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| Jockey Club STEAM Education Resources Sharing Scheme |
| **Genomic DNA Extraction from Meat Samples** |
| *Lab Manual* |

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| |  |  | | --- | --- | | Name: |  | | Class: |  | | School: |  | |
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# Laboratory Safety

For safety reasons, students must read the following rules and regulations prior to entering the laboratory and follow them while inside the laboratory:

1. No eating or drinking in the laboratory
2. Always wear long trousers or dresses, and shoes with full coverage of toes and feet. No shorts or sandals.
3. Tie your hair if it is too long.
4. Wear your basic personal protective equipment (PPE) including a lab coat, and latex or nitrile gloves before handling any chemical or biological sample.
5. Wear additional PPE such as goggles whenever instructed by laboratory staff.
6. Do not touch any of your personal belongings, such as worksheets, stationaries or electronic devices when wearing your gloves.
7. Wash your hands thoroughly after taking off your gloves or before leaving the laboratory.
8. Keep the lab bench clean and tidy. No personal belongings should be placed on the bench.
9. Dispose of all solid waste in the designated container.
10. Discard all liquid biological wastes in 1:99 diluted bleach.
11. Discard syringes, sharp glasses, or broken glassware in sharp boxes.
12. Do not leave any fire unattended.
13. Consult laboratory staff when in doubt.
14. Locate the Emergency Exits, evacuation path, and fire extinguishing devices.
15. Notify laboratory staff **IMMEDIATELY** in case of accidents or emergency.

Handwashing is one of the most important and effective ways to avoid possible contact with infectious diseases. Students are required to follow the handwashing procedures recommended by the Centre for Health Protection, Department of Health, HKSAR as shown below:

1. Wet hands under running water.
2. Apply liquid soap and rub hands together to make a soapy lather.
3. Away from the running water, rub the palms, back of hands, between fingers, back of fingers, thumbs, fingertips and wrists. Do this for at least **20 seconds.**
4. Rinse hands thoroughly under running water.
5. Dry hands thoroughly with a clean cotton towel, a paper towel, or a hand dryer. Dispose of paper towels properly.
6. The cleaned hands should not touch the water tap directly again.
7. Turn off the tap after splashing water to clean the faucet; or use paper towels to wrap the faucet.



*Source: Centre for Health Protection, Department of Health, HKSAR Government (2020). Perform Hand Hygiene Properly.*

# Genomic DNA Extraction from Meat Samples

## **Purpose**

To extract genomic DNA from the meat samples

## **Introduction**

Genomic DNA is the genetic material that allows the development, maintenance of life and reproduction of an organism. Its extraction is one of the most widely used techniques in molecular biology for purposes such as the study of biological processes and genetic testing.

In genomic DNA extraction, cells are usually lysed with detergent and lysis buffer to release the nucleic acids inside. The nucleic acids are then precipitated with ethanol, purified from proteins, RNA and cell debris, and finally dissolved in nuclease-free water. Nowadays, research and testing laboratories routinely use commercially available DNA extraction kits for extracting nucleic acid.

In this experiment, students are going to extract the genomic DNA from meat samples using a commercially available DNA extraction kit. After that, the extraction of DNA will be confirmed by performing an agarose gel electrophoresis.

## **Experiment Objectives**

* To extract genomic DNA from the meat samples
* To analyze the extracted DNA by agarose gel electrophoresis

## **Equipment and Materials**

### For DNA Extraction

* + - 1.5mL centrifuge tubes
    - Absolute ethanol
    - Autopipettes (20 – 200 µL, 100 – 1000µL)
    - DNA extraction kit\*
* Proteinase K
* Buffer AL
* Buffer ATL
* Buffer AW1
* Buffer AW2
* Buffer AE
* 2mL collection tube
  + - Meat samples (Salmon)
    - Microcentrifuge
    - Quick spin
    - Pipette tips (DNAse and RNAse-free) – 200µL, 1000µL
    - Vortex
    - Water bath at 56˚C

