Wheat DNA extraction in 96-well plates

The following protocol is adapted from Pallota 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) p789-791.
This is a high-throughput protocol yielding good amounts of genotype quality DNA (i.e. low molecular weight DNA); If performed carefully can also yield high molecular weight DNA, although other protocols are better suited for that (DNA extraction protocol for HMW DNA).

a) Sample preparation:

Young leaf tissue at 2 leaves stage (2 x 2.5 cm sections) is harvested and placed in 1.2mL 96-well plate (ABgene AB-0564, sealing mats AB-0566) containing 1 x 3 mm tungsten bead (Quiagen 69997). Everything is then freeze-dried O/N. The samples are grinded to a fine powder in Spex GenoGrinder 2000 for 2' at 160 strokes (20Hz) and quick spinned at 2700rpm.

b) Reagents:

1. Extraction Buffer (0.1M Tris-HCl pH 7.5, 0.05 EDTA pH 8.0, 1.25% SDS). Place at 65°C.
For 1 litre:
100mL 1.0 M Tris-HCl pH 7.5
100mL 0.5 EDTA pH 8.0
125mL 10% SDS
675mL ddH2O

2. 6M ammonium acetate, stored at 4°C. MW ammonium acetate: 77.08 g/mol; 231.24g in 0.5L of water.

3. 70% ethanol

4. Prepare 1.2mL round collection tubes (ABgene AB-0564, sealing mats AB-0674) containing 360µL of propan-2-ol in each well. Store at -20°C.

c) Extraction:

1. Preheat extraction buffer to 65°C (50mL per plate) + RNAse A
2. Add 500 µL of extraction buffer to each tube and mix by pipetting; seal the plates with clear silicone sealing mats (e.g. AB0566) from ThermoFisher. Incubate the plate at 65°C for 30'-1h.

3. Place the plates in the fridge to cool them down to room temperature (about 15’) before adding 250µL 6M ammonium acetate, which is stored at 4°C. Mix by pipetting and leave 15’ in the fridge.
4. Centrifuge the plate for 15’ at 5000rpm in a Sigma 4-15 centrifuge to precipitate proteins and plant tissue.
5. Transfer 600µL of the supernatant into a new 1.2mL 96-well plate containing 360µL of propan-2-ol. Mix thoroughly by pipetting and allow the DNA to precipitate for 5’.
6. Centrifuge the samples for 15’ at 5000rpm 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 plate onto a piece of paper towel. Only invert the plate for less than 1’ otherwise you might loose the DNA pellets.
7. Wash the pellet in 500µL of 70% ethanol.
8. Centrifuge the plate for 15’ at 5000 rpm and again discard the supernatant. Leave without leads to dry the pellet for 2 hours (or O/N) or incubate for 30’ at 65°C.
9. Resuspend the pellet in 200µL of ddH2O and seal the plate. Vortex thoroughly (DNA might be trapped under cell/tissue debris) and incubate at 65°C for 15’ to dislodge the pellet.
10. Vortex the plate again and then spin down the un-dissolved cellular debris by centrifuging the plate for 20 minutes at 5000 rpm.
11. Quantify DNA concentration on gel and random samples at the spectrophotometer. The total yield is estimated at 50ng/µL.
12. Optional. If there is a lot of green pellet and dirt, transfer approximately 150-200µL into a new plate after centrifugation. Avoid pipetting any debris at the bottom of the well.