Lab Two: Plant DNA extraction

Introduction

Purpose of DNA Extraction:

To obtain DNA in a relatively purified form, which can be used for further investigations, i.e. PCR, sequencing, etc.

- **Most DNA extraction protocols consist of two parts:**

1. A technique to lyse the cells gently and solubilize the DNA
2. Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules

- **Four steps are used to remove and purify the DNA from the rest of the cell.**
- Lysis
- Precipitation
- Wash
- Resuspension

Lysis: grind in Liquid N_2 or use detergent (this step commonly refers to the breaking of the cell wall and cellular membranes (most importantly, the plasma and nuclear membranes)

- The cell wall (made of cellulose) is disrupted by mechanical force (for example, grinding the leaves)
- Then the addition of a detergent in the which breaks down the cell membranes
- The result of LYSIS is that the contents of the plant cells are distributed in solution.

Precipitation Part I: This a series of steps where DNA is separated from the rest of the cellular components. The first part of precipitation uses phenol/chloroform to remove the proteins from the DNA

- Phenol denatures proteins and dissolves denatured proteins.
- Chloroform is also a protein denaturant

Precipitation Part II: addition of salt, the salt helps to get rid of the proteins that package the DNA tightly inside the nucleus.

Precipitation Part III: addition of alcohol (absolute ethanol or isopropanol), to pull DNA out of solution. The DNA is pelleted by spinning with a centrifuge and the supernatant removed

Washing

The precipitated DNA is laden with acetate salts. It is "washed" with a 70% ethanol solution to remove salts and other water soluble impurities but not resuspend the DNA.

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 buffer**

Checking the Quality of DNA

The quality of the extracted DNA (absence of degradation) was estimated based on the size of the DNA fragments or relative position of the DNA smears in 1% agarose gel electrophoresis.

To determine DNA concentration, total extracted DNA was quantified using Nano drop instrument **using an equation:**

DNA concentration μg/ml= O.D 260nm*50 *dilution factor

The purity was calculated according to formulae:

1-DNA purity = O.D 260nm / O.D 280nm
This ratio used to detect nucleic acid contamination with protein.
2- DNA purity = O.D 260nm / O.D 230nm
This ratio is used to detect nucleic acid contamination with RNA.

Experiment: DNA Extraction from Kiwi fruit

1- Materials:	2-chemicals
1-Two kiwi fruits 2-- Knife 3-morters 4-stainers 5-beakers 6-test tubes 7-swapes	1- washing up liquid 2-distilled water 3-table salt 4- cold 95% ethanol or isopropanol

A-Preparation of the extraction solution:

-In one of your beakers measure out about 80 mls water

- Add half a teaspoon of salt and stir until dissolved

- Add two teaspoons of washing up liquid and stir gently avoiding making too many bubbles

B-Prepare your fruit mush

Peel your kiwis and chop into small pieces

Mush the kiwi thoroughly by using mortar.

C-cellular lysis

Add the extraction solution to the fruit mush

Leave at room temperature for about 20 minutes

D- Filter the solution

E- Precipitating the DNA

-Remove the alcohol from the freezer

-Carefully pour about equal volumes alcohol to the DNA solution down the side of the tube walls. After about 10 minutes you should be able to see a mass of white stringy stuff at the top of the tube.

Benefits of additives

Detergent: The membranes of the cell and of the nucleus are rich in fats so detergent can break them down so the DNA can be released.

Salt: The salt helps to get rid of the proteins that package the DNA tightly inside the nucleus.

Advantage of solution filtration:

This gets rid of the fruit pulp and seeds and should leave a pure solution of DNA