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CTAB Extraction from Sample Ground in Liquid Nitrogen

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Jordan Beasley: I have written up this protocol shared with me by Claire Griffin, Sequencing Assistant at the Natural History Museum, London.

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

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Disclaimer

This protocol was shared with me by Claire Griffin, Sequencing Assistant at the Natural History Museum, London. Thank you Claire!

Abstract

This protocol describes the process of grinding a sample in liquid nitrogen and extracting DNA with a CTAB extraction.

Materials

1.5ml Eppendorf tubes
Pestle and mortar
Liquid nitrogen dewer
Liquid nitrogen
500µl CTAB
50µl Sarkosyl
50µl Proteinase K
200ul and 1ml tips
300µl Phenol chloroform
1ml SEVAC
400µl Isopropanol
500µl 70% Ethanol
Nuclease free water

Cryogenic Grinding and CTAB Extraction

1 Dispense some liquid nitrogen in a small dewer (small thermos size)

2 Fill a mortar with liquid nitrogen to cool it and let it boil off. When it has completely boiled away, add a small amount more into the mortar and grind with pestle to a fine powder. Scrape the powder into a 1.5ml tube.

3 Add 500µl CTAB, 50µl sarkosyl and 50µl Proteinase K to the powder and vortex to mix.

4 Place tube in a hot block at 60°C overnight or if in a rush 60-90 minutes.

5 After incubation add 300µl of phenol chloroform (This extracts the DNA into an aqueous layer).

6 Mix by inversion for 15 minutes on a rotator if you need high molecular weight. If you don't need high molecular weight, vortex well.

7 Centrifuge for 3 minutes at 16 rpm (max speed) and label a new tube.

8 Carefully remove the top aqueous layer and transfer to the new tube. Avoid disturbing any of the white protein layer.

9 Add 500µl SEVAC and mix by placing on a rotator for 15 minutes (or vortex well if you don't require high molecular weight DNA).

10 Centrifuge for 3 minutes at 16 rpm (max speed) and label a new tube.

11 Carefully remove the top aqueous layer and transfer to the new tube. Avoid disturbing any of the white protein layer.

12 Repeat steps 9-11.

13 Add 400µl isopropanol and mix by inverting - DO NOT VORTEX! You may see DNA precipitate out.

14 Centrifuge for 3 minutes at 16 rpm (max speed), you might see a pellet.

15 Remove as much of the liquid as you can without disturbing the pellet.

16 Add 500µl 70% ethanol (to wash away isopropanol)

17 Wash pellet by inverting several times

18 Centrifuge for 3 minutes at 16 rpm (max speed) and remove as much ethanol as possible without disturbing the pellet.

19 Place tube on a hot block with the lid open at 60°C for 5 minutes to evaporate off any remaining ethanol.

20 Resuspend in 30-50µl sterile water (flick the tube to help the pellet dissolve and if stubborn, pop back on the hot block for a few minutes).

21 Quantify extract on Nanodrop and/or Qubit.