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Comparison of Two Isolation Methods for Naturally Preserved DNA in Ambergris

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Abstract. DNA extraction is a fundamental initial step in numerous molecular research studies. Different extraction methods are required for different biological samples to obtain high-quality DNA. Therefore, this step is one of the limiting factors for the success of molecular analysis. There has been no research evaluating an appropriate method to extract DNA from ambergris jetsam samples. This study aims to determine an appropriate method for extracting DNA from ambergris samples. The ambergris sample was collected from the southern coast of Cilacap. DNA extraction was performed using a commercial DNA isolation kit and the Chelex® 100 method. The extracted DNA was visualized using agarose gel electrophoresis followed by quantification with a UV light trans-iluminator. The data were analyzed descriptively to determine the most effective extraction method. The success of the extraction was also assessed by measuring the DNA concentration using the Nanodrop spectrophotometer. The results showed that the commercial isolation kit failed to produce genomic DNA from whale ambergris, as indicated by the absence of stained DNA bands on the agarose gel. In contrast, the Chelex® 100 method successfully produced genomic DNA from ambergris, as evidenced by the presence of stained DNA bands on the agarose gel and a high quantity of genomic DNA after a Nanodrop measurement. It can be concluded that the Chelex® 100 method is more suitable than commercial kits for extracting DNA from ambergris samples. This finding contributes to the development of various scientific fields based on molecular data by providing evidence that each biological sample requires an appropriate method to obtain high-quality DNA.

Keywords: ambergris; Chelex100®; commercial kits; coprolite; whale

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INTRODUCTION

Field biologists find non-invasive sampling highly appealing because it is simpler and safer for both the animal and the researcher. It is a cost-effective way to increase sample sizes in studies of huge, dangerous mammals, and it has enormous potential for studying elusive species (Russello et al., 2015; Seah et al., 2020). Coprolites, or fossilized feces, offer indirect evidence of the activities of past animals in a specific region, even in the absence of their fossilized bones (Bennett et al., 2016). As valuable biological and environmental records, coprolites frequently contain preserved cellular material, including DNA fragments (Luis et al., 2022; Macleod et al., 2020). Despite the potential for DNA degradation due to age and environmental conditions, there are techniques that have enabled the extraction and analysis of DNA from coprolites in numerous instances (Jacob et al., 2024; Luis et al., 2022; Macleod et al., 2020).

High DNA quality is among the limiting factors for the success of genetic marker amplification during the polymerase chain reaction (PCR) process (Abdel-Latif & Osman, 2017; Schiebelhut et al., 2017). Furthermore, it has become the cause of the difficulty of molecular analysis of unusual samples such as ambergris samples in coprolite form. A qualified DNA from various samples of the organism remains, tissue samples or animal vomit is characterized by high molecular weight ranges from 1.8 to 2.0 in A260/280 and lack of contamination by other organic molecules (the absorbance A260/230 ranges between 1.65 and 2.00), such as RNA,

lipids, and polysaccharides (Al-Griw et al., 2017).

Ambergris is a rare substance produced in the digestive system of whales (Macleod et al., 2020). Its formation occurs in the hind stomach of whales from the remnants of bile, gastric fluids, and blood. This material is formed due to the irritation of the whale's digestive system by the remains of prey, particularly cephalopods, which are their primary food source (Brito et al., 2016). The formation process and the classification of ambergris as a coprolite make it a suitable medium for preserving DNA from the large intestine (Yang et al., 2022; Rowland et al., 2019; Rowland et al., 2017). This is because ambrein, a key component of ambergris, is hydrophobic and resistant to degradation in acidic environments (Rowland et al., 2019), even after prolonged exposure to unstable environmental conditions (Macleod et al., 2020).

