**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. Na2CO3 (Sodium Carbonate) 0.159g
2. NaHCO3 (Sodium Hydrogen Carbonate) 0.293g
3. PVP 40 (Polyvinylpyrrolidone) 2g
4. BSA (Bovine Serum Albumin) 0.2g
5. Tween 20 50μL
6. Na2S2O5 (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