DNA Extraction Protocol

Tail DNA Preparation

Day One

1. In 1.5 ml eppendorf tube containing Mouse tail clip, add 750ul of Tail buffer.
2. Add 10-15ul, 20mg/ml Proteinase K
3. Incubate Overnight at 55 degrees C

Day Two

1. Shake tubes for 2 minutes on vortex Shaker
2. Add 300ul of 5M NaCl
3. Shake tubes again for 2 minutes on Vortex Shaker
4. Spin tubes at maximum speed in microcentrifuge at room temperature for 8 minutes.
5. While tubes are spinning, take out an equal number of new 1.5ml eppendorf tubes, label them and add 500ul of Isopropanol to each tube.
6. Transfer 750ul of the supernatant from step 4 to the newly labeled tubes, be careful to avoid the pellet at the bottom and the lipid layer at the top. Keep the tubes just in case the DNA prep fails and DNA needs to be extracted from the left over supernatant.
7. Rock on the Nutator for at least 5 minutes, but it is far better to rock at least 30 minutes.
8. Spin for one minute at max speed at room temperature.
9. Gently pour off Isopropanol in the proper waste container, be careful not to loose the pellet that has spun down in the bottom of the tube.
10. Add 1ml of 70% ethanol, and spin at max speed for 1 minute
11. Gently pour off the ethanol into the proper waste container
12. Spin the tubes once more at max speed for 15 seconds
13. Pipet off the excess ethanol and let the tubes air dry for 10 or so minutes at room temperature.
14. Add 100ul of 18.2 MΩ water that has been autoclaved.
15. Allow tubes to sit overnight at 4 degrees to fully dissolve in the water.

Reagents

Tail Buffer

Reagent	Stock Concentrations	Volumes	Final Concentration
Tris HCl pH 8.0	1M	25ml	50mM
EDTA pH 8.0	0.5M	100ml	100mM
NaCl	5M	10ml	100mM
SDS	10%	50ml	1%
Sterile Water	NA	315ml	NA
Total Volume		*500ml*	

Proteinase K

Add 5mls of Sterile 18.2 MΩ water to the lyophilyzed stock proteinase K, and then aliquot into tubes and store at -20 degrees C.