Polo del Conocimiento

Pol. Con. (Edición núm. 99) Vol. 9, No 10
Octubre 2024, pp. 900-918
ISSN: 2550 - 682X
DOI: https://doi.org/10.23857/pc.v9i10.8152

DNARS: Un método de extracción de ADN seguro, ecológico y de alta calidad adecuado para diversas muestras biológicas

DNARS: A safe, environmentally friendly and high-quality DNA extraction method suitable for various biological samples

DNARS: Um método de extração de ADN seguro, amigo do ambiente e de alta qualidade, adequado para diversas amostras biológicas

Cesar David Guerra-Naranjo [I]
dnaranjo@clonallyxcorporation.org
https://orcid.org/0000-0001-6651-9591

Tanira Alessandra Silveira-Aguirre [II]
tanira@ufcspa.edu.br
https://orcid.org/0000-0001-7178-6686

Ana Paula Guedes Frazzon [III]
ana.frazzon@ufrgs.br
https://orcid.org/0000-0002-1029-5776

Correspondencia: dnaranjo@clonallyxcorporation.org

Ciencias Técnicas y Aplicadas
Artículo de Investigación

*** Recibido:** 12 de agosto de 2024 ***Aceptado:** 24 de septiembre de 2024 *** Publicado:** 10 de octubre de 2024

I. Postgraduate Program in Biosciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre. Brazil; Department of Microbiology, Immunology and Parasitology, Porto Alegre, RS, Brazil, Department of Molecular Biology, Center for Biosciences, Clonallyx Corporation, Quito, Ecuador.
II. Postgraduate Program in Biosciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil.
III. Postgraduate Program in Biosciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre. Brazil; Department of Microbiology, Immunology and Parasitology, Porto Alegre, RS, Brazil.

Resumen

Las relaciones funcionales e interaccionales entre los ácidos nucleicos y las proteínas forman el marco fundamental para los estudios de biología molecular. En este contexto, la obtención de ácidos nucleicos de alta calidad es un paso crucial para el éxito de las aplicaciones posteriores. Este estudio tuvo como objetivo desarrollar un método de extracción de ADN rentable, ecológico y versátil que produzca ADN de alta calidad a partir de diversas muestras biológicas. Utilizamos tres tipos de vidrio de laboratorio reciclado a base de borosilicato como fuente de sílice, combinado con yoduro de sodio como agente caotrópico, creando un sistema eficiente para la lisis celular y la extracción de ADN.

El control de calidad se realizó evaluando la concentración y la pureza del ADN extraído mediante espectrofotometría, y los resultados se compararon con los obtenidos con un kit comercial. La integridad del ADN se evaluó mediante electroforesis en gel de agarosa. Para verificar la idoneidad del ADN extraído para aplicaciones posteriores, realizamos amplificaciones por PCR de ARNr 16S e ITS.

Nuestros hallazgos demostraron que nuestro método de extracción de ADN produjo rendimientos significativamente mayores, mejor pureza y mayor integridad en comparación con el kit comercial. Además, el ADN extraído fue fácilmente aplicable en procedimientos basados en PCR, lo que confirma la eficacia del método para aplicaciones de biología molecular.

Palabras clave: Extracción de ADN; Sal caotrópica; Sílice; PCR.

Abstract

The functional and interactional relationships between nucleic acids and proteins form the foundational framework for molecular biology studies. In this context, obtaining high-quality nucleic acids is a crucial step for successful downstream applications. This study aimed to develop a cost-effective, eco-friendly, and versatile DNA extraction method that yields high-quality DNA from diverse biological samples. We utilized three types of borosilicate-based recycled laboratory glass as a silica source, combined with sodium iodide as a chaotropic agent, creating an efficient system for cell lysis and DNA extraction.

Quality control was performed by assessing the concentration and purity of the extracted DNA using spectrophotometry, and the results were compared to those obtained with a commercial kit. DNA integrity was evaluated via agarose gel electrophoresis. To verify the suitability of the

extracted DNA for downstream applications, we conducted 16S rRNA and ITS PCR amplifications.

Our findings demonstrated that our DNA extraction method produced significantly higher yields, better purity, and greater integrity compared to the commercial kit. Moreover, the extracted DNA was readily applicable in PCR-based procedures, confirming the method's effectiveness for molecular biology applications.

Keywords: DNA extraction; Chaotropic salt; Silica; PCR.

Resumo

As relações funcionais e interacionais entre os ácidos nucleicos e as proteínas formam a estrutura fundamental para os estudos de biologia molecular. Neste contexto, a obtenção de ácidos nucleicos de elevada qualidade é um passo crucial para aplicações a jusante bem-sucedidas. Este estudo teve como objetivo desenvolver um método de extração de ADN económico, ecológico e versátil que produza ADN de alta qualidade a partir de diversas amostras biológicas. Utilizámos três tipos de vidro de laboratório reciclado à base de borossilicato como fonte de sílica, combinado com iodeto de sódio como agente caotrópico, criando um sistema eficiente para a lise celular e extração de ADN.

