**A. Ancheta**

**Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_**

**DNA extraction lab**

**Prelab Questions:**

1. Draw the basic cell structures of a plant, an animal, and a bacterium. Label its parts.

1. How are the plant, animal, and bacterial cells alike or different? Complete the table below.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Plant | Animal | Bacteria |
| Cell wall | Present | Absent | Present |
| Cell membrane |  |  |  |
| Nucleus |  |  |  |
| Genomic DNA(Chromosomal) |  |  |  |
| Plasmid DNA |  |  |  |
| mitochondria |  |  |  |
|  |  |  |  |
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1. What are the four basic steps in DNA extraction? (refer to the background information).

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1. Does a liver cell contain the same chromosomes as a cheek cell? Why or why not?

**Purpose**:

* + To compare and contrast the DNA extraction methods.
* To be able to extract DNA from strawberry, human cheek cell, and *E. coli* and to compare their DNA.

**Safety:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Procedure:**

Procedure Flow Chart

Method #1: The DNA o f Bacteria (*E. coli*)

Pre-Procedure:

* Prepare sterile LB agar plates and incubate overnight.
* Prepare sterile LB broth and store in refrigerator.
* Prepare 50% dishwashing detergent solution.
* Learn plate streaking method (text – DNA science)

Procedure:

1. Culture (streak) E. coli on a Luria agar plate to obtain individual colonies. Incubate overnight at 37 °C. Note: Other species of bacteria can be used. Use sterile technique to suspend one *E. coli* colony from the plate in a 15 mL culture tube containing 5 mL sterile Luria broth. Let incubate overnight at 37 °C (incubator or shaking water bath) or 3 days at room temperature.
2. Make a 50% dishwashing detergent solution by adding 1.5mL of distilled water to 1.5mL of liquid dishwashing detergent. Add 3 mL of the 50% liquid dishwashing detergent solution to *E. coli* suspension. Shake the suspension to rupture (lyse) the cells and release DNA.
3. Place the tube of lysed *E. coli* suspension in 65-75°C water bath for 15 minutes.
4. Carefully layer about 5mL of ice cold 95% ethanol onto the lysed suspension. Let sit 10-15 minutes before spooling.

**Staining and observing under the microscope (optional)**

1. On a clean microscope slide, place the extracted DNA and apply two or three drops of HCl.
2. Holding the slide with a clothespin, pass it through the flame of a Bunsen Burner for five seconds. Note: *do not hold the slide over the flame*.
3. Without harming the DNA, blot the specimen with a paper towel to remove the excess HCl.

Note: you may wish to touch a corner of the paper towel to the drop on the slide.

1. Add a few drops of 0.5% aqueous toluidine blue stain, covering the DNA.
2. Pass the slide through the flame of a Bunsen burner for one to two minutes. Le the slide stand for one minute. Without disturbing the specimen, use a paper towel to remove the excess stain.
3. Cover with cover slip. View under 400x and draw your observation.

Method #2: The DNA o f Plant (Strawberry or banana)

Procedure:

1. Cut strawberry into chunks. Place fruit into mortar and smash well with pestle, then pour into 100 mL beaker.
2. Pour soap/salt solution into beaker just enough to cover fruit. Stir gently to mix.
3. Place beaker with fruit into 50-60 C hot water bath for 10minutes. Be sure the water temp stays within range of 50-60 C. You can prepare the hot water in 1000 mL beaker using the microwave oven. Mash fruit against the side of beaker every few minutes while in hot water bath.
4. While waiting, use graduated cylinder to measure and mark 5 & 10 mL on test tube.
5. Transfer the beaker from the hot water bath to ice water bath, and leave in ice water bath for 3 minutes. Continue to mash fruit every few minutes.
6. While beaker is in ice water bath, set up filtering apparatus.
7. After 5 minutes in the ice bath, pour the cold mixture into the funnel and let the liquid filter for 5 minutes or longer, until 5mL liquid have filtered through into test tube (from step 4). Gently swirl the filtrate in the test tube (DNA may be on bottom).
8. Add 5 mL of alcohol to the same test tube by trickling it down the side of the tube at a 45° angle.
9. Observe the test tube for a few minutes until DNA becomes visible between the two layers. DNA looks like white mucus. This “white mucus” consists of millions of DNA strands clumped together.
10. Place the test tube with collected DNA in the refrigerator.

