

Jockey Club STEAM Education Resources Sharing Scheme

# To be a Food Detective

*Learning Portfolio (Teacher Version)*

Name: \_\_\_\_\_

Class: \_\_\_\_\_

School: \_\_\_\_\_

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## Laboratory Safety

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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 when 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 all solid waste in the designated container.
- (10) Discard all liquid biological wastes in 1:99 diluted bleach.
- (11) Discard syringe, 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 paper towel 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 using the paper towel to wrap the faucet.



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

## Background

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Food adulteration means the inclusion of impurities or food contents that are not listed in food labelling or as claimed during the sale of the food. Food adulterants can be heavy metals, an ingredient of a quality inferior to as claimed in food advertisement, or species of meat that is not listed in the food labelling. Apart from leading to a discrepancy in the monetary value of the food, food adulteration may lead to food safety issues. For example, adulterating nuts in food may lead to accidental consumption by customers allergic to nuts, which may result in death. As a result, it is important to prevent food adulteration. Multiple methods are useful in detecting food adulteration. In this module, you will determine the species of constituting meats in different processed meat products by DNA fingerprinting and determine if the food samples are adulterated.

This module includes five units. In Unit 1, students will build a thermocycler using Arduino. Students will also decorate the casing of the thermocycler. The thermocycler will be used for the PCR of Unit 3. In Unit 2, students will extract DNA of the constituting meat from the processed meat samples. In Unit 3, students will amplify the cytochrome gene from the DNA extracted in Unit 2 by PCR, using the thermocycler built in Unit 1 or thermocycler of other brands. Students will also perform an agarose gel electrophoresis to confirm whether the gene is successfully amplified. In Unit 4, students will digest the amplified cytochrome gene using the technique of restriction fragment length polymorphism. Students will perform another agarose gel electrophoresis of the digested DNA for visualising the digestion pattern of the gene (DNA fingerprinting). Teachers will also assess if the students have met the learning outcomes.

# Unit 1 – Building a Thermocycler

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## 1. Purpose

To build a simple functional light bulb thermocycler for the subsequent experiment.

## 2. Introduction

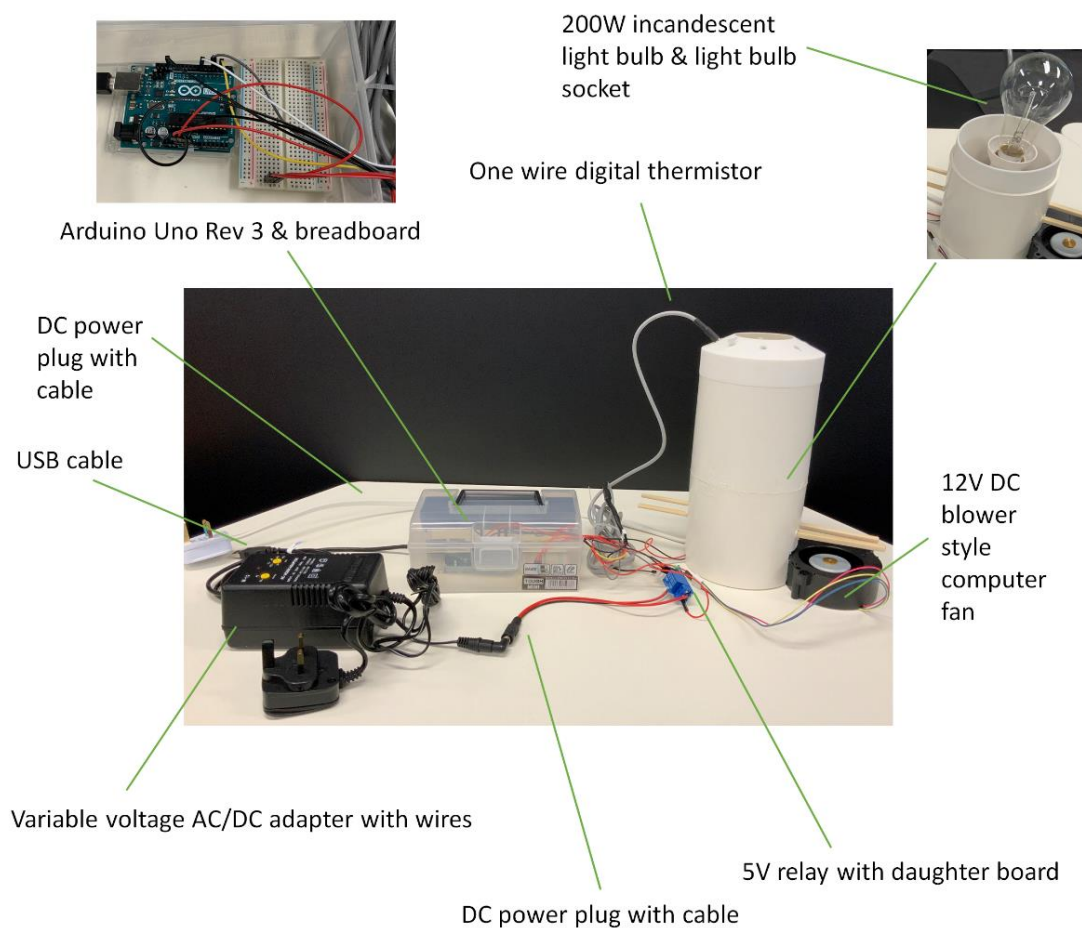
Polymerase Chain Reaction (PCR) is a common molecular biology technique used for gene detection and gene amplification. Polymerase chain reaction involves multiple cycles of temperature changes, which allow (1) the denaturation of double-stranded DNA, (2) the annealing of primer to a specific DNA region, and (3) the extension of primers to form a new DNA strand by DNA polymerase. The optimal reaction temperatures of the three steps are 96°C, 50 – 65°C, and 72°C respectively.

