Prepare CTAB solutions to extracting DNA from Plant

By

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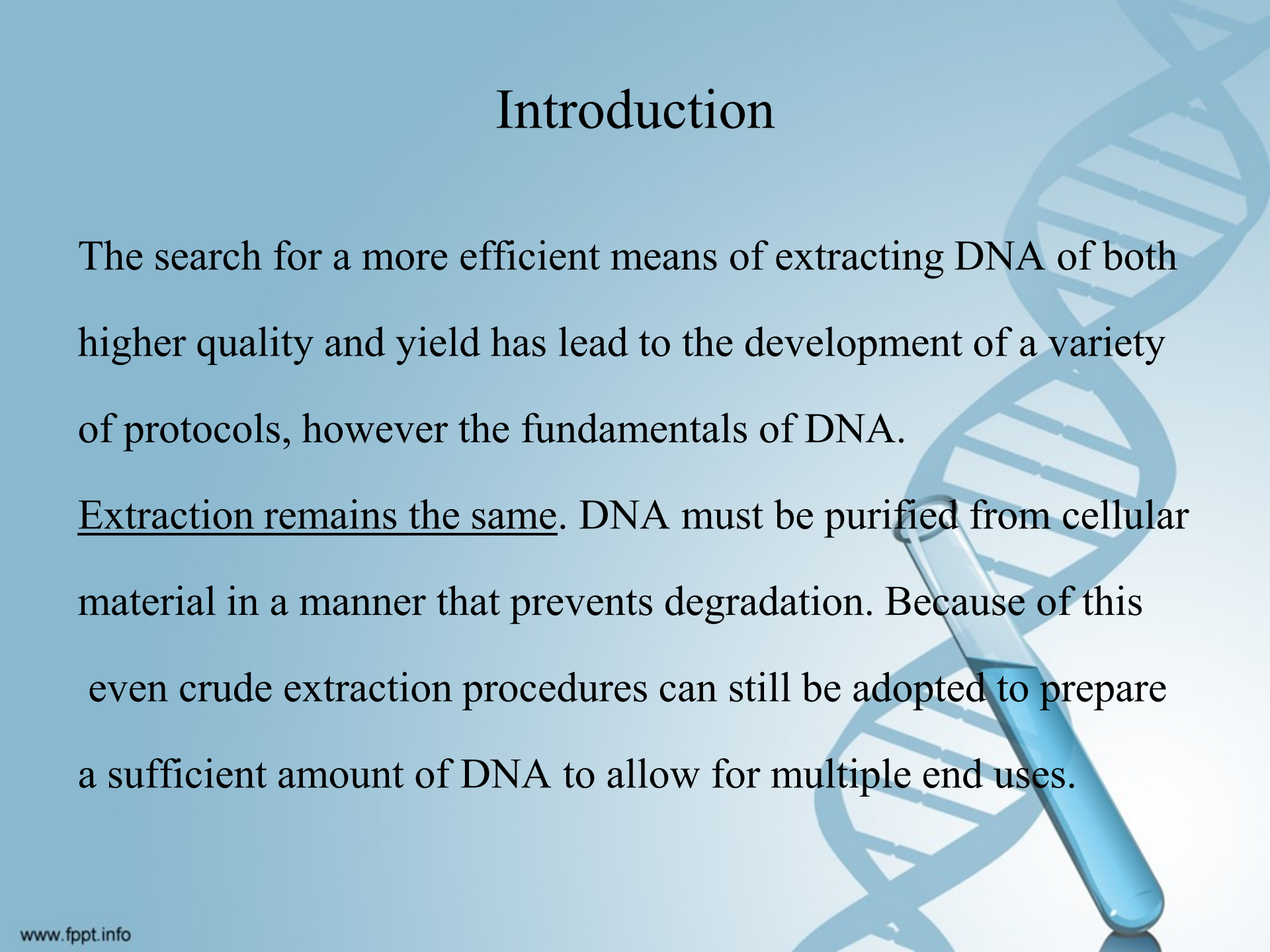
Introduction

The search for a more efficient means of extracting DNA of both higher quality and yield has lead to the development of a variety of protocols, however the fundamentals 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.



