Tail DNA Preparation for Analysis of Transgenic Mice

1. With mouse firmly restrained, cut off approximately 0.5 cm from the tip of the tail using a pair of surgical scissors. Any bleeding which results from this procedure can be prevented by treating the wound with silver nitrate applicators which have been dampened in water (the applicators are available from Butler, Columbus, OH 43228; catalog #40606). A smaller sample of tail is required from older mice due to the tail's larger size.

   Note: Check current IACUC guidelines for use of anesthesia when tail biopsies are performed. Generally, if the tail samples are obtained at less than 14 days of age, anesthesia is not required. Older mice, however, will require anesthesia.

2. Each tail sample is cut into smaller pieces (1-2 mm) with a razor blade and placed into a 1.5 ml microcentrifuge tube. **For younger mice (less than 14 days), the tail sample does not need to be cut into smaller pieces.**

3. Add 750 µl of isolation buffer to each tail sample and incubate at 55°C overnight. (It sometimes helps to shake the samples a few times during the first hour or two of incubation.)

4. Shake samples vigorously the following morning to produce a uniform solution (everything should be digested except the hair). Add more proteinase K (100 µg/ml) if there are still pieces of tail visible, and incubate at 55°C again until the sample is fully digested (approximately 1-2 hrs.).

5. Add 650 µl of phenol (equilibrated with 200 mM Tris, pH 8.5). Shake for 3-5 minutes.

6. Separate layers by centrifugation in a microcentrifuge for 3 minutes at room temperature. Remove the top aqueous layer to a new microcentrifuge tube.

7. Add 650 µl phenol:chloroform (1:1) and shake for 3 minutes.

8. Separate layers by centrifugation in a microcentrifuge for 3 minutes at room temperature. Remove the top aqueous layer to a new microcentrifuge tube.

9. Add 650 µl chloroform and shake for 3 minutes.

10. Separate layers by centrifugation in a microcentrifuge for 3 minutes at room temperature. Remove the top aqueous layer to a 5 ml polypropylene tube containing 1 ml of 100% ethanol/0.3M sodium acetate (pH 6.0). Close and invert the tube several times to allow the DNA precipitate to form. (The samples can be left on ice for up to an hour at this point, if necessary.)

11. Carefully remove the DNA sample using a P200 pipetman, and transfer to a new tube containing 150 µl of cold 70% ethanol. Vortex <u>very</u> gently. (Again, this is a good stopping point since the samples can be left in the 70% ethanol on ice, if necessary).

12. Pellet the DNA by centrifugation at 4°C for 10 minutes. Carefully remove the supernatant from each tube with a 200 µl pipetman. (The pellets will sometimes float off the tube so be very careful at this step.)

13. With a P200 pipetman add 100 µl of TE to each pellet and resuspend at 55°C overnight. Keep samples at 4°C once they are completely resuspended.

14. Remove 5µl from each tube into 750 µl of dH_2O in a microcentrifuge tube. Close each tube, vortex gently and measure the A_{260}. The DNA concentration (µg/µl) in the stock sample is 7.5x the A_{260} measurement.

15. DNA samples prepared by this method can be used for PCR, dot blots or Southerns.

Buffers:

A. Isolation buffer:

0.1M EDTA
0.05M Tris, pH 8.0
0.5% SDS

Add proteinase K to 500 µg/ml immediately before adding the isolation buffer to the tail samples.

B. 100% ethanol/0.3M sodium acetate (pH 6.0):

45 ml ethanol
5 ml 3M sodium acetate (pH 6.0)

C. TE is 10mM Tris (pH 7.4), 1mM EDTA