A Simplified Protocol for Genomic DNA (gDNA) Preparation from Bone Tissue

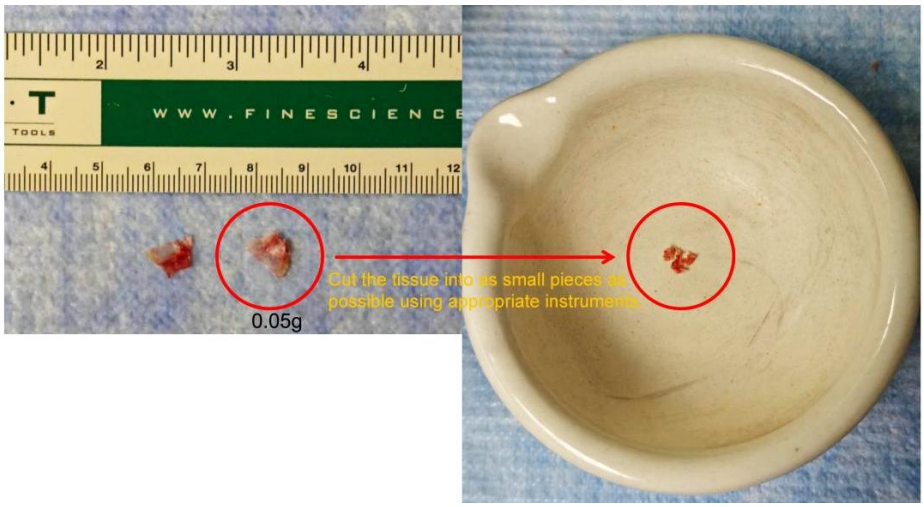
(Ruiliang Chu @ 08/17/2026; TCH Comments)

1) Thoroughly clean/rinse the mortars and pestles with ddH_2O to remove any contaminating DNA (NOTE: No need to be sterile or autoclaved).

2) Pre-cool the mortar and pestle or airdry before use.

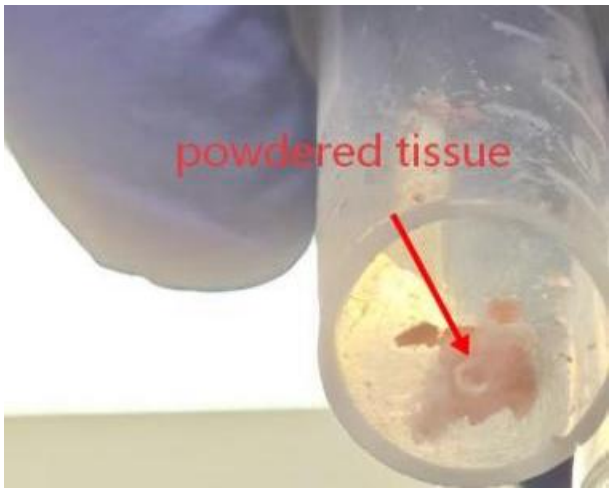
3) Prepare the tissue sample and cut it into approximately 1 mm^3 pieces. Place the tissue pieces in the mortar and add enough liquid nitrogen to completely cover the tissue.

4) Grind the frozen tissue vigorously with the pestle until it becomes a fine powder. If the



liquid nitrogen evaporates during grinding, add more as needed to keep the sample frozen.

5) Transfer the powdered tissue into a 1.5 mL microcentrifuge tube. Add **500-700µl** of the homemade **Genomic DNA Extraction Buffer** (see below)**.**



NOTE: *Pre-warm the buffer to 37°C before use to dissolve any precipitated SDS. The buffer should be completely clear before use.*

TIP: *Use approximately 0.7 mL of extraction buffer per 50 mg of tissue.*

6) Mix thoroughly for 3 minutes by inverting the tubes.

7) Centrifuge at the maximum speed of the microcentrifuge (approximately 14,000 rpm) for 5 minutes.

8) Carefully transfer the supernatant (~550µl) to a new 1.5ml tube. Do not disturb or aspirate the pellet or any insoluble debris.

