

**EXTRACTION OF DNA FROM CALF THYMUS CELLS**

Revised 2/1/96

**Introduction**

Cells may be classified into two primary types depending on whether they have a discrete nucleus (eukaryotic) or do not (prokaryotic). Prokaryotes include bacteria, such as *E. coli*, and the blue-green algae. Examples of eukaryotes include higher plants and animals, protozoans, as well as the true algae and fungi. The cellular machinery required for the complex metabolic processes of both eukaryotic and prokaryotic cells is surrounded by a plasma membrane. However, in contrast to prokaryotic cells, the internal structure of eukaryotic cells is quite complex. They contain a number of different types of intracellular organelles such as mitochondria and specialized membrane structures in addition to a nucleus all within the plasma membrane. Prokaryotic cells, on the other hand, are relatively simple and lack discrete organelles. Various methods have been developed for isolating the organelles of eukaryotic cells. Most of these involve, as a first step, disrupting the plasma membrane to form a homogenate. This can be accomplished in several ways such as mechanical breakage (e.g. stirring at high speed in a blender, or exposing to high intensity sound waves), treatment with certain types of detergents, or osmotic shock. Subsequently, the organelle can be separated by techniques such as centrifugation which take advantage of their differences in size and/or density.

Most or all of the DNA of prokaryotic cells is contained in a single large circular molecule located in the central region of the cell, but may be attached to the plasma membrane at some points. The DNA of eukaryotic cells is divided into two or more chromosomes which, except during cell division, are contained within a membrane-enclosed nucleus. Each chromosome is believed to contain a single linear DNA molecule which is much larger than the DNA of a prokaryotic cell. For example, the DNA of a single human chromosome is about 30 times larger than the DNA of *E. coli*. Human cells contain 46 chromosomes and the average length of each DNA molecule if stretched out is estimated to be 4 cm. Therefore the length of all the DNA molecules in a human cell combined is nearly 2 meters. If the human body contains approximately  $10^{14}$  cells, the total length of the DNA would be equivalent to 100 round trips from the earth to the sun! Obviously the DNA must be packed in a very condensed form within each chromosome.

During a large portion of the life cycle of a eukaryotic cell (called the interphase), the chromosomes are in a diffuse form within the nucleus and this form is referred to as chromatin. At the time of cell division, during prophase, metaphase and anaphase, the chromatin condenses into the organized structures we can visualize as chromosomes under the microscope. Chromosomal DNA is associated with several different positively charged proteins called histones forming nucleoproteins which are involved in the organization of the long DNA molecules into a compact structure. If these proteins are dissociated from DNA, the DNA becomes stretched out into very long molecules resulting in an increase in viscosity of a solution containing the DNA.

In this laboratory, you will be isolating DNA from the nuclei of calf thymus cells.

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**LABORATORY OVERVIEW**

**Part I**

In the first part of the laboratory you or your instructor will first isolate nuclei from calf thymus cells. This can be done rather simply by mechanically chopping up calf thymus tissue in a blender with a buffer containing a mild detergent to break the plasma membrane of the cells. The resulting mixture will be centrifuged in order to separate the large dense nuclei from the remaining organelles, fragments of membranes and soluble components. The centrifugal force developed as the tubes are spun in a centrifuge will cause the nuclei to collect at the bottom of the tube. These concentrated nuclei will then be used in Part II of the laboratory.

**Part II**

In this part, you will be disrupting the nuclear membranes and liberating the DNA from the proteins found in chromatin. These nucleoprotein complexes will be dissociated using a detergent (sodium dodecyl sulfate) and the DNA will be precipitated in the form of long fibers using alcohol. In the presence of alcohol, DNA molecules precipitate as long fibers, whereas contaminating RNA molecules and proteins precipitate as finer particles. In this experiment you will take advantage of this to recover the DNA by winding the fibers onto a glass rod. In other experiments, where you may be using DNA molecules which have been broken into smaller sizes, you will find that the DNA cannot be "spooled" but will form a finer precipitate.

**Part III**

In part III, you will stain cells you obtain from thymus tissue as well as nuclei from the nuclear suspension made in Part I. You will then compare the nuclei from each using a light microscope. In this part of the lab, it will be necessary to make detailed drawings of what you observe under the microscope.

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**PROCEDURE:**

**Part I: Isolation of Nuclei**

1. Dice about about 10-12 grams of thymus tissue into 1 centimeter cubes and place the pieces into a 500 mL beaker. Return the remaining thymus into the freezer.
2. Add 70 mL of cold nuclear buffer to the beaker containing the tissue.
3. Pour the mixture into a prechilled blender. Add about 10 mL of ice chips and blend for 60 seconds. (This should mechanically break down the plasma membranes of the cells, leaving the nuclear membranes intact.
4. Filter the blended mixture through 2 layer of cheese cloth into a clean beaker. Pour the filtered mixture into centrifuge tubes and centrifuge for 3 minutes or until pellets are formed.
5. Carefully pour off (and throw away) the liquid above the pellet (discard the supernatant).
6. Transfer the pellet into the chilled blender and add 70 mL of cold nuclear buffer.
7. Blend for 10 seconds, then filter through 2 layers of cheese cloth again. Store the nuclear suspension chilled.