O controlo de qualidade foi realizado através da avaliação da concentração e pureza do ADN extraído por espectrofotometria, e os resultados foram comparados com os obtidos com kit comercial. A integridade do ADN foi avaliada por eletroforese em gel de agarose. Para verificar a adequação do ADN extraído para aplicações a jusante, realizámos amplificações de 16S rRNA e ITS PCR.

As nossas descobertas demonstraram que o nosso método de extração de ADN produziu rendimentos significativamente mais elevados, melhor pureza e maior integridade em comparação com o kit comercial. Além disso, o ADN extraído foi prontamente aplicável em procedimentos baseados em PCR, confirmando a eficácia do método para aplicações em biologia molecular.

Palavras-chave: Extração de ADN; Sal caotrópico; Sílica; PCR.

Introduction

Several methods for DNA extraction have already been reported (Breeding et al., 2004; Mäki et al., 2017), but each has limitations that must be considered before selecting a DNA extraction kit, such as cost, time, processing infrastructure, and the need for additional reagents (Chapela et al., 2007; Salimans & Sol, 1990). These factors highlight the importance of developing new, efficient alternatives. An effective DNA extraction method should ensure robust cell disruption, nuclease inactivation, removal of inhibitors, and high DNA yield (Jacobsen et al., 2009; Sajali et al., 2018; S. K. Verma et al., 2017).

Current DNA extraction techniques can be classified into mechanical and non-mechanical methods, either used separately or in combination (Jahanshahi & Najafpour, 2007; J. Kumar et al., 2016). Mechanical methods involve contact-based processes using shear forces, such as bead beating, high-pressure homogenization, or microfluidization (Harrison, 2011; Islam et al., 2017). Non-mechanical methods rely on physicochemical, chemical, or biological agents that disrupt the integrity of the cell wall and membrane without the use of shear force (Dowd & Kelley, 2011). Chemical agents, including organic solvents, surfactants, chelating agents, and chaotropic salts, are commonly used. Physical agents like heat or osmotic shock, and biological agents such as enzymes, are also employed (Günerken et al., 2015; Gupta, 2019).

It is important to note that some common DNA extraction methods use hazardous reagents, such as phenol-chloroform (PCL) and cetyltrimethylammonium bromide (CTAB). The European Chemicals Agency (ECHA) classifies these reagents as toxic, corrosive, and harmful to both human health and the environment (Breeding et al., 2018; Goud et al., 2018; J Shetty, 2020). To mitigate these risks, alternative approaches using chaotropic agents have been proposed (Hosomi et al., 2017; Zainabadi et al., 2019).

Chaotropic salts destabilize non-covalent interactions—such as hydrogen bonds and Van der Waals forces—in aqueous solutions, disrupting biological assemblies like cellular membranes. This leads to protein denaturation by destabilizing their native state and enhancing hydrophobic interactions (Sawyer & Puckridge, 1973). Protein inactivation in this step is crucial, as it allows for protein solubilization with surfactants (Jariwalla et al., 1977; Salvi et al., 2005).

After cell lysis, extracting and purifying the genetic material is essential. Common extraction methods include density gradient centrifugation, ion-exchange chromatography, reverse-phase chromatography, salting out, adsorption, and precipitation using membranes (Pouseele & Supply,

2015; Wohlgemuth, 2019). Among these, silica-based columns are an effective, fast, and safe method for DNA extraction. Unlike PCL and CTAB, silica-based methods pose no health risks [26–28]. Silica adsorption can be enhanced by chaotropic salts under specific pH and ionic conditions, which disrupt nucleic acid-water interactions and promote DNA binding to silica (Alonso, 2013). Studies have also demonstrated that glass matrices can serve as a silica source, yielding successful DNA extractions (Hoyos et al., 2017; Katevatis et al., 2017; P. V. Verma et al., 2018).

Building on these findings, our research aimed to develop a DNA extraction method using recycled laboratory glass as a silica source, combined with sodium iodide (NaI) as a chaotropic salt. This approach offers a fast, simple, environmentally friendly, and safe alternative for obtaining high-quality DNA, with minimal risk of operator exposure

Materials and Methods

Samples

A total of 14 samples were tested, including bacterial and fungal strains, as well as fecal samples. Four Gram-positive bacterial strains (Bacillus cereus, Enterococcus casseliflavus, Enterococcus faecalis, and Staphylococcus epidermidis) and four Gram-negative strains (Escherichia coli, Klebsiella oxytoca, Citrobacter freundii, and Serratia marcescens) were cultured in Tryptic Soy Broth (TSB) (Liofilchem®, Italy) for 24 hours at 37ºC. Additionally, four fungal strains (Rhodotorula mucilaginosa, Penicillium citrinum, Exophiala phaeomuriformis, and Saccharomyces cerevisiae) were cultured in Sabouraud Dextrose Broth (SDB) for 48 hours at 30ºC. Two fecal samples from canines and felines were collected in 50 mL sterile conical tubes and stored at 4ºC until processing.

Preparation of the Glass powder solution

Three types of borosilicate-based recycled laboratory glass (clear, green, and amber) were collected from the Institute of Basic Health Sciences at the Federal University of Rio Grande do Sul. Each type of glass was pulverized using a mortar and pestle until a fine powder was obtained. The glass powder solution was prepared by resuspending 2 g of each glass powder in 10 mL of ultrapure water, followed by sterilization through autoclaving.

