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 We use this protocol and it's working

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CTAB DNA Extraction for genotyping

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Mimulus

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ATTACHMENTS

[CTAB DNA Extraction \(Regular\).pdf](#)

MATERIALS

MATERIALS

- ✂ Liquid Nitrogen **Contributed by users**
- ✂ 70% Ethanol **Contributed by users**
- ✂ CTAB DNA Extraction buffer **Contributed by users**
- ✂ Chloroform: IsoAmyl Alcohol (24:1) **Contributed by users**
- ✂ 7.5M Ammonium acetate **Contributed by users**
- ✂ 100% Ethanol **Contributed by users**
- ✂ dH2O **Contributed by users**

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).

Keywords: DNA extraction,
Mimulus

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

Note

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 inverting the 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 .

Note

You should see a pellet at the bottom (align the tube so 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 μ L 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)

11.1 Add 500 μ L 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).

Note

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.