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CTAB DNA Extraction for genotyping

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Protocol status: Working
We use this protocol and it's working

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Attachments

CTAB DNA Extraction ...

26KB

Materials

MATERIALS

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

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).

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 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 .

Note

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)

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).

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.