Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Short communication Open access

Rapid and Simple DNA Extraction Method for Polymerase Chain Reaction Using Contact Lens Solutions

Ryosuke Ishii, Shugo Takaoka

Graduate School of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kagawa 761-0795, Japan

DOI: 10.31383/ga.vol8iss2ga04

***Correspondence**

E-mail: ryoten141@gmail.com

Received

November, 2024

Accepted

December, 2024

Published

December, 2024

Abstract

DNA extraction is an important step for DNA amplification by polymerase chain reaction (PCR), but existing methods often require complex procedures, expensive commercial kits, or harmful organic solvents. This study addresses current issues related to safety, cost, and processing time in DNA extraction by presenting a rapid and cost-effective DNA extraction method that utilizes commercially available contact lens solutions (CLS). A simple protocol involving tissue crushing, heat treatment and centrifugation was developed to extract DNA from blood-sucking mosquito *Aedes albopictus* Skuse, 1894. This process is completed within 30 minutes and requires no specialized equipment, making it highly accessible. The quality of the extracted DNA was sufficient for the amplification of 700 bp of mitochondrial cytochrome c oxidase subunit I (COI) gene segment. The proposed method showed superior efficiency compared to the extraction control in which insect samples were treated with sterile water, and clear PCR amplification products were obtained in all DNA samples extracted by CLS. These results suggest that protein-degrading agents or surfactants in CLS may function in DNA isolation. Moreover, since CLS are designed for direct ocular contact, the safety of this method is an advantage over common extraction techniques that use organic solvents. Although the present study focused on insect specimens, the method could potentially be widely applied to various types of biological specimens. By providing a low-cost, rapid, and user-friendly DNA extraction technique, this method can substantially reduce barriers in DNA extraction for PCR. This method may be a preferable option for school educators seeking safe and time-efficient experimental approaches.

Keywords

PCR, nucleic acids extraction, cost-effective, non-toxic, Aedes albopictus

Introduction

Various methods have been developed for preparing template DNA for polymerase chain reaction (PCR). The most conventional method involves treating tissues with a lysis solution containing SDS and salts, followed by phenol-chloroform extraction and cold ethanol precipitation. However, this approach presents challenges regarding human toxicity and handling complexity due to organic solvent use. To address these issues, alternative methods avoiding organic solvents have been developed. Commercial DNA extraction kits represent a notable alternative approach. While these kits efficiently isolate high-purity, high-molecular-weight DNA, they cost several dollars per sample, making them less suitable for applications involving numerous samples. Other simplified methods have been proposed, including the HotSHOT method (tissue lysis with sodium hydroxide solution followed by neutralization with 1 M Tris-HCl) and modified SDS method (cell lysis with SDS followed by protein removal with 10 M ammonium acetate and ethanol precipitation) (Truett et al., 2000; Ikeda, 2019). While these methods have made DNA extraction less expensive, there remains room for improvement in avoiding dangerous substances and multi-step processes. These challenges are particularly problematic in educational settings, such as school experimental classes, where cost and time constraints are significant, and ensuring a high level of safety is paramount.

This study proposes a simple DNA extraction method using readily available and inexpensive commercial contact lens solutions (CLS). Conventional DNA extraction methods, such as those described above, commonly employ reagents like NaOH or high concentrations of sodium dodecyl sulfate, which can pose risks of skin irritation or other dermatological issues upon exposure. In contrast, CLS, being a product specifically designed for direct contact with the eye, is considered a potentially safer alternative to traditional methods that rely on these reagents and organic solvents. The effectiveness of CLS method was evaluated by DNA extraction and PCR using insect samples.

This method was designed based on experimental observations suggesting that protein-degrading agents in CLS contribute to DNA isolation (Nakayama & Maekawa, 1998; Oi et al., 2019). For example, Oi et al. (2019) utilized CLS in combination with dishwashing detergent and salt as a protein-degrading agent for DNA extraction from tissue. Their study focused on relatively large tissue samples, rather than the small-scale samples, such as a few milligrams, typically used in DNA extraction. Moreover, their method involved multiple steps to combine CLS with other reagents and carry out the extraction. In this study, we investigated a DNA extraction method for PCR that significantly reduces cost and time by utilizing a single material and optimizing the procedure.

Material and methods

Sample Collection and Storage

The blood-sucking mosquito *A. albopictus* was used for DNA extraction. Specimens were collected in Miki-cho, Kagawa Prefecture in August 2024 and stored at -20℃ in sealed test tubes for 3 months before the experiment.

DNA Extraction

DNA was extracted by immersing the insect sample in 200 µL of CLS placed in a 1.5 mL tube. The tissue was disrupted using a pipette tip, followed by incubation at 80°C for 10 minutes.

