1. Grind the bone into a fine powder using a metal blender half filled with liquid nitrogen. Place ≤100 mg in a 1.5 mL centrifuge tube.

2. **Add 200 µL of buffer TI, and then Add 20µL OB Protease solution, vortex to mix well, and incubate at 55°C in a shaking waterbath to effect complete lysis**. Allow lysis to proceed overnight.

3. **Centrifuge at 13,000x g for 2 minutes to pellet any undigested particles. Aspirate or pipette off the supernatant into a clean 1.5ml microfuge tube.**

4. **OPTIONAL:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 5µL (assuming a sample size of 10mg) RNase A (25mg/mL) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol.

5. **Add 220µL Buffer BL and vortex to mix well. Incubate at 70°C for 10 minutes. Linear Acrylamide is needed, add 1µL ofLinear Acrylamide to 220µL BL Buffer.**

   Note: Step 7 can be performed during incubation time.

6. **Add 220µL absolute ethanol and mix thoroughly by vortexing for 15 seconds at max speed. Briefly centrifuge to bring down any liquid from the top of the lid.**

7 **Insert a HiBind® DNA Mini Column into a 2 ml collection tube (**provided). **Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds**

8. **Assemble a HiBind® MicroElute column in a 2mL collection tube (provided). Transfer the entire solution from Step 6 into the column including any precipitate that may have formed. Centrifuge at 13,000 x g for 30 to 60 seconds to bind DNA and discard the flow-through.**

9. **Add 500µL of Buffer HB. Close the lid and centrifuge at 13,000x g for 30 to 60 seconds and discard the flow-through.**

10. **Add 700µL of DNA Wash Buffer diluted with ethanol. Centrifuge at 13,000x g for 30 to 60 seconds and dispose of flow-through.**

    Note: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions or on Page 3 for preparation.

    Optional Step: Repeat step 10

11. **Centrifuge the column at 13,000x g for 2 min to dry the HiBind® membrane.**

NOTE: This step is crucial for ensuring optimal elution in the following step.

12. **Place the column into a nuclease-free 1.5ml microfuge tube (Not supplied). Add 10-50µL of preheated (70°C) Elution Buffer onto the center of membrane. Allow to sit for 3 min at room temperature.**

13. **To elute DNA from the column, centrifuge at 13,000x g for 1 min.**

NOTE: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yields per elution. Alternatively the second elution may be performed using the first eluate or using the second 10-100µL of preheated Elution Buffer.