DNA extraction method design and standardization

For our study, we designed a four-step workflow for DNA extraction, which includes cell lysis, DNA extraction, DNA purification, and DNA elution. To standardize the process, we used an E. coli strain cultured in TSB, with clear glass powder solution serving as the sole source of silica.

Cell lysis process

Since our DNA extraction model primarily consists of a chaotropic salt and a glass powder solution, determining the ideal concentration between these two agents was essential. Cell lysis was performed using 1 mL of the StartUp Buffer (X M NaI, 20 mM EDTA, 10 mM Tris-HCl, 5% Triton X-100, pH 8.0), where X represents the five different NaI molarities tested (6 M, 7 M, 8 M, 9 M, and 10 M). The sensitized cell walls and membranes released the intracellular content, primarily due to the action of sodium iodide and Triton X-100. Subsequently, the glass powder solution was added in varying quantities (20 mg, 40 mg, 60 mg, and 80 mg) and mixed by vortexing to enhance cell disruption.

DNA Extraction

The DNA bound to the silica was collected by centrifugation at 10,000 x g for 1 minute, and the supernatant was discarded. The pellet was then resuspended in 1 mL of the Next buffer (4 M NaI, 10 mM EDTA, 30 mM Tris-HCl, 5% Tween 20, 0.5% Triton X-100; pH 8.0) and mixed by vortexing. At this stage, denatured and inactivated proteins, along with cell debris, are removed through solubilization with the surfactants. (Wang et al., 2008).

DNA Purification

The DNA purification process involved removing any residual cell debris or reagents through a double wash with the OH buffer (100 mM NaCl, 10 mM Tris-HCl, 70% ethanol; pH 8.0). The higher ethanol concentration facilitated dehydration, allowing the DNA to remain bound to the silica in a more compact, folded structure. DNA was then eluted from the silica using 1 mL of absolute ethanol (Green & Sambrook, 2017). In this fully dehydrated state, ethanol neutralizes the DNA's electrostatic charge, enabling its release from the silica. The DNA precipitates due to

structural supercoiling (Oda et al., 2016). Finally, any residual ethanol was removed by drying at 56ºC for 15 minutes.

DNA Elution

The dried silica was resuspended in 100 µL of Recovery Buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) and eluted by incubating at 56ºC for 5 minutes. After the incubation period, the glass particles were removed by centrifugation at 13,000 x g for 5 minutes. The supernatant containing the DNA was carefully collected and transferred into a new 1.5 mL sterile microtube. Finally, all extracted DNA samples were stored at -20ºC.

Optimization of working conditions

As part of the standardization process, it was crucial to evaluate whether variations in pH and temperature could optimize our working conditions. For this, the clear, green, and amber glass powder solutions were individually tested for method optimization.

Since cell lysis is a key step in releasing intracellular content, we examined whether the cell lysis and DNA extraction solutions were effective under different pH conditions of 8, 9, and 10. Previous studies have shown that DNA remains more stable in alkaline conditions (Dinis et al., 2020), which can enhance extraction and purification. Additionally, temperature plays a critical role in increasing cell membrane sensitization (Dowd & Kelley, 2011). Therefore, various temperature ranges (56°C, 61°C, 66°C, and 68°C) were also tested to determine their effect on the extraction process.

DNA extraction from diverse samples

DNA from diverse samples, including Gram-positive and Gram-negative bacteria, fungal cultures, and animal feces, was extracted following the protocol illustrated in Figure 1. All extraction procedures were performed in duplicate, utilizing each type of glass powder solution respectively. For DNA extraction from liquid bacterial and fungal cultures, 1 mL of each culture was transferred into individual 1.5 mL sterile microtubes. The cells were collected by centrifugation at 6000 x g for 5 minutes using a High-Speed Micro-Centrifuge D3024R (DLAB Scientific Inc., Riverside, CA, USA), and the supernatant was discarded. For the fecal samples, between 100 to 125 mg of

animal feces were placed into 1.5 mL sterile microtubes, resuspended, and homogenized by vortexing in 300 µL of Recovery buffer prior to processing.

In parallel, DNA was also extracted from all samples using NewGene PREP and NewGene PREAmp, commercial DNA extraction kits, following the manufacturer's guidelines to serve as controls.

Spectrophotometry assay: Purity and Yield of the extracted DNA

The yield and purity of the extracted DNA samples were determined by measuring the absorbance at A260 for yield and the A260/280 ratio for purity using a NanoDrop™ Lite Spectrophotometer (NanoDrop Technologies, Inc.).

DNA Integrity

To assess DNA integrity, 3 µL of each extracted sample was subjected to electrophoresis in a 1% (w/v) agarose gel stained with 1X Gel Red loading buffer (QuatroG, Pesquisa e Desenvolvimento Ltda.), and run in 1X TAE (Tris Acetate-EDTA). The gel was visualized using a Gel Documentation System L-PIX TOUCH (Loccus do Brasil LTDA, Santa Mônica, SP, Brazil).

