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Optimized DNA Extraction from Blood of Various Turtle Species Using QIAamp Blood Mini Kit

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We use this protocol and it's working

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Disclaimer

All authors declare no conflict of interest.

Abstract

Turtle blood, in contrast to mammalian blood, contains nucleated erythrocytes, which present distinct challenges for the development and application of DNA extraction protocols. To address these challenges, the QIAamp Blood Mini Kit was modified to facilitate the extraction of high-quality genomic DNA from turtle blood from three species: loggerhead, green, and hawksbill turtles. This adaptation involved optimizing the sample dilution step, specifically diluting 20 μ L of whole blood with 180 μ L of PBS prior to the lysis stage. This adjustment effectively minimized clot formation while maximizing the yield of genomic DNA. The resulting stepwise protocol is tailored for use with preserved blood samples and produces DNA of sufficient quality and purity for downstream applications, including PCR amplification and sequencing.

Guidelines

Turtle blood, in contrast to mammalian blood, contains nucleated erythrocytes, presenting distinct challenges for DNA extraction protocols. Due to their nucleated erythrocytes, turtles can carry oxygen more efficiently, which is crucial for their survival in a variety of habitats, including aquatic habitats with low oxygen levels. Moreover, turtle blood contains more genetic material due to its nuclei, which is advantageous for studies of evolutionary biology and conservation genetics. Nonetheless, nucleated erythrocytes complicate the process because their nuclei add to the amount of cellular material to be broken down before pure DNA can be separated leading to clot formation. In this case, the extraction process becomes more time-consuming and requires additional purification steps because the DNA yield is reduced due to contamination. In response to these challenges, the QIAamp Blood Mini Kit, frequently used for DNA extraction from human blood, was modified to facilitate the extraction of high-quality genomic DNA from turtle blood by refining the dilution procedure used for sampling. Blood samples were obtained from three species: loggerhead (*Caretta caretta*), green (*Chelonia mydas*), and hawksbill turtles (*Eretmochelys imbricata*), from the National Aquarium Abu Dhabi's rescue program which is supervised by Abu Dhabi's Environmental Agency. Our initial trials using undiluted blood frequently resulted in poor yield and sample clotting during the extraction process (Figure 1). To address this, we tested various dilutions and found that a 1:9 dilution (20 μ L of blood with 180 μ L of phosphate buffered saline - PBS) consistently prevented clot formation while yielding high-quality DNA. This carefully optimized, stepwise protocol is designed for use with preserved blood samples and produces DNA of sufficient quality for downstream applications, including PCR amplification and sequencing. We believe that this protocol outlines a reliable, reproducible, and kit-based method suitable for genomic studies of turtle species and can be adapted to other non-mammalian vertebrates with similar blood composition.

Materials

1. Reagents:

- QIAamp Blood Mini Kit (Qiagen, Cat. No. 51104)
- Proteinase K (supplied in kit)
- Buffer AL, AW1, AW2, AE (supplied in kit)
- Ethanol (70%)
- Phosphate-buffered saline (PBS), sterile, 1×

2. Equipment:

- Microcentrifuge capable of $\geq 20,000 \times g$
- Heat block or water bath (set to 56°C)
- Vortex mixer
- Pipettes and sterile filter tips
- 1.5 mL and 2 mL microcentrifuge tubes

Procedure

- 1 Sample preparation:
 - 1.1 Mix 20 μ L turtle blood with 180 μ L PBS in a sterile 1.5 mL microcentrifuge tube. (Note: This dilution prevents clotting while preserving sufficient cellular material for DNA extraction.)
- 2 Lysis and binding:
 - 2.1 Add 20 μ L Proteinase K to the diluted sample.
 - 2.2 Add 200 μ L Buffer AL, vortex to mix.
 - 2.3 Incubate at 56°C for 10 minutes.
 - 2.4 Add 200 μ L ethanol (70%), vortex to mix.
- 3 Column purification:
 - 3.1 Transfer the mixture to a QIAamp spin column (placed in a 2 mL collection tube). Centrifuge at 6000 $\times$ g (8000 rpm) for 1 minute. Discard flow-through.
 - 3.2 Add 500 μ L Buffer AW1, centrifuge at 6000 $\times$ g (8000 rpm) for 1 minute. Discard flow-through.
 - 3.3 Add 500 μ L Buffer AW2, centrifuge at 16,850 $\times$ g (13,400 rpm) for 5 minutes.
 - 3.4 (Recommended) Spin again at full speed for 1 minute to remove ethanol.

