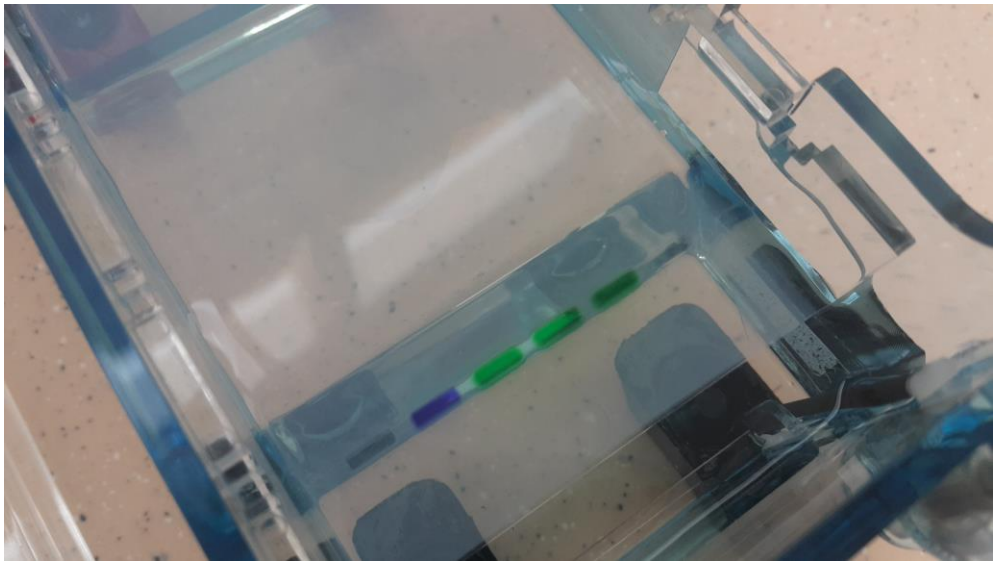
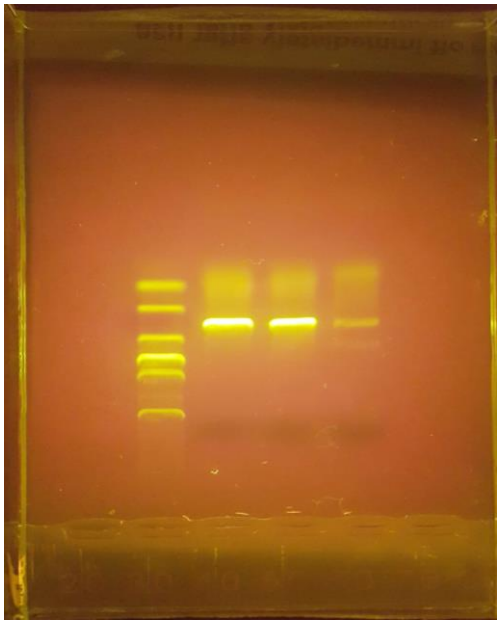
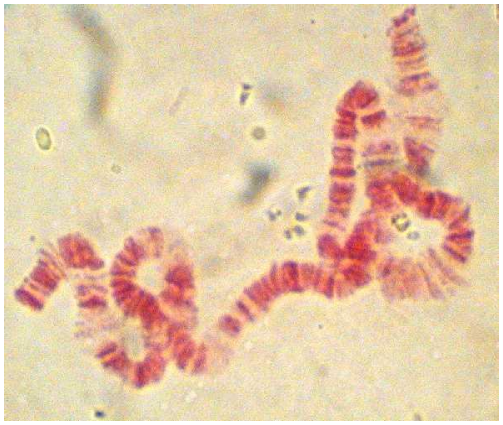
CARITAS CHAN CHUN HA FIELD STUDIES CENTRE







Biotechnology (Advanced) – DNA Extraction, Gel Electrophroesis & Observation of Polytene Chromosomes

School Name / Group	
Student Name	

DNA Fingerprinting

Deoxyribonucleic acid (DNA) is the genetic material that acts as the blueprint for protein synthesis by cells. In mammals, a large fraction of the total DNA does not code for proteins. Polymorphic DNA refers to chromosomal regions that vary among individuals. By examining several of these regions within genomic DNA, one can determine a "DNA Fingerprint" for an individual. DNA polymorphisms are now widely used for determining paternity/ maternity, identification of human remains and to determine the genetic basis of various inherited diseases. The most widely used and far-reaching application has been to field of criminal forensics. DNA from crime victims and offenders can now be definitively matched, affecting outcomes of criminal and civil trials.