The challenge of obtaining high-quality DNA from ambergris arises from its high lipid content, which, while making it an excellent medium for DNA preservation, also complicates the extraction process (Macleod et al., 2020). DNA extraction is a critical initial step in many molecular research studies. In this study, we compare two DNA extraction methods: the Chelex100® isolation method (Walsh et al., 1991) and commercial DNA isolation kits. While commercial DNA isolation kits offer standardized and efficient processes (Wangiyana et al., 2024; Ringbauer et al., 2024), they often come with higher costs (Pereira et al., 2016; Al-Griw et al., 2017). Conversely, the Chelex100® method, though simpler and more cost-effective, may yield suboptimal extraction results (Walsh et al., 1991).

Several techniques, including the Chelex isolation method and commercial DNA isolation kits, have been employed to extract DNA from coprolite or animal fecal materials (Abdykerymov et al., 2024; Catalano et al., 2024). While commercial kit techniques offer standardized and efficient processes, they often come at a higher cost. Conversely, the Chelex approach, though more straightforward and cost-effective, may yield less optimal extraction results. Previous studies have shown that the Chelex100® method is cheaper, faster, and more effective than the commercial DNA isolation kit method (Costa et al., 2016; Al-Griw et al., 2017). Given that our ambergris samples have high-fat content and have likely been in the water for an extended period, it is crucial to determine the most effective method for DNA isolation. This comparison is significant as it can provide insights into the best practices for DNA isolation from challenging samples like ambergris, thereby contributing to marine molecular biology and paving the way for future research in this field.

This study aims to determine an appropriate method for extracting DNA from ambergris samples. This finding provides valuable insights for the development of various scientific fields based on molecular data by demonstrating that each biological sample requires an appropriate method to obtain high-quality DNA.

METHODS

Research location, sampling technique, and time

This research was conducted in separate and dedicated rooms to prevent contamination from present DNA as suggested by Fulton & Saphiro (2019), at the Animal Taxonomy Laboratory and the Genetics and Molecular Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, as well as the Universitas Jenderal Soedirman Research Laboratory, over a period of one month from July to August 2024. Ambergris samples were collected from the coastal waters around Cilacap Regency, Central Java, Indonesia (Figure 1), through incidental sampling by local fishermen. After collection, ambergris samples were stored at -80°C to prevent further DNA degradation.

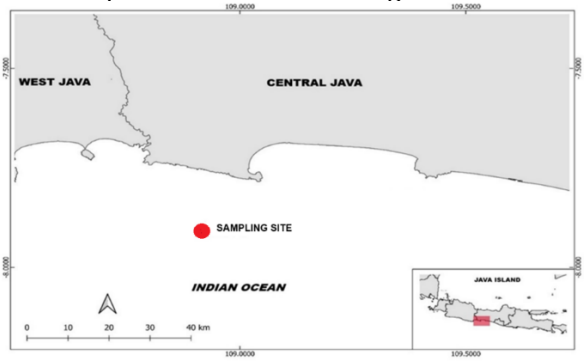

Figure 1. A map of Central Java showing sampling site (red dot) in the south-coast Cilacap (7° 54' 33.12" S; 108° 54' 53.6184" E)

DNA isolation

This study used two methods for genomic DNA isolation, i.e., Chelex100® and commercial Ecotech EcoPURE DNA purification kit. The modified Chelex100® procedure was as follows: The procedure begins by weighing 80 mg of ambergris sample (Figure 2), which has been grinded into powder by mortar and pestle and placed into a 1.5 mL sterile microcentrifuge tube. Next, 500 µL of Chelex 5% solution, 0.1 M DTT,

and Proteinase K (20 mg/mL) are added. The mixture is then incubated overnight in a thermomixer with agitation at 1000 rpm. Following incubation, the mixture is heated at 95°C for 10 minutes to allow for protein denaturation and inactivation of Proteinase K. After heating, the mixture is briefly vortexed and centrifuged at 12,000 rpm for 5 minutes to separate the supernatant from the Chelex beads and debris. The supernatant containing DNA is carefully transferred to a new sterilized microcentrifuge tube, ensuring no Chelex or debris is carried over, and the DNA is stored at 20°C.

Figure 2. The ambergris used in this study was found on the coastal waters of Cilacap, Central Java.

