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To beat or not to beat a tick: Comparison of DNA extraction methods from ticks (*Ixodes scapularis*)

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ABSTRACT

Background. Blacklegged ticks (Ixodes scapularis) are important disease vectors in the United States, known to transmit a variety of pathogens to humans, including bacteria, protozoa, and viruses. Their importance as a disease vector necessitates reliable and comparable methods for extracting microbial DNA from ticks. Furthermore, to explore the population genetics or genomics of this tick, appropriate DNA extraction techniques are needed for both the vector and its microbes. Although a few studies have investigated different methods of DNA isolation from ticks, they are limited in the number and types of DNA extraction and lack species-specific quantification of DNA yield.

Methods. Here we determined the most efficient and consistent method of DNA extraction from two different developmental stages of I. scapularis – nymph and adult - that are the most important for disease transmission. We used various methods of physical disruption of the hard, chitinous exoskeleton, as well as commercial and non-commercial DNA isolation kits. To gauge the effectiveness of these methods we quantified the DNA yield and confirmed the DNA quality via PCR of both tick and microbial genetic material.

Results. DNA extraction using the Thermo GeneJET Genomic DNA Purification kit resulted in the highest DNA yields and the strongest, most consistent PCR amplification. We also found that physical disruption of the tick exoskeleton was most effective using cross-sectional cutting compared to any type of bead-beating matrices used. Storing ticks at -80°C resulted in considerably higher DNA yields than those from ticks stored in ethanol.

Discussion. We contrasted a variety of readily available methods of DNA extraction from single individual blacklegged ticks and presented the results through a quantitative and qualitative assessment.
INTRODUCTION

Blacklegged ticks (Ixodes scapularis Say, 1821) are hard-bodied, hematophagous arthropod (Arachnida, Ixodida) ectoparasites of vertebrates in North America. During its two-year life cycle, the tick acquires a bloodmeal at each developmental stage (i.e., larva, nymph, and adult) prior to molting or egg-laying, in the case of adult females. These ticks are of great public health importance as disease vectors because they carry and transmit a variety of human disease agents, such as Borrelia burgdorferi, the causative agent of Lyme disease, Anaplasma phagocytophilum which causes granulocytic anaplasmosis, and Babesia microti, a protozoan responsible for the malaria-like illness, babesiosis (Speilman, 1976; Steere et al., 1978; Pancholi et al., 1995). Recently, I. scapularis has been found to transmit Borrelia miyamotoi (Scoles et al., 2001) and Powassan virus lineage 2 (a.k.a., Deer Tick Virus) (Telford, 1997). Their importance as human disease vectors necessitates research that involves successful isolation of genetic material needed in investigations of both the vector itself and of the wide range of pathogens that they carry. However, DNA isolation in ticks is challenging due to the hard chitinous exoskeleton and the small amount of microbial nucleic acids present (Halos et al., 2004). Furthermore, tick DNA is susceptible to degradation (Hubbard et al., 1995; Hill & Gutierrez, 2003; Halos et al., 2004) and PCR can be challenged by inhibitors (Halos et al., 2004). With respect to public health, the unexplored potential for aerosolization of pathogen DNA may likewise pose a risk (Borst, Box & Fluit, 2004).

Although DNA extraction from ticks for both pathogen isolation and tick genetic and genomic research is performed routinely by researchers, there is no consensus regarding the most effective method of DNA isolation from any tick species. A few such studies have been conducted (Hill & Gutierrez, 2003; Halos 2004; Crowder et al., 2010), however, they are limited to a handful of extraction techniques, and quantitative data on DNA concentration is lacking. In this study we aim to identify the optimal DNA isolation procedure for both tick and microbial DNA from an important tick disease vector, the blacklegged tick.
MATERIALS & METHODS

Tick collection

Nymphal and adult female blacklegged ticks are important life stages in the transmission of disease agents compared to larvae, which are rarely infected with human pathogens, and adult males, whose brief feeding bouts minimize the risk of pathogen transmission (Piesman et al., 1986; Falco & Fish, 1988; Falco et al., 1999). Thus, the ability to dependably extract DNA, and in particular microbial DNA, from nymphal and adult female blacklegged ticks is of importance to tick-borne disease research and constitutes the focus of this study.

