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Updated on 11/22/2011

DNA Isolation from Mouse Biopsies – for genotyping

1) Lysis
    a. Incubate mouse tissue in 500 μl tail lysis buffer + 10 μl proteinase K (10 mg/ml stock) overnight at 55°C
2) Removal of contaminants
    a. 200 μl of 8M ammonium acetate to precipitate any soluble proteins.
    b. Incubate samples on ice for 20 minutes, invert tubes 3x, and then centrifuge at 4ºC at 12kRPM for 20 minutes to pellet out the proteins.
3) DNA precipitation (can do this at room temperature)
    a. Transfer 500 μl your cold liquid lysate into 500 μl of 100% isopropanol. Mix by repeated inversion. You should be able to see some precipitate form here. Centrifuge at 4ºC for 10 minutes at high-speed. The DNA will precipitate at the bottom of the tube (you should see a small, translucent pellet).
        i. Decant the isopropanol.
    b. Wash the pellet in 1 ml of 70% ethanol. Centrifuge at 4ºC for 10 minutes at high-speed. The DNA will precipitate at the bottom of the tube ((you should see a small, white pellet).
        i. Decant the 70% ethanol, air-dry the pellet, and resuspend the DNA pellet in 50-100 μl TE solution or ddH_2O.

Reagents:

1) Proteinase K (from Roche): stored at 10 mg/ml stocks at -20 °C
2) Tail lysis buffer: (10 mM Tris pH 8.0, 100 mM NaCl, 10 mM EDTA pH 8.0, 0.5% SDS)
    To make 500 ml:

Stock soln	mL needed	Final conc
1 M Tris pH 8.0	5	10 mM
5 M NaCl	10	100 mM
0.5 M EDTA pH 8.0	10	10 mM
10 % SDS	25	0.5 %
dH_2O	450 mL	

3) 8M Ammonium Acetate (from Fisher Scientific #A637-500, F.W. 77.08)
    To make 250 ml:
    a) Add 154.16g of Ammonium Acetate to 150 ml of dH_2O
    b) Stir to dissolve
    c) Fill to 250 ml with of dH_2O
4) Tris-EDTA (TE) buffer (10 mM Tris pH7.5, 1 mM EDTA pH 8.0)