Downstream process: PCR amplification

Extracted DNA from bacterial samples underwent PCR targeting a region of the 16S rRNA gene, using the primers FC27 (5´-AGAGTTTGATCCTGGCTCAG-3´) and R530 (5´-CCGCGGCTGCTGGCACGTA-3´), following the PCR conditions described by (Gontang et al., 2007).

For fungal DNA, the universal fungal barcode sequence ITS (Interspaced Transcribed Spacer) was amplified using the primers ITS1 (5´-TCCGTAGGTGAACCTGCGG-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´), under PCR conditions from (Menezes et al., 2010). Fecal samples were subjected to both 16S rRNA and ITS PCRs under the same conditions as previously described, with reactions performed on a 2720 Thermal Cycler (Applied Biosystems - Thermo Fisher Scientific, Massachusetts, USA).

Statistical analysis

Statistical analysis was performed using Two-Way ANOVA to assess the main effects and interactions of independent variables, followed by post hoc Tukey's test for multiple comparison analysis to identify significant differences. Statistical significance was considered at ($p < 0.05$). All analyses were conducted using the RStudio software.

Results

Standardization of the DNA extraction method

DNA extracted from an E. coli strain was used to standardize the novel DNA extraction method by determining the optimal conditions for NaI molarity and glass powder concentration, both essential for cell lysis and DNA extraction. The results, shown in Table 1, were analyzed using Two-Way ANOVA. The interaction between glass powder concentration and NaI molarity had a significant effect on the DNA yield ($p < 0.05$). Post hoc Tukey's test revealed that only glass powder concentrations of 20 mg and 40 mg significantly influenced DNA yield ($p < 0.05$).

Similarly, the test indicated that DNA concentration was significantly different between the NaI molar concentrations of 6, 7, 8, 9, and 10 ($p < 0.05$). However, the purity of the DNA (A260/280) was not significantly affected by either NaI molarity or glass powder concentration ($p > 0.05$), indicating that neither variable influenced the DNA purification process.

Based on these findings, we determined that 8 M NaI and 20 mg of glass powder were the optimal conditions for further refinement of the DNA extraction process.

pH and temperature influence on DNA quality

As described earlier, the extracted DNA did not meet the desired purity range, A260/280: 1.8-2.0, so it was necessary to evaluate whether factors such as pH and temperature could improve the DNA extraction process.

First, the impact of pH on cell lysis and DNA extraction was assessed. As shown in Table 2, a Two-Way ANOVA indicated a significant difference in both DNA concentration and purity based on pH ($p < 0.05$). A post hoc Tukey's test further revealed statistical differences between pH levels 8, 9, and 10, with more alkaline conditions (pH 10) yielding higher DNA purity values.

Temperature was another critical factor in standardizing the novel DNA extraction method. Table 3 shows that temperature significantly influenced both DNA concentration and purity ($p < 0.05$),

as determined by Two-Way ANOVA. Post hoc Tukey's test showed that temperatures of 56°C, 61°C, 66°C, and 68°C led to statistically significant improvements, with higher temperatures correlating with better DNA quality.

In conclusion, the optimal conditions for DNA extraction and cell lysis were achieved at pH 10 and a reaction temperature of 68°C, where the highest DNA concentration and purity were obtained.

Quality control of extracted DNAs: Concentration, purity, and integrity

All DNA extracted from the various biological samples described in the Materials and Methods section was quantified for concentration and purity, as well as assessed for integrity. The values presented in Table 4, analyzed via Two-Way ANOVA, demonstrated that the interaction between extraction methods and biological samples did not significantly affect DNA concentration ($p > 0.05$). However, post hoc Tukey's test revealed that the DNA concentration differed significantly between the various types of biological samples ($p < 0.05$) (Figure 2). This indicates that while the extraction methods (M1, M2, M3, and MC) did not have a significant influence on DNA concentration, the type of biological sample did.

In contrast, the purity of the DNA (A260/280) was significantly affected by the extraction method, as shown by Two-Way ANOVA ($p < 0.05$). Post hoc Tukey analysis indicated that methods M1, M2, and M3 were significantly different from MC ($p < 0.05$) (Figure 3), with the purity values falling within the reference range for DNA purity (1.8 - 2.0).

Regarding DNA integrity, another crucial quality parameter, agarose gel electrophoresis revealed that methods M1, M2, and M3 produced intact DNA (Figure 4). However, DNA extracted using the MC control method showed fragmented DNA, with no visible full-length DNA bands in any of the eight samples.

16S rRNA and ITS regions PCR gene amplification

As a downstream application, DNA extracted from Gram-positive and Gram-negative microorganisms was subjected to PCR to amplify the 16S rRNA gene, which is commonly used for prokaryotic taxonomy. As shown in Table 5, DNA extracted with methods M1, M2, and M3 successfully amplified the 16S rRNA gene, producing a PCR product of approximately 530 bp, as confirmed by agarose gel electrophoresis.

For fungal samples, including both filamentous fungi and yeast, the ITS region was targeted for identification via PCR. Similarly, PCR amplification using methods M1, M2, and M3 produced a product of approximately 560 bp, which was visualized on agarose gel.

Due to the microbial diversity in fecal samples, total DNA extracted from feces was subjected to 16S rRNA and ITS gene amplification. Both feline and canine stool samples showed positive amplification for these genes, demonstrating the presence of diverse prokaryotic and fungal species.

