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Kelly Lab
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Extraction of Genomic DNA from tails

1. Cut approximately 1 cm of tail from each mouse after ear clipping and store at +4.
2. Add 400 ul of digestion buffer to each tail. Add 18 ul of 20 mg/ml Proteinase K (dissolve PK in water and store stocks at –20).
3. Place samples in 55 C bath overnight.
4. Add 200 ul of phenol and 200 ul of chloroform with barrier tips. Slowly invert.
5. Put in microfuge for 10 minutes at full speed. If you can smell phenol during the spin, use the microfuge in the cold room in order to prevent contaminating the lab.
6. Pipet off bottom layer using barrier tips and discard. Keep top! Do not disturb interface.
7. Add 200 ul of chloroform with barrier tips to TOP layer. Slowly invert.
8. Microfuge for 5 minutes at full speed.
9. Transfer top phase to a new tube trying not to bring debris along.
10. Add 45 ul of 7.5 M ammonium acetate pH 7.4 and 1 ml of 100% ETOH to TOP phase. Slowly invert. You should see stringy precipitate develop. Do not let DNA stay in ETOH/salt overnight.
11. Using sterile pipet tip to remove precipitate and put into new tube containing 500 ul of 70% ETOH.
12. Microfuge at full speed for 5 minutes. Decant supernatant and pipet out any excess liquid. Air dry.
13. Resuspend in 50 ul of TE buffer. Incubate at 37° for 1 hour then at +4 overnight to completely dissolve.
14. Resuspend well with a pipet tip and read OD.

Tail Digestion Buffer		**TE Buffer pH 7.9**
25 mls of 1 M Tris pH 8.0	**(50 mM)**	10 mM Tris
10 mls of 5 M NaCl	**(100 mM)**	1 mM Edta
100 mls of .5 M Edta pH 8.0	**(100 mM)**	
315 mls of H20		
Autoclave then add 50 mls of 10% SDS	**(1%)**	
(Sterile filtering is also fine)		