**Min’s Lab Genomic DNA Extraction Protocol**

**on Fagaceae Samples**

**(30 Nov. 2017)**

**Reagents:**

**1. CTAB extraction buffer Stock (500 mL)**

CTAB: 10 g

NaCl: 41 g

1M Tris-HCl (pH 8.0): 50 ml

EDTA-Na2: 3.7g.

*Add distilled water to 500 ml*

*Autoclave 120℃ 20 min, Store at RT.*

**2. Lysis Buffer**

**N-Lauroyl Sarcosine Sodium Salt (mw 293.28): 20g**

1M Tris-HCl (pH8.0): 20 ml

0.5M EDTA: 8 ml

*Add distilled water up to 200 ml*

*Autoclave 120℃ 20 min, Store at RT.*

**3. Wash Buffer (1 ml/sample)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Wash buffer volume** | **PVP** | **Ascorbic Acid** | β-mercaptoethanol. | **Distilled water** |
| 5 ml | 51 mg | 45 mg | 100 ul | up to 5 ml |
| 10 ml | 102 mg | 90 mg | 200 ul | up to 10 ml |
| 20 ml | 204 mg | 180 mg | 400 ul | up to 20 ml |
| 50 ml | 510 mg | 450 mg | 1000 ul | up to 50 ml |
| 100 ml | 1020 mg | 900 mg | 2000 ul | up to 100 ml |

**Please prepare this reagent in fresh. Wash step might need to take 2-3 times [or more], if the sample is sticky.**

**Steps:**

1. Adding 200-300 mg fine powder of leaves into a 2 ml tubes, then adding 1 ml Wash Buffer. Votex and mix well.

### Spin at 10.000 rpm, 5 min at 16-20℃. Discarding the upper liquid using [pipette](https://www.sigmaaldrich.com/catalog/product/aldrich/z683922?lang=en&region=US). If the sample is very “sticky” [e.g. the boundary of solid and liquid is blunt, wash and spin again, until it is clear.]

1. Adding 800 ul ***DNA extraction Buffer*** to each tube

***DNA extraction buffer:***

***CTAB stock: Lysis buffer: distilled water*: β-mercaptoethanol [added just before use]** = 100: 10: 89: 1

**Pre-warm CTAB stock-Lysis buffe-distilled water mixture at 60** ℃

1. Incubate at 60℃ for 40 min. Shaking up and down 20 times every 5 min.
2. Add 800 ul Chloroform/Isoamyl alcohol (24:1) to the tubes, then shaking fast [ca 40 times up and down]. Spin at 15,000 rpm, 10 min, at RT.
3. Transfer the supernatant to a new 1.5 ml tube [about 600-700 ul];
4. Add 700 ul Chloroform/Isoamyl alcohol (24:1) to the tubes.
5. Shaking fast [ca 40 times up and down]. Spin at 15,000 rpm, 10 min, at RT.
6. Transfer the supernatant to a new 1.5 ml tube. (\*if the boundary between two liquid with a white thick layer, repeat step 7 to 9 again)
7. Adding -20℃ pre-chilled isopropanol 600 ul and store at -20℃ for 20 min. Meanwhile, pre-chilled the centrifuge to 4℃.
8. Spin at 15.000 rpm, 4 ℃ for 10 min
9. Get rid of supernatant liquid and wash 3 times with pre-chilled 70% ethanol.
10. Resuspend DNA in 50 ul TE buffer or distilled water.
11. Add 0.5 ul 10mg/ml RNAse into the DNA liquid, mixture well and incubate at 37℃ for 60 min;
12. Add 5 ul 3M NaAC (pH 5.2), and >3 total volume of pre-chilled 100% ethanol, mix well and store at -20℃ for 30 min;
13. Spin at 10,000 rpm at 4 ℃ for 10 min
14. Get rid of upper liquid, wash the pellet with 400 ul pre-chilled 70% ethanol for 3 times, then with 500 ul 100% pre-chilled ethanol;
15. The tube with caps open at RT for ca. 15 min to dry (but not too dry)
16. Resuspend the pellet in 100 ul of TE.
17. 0.65% Agarose gel checking

**Trouble shootings:**

**Sticky sample**

1) If having the chance, place the branch into zip bag and place it into dark place for 3-7 days before the extraction;

2) Using Wash Buffer 2-4 times before moving to CTAB extraction step;

**Browning issue**

1) Using Wash Buffer more times and double the dose of **Ascorbic Acid** in the Wash buffer;

2) Reduced the leaf powder for extraction to 100 mg. [but need to more tubes per sample, to eventually get enough DNA for downstream experiments!]

3) Less time when 60℃ incubation. If using 20 ml extracting system dealing with 1 g leaf powder, After adding preheated 20 ml 60 ℃ CTAB DNA extraction buffer, no incubation needed, just shake up and down for 60 times, then the sample will be good to move to the 24:1 extraction step!

4) Doubling the volume of β-mercaptoethanol in CTAB extraction buffer might be helpful to avoid the sample turning to brown, but it will reduce about 25% of DNA yield.

**Final solution** on sticky browning samples **[but will lost about 40-50% DNA yield]:**

NaCl-glass milk biding protocol to purify DNA. If you have a lot of fresh leaves, it might be an easier way to do. Just following the standard CTAB protocols after resolved DNA pellet in to TE buffer [step 13], the do the biding purification.