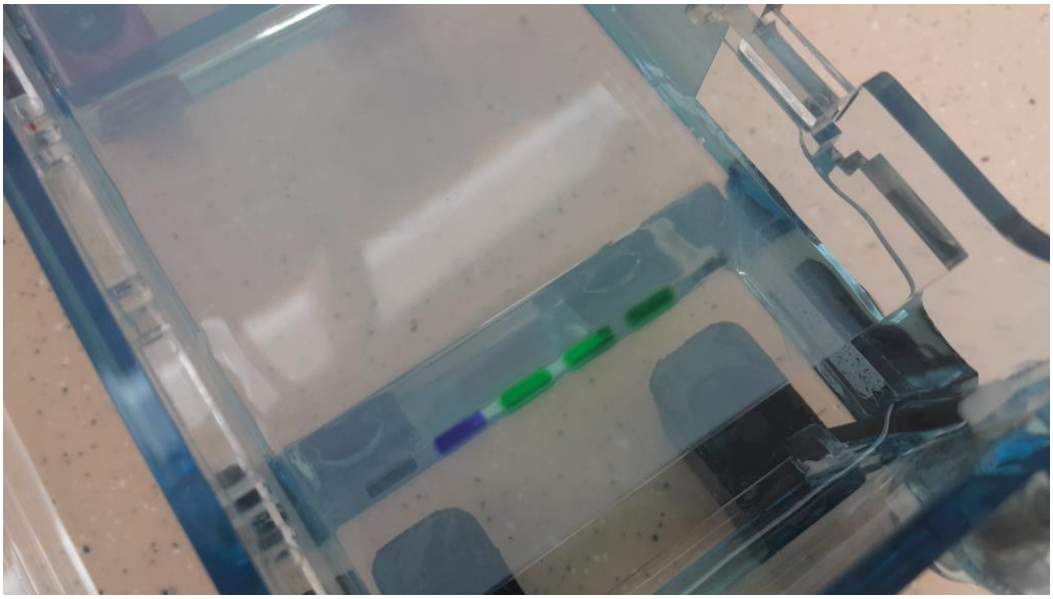
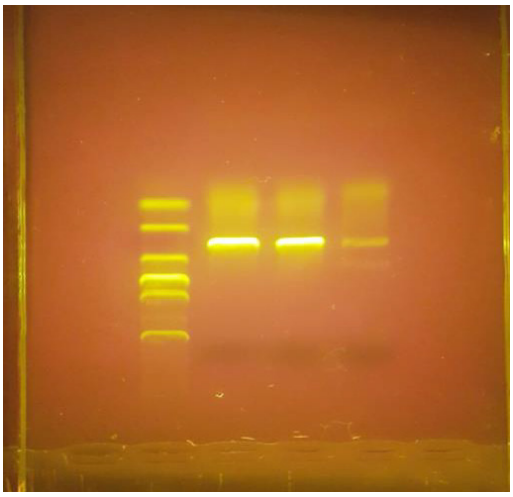
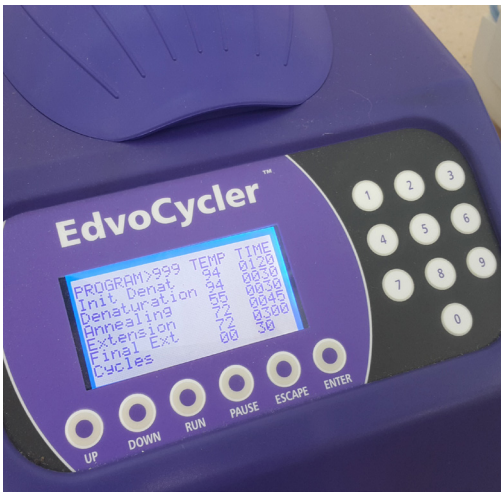
Biotechnology（Advanced）
DNA Extraction, Polymerase Chain Reaction & Gel Electrophroesis







School Name / Student Name / Group

Site / Date / Time / Recent Weather Conditions

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

250 ml conical flask 1

Cooling basin 1

Digital thermometer 1

Electronic balance ..... Share

Gel bed with comb ..... 1 set
(Close off the open ends by paper tape)

Microwave oven ..... Share

Microwave oven wrap ..... Share

Latex gloves ..... 1 pair

Pipettement and tips ..... 1 set

Chemicals

Agarose ..... Share

TAE buffer(1X) 50ml

Lysate Protocol

1. Set a water bath at 55°C.
2. Place 1g tissue into a sterile microcentrifuge tube
3. Add **180 µl Genomic Digestion Buffer and 20 µl Proteinase K** to the tube. Ensure the tissue is completely immersed in the buffer mix.
4. Incubate at 55°C with occasional vortexing until lysis is complete (~1 hour).
5. To remove any particulate materials, centrifuge the lysate at maximum speed for 3 minutes at room temperature.
6. Transfer supernatant to a new, sterile microcentrifuge tube.
7. Add **20 µl RNase A** to lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
8. Add **200 µl Genomic Lysis/Binding Buffer** and mix well by vortexing to yield a homogenous solution.
9. Add **200 µl 99% ethanol** to the lysate.
10. **Lysate Prepared.**