Unfed, host-seeking nymphal and adult blacklegged ticks were collected by dragging a 1m² flannel cloth along the forest floor or along low vegetation, respectively, during each life stage’s peak activity period. Ticks were collected from sites in Westchester County, Putnam County, and Orange County in New York state in the summer and fall of 2009-2011, and were subsequently stored in 70% v/v ethanol until DNA isolation. Additionally, a subset of nymphs and adult females was collected during the summer and fall of 2013-2014 at the Louis Calder Center–Biological Field Station of Fordham University in Armonk, NY and frozen at -80°C until DNA isolation.

DNA isolation

We contrasted the efficiency of extracting DNA from ticks stored in 70% ethanol using five different DNA isolation procedures coupled with either cross-sectional division or bead-based physical disruption of the tick body. These procedures included four commercially available DNA extraction kits: DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA), GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA), Tissue & Insect DNA MicroPrep (Zymo Research, Irvine, CA), PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA); and one noncommercial DNA extraction method using ammonium hydroxide (NH₄OH) and heat, which has been primarily used for DNA
isolation from the European sheep tick, *Ixodes ricinus* (Guy & Stanek, 1991; Rijpkema et al., 1996; Pichon et al., 2003; Humair et al., 2007; Pangrácová et al., 2013). All commercial kits examined here use a silica-based column procedure and have either been used in previous studies for DNA isolation in ticks or are marketed for efficient microbial DNA recovery or insect DNA isolation (Table 1). DNA extraction kits were used according to the manufacturers’ recommended protocols with few exceptions (Table 1), including a final elution completed following a 5-min room-temperature incubation in 100 µl of deionized, sterilized, distilled water (sdH2O) for consistency. Each tick was air-dried to evaporate the ethanol prior to DNA extraction, as ethanol may inhibit PCR (Hubbard, Cann & Wright 1995; Bessetti et al., 2007; Schrader et al., 2012).

The NH4OH method included adding 150 µl of a 0.7-M NH4OH solution to the tick sample in a 1.5-ml snap cap tube, and heating to 100ºC for 15 min. The solution was briefly centrifuged to concentrate fluid at the bottom and then was evaporated to 70-100 µl by opening the tubes and heating at 100ºC for an additional 15 min. The solution was then centrifuged for 10 min at 10,000× g and the supernatant was collected and respun for 2 min at 10,000× g. The new supernatent was collected and stored at -20ºC.

To determine the most effective method of physical disruption of the hard, chitinous exoskeleton of ticks prior to DNA extraction, we compared cross-sectionally dividing ticks (bisection for nymphs and quadrisection for adult females) and crushing the entire tick in a variety of bead matrices using the BeadBlaster 24 (Benchmark Scientific, Edison, NJ) (Table 1). The MoBio and Zymo kits included their own bead matrices (Table 2). The beads from these two kits were used according to the manufacturers’ instructions with either whole or cut ticks. MoBio-processed samples were beaten on a GeneMate vortex mixer (BioExpress, Kaysville, UT) at maximum speed (3200 rpm) for 10 min and Zymo-processed samples were beaten on the BeadBlaster 24 for 10 min at 4 m/s. For DNA extraction methods that did not include bead matrices (i.e., QIAGEN, Thermo, and NH4OH), we used six different MP Bio Lysing Matrices, which were either marketed for tough samples or, as in the case of Matrices H and I, were marketed specifically for ticks. We beat the ticks with the MP Bio Lysing Matrices for 1.5 and 4 min at 4 m/s (Table 2).
A subset of nymphs and adult females stored at -80°C was bead-beaten for 1.5 min at 4 m/s with each of the MP Bio matrices and then the DNA was extracted with the QIAGEN kit to assess the effect of storage method on DNA isolation. All DNA extraction and physical disruption methods were conducted on three individual nymphs and three individual adult female blacklegged ticks.

**DNA quantification**

The resulting DNA yields were quantified via double-stranded DNA (dsDNA) fluorometric quantitation on a Qubit 2.0 fluorometer (Life Technologies, Norwalk, CT) using 10 µl of extracted DNA template in 190 µl of the High Sensitivity (HS) dsDNA assay.

