Drosophila gDNA isolation in microcentrifuge tubes

Materials

- Samples: frozen whole drosophila.
- Bead lysis kit: 1.5 mL RED RINO/Eppendorf Lysis Kit or 2 mL tube with beads.
- DNA isolation kit: magNEAT Tissue DNA Extraction Kit (Kit ID: 2TDMN-50) from Microzone $^{\mu}$.

Method: Homogenization

1. Add 500 µL Lysis Buffer, 20 µL proteinase K, and 10 µL FoamBlocker into each lysis kit tube.
2. Weigh and transfer 5 whole drosophila into the buffer-filled tubes.
3. Set the Bullet Blender at speed 12, time 2 minutes and homogenize the samples. If using other homogenizer models, refer to the manufacturer's instructions for appropriate settings.
4. Remove the tubes and visually inspect the samples to confirm complete homogenization.

Method: Extraction

RNase Treatment & Binding:

1. Prepare new microtubes containing 200 µL PBS.
2. Transfer 320 µL of the homogenate to the new tubes containing PBS.
3. Add 5 µL RNase A to each sample, mix well by inversion and leave at room temperature for 10 minutes, mixing again halfway through the incubation.
4. Centrifuge tubes at 13,000 x g for 5 minutes.
5. Transfer 450 µL of supernatant into new microfuge tubes, being careful not to disturb the pellets or transfer any debris. Place the tubes on the tube rack.
6. Add 450 µL Binding Buffer to the supernatant in each tube and mix thoroughly by inversion.
7. Add 30 µL of magNEAT magnetic beads to each sample tube. Mix thoroughly by inversion until the sample is homogenous. Allow to stand for 5 minutes, mixing again halfway through.
   Note: *Vortex magNEAT beads thoroughly before adding to ensure they are resuspended.*
8. Transfer the tube rack to the magnetic stand and allow to sit for 1-2 minutes, until the magnetic beads are separated.
9. Discard the supernatant and remove the tube rack from the magnetic stand.

Washing:

10. Add 700 µL Wash Buffer 1, mix thoroughly by inversion, ensuring all beads have been detached from the tube walls.
11. Place the tube rack onto the magnetic stand and allow the magnetic beads to separate.
12. Discard the supernatant then remove the tube rack from the magnetic stand.
    Note: *Be careful not to disturb the magnetic beads.*
13. Add 700 µL Wash Buffer 2, mix thoroughly by inversion to completely resuspend the beads.

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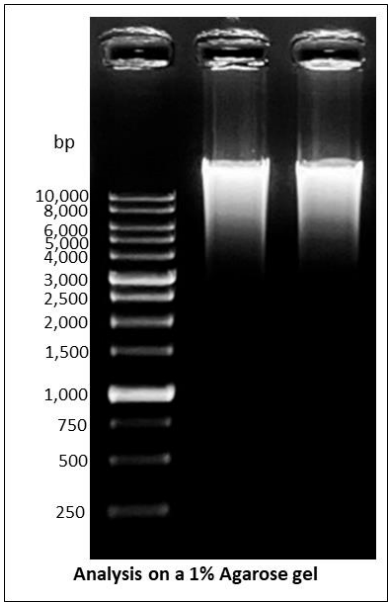
14. Transfer the tube rack to the magnetic stand and allow the magnetic beads to separate.
15. Discard the supernatant and remove the tube rack from the magnetic stand.
    Note: *Be careful not to disturb the magnetic beads.*
16. Repeat steps 13-15 for a total of 2 washes with Wash Buffer 2.
17. Centrifuge samples at 2,000 x g for 3 minutes.
18. Use a 20 µL pipette to remove any remaining wash buffer from the tubes. Do not disturb the magnetic beads.
19. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes.
    Note: *Confirm there is no ethanol present before proceeding to elution steps.*

Elution:

20. Add 100 µL of DNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
21. Incubate at 60°C for 5 minutes with caps closed.
22. After incubation, place the tubes back on the tube rack and then transfer onto the magnetic stand to allow the beads to separate.
23. Transfer the clear supernatant containing the gDNA into new microfuge tubes for downstream processing. Be careful not to disturb the magnetic beads.
24. Analyze quality (OD_{260}/OD_{280}) and yield using NanoDrop and agarose gel (as shown in Figure 1 and Table 1).
25. Isolated DNA can be stored at 4°C for up to a week or at -20°C for long term storage.

Figure 1. In-house data on agarose gel.



Analysis on a 1% Agarose gel

Table 1. In-house data using NanoDrop.

Tissue	Yield: µg/mg Tissue	$OD_{260/280}$
Drosophila 1	0.888	1.77
Drosophila 2	0.906	1.75