# Protocol 5

## Preparation of Genomic DNA from Mouse Tails and Other Small Samples

OVER THE YEARS, MANY PROTOCOLS FOR THE EXTRACTION OF DNA from mouse tails have been published, almost all of them descendants of the original method developed by Richard Palmiter and Ralph Brinster in 1985 (Palmiter et al. 1985). Palmiter's laboratory was in Seattle, while Brinster and his thousands of mice were 3000 miles away in Philadelphia. Brinster would snip fragments of tails from the mice, place them in a solution of SDS and proteinase K, and, in those pre-FedEx days, would ship them to Palmiter by U.S. Mail. After their 2–3-day journey at ambient temperature, the samples were extracted with phenol:chloroform, and the genomic DNA was recovered by precipitation with ethanol. Success with this method fortunately does not require entrusting semidigested mouse parts to the care of the U.S. postal system. Instead, the digestions can be more conveniently carried out overnight at 55°C, without transportation. Each tail snippet generates 50–100 μg of DNA that can be used in dot or slot blotting to detect a transgene of interest, in Southern hybridization to detect DNA fragments that are <20 kb in size, and, more expediently, as a template in PCRs. This simple protocol continues to be used in hundreds of laboratories for genotyping transgenic and knock-out mice and for extracting DNA from small numbers of cultured cells or from fragments of tissue.

Two variants of the basic protocol are useful when processing very large numbers of samples. The method of Laird et al. (1991) omits extraction with phenol:chloroform, whereas the protocols described by Thomas et al. (1989) and Couse et al. (1994) use commercially available gel-barrier tubes to eliminate the tedious transfer of samples during serial extraction with organic solvents. These variations are described in the alternative protocols at the end this protocol.

## MATERIALS

**CAUTION: Please see Appendix 12 for appropriate handling of materials marked with <!>.**

### Buffers and Solutions

**Please see Appendix 1 for components of stock solutions, buffers, and reagents. Dilute stock solutions to the appropriate concentrations.**

*Ethanol*
*Isopropanol*
*Phenol:chloroform:isoamyl alcohol (25:24:1 v/v)* **<!>**
*Phosphate-buffered saline*

*SNET*

20 mM Tris-Cl (pH 8.0)
5 mM EDTA (pH 8.0)
400 mM NaCl
1% (w/v) SDS

Sterilize the solution by filtration through a 0.45-μm nitrocellulose filter. Store the sterile solution in 50-ml aliquots at room temperature.

*TE (pH 8.0)*

## Enzymes and Buffers

*Proteinase K (20 mg/ml)*

Please see Appendix 4.

## Centrifuges and Rotors

*Sorvall H1000B and SH-3000 rotors or equivalents*

## Special Equipment

*Polypropylene tubes (17 x 100 mm)*
*Rocking platform at room temperature and 4°C*
*Rocking platform or shaking incubator, preset to 55°C*
*Shepherd's crook (for a description, please see Steps 5–7 of Protocol 3)*

## Cells and Tissues

*Cultured Cells*

Monolayer cultures, grown to confluence or semiconfluence in 100-mm dishes, should be washed twice with ice-cold phosphate-buffered saline and then immediately lysed by addition of 1 ml of SNET containing 400 μg/ml proteinase K, as described in Step 1.

Cells growing in suspension should be recovered by centrifugation, washed twice in ice-cold phosphate-buffered saline, and then resuspended in TE (pH 8.0) at a concentration of 5 x $10^7$/ml. Aliquots of the suspension (0.2 ml) are then transferred to a series of 17 x 100-mm Falcon polypropylene tubes and the cells are immediately lysed with SNET containing 400 μg/ml proteinase K, as described in Step 1.

*Mouse tails or mouse tissue*

Samples of mouse tails are generally cut from 10-day old suckling animals or at the time of weaning (~3 weeks of age). In the former case, the distal one third of the tail is removed and transferred into a microfuge. In the latter case, 6–10 mm of the tail is removed under anesthesia and transferred to a 17 x 100-mm Falcon polypropylene tube. Under rare circumstances, where obtaining a result rapidly is of paramount importance, the entire tail can be removed from newborn animals and transferred to a microfuge tube.

To isolate DNA from mouse tissue (other than tail snippets), transfer ~100 mg of the freshly dissected tissue to a 17 x 100-mm Falcon polypropylene tube.

