Log of times performed on back

Isolation of gDNA from C. elegans

1. Label the side of one PCR tube for each sample. Include your initials, gDNA, the date, and the worm strain. PCR tubes with your initials and number them.

3. Use a micropipet with a fresh tip to add 25 - 50 µL of lysis buffer to each tube. (You can scale this protocol up or down, as appropriate.)

4. Use a sterilized worm pick to transfer 4 to 5 adult worms of the appropriate genotype to each tube. Swish the tip directly in the lysis buffer to dislodge the worms from the pick.

5. Observe the PCR tube under a dissecting microscope. Verify that at least 3 worms are alive and writhing in the buffer.

6. Fit each of your PCR tubes into a 1.5-mL tube to act as an adaptor. → spin 10s @ 15k xg

7. Place your tubes in a balanced configuration in a microcentrifuge, and spin for 5-10 seconds at full speed. This will pellet the worms.

8. Freeze your tubes in liquid nitrogen, on dry ice, or in a –80°C freezer for 10 minutes. (Freeze-thawing cracks the tough outer cuticle of the worm.) → ~~oops... Didn't do for fully 10 mins. did until Liquid N2 stopped bubbling~~ SMG 230720 went back and froze for 10min

9. Place your PCR tubes in a thermal cycler that has been programmed for one cycle of the following profile. The profile may be linked to a 4°C hold program. → "GDNA EXTR" program

Incubating step: 65°C 90 minutes
Boiling step: 95°C 15 minutes

(Proteinase K in the lysis buffer digests protein in the cuticle and helps to liberate individual cells by digesting protein fibers of the extracellular matrix that bind cells together. It also inactivates cellular proteins, including DNases that interfere with PCR amplification. The near-boiling temperature lyses individual cells and inactivates the proteinase K.)

*Note: The procedure can be done in 1.5 ml tubes using heat blocks and/or water baths, but the PCR machine does the temperature changes for you!

10. Store your sample on ice or at –20°C until you are ready to continue.

Lysis buffer (45 µL per experiment). ← for 4x samples
85 µL deionized water ~~170 mL~~ ~~255 mL~~ 170 mL
10 µL 10X PCR buffer (100 mM Tris, 500 mM KCl, 15 mM MgCl2, pH 8.3) ~~30 mL~~ 20 mL
5 µL 20 mg/mL proteinase K. (stock in refrigerator) ~~15 mL~~ 10 mL

Prepare lysis buffer fresh on the day of the experiment; store aliquots on ice.