Genomic DNA Purification Student Laboratory Manual

I. Purpose

The purpose of this laboratory is to isolate DNA from cells and, in the process, become familiar with the reagents and steps necessary for DNA purification. You will isolate DNA from bananas using household solutions to demonstrate the fundamental steps involved, then apply this knowledge to the purification of genomic DNA from cultured cells using a commercially available kit that uses a different DNA purification mechanism.

II. Introduction

In a cell, DNA acts as the genetic repository of information, and for this reason, scientists often study DNA to learn more about cellular biology. To manipulate or amplify DNA, scientists often must remove other cellular components that might interfere with their experiment, such as proteins, RNA and lipids, without damaging the DNA. The first scientist to purify DNA was a Swiss chemist named Johann Friedrich Miescher (1844–1895).

Miescher was working to isolate intact leukocytes from used hospital bandages. His initial attempts were unsuccessful and yielded a viscous lysate that was impossible to handle. However, by optimizing the composition of the salt solution in which the bandages were soaked, he was able to obtain intact cells. He then went on to develop a protocol to isolate intact nuclei from these celll. From these intact nuclei, Miescher extracted an acid-insoluble, alkali-soluble substance, which he named nuclein (1,2). At the time, scientists believed that cells were made up largely of protein, but Miescher determined that nuclein was not made up of protein because it was not digested by protease. Miescher soon demonstrated that nuclein was found in many other cells.

The term “leukocyte” refers to a broad class of white blood cells, which are involved in a body’s immune response to infection and disease.

Nuclein was later renamed nucleic acid. Today we refer to it as deoxyribose nucleic acid (DNA) to distinguish it from other types of nucleic acids such as ribonucleic acid (RNA).

Miescher’s original DNA purification protocol was crude, and thus, the resulting DNA preparation was not pure, preventing an accurate chemical analysis. One of his main concerns was protein contamination. Miescher set out to modify his original protocol to obtain pure nuclein relatively free of interfering protein. He turned to pepsin, a proteolytic enzyme that he extracted from pig stomachs, to eliminate proteins in the cells’ cytoplasm. With this modified protocol (Figure 1; 1,2), he obtained nuclein with sufficient purity and in sufficient quantities to perform chemical analyses and show that nuclein contained carbon, hydrogen, oxygen and nitrogen but, unlike protein, large amounts of phosphorus and no sulfur.

Since Miescher’s early experiments, much work has been done to optimize and simplify DNA purification. Today, DNA purification is often considered a routine laboratory process, and most scientists use existing protocols, which take advantage of the chemical and physical properties of DNA. These protocols allow scientists to focus their time and energy on other experimental goals.

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Wash used hospital bandages with a dilute solution of sodium sulfate [1:10 dilution of cold saturated Glauber's salt ($Na_2SO_4 \cdot 10\ H_2O$) in water]. Filter the wash solution, and examine the cells under a microscope to ensure that leukocytes are intact.

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Wash leukocytes three or four times with "warm alcohol".

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Prepare pepsin solution by washing pig stomachs with a mixture of 10 ml of fuming hydrochloric acid and 1 liter of water. Filter the solution until it is clear.

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Digest the washed leukocytes with the pepsin solution at 37–45°C for 18–24 hours. Change the pepsin solution twice during this time. Allow the gray sediment to settle, then remove the aqueous layer.

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Add a mixture of water and ether to the sediment, and shake several times to obtain intact nuclei free of cytoplasm. Collect the resulting nuclei by filtration, and wash them with water until there is no trace of protein present.

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Add a 1:100,000 dilution of sodium carbonate to the nuclei, then add an excess of acetic or hydrochloric acid to obtain an acid-insoluble, flocculent precipitate (nuclein).

Figure 1. Miescher's modified DNA purification protocol.

