Cucurbit Mitochondrial DNA Isolation

Ward et al. (1981) Cell 25:793-803.

1. Shoot tissue from seedlings grow 7 to 10 days in the dark at 28°C. Surface sterilize for 5 minutes and rinse with sterile distilled water.

2. Homogenize with three 10 sec bursts in a Waring blender in 4 ml of buffer A per gram of tissue.

Buffer A			To make:			
	Stock or MW	Unit	0.5 L	1 L	2 L	4 L
0.3 M Mannitol	182.2	g	27.3	54.7	109.3	218.6
0.2% BSA		g	1.0	2.0	4.0	8.0
50 mM Tris (pH 7.6)	1.0 M	ml	25.0	50.0	100.0	200.0
3 mM EDTA	0.5 M	ml	3.0	6.0	12.0	24.0

3. Filter through a couple of layers of Miracloth.

4. Spin at 1500 × g (4000 rpm in GSA rotor) for 10 min.

5. Harvest supernant and spin again at 12,000 × g (11,000 rpm in GSA rotor) for 20 min.

6. Resuspend pellet in Buffer B.

Buffer B			To make:			
	Stock or MW	Unit	0.5 L	1 L	2 L	4 L
0.3 M Mannitol	182.2	g	27.3	54.7	109.3	218.6
0.2% BSA		g	1.0	2.0	4.0	8.0
50 mM Tris (pH 7.2)	1.0 M	ml	25.0	50.0	100.0	200.0

7. Repeat 1500 × g and 12,000 × g centrifuge steps.

8. Resuspend pellet in Buffer B. Put into 50 ml Oakridge tubes or smaller, depending on volume. Add $MgCl_2$ to 10 mM.

9. Add 50 to 100 μg/ml DNase and incubate on ice for 1 to 2 hours. Add EDTA to 20 mM and repeat steps 4 and 5.

10. Lyse mitochondria at 0° to 2°C in Buffer C.

Buffer C			To make:			
	Stock or MW	Unit	0.5 L	1 L	2 L	4 L
0.8% Sarkosyl		g	4.0	8.0	16.0	32.0
200 mM NaCl	5.0 M	ml	2.0	4.0	8.0	16.0
30 mM EDTA	0.5 M	ml	3.0	6.0	12.0	24.0

Extraction with phenol/chloroform and EtOH precipitation may be necessary. Wash with 70% EtOH and dry to remove EtOH.

11. Dissolve DNA in 50 mM Tris (pH 8.0) and 20 mM EDTA. Add solid CsCl (1.1 g/ml) to density of 1.57 g/cm^3.

12. Spin at 17,000 × g (15,000 rpm in SS34 rotor) for 20 to 30 min to remove impurities. Harvest supernant and add EtBr to 300 μg/ml.

13. Centrifuge at 40,000 rpm in Vti65 for 15 to 20 hours. Harvest band with syringe, partition against alcohol to remove EtBr, and dialyze to remove CsCl.