**PCR validation**

The isolated DNA was validated using PCR amplification of tick mitochondrial and nuclear loci. We amplified the tick DNA using (1) the cytochrome c oxidase subunit 1 (Cox1) DNA barcode region (~650bp) located on the mitochondrial genome with the HCO/LCO primers (Folmer et al., 1994), and (2) a dinucleotide (CA)$_n$ microsatellite repeat located on the nuclear genome with the bac7ea/bac7eb primer pair (139-197bp) (Chan, 2012). We targeted the genus *Rickettsia* using PCR to validate the successful extraction of microbial DNA from inside the tick, which is necessary for studies involving PCR detection of human pathogens transmitted by this tick species. Members of this genus are obligate intracellular bacteria and are abundant in blacklegged ticks (Benson et al., 2004; Clay & Fuqua 2010; Moreno et al., 2006; Noda, Munderloh & Kurtti, 1997; Steiner et al., 2008; CP Zolnik, unpublished data). We targeted a 532-bp fragment of the *ompA* gene using *Rickettsia*-specific primers (Vitorino et al., 2007).
All thermal cycling conditions were slightly modified from their published protocols and are detailed below. The thermal cycling conditions for the Cox1 DNA barcode region began with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s, and then a final extension at 72°C for 7 min (Folmer et al., 1994). The microsatellite region was amplified using a touchdown PCR with an initial denaturation at 95°C for 1 min, then 5 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 30 s; 30 cycles of 95°C for 20 s, 50°C for 25 s, and 72°C for 30 s; and a final extension for 5 min at 72°C (Chan, 2012). The Rickettsia ompA locus was amplified with an initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 30 s, and a final extension for 7 min at 72°C (Vitorino et al., 2007). All PCR reactions were performed using a Techne Prime Elite Thermal Cycler (Bibby Scientific, Burlington, NJ).

Each PCR was performed in a final 25-µl volume with 6.25 µl 2× MyTaq HS Mix (Bioline, Taunton, MA) and 0.2 µM of each forward and reverse primer. The PCRs targeting nuclear and mitochondrial tick DNA were carried out using 16.25 µl sdH2O and 1.5 µl DNA template. To account for the lower DNA concentrations of microbial DNA within this tick, and specifically for nymphs, the PCRs targeting the Rickettsia ompA fragment were carried out with 15.75 µl sdH2O and 2 µl DNA template from nymphs, and 16.75 µl sdH2O and 1 µl DNA template for adult females. PCRs were confirmed by agarose gel electrophoresis on a 1.5% w/v gel.

**Statistical analysis**

Given the substantial differences in size and DNA yield between nymphs and adult females, statistical analyses of the DNA concentration data from the two sets of samples were performed separately. Following a one-way ANOVA, Tukey’s HSD test was used for post-hoc analysis of the average DNA quantification values resulting from nymph bisection or female quadrisection across the five DNA isolation methods (QIAGEN,
Thermo, MoBio, Zymo, and NH₄OH). The same procedures were also used to compare
the DNA yields resulting from the different MP Bio Lysing Matrices (G, H, I, M, S, and
Z) and nymph bisection or female quadrisection methods using data from the two
highest-yielding DNA extraction methods. A two-tailed Student’s $t$-test was used to
compare the average DNA concentrations resulting from bead beating for 1.5 min with
those resulting from bead beating for 4 min using the data from the two highest-yielding
DNA extraction methods. A $t$-test was also used to compare DNA yields following 1.5
min bead beating and DNA extraction with the QIAGEN kit from ticks stored in 70% v/v
ethanol with DNA yields from ticks stored at -80°C.

RESULTS AND DISCUSSION

DNA extraction method comparison

The comparison of DNA concentrations resulting from nymph bisection and female
quadrisection across the five DNA extraction methods yielded no significant difference
between the QIAGEN DNEasy Blood & Tissue Kit and the Thermo GeneJET Genomic
DNA Purification Kit (Fig. 1). However, for both nymphs and adult females, the
QIAGEN and Thermo methods resulted in significantly higher DNA yields than the
Zymo Research Tissue & Insect DNA MicroPrep and the MoBio PowerSoil DNA
Isolation Kit (P<0.01) (Fig. 1). QIAGEN and Thermo also had significantly higher DNA
yields than the NH₄OH method for both nymphs (P<0.05) and females (P<0.05, P<0.01)
(Fig. 1). There was no significant difference among the DNA extraction concentrations
resulting from the NH₄OH method and Zymo and MoBio kits for both nymphs and
females, except for the NH₄OH adult female DNA extraction, which was significantly
better than the Zymo method (P<0.05) (Fig. 1). Of the five methods, QIAGEN and
Thermo generated the highest DNA yields overall.
Physical disruption methods