References

1. Miescher, F. (1869) Letter I; to Wilhelm His; Tübingen, February 26, 1869 In: W. His *et al*. Editors, *Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher—Aus dem wissenschaftlichen Briefwechsel von F. Miescher* vol. 1, F.C.W. Vogel, Leipzig, 33–38, as cited in: R. Dahm (2005) Friedrich Miescher and the discovery of DNA. *Dev. Biol.* **278**, 274–88.
2. Miescher, F. (1871) Ueber die chemische Zusammensetzung der Eiterzellen, *Med. Chem. Unters.* **4**, 441–460, as cited in: R. Dahm (2005) Friedrich Miescher and the discovery of DNA. *Dev. Biol.* **278**, 274–88.

III. DNA Purification from Bananas

Wear gloves, lab coats, closed-toe shoes and protective eyewear whenever you are working in a laboratory.

Materials Required

- table salt
- distilled water
- clear liquid detergent containing EDTA
- 100 g piece of peeled banana
- mortar and pestle
- 60°C water bath
- ice-water bath
- beakers
- rubber band
- 100 ml graduated cylinder
- pipets or graduated cylinders capable of measuring volumes of 1–15 ml
- coffee filter
- fresh pineapple (canned pineapple cannot be substituted)
- test tubes
- test tubes containing 15 ml of ice-cold isopropyl alcohol, kept on ice during the DNA purification
- rubber policeman or other stirring implement
- glass rod or wooden stir stick

Preparation of Detergent Solution

1. Combine 3 g of table salt and 80 ml of distilled water. Mix to dissolve the salt.
2. Add 10 ml of liquid detergent. Add water to bring the final volume to 100 ml. Mix gently to avoid foaming.

DNA Purification

Preparing the Lysate

1. Mash 100 g of banana in a mortar and pestle until you obtain a smooth pulp. Place the pulp in a 250 ml beaker, and add 100 ml of detergent solution. Mix gently but thoroughly.

   Note: Alternatively, the banana can be mashed on a clean, hard surface using a fork.
2. Place the beaker containing the homogenized banana in a 60 °C water bath for 15 minutes, mixing frequently.
3. Remove the beaker to an ice-water bath for 5 minutes to cool the pulp.
4. Insert a coffee filter into a clean beaker, fold the edge of the filter over the beaker rim and secure the coffee filter with a rubber band. The bottom of the coffee filter should be about an inch from the bottom of the beaker.
5. Carefully pour approximately 25 ml of homogenized banana into the coffee filter. After 5–10 minutes, you should have at least 5 ml of filtered solution. If the volume is less than 5 ml, add more homogenized banana into the coffee filter, being careful not to tear the coffee filter.

Removing Proteins (Optional)

6. Squeeze or crush a slice of fresh pineapple in a clean mortar and pestle to obtain at least 1 ml of juice. Pipet 1 ml of pineapple juice into a test tube.
7. Add 5 ml of filtered solution from Step 5 to the pineapple juice, and mix. Place the tube at room temperature for 2–3 minutes.

Precipitating the DNA

8. Tilt the test tube containing 15 ml of ice-cold isopropyl alcohol, and gently pipet the pineapple juice mixture down the side of the tube so that the mixture forms a layer on top of the isopropyl alcohol. Do not mix.
9. Incubate the test tube at room temperature for 4–5 minutes or until the DNA begins to precipitate. Record the appearance of the DNA in your laboratory notebook.
10. Insert the glass rod or stir stick into the tube, and slowly rotate it to spool the DNA onto the rod. Carefully remove the glass rod, and observe the purified DNA. Record the appearance of the DNA in your laboratory notebook.

