

DNA isolation from Filarial Parasites¹

1. To every 250 μ L worms in buffer add 100 μ L lysis buffer (0.1M Tris, 0.1M EDTA, 1% SDS, 0.5% DEPC).
2. Add one 5mm stainless steel BB to each 2mL round-bottom tube. Vortex on high speed for 45 min. This can be done with either an adaptor for the vortex (ex. Vortex genie adaptor Molecular Bio Laboratories Inc catalog #13000-VI) or by taping the tube to the flat portion of a regular vortex mixer platform.
3. Spin tube briefly in nanofuge. Incubate at 70 °C for 30 minutes.
4. Add 25 μ L of 5M KoAc, Mix by inversion. Incubate on ice for 30 minutes.
5. Briefly spin tube in your nanofuge and transfer entire sample, except for the BB into a new 1.5 mL tube.
6. To pellet cellular debris, centrifuge sample at 20,400 x g for 15 minutes at 4 °C. Transfer supernatant to a new 1.5 mL tube.
7. To precipitate your DNA, add 375 μ L of cold isopropanol. Mix by inversion.
8. Centrifuge for 20 minutes at 20,400 x g at 4 °C.
9. Carefully extract isopropanol, being careful not to disturb pellet. This is your DNA!
10. Wash pellet with 1mL of cold 75% EtOH.
11. Centrifuge for 10 minutes at 15,000 x g at 4 °C. Carefully remove EtOH. Air-dry pellet for 15 minutes.
12. Resuspend DNA in 50 μ L 0.1X TE. To completely redissolve your DNA, incubate at 55°C for 10 minutes.
13. To remove excess salt, dialyze² against 250 mL of 0.1X TE for 4 hours to overnight.

¹ Protocol modified from Aonuma et al: **Loop-mediated isothermal amplification applied to filarial parasites detection in the mosquito vectors: *Dirofilaria immitis* as a study model.** *Parasites & Vectors* 2009, 2:15

² We will be using a 0.025 μ m membrane from Millipore, catalog number VSWP