Promega

Product Application

Automated Purification of DNA from Insects and Zebrafish

Purify DNA from insects and non-mammalian organisms using the Maxwell® RSC Instrument and the Maxwell® RSC Tissue DNA Kit. Options are presented for mechanical and enzymatic disruption of organisms.

Kit: Maxwell® RSC Tissue DNA Kit (Cat.# AS1610)

Analyses: UV absorbance, dye-based quantitation, and qPCR

Sample Type(s): Mosquito (*Aedes aegypti*)
Tick (*Ixodes scapularis*)
Fruit fly (*Drosophila melanogaster*)
Brine shrimp (*Artemia franciscana*)
Zebrafish embryos (*Danio rerio*)

Input: 1-3 whole organisms, depending on organism size

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM476, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Materials Required:

- For enzymatic disruption
  - Tissue Lysis Buffer (TLA) (Cat.# A5091)
  - Proteinase K Solution (Cat.# MC5005)
- For mechanical disruption
  - Fisherbrand™ RNase-Free Disposable Pellet Pestles (e.g., ThermoFisher, Cat.# FS7495211590 or similar)

Protocol:

Different pre-processing may be desired based on cost, throughput, or a need for maximum yield. Options are presented below for mechanical, enzymatic, or combined pre-processing.

1. Remove storage solution (if present). Disrupt the organism(s) using one of the methods below. Different methods may yield different amounts of DNA, depending on the organism.
   a. For mechanical disruption, add 80µl of 1X TE Buffer (included with the kit) to the organism(s) and manually homogenize using a disposable pestle or similar.
   b. For enzymatic disruption, add 300µl of TLA Buffer and 30µl of Proteinase K to the organism(s). Incubate at 56°C for 1 hour with shaking. Digestion may not be complete.
   c. For combined mechanical and enzymatic disruption, add 300µl of TLA Buffer and 30µl of Proteinase K to the organism(s). Manually homogenize using a disposable pestle or similar. Then incubate at 56°C for 1 hour with shaking.
2. Proceed with the protocol in Technical Manual TM476 to purify the DNA using the Maxwell® RSC Tissue DNA Kit. Load the entire lysate volume into well #1 of the cartridge.

Results:

DNA was purified from mosquito (*Aedes aegypti*), tick (*Ixodes scapularis*), fruit fly (*Drosophila melanogaster*), brine shrimp (*Artemia franciscana*), and zebrafish embryos (*Danio rerio*) using the above method and either enzymatic disruption, mechanical disruption, or both. Purified DNA yield (Figure 1) and amplifiability (Figure 2) were compared to samples purified in parallel using the Qiagen DNeasy Blood & Tissue Kit, which employs both mechanical and enzymatic disruption. In most cases, the highest DNA yields are achieved using a combination of enzymatic and mechanical disruption followed by purification with the Maxwell® RSC Tissue DNA Kit. However, reduced DNA yields could be purified from most insects with either pre-processing method alone, allowing the user to select the method to suit their throughput and cost needs. Insects with chitinous exoskeletons, such as ticks, may require both enzymatic and mechanical pre-processing for sufficient DNA yields.

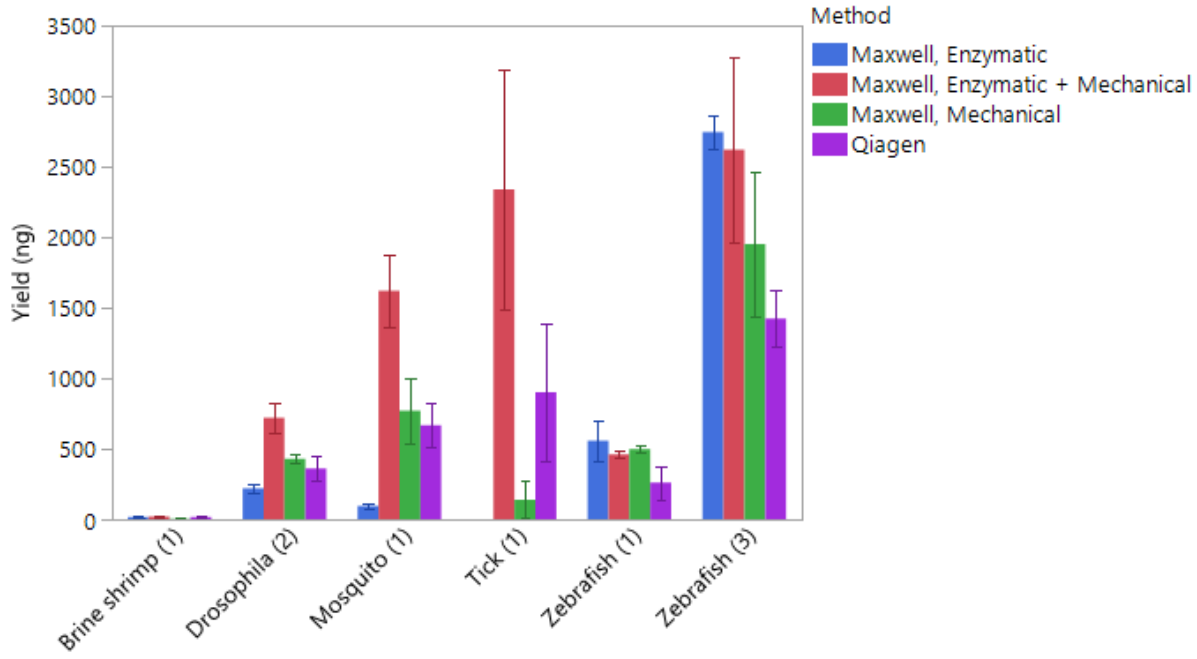

Figure 1. Total DNA yield purified from insects and non-mammalian organisms using the Maxwell® RSC Tissue DNA Kit on the Maxwell® RSC Instrument or the Qiagen DNeasy Blood & Tissue Kit. The number of organisms used per purification is indicated in parentheses. Zebrafish embryos were stored in methanol at -20°C and methanol was removed prior to purification. All other samples were stored at -20°C. Yields are calculated from the concentration measured with the QuantiFluor® ONE dsDNA System (Cat.# E4871) and the measured eluate volume. Data represent the mean ± STD of N=3.

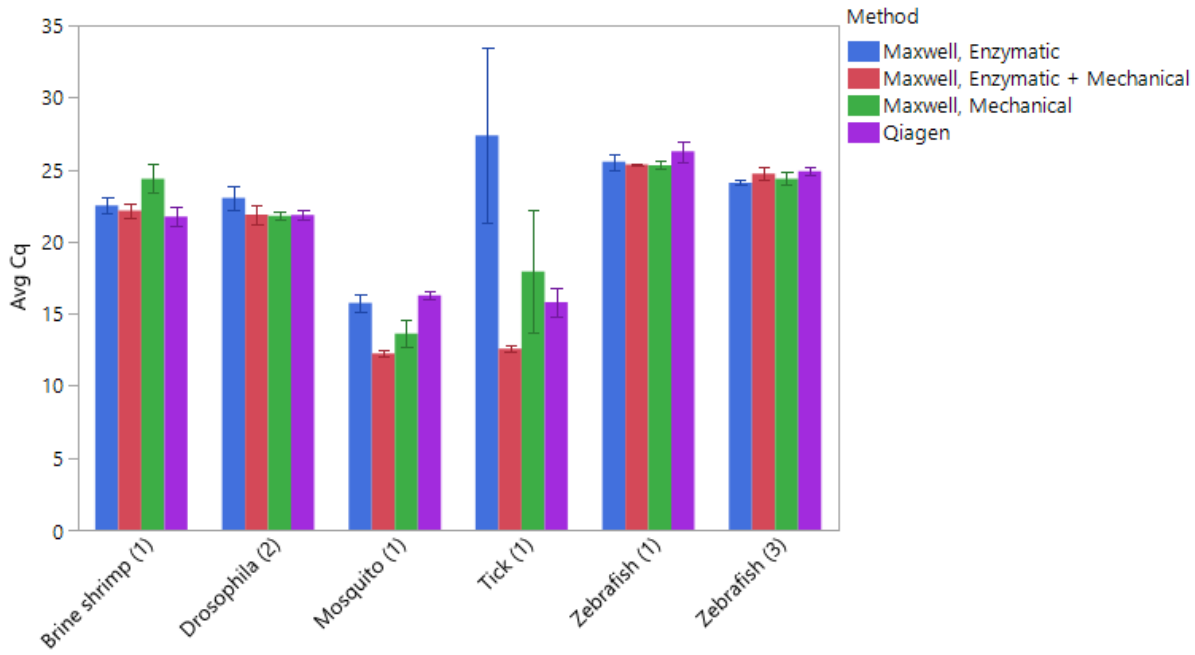

Figure 2. Cq values from insect and non-mammalian organism DNA purified using the Maxwell® RSC Tissue DNA Kit on the Maxwell® RSC Instrument or the Qiagen DNeasy Blood & Tissue Kit. DNA was extracted in triplicate from each organism using the indicated pre-processing method and chemistry. DNA from each sample was amplified in duplicate using species-specific primers and the GoTaq® qPCR Master Mix (Cat.# A6001) on a Bio-Rad CFX96 Real-Time Instrument. Input DNA volume varied between organisms but was adjusted to account for differences in eluate volumes between the Maxwell® and Qiagen purifications. Cq values should only be compared between pre-processing methods, not between organisms. Data represent the mean ± STD of N=6 (3 purifications, 2 amplifications each).