# Experiment (2): Genomic DNA Extraction from Plant Tissue

## Aim:

- To isolate pure genomic DNA from plant tissue.

## Introduction

Studying plant genome allows us to characterize and modify plant genes and metabolic pathways, as well as understanding the genetic variation in species. Transgenic (GM) plants are those that have been genetically modified using recombinant DNA technology. This may be to express a gene that is not native to the plant or to modify endogenous genes. The protein encoded by the gene will confer a particular trait or characteristic (eg: growth or survival) to that plant. The technology can also be used to improve the nutritional content of the plant, as well as production of some industrial products, such as monoclonal antibodies, vaccines, plastics and biofuels.

Method used for extracting DNA from plants is different from extracting DNA from animal sources as the plant contains hard cellulose cell wall and large DNA molecule. Essentially, breaking the cell wall and cellular membranes (lysis) using mechanical or non-mechanical methods to allow access to nuclear material, without its degradation. For this, usually an initial grinding step is employed to break down cell wall material and allow access to DNA while cellular enzymes are inactivated.

The main goal of developing number of protocol for isolating DNA from plant, is to extract DNA with pure and high quality. DNA must be purified from cellular material in a manner that prevents degradation.

PAUSE AND THINK ➔ How extraction of plant DNA differ from animal DNA?

## Principle:

Breaking the cell wall and cellular membranes (lysis) by using mechanical or non-mechanical methods. Mechanical method, is applying force to the **cell wall** to open and spilling the contents. On the other hand, non-mechanical method is the addition of enzymes or chemicals that specifically break down cell wall components in combination with mechanical force. The advantage of mechanical disruption over the non-mechanical is that no chemicals are introduced that might interfere with the extracted substance, and these chemicals need to be removed from the sample afterwards.

After lysis, small cracks were formed in the **cell membrane** for accessibility of detergents. Detergents will break down the cell membranes due to the amphipathic (having both hydrophilic and hydrophobic regions) nature of both cellular membranes and detergent molecules.

The DNA is then precipitated from the protein in a subsequent step with isopropanol or ethanol. The clean DNA is now suspended in a 1xTE buffer or dd.H2O.

## Materials:

### Chemical

Strawberry, Extraction solution, 96% Cold ethanol or isopropanol, and TE buffer or double distilled water.

### Preparation of Extraction Solution

Add 100 ml detergent to 750 ml of distilled water and then add 11 g NaCl. Make up the volume to 1 L with distilled water.

### Equipment and Glassware:

Balance, Microfuge centrifuge, Razor blade, Mortar and pestle, Cheesecloth, Funnel, Gradual cylinder 25 ml, Beaker 50 ml, Test tube, centrifuge tube, and Pasteur pipette.

## Protocol:

1. After removing the green leaf of the strawberry, weight the plant using sensitive balance.
2. Place the plant onto a mortal. Chop it into small pieces using a clean razor blade.
3. Add the DNA extraction buffer on a 1:1 ratio (e.g. if the plant weight 20 g, we will add 20 ml of the solution).
4. Then mix the chopped strawberry pieces using a pestle for 5 minutes.
5. Pour the mixture through cheesecloth into clean beaker
6. Pipette 2 ml of the mixture into a clean test tube.
7. On the same tube, add 2 ml of cold ethanol slowly and Do NOT mix.
8. DNA will appear as a clear white thread.
9. Using a clean Pasteur pipette, spool the DNA onto the hooked end.
10. Immediately transfer the DNA to centrifuge tube, and spin at 6000 rpm for 5 minutes.
11. Gently remove the supernatant (ethanol layer) without disrupting the DNA pellets, and leave it to dry
12. Suspend the pellet in 0.5-1.5 ml TE or double distilled water.

## Results:

Cloudy precipitation can be seen by the naked eye, and it represent the isolated DNA.

## References:

1. Surzycki S. basic techniques in molecular biology. Springer. (2000).