High Throughput DNA extraction procedure for Populus

Upendra Kumar Devisetty
04/14/2015
V1.0

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 (Cat no# A1302-100ML Store at room temperature)

Working solution:

Lysis/binding buffer (**LBB**) (Store at 4C 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	5% w/v	10 ml
5 mM DTT	0.5 M	500 µl
Antifoam A (optional)		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:

- Deep well 96 well plates (Cat no # 780201 VWR (Greiner Bio-one))
- Strip cap tubes (Cat no# CA60872-494, T100-35)
- 100% Isopropanol (Store at room temperature)
- 3M Sodium Acetate (Store at room temperature)
- 70% Ethanol (Store at -20C)
- 1X TE buffer (Store at room temperature)

Procedure:

1. Grind the samples (~100mg) in deep well plates in liquid N2 with strip caps tubes on and add 0.4 ml of LBB and vortex thoroughly to homogenize in Genogrinder (settings: speed - 1700rpm and 15s x 3).

2. Vortex the plate thoroughly for 1-2 minutes and incubate at 65C for 45 minutes. Vortex and invert the plate occasionaly.

3. Spin the plate in bucket centrifuge at max (4000rpm) for 20 min at RT.

4. Separate 200ul of the lysate and add 50ul of RNase to it and cap it. Put the plate in a shaker for 5 minutes and incubate at 37C for 1 hour.

5. Add 250ul of Isopropanol to 250ul of tissue lysate in a 96 deep well plate. Cap it and mix the contents gently on shaker for 5 min

6. Spin the plate in bucket centrifuge at max (4000rpm) for 15 min at RT.

7. Carefully discard the supernatant

8. Wash the DNA pellets with 600ul of 70% EtOH and centrifuge at 4k rpm on plate centrifuge at RT for 15 min

9. Discard the EtOH using pipette and remove any traces of remaining EtOH by inverting the blocks onto absorbent paper towel. Put the block on a clean bench and air dry for 30 min

10. Dissolve the DNA pellet with 60ul of 0.1X TE (Use pre-warm TE at 65C)

11. Centrifuge at 4k rpm for 15 min at RT

12. Carefully transfer 50ul of supernatant from each well to new 96-well plate