DNA extraction procedure for Populus

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V3.0

Stock solutions:

- 1 M Tris-HCl pH 8 (Store at room temperature)
- 8 M LiCl (Store at room temperature)
- 500 mM EDTA pH 8 (Store at room temperature)
- 5% W/V SDS (Store at room temperature)
- 500 mM DTT (make 500 µl aliquots and store at -20 C)
- Antifoam A (Store at room temperature)

Working solution:

- Lysis/binding buffer (**LBB**) (Store at 4 C and warm up to RT before use by placing at 37 C for several minutes)

Solution components	stock solutions	volumes for 50 ml
100 mM Tris-HCl	1 M pH 8	5 ml
500 mM LiCl	8 M	6.25 ml
10 mM EDTA	500 mM pH 8	1 ml
1% SDS (or LiDS)	5% w/v	10 ml
5 mM DTT	0.5 M	500 µl
Antifoam A		750 ul
		RNAse-free H2O to 50 ml

Immediately before adding to ground tissue add 5 µl/ml 2-Mercaptoethanol. Ensure salt crystals are fully dissolved and Antifoam A is fully homogenized in solution prior to each use.

Other reagents:

- 100% Acetone (Store at room temperature)
- 80% Ethanol (Store at room temperature)
- 100% Isopropanol (Store at room temperature)
- 3M Sodium Acetate (Store at room temperature)
- 95% Ethanol (Store at -20C)
- 70% Ethanol (Store at -20C)
- 1X TE buffer (Store at room temperature)
- 10 mM Tris-HCl pH 8 (Store at room temperature)

Procedure:

● Grind the samples (>100mg) in liquid N2 and add 1 ml of LBB and vortex thoroughly to homogenize.

If any fragments of tissue are visible, place tubes in room temperature bead beater block and bead beat for an additional 1-minute to fully homogenize tissue lysate. (This is particularly important for small tissue samples)

● Incubate at 65C for 45 minutes. Occasionally invert each tube several times

● Centrifuge for 10 minutes at maximum speed at RT.

● Carefully transfer supernatant (900ul) to fresh tubes avoiding transfer of tissue debris.

● From 900ul of LBB lysate, transfer 200ul to a fresh 1.5ul tube and add 1 volume (200ul) of 100% Isopropanol. Store the rest of the LBB lysate at -80C

● Invert several times to mix completely, let stand 5 minutes and place in centrifuge on maximum for 5 minutes at maximum speed

● Remove and discard supernatant without disturbing pellet (decanting is ok)

● Add 400 µl of 100% Acetone to each tube. Let stand at room temperature for 10 minutes with occasional gentle inversion (every 2 minutes)

You should be able to see acetone soluble substances dissolving into the solution and the acetone taking on the color of the pellet. You should be able to see when this process has gone as far as it is going to.

● Carefully remove supernatant avoiding disturbing pellet (Do not decant). The pellet may have become dislodged so be careful not to suck it up.

● Add 400 µl of 100% Acetone for second wash. Let stand at room temperature for 2 minutes with occasional gentle inversion. You will see when the pellet is fully de-colored.

● Centrifuge 1 minute on maximum and discard the supernatant.

● Allow pellet to dry fully and add 100 µl 10 mM Tris-HCl pH 8. (Drying of Acetone occurs quickly.)

● Allow DNA to re-suspend into solution. Place at 65 C (Agarose oven or heat block) for 10 mins to speed the process. Gently flick the bottom of the tubes occasionally to homogenize the solution without grinding the pelleted junk into unmanageably small pieces (Do not vortex).

● Centrifuge samples on maximum for 1 minute.

● Transfer up to ~100 µl of each sample to fresh 1.5 ml Eppendorf tubes being careful to avoid chunks of pellet debris.

● Add 10 µl of 3M Soduium acetate to each sample and mix.

● Add 250 µl of 100% EtOH to each sample and mix by inverting several times and let stand 5 minutes.

● Centrifuge on maximum for 2 minutes.
If you can see precipitate forming quickly then shorter centrifuge times are fine but if the amount of DNA is very small longer times are recommended.

● Carefully remove and discard supernatant with a 200 µl pipette (decanting is ok). The DNA pellet should be very clean and thus totally invisible so be careful to avoid the areas of the tube where the pellet would form. When the supernatant is removed the DNA should be as transparent as glass.

● Wash DNA pellet by adding 400 µl of 80% EtOH to each tube and mix by inverting. Allow to stand 2 minutes with occasional inverting to remove excess Ammonium acetate.

● Centrifuge on maximum for 30 seconds.

● Carefully discard the supernatant and dry samples on the bench or in hood or speed-vac.

● Add 50 µl 10 mM Tris to each pellet to re-suspend DNA.

RNase treatment:

● Add 50ul of RNase (10ug/ml) and Incubate at 37C for 1 hr.

● Add $1/10^{th}$ volume (10ul) of 3M NaOAc and 2 volumes (200ul) of ice cold 95% ethanol

● Let rest in freezer for 1 hr

● Spin for 10 minutes at 13,000 rpm

● Remove ethanol (decant is ok) and add 100ul of ice-cold 70% ethanol

● Spin for 5 minutes at 13,000 rpm. Remove ethanol (decant is ok)

● Dry samples. Resuspend DNA in 40ul of Tris-Hcl (pH 8.0)

Gel running:

● 2ul of 6X LB + 5ul of DNA