After incubation, the sample was centrifuged at 12,000 rpm for 5 minutes, and the resulting supernatant was used as the template for PCR.

"Soft lens care N-1 Next Fresh" (SEED, Japan) was used as the CLS. The composition of the CLS used is not disclosed in detail but includes the following: Polyhexanide hydrochloride (1.0 ppm), buffers, metal sequestering agents (EDTA; ethylenediaminetetraacetic acid), isotonicity agents, wetting agents, and surfactants. To evaluate whether the observed results were due to the composition of CLS rather than solely the effect of heat treatment, a control experiment was performed using sterile water under identical conditions. Three of the five specimens were used for CLS experiments, and two for control experiments. DNA extracted from *A. albopictus* using the Wizard® Genomic DNA Purification Kit (A1120, Promega, USA) served as a positive control for PCR.

PCR Amplification

The quality of DNA extract for downstream applications was verified by amplification of mtDNA cytochrome c oxidase subunit I (COI) gene. Universal invertebrate primers L1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and H2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) were used (Folmer et al., 1994). This primer set amplifies a 710 bp amplicon.

PCR was performed in a total volume of 5.0 μL with the following composition: 0.25 U of Taq DNA Polymerase (02-002, BioAcademia, Japan), 0.5 μL of 10×Robust buffer, 200 μM of each dNTP, 200 nM of each primer, 1.0 μL of template DNA, and sterile water to adjust the total volume to 5.0 μL. Positive and negative controls for the PCR consisted of DNA extracted from *A. albopictus* using the Wizard® Genomic DNA Purification Kit and sterile water, respectively.

PCR conditions were as follows: 30 cycles of denaturation (95℃, 5 sec), annealing (53℃, 5 sec), and extension (72℃, 10 sec); and final extension (72℃, 5 min). All PCR reactions were performed twice per sample using the thermal cycler (TP240, Takara, Japan).

PCR amplicons were stained with 6×GR Green Loading Buffer (GRG-1000, BIO CRAFT, Japan) and subjected to 2% agarose gel electrophoresis using SB buffer (Brody & Kern, 2004). A 100 bp DNA Ladder (316-06951, NipponGene, Japan) was used as a size marker, and amplicons were visualized using a blue LED transilluminator (Ishii, 2023). PCR amplicons were excised from the gel and sequenced from the L1490 primer side. Sequencing was conducted by Eurofins Genomics (Japan), and the resulting sequences were analyzed using nucleotide BLAST. (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Results and Discussion

DNA extraction using CLS was completed in a single test tube through simple steps including tissue disruption, heat treatment, and supernatant collection after centrifugation. The entire procedure was completed within 30 minutes. After incubation at 80℃, the DNA extraction solution became turbid, but the turbidity decreased after centrifugation. The precipitate after centrifugation contained leg and exoskeleton debris and white particulate matter.

Electrophoretic analysis of 15 μL extracted DNA samples revealed fragmented nucleic acids in both CLS and sterile water treatments (Figure 1). Since the 6×GR Green Loading Buffer stains both DNA and RNA, precise identification of the detected nucleic acids was difficult. Stronger fluorescence was observed in samples using CLS. This is likely due to c or surfactants in the solution promoting protein degradation and improving nucleic acids isolation efficiency. Additionally, CLS components and EDTA may have functioned similarly to TE buffer, potentially suppressing DNA fragmentation.

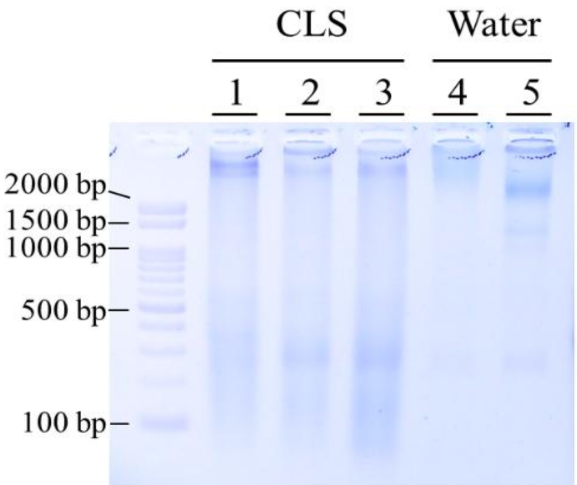

Figure 1. DNA yield using contact lens solution and amplification of COI. A: Agarose gel electrophoresis of DNA extracted using contact lens solutions (CLS) or water as a control. Lanes 1-5 represent individual mosquito samples.

DNA quantification by spectrophotometry was not conducted in this study, as the primary objective was to develop and evaluate a simplified DNA extraction method. Quantification would likely be inaccurate due to impurities like proteins and chitin.

