

High Mol. Wt. Fly DNA. (from the lab of M. McKeown)

1. Weigh adult flies (0.5 g or so)
2. Freeze in liquid N₂ in porcelain mortar.
3. Grind to fine powder
4. Pour powder into 2.5 ml of :
 - 0.2 M Tris HCl pH 8
 - 0.2 M EDTA pH 8
 - 1% Sarkosyl
 - 100 ug/ml proteinase K
5. Put in 50 ml screw cap Erlenmyer, shake 48° C 1 hr.
6. Transfer into corex tube to spin 10K 5 min.
7. Pipette super. into graduated tube,
 - + TE to 4 ml
 - + 3.7 g CSCI
 - + 0.4 ml 10 mg/ml EtBr
8. 53K 20° in VTi65 8h (w²t approx. 2.0x 10¹²)(use brake down to 5K)
9. Pull DNA band from side, slowly with 20 ga. needle
10. Butanol extract 3x.
11. EtOH ppt., wash, resuspend 1-2 ml TE.

Yields approx. 100ng/mg fly.

Michael M. says this makes DNA so large that it is hard for them to handle it , so at the end they pipetted it up & down through a 5 ml pipette to break it down a little.