96-Well Format DNA Extraction for Direct PCR

Modified from Bellstedt et al. (2010) by Matt Jones

1. Add a single ball bearing to each 1.2 ml tube containing tissue (1 cotyledon sized piece works, up to 1 small leaf normally)
2. Add 350µL grinding buffer
3. Disrupt tissue by shaking with paint shaker for 1 min. Repeat if clumps of tissue remain
4. Spin for 5min @ 3000rpm
5. Add 4µL of extract to 25µL GES buffer in PCR tube
6. 95°C for 10 min, 4°C for 5 min
7. Add 0.5µL of final extract to standard PCR reaction (20µL)

100mL Grinding Buffer

To prepare 100 mL of grinding buffer dissolve all below components except Tween 20 in double-distilled sterile water. Stir at low speed to avoid excessive foaming. Once all components have dissolved, adjust pH to 9.6 using NaOH solution. Then add Tween-20. Autoclave and store at 4°C.

1. Na_2CO_3 (Sodium Carbonate) 0.159g
2. $NaHCO_3$ (Sodium Hydrogen Carbonate) 0.293g
3. PVP 40 (Polyvinylpyrrolidone) 2g
4. BSA (Bovine Serum Albumin) 0.2g
5. Tween 20 50µL
6. $Na_2S_2O_5$ (Sodium Metabisulphite) 1g

100mL GES Buffer

1. 1M Glycine, pH9, 10mL
2. 2M NaCl, 2.5mL
3. 0.5M EDTA, pH8 200µL
4. 500µL Triton X-100

Reference

Belstedt et al (2010) American Journal of Botany, 97(7):e65-e68