

Worm gDNA extraction protocol

- Wash worms 2x with M9 buffer
- Spin down store @ -20 til use
- +250µl of worm genomic DNA lysis buffer + proteinase K
 - 200mM NaCl
 - 100mM Tris-HCl pH8.5
 - 50mM EDTA pH8.0
 - 0.5% SDS
 - + 0.1mg/ml proteinase K directly before use
- incubate at 65° for 1 hour vortexing occasionally
- incubate at 95° for 20 minutes
- + RNase A to 0.1mg/ml and incubate @ 37° for 1 hour
- add 250ul of phenol;chloroform;isoamyllic acid (stored @ 4°)
- mix by inverting
- spin @ 13,000 rpm @ RT for 15 minutes (not cold or SDS will ppt out)
- take aqueous phase to new tubes
- add 25µl of 3M Sodium acetate
- add 750µl of 100% EtOH
- (if low DNA amount add glycogen 10µl of 5mg/ml)
- put at -80° for 1 hour (can be left o/n)
- spin @ 13,000 rpm @ 4° for 30 minutes
- remove sup
- add 350µl of 75% cold EtOH
- spin @ 13,000 rpm @ 4° or RT for 10 minutes
- discard liquid
- air dry for 7 minutes
- resuspend in 30µl of TE and take o.d.
 - 10mM Tris-HCl
 - 1mM EDTA pH 8
 - final pH 7.5

pure DNA 260/280 ratio ~1.8

pure RNA 260/280 ration ~2.0

low ratios can be caused by protein or phenol contamination