9) Add **400µl** PC-8; mix the two phases slowly by inverting tube multiple times, and spin at room temp for 10 min (top speed, benchtop microfuge);

10) Recover the (top) aqueous phase(**500~550µ**l), and transfer it to a new 1.5ml tube;

11) Precipitate the gDNA: **500~550µl gDNA solution + 60µl 7.5M (NH4)OAC + 700µl isopropanol;**

12) Precipitate gDNA at top speed for **5min** (benchtop microfuge) (Note: you do not need to add glycogen although it won't hurt if you add);

13) **Wash** gDNA pellet with **700µ**l of 70% ethanol twice;

14) Aspirate liquid completely, and dissolve gDNA in **35µl ddH2O** by mixing gently @ RT (Note: your gDNA concentrations should be in the range of 0.1~0.3µg/µl).

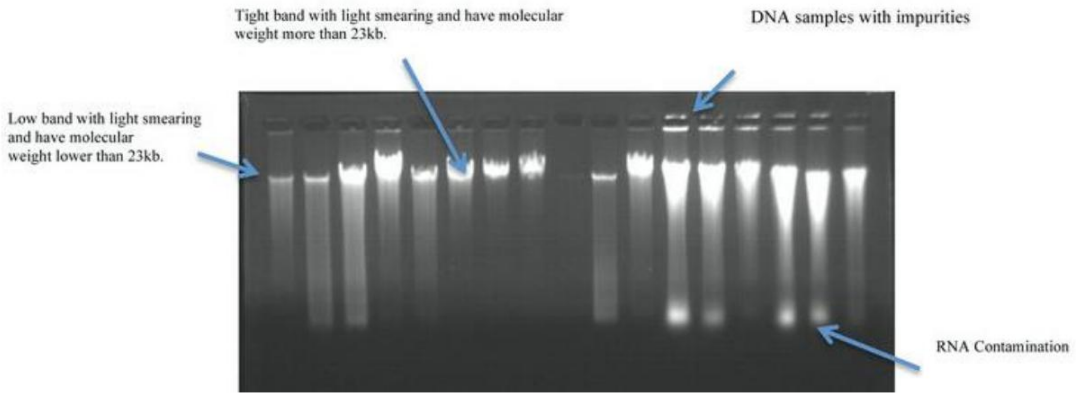
15) Use 2~3µl to run 0.6% agarose gel (see next page for quality check) (Note: You will need to perform RNase digestion or run through the Mag-Bind Beads to remove RNA completely for DNA-Seq library preparation).

Genomic DNA Extraction Buffer (GDEB)

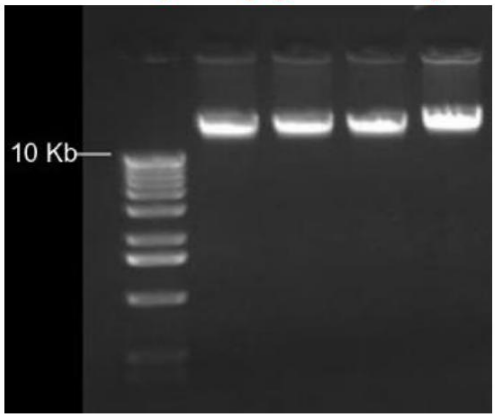
100mM NaCl
10mM Tris-HCl, pH 8.0
25mM EDTA, pH 8.0
0.5% SDS
20µg/ml RNase A (Pancreatic)

NOTE: Keep at 4°C for long-term storage.
Warm up to dissolve SDS precipitate at 37°C.

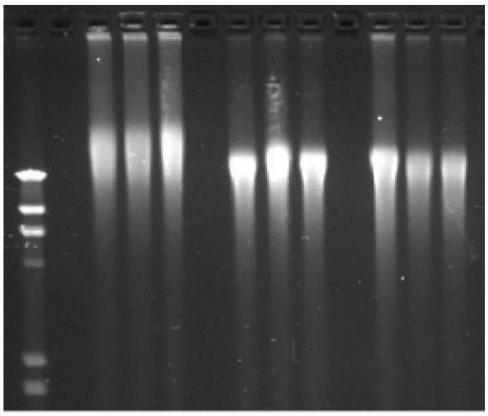
Typical Results of gDNA Preps



Best Quality gDNA Preps



Acceptable Quality gDNA Preps



Poor Quality gDNA Preps