

Table 2. Extraction Protocol for Formalin-fixed Tissue.

1. Weigh out approximately 20 mg of tissue using sterile forceps, razors, and etc.
  2. Briefly blot excess formalin from tissue that was decontaminated for 15 seconds in a Stratalinker (Stratagene Cloning Systems, La Jolla, CA).
  3. Place tissue into 10ml of 1X GTE buffer (Shedlock et al. 1997)  
100 mM glycine  
10 mM Tris-HCL pH 8.0  
1 mM EDTA
- This buffer acts a binding agent for excess formalin.
4. Wash tissue three times, once every 24-hour period for a total of 72 hours. Additional washes may be needed due to the amount of excess formalin.
  5. Air-dry or place tissue at 55°C until dry. Do not over dry tissue.
  6. Cut tissue with sterile razor blade into small pieces. Do not macerate the tissue.
  7. Place the pieces of tissue into 1.5 ml microcentrifuge tube containing 500ul Pure Gene Cell Lysis Solution, 100ul Proteinase K (20mg/ml), and 20ul 1mM DTT (dithiothreitol). Incubate at 55°C for 24 hours, invert periodically.
  8. Add another 20-50ul of Proteinase K (20mg/ml) and 10-20ul Pure Gene RNase Solution to the tube. Incubate at 55°C for another 24 hours.  
\*If the tissue is not digested after 48 hours, add 25ul of Proteinase K at 50mg/ml for another 24 hours.
  9. Once all tissue is in solution, cool sample to room temperature. Immediately, place samples on ice for 5 minutes.
  10. Add 200 ul Pure Gene Protein Precipitation Solution to the cell lysate.
  11. Gently invert tube 50 times. Do not vortex.
  12. Centrifuge at 13,000-16,000 x g for 3 minutes.
  13. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5ml microcentrifuge tube containing 600ul cold 100% isopropanol (2-propanol) and 1ul of Pure Gene glycogen solution.
  14. Mix by gently inverting about 25-50 times.
  15. Incubate at -20°C for 24-48 hours, invert periodically.
  16. Centrifuge at 13,000-16,000 x g for 20-30 minutes at 4°C.
  17. Carefully pour off supernatant.  
\*If there is an orange pellet present, add approximately 100-200ul of Cell Lysis solution to the pellet and incubate at 65°C for 1 hour. Repeat steps 9-17.
  18. Add 200ul 70% ethanol and gently invert 25-50 times.
  19. Centrifuge at 13,000-16,000 x g for 3 minutes.

20. Invert and drain the tube on clean absorbent paper and allow to air-dry 10-15 minutes or place in speed-vac for 5 minutes at medium heat.

21. Depending on the size of the pellet add 30-40ul of Pure Gene DNA Hydration Solution. Rehydrate DNA by incubating sample for 1 hour at 55°C and place on shaker (low) overnight at room temperature.

22. Store DNA at 4°C. For long term storage, place sample at -20°C or -80°C.