- 3.5 Place the column in a clean 1.5 mL tube. Add 200 μ L Buffer AE, incubate for 1 min at room temperature, then centrifuge at 6000 $\times$ g (8000 rpm) for 1 min to elute DNA.

Timing

- 4 Blood Thawing: 20 min
- 5 Sample preparation: 5 min
- 6 Lysis & incubation: 10 min
- 7 Binding & column purification: 25 min
- 8 Purity and quality check: 10 min
- 9 Total: ~70 minutes per extraction

Troubleshooting

- 10 One common issue encountered during the DNA extraction process is blood clotting. This is often caused by the absence of a proper dilution step before processing. To resolve this, it is important to dilute the sample appropriately by mixing 20 μ L of blood with 180 μ L of PBS. This helps prevent clotting and ensures smoother downstream processing. Prior to establishing the final protocol, several modifications were tested, including increasing the lysis buffer volume to 30 μ L, extending incubation times to 1 hour and 3 hours, and experimenting with dilution ratios of 1:1, 1:19, and 1:3. Among these, the 1:9 dilution ratio consistently produced the most optimal results.
- 11 Another issue that may arise is low DNA yield. A possible cause for this is incomplete lysis of cells, which can result from insufficient mixing or inadequate incubation time. To improve yield, ensure that the sample is thoroughly mixed and incubated for the full 10 minutes as specified in the protocol. This allows complete cell lysis and efficient DNA release.

Results

- 12 With no and low dilutions, we observed low levels of DNA concentrations and poor quality in turtle samples from the three species, and clots were observed with no or low dilutions as shown in Table 1.
- 13 Table 1: Results of DNA concentrations and purity of turtle blood obtained with and without PBS dilutions.
- 14 In addition, as shown in Figure 1 (left panels), blood clots are evident when no or low dilutions are used, even with increases in incubation time, proteinase K concentration, or vortexing time.
- 15 Using the modified protocol, which incorporates a 1:9 dilution of whole blood in PBS (20 μ L blood + 180 μ L PBS) and omits the optional ethanol evaporation step, genomic DNA was successfully extracted from six individual turtle blood samples representing hawksbill, green, and loggerhead species. DNA yields ranged from 36.2 to 74.7 ng/ μ L, with an average concentration of approximately 55.8 ng/ μ L. These values fall within the expected range for DNA extractions from whole blood (20–100 ng/ μ L) and are suitable for a variety of downstream molecular biology applications (Table 2).
- 16 Purity assessments using spectrophotometry showed A260/A280 ratios ranging from 1.76 to 1.87, indicating minimal protein contamination. A260/230 ratios ranged from 1.70 to 2.39, with five of the six samples exceeding the recommended minimum of 1.8 (Table 2). Although the ethanol evaporation spin step was not performed, the absence of significant carryover contaminants suggests that the dilution strategy effectively minimized inhibitor presence. The one sample with a slightly lower A260/230 value (1.70) still produced DNA of acceptable purity for most standard applications.
- 17 Table 2: Results of DNA concentrations and purity of turtle blood obtained with blood to PBS dilution of 1:9.
- 18 The extracted DNA met or exceeded the quality and quantity thresholds required for common downstream applications. For PCR-based techniques (including conventional PCR and qPCR), all samples provided ample concentration (>30 ng/ μ L) and acceptable purity ratios. For Sanger sequencing, which typically requires 20–50 ng/ μ L of clean DNA, all six samples were suitable. Furthermore, several samples, particularly those with concentrations $\geq$ 50 ng/ μ L and high A260/230 values, would also meet input requirements for next-generation sequencing (NGS) workflows, including amplicon sequencing and targeted capture protocols.
- 19 Overall, this modified extraction protocol yields DNA of sufficient quantity and quality for a range of downstream applications, with high reproducibility across different turtle species and individual samples. Reintroducing the ethanol evaporation spin may further



enhance purity and is recommended for protocols that are particularly sensitive to residual contaminants.

Conclusion

- 20 This protocol provides an optimized method for extracting high-quality genomic DNA from turtle blood using the QIAamp Blood Mini Kit, with a modified 1:9 blood-to-PBS dilution step to minimize clotting. The method yielded consistent DNA concentrations and acceptable purity ratios across multiple turtle species, demonstrating its effectiveness and reproducibility. The resulting DNA is suitable for downstream applications such as PCR, Sanger sequencing, and potentially next-generation sequencing. This protocol may serve as a useful reference for researchers working with reptilian blood or other nucleated cell-rich samples.