UCSD Transgenic Mouse and
Gene Targeting Core
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PREPARATION OF GENOMIC DNA FROM MOUSE TAIL TISSUE

Reagents

Lysis Buffer: for 1 liter

for 10mM Tris	1.21 g
for 100mM NaCl	5.84 g
for 10mM EDTA	3.72 g
for 0.5% SDS	5.00 g

balance to 1 liter with ddH_2O

-pH solution to 8.0
-filter sterilize (45 micron filter)

Proteinase K Solution: 10mg/ml

BRL catalog number 5530UA 100mg
to reconstitute one bottle add:
9.5 ml ddH_2O
0.5 ml 1M Tris pH 8.0
4 µl 2.5 M $CaCl_2$
Store at 4ºC
Any problems with this, call BRl Tech-Line @ 1-800-828-6686

Phenol-Tris saturated

1. Add 10% ddH2O
2. Melt phenol at 60°C
3. Add an equal volume of 1 M Tris pH 8.0. Stir for ten minutes, aspirate off the top layer, if two layers do not form add more Tris until phenol is saturated.
4. Repeat with an equal volume of 0.1 M Tris pH 8.0
5. Check to see if pH of the supernatant is between 7.5-8.2. If not, add more 0.1 M Tris pH 8.0 and check pH again.
6. Remove H_2O leaving 10% to protect phenol.
7. Add 1mg 8-hydroxguinoline for each 1ml of phenol.
   When all 8-hydroxguinoline is dissolved, aliquot into conical tubes and store at -20°C.

Method

1. Add 500 µl of Lysis buffer to ~1cm tail sample. Incubate 56°C O/N.

   Lysis buffer:

100 mM	Tris-HCL pH 8.5
5 mM	EDTA
0.2%	SDS
200 mM	NaCl
600-800 µg/ml	Proteinase K

2. 1X Phenol/$CHCl_3$ extract, 1X $CHCl_3$ extract.

3. Add 50µl 3M NaAc and 1 volume of Isopropanol. Spin down 3 min.

4. Wash pellet with 1 ml 70% EtOH.

5. Spin briefly to collect the pellet.

6. Dry pellet 5-10 min. at room temperature.

7. Add 80 µl TE.

8. Check concentration with Spectrophotometer. You should have about 40 µg total DNA. Use 20 µl (~10 µg) DNA for digestion in 100 µl reaction and use for a Southern Blot.