

Extraction of genomic DNA from *C.elegans* using Qiagen kit

1. Collect worms from one 60mm/ two 35mm petri plates in dH₂O or M9 buffer and transfer to 15ml tube.
2. Pellet down the worms by centrifugation at 2500 rpm at room temperature for 30 seconds.
3. Repeat steps 1 and 2 twice.
4. Wash with M9 buffer/dH₂O twice.
5. Remove the supernatant and re-suspend the worms in 200ul of **ATL buffer (Kit)**.
6. Transfer the worms to a 1.5ml Eppendorf tube. Incubate at -80 °C overnight (Worms can be stored indefinitely at -80 °C)
7. Follow 3 cycles of Freeze (-20 °C)/thaw (37 °C).
8. Add 20ul of **Proteinase K (Kit)** and incubate at 56 °C for 3 hours. Vortex in between.
9. (Optional step) Add 8ul of **RNase A (not in Kit)** (50 mg/ml) and incubate at room temperature for 5 min.
10. Add 200ul of **AL buffer (Kit)** and mix/vortex. Incubate for 10 min at 56 °C.
11. Add 200ul of **ethanol (100%) (not in Kit)** and mix/vortex.
12. Pipet the mixture into a DNeasy Mini spin column placed in a 2ml collection tube (**KIT**). Centrifuge at 8,000rpm for 1 min at RT. Discard flow-through.
13. Add 500ul **AW1 buffer (Kit)**. Centrifuge at 8,000rpm for 1 min at RT. Discard flow-through.
14. Add 500ul **AW2 buffer (Kit)**. Centrifuge at 14,000rpm) for 3min to dry the column membrane. Discard flow-through and collection tube. Spin for 2 min to get rid of wash buffer AW2
15. Place the column in a 1.5 ml Eppendorf tube and pipet 50ul of sterile dH₂O/ **AE buffer (Kit)** (or 1X TE) directly in the middle of the membrane. Incubate at room temperature for 1 min and then centrifuge for 1 min at 8,000 rpm at RT to elute.
16. Repeat the step 15 by adding another 50ul of sterile dH₂O/ **AE buffer** (or 1X TE) directly onto the membrane in the same Eppendorf tube.
17. Check quality and quantity of DNA on agarose gel. This procedure yields around 20-30ng/ul of DNA.
18. Store the DNA at 4 °C no freezing required, it is good for PCR application.

DNeasy Blood and Tissue kit cat. No 69504 and 69506, kept at RT. Above Pratibha's work bench