Sigma Extract-N-Amp Tissue PCR Kit

(Sigma-Aldrich product #XNAT2)

1. Rinse insects by soaking in a tube of PBS.
2. Remove insect from the PBS and blot on a paper towel.
3. Label one microfuge tube for each insect sample.
4. Add 100 µl of Extraction Solution, followed by 25 µl of Tissue Preparation Solution. Mix by pipeting up and down.

Note: If several extractions are to be done, sufficient volumes of Extraction and Tissue Preparation Solutions may be pre-mixed in a ratio of 4:1 up to 2 hours before use.

1. Remove a ~2mm long by ~2mm wide piece of the posterior end of the insect. If the insect is smaller than this use the entire abdomen or the entire insect. Place insect piece in the labeled eppendorf tube containing Extraction and Tissure Preparation Solution mixture.
2. Incubate at room temperature for 10 minutes.
3. Incubate at 95 degrees C for 3 minutes.
4. Add 100 µl of Neutralization Buffer to each sample and mix on a vortex. Store at 4 degrees C or use in PCR directly.
5. Add the following reagents to a thin-walled microcentrifuge tube:

Water, PCR grade x ul

Extract-N-Amp

Reaction mix 10 ul

Forward primer y ul

Reverse primer y ul

Tissue extract 4 ul

Total volume 20 ul

The final primer concentration is 0.5 uM. If less than 4ul of tissue extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction:Neutralization Solution to bring the volume of tissue extract up to 4 ul.

1. Mix gently.
2. Amplify in thermal cycler. It is recommended not to leave PCR reactions overnight on the thermal cycler to avoid DNA degradation. Store PCR samples at -20 degress C.