

E. Hyatt 05

## **Phenol/Chloroform/Isoamyl DNA isolation Protocol**

### **Digest mouse tail to obtain genomic DNA**

Add 250 µl of tail digestion buffer, incubate overnight at 45° or 55° C.

Add 250 µl phenol/chloroform/isoamyl alcohol (25:24:1), shake 2 min, do not vortex.

**(Note: Use Phenol of pH 6.6 or greater!!!!)**

Spin 10' @ 13,000 rpm

Transfer 200 µl aqueous phase to 1 ml, 100% ETOH to precipitate DNA.

Spin 1-2' @ 13,000 rpm to pellet.

Decant 100% ETOH into sink.

Dry pellet (should still look white) 10 min on bench top.

Add 100 µl TE, to resuspend (If desired, dissolve in 37° C water bath for 10 min.)

Store at 4° C until ready for use.

### **Tail Digestion Buffer(100 ml)**

1 ml, 1M TRIS, pH 7.8 (final 10 mM)

1 ml, 500 mM EDTA (final 5 mM)

10 ml, 10% SDS (final 1%)

1 ml, 20 mg/ml Proteinase K (final 200 µg/ml)

20 ml, 1.5 M NaOAc (final 0.3 M)

67 ml ddH<sub>2</sub>O autoclaved