For the two highest-yielding DNA isolation methods, i.e., QIAGEN and Thermo, in most cases there was no significant difference in the DNA concentration yields among the six different MP Bio Lysing Matrices (G, H, I, M, S, and Z) and bisection for nymphs (Fig. 2A). However, both the S matrix and bisection resulted in significantly higher yields than the G and M matrices (P<0.05, P<0.01) (Fig. 2A). For females in all cases, quadrisection resulted in significantly higher DNA concentrations than any bead beating matrix (P<0.05 for H and I; P<0.01 for G, M, S, and Z) (Table 2B).

For both nymphs and females that were physically disrupted with the MP Bio Lysing Matrices and underwent a DNA extraction with either the QIAGEN or the Thermo method, there was no significant difference between the DNA yields resulting from bead beating for 1.5 and 4 min (Fig. 3).

Tick storage method and DNA yield

DNA extractions resulting from bead beating for 1.5 min with the MP Bio Lysing Matrices and DNA isolation with the QIAGEN method were significantly better for nymphs and females that were stored at -80°C prior to DNA extraction than for nymphs and females stored in 70% ethanol (P<0.0001) (Fig. 4).

PCR validation

Although the QIAGEN and Thermo methods produced similar DNA yields, the Thermo method exhibited the strongest and most consistent gel electrophoresis PCR product bands in the case of both nymphs and females for Cox1, microsatellite, and Rickettsia sp. ompA. Increasing bead beating duration had a positive effect on PCR when DNA was isolated through the QIAGEN method, while the same effect was not evident when using the Thermo method. Among the five DNA isolation methods, Thermo ultimately produced the best-amplifiable genomic DNA.
The DNA extractions resulting from the NH$_4$OH protocol yielded strong and somewhat consistent amplification of the Cox1, microsatellite, and Rickettsia ompA loci for adult females. NH$_4$OH yielded significantly lower DNA concentration results than the QIAGEN and Thermo methods when using quadrisected adult female ticks; however, this did not affect PCR success. PCR amplification was consistently poor for nymphs treated with the NH$_4$OH protocol. Only the combination of the NH$_4$OH method with bead beating with the M matrix for 1.5 or 4 min resulted in consistent amplification of the microsatellite target for nymphs, as well as limited success for the Cox1 and Rickettsia ompA loci.

The DNA extracted using the Zymo Research Tissue & Insect DNA MicroPrep did not produce nuclear, mitochondrial, or bacterial PCR amplifications for either whole or cut nymphs, or adult females, consistent with the very low DNA yields we measured. DNA extraction from adult female ticks with the MoBio PowerSoil DNA Isolation Kit resulted in the consistent amplification of the three targeted loci, with quadrisection substantially enhancing the amplicon gel band quality in comparison with whole females. However, the very low yields for both bisected and whole nymphs extracted with MoBio corresponded with the limited success of all three PCR protocols with the MoBio method.

Among the MP Bio Lysing Matrices, the S matrix produced some of the strongest and most consistent PCR results across all targeted loci (nuclear, mitochondrial, and bacterial), DNA extraction methods (QIAGEN, Thermo, and NH$_4$OH), bead beating times (1.5 and 4 min), and life stages (nymphs and females). Although the S matrix did not produce significantly higher DNA concentrations than any other matrix for adult females, yet generated higher DNA yields than the G and M matrices did for nymphs, the PCR results were generally better for the S matrix. The H and I matrices, which the MP Bio website recommends for ticks, also produced comparably good results in terms of strength and consistency for many cases, although the bands resulting from the S matrix were slightly stronger and more consistent following 4 min of bead beating and DNA extraction with the QIAGEN and Thermo kits.
Nymph bisection and female quadrisection produced PCR results that were generally just as strong and consistent as bead beating with the S, H, and I matrices combined with DNA extraction with Thermo and NH$_4$OH for nuclear and mitochondrial DNA. However, cutting the ticks in combination with the QIAGEN methods produced substantially better results than did bead beating. Additionally, cutting generally produced better results than bead beating for the PCR amplification of rickettsial DNA.

