DNA Isolation from Tails

1. Each tail should be in a clean eppendorf tube.
2. Add 500µl of tail lysis buffer containing Proteinase K (PK) to each tube.
3. Incubate tail samples in 50-60C water bath overnight.
4. Add 250µl saturated (6M) NaCl to each tube.
5. Shake tubes vigorously (~ 20 times) and incubate tubes on ice for 10 minutes.
6. Spin tubes on low speed (#6 on Hemle centrifuge) at 4C for 10 minutes.
7. Remove supernatant and place into a clean eppendorf.
8. Add 650µl isopropanol and invert to mix. Incubate tubes at room temperature for 15 minutes.
9. recover DNA by centrifuging, max speed, 10 minutes at room temp.
10. Place tubes inverted on bench and allow to air dry 5 minutes.
11. Add 200µl of TE pH 7.5 or sterile water to each tube. Incubate in 50-60C water bath for * 10 minutes. Resuspend pellet by pipetting up and down several times.

Tail Lysis Buffer:

	Final Concentration	per 500ml
1M Tris pH 8.0	10mM	5ml
5M NaCl	100mM	10ml
0.5M EDTA pH 8.0	10mM	10ml
10% SDS	0.5%	25ml
dH_20		to 500ml

Proteinase K concentration:
Add 20µl of a 20 mg/ml stock per 1ml of tail lysis buffer.

ES Cells:

For ES Cells the protocol is very much the same except for the following:
All steps are done in a well of a 24 or 6-well dish.
The initial incubation in the lysis buffer is done at 37C for 2 hours to overnight.

Southerns:

For important southerns:
1. Dilute DNA in 400µl of water.
2. P henol/chloroform extract DNA.
3. Precipitate in 1/10 vol 3M NaOAc and equal volume of isopropanol.
4. Precipitate 15 minutes at RT.
5. Wash pellet with 70% EtoH.
6. Resuspend in water.

Hot Shot Tail DNA prep

1. Tag and tail mice as usual.

2. Cut a small piece of each tail (~2mm) and place in a 96-well PCR plate. Save remaining piece of tail at 4° C.

Note: In step 6, it says to transfer your sample to an eppendorf, so instead of wasting money on a 96 well plate, **and** instead of hogging PCR machines, it may be better to just do it in eppendorf tubes that will fit in a hot block.

3. Add 75 ul of Alkaline Lysis Buffer (see recipe below) to each sample, making sure that the tail is immersed in the buffer and that there is no air bubble at the bottom of the well. Seal plate. Important: The Tail Lysis Buffer should be prepared **fresh** just before adding to the tails.

4. Put in PCR machine and remove promptly after program has finished (30 min at 95° C, followed by 15 min at 4° C. Do not leave plate in the PCR machine.

5. Immediately add 75 ul of neutralization buffer (40 mM Tris-HCl which has not been pH'd) to the tails and mix briefly using a separate filter tip for each tail.

6. The tail preps are now ready for PCR analysis. Use 2ul in a 20ul PCR reaction. Take up 2ul of sample from the top of the 150 ul tail prep avoiding the debris at the bottom of the well. Do not spin the tail preps in an eppendorf centrifuge because spinning can cause a proportion of the DNA to be lost in the debris at the bottom of the well thereby reducing the concentration of DNA in the sample. Wrap the plate with parafilm and store the tail preps at 4° C. For long-term storage it is best to transfer the DNA samples to 0.5ml eppendorf tubes and store them at 4° C.

Alkaline Lysis Buffer: Prepare fresh before use

Mix in 15 ml tube:
10 ml sterile dWater
14 ul 50% Sodium Hydroxide
14 ul 0.5M EDTA, pH 8.0

Vortex Briefly

Neutralization Buffer (100ml)
40 mM Tris-HCl (not to be pH'd) pH=5 -- Filter Sterilize

Protocol From JAX:

DNA from tail biopsies

1. Remove 0.5 mm of tail into polypropylene microfuge tube (do not mince).
(The tubes must have tight-fitting caps, so that there are no leaks in steps 3 and 7 below.)
2. Add 0.5 ml DNA digestion buffer with proteinase K added to 0.5 mg/ml final concentration.
(0.5 mg/ml is a high concentration and can probably be reduced.)
DNA digestion buffer: 50 mM Tris-HCl pH 8.0
100 mM EDTA pH 8.0
100 mM NaCl
1% SDS
3. Incubate overnight at 50-55 °C with gentle shaking.
(At this step, mechanical agitation greatly aids complete disruption of the tail.)

4. Quick spin tubes to get solution off inside of cap.
5. Fill inside well of microfuge tube cap with vacuum grease.
(We use Dow Corning high vacuum grease and a 10cc syringe to dispense.)
6. Add 0.7 ml neutralized phenol/chloroform/isoamyl alcohol (25:24:1).
7. Mix fairly vigorously. (Do NOT vortex--We use a clinical rotator for 1 hour.)
8. Spin in microfuge at top speed 5 minutes and transfer 0.5 ml of the upper phase to new microfuge tube. (Use P1000 for transfer, and draw the aqueous phase gently through tip several times after transfer if the DNA is still in large, gelatinous mass.)
9. Add 1 ml 100% ethanol at room temperature and invert (using clinical rotator if you wish) until DNA precipitate forms. (approximately 1 minute)
10. Spin in microfuge 5 minutes and carefully remove and discard supernatant.
11. Add 0.5-1 ml 70% ethanol (-20 °C) and invert several times.
12. Spin in microfuge 5 minutes and carefully remove and discard supernatant.
13. Quick spin tubes and remove last drop of ethanol solution with 25 µl capillary tube.
14. Air dry at room temperature or in dessicator (overnight if you wish).
15. Add 100-200 µl TE buffer and incubate at 65 °C for 15 minutes to resuspend DNA. Draw DNA through P1000 tip after 65 °C incubation to aid in suspension if you wish.
16. Use 10-20 µl for restriction enzyme digest.
17. Total yield is approximately 20-50 µg DNA, 0.1-0.25 µg/µl.