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Activities Exchange

Introduction to DNA Extractions

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Notes to the teacher:

Through two weeks of numerous trials, I have learned a great deal about DNA extractions. I originally worked with eight different protocols to modify, simplify, and develop good extraction labs. My intent was to produce simple DNA extractions that use various types of cells. The materials used come from the grocery store, health food stores, and butcher shops. Several extractions require a centrifuge. I made it a point to get a centrifuge this year so that I could run the experiments. If you do not have access to a centrifuge, you might run the extractions as written and try to create ways to get around it. The centrifuge step can be skipped in the thymus experiment and good results still obtained. It's amazing what steps can be eliminated or modified.

It is best to begin collecting the materials two weeks in advance. All materials that you extract from must as fresh as possible. The two most difficult items to obtain are non-roasted wheat germ and calf thymus. Health food stores usually carry the non-roasted type of wheat germ, as do some large grocery stores. Thymus (sweetbread) will need to be ordered from a butcher shop. As butcher shops don't always know what will be slaughtered ahead of time, I had several shops trying to get it. Liver is not difficult to get but should be ordered fresh. Once purchased, thymus can be frozen until you need them. Cut them into chunks before freezing so that you can get just what you need each day. The other items can be purchased at most any large grocery store.

I like for my students to do as much of the protocol as they can. With several blenders and a little organization, all the labs can be completed in a 55 minute period or less. Each protocol produces enough lysate for 10-15 spoolings. If you are short on time and equipment, you can demonstrate the first part and then have the students complete the rest of the lab. If you want the individual students to complete all of the protocol, cut the quantities down proportionatey until you get to the spooling step. At this point, use the original protocol. The blender can handle mixing 10-15 ml of solution but you may need to cut the blending time down.

When DNA extractions are performed, you can expect three basic results.

1. No DNA
2. DNA appears fluffy which means it has sheared in the extraction process
3. DNA appears as thin threads.

Although DNA that strands is the most impressive, DNA that has sheared still shows that DNA is present.

All the experiments will yield DNA but some more than others. The lima bean bacteria, and yeast give the poorest results. I'm sure that with some more experimentation they could be improved greatly. The most impressive is the calf thymus, so I have students do it last. The long threads of DNA are easily spooled and the quantity is immense compared to the other extractions.

If you have students do many DNA extractions, you will find that their lab skills will improve. However, a problem that constantly persists occurs when they add the alcohol– the students usually pour it too fast. Rather than forming two distinct layers, they mix the two. Once that happens, there's not much that can be done.

The experiments work well as written. However the following substitutions can be used:

1. a reusable coffee filter or cheese cloth can be used to strain the materials
2. 91-99% isopropyl (rubbing alcohol) can be substituted for ethanol, although I prefer ethanol
3. fresh papaya or pineapple juice can be substituted for the meat tenderizer solution (use the same amount of ml as the meat tenderizer solution)
4. 10% SDS (sodium dodecyl sulfate) can be used in place of all the detergent solutions. It comes as a 10% solution already mixed or you can buy the powder and mix a 10% solution (5g SDS and 50ml distilled water).

A good science project for students is to run through several of the protocols. Then have them design modifications to test. They can use the substitutions, use other things to extract from, or switch solutions/protocols. You can have the students pipet the alcohol/DNA layer off and place it in a clean test tube to view later. At the end, they can compare the DNA from all the extractions or even create other labs in which to use the DNA. An excellent experiment is to have students run all the DNA extractions except yeast. Have them analyze the other extractions using the summary chart and research the characteristics of yeast. Based on their findings, have them design a protocol for yeast DNA extraction, run the experiment, and justify their results.

There are two to three basic steps in DNA extraction. The cell must be lysed (broken open) to release the nucleus. The nucleus (if present) must also be opened to release the DNA. At this point the DNA must be protected from enzymes that will degrade it, causing shearing. Once the DNA is released, it must then be precipitated in alcohol.

In order for the cell to be lysed, the lipid walls must be broken down. The detergent and salt solutions accomplish this. Cell walls, cell membranes, and nuclear membranes are also broken down by the action of the blender. In all but one protocol I eliminated the use of heat. Some references state that a temperature of 60oC is necessary to denature the DNAase enzymes that cause shearing in DNA while DNA is denatured about 80oC. Other references state that DNA can denature at 60oC. From all the experiments I ran (except for the wheat germ protocol), I had sheared DNA when I used heat. Heat may destroy the enzymes as well as the DNA. However keeping the solutions cool seems to slow the enzyme action. The prep solution uses epsom salts and buffered aspirin to further deactivate the enzymes that degrade DNA when released and stabilize the DNA (acid vs. base). Sodium bicarbonate (baking soda) also is used to buffer the solution. The meat tenderizer has papain, an enzyme that helps clean the protein from the DNA that can contaminate it. Papaya juice and pineapple juice also contains this enzyme. Finally, the ethanol is used to precipitate the DNA. In water, DNA is soluble. When it is in ethanol, it uncoils and precipitates leaving behind the other cell components that are not soluble in ethanol.

