TARDIGRADE LYSIS/PCR
*** For small-scale genomic DNA preps from mixed-stage tardigrade population**

(adapted by Jenny Tenlen from *C. elegans* protocol originally developed by Bob Barstead and modified by Barbara Page.)

1. Prepare 10X Buffer Mix in dH_2O:

100 mM	Tris, pH 8.2
500 mM	KCl
15 mM	$MgCl_2$
0.1%	gelatin

   * Freeze stocks at -20°C for long-term storage.

2. Prepare Detergent Mix in dH_2O:

4.5%	Tween-20
4.5%	Nonidet P-40 (can substitute Igepal CA-630)

   * Keep stocks at room temp, away from direct light.

3. Prepare Lysis Buffer (per ml):

100 µl	10X Buffer Mix
300 µl	Detergent Mix
30 µl	Proteinase K (10 mg/ml stock)
570 µl	dH_2O

   * Store aliquots at –70°C. They tend to go bad after a year (due to Proteinase K)

4. Thaw lysis buffer; add 80 µl lysis buffer + some crushed glass (such as from crushed cover slips) to 1.5-ml microcentrifuge tube

5. Transfer tardigrades to cap of microcentrifuge tube (minimum of 30-40 adults; more is better). Remove as much water as possible; replace with 20 µl lysis buffer.

6. Place cap on tube and centrifuge briefly.

7. Vortex for several seconds to shear tardigrades. Centrifuge briefly.

8. Freeze tubes on dry ice and transfer to –70°C for a minimum of 15 min. (can be left overnight at this point).

9. Thaw tubes; for each PCR reaction, transfer 7.5 µl of lysate to a PCR tube.

10. Heat tubes in PCR block at 65°C for 60 min, then at 95°C for 5 min. Give tubes a quick spin.

11. Proceed with PCR. Lysis of about 50 tardigrades will yield enough genomic DNA for 10 – 12 PCR reactions.