The control of the temperature of the reaction mixture was previously achieved by using water baths at three different temperatures. The reaction mixture was cycled between the three water baths at multiple short intervals of time. This is laborious and the control of the temperature mixture is not accurate.

This was changed with the invention of a thermocycler, which can heat up or cool down to a specific temperature rapidly. This provides precise control of the temperature of the reaction mixture. However, a thermocycler is expensive and may not be affordable.

In this Unit, you will build a simple functional light bulb thermocycler for the subsequent experiment.

The light bulb thermocycler consists of three layers which house the circuit board, light bulb and cooling fan, and the adapter for holding reaction vials (Figure 1). The temperature of a reaction vial is sensed by a thermistor. An Arduino UNO board loaded with programmes code will be used for controlling the temperature. The light bulb and the cooling fan will be used for heating up and cooling down the reaction vial respectively.



**Figure 1. Structure of the lightbulb thermocycler.**

### 3. Experiment Objectives

- To build a thermocycler for subsequent experiments.
- To evaluate the performance of the thermocycler.

### 4. Equipment and Materials

#### 4.1 Tools

- Flat Head Screwdriver (Small)
- Cross Head Screwdrivers (Small and Medium)
- Wire Stripper (For Jumper Wires)
- Utility Knife
- Hand Saw
- Power Drill
- Drill Bits
- Sandpapers
- Calipers
- Scissors
- Marker Pen
- Computer (With USB Port to Connect the Arduino UNO)
- 3D Printer (For Printing the 3D Printed Parts)



## 4.2 Materials

- (a) Arduino UNO Rev 3
- (b) OneWire Digital Thermistor DS18B20
- (c) 200W Incandescent Light Bulb
- (d) Light Bulb Socket
- (e) 3-Prong Plug with Wires
- (f) 12V DC Blower Style Computer Fan
- (g) Variable Voltage AC/DC Adapter with Wires
- (h) 5V Relays with Daughter Board SRD-5VDC-SL-C (Two per machine)
- (i) Breadboard
- (j) Jumper Wires (Male to Male, Female to Female and Male to Female)
- (k) USB-A 2.0 to USB-B 2.0 Cable
- (l) PVC Water Pipes Section with Inner Rail (Two per machine)
- (m) Nylon Plastic 3D Printed Couplers (Two per machine)
- (n) Nylon Plastic 3D Printed Adapter
- (o) Plastic Box
- (p) Black Tape
- (q) Aluminium Foil
- (r) Chopstick
- (s) 3A 揸玉 (Wire Linkage Plastic Bits)
- (t) Cable Ties

## 5. Preparations

- (1) For any DIY project, it is a good practice to test each component individually before putting them together. This allows early detections of possible malfunctioning components before they are mixed into the entire assembled machine, which would help alleviate a lot of hassle when troubleshooting.
- (2) When each component is separated from one another, they are the only parts that are in the equation, no other component that is malfunctioning could affect its function, so check each component carefully to look for any apparent visible damage.
- (3) After that, some components' integrity can be checked by connecting with a couple of the other components and forming some simple circuits. For example, to check whether or not the light bulb is working, we can simply connect the light bulb with the socket and connect the wires with the 3-prong plug, closing the circuit, plug it in and see if it works as intended.

## 6. Procedures

- (a) Heating Circuit
  - (1) Using the scissors, cut a roughly 1.5 inches section of wire from the original long wire.
  - (2) Using the utility knife, cut open the white outer casing of the wire on each of the 4 ends. Gently cut along the middle and then cut across it, be careful not to cut the inner wires as well.



- (3) Using the wire stripper, find the appropriate size hole by comparing it against the inner wires, and then use that hole to cut both of the colored outer casings of the inner wires on each of the 4 ends.
  - (4) Using the crosshead screwdrivers, open the 3-prong plug and connect the long wire section to the plug. Neutral wire in blue color on the left, live wire in brown color on the right, and the last one earth wire in the middle. Tighten the screw and close the plug with the crosshead screwdrivers.
  - (5) On the other end of the now attached long wire, using the medium crosshead screwdriver, loosen up the screws on the bottom side of the light bulb socket and attach the blue neutral wire and the non-sleeved earth wire to the left and right hole respectively as labeled at the bottom of the light bulb socket.
  - (6) Using the small crosshead screwdriver, loosen up the screws for the COM and NO port of the 5V relay and attach the leftover brown live wire of the long wire to the COM port.
  - (7) Then attach the brown live wire of the shorter wire section to the NO port, and the other end of it to the middle hole of the light bulb socket.
- (b) Cooling Circuit
- (1) Using the flat head screwdriver, adjust the knob on top of the AC/DC adapter to DC 12V.
  - (2) Using the small cross head screwdriver, loosen up the screws for the COM and NC port of the other 5V relay, and connect the red positive wire from the adapter to the COM port.
  - (3) Using a M to M jumper wire, connect the NC port and the positive pin of the computer fan.
  - (4) Using the flat head screwdriver, another jumper wire, and a wire linkage plastic bit, loosen up the screw of the wire linkage and connect the negative pin with the black negative wire of the adapter.
- (c) Probing Circuit
- (1) Using an M to M jumper wire, connect the 3V port on the Arduino UNO board to the positive pin of the OneWire thermistor.
  - (2) Using another M to M jumper wire, connect one of the GND ports on the Arduino board to the negative pin of the OneWire thermistor.
  - (3) Using another M to M jumper wire, connect the No. 2 port on the Arduino UNO board to the yellow signaling pin of the OneWire thermistor.
- (d) Signalling Circuit
- (1) Using an M to M jumper wire, connect the 5V port on the Arduino UNO board to one of the pins on the breadboard.
  - (2) Using an M to F jumper wire, connect the pin on the left of the first attached pin on the breadboard to the VCC pin on the 5V relay.
  - (3) Using another M to F jumper wire, connect the pin on the right of the first attached pin on the breadboard to the VCC pin on the other 5V relay.
  - (4) Using two M to F jumper wires, connect the GND pin on each of the 5V relays to the two unoccupied GND ports on the Arduino board.

