DNA PREPARATION FROM MOUSE TAILS

Day 1

1. Cut 5mm from the tails of 1-2-week-old pups and place in a 1.5mL tube
2. Add 20uL of proteinase K (20mg/mL) per 1mL tail lysis buffer
2. Add 400ul of tail lysis solution to each tube
3. Place at 60C˚ in shaker for 6-12 hours (O/N)

Day 2

4. Add 75uL of 8M potassium acetate to each tube
5. Add 500uL of chloroform to each tube and invert tubes several times
7. Spin in microfuge for 5 min at top speed, RT
8. Remove aqueous phase (~380uL) and transfer to a new tube
9. Add 1mL of 100% EtOH, invert tubes, and incubate at -20°C for at least 30min
10. Spin in microfuge for 10 min, top speed, 4°C (RT spin is OK, but 4°C improves yield)
11. Carefully pour off EtOH, so as not to lose pellet, add 1mL 70% EtOH & spin top speed 5 min, 4°C
12. Pour off supernatant carefully, pulse spin, pull off residual EtOH with P100 pipette, dry 10 min, RT
12. Resuspend pellets in 100uL TE
13. Resuspend for 10-20 min in shaker at 60°C

Mouse Tail Lysis Buffer

To make 500mL:

10% SDS	25mL
5M NaCl	10mL
1M Tris PH 8.0	25mL
0.5M EDTA PH 8.0	2.5mL
H2O	437.5mL

Filter Sterilize the Lysis Buffer using a Nalgene Rapid-Flow Filter; store at RT

Tail Lysis Buffer Final:
0.5% SDS
100mM NaCl
50mM Tris PH 8.0
2.5mM EDTA
Proteinase K, 200ug/ml added just before use

To make Proteinase K, stock:

MilliQ H2O	12.5 mL
Proteinase K	250mg

Aliquot 1mL each into the Eppendorf tubes