Purification Protocol

1. Add the lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol to the spin column.
2. Centrifuge the column at 10,000rpm for 1 minute at room temperature.
3. Discard the collection tube and place the spin column into a clean Collection Tube supplied with the kit.
4. Add **500 µl Wash Buffer 1** prepared with ethanol to the column.
5. Centrifuge column at 10,000rpm for 1 minute at room temperature.
6. Discard the collection tube and place the spin column into a clean collection tube supplied with the kit.
7. Add **500 µl Wash Buffer 2** prepared with ethanol to the column.
8. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
9. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
10. Add **100 µl of Genomic Elution Buffer** to the column.
11. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.
12. Purified DNA Obtained.

Gel Electrophroesis

Procedures

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

1. Put the conical flask on the electronic balance and set **Zero**.
2. Add **0.75g agarose** into the conical flask.
3. Add **50ml 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:

1) GM papaya
2) Non GM papaya
3) Other plants

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.....1

5ml Syringe.....1

100ml Beaker 1

Coffee filter 1

Filter funnel.....1

Pestle and Mortar 1

Fruit Sample

Test tubes 2

Chemicals

Extraction Buffer: ..... 10 ml

Detergent ..... 100 ml

Distilled water ..... 900 ml

NaCl..... 15 grams

Cold Alcohol (pre-measured)..... 4 ml

Procedures

DNA Isolation using Strawberries

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 test tube.
6. Add **4 ml** of cold ethanol. (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.

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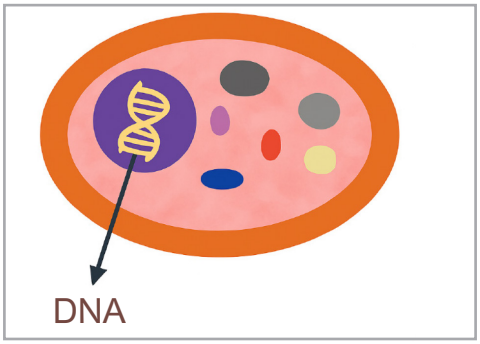
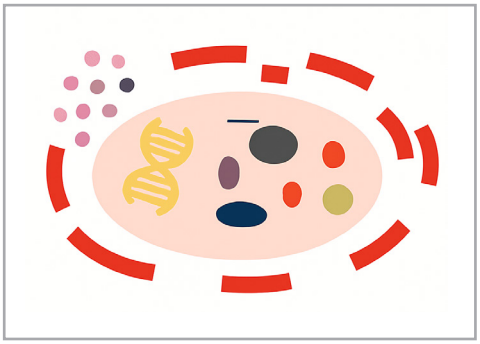
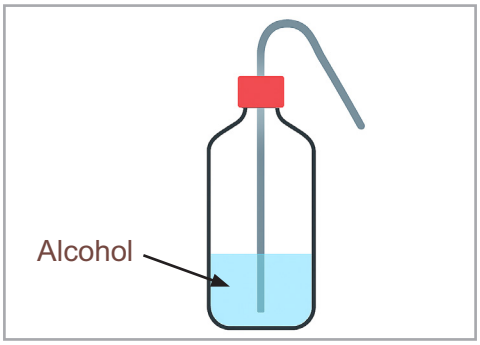
Principles



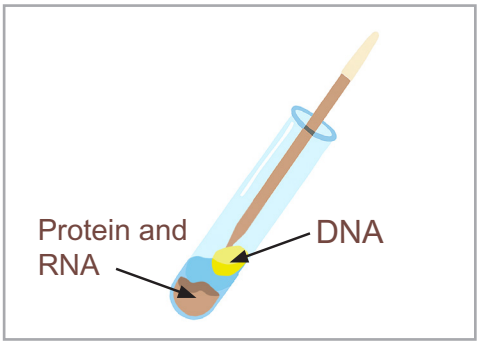
① DNA is in the nucleus of the cell



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.

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?

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6. Transfer supernatant to a new, sterile microcentrifuge tube.
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8. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to yield a homogenous solution.
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10. Lysate Prepared

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2. Centrifuge the column at 10,000rpm for 1 minute at room temperature.
3. Discard the collection tube and place the spin column into a clean PureLink™ Collection Tube supplied with the kit.
4. Add 500 µl Wash Buffer 1 prepared with ethanol to the column.
5. Centrifuge column at 10,000 × g for 1 minute at room temperature.
6. Discard the collection tube and place the spin column into a clean PureLink™ collection tube supplied with the kit.
7. Add 500 µl Wash Buffer 2 prepared with ethanol to the column.
8. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
9. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
10. Add 100 µl of PureLink™ Genomic Elution Buffer to the column.
11. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.
12. PURIFIED DNA OBTAINED