

DNA Extraction (single fly)

Cell Lysis

1. Chill **cell lysis solution**, and a 1.7 ml tube containing fly, separately on ice;
2. Add 100 μ l **cell lysis solution** to each tube;
3. Homogenize each fly (2-4 seconds) with motor-driven pestle;
4. Incubate at 65°C for 15 minutes;
5. Cool sample to RT.

RNase Treatment

6. Add 2 μ l of a 1/4 dilution of **RNase A solution** in water (= 1 mg/ml) to cell lysate;
7. Mix sample by inverting tube 25 times, and incubate at 37°C for 40 minutes;
8. Cool sample to RT.

Protein Precipitation

9. Add 33 μ l **protein precipitation solution** to the RNase-treated cell lysate;
10. Vortex on high speed for 10 seconds, and place sample on ice for 5 minutes;
11. Centrifuge at 14,000 rpm for 3 minutes.

DNA Precipitation

12. Move supernatant to a new, pre-labeled 1.7 ml tube containing 100 μ l **isopropanol**;
13. Mix by inverting tube gently 50 times;
14. Centrifuge at 14,000 rpm for 5 minutes, and gently pour off supernatant;
15. Add 100 μ l of **70% ethanol**, and invert tube a few times to wash pellet;
16. Centrifuge at 14,000 rpm for 1 minute, and remove supernatant with pipette;
17. Invert tube on absorbant paper and leave to air dry for 20-30 minutes.

DNA Resuspension

18. Add 20 μ l of sterile-filtered **1X TE (pH 8.0)**;
19. Resuspend O/N at RT.

OPTIONAL:

Agarose Gel

20. Mix 1 μ l of DNA (resuspended in 20 μ l) to 5 μ l 1X loading dye, and run on 1.5% agarose gel. Should see clear high molecular weight band (no smearing).

DNA dilution

21. Dilute DNA 1/10 with sterile-filtered dH₂O. 1 μ l diluted DNA will be suitable for a standard 5 μ l PCR.
- 19 μ l DNA + 171 μ l dH₂O = 190 μ l
 - 20 μ l DNA + 180 μ l dH₂O = 200 μ l

REAGENTS NEEDED:

Genra Systems, Puregene Cell & Tissue DNA Isolation Kit
(Kits D-5500A, D-5000A, or D-50KA)
Isopropanol (Fisher Chemical: A416)
70% Ethanol
1X TE (pH 8.0)

EQUIPMENT NEEDED:

Kontes Pellet Pestle Cordless Motor (K749540-0000)
Kontes Pellet Pestle for 1.5 ml tubes (K749521-1500)
(both distributed by Fisher Scientific)

NOTES:

- For some applications may need to resuspend DNA directly in dH₂O. If so, note that resuspension will be more difficult - incubating at 65°C for 1 hour (instead of O/N at RT) helps. Also, be aware that DNA resuspended only in dH₂O is not particularly stable, and may smear when run on an agarose gel.
- DNA should *never* be vortexed (to avoid shearing). To mix, simply flick tube.