- (5) Using another two M to F jumper wires, connect the IN pin on each of the 5V relays to the No.7 and No.4 port on the Arduino board for the heating and cooling circuit respectively.
- (e) Case and Setup
- (1) Using a hand saw, power drill, and drill bits, saw open a rectangular opening on one of the PVC water pipes that can fit the rectangular opening of the blower-style computer fan as instructed in the following steps.
  - (2) Saw in perpendicular to the bottom of the pipe twice.
  - (3) Use the power drill and appropriate size drill bits to drill two holes at each corner.
  - (4) Saw in parallel to the bottom of the pipe without cutting any portion want to left unscathed.
  - (5) Push the now nearly broken pieces off from the rest of the pipe, then use sandpapers the sand down any rough surface.
  - (6) Saw open another smaller rectangular opening on the same PVC water pipe that allows the wires to fit through, it should be leveled to the first opening, but at the opposite side of the pipe.
  - (7) Using the power drill and drill bits, drill two holes on the same PVC water pipe, they should be just above the inner rail of the pipe, and they should be about an inch apart from each other.
  - (8) Drill another two holes opposite to the first two.
  - (9) Using the power drill to drill 8 holes surrounding the center hole symmetrically on the top of the 3D printed adapter, 7 of the holes should be just about the same size as a PCR tube, one of them should be slightly larger, just fitting the thermistor probe.
  - (10) Using the aluminium foil, cover the plastic part of the light bulb socket carefully, make sure it is not covering or in contact with any metal part since it could potentially cause a short circuit.
  - (11) Using the black tape, cut a small piece that is just enough to cover the reflective part of the thermistor probe.
  - (12) Attach the light bulb to the light bulb socket.
  - (13) From the bottom side of the PVC water pipe with rectangular holes cut open, move the light bulb socket assembly into the pipe, with the wires coming out from the small rectangular opening.
  - (14) Also from the bottom side of the water pipe, move the rectangular opening of the computer fan into the larger rectangular opening of the water pipe.
  - (15) While holding the light bulb socket assembly, insert two chopsticks into the 4 drilled holes, they should form a parallel bridge that allows the light bulb socket to sit on it.
  - (16) Insert the 3D printed coupler into the inner rail of the water pipe.
  - (17) Insert the other water pipe on top of the bottom one.
  - (18) Insert another 3D printed coupler into the inner rail of the top water pipe.
  - (19) Cover the top with the 3D printed adapter.
  - (20) Insert the black tape covered with a thermistor into the slightly larger hole on top of the 3D printed adapter.
  - (21) Using the power drill, drill a small hole on the connecting part of the lid and the bottom part of the plastic box.

- (22) Also, drill a larger hole on the side of the box near the bottom, this hole should be large enough to fit through the USB-B side of the USB cable.
- (23) Put the Arduino board and the breadboard into the plastic box, line up the USB-B port with the large hole at the side of the box, and bundle all of the jumper wires coming out of the box onto the half circle at the smaller hole.
- (24) Insert the USB cable into the box at the larger hole and connect the Arduino board with a computer.
- (25) Install the Arduino IDE program on the computer.
- (26) Inside Tools, pick the UNO as the board, and the respective COM port.
- (27) Also inside Tool, in Manage Libraries, install the OneWire and the DallasTemperature library.
- (28) Copy and paste the downloaded programming code into the main editing area, replacing the default content.
- (29) Click Upload to send it to the Arduino board.

## 7. References

- [i] Grushkin Daniel. "DIY Gene Machine: A pipe that copies DNA using the heat of a lightbulb", Popular Science, Bonnier Corporation, 14th May 2013, <https://www.popsci.com/diy/article/2013-04/gene-machine/>
- [ii] Durrett, Russell. "The Light Bulb PCR Machine", 6th January 2011, <http://web.archive.org/web/20180425204818/http://www.russelldurrett.com:80/lightbulbpcr.html>
- [iii] McCabe Paul. "Coffee Cup Polymerase Chain Reaction Machine", Hackaday.io, 9th October 2017, <https://hackaday.io/project/27623-coffee-cup-polymerase-chain-reaction-machine>

# To be a Food Detective

## Unit 1 – Building a Thermocycler

### Student Worksheet

1. Set the following temperature and measure the actual temperature of the heating block of the thermocycler and the time taken to reach the temperature.

<i>Temperature (°C)</i>	<i>Actual Temperature</i>			<i>Time Taken</i>		
	<i>Reading 1</i>	<i>Reading 2</i>	<i>Reading 3</i>	<i>Reading 1</i>	<i>Reading 2</i>	<i>Reading 3</i>
100						
95						
90						
85						
80						
75						
70						
65						
60						
55						
50						
45						
40						

2. Plot a standard curve using the data in the above table using a graph paper.
3. What is the reliable temperature range of the thermocycler? Why?

