Aug 11, 2022

Modified Qiagen DNeasy Blood and Tissue extraction method (Cat. No. / ID: 69506) for eDNA extraction from filters (Nitrocellulose Mixed Ester membrane filters or similar)

Yoamel Milián-García[1]

[1]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada 50 Stone Rd E, Guelph, ON N1G 2W1

dx.doi.org/10.17504/protocols.io.8epv59y4jg1b/v1

Yoamel Milián-García

ABSTRACT

The commercial Qiagen DNeasy Blood and Tissue kit constitutes one of the most used kits in Molecular Biology Laboratories for DNA extraction from tissue samples. Here, I have adapted the Qiagen Blood and Tissue kit for eDNA extraction from filters (Nitrocellulose Mixed Ester membrane filters or similar). A list of modified steps and reasoning behind the modification is also provided.

DOI

dx.doi.org/10.17504/protocols.io.8epv59y4jg1b/v1

PROTOCOL CITATION

Yoamel Milián-García 2022. Modified Qiagen DNeasy Blood and Tissue extraction method (Cat. No. / ID: 69506) for eDNA extraction from filters (Nitrocellulose Mixed Ester membrane filters or similar). **protocols.io** https://protocols.io/view/modified-qiagen-dneasy-blood-and-tissue-extraction-ce45tgy6

CREATED

Aug 10, 2022

LAST MODIFIED

Aug 11, 2022

PROTOCOL INTEGER ID

68477

1 Allow a filter to thaw on a sterile Petri Dish (e.g., Fisher Scientific Catalog No. FB0875713 or FB0875713A) and cut into quarters (halves can be used to decrease the number of extractions per filter). Cut the filter quarters (or halves) into strips using sterile razor blades. Filter strip manipulations at this step can be supported using sterile forceps or 1 mL pipette tips or similar.

2 Place small strip pieces of each quarter (or halves) in independent 2 mL microcentrifuge tubes with approximately 250 mg of glass beads (0.75–1 mm diameter). Spin the samples down at 11,000 g for 30 seconds to avoid losing filter pieces before adding any buffer. Add 380 µL Buffer ATL, which should be enough to cover all the strip pieces if they are cut at appropriate sizes. **Note 1:** It is essential to use 2 mL tubes that will facilitate the mechanical disruption, given the shape of the tube bottom, but also will be key to containing all the adjusted volumes.

3 Mechanically disrupt every quarter piece before Proteinase K digestion using the Qiagen TissueLyser II (1 min at 30 Hz). Extended times and higher frequencies can be explored as needed (please, consult Qiagen TissueLyser manufacturer´s instructions [https://www.qiagen.com/us/products/human-id-and-forensics/automation/tissuelyser-ii/]). Spin the samples down at 11,000 g for 1 minute.

4 Add 20 µL Proteinase K. Mix thoroughly by vortexing (10 seconds), and incubate at 56°C (overnight), shaking at 700 r.p.m (e.g., Ohaus Orbital Shaker, Item Number: 30391924). Overnight incubation is suggested rather than 1-3 hours as it can maximize DNA yield and concentration. The latter can be critical, considering that eDNA retained in filters from environmental samples is generally expected to be in low concentrations. **Note 2:** ATL buffer volume was adjusted to account for enough volume to cover the filter pieces in combination with the proteinase K. Total volume adjustment of buffer ATL plus proteinase K is twice (400 µL vs 200 µL) the one suggested by the manufacturer in the standard kit uses. The amount of proteinase K was kept the same as a lower amount of tissue is expected in environmental studies using water samples.

5 Vortex for 15 seconds and spin down at 11,000 g for 30 seconds. Add 400 µL Buffer AL to the sample and mix thoroughly by vortexing (10 seconds). Incubate at 56°C (10 min), shaking at 700 r.p.m. **Note 3:** If a glass fiber filter is used, an additional step is needed to transfer all the material (lysate and remaining filter pieces, except the glass beads) from step 5 to a QIAShredder column (Qiagen Catalog No. / ID: 79656). After moving the material to the QIAShredder column centrifuge for 2 minutes at 20,000 g to recover all lysate before step 6. This step is necessary given that the fiber filter will absorb most of the buffers added to it until this point.

Citation: Yoamel MiliÃÂ¡n-GarcÃÂa Modified Qiagen DNeasy Blood and Tissue extraction method (Cat. No. / ID: 69506) for eDNA extraction from filters (Nitrocellulose Mixed Ester membrane filters or similar)
https://dx.doi.org/10.17504/protocols.io.8epv59y4jg1b/v1

6 Then, add 400 µL ethanol (96–100%) and mix thoroughly by vortexing (30 seconds). **Note 4:** Volumes of AL and ethanol are twice the amount suggested by the manufacturer in correspondence with the increased ATL buffer (refer to discussed adjustments above).

7 Pipet the mixture from step 6 (including any residue except for remaining filter pieces and glass beads) into the DNeasy Mini spin column by adding up to 650 µL at a time. Then place it in a 2 mL collection tube and centrifuge at 11,000 g for 1 min. Discard flow-through and collection tube. This step is needed twice. As an alternative to the collection tubes provided by the manufacturer, 2 mL microcentrifuge tubes can be used.

8 Place the DNeasy Mini spin column in a new 2 mL collection tube, add 500 µl Buffer AW1, and centrifuge for 1 min at 11,000 g. Discard flow-through and collection tube.

9 Place the DNeasy Mini spin column in a new 2 mL collection tube, add 500 µL Buffer AW2, and centrifuge for 5 min at 17,000 g to dry the DNeasy membrane. Discard flow-through and collection tube. **Note 5**: Following the centrifugation step, carefully remove the DNeasy Mini spin column, so it does not come into contact with the flow-through. This will result in a carryover of ethanol. If the carryover of ethanol occurs, empty the collection tube and reuse it in another centrifugation for 5 min at 17,000 g. Drying the DNeasy Mini spin column membrane is essential as residual ethanol could interfere with subsequent steps. This centrifugation ensures that no residual ethanol will be carried over during the following elution.

10 Place the DNeasy Mini spin column in a clean 1.5 mL (LoBind Eppendorf, Catalog No. 022431021) and pipet 100 µL Buffer AE (prewarmed at 70 oC) directly onto the DNeasy membrane. Incubate at room temperature for 15 minutes, then centrifuge for 5 minutes at 11,000 g to elute. Pool the DNA extracts from different quarters (or halves) into a single tube. **Note 6**: Buffer AE prewarming facilitates the elution and, combined with an extended incubation time, increases eluted yield/concentration. Elution with 100 µL (instead of 200 µL) increases the final DNA concentration in the eluate but can decrease the overall DNA yield.

11 A second elution can be repeated under the same conditions indicated in step 10, keeping the second elution separately from the first elution.

Citation: Yoamel MiliÃÂ¡n-GarcÃÂa Modified Qiagen DNeasy Blood and Tissue extraction method (Cat. No. / ID: 69506) for eDNA extraction from filters (Nitrocellulose Mixed Ester membrane filters or similar)
https://dx.doi.org/10.17504/protocols.io.8epv59y4jg1b/v1