

I-E-1 WORM GENOMIC DNA PREP

I. GROWING THE WORMS

1. Chunk worms onto a large DNA plates which have been spread with HB101 or some other non-plasmid containing strain (i.e not OP50).
Note - We have used OP50 without issues. We have also used our current regular Large NGM plates without issues.
2. Rinse off worms with H₂O after plate is close to or has starved and place in glass conical tube.
3. Wash worms twice with H₂O to get rid of bacteria, and then transfer to an eppendorf tube.
4. Rinse worms with TEN (50 mM Tris pH 7.5, 10 mM EDTA, 100mM NaCl) and then remove as much liquid as possible and freeze. 100 μ l of packed worms will yield approximately 50-100 μ g of genomic DNA. Worms can be stored at -70° a long time before prepping them.

II. PREPING THE WORMS

1. Thaw a 100 μ l pellet and
add 350 μ l TEN,
12 μ l 20% SDS,
2 μ l of 20mg/ml proteinase K in H₂O
1 μ l β -ME
2. Resuspend by inverting and place at 65°C until carcasses dissolve (1-4 hrs), mixing by inverting tube every 30 minutes. If they haven't dissolved in 4hrs you probably need to add more lysis buffer (too many worms).
3. Extract with equal volume phenol, placing the tube on a rocking platform for 10 minutes to mix. Spin 5 minutes and remove supernatant gently with a cut off 1000 μ l ependorf pipette tip.

NOTE: We have used more vigorous 30 seconds of quick rocking back and forth by hand successfully. The issue here is not shearing the DNA too much if you want long intact fragments for PCR or southernns
4. Phenol/CHCl₃ extract 2-3 times as above until interface is clear. CHCl₃ extract once.
NOTE: 2 times should be enough for phenol
5. Ethanol precipitate by adding 1/10 volume 3 M NaOAc and 2.5 volumes ethanol.
NOTE: addition of 0.7 volumes of isopropanol also works (without addition of the NaOAc). This is helpful if you needed to increase your TEN volume to digest worms.
6. Spin 10 minutes at 4C at full speed in microfuge.
7. Remove supernatant and re-suspend in 400 μ l TE, add 1 μ l 10 mg/ml RNase. Incubate 30 minutes at 37°C.

- 8 Phenol/ CHCl_3 extract and ethanol precipitate as above.
9. Wash pellet this time with 70% ethanol to remove salts.
8. Re-suspend in $100\mu\text{l}$ TE. Concentration of DNA should be approximately 0.5-1.0 mg/ml for 0.1 ml of packed worms starting material.

DNA Plates

bacto-tryptone	5g/liter	then add:	
yeast extract	2g/liter	1 ml/liter	5 mg/ml cholesterol in ethanol
NaCl	2g/liter	1 ml/liter	1 M CaCl_2
Agarose	20g/liter	1 ml/liter	1 M MgSO_4
Autoclave		25 ml/liter	1 M KH_2PO_4