CTAB

Description

This is a good method to get clean DNA from difficult organisms (mostly plants and molluscs), adding PVP to the CTAB buffer can help with nasty polysaccharides, can be combined with a column wash to give nice clean DNA.

What you need

1. Hexadecyltrimethylammonium bromide (CTAB)
2. PVP (optional)

Protocol

1. Take tissue, smash it in the ball mill
2. Add 500µl of CTAB @ 65^{o}C (see recipe below) and 4µl proK and lyse @ 55^{o}C for 3 hrs or overnight (optional, can lyse at 70^{o}C for 1hr instead)
3. Cool on ice, add 500µl (1X) Chloroform and shake for 15secs
4. Spin for 5-15 mins full speed
5. remove aqueous (top) layer to new tube, discard the organic nasties in the correct chemical disposal container. Make sure not to pipette up any solvents (chloroform), or tissue from the interphase, it will cause problems downstream, best not to be too greedy, if you get some then put it back and spin it again, if it looks dirty repeat 3-5.

6. Add equal volume EtoH, then either proceed as per below or put it through a column (after adding some binding salt ~1X)

7. Spin max speed 15mins, tip out ethanol (not pellet).

8. Add 500µl 70% EtOH

9. Spin 5 mins full speed

10. Dry ethanol off

11. Resuspend in favourite buffer (reccomend EB, 10mM tris ph8)

CTAB buffer (1 litre)

CTAB (Cetyl Trimethyl Ammonium Bromide)	20g
NaCl	81.2g
0.5M EDTA	40ml
1M Tris pH8	100ml
(optional) Polyvinylpyrolidone (PVP40) MW=40,000	20g
Clean H_2O	fill to 1l

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