All in all, the DNA extraction labs are very workable. Try some and then decide if you would like to modify any to fit your needs better. Good luck!!

Onion DNA Extraction

Materials

- fresh onions
- graduated cylinders (10ml and 100ml)
- knife
- 15 ml test tube
- blender
- test tube rack or 250 ml beaker
- strainer
- glass stirring rod

- coffee filters
- non-iodized salt
- Adolph's natural meat tenderizer
- Palmolive detergent
- beaker
- distilled water
- ice cold 95% ethanol

Solutions

Detergent/salt solution:

- 20 ml detergent
- 20 g non-iodized salt
- 180 ml distilled water

5% meat tenderizer solution:

- 5 g meat tenderizer
- 95 ml distilled water

Protocol

1. Cut an inch square out of the center of 3 medium onions. Chop and place in a blender.
2. Add 100 ml of detergent/salt solution.
3. Blend on high 30 sec-1 minute.
4. Strain the mixture into a beaker using a strainer with a coffee filter.
5. Add 20-30 ml meat tenderizer and stir to mix.
6. Place 6 ml filtrate in a test tube.
7. Pour 6 ml ice cold ethanol carefully down the side of the tube to form a layer.
8. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
9. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.

Modified from: "Isolation of DNA from Onion" Ellen Averill

Wheat Germ DNA Extraction

Materials

- 250 ml beaker
- baking soda
- hot plate
- Adolph's natural meat tenderizer

- non-roasted wheat germ
- ice cold 95% ethanol
- thermometer
- 15 ml test tube
- pH meter
- glass stirring rod
- Palmolive
- detergent
- distilled water
- test tube rack or 250 ml beaker
- graduated cylinders (10ml and 100ml)

Solutions

Baking soda solution:

- Add baking soda to distilled water until a pH of approximately 8.0 is reached.

Protocol

1. Add 100 ml distilled water to a beaker and heat to 50-60oC.
2. Add 1.5 g wheat germ and mix until dissolved.
3. Add 5 ml detergent. Maintain 50-60oC temperature and stir for 5 minutes.
4. Add 3 g meat tenderizer.
5. Add baking soda solution to bring the pH to approximately 8.0.
6. Maintain the 50-60oC temperature and stir for 10 minutes.
7. Remove from heat.
8. Add 6 ml of the solution to a test tube and cool to room temperature.
9. Pour 6 ml ice cold ethanol carefully down the side of the tube to form a layer.
10. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
11. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.

Modified from: "Wheat Germ DNA Extraction" Judy Brown

Lima Bean Bacteria DNA Extraction

Materials

- dry lima beans
- Palmolive detergent
- centrifuge
- distilled water
- centrifuge tube
- fresh papaya juice

- graduated cylinder (10ml)
- non-iodized salt
- granulated sugar
- pipet
- epsom salts
- 15 ml test tube
- bufferin (325mg)
- test tube rack or 250 ml beaker
- ice cold 95% ethanol
- glass stirring rod

Solutions

Lima Bean Bacteria Suspension: Place 1-2 handfuls of dry lima beans in a large jar and fill halfway to the top with distilled water. Cover and sit in a warm room for 2-3 days. Culturing longer than three days often results in more DNA but it usually shears. Pour through a strainer and keep the liquid for the extractions.

Prep buffer solution:

- 57 g granulated sugar
- 3 g epsom salts
- 1 buffered aspirin
- add distilled water for a total volume of 500 ml

50% detergent solution:

- 20 ml detergent
- 20 ml distilled water

Salt solution:

- 29.2 g non-iodized salt
- add distilled water for a total volume of 250 ml

Protocol

1. Add 14 ml of the bacterial suspension to a centrifuge tube and spin in a balanced centrifuge for 5 minutes.
2. Pour off the liquid (supernatant) and discard. You want to keep the pellet as this has your cells.
3. Add 5 ml of prep buffer and resuspend your cells with a pipet.
4. Add 1 ml 50% detergent solution.
5. Add 1 ml papaya juice.
6. Add 2 ml salt solution and shake for 2 minutes.
7. Place the tube in the centrifuge and spin for 5 minutes. Make sure the centrifuge is balanced.
8. Draw off 7 ml of the supernatant (liquid) as this has the DNA and place it in a

clean test tube.

