

***C. elegans* DNA by Salting Out**

by Matt Rockman (4-20-06), edited by Erik Andersen (6-10-2009)

1. Grow worms until starvation (4-6 days). We have used two 10 cm plates of worms for each prep.
2. Wash worms off plates with M9. Rinse several times in M9. In each rinse, let the worms settle without any spinning. The rinsing should extend over an hour or so, so that there are no bacteria left, including in worm intestines.
3. Concentrate the clean worms in a microfuge tube. Use a glass pipette to transfer the worms, as the worms stick to plastic tips.
4. Freeze them at -80 overnight or longer.
5. Thaw and add:
275 μ l TNE
7.5 μ l 20% SDS
15 μ l proteinase K (20 mg/ml)
6. Incubate at 55° for 10 hours.
7. Add 6 μ l RNase A (100 mg/ml), vortex, and let sit for five minutes.
8. Add 85 μ l 5M NaCl. Vortex. All the proteins and debris should precipitate.
9. Spin at 14,000 rpm for 10 minutes.
10. Carefully transfer the supernatant to a new microfuge tube.
11. Add 350 μ l ice-cold 100% ethanol from the -20°C freezer. The DNA should precipitate. Invert the tube a few times. You can put the tube at -20° overnight.
12. Spin at 14,000 rpm for 3 minutes. You may see a white pellet of DNA.
13. Pipette off the supernatant. Add 750 μ l 70% ice-cold ethanol (-20°C).
14. Spin at 14,000 rpm for 3 minutes.
15. Pipette off the supernatant, spin at 14,000 rpm for 30 seconds, pipette off the supernatant, and leave the tube out to air dry.
16. Add X μ l TE. Incubate at 37° for a few hours with agitation. For two 10 cm plates of worms, I used 100 μ L of TE.
17. Run DNA through DNeasy Blood and Tissue column to clean up excess salt.
18. Quantify DNA concentration using the broad-range fluorimetry kit with a Qbit, dilute, and enjoy.

Enzymes and Solutions:

TNE: 50 mM Tris-HCl (pH 8), 20 mM EDTA (pH 8), 400 mM NaCl

5M NaCl

100% and 70% ethanol, in the -20°C freezer

TE: 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8)

20% SDS (Don't autoclave!)

20 mg/ml proteinase K — available in the stockroom

100 mg/ml RNase A — available in the stockroom