Protocol

1. Make sure there is ethanol in the freezer.
2. Place in a 1.5ml eppendorf tube 200 ul Digsol buffer and 10 ul Proteinase K (10mg/ml).
3. Remove fly from ethanol (with forceps) and blot onto tissue. When dry, transfer to the eppendorf.
4. Disrupt the fly with electric pestle machine.
5. Wrap the eppendorf rack in tissue paper and elastic bands and place in rotating oven at 55oC (3hrs) or 50oC (overnight).
6. (Optional) Add 3 ul 10ug/ul RNase A[1] and incubate at 37oC for 45 min.
7. Add 300 ul 4M ammonium acetate to each sample.
8. Vortex several times over a period of at least 15 minutes at room temperature to precipitate the proteins.
9. Centrifuge[2] for 10 minutes at 13,000 rpm [3]. Label new eppendorfs.
10. Aspirate 500 ul supernatant into the clean labelled 1.5ml eppendorfs[4].
11. Add 1ml 100% ethanol and mix by inverting. The DNA should precipitate out of solution, but may not be visible from single flies, especially males.
12. Leave samples in freezer at -20 oC for 1h.
13. Centrifuge for 10 minutes at 13,000 rpm.
14. Pour off ethanol in a smooth movement so as not to lose DNA pellet.
15. Add 500 ul 70% ethanol and invert several times to rinse pellet.
16. Centrifuge for 5 minutes at 13,000 rpm[5].
17. Pour off ethanol in a smooth movement and stand tubes upside-down on clean tissue until they are completely dry (1 h or overnight incubation).
18. Once fully dry add Low TE. The amount added is dependent on pellet size, 20 ul results in up to 50 ng/ul DNA from a single female *D. pseudoobscura*.
19. Place tubes in shaking incubator for 30 minutes (55 oC) to dissolve pellet (flicking every 10 mins). Or leave overnight at room temperature. You are aiming for a fully homogeneous, slightly viscous solution.
20. Store at -20 oC (long term) or 4 oC (short term).

Recipes

1M Tris-Base (MW 121.1) pH 8.0

(Tris Hydroxymethyl Aminomethane)

For 200ml:

- Dissolve 24.22g in distilled water by stirring
- pH should be about 8.0
- Autoclave to sterilise

0.5M EDTA (MW 372.2) pH 8.0

(EthyleneDiamineTetraacetic Acid)

For 200ml:

- Dissolve 37.2g in distilled water by stirring.
- Will need to pH solution with NaOH whilst it is dissolving (in order for all EDTA to solubilise).

20% SDS

(Sodium Dodecyl Sulphate)

For 100ml:

- Add 20g SDS (use autoclaved water as end solution cannot be autoclaved).
- Use a fume hood and wear a mask when weighing this powder.
- It is easier to dissolve by heating at 55 oC than by shaking.

Digsol pH 8.0

(Digestion Solution - Bill Amos and Josephine Pemberton)

DIGSOL RECIPE

FINAL CONCENTRACTION	STOCK	FOR 1000ML	FOR 200ML
20mM EDTA	EDTA (0.5M, pH 8.0)	40ml	8ml
120mM NaCl	NaCl	6.85g	1.37g
50mM Tris	Tris (1M, pH 8.0)	50ml	10ml
	Distilled water	810ml	172ml
SDS (0.5%)	SDS (20%)	25ml	5ml

- Warm all constituents except SDS until dissolved
- Autoclave to sterilise
- Add SDS
- pH with HCl if necessary

4M Ammonium Acetate, pH 7.5

For 100ml

- Dissolve 30.83g Ammonium acetate[6] in distilled water.
- Autoclave to sterilise.
- If necessary pH with Glacial acetic acid (was not necessary).

T.E. pH 7.5–8.0

TE RECIPE	
FINAL CONCENTRATION	FOR 400ML
10mM Tris	4ml of 1M Tris (pH 8.5)
1mM EDTA	800 ul of 0.5M EDTA (pH 8.5)

Low EDTA T.E. Buffer pH 7.5–8.0

For resuspending DNA which will be used in PCR

LOW TE RECIPE	
FINAL CONCENTRATION	FOR 400ML
10mM Tris	4ml of 1M Tris (pH 8.5)
0.1mM EDTA	80 ul of 0.5M EDTA (pH 8.5)

- pH if necessary
- Autoclave to sterilise

— 10 mg/ml Proteinase K —

In 1ml aliquots in -20 oC freezer.

In St Andrews it is kept in special solution at 4 oC at 20 mg/ml.

1. Keeping RNA in the samples will make the nano drop quantification unreliable, there can easily be 5-10x more RNA than DNA in the final sample. However for low DNA volumes, having more nucleic acid seems to increase DNA yields because it allows for easier precipitation. ↩
2. Always mix by inverting the eppendorfs while loading to the centrifuge. ↩
3. RPM (revolutions per minute) can be converted to RCF (relative centrifugal force). A common centrifuge gives about 10,000 RCF, I have found that higher RCF (18,000) give better pellets and cleaner supernatants so it is worth finding a more powerful centrifuge is possible. ↩
4. Sometimes (depends on temperature and centrifuge) some of the salt comes out of solution and forms part of the pellet (white). Other times it does not, but it forms a thicker layer close to the bottom of the eppendorf. It can be shown to be salt by adding ethanol, and this thick part becomes white. When aspirating the supernatant be very careful not to include the thick bottom layer, which may extend to the sides of the bottom of the eppendorf. ↩
5. If very clean samples are required, repeat the 70% wash steps. However if there will be follow-up cleanup of the samples (like RNase treatment), a single wash is sufficient and reduces the risk of a lower yield. ↩
6. Ammonium acetate is hydrophilic and therefore most of the stock chemical is very wet, however this does not seem to affect the extraction process. ↩