High Molecular Weight DNA Purification from Arthropods

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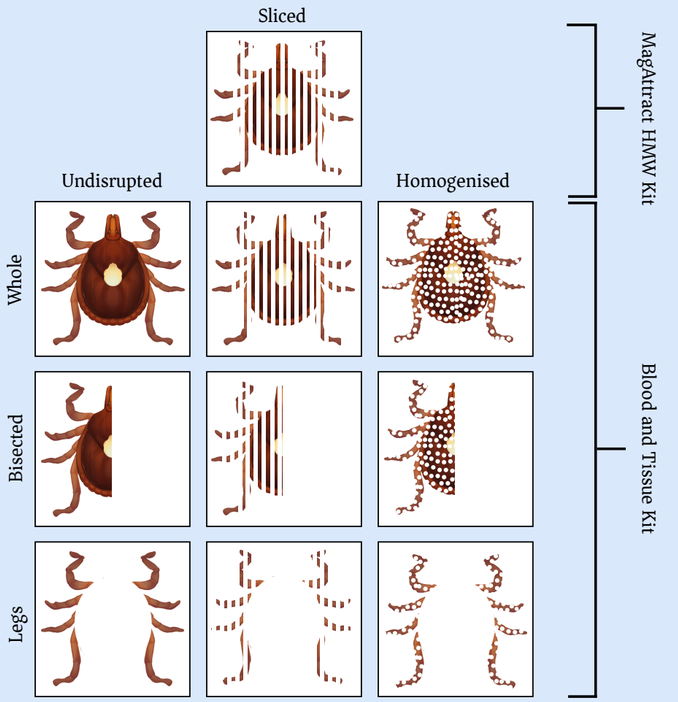
Introduction

Ixodida (Ticks) are major arthropod vectors of disease[1] with complex life histories intertwined with host dynamics, urbanisation, and climatic conditions, making genomic surveillance essential.

Existing DNA extraction methodologies for ticks and similarly sized arthropods are outdated and insufficiently optimised[2] for newer genomic sequencing technologies that demand specific DNA quality and quantity requirements[3].

This study evaluates and recommends DNA extraction methods for Ixodida and similarly-sized arthropods, considering DNA quality, quantity, and content (tick and bacteria) DNA for current Next-Generation Sequencing protocols.

Materials and Methods



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

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)

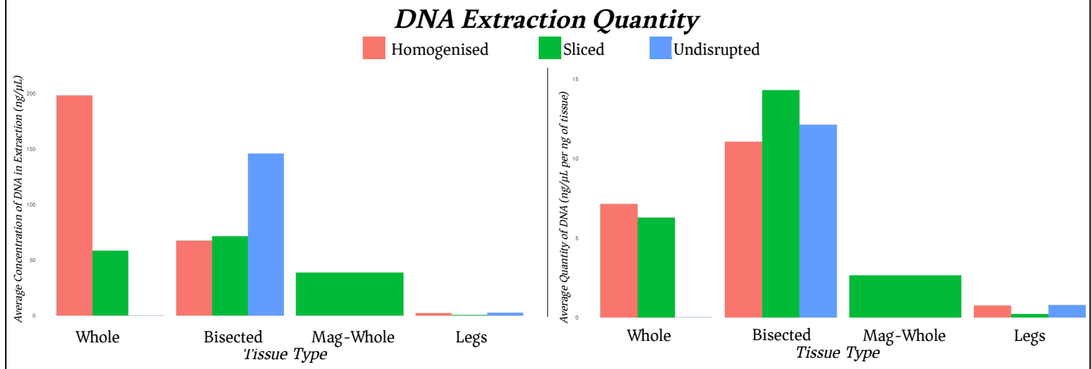
Tissue Disruption:

- ***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).

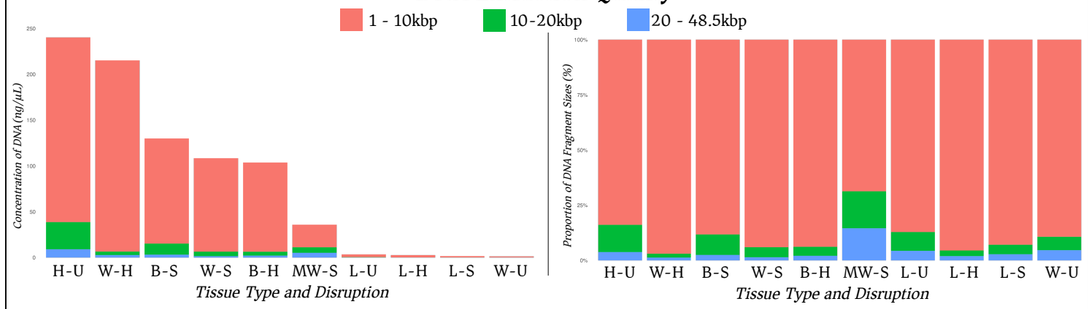
Extractions were assessed for quantity using a Qubit 2.0 Fluorometer, for quality with an Agilent 2200 TapeStation, and for content through qPCR targeting Bacteria and Ixodida, with copy number calculated by gBlock Standards.

Results



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.

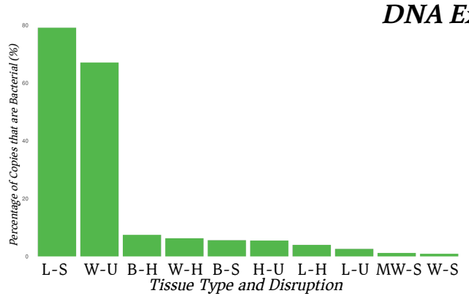
Normalized data: bisected sliced highest at 14.30 ng/µL/mg; tick legs consistently low; whole samples using homogenized and sliced both around 7 ng/µL/mg.



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, H-H, H-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



This graph 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

Efficient DNA extraction from arthropods is crucial for successful sequencing. The right method saves time, resources, and enhances experiment results.

For high molecular weight recovery a Qiagen MagAttract HMW Kit, when paired with non-destructive tissue disruption like slicing, offers optimal quality. Undisrupted specimens yield limited DNA, but bisecting them boosts extraction.

Using tick legs to minimise microbial content results in low DNA yields, unsuitable for most Next-Generation Sequencing platforms but may be viable for PCR applications.

Maximal DNA yields are achieved with whole specimens and bead homogenisation. However, this method yields shorter DNA fragments.

Whole sliced specimens produce larger DNA fragments. Normalising DNA quantity to tissue weight can give deceptive outputs due to spin column saturation, suggesting bisecting specimens for efficient extraction and conservation.

Suitable Applications

	PCR	Short Read NGS	Long Read NGS
Homogenisation	✓	~	✗
Slicing	✓	✓	✓
Undisrupted	~	✗	✗
Whole	~	~	~
Bisected	✓	✓	✓
Legs	~	✗	✗

References

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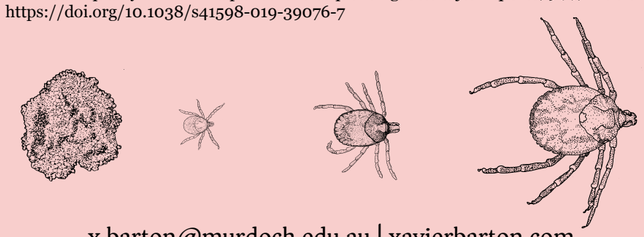
3. Tan, G., Opitz, L., Schlapbach, R., & Rehrauer, H. (2019). Long fragments achieve lower base quality in Illumina paired-end sequencing. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-39076-7



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