LABORATORY 1

DNA Isolation

OBJECTIVES

- To understand the process of tissue homogenization and fractionation.
- To isolate a DNA fraction from plant or animal tissue.

BACKGROUND

Biologists study living processes at different levels. Even cell biologists may study at the level of the tissue, cell, organelle, protein complex, or molecule. Frequently, a simpler level allows for more in-depth understanding of a process, and knowledge gained can then be applied to the cell or system. Tissues can be removed from an organism, and cells can be isolated for study. Separating subcellular components for study requires a specific set of techniques to maintain the integrity of the desired component. This lab will focus on purification and quantification of the macromolecule DNA from plant and animal tissue. Similar methods have been devised for preparation of protein, lipid, and RNA macromolecules.

During the isolation of subcellular components, several factors must be kept in mind. The cell must be disrupted to free the contents from within the cell, without damaging the component(s) of interest. The optimal technique for cell disruption may vary, depending on the properties of the specific tissue or cell being used and the component being isolated. This lab involves isolation of DNA from two very different tissue types. Degradation of the structures or molecules of interest must be kept to a minimum during the isolation process, to assure they remain in a form similar to that found in the cell. Degradation may be the result of oxidation, changes in pH, released internal enzymes (proteases, etc.), or bacterial contamination. Protease inhibitors may reduce enzyme activity, but are often toxic compounds themselves that affect biological processes, and, therefore, are not always desirable. Cooling will reduce enzyme activity, as well as bacterial growth. Cells are therefore routinely lysed or homogenized in cooled conditions and kept on ice for subsequent manipulations.

A cell lysate or homogenate contains all the components of lysed cells in a suspension buffer. The desired components must then be separated from the lysate. The method used depends on what is to be isolated. Filtration or centrifugation is frequently used for components of differing sizes or densities. When separating macromolecules, chemical and physical properties of the molecules and potential contaminants are considered in designing separation protocols. The chemical structure of DNA is fairly specific (only RNA is fairly similar) and different from the proteins and lipids. For the DNA isolation method in this exercise, solubility properties and enzymatic susceptibility of DNA compared to other macromolecules are important. Other methods may rely

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on other properties of DNA. Kits are commercially available to specifically isolate DNA or RNA from different cell types in a quick and reproducible manner.

Once an isolated fraction from a lysate has been obtained, its quality must be assessed. The yield and purity of the desired component, as well as possible contaminants, may be assessed. The contaminants may vary depending on the tissue used and the method. Assessing the presence or absence, as well as the amount of the desired component, is especially important when devising a new isolation protocol. The desired component must be identified at each step of separation. As with any technique, the limitations of the method must be understood.

MATERIALS

- Bovine or Chicken liver
- Spinach leaves
- Saline Citrate Buffer (SSC) (15 mM Na citrate, pH 7.0, 0.15 M NaCl) with 1 mg/ml proteinase K
- Microfuge tubes and micropestle
- 1 M NaCl
- 95% (v/v) ethanol
- 70% (v/v) ethanol
- TE RNase (10 mM Tris pH 8.0, 1 mM EDTA, 10 ug/ml RNase)

PROCEDURE

1. Weigh out approximately 200 mg of liver or de-veined spinach leaves. Record the exact weight for later calculations. Chop tissue finely with a razor blade. Half of students use spinach; half use liver. *Why remove veins from the spinach?*

2. Drop the pieces of liver or spinach into a precooled 1.5 ml microcentrifuge tube containing 500 ul of ice-cold citrate-saline buffer (SSC). Grind the tissue with a micro pestle for about 5 minutes, keeping the solution cold at all times. The solution should start to get viscous with the release of chromosomal DNA from the cells in the tissue. *Why do we keep this on ice as much as possible? What is the purpose of the proteinase added to the SSC?*

3. Centrifuge mixture for 2 minutes at 10,000 rpm in the micro-centrifuge. *This pellets the cell debris.*

4. Carefully pipet only the supernatant into a new 1.5 ml microcentrifuge tube. Avoid getting any pellet material. *The supernatant has the DNA, since it is soluble in SSC.*

5. Repeat Steps 3 and 4 with first supernatant to get a cleaner second supernatant.

6. Add 50 ul of 1 M NaCl to this second supernatant, and mix by inverting the tube.

7. Add 1 ml of cold 95% ethanol, allowing it to pour down the side of the tube and layer on top of the aqueous supernatant. *DNA will precipitate in salt solutions that are 70% ethanol.*

8. Cap the tube and slowly invert the tube back and forth. *You may notice cloudy strands of DNA coming out of the solution.* After you have done the mixing for a minute, allow the solution to sit on the benchtop for 5 minutes. *What slows DNA degradation at this point?*

9. Microcentrifuge for 1 minute at 10,000 rpm.

10. The pellet at the bottom of the tube is your DNA. Be careful not to disrupt it when removing and discarding the supernatent. The resultant DNA quality may vary depending on your technique. Very pure DNA pellets are hard to see (white or clear). This simple separation procedure often results in more contaminated or pigmented pellets. If your pellet is clean, skip to Steps 14-17. If your pellet is pigmented, continue with Steps 11-13 to obtain a cleaner DNA solution.

