

Worm Genomic DNA prep

Materials needed

- NGM agarose plates
- M9
- TEN (20 mM Tris, 50 mM EDTA, 100 mM NaCl, pH 7.5)
- 10% SDS
- 20 mg/ml Proteinase K in water
- Phenol/chloroform/isoamyl alcohol solution
- 3M Na acetate, pH 5.2
- 70% and 100% ethanol
- 10 mg/ml RNAaseA
- TE, pH 8.0

Protocol

1. Make NGM agarose worm growth plates. These use agarose to avoid impurities in most batches of agar, and are enriched to allow greater worm growth.

Mix:

- 5 g Bacto tryptone
- 2 g Bacto yeast extract
- 5 g NaCl
- 20 g agarose
- 1 L dH₂O

Autoclave this solution.

Add:

- 1 ml 5 mg/ml cholesterol in ethanol
- 2 ml 1M CaCl₂
- 1 ml 1M MgSO₄
- 25 ml 1M KH₂PO₄

Pour the media into large plates (about 20 ml per plate), and let dry.

Spread the plates with HB101, or another high growth strain of *E. coli*, e.g.

AMA1004

2. Add worms to the plates, and let them grow until the plates starve.

3. Rinse the worms off in M9, put them in 15 ml polypropylene tubes, and pellet them by spinning 30 sec. in a clinical centrifuge.

4. Remove the supernatant, and wash and pellet the worms in about 4 ml of TEN.

5. Remove the supernatant, and resuspend the worms in about 0.5 ml TEN. Freeze at -20°C. Frozen worms seem to be easier to digest. (The worms can now be stored at -20°

indefinitely, so this is a good place to synchronize DNA preps of different strains of worms.)

6. Transfer the worms in TEN to a 1.5 ml eppendorf tube.

Add:

25 μ l 10% SDS (\geq 0.5% SDS)

2.5 μ l 20 mg/ml Proteinase K ($>$ 0.1 mg/ml)

7. Incubate the tubes at 50°C to 60°C for one hour. Resuspend the pellet at 10 min., 20 min., and 30 min.

8. Add another 2.5 μ l of Proteinase K and incubate for another hour.

9. Phenol extract the solutions with 0.5 ml phenol. Mix well by inversion.

10. Phenol/CIA extract the solutions. (CIA= 24:1 Chloroform: isoamyl alcohol)

11. CIA extract the solutions.

12. Add 45 μ l of 3M NaOAc, and 0.8 ml ethanol at room temperature. The DNA should precipitate almost immediately. Mix the tubes by inversion, and flick them with your finger to wind the DNA strands.

13. Spin very lightly (2-5 sec.) in a microfuge to precipitate the DNA.
(Longer spins may bring down other stuff as well.)

14. Drain the ethanol, and wash the pellet in ice cold 70% ethanol.

15. Drain the wash and let the tubes dry completely. Aspirate off any drops of ethanol with a pulled-out pasteur pipette.

16. Resuspend the pellets in 0.5 ml TEN. (This is often a difficult resuspension. Heating to 50°C may help. Time also helps, so this is a good place to stop for the day, and put your tubes at 4°C.)

17. Add 2 μ l of 10 mg/ml RNase A ($>$ 40 mg/ml) .

18. Incubate at 37°C for one hour.

19. Phenol extract the solutions.

20. Phenol/CIA extract the solutions.

21. CIA extract the solutions.

22. Add 45 μ l of 3M NaOAc, and 0.8 ml ethanol at room temperature. The DNA should precipitate almost immediately. Mix the tubes by inversion, and flick them with your finger to wind the DNA strands. You may want to leave the tubes at -20° for 30 min. to overnight at this point.

23. Spin the tubes 30 sec. in a microfuge.

24. Drain the ethanol, and wash the pellet with ice cold 70% ethanol.

25. Drain the wash and let the pellet dry completely. Aspirate off any liquid. Resuspend the pellet in 100 μ l of TE.