Wheat and Barley DNA Extraction Protocol (96-well plate format) (updated October, 2009 kim)

NOTE: The original reference that this protocol is based on is:
Pallotta, MA, P Warner, RL Fox, H Kuchel, SJ Jefferies and P Langridge (2003) Marker assisted wheat breeding in the southern region of Australia. Proceedings of the Tenth International Wheat Genetics Symposium (1-6 September, 2003, Paestum, Italy) p.789-791. Contact person: Patricia Warner at patricia.warner@adelaide.edu.au.

1a. tissue is harvested into 1.1 ml collection tubes with stainless steel beads and frozen at -80°C. Samples are ground frozen for 2 minutes at 400 on the Spex GenoGrinder. [frozen ½ seed 2-5 min at 500 (1500strokes/min)]

1b. tissue is harvested into 1.1 ml collection tubes with ~0.25g of silica gel (6-12 mesh). Dry samples at room temp. Samples are ground in silica gel using Spex GenoGrinder for 5-10 min at 500.

2. Preheat extraction buffer to 65°C.

Extraction Buffer (0.1M Tris-HCl pH 7.5, 0.05 EDTA pH 8.0, 1.25% SDS).

For 1 litre:	100 ml 1.0 M Tris-HCl pH 7.5
	100 ml 0.5M EDTA pH 8.0
	125 ml 10% SDS
	675 ml ddH_2O
~16 plates	add 200µl 10mg/ml RNase after heating to 65°C for 1L
~8 plates	add 100µl 10mg/ml RNase after heating to 65°C for 0.5L
~1 plate	add 12.5µl 10mg/ml RNase after heating to 65°C for 62.5ml

3. Add 500µl of extraction buffer to each tube, seal the plates with caps and shake thoroughly. Incubate the plate at 65°C for 30 minutes – 1hour.

4. Place the plates in the -20°C to cool them down to room temperature (about 15 minutes) before adding 250µl 6M ammonium acetate, which is stored at 4°C. Vortex vigorously to mix in the ammonium acetate and then leave to stand for 15 minutes @ 4°C.

5. Centrifuge the plate for 15 minutes at 5,000 rpm @ 4ºC to collect the precipitated proteins and plant tissue.

6. Recover 600µl of the supernatant into new collection microtubes containing 360µl of Isopropanol in each well. Mix thoroughly and allow the DNA to precipitate at -20°C for 10 minutes to overnight. (we use a 96-well plate mat at this point)

7. Centrifuge the samples for 15 minutes at 5,000 rpm at 4ºC in order to pellet the DNA and then tip off the supernatant. Allow the remaining fluid to drain off the DNA pellet by inverting the tubes onto a piece of paper towel. Only invert the tubes for less than 1 minute otherwise you will lose the DNA pellets.

8. Wash the pellet in 1000µl of 70% ethanol.

9. Centrifuge the plate for 20 minutes at 5,000 rpm at 4ºC and again discard the supernatant. **(Repeat steps 8-9)**

10. Wash the pellet in 1000µl of 95% ethanol.

11. Centrifuge the plate for 20 minutes at 5,000rpm at 4°C and again discard the supernatant.

12. Allow samples to dry overnight while resting on their sides.

13. Resuspend the pellet in 100µl of 0.1M (1/10th) TE + 100µl Sigma H^2O (200µl total solution per sample)

[for 1 litre of 1/10th TE: 1ml 1M Tris-HCl pH 7.5; 200µl 0.5M EDTA pH 8.0; 1 L Sigma H2O (W4502) minus 1.2 ml] Store at 4°C.