Biotechnology in the Classroom

PARTNERSHIP FOR BIOTECHNOLOGY & GENOMICS EDUCATION

UNIVERSITY OF CALIFORNIA DAVIS

Basic Biotechnology Kit

DNA EXTRACTION FROM STRAWBERRY

Partnership for Biotechnology and Genomics Education

Barbara Soots
Education Coordinator
University of California Davis
530 752-6552
530 754-4410 (fax)
besoots@ucdavis.edu

Linda Curro
Assistant Education Coordinator
University of California Davis
530 752-6613
530 754-4410 (fax)
llcurro@ucdavis.edu

This program is made possible through the generous support of:

UCDAVIS OFFICE OF RESEARCH

novozymes Rethink Tomorrow

UCDAVIS COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

DNA EXTRACTION
Strawberry

Teacher Information

Introduction

This lab provides an introduction to DNA (deoxyribonucleic acid) and the field of biotechnology. Students will extract DNA from a strawberry.

Attached readings and activities help illustrate both the structure and function of DNA. The basic information learned here is vital to the understanding of later biotechnology activities.

Objectives

1. Understand DNA is in the cells of all living organisms.
2. Determine how each of the ingredients in the protocol helps to extract DNA.
3. Extract a visible mass of DNA from strawberry tissue.

National Science Standards

Every cell is surrounded by a membrane that separates it from the outside world. Inside the cell is a concentrated mixture of thousands of different molecules which form a variety of specialized structures that carry out such cell functions as energy production, transport of molecules, waste disposal, synthesis of new molecules, and the storage of genetic material.

Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.

In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T).

Materials

For Each Lab Group

- 10 ml DNA extraction buffer *
- 1 strawberry*
- 1 quart-size zip loc freezer bag *
- test tube *
- test tube rack *
- funnel *
- 10 ml graduated cylinder *
- cheesecloth
- glass rod or plastic loop

Common Materials

- 5 ml 95% ethanol or isopropyl alcohol * *keep on ice*

** Materials provided by instructor.*

Advance Preparation

1. Make DNA Extraction Buffer - *100 groups*
   - 100 ml (3/8 cup) of shampoo without conditioner or liquid dishwashing soap
   - 15 grams (2 teaspoons) of salt
   - 900 mls water
2. Refrigerate ethanol overnight and place on ice before class.
3. Obtain strawberries.

Teacher Notes

Use thick zip-loc bags – they are thicker and resist breaking much better than the sandwich type.

Strawberries can be fresh or frozen. If using frozen strawberries, thaw them out before the lab.

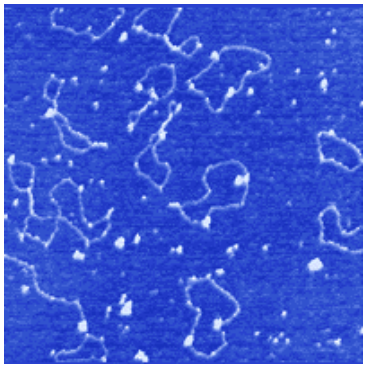
Answers to Student Activity

1. DNA directs the synthesis of every protein and contains all the genetic information that is passed on to new cells.
2. DNA is located inside the nucleus of all eukaryotic cells and in the cytoplasm of prokaryotes. It is also located in some cell organelles like the chloroplasts, mitochondria, and ribosomes.
3. 1) lyse the cell membrane, 2) lyse the nuclear membrane, 3) precipitate the DNA
4. Since bacteria no not have nuclei, the second step of the extraction process is omitted.
5. The salt solution contributes positively charged atoms that are attracted to the negative charge of DNA, effectively neutralizing the DNA's electric charge. This neutralization allows the DNA molecules to aggregate with one another.
6. Detergent breaks apart the cell and nuclear membrane by disrupting the lipid and protein structure. The cellular contents, including DNA, are then released.
7. When the ethanol is added, the DNA clumps together and precipitates at the water/ethanol interface because the DNA is not soluble in ethanol.
8. The DNA extracted in this experiment is very impure. Many proteins precipitated out along with the DNA. Additional steps would be required to purify the DNA for further study.

