DNA isolation & extraction

CTAB TECHNIQUE / Method / Schedule / Protocol FOR DNA ISOLATION / DNA EXTRACTION FROM PLANT LEAF / LEAVES SAMPLES

(see also DNA RNA double isolation procedure if both DNA and RNA are needed)

Reagents needed

CTAB buffer

2% CTAB 20gm CTAB

20mM EDTA 40ml EDTA stock (0.5M)

100mM Tris-Cl pH 8.0 100ml Tris-Cl stock (1M)

1.4M NaCl 280ml NaCl stock (5M)

make up to 1 Litre with water, pH 7.5 - 8.0, and autoclave

+ 0.2% Mercaptoethanol

Wash Buffer

76% Ethanol

10mM NH 4 Ac

DNA Extraction

1. Preheat 5ml CTAB (add 10µl mercaptoethanol to each 5ml CTAB) in a blue-topped 50ml centrifuge tube at 60-65 o C. Remove and discard midribs, and wrap laminae in aluminium foil and freeze in liquid nitrogen. 0.5 – 1.0 gm tissue/5ml CTAB

(Can store leaf material after liquid Nitrogen – 1-2 days at –20 or –80 for longer periods)

2. Gently crumble leaf tissue over cold pestle of liquid nitrogen. Grind frozen leaf with one spatula of fine sand add 0.5 spatula of PVPP powder after grinding.

3. Scrape powder into dry tube and add pre-heated buffer and mix gently. Avoid leaving dry material around rim of tube. Adjust CTAB volume to give a slurry-like consistency, mix occasionally.

4. Incubate for 60 min at 60 o C

5. Add equal volume of chloroform/iso-amyl alcohol (24:1), Mix for about 3min, then transfer contents to narrow bore centrifuge tubes. Balance by adding extra chlor/iso. Spin 5,000rpm for 10min (ensure correct tubes used), brake off. (For extra pure DNA isolation - spin and retain supernatant before chloroform extraction).

6. Remove supernatant with wide-bore pastette (cut off blue tip) to clean tube, repeat chloroform extraction once. Supernatant should be clear, though may be coloured.

7. Precipitate DNA with 0.66 vol. of cold isopropanol - can leave overnight. Spool out or spin down DNA, 2min at 2,000rpm.

8. Transfer to 5ml wash buffer for 20min.

9. Dry briefly and resuspend in 1ml T.E. (can be left overnight)

10. Add 1µl 10mg/ml RNAse to each 1ml T.E./DNA mixture and incubate for 60min at 37 o C. (If RNase in the sample doesn't matter – stages 11 and 12 may be omitted)

11. Dilute with 2 volumes TE and add 0.3vol 3M Sodium acetate

(pH 8) + 2.5 vol cold 100% ethanol,

12. Spool DNA out. Air dry and resuspend in 0.5 to 1ml TE or water (takes time) and freeze until required.

DNA Quantification

An approximate way to determine DNA concentration is to look at the viscosity of the solution: not accurate to 10% but, unlike spectrophotometry, you will not get results which are 10 or 100 times wrong!

In a microcentrifuge tube, DNA solutions stronger than 0.1 ug/ul will show a reluctance to pour when you tilt the tube. From about 0.5 ul/ug and above, you can tilt the tube - very gently - and the solution will stay at the end. If you dip a 10-200 ul (yellow) pipette tip into the solution and pull it away, a solution of 1ug/ul will form a distinct string from the surface to the tip which breaks when about 1 to 2 mm long.

Make a 0.8% agarose gel with 1x TAE and 0.1µl of Ethidium bromide (10mg/ml) per 10ml solution. Load samples undiluted and at a 1 in 10 (1+9) dilution., with 3µl loading buffer. Also include a Lamda ladder cut with HindIII and EcoRI. This contains 100ng of DNA per microlitre and use as follows:

1µl ladder + 4µl water + 2µl loading buffer

2µl ladder + 3µl water + 2µl loading buffer

The different bands of the ladder are of known molecular weight and known DNA concentration. Match the brightness of your samples with those of the two dilutions of the ladder. Refer to the diagram to match the band with the concentration. Remember that although the ladder concentrations are absolute, you have loaded 5µl of sample and also diluted some of them. This must be taken into account when calculating the strength of the sample s in ng/µl.

Pestles and mortars washed for 20-30min in 0.25M HCl, rinsed in water and air-dried, all mess to be tidied up and tubes washed and left to drain.

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