9. Pour 7 ml of ice cold ethanol carefully down the side of the tube.
10. Let the mixture sit undisturbed 2-3 minutes until the bubbling stops.
11. The DNA will float in the alcohol. Swirl a glass rod at the interface of the two layers. You may see some tiny threads of DNA but are more likely to see fluffy, white sheared DNA.

Modified from: "Generic, All Purpose DNA Extraction from Meat Protocol" Judy Brown

"Mammalian DNA Extraction" Theresa Knapp

Yeast DNA Extraction

Materials

- dry yeast
- Adolph's natural meat tenderizer
- beaker
- distilled water
- non-iodized salt
- glass stirring rod
- Palmolive detergent
- graduated cylinders (10ml and 100ml)
- blender
- 15 ml test tube
- ice cold 95% ethanol
- test tube rack or 250 ml beaker

Solutions

detergent/salt solution:

- 20 ml detergent
- 20 g non-iodized salt
- 180 ml distilled water

5% meat tenderizer solution:

- 5 g tenderizer
- 95 ml distilled water

Protocol

1. Mix 1 package of dry yeast with 40 ml of 50oC hot tap water to dissolve the yeast in a beaker. Keep mixture covered and warm for about 20 minutes.

2. Add 40 ml detergent/salt solution.
3. Place mixture in a blender and blend 30 sec-1 minute on high.
4. Pour mixture back into the beaker, add 15 ml of meat tenderizer solution, and stir to mix.
5. Place 6 ml of mixture into a test tube.
6. Pour 6 ml of ice cold ethanol carefully down the side of the tube to form a layer.
7. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.
8. You will see a precipitate in the alcohol. Swirl a glass stirring rod at the interface of the two layers. The precipitate is DNA.

Modified from: "Isolation of DNA from Onion" Ellen Averill

Thymus DNA Extractions

Materials

- fresh thymus
- blender
- beaker
- sugar
- pipet
- centrifuge tube with cap
- bufferin (325mg)
- knife
- graduated cylinders (10ml,100ml)
- epsom salts
- distilled water
- centrifuge
- 95% ice cold ethanol
- 15 ml test tube test tube rack or beaker
- Palmolive detergent
- non-iodized salt

Solutions

prep buffer solution:

- 57 g granulated sugar
- 1 buffered aspirin
- 3 g epsom salts
- add distilled water for a total of 500 ml

10% detergent solution:

- 90 ml distilled water

- 10 ml Palmolive detergent

salt solution:

- 29.2 g non-iodized salt
- add distilled water for a total volume of 250 ml

Protocol

1. Cut out a chunk of liver or thymus 1 inch square and place in the blender.
2. Add 100 -150 ml prep buffer and 10 ml detergent solution to the blender.
3. Blend for 1 minute or until the mixture is smooth.
4. Pour the mixture into a beaker.
5. Transfer 1 ml of the mixture to a centrifuge tube.
6. Add 2 ml of salt solution, cap, and shake for 2 minutes.
7. Centrifuge for 7 minutes in a balanced centrifuge.
8. Carefully remove the tube from the centrifuge and note the two layers:
    - lower layer - pellet
    - *upper layer - liquid (supernatant) and what has the DNA in it.
9. Pipet or carefully pour the liquid into a clean test tube.
10. Pour 5 ml ice cold ethanol carefully down the side of the tube to form a layer.
11. Let the mixture sit undisturbed for a minute or two.
12. The DNA will float in the alcohol. The DNA of the thymus will be long threads that easily spool.

Modified from: "Generic, All Purpose DNA Extraction from Meat Protocol" Judy Brown

"Mammalian DNA Extraction" Theresa Knapp

DNA Extraction Summary Chart

QUESTIONS	ONION	WHEAT GERM	BACTERIA	YEAST	THYMUS
What are the cell characteristics?					
What lyses the cell and nucleus?					
What protects the DNA?					
What precipitates the DNA?					
Amount of DNA					
Description of DNA					

Changes in protocol					

Brief procedure
Experimental Design: Yeast Extraction

Task:

You are to design an experiment to extract DNA from yeast, run the experiment, and then do a lab write-up with your results.

