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CTAB DNA Extraction for high quality/molecular weight DNA

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High molecular weight D... | Mimulus

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

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Attachments

PDF

CTAB DNA Extraction ...

28KB

Guidelines

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

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

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).

Note

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 .

Note

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).

Note

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.

Note

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).