

TAIL DNA ISOLATION/PURIFICATION

TAIL BUFFER

1% SDS (50ml of 10%)
0.1M NaCl (10ml of 5M)
0.1M EDTA (100ml of 0.5M)
0.05M Tris (pH8) (25ml of 0.5M)
315ml sterile ddH₂O

-store buffer at room temperature
-heat buffer before use @ 55°C if SDS precipitation forms

OTHER SOLUTIONS NEEDED

Proteinase K (20mg/ml)
Potassium Acetate (5M)
Isopropanol
Ethanol (70%)
TE (pH 7.6)

PROCEDURE

1. Add 450ul of tail buffer to ½ inch tail clipping.
2. Add 15ul proteinase K. Shake well.
3. Let sit in 55° water bath overnight or until tail dissolves.
4. Add 200ul potassium acetate. Shake well and let sit for 1-2 minutes.
5. Spin in centrifuge at 12,000rpm for 2-3 minutes. You should see a large pellet of protein at the bottom of your tube.
6. Remove supernatant and transfer to fresh tubes (should get approx. 500ul).
7. Add approx. 700-800ul of isopropanol to the supernatant (amount of isopropanol should be about 1.5-2x the amount of supernatant).
8. Shake well and let sit for 1-2 minutes.
9. Spin in centrifuge at 12,000rpm for 2 minutes. You should see a small pellet at this point.
10. Remove and discard supernatant, being very careful not to discard the pellet.
11. Add 600ul ethanol. Gently invert tube several times to wash the pellet.
12. Spin in centrifuge at 12,000rpm for 2 minutes.
13. Remove and discard the supernatant.
14. Let the pellet air dry (15-20min.) by leaving the caps of the tubes open.
15. Resuspend in approx. 50ul TE and store at 4°.

NOTES

-do no let the pellet completely dry as it will be less likely to dissolve in the TE
-if tail does not dissolve overnight, add more tail buffer and proteinase K