Method #3: The DNA of Animal (human cheek cell)

Procedure:

1. Obtain 15 ml tube containing 3 ml water from your instructor. Label the tube with your initials. Gently chew the insides of your cheeks for 30 seconds. It is NOT helpful to draw blood! Take the water from the 15 ml tube into your mouth, and swish the water around vigorously for 30 seconds. Carefully expel the liquid back into the 15 ml tube.
2. Obtain the tube of lysis buffer and add 2 ml of lysis buffer to your tube. Place the cap on the tube, and gently invert the tube 5 times (don’t shake your tube!). Observe your tube — do you notice any changes? If you do, write them down.
3. Obtain the tube of protease (**prot**) at your workstation. Add 5 drops of protease to your tube. Place the cap on your tube, and gently invert it a few times.
4. Place your tube in a test tube rack or beaker in the water bath and incubate at 50°C for 10 minutes. Remove your tubes from the water bath.
5. Obtain the tube of cold alcohol from the refrigerator. Holding your tube at a 45° angle, fill your tube with cold alcohol, by adding approximately 3 mls to your tube. It will take repeated additions to add 3ml of the cold alcohol using the disposable plastic transfer pipet.
6. Place your cap on your tube, and let it sit undisturbed for 5 minutes. Write down anything you observe happening in the tube.
7. With a disposable plastic transfer pipet, carefully transfer the precipitated DNA along with approximately 750 μl of the alcohol solution into a small glass vial provided.

**Instructions for DNA necklace (human cheek cell only)**

1. Using a disposable plastic transfer pipet, carefully transfer your DNA in alcohol into the glass vial, leaving enough space for the plastic stopper cap. The glass vial should be filled with alcohol no higher than ½ cm from the top of the neck of the vial. You can share transfer pipets.

2. Firmly push the plastic stopper cap into the neck of the vial to seal the glass vial. Note: cut the sides of stoppers for better cap fit.

3. Apply a small drop of glue into the inside of the silver cap. Apply a small amount of glue around the rim of the glass vial/plastic stopper cap. Do not apply too much glue as it may interfere with the drying process.

4. Place the silver cap onto the top of the glass vial and press down firmly for 30 seconds. Allow the glue to dry for 10–15 min and then check for complete seal. Make sure your heart lines up with holes so it will lie flat on the neck.

5. After the glue has dried, slip the waxed cord through the silver cap and tie the cord.

**Data**: qualitative data - Drawing and description of your DNA from three organisms (in the vial with alcohol & under the microscope). Include other observations during the experiment (i.e. Method 3 – step 2).

**Conclusion:** Start by restating the purpose, “The purpose of this lab was to \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_” then write briefly compare and contrast the three methods. Finally write your observation of DNA (compare DNA from three different organisms).

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**Questions:**

1. What does each letter in DNA stand for?

2. Draw the chemical structure of two DNA molecules attached in 5’🡪 3’ orientation.

3. Human Cheek Cell DNA: Match the outcomes on the left with the laboratory steps on the right.

|  |  |
| --- | --- |
| \_\_\_\_Harvest the cells | A. Scrape a brush against the inside of your cheek |
| \_\_\_\_Dissolve cell membrane | B. Add protease, incubate at 50C |
| \_\_\_\_Precipitate the DNA | C. Mix in a detergent solution |
| \_\_\_\_Break down proteins | D. Layer cold alcohol over cell extract |
| \_\_\_\_Make DNA less soluble in water | E. Add salt |

**DNA Extraction Lab Background Information**

 DNA (deoxyribonucleic acid) is an immensely long molecule. It is found in every cell with a nucleus, whether it is a plant, fungal, protist, or animal cell. DNA is also found in all bacterial cells and some viruses. In humans, a cell that may be only about one millimeter long will contain about 2 meters of DNA divided among the 46 chromosomes. In order to pack all this DNA into a cell, it is tightly wound around tiny proteins called histones. The 2 meters of DNA contain about 6 billion base pairs. In the cell, DNA appears like a tangled mass of thread. There are techniques that allow you to extract the DNA from cells so that you can see it and work with it. It is a brittle molecule; it can bend just so much before it breaks. This is why it must be handled gently. Even though DNA appears like an unpromising whitish goop, it is the molecule of life; containing the genetic code that directs all the functions of the cell.

