DNA isolation from insect tissue (customized phenol-chloroform DNA extraction protocol)

1. Grind insect tissues in liquid nitrogen using mortar and pestle or metallic beads and tissuelyzer
2. Add 0.1g of tissue per 1ml of DNA extraction buffer*
3. Add 500ul phenol, vortex
4. Add 300ul chloroform, vortex
5. Centrifuge at 8000rpm for 60 minutes at 4 degrees Celsius
6. Transfer upper aqueous layer to new tube
7. Add 200ul phenol and 200ul chloroform, vortex and centrifuge 8000rpm for 10 minutes at 4 degrees Celsius
8. Transfer upper aqueous layer to new tube
9. Add 400ul chloroform, vortex and centrifuge 8000rpm for 10 minutes at 4 degrees Celsius
10. Repeat steps 9 and 10 once or twice until white interface disappears
11. Transfer upper aqueous layer to new tube
12. Add 0.1 times current volume of 3M NaAc and 2 volumes of absolute EtOH
13. Invert tube several times, incubate at -20 degrees Celsius overnight
14. Centrifuge 8000rpm for 30 minutes at 4 degrees Celsius, remove EtOH
15. Wash with 70% EtOH
16. Dry DNA pellet thoroughly and resuspend in distilled water or buffer
17. Perform RNase clean-up step if necessary

*DNA extraction buffer:

200mM Tris-HCl (pH 8.0)

150mM NaCl

25mM EDTA (pH 8.0)

0.5% SDS