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PREPARATION OF GENOMIC DNA FROM PLANT TISSUES

AIM

To isolate pure DNA from plant

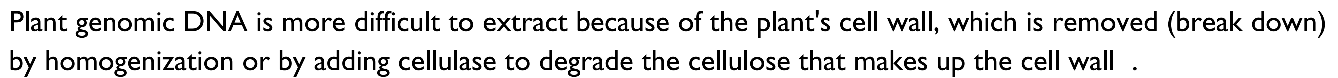
Introduction

Plant cells are distinguishable from animal cells by containing a hard cell wall and organelles like the chloroplasts. They also contain proteins and enzymes that play a key role in photosynthesis. Some plant cells are polyploidy, they have more than one copy of each chromosome per cell. Like mitochondria, plants contain chloroplasts that have their own DNA. The differences between plant and animal DNA lie in the sequence of bases in the helix. Some proteins found in plant cells are absent in animal cells, and DNA base sequences reflect this, as the genomic plant DNA is often larger than animal DNA.

It is important to study plant DNA, It allowed the characterization and modification of genes and metabolic pathways, as well as the use of genetic variation for studies in species diversity.

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 to that plant. The technology can be utilized in a number of ways, that would normally prove detrimental to plant growth or survival. The technology can also be used to improve the nutritional content of the plant, re now also being developed for the production of recombinant medicines and industrial products, such as monoclonal antibodies, vaccines, plastics and biofuels.

Method used for extraction of DNA from the plants is different from extracting DNA from animal sources as the plant contains hard cellulose cell wall. A number of protocol for isolating DNA from plant sources are available which ranges from using simple chemicals in the lab to a more sophisticated Isolation protocol by using kits. The main goal of developing all these protocol is to search, for a more efficient means of extracting DNA of both higher quality and yield. However the fundamental of DNA extraction remains the same. DNA must be purified from cellular material in a manner that prevents degradation. Because of this, even crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow for multiple end uses.



Plant genomic DNA is more difficult to extract because of the plant's cell wall, which is removed (break down) by homogenization or by adding cellulase to degrade the cellulose that makes up the cell wall .

Principle of DNA Extraction from plant:

1-LYSIS:
In DNA extraction from plants, this step commonly refers to the breaking of the cell wall and cellular membranes

1. MECHANICAL METHODS OF CELL DISRUPTION

Mechanical cell disruption is really just that: forcing open the cell wall and spilling the contents. The advantage to mechanical disruption is that no chemicals are introduced that might interfere with the substance you want to extract.

Eg.Mortar and Pestle, Sonication, Freezing,

2. NON-MECHANICAL METHODS

Non-mechanical methods involve the addition of enzymes or chemicals that specifically break down cell wall components. They are often used in combination with mechanical force to ensure complete disruption of the cell. The disadvantage to their use is that they often have to be removed from the sample afterwards.

2-Then the addition of a detergent in the which breaks down the cell membranes

Detergents are able to disrupt membranes due to the amphipathic (having both hydrophilic and hydrophobic regions) nature of both cellular membranes and detergent molecules. The detergent molecules are able to pull apart the membranes

3- PRECIPITATION

The DNA is then precipitated from the protein in a subsequent step with isopropanol or ethanol
In the presence of cations, ethanol induces a structural change in DNA molecules that causes them to aggregate and precipitate out of solution.

4- Resuspension:

The clean DNA is now resuspended in a buffer to ensure stability and long term storage.
The most commonly used buffer for resuspension is called 1xTE

Materials:

Chemicals:

- Strawberry
- Extraction solution
- 96% Cold ethanol or isopropanol
- TE buffer or double distilled water

Extraction Solution:

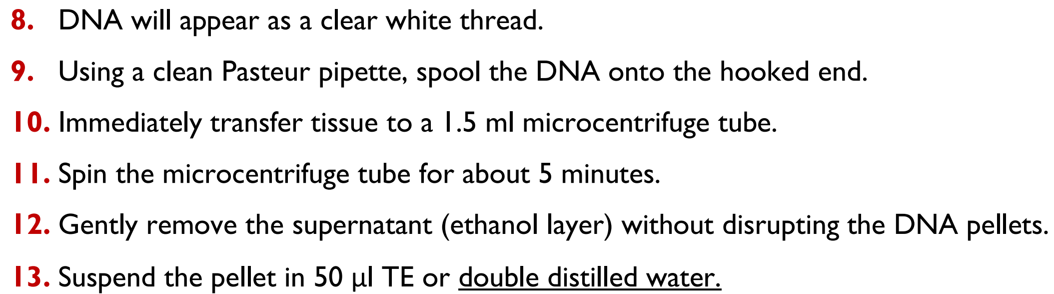
Add 100 ml detergent to 750 ml of distilled water and then add 11 g NaCl. Make up the volume to 1 L with distilled water.

Equipment and Glassware:

- Microfuge centrifuge
- Razor blade
- Mortar and pestle
- Cheesecloth
- Funnel
- Gradual cylinder 25 ml
- Beaker 50 ml
- Test tube
- Microcentrifuge tube
- Pasteur pipette

Experimental 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.



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 tissue to a 1.5 ml microcentrifuge tube.
11. Spin the microcentrifuge tube for about 5 minutes.
12. Gently remove the supernatant (ethanol layer) without disrupting the DNA pellets.
13. Suspend the pellet in 50 µl TE or <u>double distilled water.</u>

Results:

Add a picture of your extracted DNA.

Discussion:

Discuss the purpose of each step that you done.

<u>Questions:</u>

1. DNA from other sources like mitochondrion and chloroplast can precipitate out with your genomic DNA, discuss how you can overcome this problem?
2. What are the sources the can possibly contaminate your DNA sample?
3. What precautions you should use while isolating DNA?
4. Why it is important to remove the ethanol completely before resuspending DNA?