CONCLUSIONS

Successful DNA extraction from tick species is important for both genetic and genomic studies of the tick vector itself, as well as for studies aimed at detecting pathogen presence in these tick vectors. This study was designed to determine the most reliable and efficient method of DNA extraction, including physical disruption of the tick exoskeleton.

We determined that the most reliable and consistent DNA extraction method for both nymphal and adult female ticks was the Thermo GeneJET Genomic DNA Purification Kit, which resulted in the highest DNA yields and the strongest and most consistent PCR amplification, demonstrated as PCR bands on electrophoresis agarose gels. The QIAGEN DNeasy Blood & Tissue Kit, though perhaps the most commonly used kit, produced less intense yet consistent results. Cross-sectional cutting was more effective than any type of bead beating matrices for the Thermo and QIAGEN extraction kits.

Both the MoBio and Zymo kits were poor choices for extracting DNA from nymphs and adult female ticks, despite the fact that the MoBio kit is marketed for enhanced microbial DNA extraction and the Zymo kit is marketed for DNA extraction from insects (as per the manufacturers’ websites). The NH$_4$OH extraction method is an inexpensive alternative to commercially available kits and produced high-quality PCR products for adult females, although the DNA yield was generally lower than that of commercial kits. However, this method was not useful for DNA extraction from nymphs, resulting in low DNA yield and poor to non-existent PCR amplification, despite the frequent use of this
method in studies on nymphal *Ixodes ricinus* in Europe (Guy & Stanek, 1991; Rijpkema et al., 1996; Pichon et al., 2003; Humair et al., 2007; Pangrácová et al., 2013).

Although bead beating greatly reduces the time needed for physical disruption of the tick’s chitinous exoskeleton and eliminates direct handling of samples, which itself may diminish potential contamination or aerosolization of DNA, we recommend nymphal bisection and female quadrisection for accessing nuclear and mitochondrial tick DNA and internal microbial DNA using the QIAGEN, Thermo and NH₄OH DNA extraction protocols. Cutting the ticks resulted in the strongest and most consistent DNA yields and PCR products. However, if bead beating is used, we recommend using the MP Bio Lysing Matrices S, H, or I with a 4-min beating time. These results confirm MP Bio’s recommendation of the H and I matrices for ticks, although the S matrix also results in DNA yields of comparable or slightly better quantity and quality (http://www.mpbio.com/index.php?cPath=2_77_425&country=223).

Finally, we explored the impact of storing ticks at -80°C in comparison with ethanol-based storage methods (70% ethanol) for a subset of ticks that was extracted using the QIAGEN method following cross-section or bead beating. We found that freezing resulted in significantly higher DNA yields than ethanol storage, and we suggest the use of -80°C storage when possible. Our study expands on previous work that determined DNA extraction success from ticks based on PCR amplification alone, without a DNA quantification assessment (Halos et al., 2004). While a recent study quantified DNA yield, the reported values were averaged across multiple tick species and focused only on one developmental stage, adults (Crowder et al., 2010). In order to test the efficiency of the DNA extraction techniques, we kept certain variables constant, such as the time for incubation in lysis buffer, bead beating speed, elution volume, and incubation time prior to elution. Alteration of these variables may result in increased DNA yield and should be considered when DNA concentration is important in downstream applications, such as gene expression, pathogen surveillance, and microbial community profiling.
ACKNOWLEDGEMENTS

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REFERENCES


Moreno CX, Moy F, Daniels TJ, Godfrey HP, Cabello FC. 2006. Molecular analysis of microbial communities identified in different developmental stages of *Ixodes scapularis* ticks from Westchester and Dutchess counties, New York. *Environmental Microbiology* 8:761-772.

to *Wolbachia* spp. and tick-borne pathogens of human and animals. *Applied and Environmental Microbiology* 63:3926-3932.


