RAPID DNA EXTRACTION

1. Prepare grinding sticks from blue pipette tips by twisting molten tip ends into 1.5ml eppendorf tubes.

2. Harvest tissue (preferably 5+ small Arabidopsis leaves, but more tissue=more DNA) and flash freeze in an eppendorf in liquid N_2.

3. Homogenise tissue in eppendorf using grinder until ground to paste, and immediately add 250 µl **DNA EX buffer**. (IMPORTANT : complete and vigorous grinding is essential for efficient DNA extraction).

4. Heat at 65°C for 10'. Shake tubes.

5. Add 65 µl 5M KOAc and invert tube 5 times.

6. Leave on ice for 5'.

7. Spin 13K 5' and remove supernate to new eppendorf, as best as is possible.

8. Spin 13K 5' and remove supernate again (should be very clean now).

9. Add 320 µl isopropanol and 30 µl 3 M NaOAc pH 4.5 to supernate and
   invert 5 times.

10. Leave –20°C for 10'.

11. Spin 13K 10'. Remove supernate and wash pellet in 70% EtOH.

12. Spin 13K 5'. Remove supernate and air-dry pellet 10'.

13. Resuspend in 41 µl H_2O.

DNA EX buffer (10 ml)

100mM Tris-HCl pH 8.0	0.5 ml 2 M
50mM EDTA	1 ml 0.5 M
500mM NaCl	1 ml 5 M
10mM β-mercaptoethanol	6.9 µl
1.4% SDS	1.4 ml 10%

This method was originally designed to produce low quality and quantity DNA from small quantities of starting tissue for PCR reactions. However, quality Southerns can be conducted using DNA prepared using this protocol. Simply resuspend in 41 µl – enough for 2 Southern digestions as follows:

20.4 µl DNA
3 µl 10 x Buffer
3 µl Enzyme
3 µl 10mM spermidine
0.3 µl 10mg/ml BSA
0.3 µl 10mg/ml RNAase A

37°C 4-5 hours.