

**DNA Extraction of Small Tail Snip**

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Hammer Lab

**I. DNA Extraction**

1. From a 21 day old mouse, place the small snip of tail tip (~0.003g) into a 1.5ml Eppendorf centrifuge tube. Add 275 µl SNET buffer + 6 µl Proteinase K stock solution (see next page).

2. Place tubes in USA Scientific Mixer HC for 4 hours to overnight (55oC, 500rpm).

3. Pipette 275 µl phenol/chloroform / isoamyl alcohol (25/24/1) (Invitrogen # 15593-031) into the 1.5 ml Eppendorf tube digested tail solution clearly marked with animal/tail identification number.

4. Vortex vigorously.

5. Centrifuge (14K) for 5 mins at room temp to separate the aqueous phase containing the DNA (top) and the organic (bottom) phase.

6. Clearly label a new set of 1.5 ml tubes and pipette 380 µl of cold 100% EtOH into each tube. Remove 190 µl from the top (aqueous) layer of the centrifuged digested tail preparation and transfer it into the appropriate 1.5 ml Eppendorf tube containing the EtOH. Vortex the tube briefly and place it into a -20°C freezer for at least 2 hours (to overnight) to permit precipitation of the genomic DNA. Note: Addition of salt is not necessary since the SNET contains 400 mM NaCl.

7. Pellet the precipitated DNA by centrifuging for 15 mins (14K, 4oC).

8. Carefully pour off EtOH – watch to make sure that the pellet doesn't slip out. To avoid extra work resuspending the pelleted DNA, do not allow the pellets to dry completely.

9. Resuspend the pellet in 50 µl of deionized, autoclaved PCR-dedicated water.

**Solutions**

 1. SNET Buffer (1L)

 820 mls H20

 100 mls 10 x SET Buffer

 80 mls 5M NaCl

 2. 10 x SET Buffer (2L)

 100 mM Tris-HCl pH 8.0

 50 mM EDTA pH 8.0

 200 g SDS

 3. Proteinase K (Fisher # M24568-2) 100 mg/vial

 Resuspend entire 100 mgs in 10 mls TE (10mM Tris-HCl ,pH8.0; 1mM EDTA).

 Final Proteinase K Conc. is 10mg/ml. The bottle should be stored at 4°C.