Discussions

The development of the proposed DNA extraction method involved optimizing key parameters for standardization. During this process, it was demonstrated that NaI concentration significantly influenced DNA yield but not its purity. The A260/280 absorbance ratios suggested the presence of RNA contamination in the extracted samples. This observation was confirmed through agarose gel electrophoresis, where the presence of residual RNA was visible. The gel also provided evidence of the integrity of the extracted DNA Appendix 1 (Shamsi et al., 2011). It is noteworthy that as the molarity of NaI increased the presence of RNA decreased. This effect can be attributed to the hydrolysis of RNA, which is influenced by factors such as pH, temperature, and ionic strength—conditions that directly impact RNA's molecular stability (K, 1999). This suggests that the use of NaI as a chaotropic salt in DNA extraction processes could be an optimal alternative, especially considering that conventional extraction methods often rely on highly toxic chemical agents like PCL and CTAB (Loparev et al., 1991).

As previously mentioned, the DNA purity did not reach the ideal range, A260/280: 1.8–2.0, likely due to the residual presence of RNA in the samples during quantification. This was further confirmed by agarose gel observation. Studies have reported that physicochemical factors, such as pH and temperature, can directly influence the stability and quality of DNA (Scorsato & Telles, 2011). To optimize the method, it was necessary to evaluate whether different pH and temperature conditions could improve DNA purity without the need for enzymes such as RNases, which are costly and increase operational expenses (Earl et al., 2018). The results showed that as the pH becomes more alkaline, the purity values reach their ideal range, while the presence of RNA decreases substantially (Appendix 1). This occurs because RNA exhibits low stability under alkaline conditions and in the presence of high ionic strength from NaI. These factors make RNA

highly reactive, leading to the cleavage of its phosphodiester bonds. Additionally, the hydroxyl group at the 2' position of the sugar in RNA increases its reactivity in alkaline environments. (Lemire et al., 2016). Consequently, the formation of these alkoxide ions allowed the structural destabilization of RNA by hydrolysis, thus facilitating removal by solubilization and increasing the purity of DNA (Bernhardt & Tate, 2012).

Additionally, the method was evaluated under different temperature conditions, confirming that temperature had a significant influence on both the concentration and purity of the DNA. This effect is due to the role of temperature during the cell lysis process, where increased heat, combined with chemical agents, enhances the sensitization of the cell structure and membrane. This leads to increased permeability and more efficient cellular disruption (Ren et al., 2007). By optimizing both pH and temperature together, the hydrolysis kinetics of RNA were enhanced, and the increased sensitivity and cell disruption facilitated the removal of denatured proteins and other cellular components through solubilization, resulting in high-quality DNA (F. Ma et al., 2018; J. Ma et al., 2020; Peach et al., 2015).

Finally, the DNA extracted from various biological sources showed that the yield was not statistically different between the proposed extraction method and the commercial method used. However, an internal analysis of the DNA concentration revealed a significant difference among the different sample types. Gram-negative samples yielded the highest DNA concentration values. This is likely due to the reduced peptidoglycan content in the Gram-negative cell wall, making these cells more permeable and susceptible to osmotic and mechanical stress factors such as ionic strength, pH, and temperature during the cell lysis process. (Dik et al., 2021). Additionally, the cell lysis process appears to be enhanced when using glass powder particles, likely because the irregular edges of the particles mechanically apply shear forces that sensitize the cells, facilitating the release of cellular content. On the other hand, DNA concentration from fungal samples was significantly lower compared to other samples (Figure 2). This is due to the structural composition of fungal cell walls, which consist of glycan, chitin, chitosan, and glycosylated proteins. These components contribute to the rigidity of the fungal cell wall, generally making DNA extraction more challenging. More rigorous treatments, such as the use of liquid nitrogen and grinding with a mortar and pestle, are often required to achieve sufficient cell wall disruption in fungi (Garcia-Rubio et al., 2020; M. Kumar & Mugunthan, 2018).

Additionally, DNA purity was a crucial factor in the study. When compared to the control method, the proposed extraction method consistently produced high-quality DNA, regardless of the type of glass powder used (Figure 3). It is important to note that DNA integrity is a key quality standard. Agarose gel comparisons revealed that the DNA extracted by the control method was highly fragmented (Figure 4), making it difficult to use for subsequent applications such as PCR. It is also worth mentioning that DNA quantification methods alone are not sufficient to verify the integrity of extracted DNA molecules (Sedlackova et al., 2013)

Finally, the study demonstrated the successful reuse of recycled materials, such as glass as a source of silica and sodium iodide as a chaotropic salt, enabling the development of a bioproduct with optimal quality for DNA extraction. This suggests that the method is both economical and safe. Importantly, the process did not require the use of enzymes or specialized equipment. Its performance in downstream applications, such as PCR, was particularly significant, showing excellent results and confirming its applicability for future processes, including the PCR tested in this study.

Acknowledgments

We would like to express our sincere gratitude to the Universidade Federal de Ciências da Saúde de Porto Alegre and the Universidade Federal do Rio Grande do Sul for providing laboratory facilities and resources for this research. We are also thankful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support. Special thanks to the Center for Biosciences, Clonallyx Corporation for funding this research and supporting its publication.

