CTAB DNA Extraction for genotyping

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Works for me
dx.doi.org/10.17504/protocols.io.3rrgm56

Mimulus

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

ATTACHMENTS
CTAB DNA Extraction (Regular).pdf

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PROTOCOL CITATION
Yaowu Yuan 2019. CTAB DNA Extraction for genotyping. protocols.io
https://dx.doi.org/10.17504/protocols.io.3rrgm56

KEYWORDS
DNA extraction, Mimulus

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CREATED
Jun 05, 2019

LAST MODIFIED
Jun 12, 2019

OWNERSHIP HISTORY
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Jun 12, 2019  Andrea Sweigart  University of Georgia

PROTOCOL INTEGER ID
24081
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 (Safety Data Sheet).

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 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.

   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).

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)

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