**Genomic DNA**

**Lysis Buffer:**

50 mM Tris-HCl, pH 8.0

50mM EDTA

1% SDS

10mM NaCl

1. Cut tissue into small pieces. For liver or other soft tissue, do not worry about making fine pieces. Add to pre-cooled (dry ice) mortar, immediately add liq. N2. Begin to grind tissue into fine powder. Add more Liq. N2 as needed. Transfer cold powder to 30 ml tube, leave uncapped so N2 can evaporate.

2. Add 9 ml of per-warmed (5°C ) Lysis buffer and gently resuspend powder.(**\*\* see step** **10**). Add 100 ul of 10mg/ml of Proteinase K, (i.e,100 ug in solution). Incubate 55°C 1-18 hours. Mix gently periodically.  Add 100 ul of Proteinase K after first 2 hours, Optimum: 3 hours adding Proteinase K at 1.5 hours.

3. Treat sample very gently. Add 1 ml of 3M Na0Ac (pH 4.0), 10 ml of warm(55°C ) phenol, mix gently but thoroughly for 5-10 minutes.

4. Centrifuge, 15 minutes.

5. Repeat warm phenol extraction.

6. Extract with equal volume (10ml) phenol: CIA (1 part phenol:1 part CIA:CIA= 23 parts chloroform:1 part isoamyl alcohol)

7. Extract with equal volume (10ml) CIA extraction

8. Upper phase should be relatively clear, If it contains large white clouds, or is very milky, (i) incubate at 68°C 15 min., and re-phenol extract,(ii) start again, but incubate longer with P-K.

9. Transfer upper phase to new tube. Add 2 volumes of cold ethanol and mix gently (Salt: Na0Ac is already in solution). Using a hook and sealed Pasteur pipette spool out DNA. Wipe off excess ethanol on the side of the tubes. Resuspend in 2-5 mls of TE. Make sure DNA is completely dissolved (leave on gentle shaker.)

10. Add RNase mix (100 ug of RNase A, 10U of RNaseT?). Incubate 30 min 37°C.**\*\*** You could add the RNase before the proteinase K, incubate at 37°Cfor 1 hour and then start proteinase digestion. You could then skip step10-15.

11. Add 100 ug of Proteinase K, incubae 37°C 30 minutes.

12. Add 1/10 vol. 3M Na0Ac, Phenol extract once, Phenol:CIA extract twice, CIA extract once.

13. Add 2.5 volumes of EtOH, put in -20°C 30 min. Centrifuge 20 min.

14. Wash well with 70%, Remove all EtOH. If you dry, **Do Not over DRY**.

15. Resuspend carefully, slowly in1-2mls T.E. (1x or 0.1x).