DNA isolation procedures using the Ecotech EcoPURE DNA Purification Kit method are as follows. 120 mg ambergris sample is subsampled and macerated using a mortar and pestle to facilitate lysis. The sample is then transferred to a 1.5 mL microcentrifuge tube, followed by the addition of 200 µL EcoPURE Resuspension Buffer, 200 µL EcoPURE Tissue Lysis Buffer, and 20 µL EcoPURE Proteinase K, and incubated at 56°C overnight. Afterward, 40 µL EcoPURE RNase A is added and incubated at room temperature for 3 minutes. Next, 800 µL EcoPURE Binding Buffer and 400 µL absolute ethanol are thoroughly mixed. The mixture is transferred to an EcoPURE Column and centrifuged. The flow-through is discarded, and the column is washed twice, first with 400 µL EcoPURE Wash Buffer 1 and then with 500 µL EcoPURE Wash Buffer 2, followed by an additional wash with 200 µL EcoPURE Wash Buffer 2. The column is transferred to a sterile microcentrifuge tube, and 30-50 µL EcoPURE Elution Buffer is added to the column membrane and incubated for 5 minutes. The purified DNA is eluted by centrifugation at 13.000 rpm for 2 minutes and stored at -20°C.

DNA quality measurements

The DNA quality was measured using two techniques, i.e., agarose gel electrophoresis UV ligth visualization and nano drops spectrophotometer. A 1% agarose gel electrophoresis was performed in a TAE IX buffer to visualize the DNA fragments obtained from both isolation methods. The gel was stained with FluoroVue™ Nucleic Acid Gel Stain for enhanced visualization under UV light. Electrophoresis was carried out at a constant voltage of 100 V and a maximum current of 400 mA for 25 minutes.

A nano drop spectrophotometer was initially powered on and allowed to stabilize for a period of 10-15 minutes. Subsequently, a blank sample consisting of 1-2 µL of Dnase-free was pipetted onto the measurement pedestal, and the “Blank” function was activated to establish a baseline. Thereafter, 1-2 µL of the extracted DNA sample was applied to the measurement pedestal, and the “Measure” function was initiated to quantify the DNA concentration. Once the spectrophotometer has stabilized, the blank and the sample absorbance readings should be noted.

Data analysis

This study obtained qualitative data through gel visualization pictures showing the stained DNA. The data was analyzed descriptively to determine the best extraction method. The extraction success was also determined by measuring DNA concentration in a nanodrop spectrophotometer.

RESULTS AND DISCUSSION

Previous research by Macleod et al. (2020) has shown that total genome isolation from ambergris material that is already in the form of coprolite is very difficult to do. This condition is because ambergris contains more lipids and proteins, and the amount of DNA contained is very small and unevenly distributed. Two DNA extraction methods have been carried out: the isolation method using a commercial DNA isolation kit and the Chelex100® method. The results of DNA visualization using 1% gel electrophoresis for a commercial DNA isolation kit method are presented in Figure 3.

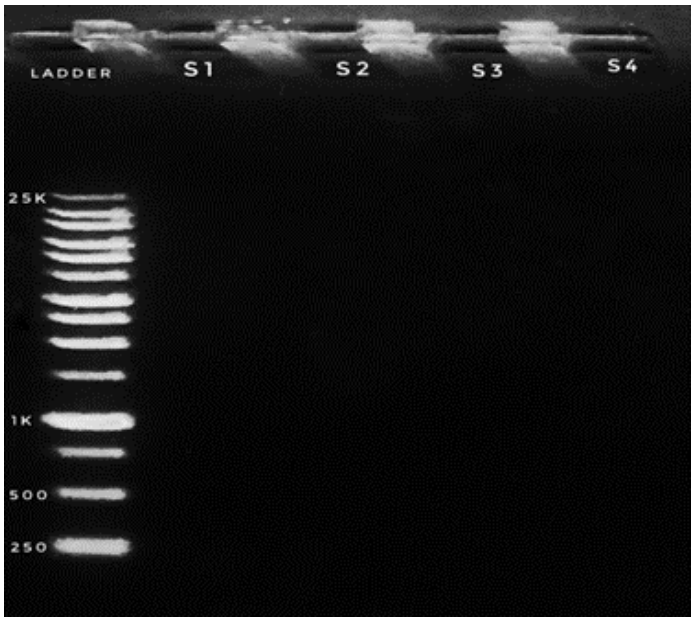

Figure 3. Gel electrophoresis visualization of DNA extracted using the Ecotech Ecopure Genomic DNA Kit. Note: S1, S2, S3, and S4 represent the first, second, third, and fourth replicates.