Mouse tails or other tissues can be stored for a few weeks at –70°C in tightly closed tubes before adding SNET and proteinase K. However, it is better to proceed without delay to digest the samples with proteinase K (Steps 1 and 2). The completed digests can then be stored indefinitely at –20°C before phenol:chloroform extraction.

All experiments carried out on laboratory mice, including removing sections of tail, require prior authorization from the appropriate institutional ethics committee.

# METHOD

1. Prepare the appropriate amount of lysis buffer (see Table 6-1) by adding proteinase K to a final concentration of 400 μg/ml in SNET. Add lysis buffer to the mouse tails or other tissues.

   This procedure also can be used to isolate DNA from monolayers of cultured mammalian cells. In this case, 1 ml of SNET containing 400 μg/ml proteinase K is added directly to 100-mm monolay-

**TABLE 6-1 SNET Lysis Buffer Volumes**

| Age of Mouse | Amount of Tissue | Type of Tube | Volume of SNET Lysis Buffer (ml) |
|---|---|---|---|
| Newborn | entire tail (1 cm) | microfuge | 0.5 |
| 10 days old | distal one-third | microfuge | 0.5 |
| Weanling (3–4 weeks) | 6–10-mm | 17 x 100-mm polypropylene | 4.0 |
| Any age | 100 mg of fresh tissue | 17 x 100-mm polypropylene | 4.0 |

ers that have been rinsed twice in phosphate-buffered saline. The viscous cell slurry is scraped from the dish with a rubber policeman, and transferred to a 17 x 100-mm polypropylene Falcon tube.

Cells growing in suspension that have been washed twice in phosphate-buffered saline are resuspended in TE and lysed with SNET containing 400 µg of proteinase K (1 ml per $10^9$ cells).

2. Incubate the tube overnight at 55°C in a horizontal position on a rocking platform or with agitation in a shaking incubator.

   It is important that the sample be mixed adequately during digestion. After overnight incubation, the tissue/tails should no longer be visible and the buffer should be a milky-gray.

3. Add an equal volume of phenol:chloroform:isoamyl alcohol, seal the top of the tube, and place it on a rocking platform for 30 minutes at room temperature.

   Protocols differ in their use of vortexing at various stages of the protocol. Some protocols state flatly not to vortex. Others say that vortexing ensures a greater yield of DNA composed of fragments up to 20 kb in length that can be detected by Southern hybridization, dot and slot blotting, and PCR analysis. If DNA of higher molecular weight is required, take care to minimize shearing forces (please see the information panel on **MINIMIZING DAMAGE TO LARGE DNA MOLECULES** in Chapter 2).

4. Separate the organic and aqueous phases by centrifugation. Centrifuge the samples in 17 x 100-mm polypropylene tubes at 666*g* (1800 rpm in a Sorvall H1000B rotor with swinging buckets or 1600 rpm in a Sorvall SH-3000 swinging bucket rotor) for 5 minutes at room temperature. Alternatively, for smaller sample volumes, centrifuge the samples in microfuge tubes at maximum speed for 5 minutes at room temperature in a microfuge. Transfer the upper aqueous phase to a fresh Falcon or microfuge tube.

5. Precipitate the DNA by adding an equal volume of isopropanol. Collect the precipitated DNA by centrifugation at 13,250*g* (8000 rpm in a Sorvall SH-3000 swinging bucket rotor or maximum speed in a microfuge) for 15 minutes at 4°C.

6. Carefully remove the isopropanol. Rinse the pellet of DNA with 1 ml of 70% ethanol. If the pellets are loose, centrifuge the samples again for 5 minutes. Remove the 70% ethanol, and allow the pellets to dry in air for 15–20 minutes at room temperature.

   Do not allow the DNA pellets to dry completely or they will be very difficult to dissolve.

7. Dissolve the nucleic acid pellet by rocking it gently overnight in 0.5 ml of TE (pH 8.0) at 4°C.

8. Transfer the solution to a microfuge tube and store it at room temperature.

   Between 100 µg and 250 µg of genomic DNA is typically isolated from 1 cm (~100 mg) of mouse tail.

   The addition of bovine serum albumin at a concentration of 100 µg/ml to restriction enzyme digests of genomic DNA prepared by this method will absorb residual SDS and reduce the possibility of incomplete digestions. If problems persist, re-extract the samples once more with phenol:chloroform and precipitate the DNA with 2 volumes of ethanol.