High Quality *C. elegans* Genomic DNA [P1.1]

Collect worms

Grow worms on a 6 cM plate with a large lawn until starved. Eggs will not lyse during prep so higher yields will be obtained from plates where adults have laid all their eggs and the eggs have hatched.
Add 1.2 ml of water[1] to plate and transfer to a 1.5 ml Eppendorf tube.
Spin at 5000 rcf for 30 seconds.
Remove all but ~ 50 µl of liquid.
Freeze at -80° C until ready to work up. We find long term storage at -20°C is very bad for prep quality.

Make Genomic DNA

1. Freeze pellet in liquid nitrogen for 5 second (if worms have not been previously frozen at -80 °C)
2. Thaw ~ 50 µl worm pellet.
3. Add 175 µl of Lysis buffer and 1 µl Proteinase K (10 mg/ml)
4. Incubate at 56° C on Eppendorf Thermomixer R at 1400 rpm for 20 minutes.[2]
5 Spin for 1 min at 15,000 g to pellet undigested worm cuticles and eggs.
6. Add 400 µl Binding Buffer to a Monarch genomic purification column (or equivalent)[3].
7. Quickly 200 ul of supernatant (everything except the last 20 ul or so) to column and pipette to mix[4].
8. Spin at 200 rcf for 4 min, then at full speed for 30 sec. Improves yields ~ 1.5- fold over a direct \fast spin.
8B. [Preheat TE to 65° C while spinning].
9. Dispose of flow-through and add 750 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 and then remove flow through
13 Spin on high speed for 1 min.
16. Place the spin column in a 1.5 ml Eppendorf tube.
19. Add 100 µl of TE preheated to 65° C to column and incubate at room temperature for 1 min.
19. Spin on high speed for 1 min.

Total yield: ~ 1.5 - 2 µg genomic DNA. 1/2 – 1ul µl is ideal for a genomic PCR.

Solutions

Lysis Buffer
10mM Tris, pH 8.0
50 mM EDTA
0.5% SDS
0.5% Triton X-100

Proteinase K
10 mg/ml proteinase K in H2O

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

Binding buffer
10 mM Tris pH 8.0
4.8 M Guanidine thiocyanate
1 % Triton X-100
5 % Isopropanol
[Currently made using 85% Qiagen QG buffer, 10% Triton X-100, and 5% Isopropanol]

TE
10 mM Tris pH 8.0
0.1 mM EDTA

[1] Using 10 mM EDTA as collection buffer might allow for long term storage of worms at –20°C before DNA prep.
[2] If a Thermomixer is not available, incubate at 55-60°C and vortex every 5 minutes until lysis is complete. May take longer.
[3] NEB Monarch genomic columns ($1.32 ea) give highest yields and Monarch plasmid columns ($0.92 ea) (90% yield). Epoch columns ($0.42 ea), Qiagen plasmid (blue) and QIAquick purple columns all work fine (all ~75% of Monarch genomic yield).
[4] We mix the binding buffer and lysis buffer directly in the column to save on plastic, but this could be done in a separate tube.