DNA extraction using the commercial Ecotech Ecopure Genomic DNA Kit on ambergris samples yielded no visible DNA bands in all four replicates. This result is strongly suggested to be due to significant DNA degradation caused by environmental exposure, as well as the presence of organic and inorganic compounds that can inhibit the performance of commercial extraction kits (Barsch et al., 2023; Yang et al., 2024; Shillito et al., 2020). Coprolites such as ambergris, as ancient biological materials, often contain fragmented DNA due to natural degradation processes (Macleod et al., 2020). Additionally, the constituents of ambergris, such as triterpenoid alcohols, ketones, fatty acids, and steroids like epi-coprosterol, can interact with proteins and other contaminants, hindering the DNA purification process (He et al., 2024; Rowland & Sutton, 2017).

The results of DNA visualization using 1% gel electrophoresis for Chelex100® methods are presented in Figure 4.

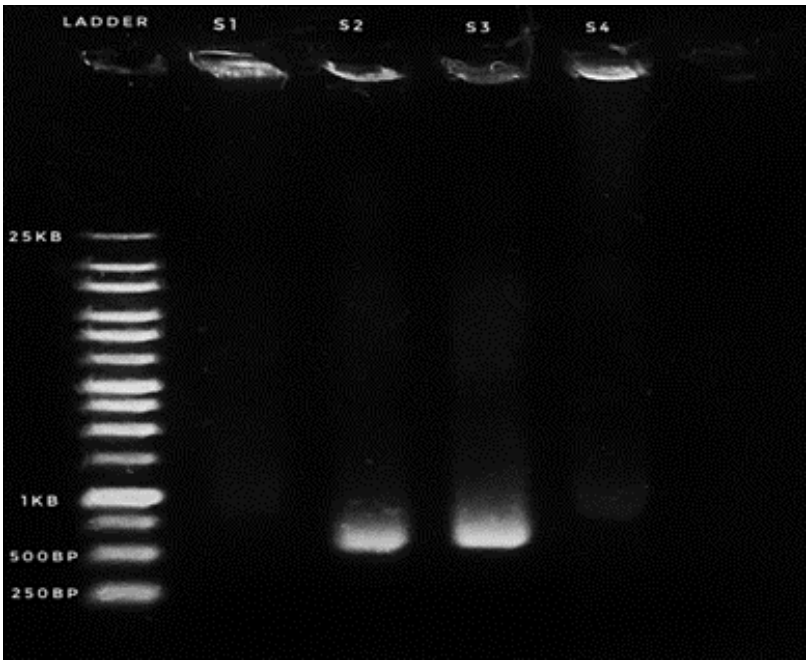

Figure 4. Gel electrophoresis visualization of DNA was extracted using the modified Chelex 5% method. Note: S1, S2, S3, and S4 represent the first, second, third, and fourth replicates.

Table 2. Results of DNA quantification obtained from samples extracted using the 5% Chelex method, as determined by UV-Vis Nanodrop spectrophotometry.

Sample	A230	A260	A280	A320	A260/A280	A260/A230	DNA Concentration (ng/ µL)
S1	1.195	0.562	0.570	0.201	0.978	0.363	181
S2	1.473	0.916	0.837	0.343	1.160	0.507	286
S3	1.505	0.819	0.747	0.305	1.163	0.428	257
S4	1.290	0.537	0.585	0.187	0.879	0.317	175