4. How to solve the problem if it is not reliable?

## Unit 2 – DNA Extraction

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### 1. Purpose

To extract DNA from the meat samples for the investigation on the presence of the adulteration

### 2. Introduction

The first step of most molecular biology experiments is the extraction of nucleic acids, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Cells are usually lysed with detergent and lysis buffer to release the nucleic acids inside. The nucleic acid is then precipitated with ethanol and dissolved in nuclease-free water. In modern days, research and testing laboratories routinely use commercially available DNA/RNA extraction kits\* for extracting nucleic acid.

In this experiment, students will extract the DNA using a commercially available DNA extraction kit from the food samples to be tested in subsequent experiments. The extraction of DNA from food samples will be confirmed by performing an agarose gel electrophoresis.

### 3. Experiment Objectives

- (a) To extract DNA from the food samples to be examined for the use in following modules.
- (b) To confirm the extraction of DNA from food samples by electrophoresis

### 4. Equipment and Materials

#### **For DNA Extraction**

- (a) Meat samples (Pure chicken, pork, beef, and packaged meat product as food samples)
- (b) Microcentrifuge
- (c) Mini spin
- (d) Vortex
- (e) Water bath at 56°C and 70°C
- (f) Autopipettes (20 – 200 µL, 100 – 1000µL)
- (g) Ice Box
- (h) Ethanol (absolute grade) #
- (i) Phosphate-buffered saline (PBS) – *(optional)*
- (j) Pipette tips (DNase and RNase-free) –200µL, 1000µL
- (k) 1.5mL centrifuge tubes (one for each sample and pure meat control)

- (l) DNA extraction kit\*
  - i. 2mL collection tube (included in the kit) #
  - ii. Proteinase K (included in the kit) #
  - iii. Buffer AL (included in the kit) #
  - iv. Buffer ATL (included in the kit) #
  - v. Buffer AW1 (included in the kit, require preparation) #
  - vi. Buffer AW2 (included in the kit, require preparation) #
  - vii. Buffer AE (included in the kit) #

\* Qiagen DNeasy Blood and Tissue Kit (50) (69504) is used in this experiment. The DNA extraction kit can also be purchased from other suppliers such as Invitrogen, Applied Biosystem, and other local suppliers. Please refer to the manufacturers' instructions if other DNA extraction kits are used.

# Please refer to the instruction manual of the kit for amount/volume needed.

### **For Agarose Gel Electrophoresis**

- (a) Conical flask x 1 µL
- (b) Parafilm x 1 µL
- (c) Agarose x 0.2 g
- (d) TBE Buffer x 20 mL
- (e) 6x loading dye x 1 µL
- (f) BenchTop 100bp DNA Ladder (ready to use) x 1 µL

## **5. Procedures**

### **DNA Extraction**

- (a) Put some ice in the ice box for placing the meat samples (Pure chicken, pork, beef, and food samples tested).
- (b) Label all tubes and put them in an organised manner on a rack.
- (c) Take out the meat sample stored at -20°C. Place it on the ice.
- (d) Weigh ~0.025g (but no more than 0.025g) frozen meat tissue sample in a 1.5mL microcentrifuge tube.
- (e) Add 180µL Buffer ATL to the 1.5mL microcentrifuge tube, mix by the vortex.
- (f) Add 20µL Proteinase K and mix by inverting the tube upside down several times. Incubate the tube at 56°C until the tissue is completely lysed (usually complete in 1- 3 hours, can take up to overnight.)^
- (g) Collect lysed meat samples prepared by laboratory technician.
- (h) Briefly centrifuge the 1.5mL microcentrifuge tube to ensure all drops of solution go back to the bottom of the tube.
- (i) Add **200µL Buffer AL** to the sample and mix the sample touch-vortexing for 15 seconds. Incubate at 70°C for 10 minutes. Briefly centrifuge the 1.5mL microcentrifuge tube to ensure all drops of solution go back to the bottom of the tube.
- (j) Add **200µL Ethanol (absolute)** to each sample. Mix the sample by touch-vortexing for 15 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube to ensure all drops of solution go back to the bottom of the tube.
- (k) Label the sample ID on a QIAamp Mini spin column fitted on a 2mL collection tube.
- (l) Load all sample mixture (including the white precipitate) to the QIAamp Mini spin column. Close the cap. Centrifuge the tube at 6,000 g for 1 minute.



- (m) 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, add **500µL Buffer AW1** without wetting the rim. Close the cap. Centrifuge the tube at 6,000 g for 1 minute.
- (n) 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, add **500µL Buffer AW2** without wetting the rim. Close the cap. Centrifuge the tube at 20,000 g for 3 minutes.
- (o) Place the QIAamp Mini spin column in a **new** 2mL collection tube and discard the tube containing the filtrate. Centrifuge the tube at 20,000 g for 1 minute.
- (p) Place the QIAamp Mini spin column in a **clean 1.5mL microcentrifuge tube** and discard the tube containing the filtrate. **Carefully** open the QIAamp Mini spin column, add **100µL Buffer AE**. **Incubate** at room temperature for **5 minutes**, and then centrifuge at 6,000 g for 1 minute.

