DNA EXTRACTIONS FOR PCR

Extraction buffer-50 mls

2M Sorbitol	3.5 mls	0.14M
1M Tris pH 7.5	11.0 mls	0.22M
0.5M EDTA pH 8.0	2.2 mls	0.22M
5M NaCl	8.0 mls	0.8M
CTAB	0.4 gms	0.022M
N-lauroyl. sarcosyl	0.5 gms	0.034M
dd H20	25 mls	
Na bisulfate	0.1g/30 mls	

Where volumes are specified assume that 1 ml is equal to 1 gram. Weigh solutions into a new sterile tissue culture bottle. It is best done the night before so that the CTAB and sarcosyl have plenty of time to dissolve. Usually I leave it in the tissue culture growth rooms. Approximately 2 mls of buffer are required for each sample.

1. Collect sample into plastic bag (1 leaf tip for onion).

2. Roll sample with a wall paper roller or lead pipe and then add 1.5 mls of extraction buffer (2X 750µls). Roll again with the roller to ensure the buffer and plant material are well mixed.

3. Pipette 300 µl of chloroform into tube. Transfer sample from the bag into the tube by pouring from one corner.

4. Invert to mix and incubate at 65°C for 30 minutes.

5. Invert tube to ensure chloroform and sample are well mixed. Centrifuge at 13,000 rpm for 5 minutes.

6. Pipette 600 µl of isopropanol into a new tube. Pipette up to 1 ml of supernatant into this tube.

7. Invert to mix and centrifuge at 13,000 rpm for 5 minutes to pellet DNA.

8. Pour off supernatant and wash pellet with 1 ml of 70% ethanol (room temperature).

9. Optional centrifugation after the 70% wash if the pellet has become dislodged; 13,000 rpm for 2-3 minutes.

10. Dry pellet in laminar flow unit. Tubes dry faster if laid on their side.

11. When pellets are nice and clear, resuspend in 200-400µl PCR ddH20. (50 -100µ TE for onion?)

12. Quantitate DNA on a 1% agarose gel. Dilute 1µl of sample into a mixture of 15µl dd H20 + 4µl of sample buffer. Mix well and load 5µl on the gel (= 1/4 of a µl). NB: Samples must be completely dissolved before quantitation.