The modified 5% Chelex method yielded superior DNA extraction results to conventional DNA kit isolation methods, with replicates S2 and S3 exhibiting thick and distinct bands within the 500-750 bp range. Smearing was observed in replicates S1 and S4, and a smear was also present in replicates S2 and S3, indicating fragmented DNA. The differential visualization between S2 and S3 with clear bands and S1 and S4 without clear DNA bands demonstrates the uneven distribution of DNA within coprolite samples such as ambergris, unlike tissue samples, as Luis et al. (2022) suggested. The success of the modified 5% Chelex method in isolating DNA from ambergris can be attributed to the addition of dithiothreitol (DTT). The DTT functions as a reducing agent, breaking disulfide bonds in proteins. By doing so, DTT helps maintain protein structure and prevents DNA degradation by nucleases (Sáenz & Seifert, 2024). The chelating resin Chelex also exhibits a strong affinity for polyvalent metal ions. It comprises iminodiacetic acid ions that serve as chelating groups within a styrene-divinylbenzene copolymer (Kampmann et al., 2025; Yang et al., 2024). Furthermore, chelating metal ions during boiling inhibits DNA degradation (Gimmoneau et al., 2024). The Chelex-based approach has proven to be a rapid, simple, cost-effective, and efficient method for DNA extraction, making it particularly suitable for isolating DNA from coprolites like ambergris.

DNA quantification using a Nanodrop spectrophotometer revealed several significant findings regarding the quality and purity of DNA extracted from samples S1 to S4. The A260/A280 ratio, used to assess DNA purity—particularly with respect to protein contamination—showed that all samples had values below the ideal range of 1.8–2.0. Samples S1 (0.978), S2 (1.160), S3 (1.163), and S4 (0.879) indicated the presence of protein contamination, which can inhibit subsequent PCR applications and hinder gDNA banking, as suggested by Al-Griw et al. (2017), with replicate S4 exhibiting the lowest value and thus the highest level of contamination. Furthermore, the A260/A230 ratio, used to detect contamination from salts, carbohydrates, or other organic compounds, also showed low values for all samples, with ideal values typically above 2.0. Samples S1 (0.363), S2 (0.507), S3 (0.428), and S4 (0.317) indicated substantial contamination, likely originating from inhibitory substances such as salts co-extracted during the Chelex100® extraction process. DNA isolation from marine-derived materials such as ambergris often encounters challenges related to salt contamination, as salts naturally present in the tissues of marine organisms can be carried over during the DNA extraction process (Lear et al., 2018). To prevent this in future research related, we recommend washing the samples with buffer solutions (such as TE buffer or PBS) prior to extraction to help reduce salt contamination, which is a common issue with marine samples due to their high salt content as suggested by Boessenkool et al. (2017).

The extracted DNA exhibited relatively high concentrations, ranging from 175 ng/µL (S4) to 286 ng/µL (S2). While these concentrations are adequate for downstream applications such as PCR, the detected contamination indicated by the A260/A280 and A260/A230 ratios warrants attention as it may compromise the efficiency of subsequent processes. For instance, although sample S2 displayed the highest DNA concentration, its purity ratios still indicated significant contamination. The elevated A230 absorbance values in several samples, particularly S2 (1.473) and S3 (1.505), further corroborate the presence of organic compounds. Concurrently, the relatively high A280 absorbance values in samples S2 (0.837) and S3 (0.747) suggest a greater presence of protein as stated by Armbrecht & Eppendorf (2013). While the obtained DNA concentrations were relatively high, DNA purity remains a significant concern.

Although this study did not assess the performance of DNA extracts from both methods for PCR applications to determine whether a small amount of genomic DNA (gDNA) could be used

for PCR profiling, Walsh et al. (1991) proposed that native high molecular weight DNA is not essential for amplifying target sequences. Instead, only intact target sequences are required, making partially degraded or denatured DNA suitable for successful PCR applications (Al-Griw et al., 2017; Walsh et al., 1991).

CONCLUSION

This study proved that the Chelex® 100 method is more appropriate than the Ecotech EcoPURE commercial kits for extracting DNA from ambergris samples. Since this study used only one commercial kit. Further study is still necessary to evaluate the most appropriate method for DNA extraction from ambergris samples by applying more commercial kits.

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