*^ As this step will take a long time to complete, the lab technician can complete Step (a) – (f) a day before the experiment, and students work from Step (g) below. Or lab technician can prepare one more set of lysed meat samples (i.e. completing Step (a) – (g). The prepared DNA samples can be distributed to students after students completing Step (f) and students can continue their experiment without waiting.*

### **Agarose Gel Electrophoresis**

- (a) Weigh 0.2g of agarose powder in a conical flask.
- (b) Add 20mL 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.
- (c) Cool the agarose solution slightly under running water.
- (d) Add 2µL of gel red to the solution and mix the solution gently.
- (e) Place the comb into the casting tray and pour the agarose solution in the casting tray carefully. Remove any bubbles formed with a p200 pipet tip.
- (f) Wait for 25 minutes until the agarose solidifies into an opaque gel.
- (g) Pull out the comb carefully to form the wells and place the solidified gel in a buffer chamber.
- (h) Pour TBE Buffer into the chamber until the gel is completely submerged.
- (i) Cut a piece of parafilm and pipet five drops of 1µL of DNA loading dye.
- (j) Mix 4µL of samples or negative control with the loading dye slowly by pipetting the mixture up and down until the mixture is uniformly coloured.
- (k) Carefully load 4µL of the sample mixture and 1µL of DNA ladder into the well
- (l) Connect the electrodes from the gel tank to the power supply.
- (m) Electrophorese the samples at 120 – 150V for 25 – 40 minutes.
- (n) After electrophoresis, visualise and photograph the gel under UV light.

### **Remark:**

- **Form groups of 4 students**
- **Go through the respective PPT and the learning portfolio with the students, in particular, the safety items**

## To be a Food Detective

### Unit 2 – DNA Extraction

#### Student Worksheet

*(With suggested answers and additional resources)*

#### 1. Identifying Societal Needs and Possible Scientific Solution

Please find out the current phenomena of food adulteration, in particular, the adulteration of meat products on the internet and the possible scientific solution.

Relevant Internet URL(s):

e.g. <https://hk.appledaily.com/international/daily/article/20130227/18178415> (Chinese)

e.g.

<https://topick.hket.com/article/2369932/%E5%8D%88%E9%A4%90%E8%82%89%E6%88%90%E4%BB%BD%E5%A4%A7%E6%AA%A2%E6%B8%AC%E3%80%8010%E6%AC%BE%E5%8D%88%E9%A4%90%E8%82%891%E6%AC%BE%E9%A9%97%E5%87%BA%E5%90%AB%E7%BE%8A%E5%9F%BA%E5%9B%A0> (Chinese)

e.g. <https://www.theguardian.com/uk/2013/feb/15/horsemeat-scandal-the-essential-guide> (English)

e.g. <https://www.wired.co.uk/article/food-fraud-dna-testing-horsegate> (English)

e.g. <https://www.rssl.com/food-and-consumer-goods/ingredient-and-product-analysis/authenticity/meat-and-fish-authenticity-species-identification-and-detection> (English)

e.g. <https://www.eurofins.co.uk/food-testing/food-authenticity/meat-species-testing/> (English)

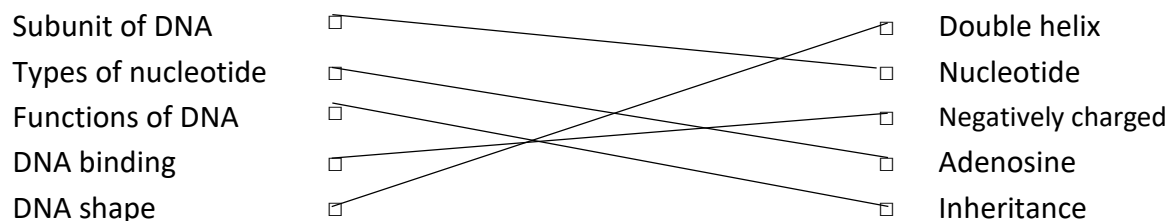
e.g. [http://scitechreports.blogspot.com/2015/04/blog-post\\_8.html](http://scitechreports.blogspot.com/2015/04/blog-post_8.html) (Chinese)

e.g. [https://www.hkctc.gov.hk/tc/tcsector/ba/food\\_a.html](https://www.hkctc.gov.hk/tc/tcsector/ba/food_a.html) (Chinese)

