

Worm Genomic DNA Preparation

Reagents:

M9	3M sodium acetate
genomic DNA lysis buffer	100% ethanol
proteinase K (10 mg/mL)	70% ethanol
phenol/chloroform	ddH ₂ O or Buffer TE
RNAse A (10 mg/mL)	

Procedure:

1. Grow 10 cm plates of N2 worms on NGM plates overlaid with 1% agarose or NGM-Rich plates (agar contains material that can inhibit subsequent enzymatic manipulations of DNA).
NOTE: You will probably need to feed the plate at least once to get a lot of worms. Put a few drops of concentrated OP50 on each plate every few days.
2. Wash the worms from the plate with M9 and pellet the worms by spinning at full speed for about 1 minute or by letting worms settle (~10 minutes).
3. Aspirate off supernatant.
4. Add fresh M9, spin, and aspirate supernatant.
5. Repeat step 4 several more times if needed.
6. Flash freeze pellet in liquid nitrogen. ←stopping point
7. Add five volumes of worm genomic DNA lysis buffer with proteinase K (common stock in -20°C stock freezer).
8. Incubate at 65°C for 1-2 hours.
9. Incubate at 95°C for 20-30 minutes to deactivate the proteinase K (0.1mg/mL).
10. Add RNAse A (common stock in -20°C stock freezer) to 0.1mg/mL and incubate at 37°C 1 hour.
11. *In the hood*, add 1 volume of phenol/chloroform buffered with Tris.
12. Mix gently and spin at 4,000 rpm for 5 minutes at room temperature (if done in the cold room the SDS may precipitate out).
13. Transfer the aqueous phase to a new tube avoiding the phenol phase and interface.
14. Repeat steps 11 through 13 two more times.
NOTE: 3x phenol extraction should be sufficient, but if a white precipitate is still visible at the aqueous/organic interface, repeat extractions until it is no longer visible.
15. Add 0.1 volume of 3M sodium acetate and >2 volumes of 100% ethanol and mix.
16. Incubate at room temperature or in the freezer for at least one hour. ←stopping point
17. Pellet DNA by centrifugation at 14,000 rpm for 15 minutes.
18. Carefully remove most of the supernatant.
19. Wash the pellet with 70% ethanol and remove supernatant. *Do not vortex.*
20. Repeat step 19 several times.
21. Air dry and then resuspend in ddH₂O or TE.

- NOTE: If there is a white precipitate, let the suspension sit for ~15 minutes and then remove the supernatant, leaving the white pellet.
22. Take OD readings at 260nm (nucleic acids) and 280nm (proteins). For a good preparation of DNA, OD260/OD280 should be about 1.8. Contamination with protein or phenol will decrease this ratio.
 23. Optional: digest genomic DNA with PvuII.

NOTE: This prep is typically clean enough for PCR or restriction digests. If DNA is to be used for injection into worms, you may wish to clean up DNA over a spin column after restriction digest.

Recipes:

Worm Genomic DNA Lysis Buffer

- * Common stock in -20°C stock freezer.
- 200mM NaCl
- 100mM Tris-HCl (pH 8.5)
- 50mM EDTA (pH 8.0)
- 0.5% SDS
- Add 0.1mg/mL of proteinase K before use.

M9 (1L)

- * Common stock in worm room.
- 5.8g Na₂HPO₄•7H₂O
- 3.0g KH₂PO₄
- 5.0g NaCl
- 0.25g MgSO₄•7H₂O
- ddH₂O to 1L
- Filter (0.22µm) and bottle.

References:

Modified from Silverman lab protocol