PREPARATION OF GENOMIC DNA FROM *Drosophila melanogaster*

This protocol enables isolation of highly pure genomic DNA from 40-100 mg of adult flies (a fly weighs ~1 mg). First, the flies are ground in a buffer under conditions that nuclei remain intact, then SDS is used to liberate DNA from broken tissue. Next, routine phenol extraction (to remove protein) and chloroform extraction (to remove phenol) is performed and the nucleic acids are precipitated with ethanol. Following centrifugation (removes lipids and small cellular molecules), the nucleic acid pellet is dissolved and digested serially with RNaseA (degrades RNA) and Proteinase K (degrades RNaseA and other proteins). Additional phenol/chloroform precipitation and ethanol precipitation yields highly purified genomic DNA.

Our objective is intact genomic DNA – Avoid shearing the DNA by excessive pipetting and vortexing.

1. Place 50 adult flies in a 1.5 ml microtube fitted with a micropestle and thoroughly grind in 500 μl of Buffer A. Rinse the pestle with 500 μl of Buffer B, adding the rinse to the homogenate; mix gently by inverting the microtube. Incubate 1 hr at 37°C.

2. Cut off the tip of a P1000 micropipette tip and use it to transfer one-half of your homogenate (500 μl) to a second microtube. Phenol extract your samples by adding an equal volume (500 μl) of TE-saturated phenol to each tube, cap, and mix. Centrifuge 5 min.

3. Use a cut-off P200 tip to draw the clear top layer (aqueous phase) to two new microtubes (~400 μl each). *Avoid drawing interface material.* Re-extract your samples with phenol by adding an equal volume (500 μl) of phenol to each tube, cap, and mix. Centrifuge 5 min.

4. Use a cut-off P200 tip to draw the clear top layer (aqueous phase) to two new microtubes (~400 μl each). *Avoid drawing interface material.* Chloroform extract your samples by adding an equal volume (500 μl) of chloroform to each tube, cap, and mix. Centrifuge 1 min.

5. Use a cut-off tip to draw the clear top layer (aqueous phase) to two new microtubes (~400 μl each). Add NaCl to 0.1M final concentration. Ethanol precipitate your samples by adding 2 volumes (~850 μl) of EtOH to each microtube; mix gently. Observe the precipitation of nucleic acids. Place the microtubes at -20°C overnight to encourage the precipitation.

6. Centrifuge 10 min. Discard the supernatant; briefly dry the pellet in the SpeedVac (use will be demonstrated).

7. Combine the samples into a single microtube as follows. Add 500 μl TE buffer to one tube then, using a cut-off P200 tip, draw up the pellet and transfer it and all

of the TE buffer to the second tube. Use the same tip to suspend the pellet in this second tube. Vortex both tubes to encourage the pellets to dissolve

8. RNaseA-treat your sample by adding NaCl to 0.1 M. Add RNaseA to 100 μg/μl final concentration; mix. Incubate 30 min. at 37°C. *You should vortex your microtubes every 10 min to ensure complete dissolution of the pellets.*

9. Proteinase K-treat your sample by adding Proteinase K to 100 μg/μl final concentration; mix. Incubate 30 min. at 37°C.

10. Phenol extract your sample.

11. Extract your sample with phenol-chloroform (1:1 mixture).

12. Chloroform extract your sample.

13. EtOH precipitate your sample by adding 2 volumes (~1 ml) of ethanol; mix. Allow to precipitate overnight at -20°C.

14. Centrifuge 20 min. Discard the supernatant. Wash the pellet by adding 1000 μl of 80% EtOH, vortexing and centrifuging 5 min.

15. Discard the supernatant and briefly dry the pellet. Dissolve the pellet in 50 μl of TE buffer (*this may require some vortexing and incubation at 37°C*).

16. Determine the concentration of your DNA sample as follows. Carefully prepare a 1/200 dilution of your DNA sample by pipetting 4 μl into 800 μl of TE; mix. Measure A_{260} and A_{280} values using a spectrophometer. For a clean DNA sample, the A_{260} value should be at least twice the A_{280} value. Calculate your DNA concentration assuming an extinction value of 1 A_{260} = 20 mg/ml:

[your A_{260} value ÷ 20] X 200 (dilution) = μg DNA / μl of your sample

Label and store your DNA at 4°C.

Buffer A: 10 mM Tris-Cl, pH 7.5
60 mM NaCl
10 mM EDTA
150 μM spermine
150 μM spermidine
200 μg Proteinase K/ml

Buffer B: 200 mM Tris-Cl, pH 7.5
30 mM EDTA
2% SDS
200 μg/ml Proteinase K

TE buffer: 10 mM Tris-Cl, pH 7.5
1 mM EDTA