DNA extraction from plant tissue can vary depending on the material used. Essentially any mechanical means of breaking down the cell wall and membranes to allow Access to nuclear material. without its degradation,

<u>For this !!</u>

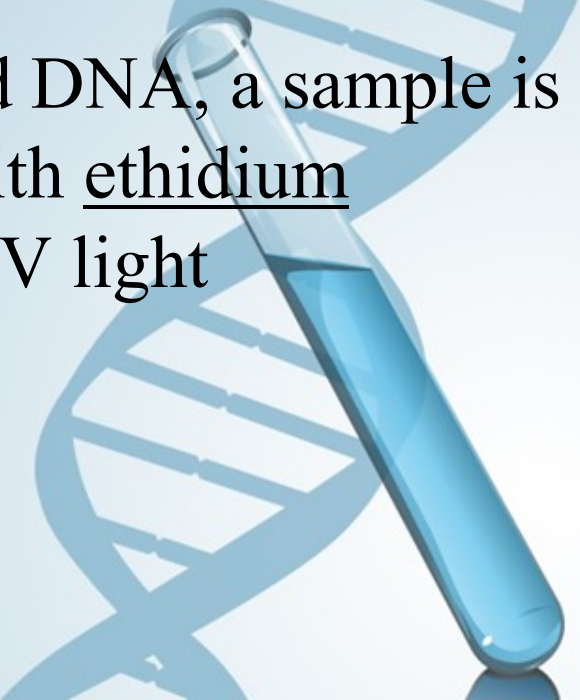
1. Initial grinding stage with liquid nitrogen (to break down cell wall material and allow accessto DNA).
2. Be resuspended in a suitable buffer, such as CTAB.
3. In order to purify DNA, insoluble particulates are removed through centrifugation while soluble proteins and other material are separated through mixing with chloroform and centrifugation.

4- DNA must then be precipitated from the aqueous phase and washed thoroughly to remove contaminating salts.

5-The purified DNA is then resuspended and stored in TE buffer or sterile distilled water.

6- This method has been shown to give intact genomic DNA from plant tissue.

To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualised under UV light



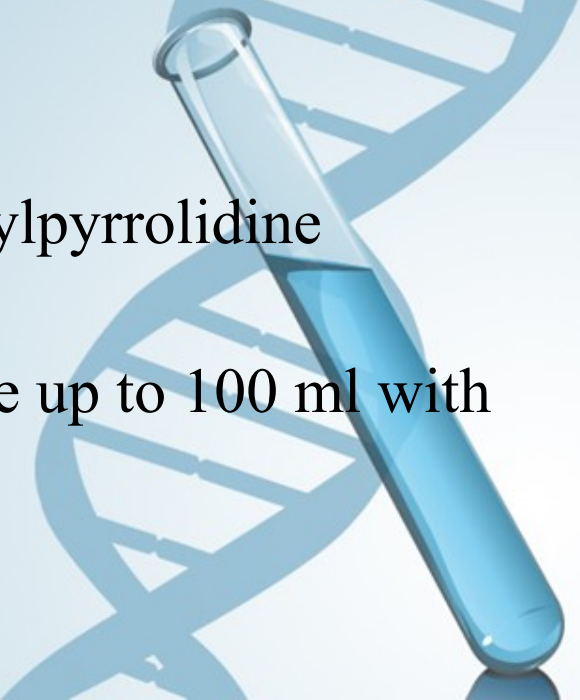
Materials

- CTAB buffer
- Microfuge tubes
- Mortar and Pestle
- Liquid Nitrogen
- Microfuge
- Absolute Ethanol (ice cold)
- 70 % Ethanol (ice cold)
- 7.5 M Ammonium Acetate
- 55° C water bath
- Chloroform : Iso Amyl Alcohol (24:1)
- Water (sterile)
- Agarose
- 6x Loading Buffer
- 1x TBE solution
- Agarose gel electrophoresis system
- Ethidium Bromide solution

Preparation of solutions

❖ **CTAB buffer 100ml**

- 2.0 g CTAB (Hexadecyl trimethyl-ammonium bromide)
- 10.0 ml 1 M Tris pH 8.0
- 4.0 ml 0.5 M EDTA pH 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)
- 28.0 ml 5 M NaCl
- 40.0 ml H2O
- 1 g PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) Mw 40,000)
- Adjust all to pH 5.0 with HCL and make up to 100 ml with H2O.



