High Molecular Weight DNA Purification from Arthropods

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Introduction

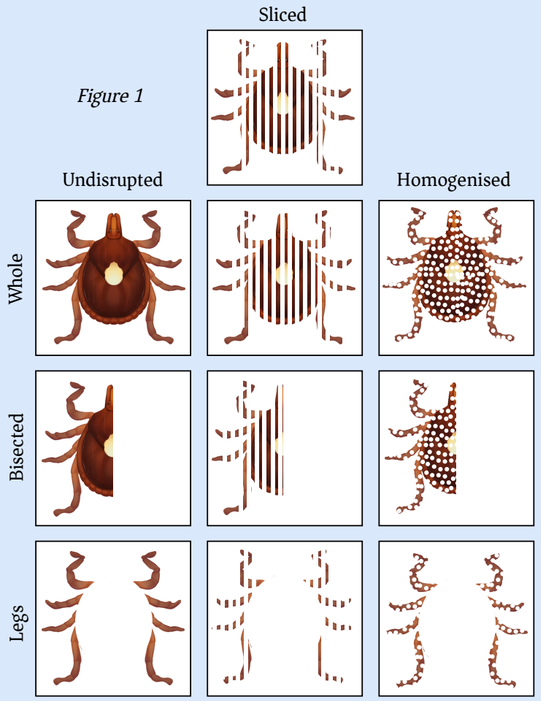
Ixodida (ticks) are major arthropod vectors of disease[1] with complex life histories intertwined with host dynamics, urbanisation, and climatic conditions. The complexity and risks involved necessitate improved genomic surveillance tools.

Existing DNA extraction methodologies for ticks and similarly sized arthropods are insufficiently optimised[2] for advanced genomic sequencing technologies that demand specific DNA quality and quantity requirements[3].

This study compares DNA extraction methods for ticks and similar sized arthropods, evaluating DNA quality, quantity and organism content for next-generation sequencing, and provides recommendations for future research.

Materials and Methods

Figure 1



Female *Amblyomma triguttatum* tick tissue types underwent three tissue disruption methods in triplicate.

Tissue type:

- ***Whole tick:*** A whole specimen (W)
- ***Bisected tick:*** A midsagittally bisected specimen (B)
- ***Tick legs:*** Only the legs of the specimen, removed with forceps (L)

Tissue disruption:

- ***Undisrupted:*** No tissue disruption performed (U)
- ***Sliced:*** Specimen sliced with a scalpel blade (S)
- ***Homogenised:*** Sample homogenised with liquid nitrogen and 3mm stainless steel bead on a TissueLyser (H)

Samples were then extracted using a Qiagen Blood and Tissue Kit. Whole-Sliced specimens were also processed using a Qiagen MagAttract HMW Kit (Mag / M).

Extractions were assessed for quantity using a Qubit 2.0 Fluorometer, for quality with an Agilent 2200 TapeStation, and for content through qPCR targeting Bacteria and Ixodida, with copy number calculated by gBlock Standards.

Results

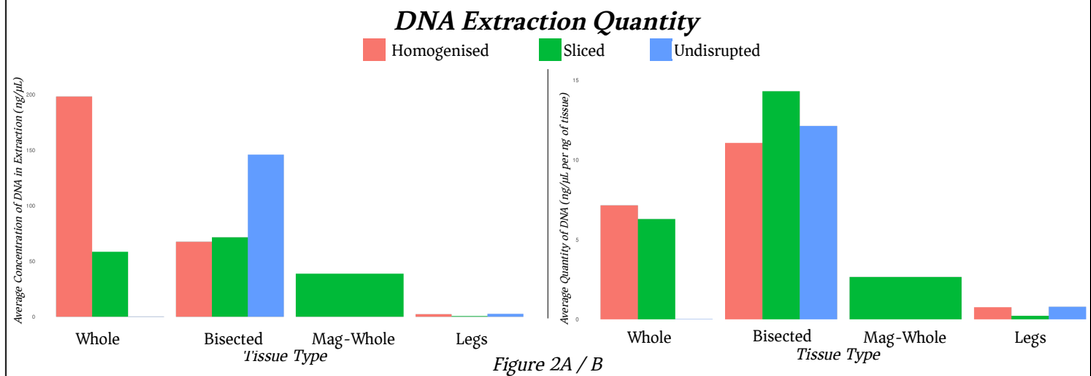


Figure 2A / B

Whole tick samples: homogenized yielded highest at 198.22 ng/µL; undisrupted lowest at 0.02 ng/µL; sliced intermediate at 6.29 ng/µL. Bisected ticks: homogenized and sliced similar around 70 ng/µL, undisrupted higher at 145.94 ng/µL. Tick legs: lower outputs, undisrupted at 2.65 ng/µL, homogenized at 2.31 ng/µL, sliced at 0.64 ng/µL. Blood and Tissue Kit outperformed MagAttract Kit for sliced specimens.

Normalized data: bisected sliced highest at 14.30 ng/µL/mg; tick legs consistently low; whole samples using homogenized and sliced both around 7 ng/µL/mg.