To extract the DNA from cells, these are the basic steps:

A. Obtain a large number of nucleated cells, the more cells, the more DNA.

B. Lyse (break open) the cell membranes. This is done using some kind of surfactant or detergent. This works because the cell membrane is made up of lipids (fats) and detergents will emulsify or break apart fats. Often shaking is advised to increase the rate of cell lysing. An odd ball, Halobacterium normally lives in a high-salt environment, so adding distilled water causes the cells to rupture due to osmosis.

C. Destroy the proteins that are present in the cells and bind to the DNA. This is done using some kind of proteolytic (protein-digesting) enzyme and/or heating the solution about 60°C is needed to destroy the proteins, but temperatures of over 80°C will denature the DNA.

D. Precipitate the DNA. Up to this point the DNA is dissolved in an aqueous solution; DNA is soluble in water but is insoluble in alcohol. This is done by slowly, carefully layering ethanol (ethyl alcohol) on top of the DNA-containing aqueous solution. The DNA appears as a whitish mass floating at the interface of the alcohol and aqueous solutions.

E. Work with the DNA. At this point you can do several things

1. Spool the DNA onto a glass stirring rod. Place an ethanol cleaned stirring rod into the solution and twirl the rod to spool the DNA. DON’T mix the two layers.
2. Extract the DNA from the interface using a pipet.
3. Dry the DNA on the filter paper which later can then be dissolved in distilled water with a small amount of sodium chloride (4%) or in TE buffer. This will yield slightly acidic solution that can be verified using pH paper or a pH meter. Test the DNA with a DNA in a tube containing 3mL of 4% NaCI solution or TE buffer. Add 3 mL of diphenylamine solution to this tube and mix. Place the tube in a boiling water bath for 10 minutes. Diphenylamine reacts with the deoxyribose sugar of DNA to produce a blue color. A DNA positive control and distilled water negative control should be tested along with your DNA sample.

**Cheek Cell DNA Extraction**

Additional Information

1. **Cell Collection**
Gently chewing the inside of the mouth combined with a water mouth wash is used to dislodge epithelial cells lining the mouth.
2. **Lysis Buffer**
What is Lysis Buffer?• 50 mM Tris-HCI, pH 8.0
• 1% SDS
Tris buffer to maintain the pH of the solution at a level where DNA is stable.

1% SDS to break open the cell and nuclear membranes, allowing the DNA to be released into the solution (SDS also denatures and unfolds proteins, making them more susceptible to protease cleavage).

1. **Why Add Protease?**

Protease is added to destroy nuclear proteins that bind DNA and cytoplasmic enzymes that breakdown and destroy DNA.

• Protease treatment increases the amount of intact DNA that is extracted.

1. **Adding Salt**

• The protease solution already contains salt.

• Na+ ions of NaCl bind to the phosphate groups of DNA molecules, neutralizing the electric charge of the DNA molecules.

• The addition of NaCl allows the DNA molecules to come together instead of repelling each other, thus making it easier for DNA to precipitate out of solution when alcohol is added.

**5. Adding Ice Cold Alcohol?**

• DNA does not dissolve in alcohol.

• The addition of cold alcohol makes the DNA clump together and precipitate out of solution.

* Precipitated DNA molecules appear as long pieces of fluffy, stringy, web-like strands.
* Microscopic oxygen bubbles “aggregate,” or “fuse” together, as the DNA precipitates.
* The larger, visible air bubbles “lift” the DNA out of solution, from the aqueous into the organic phase.