Wheat high molecular weight DNA extraction

This protocol aims to of high quality Genomic DNA from Cereals (method originally for RFLP analysis, but it is suitable for other applications).

a) Equipment and reagents:

Equipment

Liquid nitrogen flask; Mortar & Pestle; Waterbath at 65°C; Benchtop centrifuge; Glass hooks for spooling.

Materials

Liquid nitrogen (LN_2)
Trizma Base (Sigma) plus HCl for pH adjustment
NaCl
Ethlene Diamine Tetra-acetic acid (EDTA)
Sodium Dodecyl Sulphate (SDS)
Phenol-Chloroform-Isoamyl alcohol (25:24:1) (Sigma)
Chloroform-Isoamyl alcohol (24:1) (Sigma)
Absolute Ethanol
70% Etahnol
Isopropanol (Propan-2-ol)
Sodium acetate plus Acetic Acid for pH-ing
Proteinase K (Sigma)
RNase A (Sigma)

b) Extraction:

1. Grind fresh leaf/plant material (up to 5g; the greener the better) in LN_2 till a fine powder (2-3 cycles)
2. Add to 20mL buffer "S" in a 50mL polypropylene tube

   Buffer "S": 100mM Tris.Cl pH 8.5
   100mM NaCl
   50mM EDTA pH8.0
   2% SDS

3. Add 100µL of Proteinase K solution (10mg/mL in water)

4. Incubate 65°C for 1-2 hours with occasional gentle in version. Treat gently to avoid shearing.
5. Add 20mL phenol-chloroform, mix completely by gentle inversion
6. Centrifuge 2000rpm for1 5min in benchtop centrifuge. Remove supernatant to fresh tube.
7. Add 0.6 vol (approx 15ml) of isopropanol & mix by inversion. Spool out precipitated DNA on a glass hook
8. Whilst still on hook, rinse in 2 changes of 70% ethanol (eg 5mL each in 2x 15ml polypropylene tubes)
9. Allow DNA on hook to air-dry for 5minutes, then resuspend in 5mL 1xTE (10mM Tris.Cl, pH8.0, 1mM EDTA). The pellet may take 1-2 days to resuspend.
10. Add 10µl of RNAse A (10mg/ml in H_2O), incubate 37°C for 1 hour 11. Add 5mL phenol-chloroform, mix completely but gently, centrifuge
11. 2000rpm for 15 minutes. Remove upper phase to fresh tube.
12. Add 5mL Chloroform, mix completely but gently, centrifuge 2000rpm for 15 minutes. Remove upper phase to fresh tube. Repeat this step if interface is excessive.
13. Add 1:10 vol sodium acetate pH 5.2 and 2 volumes cold absolute ethanol. Mix. Spool out DNA on glass hook.
14. Rinse in an excess of 70% ethanol (see step 8), then dislodge DNA into a 2ml microfuge tube (use sterile toothpick if necessary).
15. Spin the tube briefly at top speed in a microfuge and remove excess 70% ethanol.
16. Air dry pellet overnight at room tempreature in tube.
17. Add 0.5-2.0ml 1xTE (depending on size of pellet) and leave to dissolve at 4°C for several days.