Procedure:

1. Investigate what type of cells yeast are as well as their cell characteristics.
2. Study the protocols for onion, wheat germ, bacteria, and thymus.
3. Based on what you have learned about yeast and its characteristics, design an experiment to extract DNA from your yeast.
4. Run the experiment.
5. Complete a lab write-up that includes:

   A. Protocol
   B. Justification of protocol used
   C. Results and conclusions

Credits and References

- "Isolation of DNA from Onion"
  Ellen Averill
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  6015 Georgetown Drive
  Columbus, GA 31907
  *Published in Woodrow Wilson's Biology Module 1993 *A Further Look at Biotechnology*
- "Wheat Germ DNA Extraction"
  "Onion DNA Extraction"
  "Generic All Purpose DNA Extraction from Plants Protocol"
  "Generic All Purpose DNA Extraction from Animal Protocol"
  "DNA Yeast Extraction"
  Judy Brown
  Edison Career Center
  Montgomery County, Maryland
- "Mammalian DNA Extraction"
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  Stevenson High School

Lincolnshire, IL 60069

- "E. coli DNA Extraction"
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  Lana Hays
  Simon Kenton High School
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  and
  Anthony Bertino
  Canandaigua Academy
  Canandaigua, NY 14424
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  A Further Look at Biotechnology

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DNA Extraction

Purpose:

The purpose of this lab is to extract DNA from a variety of cells and see DNA molecules (this procedure actually took scientists many years to discover). This will show that, contrary to popular opinion, DNA is not just found in blood cells, but in a variety of tissues (try at least one plant tissue and one animal tissue). Prior knowledge should include the fact that cell membranes are layers of lipids, or fat molecules, that DNA is found in the nucleus of a cell, and that enzymes speed up chemical reactions. The plant and animal cells will be chemically treated to break open the cell and nuclear membranes. The part of the cell mixture containing

DNA will be separated from the cell membranes and associated proteins (gloppy portion), and the solution containing the dissolved DNA will be altered so that the DNA can no longer remain dissolved. It will be "precipitated," and observable with the naked eye.

Supplies:

plant or animal tissues--liver and onions, for example
blender
salt
clear liquid dish soap
warm water
clear glass
strainer
toothpicks
rubbing alcohol

Cut up a small amount of the cell source.

Add it into a blender and fill it with enough warm salty water to cover it (use several pinches of salt--you may experiment with what works best).

Blend for 5-10 seconds, but don't totally liquefy. Pour through the strainer into a clear glass, filling about half full.

Gently stir in about 2-3 teaspoons of the soap (again, you may want to experiment with the amount to see what works best). Stir very gently, trying not to make bubbles.

(Be careful when pouring and make sure not to use too much soap...)

Slowly pour the alcohol into the glass, pouring it down the side of the glass so that it forms a separate layer on top of the soapy cell mixture. Fill nearly to the top. Let it sit for 3 - 5 minutes, observing what happens.

The DNA will slowly rise from the watery lower layer up into the alcohol layer above it. The DNA will look stringy and have small bubbles attached to it. It will be a clear, "snotty" substance, and may be hard to see. Slowly twist substance onto a toothpick. (Do not scoop up cell scum from the lower layer.) Congratulations--you have extracted DNA!

Mmmmmm! Tasty! (Do not actually drink it.)

You can try this experiment with a variety of materials.

(When working with liver, make sure it is actually dead.)

Make sure to chop up liver before blending it.

Again, the DNA will float to the top.

Bon Appetite! (Do not actually drink it.)

Results, Questions:

1. What does the salt do? *(Salt provides the DNA with a favorable environment; it contributes positively charged atoms that neutralize the normal negative charge of DNA.)*

2. What does the blender do? *(help break down the cell walls)*

3. When you mix the blended cell source with the soap, what is happening? *(In the experiment, the enzymes in the soap are breaking down the lipid molecules of the cell and nuclear membranes, releasing the contents of the cell, including the DNA. These enzymes in the soap are what break down grease while washing dishes.)*

4. What does the alcohol do? Why does the DNA rise to the top after adding alcohol? *(DNA will not dissolve in this alcohol, so the DNA comes out of the solution, or precipitates. It is less dense than water or cell scum--which is what settles to the bottom of the glass--so it*

floats up into the alcohol layer, where you see it as a snotty, string-like substance, with small bubbles formed on it.)

5. If you try a seed food such as peas, there will be more protein residue in the liquid. Why? *(Because protein is stored in them for the nutrition of the new plant.)*

6. Why can't you see the double helix? *(It is too small to be seen with the naked eye. What you extracted is millions of strands of DNA.)*

7. What part of the cell did the DNA come from? *(99% is from the nucleus.)*

Applications:

1. If you did the experiment with both plant and animal cells, how do their DNA compare?