QIAGEN Supplementary Protocol:

Purification of total DNA from ticks using the DNeasy® Blood & Tissue Kit for detection of *Borrelia* DNA

This protocol provides recommendations for DNA purification from ticks, for use in real-time PCR detection of *Borrelia* spp., using, for example, the QIAGEN® *artus*® Borrelia LC PCR Kit (cat. no. 4551063 or 4551065, not available in the USA).

Introduction

Due to generally low numbers of *Borrelia* in ticks, both complete digestion of tick tissue (except the exoskeleton) and removal of inhibitors are crucial to guarantee the highest possible sensitivity in downstream PCR.

In general, for DNA preparation single, whole ticks are used, except for ticks that are engorged with blood. Preparation of the whole tick is only useful if the body does not exceed 5 mm. Larger ticks most probably have recently sucked blood and therefore may inhibit downstream PCR analysis due to the release of inhibitors from digested blood.

Therefore, use only the head section of large and often dark colored ticks (females). Cut off the abdomen containing most of the digested blood with a sharp scalpel. Frequently, relatively small ticks (males) can be found fixed to the ventral body of a much bigger female. In this case only the females (head) have to be analyzed, because the males did not have contact with the host.

IMPORTANT: Please read the *DNeasy Blood & Tissue Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

DNeasy Blood & Tissue Kits: For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. The *artus* Borrelia LC PCR Kit is not available in the USA. Purification of total DNA from ticks using the DNeasy Blood & Tissue Kit and detection of *Borrelia* DNA using the *artus* Borrelia LC PCR Kit is a research application.

Equipment and reagents required

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- DNeasy Blood & Tissue Kit (cat. no. 69504 or 69506)
- Pipets and pipet tips
- Scalpel
- Vortexer
- Microcentrifuge tubes (1.5 ml or 2 ml)

- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer, shaking water bath, or rocking platform for heating at 56°C and 70°C
- Ethanol (96–100%)*
- Carrier RNA solution, 10 mg/ml (e.g., poly A RNA Homopolymer, Amersham Biosciences, cat. no. 27-4110-01)†
- Optional: Internal control DNA (e.g., as provided in the QIAGEN *artus* Borrelia LC PCR Kit, cat. no. 4551063 or 4551065, not available in the USA)

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" in the *DNeasy Blood & Tissue Handbook*.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

Things to do before starting

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 4.
- If using frozen samples, equilibrate the sample to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

Procedure

1. **Place the whole tick or the head in a 1.5 ml microcentrifuge tube with 180 µl Buffer ATL. Vortex thoroughly.**
2. **Draw the moistened body of the tick with the tip of a pipette to the upper rim of the opened microcentrifuge tube and cut it with a scalpel once longitudinally and once diagonally (depending on the sample size, further cuts may be necessary).**

   Cutting the tick is necessary so that the tick tissue is thoroughly lysed and the *Borrelia* contained in it can be quantified.
3. **Close the microcentrifuge tube and centrifuge briefly to collect all tissue pieces at the bottom of the tube.**

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

4. **Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed (only the exoskeleton remains). Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking shaker.**

   Lysis time varies depending on the size of the tissue pieces. Lysis is usually complete in 30–60 min. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

   Heat the thermomixer, shaking water bath, or rocking shaker to 70°C after this step if it will be used for the incubation in step 5.

5. **Vortex for 15 s. Add 200 µl Buffer AL (without added ethanol) to the sample, and mix thoroughly by vortexing. Incubate at 70°C for 10 min.**

   Ensure that ethanol has not been added to Buffer AL (see "Buffer AL" in the *DNeasy Blood & Tissue Handbook*). Buffer AL can be purchased separately.

   It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL. This precipitate does not interfere with the DNeasy procedure.

6. **Add 1 µl of carrier RNA (10 mg/ml), and mix thoroughly by vortexing. Then add 230 µl ethanol (96–100%), and mix again thoroughly by vortexing.**

   Optional: Add internal control DNA with the carrier RNA. Adjust the amount of internal control DNA according to the final elution volume. For very small ticks (<5 mm), a final elution volume of 60 µl is recommended. For larger ticks (or part of one), use elution volumes up to 200 µl. When using the *artus* Borrelia LC PCR Kit, add 6 µl or up to 20 µl, respectively, of the Internal Control provided in the kit.

   It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. This precipitate does not interfere with the DNeasy procedure.

7. **Pipet the mixture from step 6 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.***

8. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow-through and collection tube.***

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See *DNeasy Blood & Tissue Handbook* for safety information.

9. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

   It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

   Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x *g* (14,000 rpm).

10. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 35 µl Buffer AE (for small ticks, <5 mm) or 105 µl Buffer AE (for large ticks) directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

11. **Pipet another 30 µl Buffer AE (for small ticks, <5 mm) or 100 µl Buffer AE (for large ticks) directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

    This step leads to increased overall DNA yield.

    Eluting with 35 µl + 30 µl Buffer AE results in an eluate volume of approximately 60 µl. Eluting with 105 µl + 100 µl Buffer AE results in an eluate volume of approximately 200 µl.

    The microcentrifuge tube from step 10 should be reused for this elution step.

    Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *DNeasy Blood & Tissue Handbook*.

QIAGEN kit handbooks can be requested from QIAGEN Technical Services or your local QIAGEN distributor.

Selected kit handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/msds.aspx .