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CTAB DNA Extraction for high quality/molecular weight DNA

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Mimulus

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EXTERNAL LINK

http://mimubase.org/FTP/Protocols/DNA_extraction/CTAB%20DNA%20Extraction%20(High%20Molecular%20Weight).pdf

CTAB DNA Extraction (High Molecular Weight).pdf

GUIDELINES

For Safety Warnings and Hazard Information please refer to the SDS (Satety Data Sheet).

MATERIALS

NAME	CATALOG #	VENDOR
liquid nitrogen		
70% Ethanol		
CTAB DNA Extraction buffer		
Chloroform: IsoAmyl Alcohol (24:1)		
7.5M Ammonium acetate		
100% Ethanol		
dH2O		

MATERIALS TEXT

CTAB DNA Extraction Buffer (Recipe to make 100 mL)

10 mL 1 M Tris Buffer
8.3 g NaCl (1.4 M)
0.744 g EDTA
2 g CTAB
2 g PVP
0.088 g Asorbic acid

1 Grind plant tissue in a mortar cooled with liquid nitrogen.

2 Add **750 µl** **CTAB DNA Extraction buffer**.

3 Wait until it warms up and becomes a green paste, then transfer to an eppie tube.

4 Incubate the CTAB/plant extract mixture for **00:15:00** at **55 °C** in the heat block and invert to mix throughout the 15 minutes.

5 Add **500 µl** **Chloroform**: IsoAmyl Alcohol (24:1) in the hood and mix the solution by inverting the tubes (**do not vortex**).

6 Centrifuge at **13000 rpm** for **00:10:00**.

7 Transfer the upper aqueous phase **only** to a new eppie tube (~ **500 µl**).

8 Add RNase A (10 µg/ml).

5 µl of 1mg/ml stock if you have **500 µl** of sample.

9 Incubate at **37 °C** for **00:30:00**.

10 Add **50 µl** **7.5M Ammonium acetate** followed by **500 µl** **ice cold 100% ethanol** and invert to mix.

11 Put tubes in **-20 °C** **freezer** for 1 hour (or longer) to precipitate the DNA.

12 Centrifuge at **13000 rpm** for **00:15:00**.

You should see a pellet at the bottom (align the tubes so that you know where the pellet is in case you can't see it very well).

13 Remove the supernatant and wash the DNA pellet as follows. (1/2)

13.1 Add **500 µl ice cold 70% ethanol** . (1/2)

13.2 Centrifuge at **13000 rpm** for **00:05:00** . (1/2)

14 Remove the supernatant and wash the DNA pellet as follows. (2/2)

14.1 Add **500 µl ice cold 70% ethanol** . (2/2)

14.2 Centrifuge at **13000 rpm** for **00:05:00** . (2/2)

15 Remove all the supernatant and allow the DNA pellet to dry in the hood (approx. **00:20:00**).

Do not over dry the pellet since it will be hard to re-dissolve.

16 Resuspend the DNA in **50 µl dH2O**.

17 NanoDrop the sample to estimate the concentration.

Alternatively the DNA can be run on a gel to estimate the concentration or size of the DNA.
Running a 0.4% gel overnight with the lambda DNA mono-cut ladder can give you an estimate of the size (it still doesn't separate the large bands very well).