DNA extractions using the *E.Z.N.A.* kit

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Modified instructions specific for extracting DNA from mouse liver samples

Part 1

Before beginning:

- Set heat block to 55ºC.
- Fill an ice pan with ice.

Instructions:

1. Transfer ~20 mg tissue to a 1.5 mL microcentrifuge tube. Keep sample in ice pan when not in use.
2. Add 200 μL TL Buffer.
3. Grind sample using disposable pestle and motor.
4. Add 25 μL OB Protease Solution. Vortex to mix thoroughly.
5. Incubate at 55°C in the heat block, and vortex the sample every 20-30 minutes (when possible). Allow sample to incubate overnight, or at least 3 hours if you're in a rush.

Part 2

Before beginning (~1 hr before starting):

- Set water bath to 70ºC, fill with distilled water, and allow it to reach 70ºC.
- Vortex Elution Buffer and transfer enough of it for all samples ([# samples $+$ 1] $\times$ 100μL) to microcentrifuge tube(s). Place in heated water bath.
- Dilute HBC Buffer with isopropanol (see bottle for instructions).
- Dilute DNA Wash Buffer with ethanol (see bottle for instructions).

Instructions:

Note: "HiBind DNA Mini Column" and "Collection Tube" are those provided in the *E.Z.N.A.* kit.

1. Remove sample from heat block, vortex to mix, and briefly spin down to remove moisture from inside of the lid.
2. Add 5 μL RNase A, and let sit at room temperature for 2 minutes.
3. Centrifuge at maximum speed (≥10,000 $\times$ *g*) for 5 minutes at room temperature.
4. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

5. Add 220 μL BL Buffer. Vortex to mix thoroughly. A wispy precipitate may form upon the addition of BL Buffer. This does not interfere with DNA recovery.
6. Incubate at 70°C in water bath for 10 minutes.
7. Add 220 μL 100% ethanol. Vortex to mix thoroughly.
8. Insert a HiBind DNA Mini Column into a 2 mL Collection Tube.
9. Transfer the entire sample in the microcentrifuge tube to the HiBind DNA Mini Column, including any precipitates that may have formed.
10. Centrifuge at maximum speed for 1 minute. Discard the filtrate; reuse the collection tube.
11. Add 500 μL HBC Buffer.
12. Centrifuge at maximum speed for 30 seconds. Discard the filtrate *and* collection tube.
13. Insert the HiBind DNA Mini Column into a new 2 mL Collection Tube.
14. DNA wash:
    a. Add 700 μL DNA Wash Buffer.
    b. Centrifuge at maximum speed for 30 seconds.
    c. Discard the filtrate; reuse the collection tube.
15. *Run a second DNA wash.*
16. Centrifuge the empty HiBind DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
17. Transfer the HiBind DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.
18. DNA elution:
    a. Add 50 μL Elution Buffer heated to 70°C.
    b. Let sit at room temperature for 2 minutes.
    c. Centrifuge at maximum speed for 1 minute.
19. *Run a second DNA elution.*
20. Store eluted DNA at -20°C.