

CTAB DNA extraction from pink berries.

Wilbanks

7/29/2010

Protocol devised drawing on protocols from Arsneau et al. 2017 and the Current Protocols in Molecular Biology (2003, ISBN: 047150338X; in our google drive; Protocols/Molecular Biology).

(<https://onlinelibrary.wiley.com/doi/full/10.1111/1755-0998.12616>)

Protocol as yet unverified. Results pending!

Materials (this might be incomplete, read through protocol 1st!)

- See solution recipes at end of document.
- 80% Ethanol
- Liquid nitrogen
- Ceramic mortar and pestle
- Spoon-like spatula for scraping out mortar
- Chloroform: Isoamyl alcohol (24:1)
- Lysozyme
- Proteinase K
- Water bath or oven @ 37C
- Water bath or oven @ 55C
- Water bath or oven @ 65C (for CTAB)
- Rotary mixer
- CTAB
- NaCl
- Tris HCl
- SDS
- EDTA
- ammonium acetate
- Nanodrop
- Qubit BR dsDNA kit & Qubit tubes
- TapeStation access and/or gel electrophoresis and/or Pulse field gel electrophoresis
- DNA Ladders: Ideally NEB Lambda DNA Mono cut for regular gel electrophoresis; 1kb extend if not that.

Protocol:

1. Weigh out 0.5 – 1.5 g wet weight of berries. These should either be fresh or should have been frozen and *kept frozen*. It is important to keep material frozen and only thaw it in lysis buffer (where EDTA will chelate nucleases). Otherwise your DNA will be too degraded for a HMW prep.
2. Cool down a ceramic mortar and pestle by pouring liquid nitrogen in and allowing it to boil off at least 2 times.
 - a. (note: lizzy actually finds working with the blue cryo gloves more dangerous as you can accidentally get your fingers in LN more easily. In thin gloves you at least know! Consider using nitrile gloves with a rag or towel to hold the mortar instead of cryo gloves)
3. With mortar less than half full of liquid nitrogen add berries. If fresh, allow them to freeze prior to grinding.

4. Grind berries with pestle. Start slowly to avoid splashing them out the sides.
 - a. You may want a friend to help with this and might want to carefully add in some more liquid nitrogen if you find it takes you a while to grind, though if you've precooled mortar twice this usually isn't necessary.
 - b. Grind until it is a fine pink powder. It will go from liquid to "wet" looking to dry as the liquid nitrogen evaporates off. This is good. It's easiest to transfer in its "dry" frozen form
5. Chill the "spoon like" end of your metal spatula in liquid nitrogen. Chill a 15 mL falcon tube in liquid nitrogen (or smaller if scaling down). Use this cold spoon to collect your powder and transfer carefully to your pre-chilled tube. (A friend here or quick hands is helpful).
 - a. This powder **must** be kept frozen from now until it lysis buffer is added, but can be stored in the -80 freezer and extracted later.
6. Just before beginning extraction freshly prepare a 100 mg / mL solution of lysozyme (in water) See below for amount needed.
7. Add 2 mL of Tris Lysis Buffer with Extra Extra (TLBE) to your powdered material.
 - a. Depending on your volume of material, if you have trouble resuspending in this volume, you can add more here and steps 11 and later accordingly
8. Add 100 uL lysozyme.
9. Vortex quickly and thoroughly.
 - a. NOTE: This is the only time you'll vortex in this protocol but its very important that this lysis solution is well mixed. DNA will not shear as it is still protected inside the cells. Any time after this though vortexing will ruin your chances of isolating HMW DNA.
10. Incubate at 37C for 1 hour.
11. Add 3 mL (1 volume) of warm CTAB lysis buffer and 100 uL of Proteinase K (~20 mg/mL; I used NEB P8107S).
12. Mix gently by inverting slowly end over end 3 times.
13. Incubate for 3 hours at 55C, mixing end over end 3x every 30 minutes. By end, lysate should have a pinkish or rusty supernatant with visible clumps.
14. Cool to room temperature.
15. Add 5 mL (1 volume) chloroform:isoamyl alcohol (24:1)
16. Mix for 10 minutes on rotary mixer at 20 rpm (~3 seconds per rotation) until a fine emulsion forms. If not emulsion, slowly increase speed and continue mixing.
17. Spin @ 5000 g for 15 minutes (10 would probably be enough). Use 4C if easy / possible.
18. Remove aqueous (top) layer to a fresh 15 mL falcon. Pipet gently using p5000 and p1000 for last bit. Be very careful to avoid the white layer at interface. Better to leave some behind than to contaminate!
19. Add 1/10 volume (~500 uL) of CTAB extraction buffer and 5 mL (1 volume) chloroform:isoamyl alcohol (24:1) and extract again as in steps 15 – 18.
20. Remove aqueous layer to clean 50 mL falcon.
21. Add 2 volumes (10 mL) warm CTAB precipitation buffer.
22. Mix gently by inversion.
 - a. This precipitate is your CTAB-DNA complex. Contaminating carbohydrates stay in solution at this step.

