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CTAB DNA Extraction for genotyping

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Mimulus

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EXTERNAL LINK

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

CTAB DNA Extraction (Regular).pdf

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

SAFETY WARNINGS

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

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1 Grind fresh plant tissue with liquid nitrogen or silica-gel dried tissue in a 1.5 ml Eppie tube.

A little silica gel grains in the tube actually helps the grinding.

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

3 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.

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

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

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

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

8 Put tubes in **-20 °C** freezer for **00:30:00** (or longer) to precipitate the DNA.

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

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

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

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

10.2 Centrifuge at **130000 rpm** for **00:05:00** . (1/2)

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

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

11.2 Centrifuge at **130000 rpm** for **00:05:00** . (2/2)

12 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.

13 Resuspend the DNA in **100 µl dH2O**.

14 Run the DNA on a 1.0% agarose gel to check the quality of the DNA.

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