**DNA Isolation using Promega Wizard SV Genomic DNA**

**Purification System #A2360**

1. Prepare Digestion Solution Master Mix:

Nuclei lysis solution 200 µl per sample

0.5M EDTA, pH 8.0 50 µl per sample

Proteinase K, 20mg/ml 20 µl per sample

RNase A Solution , 4ng/ml 5 µl per sample

 Total Volume 275 µl per sample

1. Blot excess ethanol off insect with a Kim wipe, rinse with PBS and blot again.
2. Place insect in a 1.5 ml microfuge tube.

4. Add 275 µl of prepared Digestion Solution Master Mix to each sample ***one at a time***. Use a pestle to ***completely*** macerate each insect in turn. For large insects use only 2mm of the abdomen; for small insects use the entire body.

 5. When all samples are macerated place them in a 55° C heating block or water bath for 10 minutes.

*Note:* Samples may be frozen at -20° at this point if necessary. They must be heated to 55° C before processing.

 6. Centrifuge to pellet insect debris, 2K for 1 minute.

 7. Transfer lysate to a new labeled 1.5 ml microfuge tube.

 8. Add 250 µl Wizard SV Lysis Buffer (comes prepared in kit) to each tube and

 vortex to mix.

 9. Transfer the entire sample lysate from the 1.5 ml microfuge tube to a Wizard SV Minicolumn assembly.

10. Centrifuge at 13K for 3 minutes.

11. Discard liquid in the collection tube for each sample.

12. Wash each column with 650 µl of Wizard SV Wash Solution. Centrifuge at 3K for 1 minute. Discard liquid.

13. Repeat above for a total of **4** column washes.

14. Dry the column by centrifuging at 13K for 2 minutes after the fourth wash.

15. Move column to a new labeled microfuge tube.

16. Add 150 µl of 65°C nuclease-free water to each column. Incubate at room temperature for 2 minutes.

17. Centrifuge at 13K for 1 minute to elute DNA.

18. Do a second elution with 150 µl of 65°C nuclease-free water. Both eluted samples may be combined.

19. Recommend using 15 µl of insect DNA in PCR samples.