Lab III-5

Extracting, Isolating, and Visualizing DNA

Equipment and Materials

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

Materials from Kit

- □ Goggles
- □ Centrifuge tubes, 50 mL
- □ Coverslips
- □ Eosin Y stain
- □ Funnel
- □ Graduated cylinder, 10 mL
- □ Methylene blue stain
- □ Microscope slides (flat)
- □ Pipettes
- □ Reaction plate, 96-well
- □ Scalpel
- □ Sodium dodecyl sulfate, 10%
- □ Spatula
- □ Stirring rod
- □ Test tubes
- □ Test tube rack
- □ Yeast (optional)

Materials You Provide

- □ Gloves
- □ Balance (optional)
- □ Beef or pork liver, raw (or yeast)
- □ Cheesecloth (or muslin, etc.)
- □ Isopropanol (see text)
- □ Freezer
- □ Microscope
- □ Paper towels
- □ Saucer
- □ Table salt
- □ Teaspoon
- □ Toothpick
- □ Water, distilled

Background

All organisms are made up of cells, from the tiniest species such as single-cell bacteria and protozoa to the largest animals and plants, which are made up of trillions of individual cells. Every cell contains DNA, which is the hereditary genetic material that allows cells and organisms to function and to reproduce themselves.

With very few exceptions (research *chimera* on the Internet) DNA taken from any cell of a

particular individual of any species is identical to that taken from any other cell from that individual, and is unique to that individual. DNA found in unrelated members of the same species—for example, you and your best friend (assuming your best friend isn't a dog or a diamond...) or two rosebushes—is nearly (but not quite) identical. DNA found in different but closely related species—for example, lions and tigers or wolves and coyotes—has greater differences, but is still extremely similar. DNA found in different, unrelated species—for example, a human and a cucumber—has still greater differences, but remains closely similar.

But what does DNA actually look like? Let's find out. In this lab session, we'll extract liver-cell DNA into solution, isolate that DNA, and stain it to visualize it.

Procedure III-5-1: Extracting and Visualizing DNA

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Transfer 5 drops of methylene blue stain and 5 drops of eosin Y stain to one well of the reaction plate. Use a toothpick to mix the solutions thoroughly, and place the reaction plate aside for later use.
3. Make up 50 mL of *normal saline* by dissolving 0.45 g (one slightly rounded spatula spoon) of table salt in 50 mL of distilled water in a 50 mL centrifuge tube, and place the tube aside for later use.
4. Transfer about 40 mL of isopropanol to a second 50 mL centrifuge tube. Cap the tube and place it in the freezer to cool.

   99% isopropanol yields the best results, but 91% or even 70% isopropanol is usable. You can also substitute ethanol for isopropanol.

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5. Obtain a teaspoon-size specimen of fresh beef or pork liver.

   It's best to use fresh liver from a butcher or fresh market. Supermarket liver can be used but may yield inferior results, depending on its age. Also, you may substitute dried baker's or brewer's yeast for the liver, although *lysis* is more difficult to observe with yeast.

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6. Use the scalpel to cut the liver into very small pieces, and transfer the pieces to the saucer.
7. Add about 10 mL of normal saline solution to the saucer, and use the teaspoon to grind and mash the bits of liver to produce a suspension of liver cells in the saline solution.
8. Place a clean test tube in the rack, with the funnel atop it. Fold and refold a small piece of cheesecloth to provide four layers. Place the cheesecloth in the funnel and pour the solution from the saucer through the cheesecloth into the tube to filter out most of the solid material.

   If you don't have cheesecloth, you can substitute muslin or a similar cotton fabric; we used old, well-washed cloth from cotton underwear. Do not substitute a paper towel, which will filter out many of the liver cells in addition to the larger solids.

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9. Use the stirring rod to transfer one drop of the liver cell suspension to a microscope slide and position a coverslip over the specimen. Observe the specimen at low, medium, and high-dry magnifications. Record your observations in your lab notebook. Include a sketch of a representative liver cell and its nucleus.
10. Add one drop of the mixed stains at the edge of the coverslip, and use the corner of a paper towel to draw the stain under the coverslip. Observe the specimen at low, medium, and high-

dry magnification. Record your observations in your lab notebook. Include a sketch of a representative liver cell and its nucleus.