Figure 1. DNA concentrations (ng/µl) resulting from the five DNA extraction methods following nymph bisection and female quadrisection as determined using the Qubit HS dsDNA Assay. Each sample set consisted of three individual tick DNA extractions. The QIAGEN and Thermo methods did not differ significantly. Note the difference in scale between the life stages. A. For nymphal ticks, both the QIAGEN and Thermo DNA concentrations were significantly different from those of NH₄OH (P<0.05), and Zymo and MoBio (P<0.01). B. For adult female ticks, the QIAGEN and Thermo concentrations were significantly different from that of NH₄OH (P<0.05 and P<0.01, respectively).
QIAGEN and Thermo concentrations were significantly different (P<0.01) from those of MoBio and Zymo yields. The NH$_4$OH yield differed significantly (P<0.05) from the Zymo yield.
Figure 2. DNA concentrations (ng/µl) resulting from the QIAGEN and Thermo DNA extraction methods following the bead beating of whole ticks with each of the MP Bio lysing matrices (G, H, I, M, S, and Z), nymph bisection, and adult female quadrisection, as determined using the Qubit HS dsDNA Assay. Twelve individuals were used for each bead beating treatment. Six individuals were used for each cutting treatment. 

A. For nymphs, bisection was significantly different (P<0.01) from the G and M matrices. Matrix S was significantly different from matrices G (P<0.05) and M (P<0.01).

B. For adult females, quadrisection produced significantly higher DNA yields than all bead beating matrices (P<0.05 against H and I; P<0.001 against G, M, S, and Z).
Figure 3. DNA concentrations (ng/µl) resulting from the QIAGEN and Thermo DNA extraction methods following the bead beating of whole ticks with each of the MP Bio lysing matrices (G, H, I, M, S, and Z) for 1.5 or 4 min, as determined using the Qubit HS dsDNA Assay. Eighteen individuals were used in each set. The duration treatments were contrasted using a two-tailed Student’s t-test. **A.** Bisected nymphs (P>0.05). **B.** Quadrisection adult females (P>0.05).
Figure 4. DNA concentrations resulting from DNA extraction from ticks stored at -80°C or in 70% v/v ethanol using the QIAGEN method, following cutting or bead beating of whole ticks with the MP Bio lysing matrices (G, H, I, M, S, and Z) for 1.5 min, as determined using the Qubit HS dsDNA Assay. Eighteen individuals were used in each set. The sample storage treatments were contrasted using a two-tailed Student’s t-test. A. Ethanol-stored vs. frozen nymphs (P<0.0001). B. Ethanol-stored vs. frozen, quadrisected adult females (P<0.0001).
Table 1. Methods of DNA isolation and physical disruption of tick samples. Only samples treated with the MoBio kit were processed on the GeneMate vortex mixer (BioExpress), while all remaining bead beating took place on the BeadBlaster 24 (Benchmark Scientific).

