Phenol / Chloroform DNA extraction protocol for PCR
ideal for tortoise blood, also can be modified for tissue; modified from original Sambrook protocol.

DNA Isolation

1. To empty tubes add 500 ul extraction buffer (10mM Tris-HCL pH 8.0, 100mM NaCl, 50mM EDTA, 0.75% SDS)
   ****optional 0.2% BME (beta mercaptoethanol or 2-mercaptoethanol)
2. BLOOD: Add blood or concentrated red blood cells
   If using concentrated red blood cells (RBC), suspend ~20µl of RBC in 500µl of extraction buffer, (or 2-4 drops of whole blood) and shake vigorously to lyse the cells.
   TISSUE: Use a small chunk of tissue about this size: O
   If it has been in ETOH or other buffer, dry on a paper towel for a minute, mince with a sterile razor blade and add tissue bits to the 500 ul of extraction buffer.

3. Add 25 µl of 10 mg/ml Proteinase K (or more if necessary – i.e. tough tissue). And vortex gently

4. (Optional) Add 10 µl of Rnase A (10 mg/ml) OPTIONAL

5. Incubate at 50°C for 2 hours to overnight OR at 65°C for 20 min. until tissue is completely homogenized, vortexing occasionally.

6. Add 500µl of Phenol:Chloroform:Isoamyl (25:24:1) and shake vigorously for 1-2 min. The phenol and chloroform are organic and hydrophobic therefore they bind to lipids such as the cell membranes and hydrophobic proteins, but not to the DNA which is hydrophilic. ***where 2 pairs of nitril gloves so you can peel top layer off if necessary.

7. Centrifuge for 15 min. at top speed (14,000 - 16,000 rmp).

8. You should see 3 layers. The bottom layer is the hydrophilic phenol/chloroform with the proteins. The thin whitish (interface) layer in the middle is the lipids, the top (hopefully clear) layer is the hydrophilic layer with the DNA. Remove top layer (the hydrophilic layer containing the DNA) and place in a clean, labeled 1.5 ml eppy tube. Avoid getting any of the interface (mainly lipids and the cell membranes). Discard the bottom phenol layer and interface.

9. IF the top layer is still quite dirty. – repeat steps 6-8.

12. Add 500 µl of Chloroform:Isoamyl (Sevag; 24:1) to each tube and mix gently for about 1 minute.

13. Centrifuge for 15 min. at top speed (14,000 - 16,000 rmp). Keep top layer again, put into a clean tube and discard bottom layer.

14. Add 1/10 volume of 3 M Soduim Acetate (NaOAc) OR 20 µl of 5 M NaCl and fill the remainder of the 1.5 tube with ice cold 95% ethanol (ETOH). Invert several times - you may be able to see stringy DNA in the tube at this point.

15. Incubate at -20°C for 15 min. to 1 hour.

16. Centrifuge tubes at high speed for 15 min. to pellet the DNA.

17. Wash the pellet once with 1 ml of ice cold 70% ETOH.

18. Dry the pellet under the speed vac approximately 4 min.; do not over dry. Otherwise allow to air dry for about 2 hours.

19. Resuspend DNA pellet in 50 µl of 1X TE overnight, or put at 65ºC for about 20 min. if you want to use the DNA right away.

20. Run 2 µl on a 0.8% gel along with a 1 KB ladder, to visualize the DNA (optional).

21. Spec the DNA using 1XTE at 260 and 280 nm to determine quantity and purity.