\* *Qiagen DNeasy Blood and Tissue Kit (50) (69504) is used in this experiment.*

### For Agarose Gel Electrophoresis

* 6x loading dye
* Agarose
* Autopipette (1µL – 10µL)
* BenchTop 100bp DNA Ladder (ready to use)
* Casting tray for DNA gel
* Conical flask
* Electrophoresis chamber
* GelRed® Nucleic Acid Gel Stain
* Measuring cylinder
* Parafilm
* Pipette tips (DNAse and RNAse-free) – 10µL
* PowerPac
* TBE Buffer

## **Procedures**

### Prepare agarose gel

* 1. To prepare a 1% agarose gel, weigh 0.25g of agarose powder in a conical flask.
  2. Add 25mL TBE Buffer to the conical flask. Heat the mixture in a microwave oven until all the agarose powder is dissolved and the solution becomes clear.
  3. Cool the agarose solution slightly under running water.
  4. Add 2.5μL of GelRed to the solution and mix the solution gently.
  5. Place the comb into the casting tray and pour the agarose solution into the casting tray carefully. Remove any bubbles formed with a p200 pipet tip.
  6. Wait for 25 minutes until the agarose solidifies into an opaque gel.

### DNA Extraction

1. Label all tubes and arrange them in an organised manner on a rack.

|  |  |
| --- | --- |
|  | Label |
| 1 microcentrifuge tube | “Lysis” + your group number |
| 1 microcentrifuge tube | “Elution” + your group number |
| 1 column | Your group number |

1. Dice the meat to the smallest possible pieces.
2. Weigh ~0.025g (but no more than 0.025g) of meat tissue sample in **“Lysis” tube**.
3. Add 180µL of Buffer ATL to the sample, mix by the vortex.
4. Add 20µL of Proteinase K and mix by inverting the tube upside down several times. Incubate the tube at 56oC for 15 mins.
5. Pipette up and down several times to mix the sample.
6. Remove the meat.
7. Briefly centrifuge the 1.5mL microcentrifuge tube to ensure all drops of solution return to the bottom of the tube (quick spin).
8. Add **200µL of Buffer AL** to the sample and mix the sample by touch-vortexing for 15 seconds. Quick spin.
9. Add **200µL of absolute ethanol** to the sample. Mix the sample by touch-vortexing for 15 seconds. Quick Spin.
10. Transfer the sample to a column.
11. Centrifuge the tube at **8,000 rpm for 1 minute**.
12. Place the QIAamp Mini spin column in a **new 2mL collection tube** and discard the tube containing the filtrate. Carefully open the QIAamp Mini spin column, and add **500µL** of **Buffer AW1** without wetting the rim. Close the cap. Centrifuge the tube at **8,000 rpm for 1 minute**.
13. Place the QIAamp Mini spin column in a **new 2mL collection tube** and discard the tube containing the filtrate. Carefully open the QIAamp Mini spin column, and add **500µL of Buffer AW2** without wetting the rim. Close the cap. Centrifuge the tube at **14000 rpm for 3 minutes**.
14. Clean up the flow-through and put the column back into the collection tube.
15. Centrifuge at **14000 rpm for 1 minute**.
16. Place the column in the **“Elution” tube**.
17. Add **50µL of Buffer AE**.Incubate at room temperature for **5 minutes**, and then centrifuge at **8,000 rpm for 1 minute** to elute the DNA.
18. Check the concentration of the extracted DNA using NanoDrop.

### Agarose Gel Electrophoresis

1. Pull out the comb carefully to form the wells and place the solidified gel in a buffer chamber.
2. Pour TBE Buffer into the chamber until the gel is completely submerged.
3. Calculate the volume of 200ng DNA and water amount for the total volume of 5mL.
4. Cut a piece of parafilm and pipet a drop of 1μL of DNA loading dye.
5. Add the calculated amount of DNA and water to the loading dye. Now the total volume should be 6μL.
6. Mix the mixture up and down by pipetting until the mixture is uniformly coloured.
7. Carefully load the sample mixture and add 1μL of DNA ladder into the well respectively.
8. Connect the electrodes from the gel tank to the power supply.
9. Electrophorese the samples at 120 V for ~ 30 minutes.
10. After electrophoresis, visualize and photograph the gel under UV light.

Examples:

