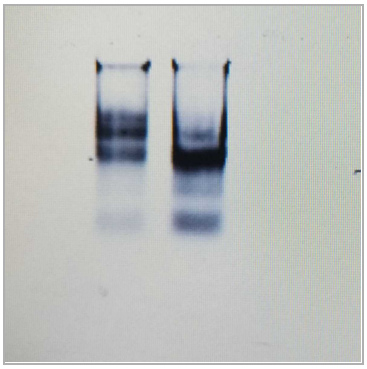

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Efficient insect DNA extraction protocol.

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We use this protocol and it's working

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ABSTRACT

Extracting DNA from insects can be a difficult process due to a number of factors. Some of these factors include the size and quantity of the insect, the presence of enzymes that degrade DNA, as well as the presence of chemical compounds that interfere with DNA extraction.
Some insects can be very small, making it difficult to collect enough tissue to extract DNA. In addition, insect tissues contain several enzymes that can rapidly degrade DNA, which can make it difficult to obtain DNA fragments long enough for molecular analysis.
Another factor that can make it difficult to extract DNA from insects is the presence of chemical compounds that interfere with the extraction process. For example, many insects produce chemical compounds to protect themselves from predators, which can interfere with DNA extraction techniques.
To overcome these challenges, researchers may need to optimize their DNA extraction techniques to meet the specific needs of the insect in question. This may involve using different chemicals and extraction protocols to remove chemical compounds and enzymes that interfere with DNA extraction.
Here is an example of an optimized and efficient insect DNA extraction protocol.

MATERIALS

1- Insects
2- 70% ethanol
3- Lysis buffer solution CTAB 2% (e.g., 2% CTAB, 1M Tris-HCl pH 8.0, 1,4 M NaCl, 20 mM EDTA, 0,2 % Beta mercapethanol for 200 mL water).
4- Water bath
5- chloroform
6- Isopropanol
7- Microcentrifuge tubes
8- Micropipettes and tips
9- Centrifuge
10- NanoDrop

Buffer preparation.

1 Lysis buffer solution CTAB 2% (4 g of 2% CTAB, 20 mL of 1M Tris-HCl pH 8.0, 16,4 g of 1,4 M NaCl, 8 mL of 20 mM EDTA, 400 mL of 0,2 % Beta mercapethanol for 200 mL water).

Note: Betamercaptoethanol (BME), added on day of use.
BME helps remove polyphenolic compounds, tannins, and proteins.
BME will inhibit RNase activity.If using RNase to clean up RNA contamination of your DNA extraction, RNase will need to be added after your DNA has been precipitated and resuspended in the final storage buffer. RNase added to a CTAB solution containing BME will neutralize the RNase activity.

Cell lyses.

2 1- Collect the insects and wash them in distilled water to remove debris and residues.
2- Blot excess water from the insects with a paper towel.
3- Place the insects in a microcentrifuge tube and add enough 70% ethanol to completely cover the insects.
4- Gently shake the tube and leave the insects in 70% ethanol for at least 30 minutes. This dehydrates the insects and makes them more permeable to the lysis solution.
5- Weigh 50 mg of insects and macerate with a pestle in liquid nitrogen.
6- After maceration, add 750 µL of 2% CTAB lysis buffer, stirring gently.
7- Then, incubate in a water bath at 65 °C for 30 minutes.

Phase separation.

3 8- Add 650 µL of ice-cold chloroform and shake (pour) the tubes gently.
9- Centrifuge at 13,000 RPM for 10 minutes, collect the supernatants and transfer to new tubes at 4°C.

DNA precipitation.

4 10- Add 600 µl of ice-cold isopropanol and hold for 30 minutes.
11- Centrifuge for 10 minutes at 13,000 RPM at 4°C, observe the formation of a pellet at the bottom of the tube.
12- The supernatant will be discarded and the pellet washed with 1 mL of 70% ethanol.
13- Centrifuge again for 10 minutes at 13,000 RPM at 4ºC and discard the supernatant.

Resuspend DNA.

5 14-Leave the pellet to dry in the hood for 10 minutes and resuspend in 20 µL of deionized water.
15- Allow the pellet to dry in the hood for 10 minutes, resuspend in 20 µL of deionized water and store at -20°C or -80°C until molecular analysis.
16- Measure the quality and quantity of your DNA using a NanoDrop. (Usually, I get around 25µg of genomic DNA from 50mg of insect sample).