Inquiry Extension Activities

1. Some options for proving you have obtained DNA include the following:
   Staining the potential DNA with ethidium bromide, sybr green, or methylene blue to see if the substance takes up the stain.
   Attempting to cleave the potential DNA with restriction enzymes and run on a gel to see if fragmentation occurs. Further purification would be required for this experiment.

2. To further purify the sample would require chemicals to clean off additional proteins and a way of separating out cellular components. Papain (found in meat tenderizer) works well to clean off excess protein. A high-speed centrifuge would work to separate the DNA from other cellular contents.



Protein/DNA complex
Digital Instruments

DNA EXTRACTION
Strawberry

Laboratory

Background

DNA (deoxyribonucleic acid) is located in the cells of all living organisms. In its strands lies the blueprint for life. The DNA molecule directs the synthesis of every protein and contains all the genetic information that is passed on to new cells.

In complex eukaryotic cells such as those from plants, animals, fungi and protists, most of the DNA is located in the cell nucleus (chloroplasts, mictochondria, and ribosomes also carry some DNA). By contrast, in simpler cells called prokaryotes, including the eubacteria and archaea, DNA is not separated from the cytoplasm by a nuclear envelope.

Although DNA is an incredibly small molecule, in large quantities, it can be seen. In this activity, you will extract DNA from a strawberry.

One of the reasons strawberries work so well is that they are soft and easy to pulverize. Also, ripe strawberries are producing pectinases and cellulases which are already breaking down the cell walls. Most interestingly, strawberries have enormous genomes. They are octoploid, which means they have eight of each type of chromosome.

There are three basic steps in DNA extraction. First, the cell must be lysed (broken open) to release the nucleus. Next, the nucleus must also be opened to release the DNA. Lastly, once the DNA is released, it must be precipitated out of solution.

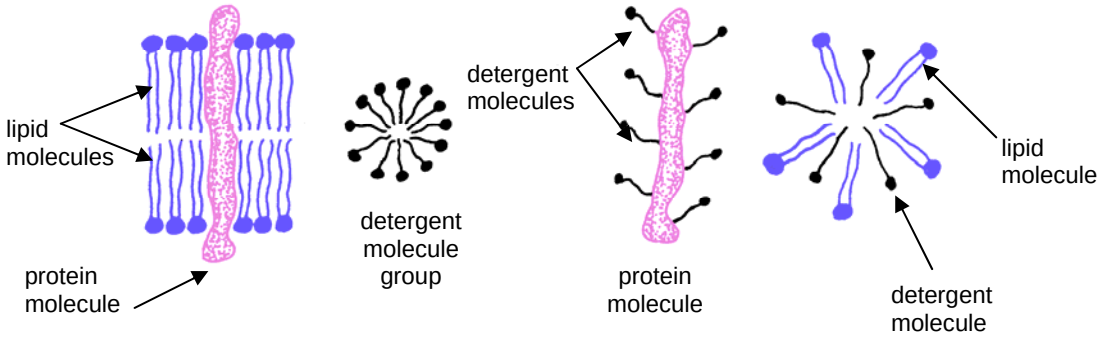
Several reagents are required to complete the extraction procedure—salt, detergent, and alcohol.

Both the cell and nuclear membranes are composed primarily of lipids. In order for the cell to be lysed, the lipid walls must be broken down. The manual grinding and detergent solutions accomplish this. Soap molecules mix with fats or lipids, causing structures made of lipids to break apart (see diagram below).

Ethanol is used to precipitate the DNA. In water, DNA is soluble. When it is in ethanol, it uncoils and precipitates

The addition of salt solution provides the DNA with a favorable environment by contributing positively charged atoms that neutralize the normal negative charge of the DNA, allowing the DNA to clump together.

