Mouse Bone gDNA Isolation in Microcentrifuge Tubes

Materials

- Navy Bead Lysis Kit
- FoamBlocker
- MAGneat Tissue DNA Extraction Kit

MAGneat Tissue DNA Extraction Kit Contents	
Lysis Buffer	27.5 mL
Binding Buffer	7.3 mL
MAGneat Magnetic Beads	1.65 mL
Wash Buffer 1	15 mL
Proteinase K	22 mg
RNase A	2.75 mg
DNA Elution Buffer	5.5 mL

Equipment and Reagents 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 17 mL isopropyl alcohol to the DNA 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 1.4 mL per sample with 10% excess.
- DNA 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.
- Aliquot into required volumes and store at -20°C. Avoid multiple free thaw cycles.

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RNase A:

- Bring RNase vial to room temperature.
- Remove bung and add 500 µL of molecular grade water to the RNase vial.
- Replace bung and incubate at room temperature for 15 minutes, mixing well by inversion.
- 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

1. Add 500 µL Lysis Buffer, 20 µL proteinase K, and 10 µL FoamBlocker into each lysis kit tube.
2. Weigh up to 50 mg of tissue samples and transfer to the buffer-filled tubes.
   Note: *Cut sample into small pieces for more thorough homogenization.*
3. Set the Bullet Blender at speed 10, time 2 minutes and homogenize the samples dry. If using other homogenizer models, refer to the manufacturer's instructions for appropriate settings.
4. After homogenization, add 500 µL Lysis Buffer, 20 µL proteinase K, and 10 µL FoamBlocker into each lysis kit tube.
5. Remove the tubes and visually inspect the samples to confirm complete homogenization.

Extraction

Mixing thoroughly *ensure they are resuspended.*

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.
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*

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8. Transfer the tubes to a magnetic separation device and allow to sit for 1-2 minutes, until the magnetic beads are separated.
9. Discard the supernatant and remove the tubes from the magnetic separation device.

Washing:

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.

Add wash buffer to the magnetic beads

3. Discard the supernatant then remove the tubes from the magnetic separation device.
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. Be careful not to disturb the magnetic beads.
7. Repeat steps 4-6 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.
Note: *Confirm there is no ethanol present before proceeding to elution steps.*

Elution:

Magnetic beads separate and the DNA is eluted in the buffer

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

Questions? Contact us
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Results:

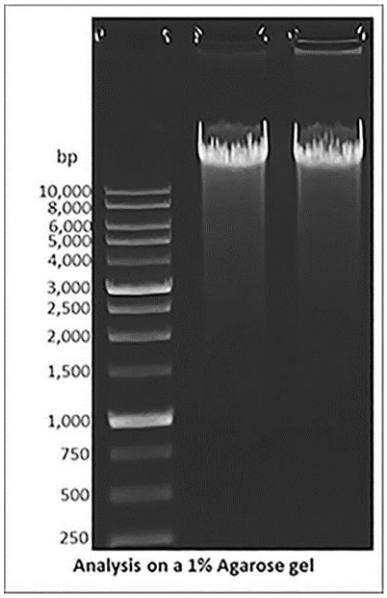

Figure 1. In-house data on agarose gel.

Tissue	Yield: µg/mg Tissue	$OD_{260/280}$
Bone 1	0.992	1.80
Bone 2	1.007	1.80

Table 1. In-house data using NanoDrop.