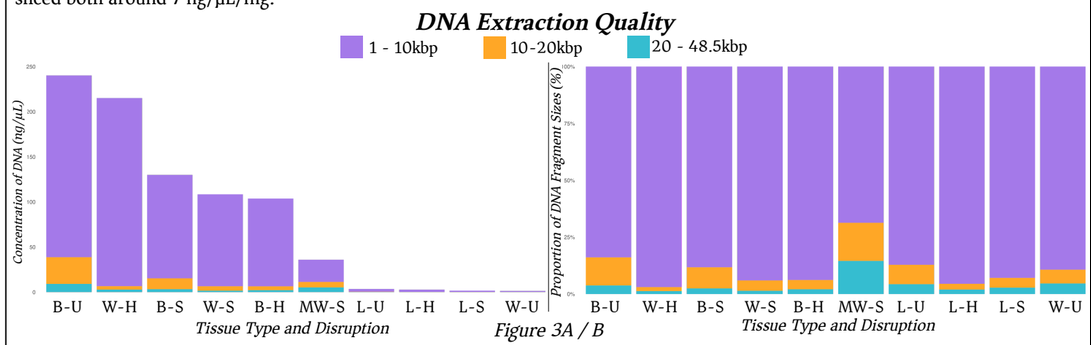


Figure 3A / B

Regarding total DNA concentrations, bisected undisrupted (B-U) had the highest fragments >10kbp at 38.69 ng/µL. Leg samples (L-U, L-H, L-S) and W-U showed low concentrations, with fragments >20kbp yielding between 0.05 to 0.15 ng/µL. W-H, W-S, B-H, B-S, and MW-S had comparable concentrations for fragments >20kbp, ranging between 1.57 and 5.25 ng/µL.

Proportionally, Whole tick sliced with MagAttract kit (MW-S) had the highest large DNA fragments: 31.30% >10 kbp and 14.58% >20 kbp. Most samples, including W-H, W-S, B-S, L-H, and L-S, primarily ranged 1-10 kbp with percentages between 92.91% and 96.95%. B-U, B-S, L-U, and W-U also predominantly fell in the 1-10 kbp range.

DNA Extraction Content

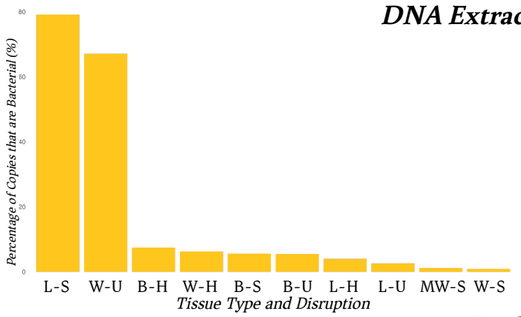


Figure 4

Figure 4 represents the proportion of Bacterial Copies, expressed as a percentage, across each sample type.

The Legs-Sliced and Whole-Undisrupted samples have high bacterial percentages at 79.12% and 67.07%.

In contrast, Whole-Sliced and Mag-Whole-Sliced samples are minimal at 0.91% and 1.19%.

Bisected-Homogenised and Undisrupted and Whole-Homogenised samples lie between at 5% to 8%.

Highlights

Qiagen MagAttract HMW Kit produced the highest proportion of >10kbp fragments (31.30%)

Whole-Homogenised samples produced the highest overall output (198.22 ng/µL)

Tick-Legs produced negligible extracted DNA (0.22 - 0.79 ng/µL of DNA for mg of tissue)

Conclusions

For long-read sequencing, researchers should use the Qiagen MagAttract HMW DNA Kit and slice specimens to obtain high molecular weight DNA, using only tick legs will produce an insufficient quantity.

For short-read NGS, whole or bisected specimen bead homogenisation is the method of choice, providing high DNA yields with fragment size suitable for this type of sequencing.

In PCR applications, all methods will likely work. Using tick legs may work, although this results in lower DNA quantities and may not amplify.

Generally, slice specimens to produce larger DNA fragments and bisect them to prevent column saturation (Figure 2) thus ensuring efficient extraction.

Suitable Applications

Table 1

Tissue Disruption Type \ Application	PCR	Short-Read NGS	Long-Read NGS
Homogenisation	✓	~	✗
Slicing	✓	✓	✓
Undisrupted	~	✗	✗
Whole	~	~	~
Bisected	✓	✓	✓
Legs	~	✗	✗

References

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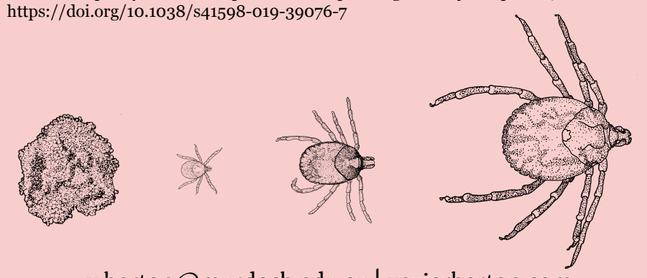
3. Tan, G., Opitz, L., Schlapbach, R., & Rehrauer, H. (2019). Long fragments achieve lower base quality in Illumina paired-end sequencing. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-39076-7



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