

Phenol-Chloroform Extraction of Tail DNA

DIGESTION

1. Cut 1 cm long tail into 3 fragments
2. digest in PK (3 microli.) per tail solution (100 microli.) in a total volume of 200 μ l overnight
3. stir digestion next morning

EXTRACTION

4. add 200 μ l phenol-chloroform-isoamyle ethanol (equal volume)
5. vortex briefly or shake by hand 15 min
6. spin at top speed for 10 min. at 4 C
7. transfer aqueous phase to fresh microfuge tube
8. repeat steps 4-7

ETOH PRECIPITATION

9. add 15 μ l of 5 M ammonium acetate and 500-600 μ l 100% cold ethanol (-20 C)
10. invert tube gently or tap until DNA precipitates
11. spin at top speed for 15 min at 4 C
12. aspirate supernatant without disturbing pellet
13. rinse with 500 μ l cold 75% ethanol
14. spin at top speed for 15 min at 4 C
15. aspirate supernatant, air dry pellet (do not overdry)
16. resuspend in 50 μ l of water