Worm Genomic DNA protocol

Description
A spin-column DNA extraction method using homemade buffers and silica spin columns to isolate *C. elegans* genomic DNA.

Collect worms

Grow worms on a 6 cM plate until starved. Lots of L1s is ideal.
Add 1.5 ml of water to plate and transfer to a 1.5 ml Eppendorf tube.
Spin at 3000 g for 30 seconds.
Remove the all but 50 ul of liquid.
Freeze at -20 until ready to work up.

Make Genomic DNA

1. Thaw 50 ul worm pellet, and add
2. Add 150 µl of Lysis buffer and 1.5 µl Proteinase K (10 mg/ml)
3. Incubate at 60°C with occasional shaking for 1 hour.

(optional)

4. Add 1 µl RNAse (10mg/ml)
5. Incubate at 37°C for 30 min.

6. Spin for 1 min at 15,000 g to pellet undigested worm cuticles and eggs.
7. Transfer supernatant (everything except the last 10 ul or so) to a new Eppendorf tube.
8. Add 200 µl Binding Buffer and mix (the solution will become cloudy).
9. Add 200 µl ethanol and mix (the solution will clarify).
8. Transfer all 600 µl to a Qiagen purple DNA purification column (or equivalent).
8. Spin on high speed for 10-30 sec.
9. Dispose of flow-through and add 500 µl of wash buffer.
10. Spin on high speed for 30 sec.
11. Discard flow through and add another 500 µl of wash buffer
12. Spin on high speed for 30 sec.
13. Remove flow through.
14. Spin on high speed for 1 min.
15. Place the spin column in a 1.5 ml Eppendorf tube.
16. Add 100 µl of TE to column and incubate at room temperature for 2 min.
17. Spin on high speed for 1 min.
18. Add another 100 ul TE.
19. Spin on high speed for 1 min.

Total yield: > 1ug genomic DNA.

1 ul is ideal for a genomic PCR.

Recipes

Lysis Buffer
10mM Tris pH 7.5, 2mM EDTA, 0.5% SDS

Binding buffer
3 M GuHCl, 3.75 M NH4Ac, pH 6

Guanidine Hydrochloride	28.6 g
7.5M Ammonium Acetate	50 ml

Adjust pH to 6 using glacial Acetic acid
Fill to 100 ml

Wash buffer
10 mM Tris-HCl pH 7.5, 80% ethanol

RNAse A Solution

RNAse A	100 mg
1M Tris pH7.5	100 µl
H2O	9.9 ml

After aliquoting 1ml per tube, heat @98oC for 5 minutes to remove any DNAses.

Spin Columns
The following columns have been tested with this protocol.

1) Epoch 1920-050
2) NEB Monarch Miniprep (we have not tested their Genomic columns)
3) Qiagen Miniprep (Blue)
4) Qiagen PCR Purification columns (Purple)

Among these, the Qiagen PCR columns give the best DNA as assayed by gel. These columns seem to bind large DNA better than smaller sheared fragments compared to the other columns. However, in practical terms all columns work to make DNA that works well for long Range PCR across 5kb-10kb genomic inserts.