



THE NORTHWESTERN UNIVERSITY
TRANSGENIC AND TARGETED MUTAGENESIS LABORATORY

Tail DNA Prep

1. Cut 1mm to 8mm mouse tail. Put in 1.5ml eppendorf (microcentrifuge) tube.
2. Add 500 μ l lysis buffer with proteinase K (add fresh).
3. Incubate at 55 $^{\circ}$ C with shaking (if possible) overnight.
4. Vortex briefly. If tail has been completely digested, the only observable particulate material in the tube will be hair and tail bone (if a larger tail sample was taken) in the tube. Sometimes, tail has not been completely digested.
5. Add additional proteinase K to each tube (may want to centrifuge tubes briefly to remove liquid from inside of tube cap before adding proteinase K) changing tips between tubes. If tail has been completely digested, 1ml prot.K/tube is sufficient. If tails have not been completely digested, add 2 μ l prot. K/tube. Vortex briefly to mix and incubate again at 55 $^{\circ}$ C for 1-2 hours. **This additional incubation with fresh proteinase K results in a more consistent ability to easily dissolve precipitated DNA in step 10.**
6. Spin tubes for 10 mins at 14,000rpm.
7. Pipet or carefully pour off supernatant into new tube avoiding pelleted hair.
8. Precipitate DNA with 1ml 95% EtOH. If very little DNA precipitates, incubation at -20 $^{\circ}$ C for 30 mins can help improve yield.
9. Spool DNA with a drawn out pasteur pipet (yields cleaner DNA than pelleting). BRIEFLY dry spooled DNA and tap DNA off glass pipet into 25-100 μ l of TE in a .5 ml microcentrifuge tube. Alternatively, centrifuge tubes at 4 $^{\circ}$ C for 5-10 mins at 3000rpm to pellet DNA. Pour off supernatant. Make sure DNA pellet stays at the bottom of the tube. Dry pellet BRIEFLY and add 25-100 μ l TE.
10. Incubate tubes at 55 $^{\circ}$ C for 10-30 mins. Briefly vortex warm tubes to dissolve DNA. DNA should resuspend easily. If DNA is difficult to dissolve at 55 $^{\circ}$ C, incubate tubes at 65 $^{\circ}$ C for about 10mins and vortex.

Lysis Buffer (1X) Stock:

For 100ml total volume:

5 ml of 2Mtris, pH7.5 or 8.0

2 ml of 5M NaCl

1 ml of .5M EDTA

2 ml of 10% SDS

90 ml ddH₂O

Proteinase K stock: 20mg/ml:

Use at 1:200 dilution (2.5ml/500ml lysis buffer per tail)

We use 1-2 μ l DNA/ 50 μ l PCR reaction (per tube). DNA isolated using this method is also suitable for Southern.

For higher quality or cleaner DNA, dissolve DNA in 100-200 μ l TE (step 9 & 10). Extract DNA once in an equal volume of phenol:chloroform: for 100 μ l extraction, add 50 μ l phenol and vortex. Then add 50 μ l chloroform and vortex again. Centrifuge for 5 mins at 10K.

An aliquot of DNA can be taken from the upper aqueous phase and used directly for PCR, Southern, dot blots, etc. The DNA can be stored in the tube as is or the upper phase transferred to a new tube for storage or a second EtOH precipitation.