IV. DNA Purification from Tissue Culture Cells

Materials Required

- Wizard® SV Genomic DNA Purification System
- 1.5 ml microcentrifuge tubes
- 95% ethanol
- microcentrifuge
- pipets and pipette tips
- 1X phosphate-buffered saline (PBS) [0.2 g/L KCl, 8.0 g/L NaCl, 0.2 g/L KH_2PO_4 and 1.15 g/L Na_2HPO_4]
- microcentrifuge tube rack
- 65 °C water bath (optional)
- tissue culture cells

Preparation of Solutions

Prepare the Wizard® SV Wash Solution prior to beginning the Wizard® SV Genomic DNA Purification System protocol:

Add 95% ethanol to the Wizard® SV Wash Solution bottle as directed on the bottle label. Label the bottle to indicate that ethanol has been added. Carefully seal the wash solution, and store at room temperature.

Preparation of Tissue Culture Cell Lysates

Use the following protocol to lyse adherent cultured cells grown in a tissue culture plate. Use at least 1×10^4 cells to a maximum of 5×10^6 cells per purification. The number of cells may need to be adjusted depending on cell type and function.

1. Remove the cell culture medium from the cells. Wash the cells once with 1X PBS.
2. Add 150 µl of Wizard® SV Lysis Buffer to the washed cells in the tissue culture plate. Mix the lysate by pipetting.
3. For each lysate, prepare one Wizard® SV Minicolumn assembly. Each Wizard® SV Minicolumn assembly consists of a Wizard® SV Minicolumn and a Collection Tube. Label the Collection Tube, and place the Wizard® SV Minicolumn assembly in a microcentrifuge tube rack.
4. Transfer the entire sample lysate from the tissue culture plate to a Wizard® SV Minicolumn assembly.
5. Place the Wizard® SV Minicolumn/Collection Tube assembly containing the sample lysate into a microcentrifuge, and centrifuge at 13,000 × *g* for 3 minutes. If some lysate remains on the column after the initial spin, centrifuge again for 1 minute at 13,000 × *g*.
6. Remove the Wizard® SV Minicolumn from the Minicolumn/Collection Tube assembly, and discard the liquid in the Collection Tube. Replace the Wizard® SV Minicolumn into the Collection Tube.
7. Verify that the ethanol has been added to the Wizard® SV Wash Solution as described above.
8. Add 650 µl of Wizard® SV Wash Solution to each Minicolumn/Collection Tube assembly.
9. Centrifuge at 13,000 × *g* for 1 minute.

10. Discard the liquid in the Collection Tube, and replace the Wizard® SV Minicolumn into the empty Collection Tube.
11. Repeat Steps 8–10 three times for a total of four washes of the Wizard® SV Minicolumn.
12. After the last wash, empty the Collection Tube, and reassemble the Wizard® SV Minicolumn/Collection Tube assembly. Centrifuge at 13,000 × *g* for 2 minutes to dry the binding matrix.

    Note: RNA may be co-purified with genomic DNA. To remove co-purified RNA, add 2 µl of RNase A Solution per 250 µl of Nuclease-Free Water prior to elution of genomic DNA from the column. Once eluted, incubate purified genomic DNA at room temperature for 10 minutes. Alternatively, the RNase A Solution (2 µl) may be added following elution from the Wizard® SV Minicolumn.
13. Remove the Wizard® SV Minicolumn, and place it in a new, labeled 1.5 ml microcentrifuge tube. Add 250 µl of room-temperature Nuclease-Free Water to the Wizard® SV Minicolumn. Incubate for 2 minutes at room temperature.

    Optional: To improve DNA yield, heat the water to 65 °C before adding it to the column for elution.
14. Place the Wizard® SV Minicolumn/elution tube assembly into the centrifuge, and centrifuge at 13,000 × *g* for 1 minute.

    Note: The total elution volume will be approximately 250 µl. Elution volumes of 250 µl are recommended for optimal DNA yield. Elution in volumes less than 250 µl will concentrate the DNA but will decrease the total DNA yield. A higher elution volume dilutes the DNA and does not improve yield.
15. Remove the Wizard® SV Minicolumn and discard. Cap the elution tube containing the purified genomic DNA, and record the appearance of the DNA in your laboratory notebook. If desired, store the DNA at –20 °C to –70 °C.