<table>
<thead>
<tr>
<th>DNA extraction method</th>
<th>Alterations to manufacturer protocols</th>
<th>Physical disruption</th>
<th>Bead beating – speed and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIAGEN DNeasy Blood &amp; Tissue Kit</td>
<td>Elution in 100 µl dsH2O with 5 min room temperature incubation</td>
<td>Bisection (Nymphs), Quadrisection (Females)</td>
<td>N/A</td>
</tr>
<tr>
<td>(cat. no. 69506)</td>
<td></td>
<td>MP Bio Lysing Matrices</td>
<td>4 m/s 1.5 &amp; 4.0 min</td>
</tr>
<tr>
<td>Thermo GeneJet Genomic DNA Purification Kit</td>
<td>Elution in 100 µl dsH2O with 5 min room temperature incubation</td>
<td>Bisection (Nymphs), Quadrisection (Females)</td>
<td>N/A</td>
</tr>
<tr>
<td>(cat. no. K0722)</td>
<td></td>
<td>MP Bio Lysing Matrices</td>
<td>4 m/s 1.5 &amp; 4.0 min</td>
</tr>
<tr>
<td>Zymo Research Tissue &amp; Insect DNA MicroPrep</td>
<td>Elution in 100 µl dsH2O with 5 min room temperature incubation</td>
<td>Bisection (Nymphs), Quadrisection (Females) followed by beating with Zymo beads</td>
<td>4 m/s 10 min</td>
</tr>
<tr>
<td>(cat. no. D6015)</td>
<td></td>
<td>Zymo beads</td>
<td>4 m/s 10 min</td>
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<tr>
<td>MoBio PowerSoil DNA Isolation Kit</td>
<td>Elution in 100 µl of dsH2O with 5 min room temperature incubation</td>
<td>Bisection (Nymphs), Quadrisection (Females) followed by beating with MoBio garnet beads</td>
<td>3200 rpm 10 min</td>
</tr>
<tr>
<td>(cat. no. 12888)</td>
<td>20 µl of Proteinase K added</td>
<td>MP Bio Lysing Matrices</td>
<td>3200 rpm 10 min</td>
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<td>NH₄OH</td>
<td>Initial volume of 150 µl NH₄OH Final volume of 70-100 µl dsH2O Second centrifugation for 2 min at 10,000× g</td>
<td>Bisection (Nymphs), Quadrisection (Females)</td>
<td>N/A</td>
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<tr>
<td>(Guy and Stanek, 1991, Pichon et al., 2003)</td>
<td></td>
<td>MP Bio Lysing Matrices</td>
<td>4 m/s 1.5 &amp; 4.0 min</td>
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<tr>
<td>Matrix</td>
<td>Manufacturer</td>
<td>Material</td>
<td>Suggested use</td>
</tr>
<tr>
<td>--------</td>
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<td>---------------</td>
</tr>
<tr>
<td>G</td>
<td>MP Bio</td>
<td>1.6 mm silicon carbide particles</td>
<td>Samples with tough, hard, or brittle cell membranes</td>
</tr>
<tr>
<td>H</td>
<td>MP Bio</td>
<td>2 mm glass beads &amp; 2 mm zirconium oxide beads</td>
<td>Tough, hard cells and organisms within dense exterior matrices, e.g. whole insects and ticks</td>
</tr>
<tr>
<td>I</td>
<td>MP Bio</td>
<td>2 mm zirconium beads &amp; one 4 mm ceramic sphere</td>
<td>Very tough, hard samples including chitin exoskeletons, e.g. whole insects and ticks</td>
</tr>
<tr>
<td>M</td>
<td>MP Bio</td>
<td>Two 6.35 mm zirconium oxide-coated ceramic grinding spheres</td>
<td>Tough tissues, seeds, spores</td>
</tr>
<tr>
<td>S</td>
<td>MP Bio</td>
<td>3.175 mm stainless steel beads</td>
<td>Tough tissues, seeds, spores</td>
</tr>
<tr>
<td>Z</td>
<td>MP Bio</td>
<td>2.0 mm yttria-stabilized zirconium oxide spheres</td>
<td>Tough plant and animal samples</td>
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<td>PowerBeads</td>
<td>MoBio</td>
<td>Garnet</td>
<td>Environmental samples</td>
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<td>BashingBeads</td>
<td>Zymo</td>
<td>Ceramic</td>
<td>Ticks, mosquitoes, bees, lice, and <em>Drosophila melanogaster</em></td>
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</tbody>
</table>

**Table 2.** Bead matrices and their attributes. The composition, characteristics, and recommended uses for the different bead matrices tested listed are adapted from the manufacturers’ websites.

<table>
<thead>
<tr>
<th>Method</th>
<th>Life Stage</th>
<th>Nymphs</th>
<th>Adult females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bisected</td>
<td>Whole</td>
<td>Quadrisection</td>
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<td>QIAGEN</td>
<td>Average</td>
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<td>9.41</td>
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<tr>
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<td>SD</td>
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**Table 3.** Average DNA concentration (ng/µl) of whole and cut nymphal and adult female blacklegged ticks. Average and standard deviation of the DNA concentration values determined using the Qubit HS dsDNA Assay. Unless otherwise indicated, samples were stored in 70% v/v ethanol. Three single-tick measurements were included in each treatment. All values listed as < 0.0005 ng/µl indicate a reading of “too low” from the Qubit fluorometer.
Table 4. DNA concentration (ng/µl) of whole nymphal and adult female blacklegged ticks bead-beaten with MP Bio bead matrices. The results of DNA extractions from whole ticks for the QIAGEN, Thermo, and NH₄OH are expanded here to include the individual results from each of the six MP Bio Lysing Matrices.
Table 5. DNA concentration (ng/µl) of frozen (-80°C) nymphs and adult females. A two-tailed Student’s t-test showed that cold storage at -80°C produced significantly greater DNA yields than 70% v/v ethanol storage for both nymphs and females (P<0.0001).

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<th>Method</th>
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<th></th>
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<th>Adult females</th>
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<td>H</td>
<td>I</td>
<td>M</td>
<td>S</td>
<td>Z</td>
<td>G</td>
<td>H</td>
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