PCR using CLS-extracted DNA as template produced amplicons in all samples. Comparison with size markers showed that the amplicons were slightly larger than 700 bp but smaller than 800 bp, closely matching the expected size (Figure 2).

PCR amplicons obtained through direct sequencing exhibited high homology (>96%) with registered *A. albopictus* sequences based on nucleotide BLAST analysis (e.g., QR391812, QR391813). In contrast, no DNA amplification was observed in the sterile water treatment (Figure 2). These results suggest that CLS mediated extraction method yields DNA of sufficient quality for PCR for medium size amplicons.

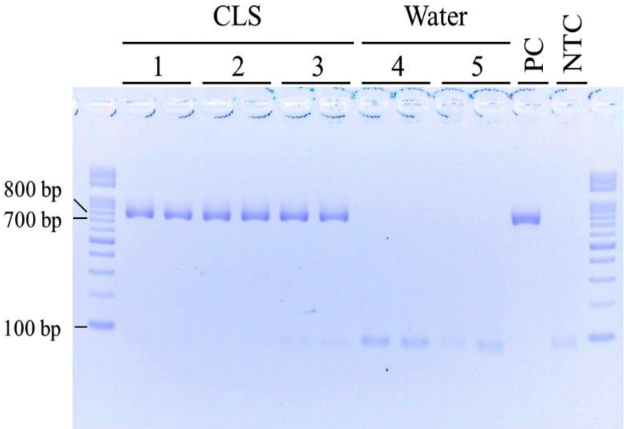

Figure 2. Agarose gel electrophoresis of PCR amplicons using the extracted DNA as templates. Lanes 1-5 correspond to the individual samples in panel A. The "PC" and "NTC" lanes indicate positive (Wizard® Genomic DNA Purification Kit) and non-template control, respectively.

This method is particularly useful in educational research settings handling numerous samples, as it requires no special reagents or expensive equipment and can be completed in a short time. Typically, an experimental class lasts approximately one hour. The DNA extraction method using CLS described here can be completed within 30 minutes, allowing sufficient time for PCR setup during the same class period. The cost per sample for the HotSHOT and modified SDS methods mentioned above is likely comparable to that of CLS. However, these methods require ordering individual reagents for preparation, which would cost at least $200. In contrast, CLS is readily available at approximately $7 for 500 mL and is easier to store, as it does not contain hazardous substances such as NaOH. Furthermore, since CLS is designed for direct ocular contact, it offers extremely high safety. Compared to conventional methods, it reduces the risk of adverse effects on human health. *A. albopictus*, the insect used in this experiment, is a small organism weighing approximately 2 mg. The successful PCR amplification from such a minimal sample size suggests the potential applicability of this method to other types of biological samples for DNA preparation. In fact, we successfully demonstrated effective DNA amplification by PCR in *Neocaridina* sp., a representative freshwater shrimp species native to Japan (data not shown).

However, this study has not completely eliminated the influence of proteins and other impurities, and further applications may be expected through quantitative DNA evaluation and the introduction of additional purification steps. Additionally, comprehensive evaluation of this method's versatility requires verification of its applicability to diverse species and samples, including plant tissues, other animal samples, and environmental samples.

Conclusion

A simple and efficient DNA extraction method using contact lens solutions (CLS) is described for PCR amplification. The method offers several notable advantages: completion within 30 minutes, no requirement for specialized equipment or harmful reagents, and significantly lower cost compared to commercial extraction kits. These features render the technique particularly suitable for implementation in school laboratories and educational research settings.

Authors' contributions

R. Ishii: Conceptualization; Investigation; Methodology; Resources; Writing – original draft; and Writing**.**

S. Takaoka: Conceptualization; Resources; Writing-review & editing.

Conflict of interest

Authors declare no conflict of interest.

References

Brody JR, Kern SE (2004) Sodium boric acid: a Tris –free, cooler conductive medium for DNA electrophoresis. *BioTechniques* 36: 214-216.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*. 3: 294-299.

Ikeda S (2019) Detection of the presence of Japanese rosy bitterling using environmental DNA. *Tech. Bull. Fac. Agr. Kagawa Univ*. 71: 27-32.

Nakayama I, Maekawa H (1998) Process of DNA Extraction by Means of a Cleansing and Preservative Solution for Hard Contact Lenses. *Jpn. J. Biol. Educ*. 38: 58-62. (in Japanese with English abstract).

Oi M, Mizuguchi T, Natsumaya Y, Ogawa Y, Miyake T (2019) Development of inexpensive and simple PCR experiment method for high school biology. *Jpn. J. Biol. Educ*. 61: 23-30. (in Japanese with English abstract).

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29: 52-54.