**Tail DNA Extraction**

***From the Jackson Laboratory:***

*Quick DNA purification protocol*

*A quick "dirty" prep is usually sufficient, while some genotyping may work better with highly purified DNA. Determine empirically which protocol works best for your genotyping.*

*NaOH extraction (quick "dirty" DNA preparation). Reference: Truett GE et al. 2000. Biotechniques 29(1):52-54*

* *Cut 2mm of tail and place into an Eppendorf tube or 96-well plate.*
* *Add 75ul 25mM NaOH / 0.2 mM EDTA.*
* *Place in thermocycler at 98ºC for 1 hour, then reduce the temperature to 15°C until ready to proceed to the next step.*
* *Add 75ul of 40 mM Tris HCl (pH 5.5).*
* *Centrifuge at 4000rpm for 3 minutes.*
* *Take an aliquot for PCR (use 2 ul undiluted, or 2 ul of a 1:100 dilution/reaction).*

*From <*[*https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genotyping*](https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genotyping)*>*

**Modified protocol for our lab:**

1. Clip tail and place in a 1.5 mL tube, store at -20 C or proceed directly to next step.
2. **Add 200 uL** of **TAIL DNA EXTRACTION SOLUTION #1** (25mM NaOH/0.2mM EDTA) to tail.
3. Mix at 1200 rpm at 98 C on the thermomixer for 35 minutes.
4. Spin down condensation by spinning for 1.5 minute at 2500 x g
5. **Add 200 uL** of **TAIL DNA EXTRACTION SOLUTION #2** (40 mM Tris HCl) to the tube
6. Mix by inverting tubes 5-7 times
7. Spin down the tail and debris by centrifuging for 1.5 minutes at 2500 x g

**NOTE:** If using a liquid handling robot, stop here and move the tubes to the robot (Wall-E or Bender).

1. Remove 200 uL from the tube and transfer to a clean 1.5 mL tube.
2. Store tube containing tail debris at -20 C if desired or discard, proceed to the next step or store the new tube (neutralized tail DNA) at -20 C until further use.
3. Dilute neutralized tail DNA 20 to 50-fold immediately prior to use, and use this as a template for genotyping reactions.

**Making TAIL DNA EXTRACTION SOLUTION #1:**

1. Add 1 grams of NaOH (MW 40.0; Sigma S8045-500G) and 58.5 milligrams of EDTA (MW 292.24; Sigma E6758-100G) to 1 liter of molecular biology grade H2O (Quality Biological 351-029-131) and mix until the NaOH and EDTA is completely dissolved in the solution.
2. Aliquot a portion of the solution and check the pH; adjust the pH of the master solution until it is close to a pH of 12.
3. Aliquot the solution into working volumes of 25 mL and freeze until use.

**Alternative with NaOH solution:**

1. Add 0.5 ml of 10M NaOH solution (MW 40.0; Sigma 72068-100ML) to 49.5 mL molecular biology grade H2O (Quality Biological 351-029-131) and mix thoroughly to generate a 100 mM solution of NaOH.
2. Add 2 mL of a 100 mM solution of NaOH to 998 mL molecular biology grade H2O (Quality Biological 351-029-131) and mix thoroughly to generate a 0.2 mM solution of NaOH.
3. Add 58.45 milligrams of EDTA (MW 292.24; Sigma E6758-100G) to 1 liter of 0.2 mM solution of NaOH and mix until the EDTA is completely dissolved in the solution.
4. Aliquot a portion of the solution and check the pH; adjust the pH of the master solution until it is close to a pH of 12.
5. Aliquot the solution into working volumes of 25 mL and freeze until use.

**Making TAIL DNA EXTRACTION SOLUTION #2:**

1. Add 6.3 grams of Tris-HCL (MW 157.6; Trizma Hydrochloride pH 3.5-5.0, Sigma T6666-50G) to 1 liter of molecular biology grade H2O (Quality Biological 351-029-131) and mix until the Tris-HCL is completely dissolved in the solution.
2. Next, adjust the pH of the solution using HCl until the pH is close to 5.
3. Aliquot the solution into working volumes of 25 mL and freeze until use.