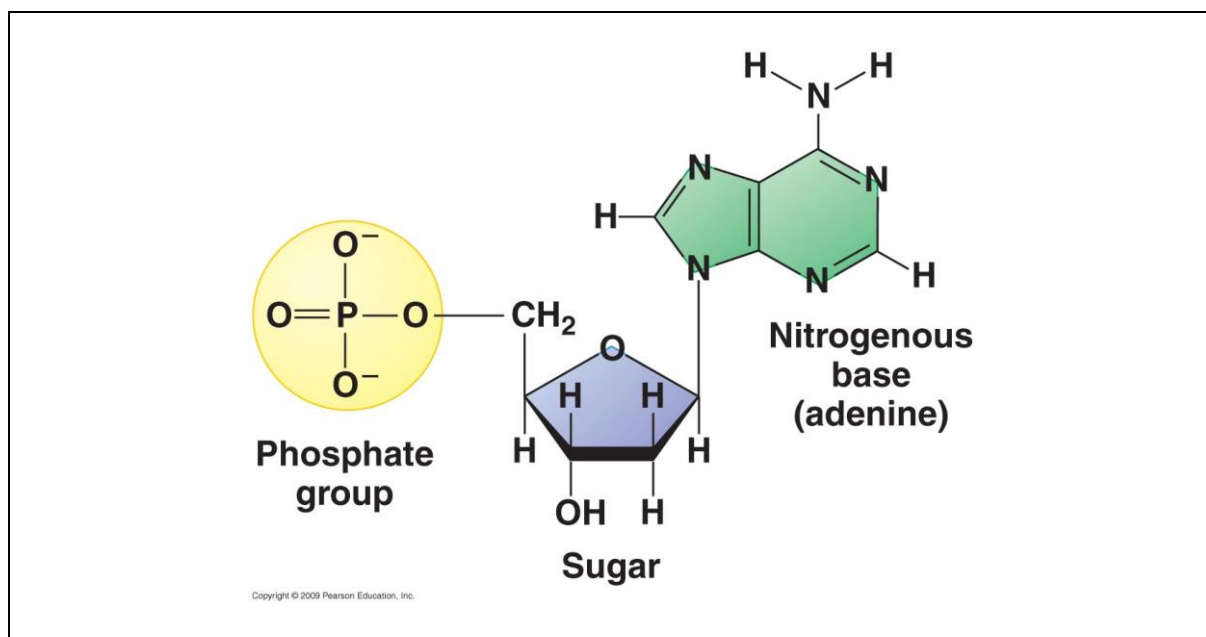
#### Suggest a Scientific Solution

Determining the species of the constituting meat by determining the DNA present in the food

2. Please match the correct pairs.



3. Draw the structure of a nucleotide.



4. What is the purpose of today's experiment?

To isolate the DNA of the constituting meat

5. Outline the workflow of DNA Extraction.

1. Break the meat into individual cells by enzymes
2. Lyse the cells to collect the cell content (including DNA)
3. Capture the DNA from the cell lysate by the column
4. Wash the DNA to remove contaminants
5. Dissolve the DNA in Dnase-free water and collect the dissolved DNA

6. What is the next step of the experiment?

To amplify the cytochrome B gene from the extracted DNA

## Unit 3 – Polymerase Chain Reaction (PCR)

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### 1. Purpose

To amplify the target using the DNA extracted from Unit 1 and determine the PCR product size by performing an agarose gel electrophoresis.

### 2. Introduction

As mentioned in previous units, polymerase chain reaction (PCR) is an important invention for advancing the development of molecular biology and is an essential technique in all molecular biology research. This simple but powerful technique has also found a number of applications in forensic investigation, environmental monitoring, and food safety monitoring.

In this experiment, you will amplify the target using the DNA extracted from the previous experiment. The result of the PCR will be assessed by performing an agarose gel electrophoresis. You will have to determine the PCR product size from the result of the gel electrophoresis. A successful PCR should result in a PCR product (a band in the agarose gel) of about 400 base pairs long.

### 3. Experiment Objectives

- (a) To amplify the cytochrome gene in the extracted DNA for restriction digestion.
- (b) To confirm the successful amplification of genes by performing an agarose gel electrophoresis.

### 4. Equipment and Materials

- (a) DNA Extracted in Unit 2
- (b) Thermocycler from the previous unit or a commercially available thermocycler
- (c) Microwave oven
- (d) Agarose gel tank with a comb
- (e) Buffer tank
- (f) Power supply
- (g) Autopipettes and tips (0.1 – 10 $\mu$ L, 20 – 200 $\mu$ L)
- (h) Gel documentation device/UV lamp
- (i) Graph paper

#### **For PCR**

- (a) Microcentrifuge tube x 2
- (b) PCR tubes (two for each sample and pure meat control)

- (c) Primers (cyt b1 and cyt b2) \*,#
- (d) Nuclease-free water #
- (e) 10X Taq Buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> #
- (f) 100µL Taq DNA polymerase (recombinant) (1U/µL) #
- (g) dNTPs (10µM) #
- (h) 50mM MgCl<sub>2</sub> #

#### **For Agarose Gel Electrophoresis**

- (g) Conical flask x 1 µL
- (h) Parafilm x 1 µL
- (i) Agarose x 0.2 g
- (j) TBE Buffer x 20 mL
- (k) 6x loading dye x 1 µL
- (l) BenchTop 100bp DNA Ladder (ready to use) x 1 µL

\* 0.025 µmole of desalted and PAGE purified primer will be sufficient for the use. Primer can be ordered from company such as Tech Dragon Limited. Primers can also be ordered from Sigma Oligo or Life Technologies. The PCR setup is based on Bio-rad iTaq DNA polymerase (1708870). Taq polymerase can also be purchased in from other biotechnology companies such as Invitrogen, and Promega.

# Refer to Scope for the volume needed.

## **5. Procedures**

### **PCR**

- (a) Label 0.2mL PCR tubes
- (b) Prepare the reaction mixture for PCR as in the following table. Note that the required volume listed is provided as one sample. Calculate the total volume of item 1 – 7 needed for their experiments and prepare a master mix solution. ^
- (c) Aliquot 24.625µL of the master mix into 0.2mL PCR tubes

^ Note for the calculation:

Master-mix solution is used for ensuring the accuracy and consistency of the PCR mixtures across different samples and is usually prepared at least 20% more master mix to allow for room for pipetting error.

