Wheat and Barley DNA Extraction Protocol (96-well plate format) (updated January, 2006)

NOTE: This protocol has been used in both Daryl Somers' research programs at Winnipeg, Canada, and the USDA-ARS Genotyping Lab in Fargo, ND. This protocol has been adapted for automation in Fargo Lab. The modifications used in these two labs are included. 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. Daryl Somers' lab: tissue is harvested, frozen and lyophilized. The tissue (2 x 2.5cm sections) is then placed in 1.2 ml collection tubes with ~200 ul glass beads (2mm) and shaken on the paint shaker for 10-15 mins to grind the tissue to a fine powder. (For SEED DNA the half seeds are crushed with pliers, placed in collection tubes with glass beads and ground on the paint shaker.)

1b. Fargo Genotyping Lab: harvest a piece of 1.5 to 2-inch leaf segment and place it in 2.2 ml 96 deep-well plates with addition of ~0.25g of silica gel. Dry samples at room temp. Samples are ground in silica gel using Spex GenoGrinder for 4 min at 1,500 strokes.

2. Preheat extraction buffer to 65°C and also allow the plates containing the tissue to warm up to room temperature if they have been stored at -20°C. (Fargo Lab stores plates at room temp.)

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

3. Add 500ul of extraction buffer to each tube, seal the plates with caps and shake thoroughly. Incubate the plate at 65°C for 30 minutes. (In Fargo lab, 600ul of buffer was added to compensate the loss of volume absorbed by silica gel. Seal the plate with a mat and shake the plate every 5 min during incubation.)

4. Place the plates in the fridge (or freezer) to cool them down to room temperature (about 15 minutes) before adding 250ul 6M ammonium acetate, which is stored at 4°C. Shake vigorously to mix in the ammonium acetate and then leave to stand for 15 minutes in the fridge. (In Fargo lab, cool the plates on ice for 15 min before adding cold ammonium acetate. Mix and incubate the plates on ice for 15 min.)

5. Centrifuge the plate for 15 minutes at 5,000 rpm in Sigma 4-15 centrifuge (Fargo Lab uses 20 min at 4,000 rpm at 10 C in Eppendorf centrifuge) to collect the precipitated proteins and plant tissue.

6. Recover 600ul of the supernatant into new collection microtubes containing 360ul of iso-propanol in each well (Fargo Lab uses 96 deep-well plates). Mix thoroughly and allow the DNA to precipitate for 10 minutes at -20 C.

7. Centrifuge the samples for 15 minutes at 5,000 rpm (Fargo Lab uses 20 min at 4,000 rpm at 10 C) 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 500ul of 70% ethanol.

9. Centrifuge the plate for 15 minutes at 5,000 rpm (Fargo Lab uses 20 min at 4,000rpm at 10 C) and again discard the supernatant. Air dry pellet for 20 min.

10. Resuspend the pellet in 300ul of ddH_2O or 100ul for seed DNA. Leave the DNA to dissolve overnight at 4 C in the fridge. Try to dislodge the pellet. (Fargo Lab uses 200ul of dd water to dissolve leaf DNA.)

11. Spin down the un-dissolved cellular debris by centrifuging the plate for 20 minutes at 5,000 rpm (This step is skipped in Fargo Lab).

12. Transfer approximately 250-300ul or 80ul for seed DNA supernatant into a 96 well microtitre plate. Avoid pipetting any debris at the bottom of the well. (This step is skipped in Fargo Lab.) Quantify DNA.

13. Fargo Lab stores DNAs in deep-well plates and checks DNA concentration and quality of random samples on agarose gel. The total yield is estimated at 20ng/microliter, or 4 micrograms total. (Total yield in Daryl's lab is 20 micrograms from leaf tissues).