

1. Etherize flies (~100-200 for each tube) and place in Marsh 1.7ml microcentrifuge tubes so that fly volume reaches 0.5 ml mark. Freeze flies in dry ice. If not processing tissue immediately, store at -80 degrees C until use.
2. Using a microcentrifuge size grinder (disposable or reusable--wash with distilled water, then ethanol before use), homogenize tissue well in 300 microliters BUFFER A.

BUFFER A Sterile filter; store at 4 degrees C.
10 mM Tris HCl pH 7.5
60 mM NaCl
10 mM EDTA
0.15 mM spermine
0.15 mM spermidine
5 % sucrose
0.5% Triton X-100

Use up and down motion combined with rotary motion to finely grind all flies. There should be no intact flies left by visual inspection.

3. Spin in microcentrifuge, 4 degrees C, 15,000 rpm, 15 minutes.
4. Remove supernatant and discard. This supernatant will have a foamy head and be either creamy or red, depending on the eye color of flies processed. The pellet is stratified: on the bottom will be fly parts (dark pellet) and on top will be a pasty layer (creamy or red, depending on eye color of flies). This pasty layer contains nuclei. Carefully resuspend this pasty layer of the pellet in 300 microliters BUFFER A by gently pipeting up and down with a P1000 pipettor. Try to not stir up too much of the fly part pellet, but strive for high yield resuspension of the nuclear pasty layer.
5. Transfer resuspended nuclear layer to a fresh microcentrifuge tube containing 300 microliters BUFFER B.

BUFFER B Sterile filter; store at 4 degrees C.
2% Sarkosyl (N-lauryl sarcosine)
0.1 M EDTA
5% sucrose

6. Incubate in tempblock or water bath, 65 degrees C, 45 minutes.
7. Phenol/Chloroform extract by adding 400 microliters phenol equilibrated with Tris buffer (pH 7-8) and 400 microliters chloroform. Vortex 10 seconds. Let sit 10 seconds. Vortex 10 seconds. Spin in microcentrifuge, room temperature, 15,000 rpm, 5 minutes.
8. As soon as spin is over, remove supernatant to a fresh tube containing 600 microliters chloroform. Vortex 10 seconds. Let sit 10 seconds. Vortex 10 seconds. Spin in microcentrifuge, room temperature, 15,000 rpm, 5 minutes.
9. As soon as spin is over, remove supernatant to a fresh tube. Add an equal volume of isopropanol. Mix by inverting tube several times. Spin in microcentrifuge, 4 degrees C, 15,000 rpm, 15 minutes.
10. Carefully remove supernatant, wash with 500 microliters 70% ethanol. Vortex 10 seconds. Spin in microcentrifuge, 4 degrees C, 15,000 rpm, 5 minutes.

11. Carefully remove supernatant. Evaporate residual ethanol in speedvac. Resuspend in 100 microliters TE2 (10 mM Tris HCl, pH 7-8; 2 mM EDTA). Let tube sit on bench for 5 minutes to aid resuspension, then gently pipet up and down to finish resuspending DNA.
12. Reprecipitate DNA by adding 200 microliters absolute ethanol. Mix gently by inverting several times. Spin in microcentrifuge, 4 degrees C, 15,000 rpm, 15 minutes.
13. Carefully remove supernatant and wash pellet with 500 microliters of 70% ethanol. Vortex 10 seconds. Spin in microcentrifuge, 4 degrees C, 15,000 rpm, 5 minutes.
14. Carefully remove supernatant. Evaporate residual ethanol in speedvac. Resuspend in 100 microliters TE2 (10 mM Tris HCl, pH 7-8; 2 mM EDTA). Let tube sit on bench for 5 minutes to aid resuspension, then gently pipet up and down to finish resuspending DNA. Add 2 microliters 10 mg/ml DNase-Free RNaseA to DNA. Store at 4 degrees C. DNA concentration will be approximately 0.3-0.5 micrograms/microliter; use 10 microliters for each digest to be analyzed by Southern blotting. Express concentration in terms of "fly equivalents," i.e. if you started with 100 flies, concentration will be 1 fly equivalent/microliter; 200 flies will have a 2 fly equivalent/microliter concentration. For 2002: Use 10 ug DNA per lane in Southern Blot.