11. Use a pipette to transfer 0.5 mL of 10% sodium dodecyl sulfate (SDS) solution to the liver-cell suspension tube, and swirl gently to to mix the solutions.

    Sodium dodecyl sulfate (SDS) is also known as sodium lauryl sulfate (SLS). If you don't have SDS or SLS, you can substitute a concentrated detergent solution, such as dish-washing liquid.

12. Repeat steps 9 and 10 to stain and view the liver cells in the presence of the SDS solution.
13. Transfer another 0.5 mL of the 10% SDS solution to the liver-cell suspension and swirl gently to mix the solutions.
14. Repeat steps 9 and 10 to stain and view the liver cells in the presence of the SDS solution.
15. Repeat steps 13 and 14 several times (about 5 repetitions should do it) and observe the liver cells in the presence of increasing concentrations of SDS. As the cell membranes break down (are *lysed*) and no longer surround the nucleus, the integrity of the cells is destroyed and the nucleus and other cell contents are exposed.
16. Using a pipette, transfer about 4 mL of the liver-cell suspension to a second test tube. Retain the excess liver-cell suspension in the first test tube until you complete the next step successfully.
17. Holding the second tube at a slight angle, very slowly and carefully trickle cold isopropanol into the tube until the liquid level reaches 2 to 3 cm from the top of the tube. The goal is to cause the isopropanol to form a separate layer on top of the liver-cell suspension. If you pour too fast, the layers will mix and you'll have to start over.
18. Place the tube in the rack and observe the interface between the liver-cell suspension layer and the isopropanol layer. You'll see a cloudy whiteness begin to form at the interface. That whiteness is DNA precipitating out of solution.
19. Carefully and gently dip the stirring rod into the test tube until it penetrates both layers. Slowly spin the stirring rod between your thumb and finger to spool the DNA precipitate onto the stirring rod. Continue until no more DNA is deposited on the stirring rod. Withdraw the rod from the test tube.
20. Place one drop of distilled water on a microscope slide. Use a toothpick to remove a tiny amount of the DNA from the stirring rod. Transfer the DNA to the water drop and position a coverslip over the specimen. Add one drop of methylene blue at the edge of the coverslip, and use the corner of a paper towel to draw the stain under the coverslip. Observe the DNA at low, medium, and high-dry magnification. Record your observations in your lab notebook, including a sketch of the stained DNA.

If you want to retain the DNA you just isolated for future use, allow it to dry and then store it in a paper back or similar porous container.

Review Questions

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1. What was the result of adding the mixed stains to the liver-cell suspension?

   Before adding the mixed stains, the liver cells were difficult to observe because there was little contrast between them and the surrounding liquid. After adding the mixed stains, the

liver cells were much more visible, with the nuclei stained blue and the cell membranes and other structures pink.

2. Methylene blue stains acidic cell structures but leaves basic cell structures unstained. Based on that information, what do you conclude about cell nuclei?

   The cell nuclei are stained by methylene blue, so they must be acidic.

3. What did you observe as you increased the amount of SDS solution you added to the liver cell suspension?

   Before adding SDS, the liver cells were mostly undamaged, each contained within its own cell membrane. As the concentration of SDS increased, more and more liver cells were lysed (broken apart), with the cell membrane being destroyed, emptying the cell contents into the surrounding fluid.

4. How did the appearance of the DNA differ before and after staining with methylene blue? What do you conclude about the acidity or basicity of DNA?

   The spooled DNA appeared to the eye as a whitish, gelatinous material, visible under magnification as whitish clumps or strings against a white background. After staining, the DNA appeared blue. Methylene blue stains only acidic materials, so DNA must be acidic.

5. What do you conclude about the relative solubilities of DNA in water and isopropanol? Propose a reason why we chilled the isopropanol.

   DNA is relatively soluble in water, as evidenced by the clear solution of DNA we produced by lysing the liver cells. DNA is nearly insoluble in isopropanol, as evidenced by its precipitating at the water/isopropanol boundary layer. We chilled the isopropanol because DNA is even less soluble in cold isopropanol than in warm or room-temperature isopropanol.