800-338-6835)
24 hour fax: (301) 340-0582
Web: www.edvotek.com
Email: edvotek@aol.com

#627 catalog

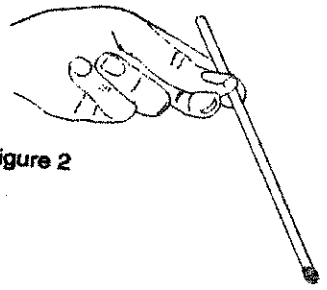


Figure 2

Gently lift the glass rod out of the solution periodically to observe the DNA material attached.

6. After spooling for a minute or so, remove the glass rod from the solution and the DNA will appear as a viscous, gelatenous-like substance adhering to the glass (figure 2).

7. Rinse the DNA on the glass rod with 95% Ethanol and allow it to dry for several minutes.

8. The DNA can be stained with a methylene blue stain, or can be rehydrated in 1 ml of 1x TE. Place the glass rod into a 1.5 ml microtest tubecontaining the TE. Stir to release the DNA and cover with parafilm or plastic wrap. Store at 4°C. This may take several days to go into solution.

9. After the DNA has been resuspended, it can be analyzed on a 0.8% agarose gel with a standard DNA marker. Chromosomal DNA from onion or other plant tissue extracted by this method may not be suitable for restriction enzyme digestion.

Troubleshooting:

If DNA does not spool well, repeat using a larger piece of tissue. There may not have been enough DNA to spool.

REAGENTS

edvotek®
The Biotechnology Education Company®

1-800-EDVOTEK (1-800-338-6835)
24 hour fax: (301) 340-0582
Web: www.edvotek.com
Email: edvotek@aol.com

#627 catalog

DNA Extraction Buffer

(250 ml)

DNA Extraction Buffer is designed to allow students to extract DNA from various plant, fruit, and vegetable tissue. The composition is safe for classroom use and is ideal for developing independent inquiry-based experiments. **Storage: Room temperature.**

All components contained in this package are intended for educational research use only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Instructions

Select onion or other plant tissue that is very fresh. The onion tissue can be soaked in cold water to hydrate if necessary. Fresh green onions, also refered to as spring onions or scallions are an excellent choice of material to use because they are usually harvested and quickly shipped to the grocery store. Other suggestions include: bananas, tomatoes, garlic, etc.

1. Carefully slice a small/medium section (~4-5 g) of tissue from the main section of the onion (not the root tip) or other tissue, and place in a test tube. A mortar and pestle can also be used to mash the tissue.
2. Pipet 4-5 ml of DNA Extraction Buffer into the tube or mortar. Mince, grind, and mash the tissuewith a pencil eraser or blunt object (this releases the cellular contents, including the DNA from the tissue).
3. Place a square of cheesecloth of a coffee filter into a funnel, place the funnel into a clean tube, and pour the tissue extract into the funnel. Squeeze out the excess juice and measure approximately 3 ml of liquid (figure 1).
4. Carefully overlay the liquid with 3 ml of 95 or 100% Isopropanol. Alternatively, use 6 ml 70% clear Isopropanol (rubbing alcohol).
5. Place a glass rod into the tube and twirl at the interface of the two liquids. The DNA should begin to spool (wrap) around the glass rod. A pasteur pipet which has been heated to melt the tip near the end to form a hook works well for spooling.

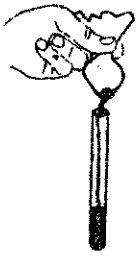


Figure 1