Conflict of Interest

The authors have no financial conflicts of interest to declare

References

1. Alonso, A. (2013). DNA Extraction and Quantification. In Encyclopedia of Forensic Sciences: Second Edition (pp. 214–218). Elsevier Inc. https://doi.org/10.1016/B978-0-12-382165-2.00039-8
2. Becker, L., Steglich, M., Fuchs, S., Werner, G., & Nübel, U. (2016). Comparison of six commercial kits to extract bacterial chromosome and plasmid DNA for MiSeq sequencing. Scientific Reports, 6(June), 10–14. https://doi.org/10.1038/srep28063
3. Bernhardt, H. S., & Tate, W. P. (2012). Primordial soup or vinaigrette: Did the RNA world evolve at acidic pH? Biology Direct, 7(1), 4. https://doi.org/10.1186/1745-6150-7-4
4. Breeding, P., Wissenschafts-verlag, B., Issn, B., Biology, P., December, R., & February, A. (2004). Short Communication A new DNA extraction method for high-throughput marker analysis in a large-genome species such as Triticum aestivum. Matrix, 356(2001), 354–356.
5. Breeding, P., Wissenschafts-verlag, B., Issn, B., Biology, P., December, R., February, A., Sajali, N., Wong, S. C., Hanapi, U. K., Abu Bakar @ Jamaluddin, S., Tasrip, N. A., Mohd Desa, M. N., Demeke, T., Jenkins, G. R., Gupta, N., Singh, U. A., Kumari, M., Iyengar, S., Vilanova, S., … Prohens, J. (2018). Method for improving the quality of genomic DNA obtained from minute quantities of tissue and blood samples using Chelex 100 resin. Biological Procedures Online, 83(1), 1–11. https://doi.org/10.1186/s12575-018-0077-6
6. Chapela, M. J., Sotelo, C. G., Pérez-Martín, R. I., Pardo, M. Á., Pérez-Villareal, B., Gilardi, P., & Riese, J. (2007). Comparison of DNA extraction methods from muscle of canned tuna for species identification. Food Control, 18(10), 1211–1215. https://doi.org/10.1016/j.foodcont.2006.07.016
7. Dik, D. A., Zhang, N., Sturgell, E. J., Sanchez, B. B., Chen, J. S., Webb, B., Vanderpool, K. G., & Schultz, P. G. (2021). A synthetic 5,3-cross-link in the cell wall of rod-shaped Gram-positive bacteria. 118, 2100137118. https://doi.org/10.1073/pnas.2100137118/-/DCSupplemental
8. Dinis, T. B. V., Sousa, F., & Freire, M. G. (2020). Insights on the DNA Stability in Aqueous Solutions of Ionic Liquids. Frontiers in Bioengineering and Biotechnology, 8(October). https://doi.org/10.3389/fbioe.2020.547857

9. Dowd, C. J., & Kelley, B. (2011). Purification Process Design and the Influence of Product and Technology Platforms. In Comprehensive Biotechnology, Second Edition (Vol. 2, pp. 799–810). Elsevier Inc. https://doi.org/10.1016/B978-0-08-088504-9.00137-9
10. Earl, C. C., Smith, M. T., Lease, R. A., & Bundy, B. C. (2018). Polyvinylsulfonic acid: A low-cost rnase inhibitor for enhanced RNA preservation and cell-free protein translation. Bioengineered, 9(1), 90–97. https://doi.org/10.1080/21655979.2017.1313648
11. Garcia-Rubio, R., de Oliveira, H. C., Rivera, J., & Trevijano-Contador, N. (2020). The Fungal Cell Wall: Candida, Cryptococcus, and Aspergillus Species. In Frontiers in Microbiology (Vol. 10). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2019.02993
12. Gontang, E. A., Fenical, W., & Jensen, P. R. (2007). Phylogenetic diversity of gram-positive bacteria cultured from marine sediments. Applied and Environmental Microbiology, 73(10), 3272–3282. https://doi.org/10.1128/AEM.02811-06
13. Goud, T. S., Upadhyay, R. C., Kumar, A., Karri, S., Choudhary, R., Ashraf, S., Singh, S. V., Kumar, O. S., & Kiranmai, C. (2018). Novel extraction of high quality genomic DNA from frozen bovine blood samples by using detergent method. Open Veterinary Journal, 8(4), 415–422. https://doi.org/10.4314/ovj.v8i4.11
14. Green, M. R., & Sambrook, J. (2017). Precipitation of DNA with isopropanol. Cold Spring Harbor Protocols, 2017(8), 673–674. https://doi.org/10.1101/pdb.prot093385
15. Günerken, E., D'Hondt, E., Eppink, M. H. M., Garcia-Gonzalez, L., Elst, K., & Wijffels, R. H. (2015). Cell disruption for microalgae biorefineries. Biotechnology Advances, 33(2), 243–260. https://doi.org/10.1016/j.biotechadv.2015.01.008
16. Gupta, N. (2019). DNA extraction and polymerase chain reaction. Journal of Cytology, 36(2), 116–117. https://doi.org/10.4103/JOC.JOC_110_18
17. Harrison, S. T. L. (2011). Cell Disruption. In Comprehensive Biotechnology, Second Edition (Vol. 2, pp. 619–640). Elsevier Inc. https://doi.org/10.1016/B978-0-08-088504-9.00127-6
18. Hosomi, K., Ohno, H., Murakami, H., Natsume-Kitatani, Y., Tanisawa, K., Hirata, S., Suzuki, H., Nagatake, T., Nishino, T., Mizuguchi, K., Miyachi, M., & Kunisawa, J. (2017). Method for preparing DNA from feces in guanidine thiocyanate solution affects 16S rRNA-based profiling of human microbiota diversity. Scientific Reports, 7(1), 1–10. https://doi.org/10.1038/s41598-017-04511-0

