**Year 10 Laboratory Assessment - DNA Extraction**

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

DNA belongs to the group of acids originally found within the nucleus of the cells, hence the name nucleic acids.

We will extract DNA from fruit to investigate how it looks and feels. This procedure is similar to what scientists have to do before they can use the information contained in this DNA. This information can be used to improve crops so that they are more resistant to disease, insect invasion or changes in climate.

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| http://www.apsnet.org/edcenter/K-12/TeachersGuide/PlantBiotechnology/PublishingImages/act1fig01.gifThe various chemicals used allow us to separate DNA from the cell. First of all, the cell wall needs to break open. The soap/detergent/extraction solution ruptures the outer part of the cell, while the salt helps separate DNA from other cellular chemicals, such as carbohydrates. The process of cooling helps protect the DNA from enzymes that normally do not bother the DNA because the DNA remains separated from the rest of the cell by the nuclear membrane. The cold temperature slows down these enzymes. Filtering removes most solid matter in the mixture. The last cooling helps the DNA solidify and precipitate. |  |
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**Objectives**

* Extract DNA from plant cells
* Understand the general structure of cells

**Materials**

* 1-small zip lock bag
* 20 ml of extraction buffer
* Skinned kiwi fruit (each fruit cut into 8 pieces) or one large strawberry (each provides ~30 g per student pair)
* 500 ml beaker (class)
* Cheese cloth
* Ice water bath
* Ice cold 95% ethanol (2 ml)
* 1 small test tube
* 1 glass hook
* Transfer pipettes

**Procedures:** You must show the teacher that you have completed each step of the procedure.

1. Cut half of one kiwi fruit or the strawberry into pieces and place in the zip lock bag.
2. Add 20mL extraction solution in the zipper bag. Close bag and CAREFULLY

squeeze out air.

1. Crush the kiwi/strawberry and extraction solution thoroughly for 5 minutes. CAREFUL

don’t break the bag!

1. Using the ice water bath, alternate cooling the mixture for one minute and continue to

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press the contents together for one minute. Repeat this three times

1. Use the cheese cloth or gauze in a funnel to filter the mixture.
2. Using the large transfer pipettes, place approximately 2 ml of the kiwi/strawberry

fruit solution into a test tube.

1. Add approximately 2 ml of ice-cold ethanol to each tube by dropping it slowly down

the side of the test tube, allowing it to rest on top of the kiwi/strawberry fruit mixture.

Do not agitate the solution.

1. Let the solution sit for two minutes without disturbing it. The DNA stands should form

at the junction between the filtrate and the isopropanol and will appear as transparent,

slimy, white mucus which can be carefully removed with the glass hook.

(2 marks)

**Safety:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

(1 mark)

**Diagram:** (Draw what you see at step 8)

(1 mark)

**Contribution to group:** (1 mark)

**Procedure Questions:** (Use the notes at the start of the experiment and the Internet to answer the questions below)

1. Why do we “crush” the kiwi/strawberry fruit? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(1 mark)

1. Why do we use shampoo? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(2 marks)

1. What does the salt do? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(2 marks)

1. Why do we need to cool the mixture? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(2 marks)

1. What does the cold ethanol do? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(1 mark)

1. Why can’t we use room temperature ethanol? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(2 marks)

**Discussion Questions**

1. Why might DNA need to be extracted from something? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(2 marks)

2. Who might want to have DNA extracted from a cell? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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(1 mark)

**Answers:** Give full marks if they have researched and given a more detailed answer. Half marks if they have only given a basic answer from the notes above.

1. **Why do we “crush” the kiwi/strawberry fruit?** Crushing the kiwi/strawberry fruit physically breaks apart the cell walls.
2. **Why do we use shampoo?** After the cell walls have been disrupted during mechanical mashing of the fruit, the detergent in the shampoo disrupts the cell and nuclear membranes of each cell to release the DNA. It does this by dissolving lipids and proteins that hold the membranes together.
3. **What does the salt do?** The salt neutralizes the negative charges on the DNA and thus enables the DNA strands to stick together. It also causes proteins and carbohydrates to precipitate.
4. **Why do we need to cool the mixture?** DNases or restriction enzymes that destroy DNA are present in the cell’s cytoplasm. They are there to protect the cell from invasion by viruses. Once the nuclear membrane is destroyed by the soap, the DNA is now susceptible to the DNases and will quickly be degraded. However, these enzymes are temperature sensitive and cooling the solution slows down the process of degradation.
5. **What does the cold ethanol do?** Everything except the DNA will dissolve in ethanol. The ethanol pulls water from the DNA molecule so that it then collapses in on itself and precipitates. The DNA will become visible as white mucous strands that can be spooled with the wooden applicator stick.
6. **Why can’t we use room temperature ethanol?** The colder the ethanol is the greater the amount of DNA that is precipitated. (You could try having some of the students use room temperature ethanol and see if the amount of DNA they can spool is the same or less than that for the groups using the ice-cold ethanol.)

1. **Why might DNA need to be extracted from something?** Look for answers such as, to identify parentage, to identify genetic disorders, to identify criminals.

(2 marks)

2. **Who might want to have DNA extracted from a cell?** Look for answers such as police, parents, scientists

(1 mark)