Equipment and Materials		**Chemicals**	
250 ml conical flask	X1	Agarose	Share
Cooling basin	X1	TAE buffer(1X)	50ml
Digital thermometer	X1		
Electronic balance	Share		
Gel bed with comb (Close off the open ends by paper tape)	X1 set		
Microwave oven	Share		
Microwave oven wrap	Share		
Latex gloves	X1 pair		
Pipettement and tips	X1 set		

Gel Electrophroesis

Procedures

Preparation of Agarose Gel (1.5% , 7 cm x 10 cm Gel)

1. Put the conical flask on the electronic balance and set **Zero**.
2. Add **0.75g agarose** into the conical flask.
3. Add **50 ml TAE buffer** into the conical flask.
4. Wrap the opening of conical flask and make a hole on it.
5. Use a microwave oven to heat the mixture until all the agarose is completely dissolved. (Clear solution can be observed)
6. Cool the agarose solution to 55℃ with a cooling basin.
7. Add 5μl SYBR red stain. (Done by teacher)
7. Pour the cooled agarose solution into the gel bed.
8. Allow the gel to completely solidify. It will become firm after approximately 20 minutes.
9. Remove the comb by slowly pulling straight up. Be careful and evenly to prevent tearing the sample wells.

Load the samples and Run the Gel

1. Load 20µl of the DNA samples* from provided tubes into the wells.
2. After DNA samples are loaded, connect the apparatus to the D.C. power source and set the power source at the required voltage.
3. Check that current is flowing properly – you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.
4. After electrophoresis is completed, proceed to DNA visualization by exposing the gel in UV light

Results

What are the band sizes of the DNA products in each well?

Well	Fragment 1 Band size	Fragment 2 Band size	Fragment 3 Band size
A			
B			
C			
D			
E			

Discussion

Background:

DNA samples are extracted from organisms below:

GM papaya; Non GM papaya; earthworm; millipede

DNA extracted are amplified using 3 different pairs of primers which amplify genes coding for:

1) Papain
2) PRSV coat protein
3) Mitochondria cytochrome oxidase subunit 1 (CO1)

The corresponding gene are amplified through Polymerase chain reaction (PCR)

1. What are the ingredients in a PCR reaction mixture?
2. What does each fragment represent?
3. Can you distinguish all the sample? What are they?
4. Is there any expected band missing in your results? What are the possible reasons?

DNA extraction from strawberries

Equipment and Materials

1ml Syringe	X1
5ml Syringe	X1
100ml Beaker	X1
Coffee filter	X1
Filter funnel	X1
Pestle and Mortar	X1 set
Fruit Sample	
Test tubes	X2

Chemicals

Extraction Buffer:		10 ml
100 ml detergent		
900 ml distilled water		
15 grams NaCl		
Cold Alcohol	(pre-measured)	4 ml

Procedures

DNA Isolation using Strawberries (wear eye protection, or be careful).

