

Protocol for Tail DNA Extraction

Materials

1. 5.0 M NaCl
2. 1.0 M Tris (pH 7.5)
3. 0.5 M EDTA (pH 8.0)
4. 10% SDS
5. 20mg/ml Proteinase K (Roche)
6. Saturated NaCl
7. Absolute ethanol
8. 70% ethanol
9. DNase and RNase free water

Tail Digestion Buffer

		<u>Final Concentration</u>
5.0 M NaCl	17.0 ml	170mM
1.0 M Tris (pH 7.5)	8.5 ml	17mM
0.5 M EDTA (pH 8.0)	17.0 ml	17mM
10% SDS	42.5 ml	0.85%
Water	<u>415.0 ml</u>	
	Total 500 ml	

Methods

1. Digest tails (~ 0.5 cm) in 500 μ l tail digestion buffer along with 20 μ l Proteinase K over night at 55°C
2. Add 250 μ l saturated NaCl solution
3. Shake tubes vigorously
4. Put on ice for 30 minutes or longer
5. Centrifuge at 7,500g for 5 minutes at room temperature
6. Take supernatant and add to a tube containing absolute ethanol. Invert tube up and down several times and place at -20°C for one hour
7. Centrifuge at 16,000 g for 5 minutes at room temperature
8. Pour off supernatant, keep pellet
9. Add 1ml 70% Ethanol to wash pellet, vortex about 1 minute
10. Centrifuge at 16,000 g for 5 minutes at room temperature
11. Pour off supernatant, keep pellet
12. Dry the pellet at room temperature
13. Resuspend the pellet in 100 μ l PCR water
14. Quantitate DNA by spectrophotometer at 260 nm
- 15.** Store DNA at 4°C