

GENOMIC DNA PREP (UREA)

1. Grind 1 - 5 g tissue in liquid nitrogen.
2. Transfer to tube (30 ml) containing 6 ml **extraction buffer**.
3. Add 3 ml phenol and then 3 ml chloroform : isoamylalcohol (24:1).
4. Mix well. Spin 10K 10'.
5. Remove supernate into a fresh tube.
6. To supernate add 3 ml 7.5 M NH₄Ac and 3.6 ml isopropanol
7. Mix well, spin 10K 5', dry in air and then dissolve pellet in 400 µl TE.
8. Add 5 µl 10 mgml⁻¹ RNAase A. 37°C 30'.
9. Extract with phenol/chloroform/IAA (25:24:1). Ethanol precipitate. Wash pellet with 70% ethanol and dry in speedvac.
10. Resuspend in 50 - 200 µl TE. Dissolve overnight at 4°C. In the morning pipette up and down again and then spin for 5' in microfuge. Jelly-like carbohydrates will pellet. Remove supernate to a fresh tube and store at 4°C. Add 50 µl TE to pellet and store at -20°C as a back up (more DNA will dissolve over time).

Extraction buffer (do not autoclave)

Urea	168 g
5 M NaCl	25 ml
2 M Tris-HCl pH8.0	10 ml
0.5 M EDTA pH8.0	16 ml
Sarkosyl (N-laurylsarcosine)	4 g
	Up to 400 ml with d H ₂ O