19. Hoyos, M., Tusso, S., Bedoya, T. R., Manrique Gaviria, A. S., & Bloor, P. (2017). A simple and cost-effective method for obtaining DNA from a wide range of animal wildlife samples. Conservation Genetics Resources, 9(4), 513–521. https://doi.org/10.1007/s12686-017-0735-z

20. Husakova, M., Kralik, P., Babak, V., & Slana, I. (2020). Efficiency of DNA isolation methods based on silica columns and magnetic separation tested for the detection of mycobacterium avium subsp. Paratuberculosis in milk and faeces. Materials, 13(22), 1–11. https://doi.org/10.3390/ma13225112

21. Islam, M. S., Aryasomayajula, A., & Selvaganapathy, P. R. (2017). A review on macroscale and microscale cell lysis methods. Micromachines, 8(3). https://doi.org/10.3390/mi8030083

22. J Shetty, P. (2020). The Evolution of DNA Extraction Methods. American Journal of Biomedical Science & Research, 8(1), 39–45. https://doi.org/10.34297/ajbsr.2020.08.001234

23. Jacobsen, N., Nielsen, P. S., Jeffares, D. C., Eriksen, J., Ohlsson, H., Arctander, P., Kauppinen, S., Hosomi, K., Ohno, H., Murakami, H., Natsume-Kitatani, Y., Tanisawa, K., Hirata, S., Suzuki, H., Nagatake, T., Nishino, T., Mizuguchi, K., Miyachi, M., Kunisawa, J., … Metzker, M. L. (2009). DNA, RNA, and protein extraction: The past and the present. Nucleic Acids Research, 4(2), 2440–2443. https://doi.org/10.1093/nar/gnh056

24. Jahanshahi, M., & Najafpour, G. (2007). Advanced Downstream Processing in Biotechnology. In Biochemical Engineering and Biotechnology (pp. 390–415). Elsevier. https://doi.org/10.1016/b978-044452845-2/50017-3

25. Jariwalla, R. J., Grossberg, S. E., & Sedmak, J. J. (1977). Effect of chaotropic salts and protein denaturants on the thermal stability of mouse fibroblast interferon. Journal of General Virology, 35(1), 45–52. https://doi.org/10.1099/0022-1317-35-1-45

26. K, K. (1999). Measurement of the rate of RNA hydrolysis in aqueous solution at elevated temperatures using a new monitoring method for hydrothermal reactions. Nucleic Acids Symposium Series, 42, 289–290. https://doi.org/10.1093/NASS/42.1.289

27. Katevatis, C., Fan, A., & Klapperich, C. M. (2017). Low concentration DNA extraction and recovery using a silica solid phase. PLoS ONE, 12(5), 1–14. https://doi.org/10.1371/journal.pone.0176848

28. Kumar, J., Kumar, M., Gupta, S., Ahmed, V., Bhambi, M., Pandey, R., & Chauhan, N. S. (2016). An Improved Methodology to Overcome Key Issues in Human Fecal Metagenomic DNA Extraction. Genomics, Proteomics and Bioinformatics, 14(6), 371–378. https://doi.org/10.1016/j.gpb.2016.06.002
29. Kumar, M., & Mugunthan, M. (2018). Evaluation of three DNA extraction methods from fungal cultures. Medical Journal Armed Forces India, 74(4), 333–336. https://doi.org/10.1016/j.mjafi.2017.07.009
30. Lemire, K. A., Rodriguez, Y. Y., & McIntosh, M. T. (2016). Alkaline hydrolysis to remove potentially infectious viral RNA contaminants from DNA. Virology Journal, 13(1). https://doi.org/10.1186/s12985-016-0552-0
31. Loparev, V. N., Cartas, M. A., Monken, C. E., Velpandi, A., & Srinivasan, A. (1991). An efficient and simple method of DNA extraction from whole blood and cell lines to identify infectious agents. In Journal of Virological Methods (Vol. 34).
32. Ma, F., Liu, F., Xu, W., & Li, L. (2018). Surfactant and Chaotropic Agent Assisted Sequential Extraction/On-Pellet Digestion (SCAD) for Enhanced Proteomics. Journal of Proteome Research, 17(8), 2744–2754. https://doi.org/10.1021/acs.jproteome.8b00197
33. Ma, J., Su, C., Hu, S., Chen, Y., Shu, Y., Yue, D., Zhang, B., Qi, Z., Li, S., Wang, X., Kuang, Y., & Cheng, P. (2020). The Effect of Residual Triton X-100 on Structural Stability and Infection Activity of Adenovirus Particles. Molecular Therapy - Methods and Clinical Development, 19, 35–46. https://doi.org/10.1016/j.omtm.2020.08.013
34. Mäki, A., Salmi, P., Mikkonen, A., Kremp, A., & Tiirola, M. (2017). Sample Preservation, DNA or RNA extraction and data analysis for high-throughput phytoplankton community sequencing. Frontiers in Microbiology, 8(SEP), 1–13. https://doi.org/10.3389/fmicb.2017.01848
35. Menezes, J. P., Lupatini, M., Antoniolli, Z. I., Blume, E., Junges, E., & Manzoni, C. G. (2010). Variabilidade genética na região its do rDNA de isolados de trichoderma spp. (Biocontrolador) e Fusarium oxysporum f. sp. Chrysanthemi. Ciência e Agrotecnologia, 34(1), 132–139. https://doi.org/10.1590/s1413-70542010000100017
36. Nouws, S., Bogaerts, B., Verhaegen, B., Denayer, S., Piérard, D., Marchal, K., Roosens, N. H. C., Vanneste, K., & De Keersmaecker, S. C. J. (2020). Impact of DNA extraction on whole genome sequencing analysis for characterization and relatedness of Shiga toxin-