11. Resuspend pellet in 300 uL of TE RNAse solution (10 mM Tris, 1 mM EDTA, 10 ug/ml RNAse) and incubate 15 minutes at room temperature. DNA is now in the solution. *Tris is a buffer. What is the RNAse for? What does EDTA do? What do you think is happening during this incubation?*

12. Microcentrifuge sample for 1 minute at 10,000 rpm to pellet undissolved debris.

13. Pipet supernatant carefully into a clean, small, microfuge tube. Sample should be a fairly clear solution at this point. Labeled tube will be stored until next lab.

14. For clean DNA pellets from Step 10 only: *Very carefully* pour out or remove the supernatant and add 1 ml of 70% ethanol to wash the DNA pellet. *This will take out much of the salt that is interacting with the DNA pellet.*

15. Microcentrifuge for 1 minute at 10,000 rpm to again pellet DNA.

16. Pour off the supernatant and leave the tube turned upside down over a Kimwipe for 10 minutes (to allow the rest of the ethanol to drain off and the DNA pellet to dry).

17. Add 300 ul of TE RNAse solution to resuspend your pellet. Store labeled tube at 4ºC until next lab. *Tris is a buffer. What is the RNAse for? What does EDTA do?*

Make sure to clearly label your tube and note any observations on specific color or physical characteristics of your DNA sample.

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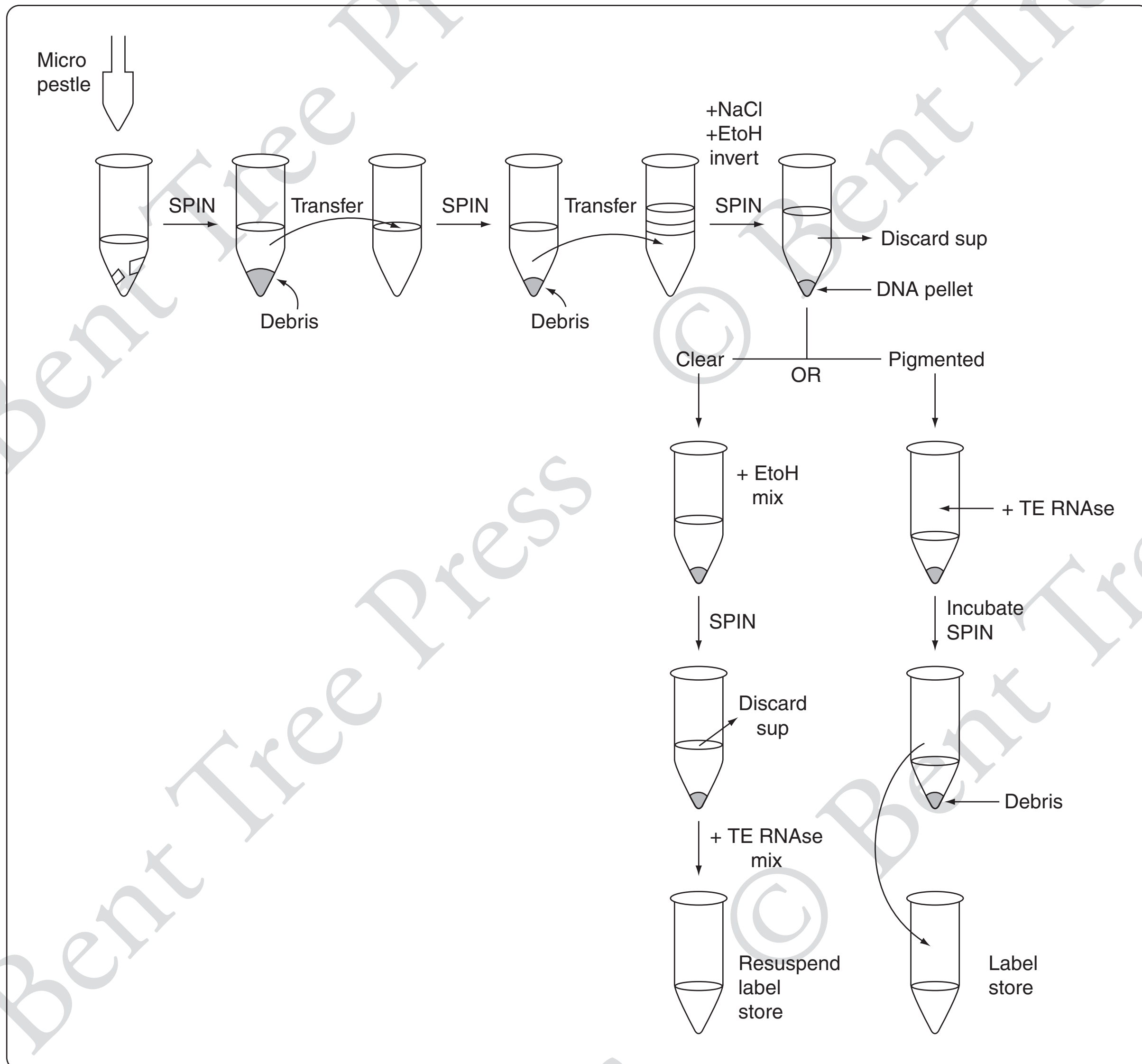

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