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| Jockey Club STEAM Education Resources Sharing Scheme |
| **DNA fingerprinting -(RFLP) workshop** |
| *Protocol* |

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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 through 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.*

# DNA fingerprinting – Restriction Fragment Length Polymorphism (RFLP)

## **Purpose**

To identify the animal species of meat in the processed meat sample by DNA fingerprinting.

## **Introduction**

Adulteration of meat products is a common food fraud committed by food manufacturers. In adulterated meat products, chicken meat and other low-cost species are commonly used to increase the overall weight of the meat, often substituting the more expensive beef. Apart from giving a false trade description, food adulteration is a public health issue, as adulterated meat products might not be suitable for human consumption. People with allergies to specific proteins may be misled to consume items that contain the allergens. Also, adherents to a specific religion may be deceived into consuming food items that contain animal species, which are forbidden by their religion. Therefore, the identification of animal species in processed meat products is vital to ensure food safety and protect consumers’ rights.

DNA fingerprinting by restriction fragment length polymorphism (RFLP) is a method to identify the species in the meat products. Some genes encoding for functional proteins, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and cytochrome b (*Cytb*), are conserved among various species. Thus, they can be used as markers in species identification. GAPDH is a key enzyme in glycolysis, which breaks down glucose for the generation of ATP. *Cytb* is one of the mitochondrial proteins that help electron transfer in cellular respiration for the generation of ATP. Due to their essential functions in cells, the DNA sequences of the genes encoding these two proteins are conserved across different species; however, slight sequential variations do exist. Hence, by comparing the restriction fragments of DNA sequences of these two genes in meat products, the constituting species can be identified.

DNA fingerprinting based on gene amplification by polymerase chain reaction (PCR) and restriction digestion of PCR products are used to determine the constituting species of a meat product. In this workshop, you are going to find out the animal species of an unknown meat product sample. You are provided with the PCR products of *Cytb* gene amplified from the DNA of reference meats (pure chicken, pure pork and pure beef) and an unknown meat product. The task that you have to do is to perform restriction digestion to cut these PCR products into fragments of different sizes. Then you have to run DNA electrophoresis and analyse the DNA fingerprint patterns of these fragments on an agarose gel. By comparing the DNA fingerprints of the reference meats, you can identify the meat composition of the unknown meat sample.

## **Experiment Objectives**

1. To cut the PCR products of the *Cytb* genes from pure chicken, pure pork, pure beef and an unknown meat sample by restriction digestion.
2. To analyse the digested DNA fragment using agarose gel electrophoresis.
3. To determine the species of meat in the food sample.

## **Equipment and Materials**

**For Restriction Digestion**

Equipment

* Micro-centrifuge
* Micropipettes (P20, P100, P200) and sterile tips
* Vortex
* Thermocycler

Materials

* 0.2 ml PCR tubes
* 70% ethanol in a spray bottle
* Biohazard bag
* Restriction enzyme BsaJI OR Restriction enzyme RsaI diluted in reaction buffer
* Disposal container
* Ice bath
* Nuclease-free H2O
* Paper towel
* PCR products (Pure chicken, pure pork, pure beef and meat sample)
* Permanent marker

**For Agarose Gel Electrophoresis**

Equipment

* Autopipettes (P10 and P20) and sterile tips
* Casting tray for DNA gel
* Conical flask
* Electrophoresis tank
* Gel documentation device / UV lamp
* Measuring cylinder
* Microwave oven
* Power supply

Materials

* 70% ethanol in a spray bottle
* Agarose
* Biohazard bag
* Disposal container
* DNA ladder
* DNA loading dye
* Paper towel
* Parafilm
* Permanent marker
* Tris-Borate-EDTA (TBE) buffer

## **Procedures**

**Restriction Digestion**

1. Label four 0.2 ml PCR tubes as indicated in Table 1, with your group name.

***Table 1***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Pure Chicken | Pure Pork | Pure Beef | Meat Product |
| Label | C | P | B | M |

1. Prepare the reaction mixture for digestion in each labelled tube as in Table 2. Note that the following required volumes listed are provided asone sample.

***Table 2***

|  |  |
| --- | --- |
|  | (per sample) |
| Nuclease-free H2O | 12.5 µL |
| Restriction enzyme BsaJI or RsaI in reaction buffer | 2.5 µL |
| PCR product | 5 µL |
| Total | 20 µL |

1. Mix the reaction mixture well by tapping the bottom of the tubes or vortexing.
2. Incubate the tubes at 37°C for 1 hour in a thermocycler.

**Agarose Gel Electrophoresis**

1. To prepare a 2% agarose gel, weigh 0.8g of agarose powder in a conical flask.
2. Add 40mL 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 4μL of gel red to the solution and mix the solution gently.
5. Place the comb into the casting tray and pour 40 mL agarose solution into each casting tray carefully. Remove any bubbles formed with a P200 pipet tip.

一張含有 室內, 牆, 個人, 藍色 的圖片

自動產生的描述

1. Wait for 25 minutes until the agarose solidifies into an opaque gel.
2. Pull out the comb carefully to form the wells and place the solidified gel in a buffer chamber.
3. Pour TBE Buffer into the chamber until the gel is completely submerged.

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自動產生的描述

1. Cut a piece of parafilm and pipet eight drops of 1μL of DNA loading dye.

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自動產生的描述

1. Mix 4μL of samples with the loading dye slowly by pipetting the mixture up and down until the mixture is uniformly coloured.
2. Carefully load 4μL of the sample mixture and 1μL of DNA ladder into the well, according to the layout shown in Table 3.

***Table 3. The layout of different samples in the agarose gel.***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Well position | Well 1 | Well 2 | Well 3 | Well 4 | Well 5 | Well 6 | Well 7 | Well 8 | Well 9 |
| Sample to be loaded | DNA ladder | Pure chicken: digested product + Dye | Pure pork: digested product + Dye | Pure beef: digested product + Dye | Meat product: Enzyme digested product + Dye | Pure chicken: PCR product + Dye | Pure pork: PCR product + Dye | Pure beef: PCR product + Dye | Meat product: PCR product + Dye |
| Volume to be loaded | 1 μl | 4 μl | 4 μl | 4 μl | 4 μl | 4 μl | 4 μl | 4 μl | 4 μl |

1. Connect the electrodes from the gel tank to the power supply. Electrophorese the samples at 120 – 150V for 25 – 40 minutes.
2. After electrophoresis, visualise and photograph the gel under UV light.

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自動產生的描述

1. From the printout of the gel electrophoreis result, check the presence of a band of the DNA samples.
2. After observation and analysis, discard the gel in the designated disposal container.

**DNA Fingerprinting (RFLP) Workshop**

## **Student Worksheet**

1. What is the purpose of today’s experiment?

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1. Complete the following revision exercise,
2. Which of the following is the correct name of RFLP?
   1. Reduced Fragment Length Polymorphism
   2. Restricted Fragment Length Polymorphism
   3. Reacted Fragment Length Polymorphism
   4. Recycled Fragment Length Polymorphism
3. Which of the following is the cause of RFLP?
   1. Different restriction enzymes used
   2. Different genes analysed
   3. Differences in the DNA sequence of the same gene
   4. Different pore sizes of agarose gel
4. A gene can be cut by the restriction enzyme EcoRI into 4 fragments. How many restriction sites are there?
   1. 3
   2. 4
   3. 5
   4. 6
5. Which of the following is NOT an application of RFLP?
   1. DNA cloning
   2. Forensic test
   3. Disease diagnosis
   4. Identification of individuals