Customer Developed Protocol

CTAB Protocol for Isolation DNA from Plant Tissue

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Contributor Research Profile

The Van Deynze lab at UC, Davis is a Plant Molecular Biology, Biochemistry and Genomics lab that works on elucidating the basis of capsaicin synthesis and resistance to phytophthora in pepper.

Read more about the Van Deynze Lab: http://biosci3.ucdavis.edu/Faculty/Profile/View/14262
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Reference

This protocol was used in:

Amanda M. Hulse-Kemp, *et al*., Reference Quality Assembly of the 3.5 Gb genome of *Capsicum annuum* from a Single Linked-Read Library, *Biorxiv*. 2017 June 20.
https://www.biorxiv.org/content/early/2017/06/20/152777

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Required Buffers and Reagents

1. AMPure XP Beads (Beckman Coulter)

2. DNA Extraction Buffer:

100 mM Tris (pH 8.0)
20 mM EDTA
2% CTAB
1.2 M NaCl
0.1% ß- mercaptoethanol (Add before use. After adding ß- mercaptoethanol, the Extraction Buffer must be used within a day)

2. Chloroform-Isoamylalcohol (CIA) 24:1 (v/v)

3. Dilution Buffer:

100 mM Tris Buffer (pH 8)
20 mM EDTA
2% CTAB

4. High Salt TE:

10 mM Tris (pH 8)
2 mM EDTA
1 M NaCl (add RNase A at 50 µg/ml)

6. Wash Buffer:

Add 300 ml TE to 700 ml ethanol

7. TE:

10 mM Tris
1 mM EDTA (pH 8)

DNA Extraction Protocol

1. For freeze-dried tissue, grinding is done using a disposable microcentrifuge pestle directly in a 2 ml microcentrifuge tube (~200 mg tissue). Keep samples cold until the DNA Extraction Buffer is added. For seeds, grinding is done using a Geno/Grinder 2010 by placing one 5 mm tungsten ball into a rounded bottom 2 ml microcentrifuge tube.
2. Add 750 µl Extraction Buffer to the ground tissue using a multichannel pipette.
3. Cap the tubes and mix well. Incubate at 60°C for 1 hour. Mix the tubes gently every 10-15 minutes during the incubation period.
4. Cool the tubes to room temperature for 5 min, then add 750 µl Chloroform:Isoamylalcohol (24:1, v/v) to each tube, mix well and centrifuge at 3000 g for 15 minutes.
5. Transfer the aqueous layer (~500 µl) to a new set of labeled tubes.

6. Add 1 ml Dilution Buffer to the aqueous phase.
7. Mix well and incubate at 60°C for ~30 minutes. (Note: Significant amounts of precipitated DNA-CTAB complex should be observed at the end of incubation).
8. Centrifuge at 4500 g for 15 minutes and discard the supernatant.
9. Add 1 ml Wash Buffer to the pellet and soak it in at room temperature for 30 minutes to remove any excess CTAB.
10. Centrifuge at 4500 g for 15 minutes and discard the supernatant.
11. Re-suspend the DNA pellet in 100 µl high salt TE with RNase A and incubate at 60°C for 30 minutes.
12. Transfer the high salt TE DNA solution to a 96-well microtiter plate, one sample per well.
13. Add 1X volume of AMPure XP beads (i.e. 100 µl sample + 100 µl AMPure XP beads).
14. Mix well by pipetting up and down.
15. Incubate the plate for 10-15 minutes.
16. Place on a magnetic stand for 2-5 minutes (or until clear) to collect beads.
17. Gently remove the solution without disturbing the bead pellet.
18. Add 150 µl 75% ethanol solution (keep plate on the magnet).
19. Wait 30 seconds and remove ethanol without disturbing the beads.
20. Repeat the ethanol wash and then let the beads air dry for 2-5 min (make sure you do not over-dry the beads as it will make re-suspension harder).
21. Add 100 µl Tris/H2O to each sample to re-suspend the DNA, mix well by pipetting up and down.
22. Incubate plate for 10-15 minutes.
23. Place on a magnetic stand for 2-5 minutes (or until clear) to collect beads.
24. Transfer the solution into a new 96-well plate (stock DNA).