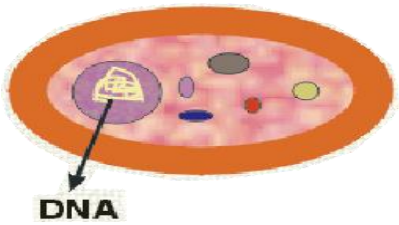
1. Mash the strawberries with pestle and mortar for **2 minutes**.
2. Add **10 ml** DNA Extraction buffer (Pre-measured).
3. Mash for 1 minute.
4. Filter solution by coffee filter into beaker
5. Transfer **2 ml** of this solution back into the two test tubes. (It will be very red)
6. Add **4 ml** (2X the volume of strawberry solution) of 75% cold ethanol and 98% cold ethanol to the test tubes respectively. (Add the ethanol slowly so that it stays on top of the strawberry solution.
7. Record the observations

NOTE:
If the tube just sits, DNA will precipitate at the interface with bubbles.
More DNA can be observed if the tube was shake in gently and eventually float to the top.

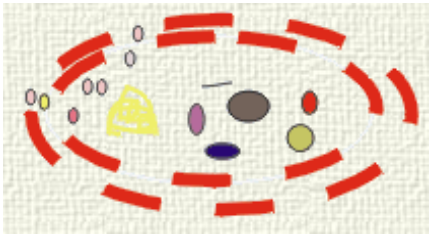
Discussion

1. What is the function of extraction buffer?
2. What is the function of alcohol? Why it should be cold?
3. Why the fruit sample should be frozen beforehand?
4. Why we usually select strawberries and Kiwi fruit as sample for DNA extraction?
5. Why the yield of DNA extraction are different within different fruit sample?
6. How to increase the yield of DNA extraction? Why?
7. Is the precipitate as a pure DNA?

Principles

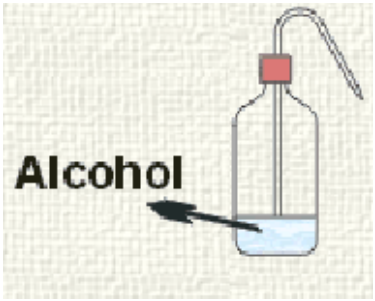


1. DNA is in the nucleus of the cell

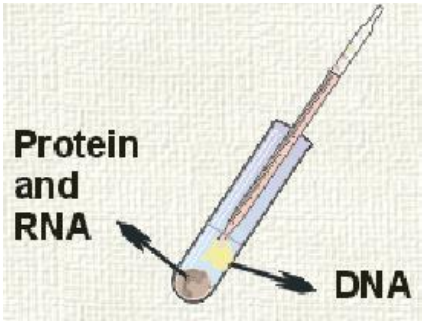


2. Cell membrane is disrupted with a detergent

`



3. Cold Alcohol is added to the tube slowly to separate DNA form other cell components. DNA moves to the alcohol layer.



4. DNA is spooled onto wooden sticks.

Observation of Polytene Chromosomes in Larva of *Chironomidae*

Polytene chromosomes are over-sized chromosomes, and are commonly found in cells which undergo repeated rounds of DNA replication without cell division, called endocyle. As the sister chromatids remain synapsed together, polytene chromosomes will be formed after multiple rounds of replication occurs. This kind of specialized cells may be found in the salivary glands of *Drosophila*.

Equipment and Materials		**Chemicals**	
Chironomidae Larva	X1 pack	Aceto-orcein	Share
Forceps	X2	Saline	Share
Filter paper	X few sheets		
Slide	X3		
Coverslip	X3		
Stereomicroscope	X1		
Light Microscope	X1		

Procedures

1. Prepare a clean slide with 2-3 drops of saline.
2. Select a large larva and place it on the slide.
3. Put the slide on a stereomicroscope.
4. Use pins or forceps to grasp the larva by its middle part and its head respectively.
5. Gently stretch the larva by pulling on it until its head separates from the rest of its body.
6. Look for the salivary glands in the head section. The glands are very small, fairly transparent, usually paired and have dark fat particles attached.
7. Soak the saline with filter paper.
8. Add 2-3 drops of aceto-orcein stain to the salivary glands and keep it for 5-10 mins.
9. After the stain has set, cover the salivary glands with coverslip.
10. Place your thumb on the over the coverslip and press down slowly.

Discussion

1. What are the benefits of having polyteny in the salivary glands?
2. What are the similarities & differences between polyteny and polyploidy?