	(per sample)	(Total Volume)
1. Nuclease-free H <sub>2</sub> O	19.125 µL	
2. 10x Taq Buffer	2.5 µL	
3. 10µM dNTPs	0.5 µL	
4. 50 mM MgCl <sub>2</sub>	0.75 µL	
5. 10 µM Forward Primer (cytb1)	0.5 µL	
6. 10 µM Reverse Primer (cytb2)	0.5 µL	
7. Taq (1U/µL)	0.125 µL	
DNA template	1 µL	

The primers and PCR program used are listed in the following:

**Primer sequences**

Forward Primer (Cyb b1): 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'

Primer Reverse (Cyb b2): 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'

**PCR program**

95°C, 3 minutes

36 cycles: 94°C, 40 seconds → 45°C, 30 seconds → 72°C, 1 minute

72°C, 10 minutes

**Agarose Gel Electrophoresis**

- (a) Weigh 0.2g of agarose powder in a conical flask.
- (b) Add 20mL 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.
- (c) Cool the agarose solution slightly under running water.
- (d) Add 2µL of gel red to the solution and mix the solution gently.
- (e) Place the comb into the casting tray and pour the agarose solution to the casting tray carefully. Remove any bubbles formed with a p200 pipet tip.
- (f) Wait for 25 minutes until the agarose solidifies into an opaque gel.
- (g) Pull out the comb carefully to form the wells and place the solidified gel in a buffer chamber.
- (h) Pour TBE Buffer into the chamber until the gel is completely submerged.
- (i) Cut a piece of parafilm and pipet five drops of 1µL of DNA loading dye.
- (j) Mix 4µL of samples or negative control with the loading dye slowly by pipetting the mixture up and down until the mixture is uniformly coloured.
- (k) Carefully load 4µL of the sample mixture and 1µL of DNA ladder into the well
- (l) Connect the electrodes from the gel tank to the power supply.
- (m) Electrophorese the samples at 120 – 150V for 25 – 40 minutes.
- (n) After electrophoresis, visualise and photograph the gel under UV light.

**To be a Food Detective**  
**Unit 3 – Polymerase Chain Reaction (PCR)**

**Student Worksheet**

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

To amplify enough genes for restriction digestion

2. Complete the following revision exercise,

(i) Which of the following is a feature of PCR?

- a. Specific amplification ☒
- b. Random binding of primer
- c. Single round of reaction
- d. Enzyme free reaction

(ii) Which of the following is NOT an application of PCR?

- a. Disease diagnosis
- b. Genetic engineering
- c. Determine DNA amount ☒
- d. Food testing

(iii) What is the correct sequence of a PCR?

- a. Annealing → Denaturation → Extension
- b. Annealing → Extension → Denaturation
- c. Denaturation → Extension → Annealing
- d. Denaturation → Annealing → Extension ☒

(iv) Which of the following is NOT a function of agarose gel electrophoresis?

- a. DNA visualization
- b. DNA size estimation
- c. Binding of DNA ☒
- d. Separation of DNA of different sizes

(v) Which is the function of electric current in agarose gel electrophoresis?

- a. Attract DNA to anode ☒
- b. Attract DNA to cathode
- c. Increase DNA energy content
- d. Visualise DNA molecules

(vi) Which of the following explains why large DNA moves slower in agarose?

- a. Large DNA receives less electric attraction
- b. Agarose has pores ☒
- c. Agarose wraps around large DNA
- d. Electrophoretic buffer traps large DNA



3. Determine the PCR product size by performing an agarose gel electrophoresis



4. Measure the distance travelled of each marker DNA molecules in your agarose gel. Record your measurement in the table below.

Size of Marker DNA (bp)	Distance Travelled (cm)
3,000	
2,000	
1,500	
1,200	
1,000	
900	
800	
700	
600	
500	
400	
300	
200	
100	

5. Plot a standard curve using the data in the above table using a graph paper.

6. Determine the size of your PCR products using the standard curve by correlating the distance travelled with the DNA size. Record your measurement in the table below. Did you amplify the correct gene?

	<i>Cattle DNA</i>	<i>Chicken DNA</i>	<i>Swine DNA</i>	<i>Sample 1</i>	<i>Sample 2</i>
DNA Size (bp)					
Target Gene Amplified? (Yes / No)					

## Unit 4 – Restriction Fragment Length Polymorphism (RFLP)

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### 1. Purpose

To digest the PCR products from the previous unit and perform an agarose gel electrophoresis of the REFP products so as to determine the presence of adulteration and the species of meat used in the previous unit.

### 2. Introduction

In this experiment, students will digest the PCR products into fragments of different sizes using different restriction enzymes. The size of the RFLP products will be visualised by performing an agarose gel electrophoresis of the products. The species of the meat used in the food sample can then be determined by comparing the gel electrophoresis pattern against that of the standards (meat of different species), i.e. DNA fingerprinting.

### 3. Experiment Objectives

- (a) To digest the PCR products.
- (b) To analyse the size of the RFLP products by agarose gel electrophoresis.
- (c) To determine the species of meat used and the presence of adulteration.

### 4. Equipment and Materials

#### **For RFLP**

- (a) Microcentrifuge x 2
- (b) Mini-spin x 1
- (c) Vortex x 1
- (d) Water bath at 56 °C or thermocycler x 1
- (e) Microwave oven x 1
- (f) Agarose gel tank with a comb x 1
- (g) Buffer tank x 1
- (h) Power supply x 1
- (i) Autopipettes (20 – 200 µL, 100 – 1000µL) x 1
- (j) Ice Box x 1
- (k) 0.2mL PCR tubes (two for each sample and pure meat control)
- (l) Restriction enzyme, **Bsa**JI, diluted in TANGO Buffer to 2U/µL (in ice box) <sup>#, ^</sup>
- (m) Restriction enzyme, **Rsa**I, diluted in TANGO Buffer to 2U/µL (in ice box) <sup>#, ^</sup>
- (n) Nuclease-free water<sup>^</sup>

