DNA Isolation using Promega Wizard SV Genomic DNA Purification System #A2360

Modified for The Wolbachia Project — Discover the Microbes Within!

1. Prepare Digestion Solution Master Mix.

Reagent	Volume per Sample (µl)	# of Samples	Master Mix Amount (µl)
Nuclei lysis solution	200		
0.5M EDTA, pH 8.0	50		
Proteinase K, 20mg/ml	20		
RNase A Solution, 4ng/ml	5		
TOTAL	275		

Master Mix Amount = (volume per sample) x (# samples)

2. Blot excess ethanol off insect with a Kimwipe, rinse with PBS or sterile water and blot again.
3. 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 at least 15 minutes.

   Note 1: For most efficient lysis, incubate for 2 hours to overnight. However, many students are successful with a 10-15 min lysis.

   Note 2: Samples may be frozen at -20° at this point if necessary. They must be re-heated to 55° C before processing.
6. Centrifuge to pellet insect debris, 2,000 *x g* 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 13,000 *x g* 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 13,000 *x g* for 1 minute. Discard liquid from collection tube.
13. Repeat above for a total of **4** column washes.
14. Dry the column by centrifuging at 13,000 *x g* for 2 minutes after the fourth wash.
15. Move column to a new labeled microfuge tube.
16. Add 50 µl of 65°C nuclease-free water to each column. Incubate at room temperature for 2 minutes.
17. Centrifuge at 13,000 *x g* for 1 minute to elute DNA.
18. Do a second elution with 50 µl of 65°C nuclease-free water. Both eluted samples may be combined.
19. Remove the column and store purified DNA in the freezer (-20° to -80 °C).