How Detergent Disrupts the Cell Membrane



Courtesy of the North Carolina Biotechnology

Objectives

1. Understand DNA is in the cells of all living organisms.
2. Determine how each of the ingredients in the protocol helps to extract DNA.
3. Extract a visible mass of DNA from strawberry tissue.

Materials

For Each Lab Group

- 10 ml DNA extraction buffer *
- 1 strawberry*
- 1 quart-size zip loc freezer bag *
- test tube *
- test tube rack *
- funnel *
- 10 ml graduated cylinder *
- cheesecloth
- glass rod or plastic loop

Common Materials

- 5 ml 95% ethanol or isopropyl alcohol * *keep on ice*

** Materials provided by instructor*.

Procedure

BREAKING OPEN THE STRAWBERRY CELLS

1. Gather all required lab supplies.

2. Place one strawberry in a self-sealing plastic zip loc bag. Press the air out and seal the bag. Mash the bagged strawberry with your fist for 2 minutes. This will break open the cells.

BREAKING OPEN THE NUCLEI OF THE STRAWBERRY CELLS

3. Add 10mL of DNA extraction buffer (made of shampoo, salt, and water) to the bag. Press the air out carefully and seal the bag. The extraction buffer will dissolve the cell membranes. Mash the bagged strawberry with the DNA extraction buffer for 1 min.

4. Filter the liquid through a cheesecloth-lined funnel and into a test tube. This will separate the organelles, broken cell walls, and membranes from proteins, carbohydrates and DNA.

5. When the test tube is about 1/4 full, take the funnel out of the test tube and discard any extra mashed strawberry pulp, the cheesecloth, and the baggie.

SPOOLING OUT THE DNA

6. Slowly drip about 5 mls of cold ethanol along the side of the test tube. The ethanol should form a layer on top of the filtered extract. This will help to clump the DNA together.

7. Dip a glass rod into the tube, right where the ethanol and extract layers are in contact with each other. Twirl the glass rod into the ethanol layer, the DNA will form fibers, somewhat like cotton candy, that will spool onto the glass rod (the DNA itself looks like mucous). Keep the tube at eye level so you can see what is happening.

7. Wash hands and clean lab station prior to leaving class.

DNA EXTRACTION
Strawberry

Student Activity

Name: _____

Please work in your lab group to answer the following questions. Attach answers on an additional paper.

1. What is the function of DNA?
2. Where is DNA located?
3. What are the three basic steps for DNA extraction?
4. How do these steps differ for extraction of DNA from bacteria?
5. What is the purpose of the salt solution in this experiment?
6. Why was detergent added to the extraction buffer?
7. Why does the DNA rise to the top after addition of the alcohol?

Inquiry Extension (requires two additional laboratory periods)

1. How can you prove that the substance you have extracted is DNA? Brainstorm with your group members and outline an experiment that could be conducted to test your hypothesis. Present your group's idea in a teacher-mediated classroom forum and hold a discussion with classmates about whether or not your chosen method would work.
2. Assuming you have extracted DNA, do you think you have pure DNA? If yes, why? If no, what other materials are present in your test tube and what steps would you take to further purify your sample?
3. With your team members, generate a step-by-step protocol for extracting DNA from another material. Be sure to include all equipment and chemical requirement as well as any safety precautions in your protocol. Have your teacher review the protocol.
4. With your teacher-approved protocol in hand, gather all necessary laboratory materials. If necessary, some items used in the DNA extraction may be obtained from home.
5. Exchange your protocol and materials with another group in the classroom. **You will perform a DNA extraction protocol written by another group.** Upon conclusion of the experiment, meet with the other group and discuss the following:
    a. Were the instructions clear?
    b. Was the DNA extraction successful? If no, why not?
    c. What alterations to the lab protocol should be made?