Thymus DNA Extraction Protocol **(Applications; Biotech Series)**

Purpose: In this lab protocol, you are going to extract the DNA from thymus gland nuclei. The thymus is a gland that is very large in immature mammals. It serves a function as a part of the immune system so there are many white blood cells with many large nuclei. Many thousands of thymus cells will be used for the extraction, so you will be combining the DNA from thousands of nuclei. In this way, you should be able to see long, combined strands of DNA. Students in Applications will be required to prepare the solutions and reagents (see page 2).

Materials for 16 lab stations:

Test tubes, 16	Test tube racks, 16	Clinical centrifuge
Prep buffer	NaCl Solution	EDTA Solution
SDS Solution	Ice cold 95% ethanol	Small cups or beakers for solutions
Thymus	Blender	15 ml cap-less centrifuge tubes, 8
Hand Strainer	250 ml beakers	Graduated cylinder, for measuring
Glass stir rods	Freezer	Refrigerator

1. Isolate nuclei. *this step will be done earlier, or as a demonstration*
    a. Puree 10 g thymus gland with ~10 ml prep buffer in blender
    b. Mix pureed thymus with 200 ml prep buffer
    c. Stir until well blended.
    d. Strain to remove large chunks of tissue – save liquid & toss solids.
    a – d can be done up to 24 hours ahead of time. Store in refrigerator.
    e. Centrifuge 4 ml of strained liquid for 5 minutes. This makes a pellet of the nuclei. (Nuclei are the heaviest part of the cell.)
    f. Pour off supernatant. (The liquid on top of the pellet.)
    g. Resuspend pellet in 2 ml (~44 drops) prep buffer. Mix well with plastic beral pipet. Make sure all of pellet is dissolved.
    h. Put ½ of solution into each of 2 test tubes, ~ 1 ml per tube. Resuspended pellet is shared by two lab groups.
    i. Prepare a slide with a drop of this solution; stain with methylene blue; observe with a microscope; diagram and describe what you see in your notebooks. This may be unsuccessful if the thymus has been frozen.
2. Lyse nuclei.
    a. Add ½ ml (~11 drops) of EDTA solution (This binds Mg and Ca ions which are needed by enzymes lurking in the cytoplasm from degrading the DNA as it is released from the nuclei.) Mix gently.
    b. Add 100 µl (~2 drops) of SDS solution. (A biological detergent similar to shampoo which solubilizes proteins and disorients fats in the cell membranes.) Mix gently.
    c. Add 250 µl (~5 drops) of NaCl solution, one drop at a time, mixing gently after each drop.
3. Precipitate DNA
    a. Gently add ~1 ml of ice cold 95% ethanol by pipetting **slowly down the side of the test tube**. The alcohol will form an overlay. You should begin to see strings of DNA precipitating at the point of the overlay and reaching up into the ethanol layer. (DNA precipitates because it is not soluble in ethanol. All of the other components of the cell are soluble in the ethanol.)
    b. Gently spool DNA threads where the ethanol and DNA mixture meet. There should be gobs of DNA. It is white, stringy and looks a bit like mucus.

Protocol for solutions and reagents:

Working Solutions:

Preparation Buffer
57.0 g Sucrose
3.1 g $MgCl_2$-$6H_2O$
0.6 g Tris·HCl
400 ml distilled H_2O
Adjust to pH 7.5 w/ 0.1 N NaOH
Bring to a final volume of 500 ml
Store in refrigerator.
Long term storage should be in freezer.

Ethylene diamine tetra acetic acid
0.72 g EDTA
200 ml distilled H_2O
Bring to a final volume of 250 ml
Store at room temperature

Sodium Dodecyl Sulfate (aka sodium laurel sulfate)
25 g SDS
distilled H_2O to a final volume of 250 ml
Caution – this is a detergent, bubbles easily
Store at room temperature

Sodium Chloride
29.2 g NaCl
Distilled H_2O to a final volume of 250 ml
Store at room temperature

Denatured 95% Ethanol
Adjust to pH 7.5 w/ 0.1 N NaOH
Store in freezer (use ice cold)

