

Chapter 3.5

96-WELL DNA ISOLATION METHOD

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The following protocol provides a high throughput, low cost method of producing a superior DNA yield of high quality which is suitable for TILLING, map based cloning or any application which requires long term DNA storage. The protocol has been designed for DNA extraction from leaf material, preferably young leaf tissue should be used as this minimises samples being contaminated with polysaccharides and phenolics.

METHOD

1. Pre-heat extraction buffer to 65°C.
2. Label collection tubes (Qiagen Cat. No. 19560) and add a single tungsten carbide bead (Qiagen Cat no. 69997) to each tube.
3. Prepare ice bucket and tube of ethanol to wash forceps after each harvest.
4. Harvest material (3 growing tips) into a single collection tube.
5. Add 400µl of extraction buffer to each tube (use a multi-pipettor). Put on lids (Qiagen Cat. No. 19566).
6. Homogenise material on the mixer mill (Retsch MM300) for 2 min/30s.
7. Incubate at 65°C for 30min to 1 hour.
8. Centrifuge for 10 minutes at full speed (Sigma 4K15).
9. Label a new rack of collection tubes.
10. Remove 300µl of supernatant into a new collection tube (use a multi-pipettor set speed to slow) with extended length tips (Matrix Cat. No. 8252).

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11. Carefully add 200µl of phenol:chloroform to each tube (THIS PROCEEDURE SHOULD BE CARRIED OUT IN THE FUME HOOD), use 200µl manual multi-pipettor with filter tips.
12. Put lids onto the tubes and invert several times, so the samples are well mixed. Centrifuge for 10-15 minutes.
13. Label a set of storage plates.
14. Using a manual multi-pipettor with filter tips very carefully remove 200µl of the upper layer to a new storage plate (AB Gene Cat. No. AB 0765) (THIS PROCEEDURE SHOULD BE CARRIED OUT IN THE FUME HOOD).
15. Using a multi-pipettor add 1/10th vol. of 3M sodium acetate (~20µl) and an equal volume of isopropanol (~220µl). Put on lids, mix well, and leave at -20°C for a maximum of 1 hour.
16. Leave plates on desk until they have reached room temperature, as spinning when still frozen at high speeds can cause the plate to crack. Centrifuge at 5600 rpm for 45 minutes.
17. Remove supernatant and add 100µl of TE containing RNase A at a final concentration of 10µg/ml and incubate 30 min at 37°C
18. Repeat the precipitation step (step 15). After centrifugation at 5600 rpm for 45 minutes remove the supernatant and add 200µl 70% ethanol. Put on lids and leave for 15 minutes or overnight.
19. Remove ethanol and leave to air dry. Add 100µl of TE and store in fridge/freezer.

BUFFERS AND SOLUTIONS

Extraction Buffer

- 200 mM Tris-HCl pH 7.5
- 250 mM NaCl
- 25 mM EDTA
- 0.5% SDS

TE

- 10 mM Tris-HCl pH 8.0
- 0.1 mM EDTA pH 8.0

TE and RNase A

- For 2 plates: 20 ml TE and 20µl DNase-free RNase A (10 mg/ml)

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