- b. Ideally you should see a white precipitate. If you don't, don't despair. Incubate at 55°C; ideally in a gently shaking incubator at ~150 rpm (slower if this seems too fast!). If no shaking incubator, then just do still in water bath with occasional mixing.
 - c. Try first for 1 hour, if still nothing or not much, incubate over night.
- 23. Spin at 16000 g for 3 minutes at room temp. (If doing this in large tubes, simply spin at max speed for your rotor).
- 24. Remove supernatant but save just in case.
- 25. Add 1 mL 80% ethanol to your pellet. Work pellet away from the side of the tube with either a gentle flick or by gentle agitating with a pipet tip. You want the pellet to be able to fully bathe in the ethanol.
- 26. Incubate 15 minutes.
- 27. Spin 3 min 16,000 g. Remove ethanol.
- 28. *(Optional; I did not do this as I was concerned about losing any more DNA. It seemed fine to skip based on our results to date.)* Re-suspend the DNA pellet in 100 µL of High-salt TE buffer and add RNase A to a final concentration of 50 µg/mL.
 - a. Incubate the samples at 56-60°C for 15 minutes to degrade RNA. Add 900 µL of 75-80% ethanol and mix the tube well by inverting. Spin down the tube at 16,000g for 3 minutes and discard the supernatant. Note: you may alternatively precipitate the DNA onto paramagnetic bead preparations (such as AMPure® XP, MagBio HighPrep™ PCR, or custom preparations) if preferred for high-throughput applications.
- 29. Wash in 1 mL 80% ethanol again. Spin. Remove supernatant.
- 30. Air dry pellet.
- 31. Resuspend in 50-200 uL EB; take care to get all the precipitate on tube sides.
- 32. Allow to rehydrate 1-2 days

Quality Control:

- 1. Make 1:10 dilution using 1 uL taken with a cut pipet tip.
- 2. Take nanodrop reading. Should have 260/280 >1.8; 260/230 >1.8 (2 and 2.2 are ideal)
- 3. Quantify using Qubit BR (buffer in BR kit is important to solubilize your DNA. Based on estimate from nan)
- 4. Check size on TapeStation if possible (easy)
- 5. If not, run a gel. Mix 1 uL of 1:10 dilution with 4 uL of EB. Add 1 uL loading dye. Be careful not to overload your gel with DNA as this will cause smearing that is independent of degradation. Run a gel, either:
 - a. 0.8% (w/v) agarose gel at 40V using either 1kb extend NEB ladder (can resolve if >10 kb, or 1kb whatever you've got) or, better,
 - b. 0.4% (w/v) agarose gel at 10V for 24 hours using the NEB Lambda DNA Mono cut ladder (can resolve if >48 kb),
 - c. or, even better than any of the above, figure out how to do pulse field gel electrophoresis (PFGE can resolve megabase sized chromosomes).

Solution recipes:

TLBE (Tris Lysis Buffer with Extra EDTA):

NaCl	100 mM
Tris HCl, pH 8	10 mM
EDTA, pH 8	100 mM
SDS	0.5% (w/v)

To make 50 mL:

1 mL	5 M NaCl
0.5 mL	1 M Tris HCl, pH 8
10 mL	0.5 M EDTA, pH 8
1.25 mL	20% (w/v) SDS

Fill with water to 50 mL; Filter sterilize.

CTAB Extraction Solution (2x)

CTAB	2% (w/v)
Tris Cl pH 8	100 mM
EDTA	100 mM
NaCl	1.4 M

For 50 mL:

CTAB	1g
1 M Tris	5 mL
0.5 M EDTA	2 mL
NaCl	4.09 g

Fill to 50 mL line in Falcon with dI water.
Heat to 65C, filter sterilize. Must be kept @ 55-65C for use.

CTAB Precipitation Solution (2x)

CTAB	1% (w/v)
Tris Cl pH 8	50 mM
EDTA	10 mM ** in future if using for extraction, try increasing to 100 mM!

(another version simply uses same as extraction but omits NaCl, this version here the one I've used)
Heat to 65C, filter sterilize. Must be kept @ 55-65C for use.

High Salt TE * don't need for this protocol but referred to as an option.

Tris HCl pH 8	10 mM
EDTA	0.1 mM
NaCl	1 M

For 50 mL:

1 M Tris	0.5 mL
0.5 M EDTA	1 uL
5 M NaCl	10 mL

Fill to 50 mL line in Falcon with dI water; filter sterilize.

EB ("Elution Buffer"):

Tris HCl, pH 8	10 mM
----------------	-------