Wheat and Barley DNA Extraction in 96-well Plates
Submitted by Shiaoman Chao and Daryl Somers

DNA extraction procedure

NOTE: This protocol has been used in Daryl Somers’ research programs at Winnipeg, Canada, and has been adapted in USDA ARS Genotyping lab in Fargo, ND recently. The modifications used in these two labs are included. The original reference for this protocol is Pallotta M.A .et al. (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 (patricia.warner@adelaide.edu.au)

Sample preparation:

Daryl Somers’ lab: tissue is harvested, frozen and lyophilized. The tissue (2 x 2.5 cm sections) is then placed in 1.2 ml collection tubes with ~200 µl glass beads (2mm) and shaken on a 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.

Fargo Genotyping Lab: harvest a piece of 1.5 inch leaf segment at a seedling stage and place it in a 2.2 ml 96 deep-well plate. After wrapping the plates with miracloth, quick freeze samples in liquid nitrogen. Plates wrapped in miracloth are then transferred to a freeze-dryer to dry samples overnight. Add one ball bearing into each well and grind samples using Spex GenoGrinder for 4 min at 1,500 strokes.

Extraction:

1. 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.)

2. Extraction Buffer (0.1M Tris-HCl pH 7.5, 0.05 EDTA pH 8.0, 1.25% SDS).
   For 1 litre:
   3. 100 ml 1.0 M Tris-HCl pH 7.5
   4. 100 ml 0.5M EDTA pH 8.0
   5. 125 ml 10% SDS
   6. 675 ml ddH2O

7. Add 500 µl of extraction buffer to each tube, seal the plates with caps (Fargo Lab uses clear plastic seals from ABI to seal plates) and shake thoroughly. Incubate the plate at 65°C for 30 minutes.

8. Place the plates in the fridge (or freezer) to cool them down to room temperature (about 15 minutes) before adding 250 µl 6M ammonium acetate, which is stored at 4°C. Shake vigorously

http://maswheat.ucdavis.edu
to mix in the ammonium acetate and then leave to stand for 15 minutes in the fridge.

9. Centrifuge the plate for 15 minutes at 5000 rpm in a Sigma 4-15 centrifuge (Fargo Lab uses 20 min at 4,000 rpm under 4°C in Eppendorf centrifuge) to collect the precipitated proteins and plant tissue.

10. Recover 600 µl of the supernatant into new collection microtubes containing 360 µl of isopropanol in each well (Fargo lab uses sterile 96 deep-well plates). Mix thoroughly and allow the DNA to precipitate for 5 minutes.

11. Centrifuge the samples for 15 minutes at 5000 rpm (Fargo Lab uses 20 min at 4,000 rpm under 4°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.

12. Wash the pellet in 500 µl of 70% ethanol.

13. Centrifuge the plate for 15 minutes at 5000 rpm (Fargo Lab uses 20 min at 4,000 rpm under 4°C) and again discard the supernatant.

14. Resuspend the pellet in 300 µl of ddH2O or 100 µl for seed DNA (Fargo lab uses 200 ul of dd water to dissolve leaf DNA). Leave the DNA to dissolve overnight at 4°C in the fridge. Try to dislodge the pellet.

15. Spin down the un-dissolved cellular debris by centrifuging the plate for 20 minutes at 5000 rpm (This step is skipped in Fargo Lab).

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

17. Fargo Lab stores DNAs in deep-well plates and quantifies DNA concentration of random samples on gel. The total yield is estimated at 50ng/microliter, or 10 micrograms total. (Total yield in Daryl’s lab is 20 micrograms from leaf tissues).

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Shiaoman Chao  
USDA ARS Biosciences  
Research Lab, 1605 Albrecht Blvd, Fargo, ND 58105  
chaos@fargo.ars.usda.gov

Daryl J. Somers  
Cereal Research Centre,  
Agriculture and Agri-Food Canada,  
Winnipeg, MB, Canada.  
somersd@agr.gc.ca

http://maswheat.ucdavis.edu