Hints

1. The proportion of thymus to prep buffer needs to be kept the same as in the original recipe. (10 g thymus into 200 ml prep buffer)
2. The proportions of extract solution to SDS, NaCl. EDTA and ethanol need to be the same as in the original recipe.
3. It is a good idea to add the prep buffer to the ground up thymus slowly. If the pink color begins to disappear, do not add the full amount.
4. When too little DNA is available, you must observe quickly. You may be able to see white threads floating up into the ethanol, but they disappear quickly, within 10 – 20 seconds.
5. The tip of the spooling rod should not go below the interface of the ethanol and the extract solution.
6. Make sure the thymus is being ground by the blender, sometimes the small chunks slide under the blades, rather than through them. Your solution should be pinkish.
7. After sitting for a few minutes, a whitish solute should be seen dropping to the bottom of the beaker. These are your nuclei.
8. If you do not have a centrifuge, you can let the solution sit for an hour or so (or overnight) and the nuclei will drop to the bottom of the beaker. You then pour off the top part of the solution. Keep refrigerated.

Troubleshooting

Problem	Why	Solution
Little to no DNA in tube	DNA is too dilute	Try spinning 8 ml of thymus in prep buffer instead of 4 ml. Use more thymus or less buffer. Stir the thymus solution before taking the 4 ml. (Because the nuclei drop, the top part of the solution becomes nuclei deficient.)
Not spooling	DNA not sticking	Try a plastic pipet, coffee stirrer, or roughen up the sides of the

	to rod	glass rod. Swirl the rod around gently, and then run it up the side of the tube. Make sure that the tip of the spooling rod does not go into the aqueous phase.
Calf thymus solution is not pink	DNA is too dilute	Grind up some more thymus and put into same buffer. Do not use <u>any</u> new buffer.
Do not need so much solution	Doing fewer experiments	Use half as much thymus <u>and</u> half as much buffer. Do the rest of the experiment the same.
Ethanol does not float on top	Ethanol solution is too dilute	Use a higher percentage alcohol.
Can't spin solution	No centrifuge	Make the thymus solution a day early and let sit in the refrigerator overnight. Carefully remove beaker from refrigerator and pour off the top part. Do not pour out any of the solute.
Can't weigh the thymus	No balance	10 g of thymus should be a little bigger than the size of your thumb. Add the prep buffer to the thymus solution slowly. If the pink starts to disappear, stop adding. Do not add more than the recipe says.

Analysis:

1. What is the purpose of centrifuging the thymus mixture after it has been pureed and strained?

2. Why do you need to bind Mg and Ca ions?

3. What common household chemicals contain SDS?

4. Why is a salt solution added?

5. What properties of DNA are responsible for its precipitation in alcohol?

6. If you looked at the spooled material under the microscope, would you be able to see the nucleotides that are the building blocks of DNA?

7. Why might freezing the thymus alter the ability to see nuclear material in the cells (step 4)?

8. Compare the general structure of an animal cell with a plant cell and a bacterial cell:
   a. what additional step would you have to take to extract the DNA of plant or bacterial cells?

   b. What could you use to accomplish that process?

The following extensions and protocol are for Applications of Biotechnology students only (junior year).

EXTENSIONS: INVESTIGATIONS OF THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF DNA:

The characteristics of DNA can be investigated using either the extracted DNA or commercially prepared DNA. The following suggestions for investigations demonstrate that DNA has characteristic properties that are conserved across phylogenetic boundaries.

Concentration- If your Biotech Lab has a spectrophotometer, you can demonstrate that DNA has an optical absorption maximum at 260nm.

Viscosity- As DNA is a large linear polymer, it tends to be very viscous in solution. As the DNA molecules are progressively broken into smaller fragments, the solution will tend to become less viscous at the same concentration. The solution's resistance to being pipetted by a fine bore pipette can assess changes in the viscosity of the solution. The greater the resistance; the more viscous the solution will be. Students can investigate the effects on a solution of:

- Mechanical shearing- due to rough handling/ multiple pipettings.
- Heating- lowers viscosity as DNA becomes single-stranded as base pairs detach.
- Heating and cooling- several cycles of heating and cooling will lead to denaturation of the DNA.
- Alkali treatment- increasing concentrations of NaOH (greater than 30mM) will denature and eventually destroy the DNA molecule.
- Nuclease treatment- causes DNA degradation

Size- DNA can be run through an agarose gel and visualised by staining to allow size determination.

Enzyme Digestion Patterns- Restriction endonucleases can be used to generate DNA 'fingerprints' for viewing via electrophoresis.

Other Tests- There are simple tests for the components of DNA (purines, phosphate, deoxyribose) given at http://tipnet.taranaki.ac.nz/~mfenton. Due to the nature of the tests, they are best carried out as professor demonstrations.