Drosophila RNA Isolation in Microcentrifuge Tubes

Materials

- Navy Bead Lysis Kit
- MAGneat Tissue RNA Extraction Kit

Included:

MAGneat Tissue RNA Lysis Kit Contents	
Lysis Buffer	27.5 mL
Binding Buffer	10.5 mL
MAGneat Magnetic Beads	1.65 mL
Wash Buffer 1	15 mL
Proteinase K	22 mg
DNase I	2.75 mg
DNase I Reaction Buffer	220 µL
RNA Elution Buffer	11 mL

Equipment and Reagent to Be Supplied by User:

- Phosphate-Buffered Saline
- 100% Ethanol
- 100% Isopropyl Alcohol
- Molecular Grade Water
- Magnetic Separation Device

Methods

Buffer Preparation

- Lysis Buffer: Ready to use.
- Binding Buffer: Add 24.5 mL isopropyl alcohol to the RNA Binding Buffer bottle.
- Wash Buffer 1: Add 22.5 mL of 100% ethanol to the Wash Buffer 1 bottle.
- Wash Buffer 2 (75% ethanol): 3 parts 100% ethanol to 1 part dH_2O, prepare fresh each day, producing 2.3 mL per sample with 10% excess.
- DNase Reaction Buffer: Ready to use.
- RNA Elution Buffer: Ready to use.

Enzyme Reconstitution

Proteinase K:

- Bring proteinase K vial to room temperature.
- Remove bung and add 1.1 mL of molecular grade water to the proteinase K vial.
- Replace bung and incubate at room temperature for 15 minutes, mixing well by inversion.

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1. Aliquot into required volumes and store at -20°C. Avoid multiple free thaw cycles.

Ensure that all solutions are at room temperature prior to use.

Homogenization

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

Extraction

Nucleic Acid Binding:


Mixing thoroughly

1. Prepare new microtubes containing 200 µL PBS.
2. Transfer 320 µL of the homogenate to the new tubes containing PBS.
3. Centrifuge tubes at 13,000 x g for 5 minutes.
4. Transfer 450 µL of supernatant into new microfuge tubes, being careful not to disturb the pellets or transfer any debris.
5. Add 450 µL Binding Buffer to the supernatant in each tube and mix thoroughly by inversion.
6. 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.
   a. ***Note:*** *Vortex MAGneat beads thoroughly before adding to ensure they are resuspended.*
7. Transfer the tubes to a magnetic separation device and allow to sit for 1-2 minutes, until the magnetic beads are separated.
8. Discard the supernatant and remove the tubes from the magnetic separation device.

Washing I:

1. Add 700 µL Wash Buffer 1, mix thoroughly by inversion, ensuring all beads have been detached from the tube walls.
2. Place the tubes onto the magnetic separation device and allow the magnetic beads to separate.

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3. Discard the supernatant then remove the tubes from the magnetic separation device.
    a. ***Note:*** *Be careful not to disturb the magnetic beads.*
4. Add 700 µL Wash Buffer 2, mix thoroughly by inversion to completely resuspend the beads.
5. Transfer the tubes to the magnetic separation device and allow the magnetic beads to separate.
6. Discard the supernatant and remove the tubes from the magnetic separation device.
    a. ***Note:*** *Be careful not to disturb the magnetic beads.*
7. Repeat steps 16-18 for a total of 2 washes with Wash Buffer 2.
8. Centrifuge samples at 2,000 x g for 3 minutes.
9. Use a 20 µL pipette to remove any remaining wash buffer from the tubes. Do not disturb the magnetic beads.
10. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes.
    a. ***Note:*** *Do not over dry the magnetic beads as this can cause RNA degradation.*

Elution and DNase I Treatment:

1. Add 100 µL of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
2. Prepare the DNase I mastermix. Each sample requires 100 µL of DNase I reaction buffer and 4 µL of DNase I. Mix by pipetting up and down. Do NOT vortex.
3. Add 104 µL of DNase I mastermix to each sample and mix by pipetting up and down to fully resuspend the magnetic beads.
    a. ***Note****: Do NOT vortex samples containing DNase I.*
4. Incubate samples at room temperature for 10 minutes.

Washing II:

Add wash buffer to the magnetic beads

1. Add 200 µL of RNA Binding Buffer to each tube. Mix well by inversion, ensuring all beads are resuspended.
2. Add 400 µL of Wash Buffer 2, mix well by inversion.
3. Place the tubes onto the magnetic separation device and allow the magnetic beads to separate.
4. Discard the supernatant then remove the tubes from the magnetic separation device.
    a. ***Note:*** *Be careful not to disturb the magnetic beads.*
5. Add 400 µL of Wash Buffer 2 and mix well by inversion. Ensure beads are detached from the tube walls.
6. Place tubes onto the magnetic separation device and allow the magnetic beads to separate.

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7. Discard the supernatant then remove the tubes from the magnetic separation device.
    a. ***Note:*** *Be careful not to disturb the magnetic beads.*
8. Centrifuge samples at 2,000 x g for 3 minutes.
9. Use a 20 µL pipette to remove any remaining wash buffer from the tubes. Do not disturb the magnetic beads.
10. Leave caps open to air dry the magnetic beads at room temperature for 5 minutes.
    a. ***Note:*** *Do not over dry the magnetic beads as this can cause RNA degradation.*

Elution II:

1. Add 100 µL of RNA Elution Buffer to each tube. Mix by tapping to ensure that the magnetic beads are completely resuspended.
2. Place the tubes back onto the magnetic separation device and allow the magnetic beads to separate.
3. Transfer the clear supernatant containing the RNA into new microfuge tubes for downstream processing. Be careful not to disturb the magnetic beads.
4. Analyze RNA quality (OD_{260}/OD_{280}) and yield using NanoDrop and gel (as shown in Figure 1 and Table 1).
5. Isolated RNA can be stored at -80°C.

Results:

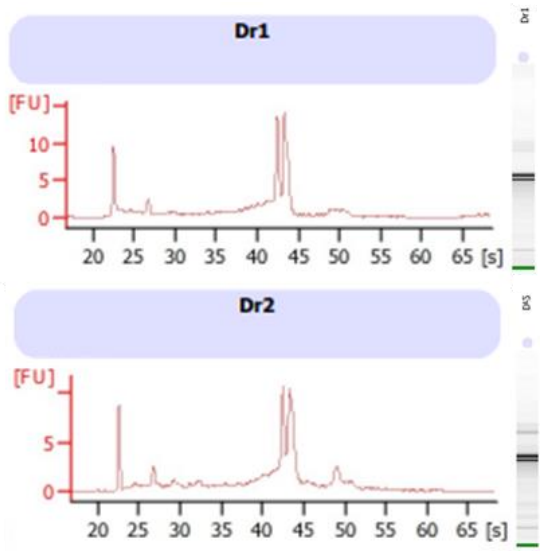

Figure 1: In-house bioanalyzer data

Tissue	Yield: µg/mg Tissue	$OD_{260/280}$
Drosophila1	1.432	1.61
Drosophila2	1.238	1.52

Table 1. In-house data using NanoDrop.