fine powder with a pestle. Add additional liquid nitrogen, as needed, to keep the tissue frozen.
2. Add frozen powder to 5 mL extraction buffer (preheated to 65°C) in a polypropylene screw-cap tube (e.g., Oak Ridge tube) and mix well.
3. Incubate at 65°C for at least 30 min (2–3 h is optimal). Rolling (e.g., in a hybridization oven) or gently mixing during incubation significantly improves DNA yield.
4. Cool samples to <50°C and add an equal volume of chloroform:isoamyl alcohol (24:1) and mix gently by inversion for ~2 min. Be sure to relieve pressure in the tubes by loosening the caps occasionally.
5. Centrifuge (10 min, ~2000*g*).
6. Transfer the supernatant (~3 mL) to a 17 × 100-mm (e.g., Falcon 2059) tube and add 2 vol of precipitation buffer.
7. Invert to mix and incubate (room temperature, 30 min). If solutions do not become cloudy, add another 1 mL of precipitation buffer and incubate longer. For dilute solutions, it is advisable to incubate overnight.
8. Centrifuge (5 min, 2000*g*) to pellet nucleic acids.
9. Discard supernatant. (**Caution:** The pellets are quite friable at this stage and are easily lost!) Aspirate as much of the remaining liquid as possible.
10. Dissolve the pellet in 200 μL of 1*M* NH_4OAc. Sometimes the pellets resist dissolving and hence this step can be more of a resuspension. In either case, transfer the nucleic acids to 1.5-mL microcentrifuge tubes.
11. Add 100 μL NH_4OAc (7.5*M*) and mix well.
12. Add 1.0 mL of isopropanol and mix well by inversion.
13. Incubate at room temperature for 5 min.
14. Centrifuge (15,000*g*, 5 min) to pellet precipitate.
15. Aspirate the supernatant and dissolve the pellet in 200 μL TE + RNase. At this point the pellet should dissolve easily. If not, or if the solution is extremely viscous, add more TE + RNase.
16. Incubate for 1–2 h at 37°C.
17. Add 100 μL NH_4OAc (7.5*M*) and mix.
18. Add 500 μL isopropanol and mix well by inversion. The nucleic acids may be visible as a fluffy, brownish-white precipitate.
19. Centrifuge (15,000*g*, 5 min) to pellet precipitate.
20. Rinse pellets with 70% EtOH and briefly dry.
21. Dissolve pellets in 100μL of TE.
22. Quantify DNA by fluorometry. Spectrophotometric analysis can be accurate, but may not be so because of contaminants. Typically, the DNA concentration will be ~0.5–1.0 mg/mL.

3.4. Genomic DNA Blot Analysis

3.4.1. Preparation of DNA for Blotting

1. Digest DNAs to completion with the appropriate restriction enzyme(s). At least the first few times DNA is isolated with this method, it is useful to confirm it has