

DNA from mouse tails:

1. Clip 1/2" of tail & keep on ice in an eppendorf tube. Cauterize tail where clipped. Freeze at -20°C if you are not digesting in tail buffer immediately. If tails are not going to be digested within 2-3 days, tails should be stored at -70°C.

2. Add 700 µl of tail buffer + 35 µl of 10 mg/ml proteinase K.

Tail buffer:	500 ml:
50 mM Tris-HCl, pH 8	25 ml of 1 M Tris-HCl, pH 8
100 mM EDTA	100 ml of 0.5 M EDTA
100 mM NaCl	50 ml of 1 M NaCl
1% SDS	50 ml of 10% SDS
	Add 275 ml of water

3. Incubate at 55°C for 8 hours-overnight.

*4. Add 700 µl of phenol and vortex well to mix. Spin in microfuge for 5 minutes. Remove aqueous phase being careful to avoid interphase. Place supernatant into a clean eppendorf tube.

5. Add 700 µl of chloroform and vortex well to mix. Spin in microfuge for 5 minutes. Remove aqueous phase being careful to avoid interphase. Place supernatant into a clean eppendorf tube.

6. Add 1000 µl of ethanol. Mix well. Spool genomic DNA using a glass rod and resuspend DNA in 50-100 µl of 0.1 X SSC.

*The following steps may be substituted for steps 4 and 5 if the restriction enzyme used for the digestion cuts well:

4. Add 700 µl of phenol/chloroform and vortex well to mix. Phenol/chloroform (1:1) is made just prior to use by adding an equal volume of phenol and chloroform together. Spin in microfuge for 5 minutes.

5. Remove ~500 μ l aqueous phase (top layer) being careful not to remove any of the interface. Place supernatant into a clean eppendorf tube.

Reagents and Supplies:

(1) equilibrated phenol: United States Biochemical- catalog # US75829. Other companies also sell equilibrated phenol (i.e. Amersham).

(2) proteinase K: Sigma, catalog # P-8044

(3) chloroform: Fisher, catalog # C298-500. Chloroform from other companies will also work.

(4) cauterizer: Reboz, catalog # RS209

(5) capillaries for spooling tail DNAs: Fisher, catalog # 12-141-8