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**Part II: DNA Isolation**

1. Place 2 mL of the nuclear suspension into a glass vial. Note the appearance and consistency of the suspension below:

Appearance: \_\_\_\_\_.

Consistency: \_\_\_\_\_.

2. Using a pipet, add 1 mL of the SDS solution to the vial and mix the contents of the vial carefully. Note the appearance and consistency of the solution below:

Appearance: \_\_\_\_\_.

Consistency: \_\_\_\_\_.

3. After 5 minutes, CAREFULLY pour the cold alcohol into the vial so that the alcohol forms a layer on top of the solution. Add enough alcohol so that the vial is nearly full.
4. Dip one end of a clean glass stirring rod through the cold alcohol and into the DNA. Slowly rotate the stirring rod and raise it back into the alcohol. Fine DNA fibers should form on the end of the rod. Produce more DNA by rotating the rod and moving it back-and-forth between the DNA and alcohol.
5. Continue until no more DNA is formed. The fibers are DNA contaminated with some proteins.
6. Keep the DNA on the stirring rod and discard the remaining solution in the vial. You may clean the vial and keep the DNA that you store in it.

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**Part III: Microscopic Analysis**

1. Get two clean microscope slides. Label one slide A and the one slide B.
2. Using a dissecting probe, scrape the surface of some thymus tissue. Smear the cells onto the center of slide A. Note: you should not get a big chunk of cells, only get enough to make a cloudy smear on the slide.
3. Using a stirring rod, transfer a drop of the nuclear suspension onto Slide B. Smear the suspension around on the center of the slide and allow the suspension to dry. A hot plate turned on the lowest setting can be used to accelerate the drying.
4. Now you need to "fix" the cells and nuclei to the slide. "Fixing" makes the cells permeable to nuclear stain and also causes them to adhere to the slide's surface so that they will not be washed away during the staining procedure. To fix the cells, apply 2-3 drops of alcohol onto the slides. Wait 2 minutes, then rinse the slides with water.
5. To stain the prepared slides, place 2-3 drops of nuclear stain onto each slide and wait 5-10 minutes.
6. Remove the excess stain from the slides by rinsing them in water. Allow the slides to dry for at least 10 minutes. Again, a hot plate set on the lowest setting can be used to accelerate drying. Cells can then be viewed under a microscope or stored until the next laboratory session.
7. To examine the cells and nuclei under the microscope, first find the cells or nuclei under LOW POWER. Then, locate the cells using the higher powered objective lenses. The nuclei should appear purple under the microscope. The cytoplasm of the cells should appear to be pale blue. Make a detailed drawing of each slide and identify the plasma membrane, cytoplasm, and nuclei of at least one cell. Attempt to identify the nucleoli in the nucleus (it should appear to be dark spot within the rest of the nucleus).
8. When you are finished examining each slide and making the drawings, clean the slides with soap and water, properly return your microscope to the cabinet, and clean your work area thoroughly.

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**Questions:**

Answer the following questions on a separate piece of paper.

1. Describe the changes in the clarity and viscosity (thickness) of the nuclear suspension before and after adding the SDS.
2. What did the blender do to the thymus cells? Why was it necessary to blend the cells?
3. DNA usually exists in cells in a loosely-packed form. However, chromosomes are supercoiled DNA and protein prior to cell division. Based on what you have learned from this lab, why do you think it is necessary for DNA to supercoil prior to cell division?
4. What did you learn from this lab?

**Reference:**

Lab procedure and portions of the introduction was obtained from Experiment (B1-2) "Cell Fractionation and DNA Isolation," John N. Anderson, 1987.

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## Teacher Supplement

Please note: The following supplies, and the amounts recommended are sufficient for a class of 24 students working in pairs. Doing Part I as a demonstration is strongly recommended. Also, set up different stations in the lab area to avoid congestion and wait time. You will need the following:

Calf Thymus (~15 grams)

The thymus should be thawed at the beginning of class by placing a bag of tissue in warm water. Thymus can be obtained from a local butcher. 12 glass vials

12 glass rods for DNA spooling

8 transfer pipets

Split the nuclear suspension into two equal parts, leaving 2 pipets per. Also split the SDS solution into two equal parts, each with 2 pipets to create a total of 4 stations (2 of each nuclear suspension and SDS).

20% Sodium dodecyl sulfate (SDS) 5 mL

Ethyl alcohol

The ethyl alcohol should be placed in the freezer at least 3 hours prior to lab. Keep in an ice bath during lab session.

Methylene blue (nuclear stain)

Cheese cloth

Nuclear buffer

The buffer should be placed in a refrigerator at least 3 hours prior to the lab. Keep buffer on ice during lab.

24 microscope slides

3 dissecting probes (for obtaining cells from thymus tissue)

Centrifuge and centrifuge tubes, 10-30 mL capacity is preferred

Funnel (for Part I)

Beakers (for Part I)

Scissors (for cutting up thymus in Part I)

Blender

Ice