Customer Developed Protocol

High Molecular Weight Genomic DNA Extraction from Grape Leaves

Contributed and Tested by:
Xia Xu, Lance Cadle-Davidson lab, USDA-ARS GGRU, Geneva, NY

Contributor Research Profile

The Cadle-Davidson lab at USDA ARS (Agricultural Research Service) addresses the critical needs for sustainable management of grapevine fungal and oomycete diseases. Their collaborative research aims to develop new grape varieties that are healthy, delicious, and disease resistant, exemplified by the VitisGen project that they co-lead.

Read more about the lab and their research: https://www.ars.usda.gov/northeast-area/geneva-ny/grape-genetics-research/people/lance-cadle-davidson/

References

This protocol is a modification of:

Rapid and Efficient Isolation of High Quality Nucleic Acids from Plant Tissues Rich in Polyphenols and Polysaccharides. Japelaghi RH, Haddad R, Garoosi GA. *Mol Biotechnol*. 2011 Oct, 49 (2): 129-37. https://www.ncbi.nlm.nih.gov/pubmed/21302150

Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. Haley A, Furtado A, Cooper T, Henry RJ. *Plant Methods*. 2014, 10:21 https://www.ncbi.nlm.nih.gov/pubmed/25053969

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10x Chromium™ *de novo* Assembly Solution - https://www.10xgenomics.com/assembly/

Notices

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Required Buffers and Reagents

1. DNA Extraction Buffer – Final Composition:

100 mM Tris-HCl pH 8.0
25 mM EDTA pH 8.0
1.5 M NaCl
2% (w/v) soluble PVP (MW 40000)
2% CTAB
1% (v/v) 2-Mercaptoethanol*

Extraction Buffer - Preparation (10 mL required per sample):

Reagent	Stock Conc.	Final Conc.	10 mL need	30 mL need	40 mL need
Tris-HCl	1 M (pH 8.0)	100 mM	1 mL	3 mL	4 mL
EDTA	0.5 M (pH 8.0)	25 mM	0.5 mL	1.5 mL	2 mL
NaCl	5 M	1.5 M	3 mL	9 mL	12 mL
PVP-40		2 % (w/v)	200 mg	600 mg	800 mg
CTAB		2 % (w/v)	200 mg	600 mg	800 mg
2-Mer.*		1 % (v/v)	100 uL	300 uL	400 uL
H2O**				~ 11 mL	~ 18 mL
Total (mL)			**10 mL**	**30 mL**	**40 mL**

NOTES: (1) DNA extraction buffer should incubate in 65°C for at least 20-30 min before use.
(2) 2-Mercaptoethanol (2-Mer.) *Add immediately before use.
(3) ** First add a small volume of H2O and keep at 65°C for a while (until the solution is clear), then add the remaining volume.
PVP-40: Polyvinylpyrrolidone (Sigma: PVP40-500G);
CTAB: Hexadecyltrimethylammoniumbromide (FW=364.46) (Sigma: H6269-500G)

2. Chloroform-Isoamylalcohol (CIA) 24:1 (v/v) (50 mL)

3. 95% Ethanol (100 mL) (store at -20°C)

4. 5 M Sodium Chloride (NaCl)

5. DNAse-free RNAse an enzyme (100 mg/mL or 10 mg/mL)

6. 70 % Ethanol (v/v) (store at 4°C)

7. EB buffer (10 mM Tris-Cl, pH 8.5) or Nuclease-Free Water, or 0.1 mM EDTA (pH 8.0).

DNA Extraction Protocol

NOTE: DNA extraction is performed at room temperature; after grinding tissue, be very gentle, do not vortex.

1. For leaves, use a ratio of 0.8 - 1.4g tissue per 10 mL extraction buffer.
2. Prepare extraction buffer and pre-warm at 65°C for at least 20-30 min. Before use, add 1% (v/v) 2-mercaptoethanol and keep warm while grinding tissue.
3. Grind samples in liquid nitrogen using a pre-chilled mortar and pestle (tissue should be ground to a fine powder). [Alternatively, use a grinder mill: Each 15-mL PC Vial, add 2 big stainless grinding balls; Use GENO/GRINDER: 1x RATE, 300 strikes/min, and 3 x 40 sec.]
4. Add the still-frozen, ground tissue into a 50 mL-tube containing 10 mL of the extraction buffer, gently invert several times to mix (use spatula to suspend the tissue clump).
5. Incubate tubes in a 65°C water bath for 20-30 min. Invert gently every 5 min.
6. Centrifuge the sample tube for 5 min at 5000 x g to pellet the leaf tissue, and transfer the supernatant into a new tube.
7. Add an equal volume of CIA (24:1 v/v) (~10 mL), invert for 5 min (we use Thermolyne Vari-Mix platform rocker at speed 25); centrifuge 10 min at 5000 x g, and transfer the aqueous phase into a new tube (never touch the middle layer; if you do, centrifuge again).
8. Add 6 µL of 100 mg/mL RNase A, and incubate at 37°C for 15 min; gently invert two times.
9. Add an equal volume of CIA (24:1 v/v) (~7.5 mL) and invert for 5 min on platform rocker; centrifuge 10 min at 5000 x g. Pipet supernatant to new 50 mL-tube (Note: It is hard to avoid taking the middle layer, so transfer some supernatant and spin down 7 min at 5000 x g again, move the clear solution to new 50 mL-tube).
10. Add 1/2 volume of 5M NaCl (~ 3.5 mL), invert gently to mix, add 3 volume equivalents of cold 95% EtOH (~ 21 mL – do not use higher concentration EtOH than 95%), and gently invert the tube several times:
    a. When you see DNA cloud form, and use pipet tip to hook the DNA, and put into 2 mL-tube with 2 mL 70% EtOH. Spin and wash one more time use 2 mL 70% EtOH, go to step 13 to air-dry DNA.
    b. For DNA left in 50 mL-tube after hooking, incubate at -20°C for 30 min (never more than 1 h), then go to step 11.
11. Collect DNA pellet by centrifugation 10 min at 5000 x g, and discard supernatant.
12. Wash the pellet with 6 mL 70 % ethanol, spin at 5000 x g for 5 min. Wash again use 2 mL 70% ethanol, and gently swirl the solution, carefully transfer to 2 mL-tube; centrifuge for 5 min at 5000 x g. Carefully discard the supernatant, spin briefly and remove any ethanol by using a P200 pipet.
13. Air-dry DNA pellet until there is no residual ethanol in the tube (up to 15 min at room temperature).
14. Gently suspend the pellet in 300 µl EB buffer (10 mM Tris.HCl, pH 8.5), or 0.1 mM EDTA solution (pH 8.0).

15. Assess the quality of the extracted DNA using a NanoDrop and 0.7 % agarose gel, looking for a single absorbance peak at 260 nm, a 260/280 absorbance ratio of 1.8-2.0, and no evidence of substantial band shearing or contamination (either RNA or polysaccharide).
16. Measure the DNA concentration using a Qubit Fluorometer.
17. Check the DNA size by running PFGE gel.
18. Store DNA at -20°C until use.

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