Protocol

1. Turn on a water bath to 55ºC.
2. Chop a small[1] amount of tissue (~ 0.5 cm^2) and chop it with a sterile scalpel blade. Transfer the sample to a labeled 1.5 ml eppendorf. Stored tissue in ethanol in should first be dried on tissue paper.
3. Add 600 µl of TNES buffer and 35 µl of Proteinase-K (20 mg/ml). Mix the sample by inverting the tube several times.
4. Close the tubes, cover with parafilm and incubate at 50ºC for 5-24 hours[2]. (Optional) occasionally mix the samples by inverting the tubes.
5. Add 3 µl RNase A (10 mg/ml) and incubate for 45 min.
6. Add 166.7 µl of 6 M NaCl[3]. Shake the samples vigorously[4] for 20 seconds.
7. Microfuge the samples at full speed for 5-10 minutes at room temperature.
8. Label new tubes whilst centrifuging samples.
9. Remove supernatent to a new, labeled 1.5 ml microcentrifuge tube. It is better to leave some supernatant than transfer cell debris.
10. Add an equal volume (~ 800 µl) of cold 100 % ethanol[5] and gently mix by inverting the tube a couple of times. White DNA should precipitate out of solution.
11. Centrifuge at full speed for 10-20 minutes (at 4ºC, if possible).
12. Pour (or pipette) off the supernatent, taking care not to dislodge the pellet of DNA.
13. Wash the DNA pellet in 200-700 µl of 100 % ethanol (add ethanol, close cap of tube and invert gently, or gently roll the tube on its side). Pour off the ethanol and briefly spin the samples to keep the pellet at the bottom of the tube.
14. Wash DNA pellet with 70 % ethanol as above. After removing the 70 % ethanol, briefly centrifuge the samples to get the last of the ethanol to the bottom of the tube; pipette off the remaining ethanol.

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[1] Using too much tissue can give poorer quality DNA and a worse yield.

[2] Overnight incubation is an option. Faster incubation can be achieved by adding more proteinase-K.

[3] NaCl is preferred to potassium acetate when DNA is dissolved in SDS because NaCl keeps SDS soluble in 70% ethanol so it won't precipitate with the DNA.

[4] Harder than inverting, but not too roughly as this can damage DNA.

[5] Ethanol is slower than isopropanol, more expensive because of taxation, but results in higher yields, especially if the sample is incubated at -20ºC for 1-2 hours.

15. Leave the sample to air dry for 10-30 min.
16. Re-suspend the DNA in 100 μl (depends on pellet size) of sterile distilled water (or Tris-EDTA).

Solutions

TNES buffer[6]

0.01 M[7] Tris, pH 7.5	10 ml 1M TrisHCl pH 7.5
0.4 M NaCl	6.67 ml 6M NaCl
0.1 M EDTA	20 ml 0.5M EDTA
0.6 % SDS	6 ml 10% SDS
	57.33 ml H_20

10% SDS	10 g SDS/100ml H_2O
6M NaCl	35.07 g NaCl/100ml H_2O (autoclave)
1M Tris-HCl (pH 7.5)	12.11 g Tris/100ml H_2O
0.5M EDTA	18.61 g EDTA/100ml H_2O (dissolves when pH is 8)

Proteinase K (20 mg/ml)

[6] Difference to squishing bufer: pH 7.5 instead of 8.2, 0.4 instead of 0.25 M NaCl, SDS included, tris 10x less concentrated here.

[7] The purpose of this is to buffer the solution, so the pH is more important than the concentration, which I find to vary more than 10-fold between protocols.

Example protocol performance

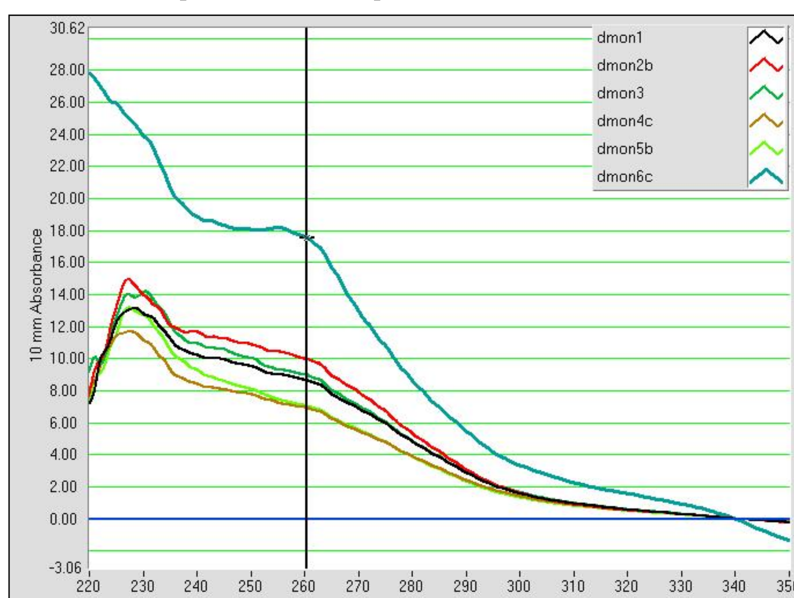
The protocol provides good yields of DNA sufficient for PCR.

The following data are from a nanodrop quantification, which may overestimate the DNA concentration by 50%. Samples 1-4 started with 10 males, while samples 5-6 with 8 males of *Drosophila montana*[8]. For all samples a small pestle was used for homogenisation.

Table 1: Absorbance of *D. montana* DNA extraction before cleanup.

Sample ID	ng/µl	A260	A280	260/280	260/230
dmon1	433.33	8.667	4.828	1.79	0.68
dmon2	499.05	9.981	5.332	1.87	0.71
dmon3	450.29	9.006	4.849	1.86	0.64
dmon4	347.65	6.953	3.891	1.79	0.62
dmon5	353.59	7.072	3.875	1.82	0.56
dmon6	881.36	17.627	8.643	2.04	0.74

Figure 1: Absorbance of the samples before cleanup.



[8] *D. montana* belong to the virilis group and a considerably larger than *D. melanogaster*.