Isolation of DNA from tail samples (the hard way)

1. Add 600µl genomic lysis buffer to eppi tubes containing sample.
    a. NOTE: Tail sample should be kept on dry ice prior to addition of lysis buffer.

2. Add 3µl Proteinase K (20 mg/ml) and incubate at 55°C overnight.
    a. NOTE: Periodically invert tube to prevent sample from sticking to bottom of tube. This is done by either using the hybridization oven, or by using the shaker located in the hot room

3. Add 3µl Rnase A (4 mg/ml) to the lysate. Mix the sample by inverting the tube several times and incubate at 37° C for 60 minutes.

4. Cool sample to room temperature. Add 200 µl Protein Precipitation Solution (or 5M NH_4OAc) to the Rnase-treated lysate solution.

5. Vortex vigorously at high speed for 20 seconds. Centrifuge at 13,000 – 16,000 x g for 3 minutes. The precipitated proteins will form a tight pellet.

6. Pipet the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml tube.

7. Add 600 µl 100% Isopropanol. Mix the sample by gently inverting the tube until white coils of DNA form.

8. Centrifuge at 13,000 – 16,000 x g for 1 minute. (DNA will form a tight pellet).

9. Remove supernatant with pipetman (or carefully pour out). Add 600 µl 70% Ethanol. Invert tube several times to wash pellet.

10. Centrifuge at 13,000 – 16,000 x g for 1 minute. Carefully remove ethanol.

11. Airdry 15 –30 minutes. Resuspend pellet in 200 µl dd H_20. Allow to re-hydrate overnight.

Genomic Lysis Solution:

	For 200 mls :	
20mM Tris-Cl, pH 8.0	2 mls	of 2M Tris
150mM NaCl	6 mls	of 5M NaCl
100mM EDTA	40 mls	of 0.5M EDTA
1% SDS	20 mls	of 10% SDS
	132 mls dd H20	