#### **For Agarose Gel Electrophoresis**

- (a) Conical flask x 1 µL
- (b) Parafilm x 1 µL
- (c) Agarose x 0.2 g
- (d) TBE Buffer x 20 mL

- (e) 6x loading dye x 1 µL
- (f) BenchTop 100bp DNA Ladder (ready to use) x 1 µL

*# Restriction enzymes (R0536S and R0101S) from New England Biolab was used in this unit. Restriction enzymes can also be purchased from other biotechnology companies such as Fermentas and Promega.*

*^ Refer to the Procedures for volume needed.*

## 5. Procedures

### RFLP

- (a) Label 0.2mL PCR tubes and restriction enzyme **Bsa**II.
- (b) Label another set of 0.2mL PCR tubes with sample ID, restriction enzyme **Rsa**I.
- (c) Prepare the reaction mixture for digestion. Note that the following required volume listed are provided as one sample. Please make a master mix solution.

	(per sample)
1. Nuclease-free H <sub>2</sub> O	12.5 µL
2. Restriction enzyme <b>Bsa</b> II or <b>Rsa</b> I (diluted)	2.5 µL
3. PCR products	5 µL
Total	20 µL

- (d) Mix it well by tapping the bottle of the tubes.
- (e) Incubate the tubes at 37°C for 16 hours in a thermocycler.
- (f) Perform agarose gel electrophoresis according to the instruction.

### Agarose Gel Electrophoresis

- (a) Weigh 0.4g of agarose powder in a conical flask.
- (b) 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.
- (c) Cool the agarose solution slightly under running water.
- (d) Add 4µL of gel red to the solution and mix the solution gently.
- (e) Place the combs into two casting tray and pour 20 mL agarose solution to each of the casting tray carefully. Remove any bubbles formed with a p200 pipet tip.
- (f) Wait for 25 minutes until the agarose solidifies into an opaque gel.
- (g) Pull out the comb carefully to form the wells and place the solidified gel in a buffer chamber.
- (h) Pour TBE Buffer into the chamber until the gel is completely submerged.
- (i) Cut a piece of parafilm and pipet five drops of 1µL of DNA loading dye.
- (j) Mix 4µL of samples or negative control with the loading dye slowly by pipetting the mixture up and down until the mixture is uniformly coloured.
- (k) Carefully load 4µL of the sample mixture and 1µL of DNA ladder into the well
- (l) Connect the electrodes from the gel tank to the power supply.
- (m) Electrophorese the samples at 120 – 150V for 25 – 40 minutes.
- (n) After electrophoresis, visualise and photograph the gel under UV light.

## To be a Food Detective

### Unit 4 – Restriction Fragment Length Polymorphism (RFLP)

#### Student Worksheet

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

To digest DNA of different meat species into the fragment of different sizes for comparison with that of control meat samples

2. Complete the following revision exercise,

- (i) Which of the following is the correct name of RFLP?

- a. Reduced Fragment Length Polymorphism
- b. Restricted Fragment Length Polymorphism ☒
- c. Reacted Fragment Length Polymorphism
- d. Recycled Fragment Length Polymorphism

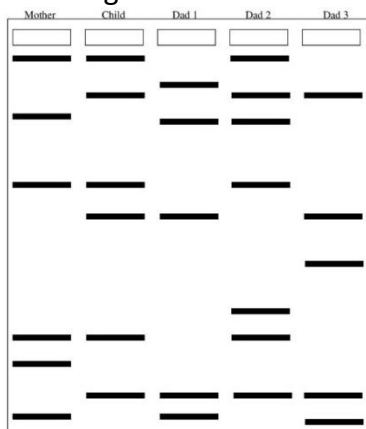
- (ii) Which of the following is the cause of RFLP?

- a. Different restriction enzymes used
- b. Different genes analysed
- c. Differences in the DNA sequence of the same gene ☒
- d. Different pore sizes of agarose gel

- (iii) A gene can be cut by restriction enzyme EcoRI into 4 fragments. How many restriction sites are there?

- a. 3 ☒
- b. 4
- c. 5
- d. 6

- (iv) The figure below shows the result of a paternity test. Who is the father of the child?



- a. Dad 1
- b. Dad 2
- c. Dad 3 ☒

- (v) Which of the following is NOT an application of RFLP?
- DNA cloning ☒
  - Forensic test
  - Disease diagnosis
  - Identification of individuals

3. Measure the distance travelled of each marker DNA molecule in your agarose gel. Record your measurement in the table below.

<i>Size of Marker DNA (bp)</i>	<i>Distance Travelled (cm)</i>
3,000	
2,000	
1,500	
1,200	
1,000	
900	
800	
700	
600	
500	
400	
300	
200	
100	

4. Plot a standard curve using the data in the above table using graph paper.
5. Determine the size of each band of each sample using the standard curve. Record your measurement in the table below.

	<i>Cattle DNA</i>	<i>Chicken DNA</i>	<i>Swine DNA</i>	<i>Sample 1</i>	<i>Sample 2</i>
DNA Size (bp)					
DNA Size (bp)					
DNA Size (bp)					

6. Determine the meat species present in Sample 1 and Sample 2. Check the ingredients listed on the food label and determine if the food is adulterated.

	<i>Sample 1</i>	<i>Sample 2</i>
Presence of Cattle? (Yes / No)		
Presence of Swine? (Yes / No)		
Presence of Chicken? (Yes / No)		
Meat Species Listed in Food Label (Cattle/Swine/Chicken)		
Presence of Food Adulteration? (Yes / No)		