Genomic DNA isolation from Filarial Parasites

Thaw worms over ice and transfer to a 2 ml round bottom tube. Add 100 l lysis buffer (per 250 l worms in buffer).

Do 3 freeze/thaw cycles: 3 minutes in Dry Ice/EtOH bath followed by 3 minutes at 65 C.

Add one 5mm BB to the 2 ml round bottom tube and attach to vortex mixer with special adaptor. Vortex on the highest speed for 45 minutes, stopping every 10 minutes to change the position of the tube to ensure all areas of the tube are equally mixed.

Centrifuge the tube quickly before opening. Add an additional 150 l of lysis buffer and vortex briefly. Note: The total volume of lysis buffer should be equal to the starting volume of worms in buffer.

Add 30 l 10% SDS

Add 2 l of -mercaptoethanol and vortex briefly

Add 60 l of Qiagen Proteinase K (20 mg/ml). Vortex gently or mix by inversion. Centrifuge the tube briefly in a nanofuge.

Incubate at 65 C for 4 hours to overnight.

Add 0.5 l RNase A (100 mg/ml) and vortex gently or mix by inversion. Spin the tube briefly in nanofuge.

Incubate at 37C for 1 hour.

Centrifuge the tube briefly. Add 1 volume Tris-buffered phenol (pH 7.9). Vortex briefly to mix.

Centrifuge at 2,000 rpm for 5 minutes.

Carefully remove the top aqueous phase and transfer to a new 1.5 ml tube.

Add an additional 1 volume phenol and vortex to mix.

Centrifuge at 2,000 rpm for 5 minutes.

Carefully extract aqueous phase and transfer to new 1.5 ml tube.

Add 1 volume chisam (24:1 Chloroform/Isoamyl Alcohol). Vortex to mix.

Centrifuge at 2,000 rpm for 5 minutes.

Carefully remove the top aqueous phase and transfer to a new 1.5 ml tube.

Using a Millipore membrane dialyze genomic DNA against a beaker (approximately 250 ml) 0.1 x TE overnight.

Measure concentration using the nanodrop spectrophotometer. It is also a good idea to run your genomic DNA on a gel to check for the presence of RNA and the integrity of genomic DNA.