Preparation of solutions

❖ **1 M Tris pH 8.0**

- Dissolve 121.1 g of Tris base in 800 ml of H2O. Adjust pH to 8.0 by adding 42 ml of
- concentrated HCL. Allow the solution to cool to room temperature before making the
- final adjustments to the pH. Adjust the volume to 1 L with H2O. Sterilize using an
- autoclave.

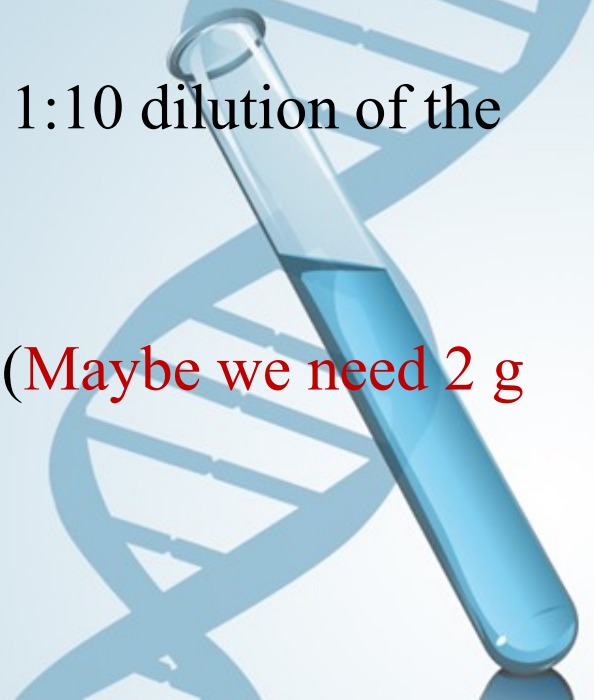
Preparation of solutions

❖ **5x TBE buffer**

- 54 g Tris base
- 27.5 g boric acid
- 20 ml of 0.5M EDTA (pH 8.0)
- Make up to 1L with water.
- To make a 0.5x working solution, do a 1:10 dilution of the concentrated stock.

❖ **1% Agarose gel**

- 1 g Agarose dissolved in 100 ml TBE. (Maybe we need 2 g Agarose).



Procedere

1. Grind 200 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.
2. Transfer CTAB/plant extract mixture to a microfuge tube.
3. Incubate the CTAB/plant extract mixture for about **15 min at 55° C in a recirculating water bath**.
4. After incubation, spin the CTAB/plant extract mixture at **12000 g for 5 min** to spin down cell debris. Transfer the supernatant to clean microfuge tubes.
5. To each tube add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, **spin the tubes at 13000 rpm for 1 min**.
6. Transfer the upper aqueous phase only (contains the DNA) to a clean microfug tube.

7. To each tube add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.
8. Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 o C after the addition of ethanol to precipitate the DNA.
9. Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution.
10. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat. (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol).

11. After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm for 1 min.
12. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). Do not allow the DNA to over dry or it will be hard to re-dissolve.
13. Resuspend the DNA in sterile DNase free water (approximately 50-400 µl H_2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 µg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 µl RNaseA in 10ml H_2O).
14. After resuspension, the DNA is incubated at 65o C for 20 min to destroy any Dnases that may be present and store at 4o C.
15. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness

DNA quality confirmation

1. Prepare a 1 % solution of agarose by melting 1 g of agarose in 100 mL of 0.5x TBE buffer in a microwave for approximately 2 min. Allow to cool for a couple of minutes then add 2.5 µl of ethidium bromide, stir to mix.
2. Cast a gel using a supplied tray and comb. Allow the gel to set for a minimum of 20 min at room temperature on a flat surface.
3. Load the following into separate wells
   - 10 µL 1kb ladder
   - 5 µL sample + 5 µL water + 2 µL 6x Loading Buffer
4. Run the gel for 30 min at 100 V – Expose the gel to UV light and photograph (demonstration)
5. Confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degredation.