High molecular weight DNA extraction protocol

Last modified by Wei Wei, March 2019

*use wide-bore tips

1. Grind about 1~2 grams of young leaf tissue in liquid nitrogen
2. Transfer the ground tissue into a 50mL centrifuge tube with 20mL extraction buffer. When adding the tissue, use one spatula (pre-chilled in liquid nitrogen) to hold the tissue, and shake slightly to let tissue fall into buffer little by little (but never let them thaw before adding). At the same time, use another spatula to stir the buffer, which helps to fully mix.

Note: check that you have a nice mix of tissue in buffer. There should be no clumps of frozen tissue. It should be "*smoothie consistency*".

3. 65C for 30min and inverse the tube carefully every 10min.
4. Cool down to room temperature. Add an equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1) pH6.7
5. Do not vortex to mix. Mix by holding the tube horizontally, with the bottom of tube facing towards you. Shake slightly back and forth to mix for 20 times. Spin at 8000g at 10C for 10min.

Note: Make sure it's mixed thoroughly. If contaminants remain a lot, you will see the DNA stuck in gel well (a bright well) when you do the electrophoresis.

6. Transfer the aqueous phase to a new tube using a wide bore 10mL tip. Add an equal volume of Chloroform: Isoamyl Alcohol (24:1). Mix in the same way as step 5. Spin at 8000g at 10C for 10min.

Note: When pipetting the aqueous phase into the new tube, I put my tip under the liquid surface or very low because I am afraid the gravity could break DNA molecules.

7. Transfer the aqueous phase to a new tube. Add 15uL 10 mg/mL RNase A (final concentration 10ug/mL). Incubate at 37C for 20min. Invert gently to mix.
8. Repeat the wash with Chloroform: Isoamyl Alcohol (24:1).
9. Transfer the aqueous phase to a new tube. Add 1/10 volume 3M NaAc. Mix by inversion. Add 0.7 volume Isopropanol and mix gently by inversion. Put in -20C for 10min.
10. At most times I can see a cloud of DNA precipitation and use a glass hook to get the precipitation out to a 2ml Centrifuge tube with 800mL 75% EtOH in it. Spin at 8000g at 4C for 10min.

Note: If no cloud of DNA precipitation seen, spin at 8000g at 4C for 10min. Discard the supernatant and add 800mL 75% EtOH to the pellet. Spin at 8000g at 4C for 10min.

11. Discard the supernatant and air dry the pellet.

Note: To facilitate the drying, I do a short spin and use a 100uL tip to remove the liquid in the bottom. But be careful not to touch the pellet.

12. Resuspend the DNA in 100 to 200uL TE buffer.

CTAB extraction buffer

2% CTAB
100mM Tris pH9.5
1.4M NaCl
1% PEG 6000
20mM EDTA

Use CTAB buffer no more than one month old; better prepare fresh every time you use
Add 50 βME to 20mls CTAB buffer before using