producing Escherichia coli isolates. Scientific Reports, 10(1), 1–16. https://doi.org/10.1038/s41598-020-71207-3

37. Peach, M., Marsh, N., Miskiewicz, E. I., & MacPhee, D. J. (2015). Solubilization of proteins: The importance of lysis buffer choice. In Western Blotting: Methods and Protocols (pp. 49–60). Springer New York. https://doi.org/10.1007/978-1-4939-2694-7_8
38. Pouseele, H., & Supply, P. (2015). Accurate whole-genome sequencing-based epidemiological surveillance of mycobacterium tuberculosis. In Methods in Microbiology (1st ed., Vol. 42). Elsevier Ltd. https://doi.org/10.1016/bs.mim.2015.04.001
39. Ren, X., Yu, D., Yu, L., Gao, G., Han, S., & Feng, Y. (2007). A new study of cell disruption to release recombinant thermostable enzyme from Escherichia coli by thermolysis. Journal of Biotechnology, 129(4), 668–673. https://doi.org/10.1016/j.jbiotec.2007.01.038
40. Sajali, N., Wong, S. C., Hanapi, U. K., Abu Bakar @ Jamaluddin, S., Tasrip, N. A., & Mohd Desa, M. N. (2018). The Challenges of DNA Extraction in Different Assorted Food Matrices: A Review. Journal of Food Science, 83(10), 2409–2414. https://doi.org/10.1111/1750-3841.14338
41. Salimans, M., & Sol, C. J. A. (1990). Rapid and Simple Method for Purification of Nucleic Acids. 495–503.
42. Salvi, G., De Los Rios, P., & Vendruscolo, M. (2005). Effective interactions between chaotropic agents and proteins. Proteins: Structure, Function and Genetics, 61(3), 492–499. https://doi.org/10.1002/prot.20626
43. Sawyer, W. H., & Puckridge, J. (1973). The dissociation of proteins by chaotropic salts. Journal of Biological Chemistry, 248(24), 8429–8433. https://doi.org/10.1016/s0021-9258(19)43151-7
44. Scorsato, A. P., & Telles, J. E. Q. (2011). Factors that affect the quality of DNA extracted from biological samples stored in paraffin blocks. Jornal Brasileiro de Patologia e Medicina Laboratorial, 47(5), 541–548. https://doi.org/10.1590/s1676-24442011000500008
45. Sedlackova, T., Repiska, G., Celec, P., Szemes, T., & Minarik, G. (2013). Fragmentation of DNA affects the accuracy of the DNA quantitation by the commonly used methods. Biological Procedures Online, 15(1). https://doi.org/10.1186/1480-9222-15-5
46. Shamsi, M. B., Imam, S. N., & Dada, R. (2011). Sperm DNA integrity assays: Diagnostic and prognostic challenges and implications in management of infertility. Journal of

Assisted Reproduction and Genetics, 28(11), 1073–1085. https://doi.org/10.1007/s10815-011-9631-8

47. Verma, P. V., Vasudevan, V., Kashyap, B. K., Samsudeen, T. I., Meghvansi, M. K., Kamboj, D. V., & Singh, L. (2018). Direct Lysis Glass Milk Method of Genomic Dna Extraction Reveals Greater Archaeal Diversity in Anaerobic Biodigester Slurry As Assessed Through Denaturing Gradient Gel Electrophoresis. Journal of Experimental Biology and Agricultural Sciences, 6(2), 315–323. https://doi.org/10.18006/2018.6(2).315.323
48. Verma, S. K., Singh, H., & Sharma, P. C. (2017). An improved method suitable for isolation of high-quality metagenomic DNA from diverse soils. 3 Biotech, 7(3), 1–7. https://doi.org/10.1007/s13205-017-0847-x
49. Wang, W., Wang, Y. J., & Wang, D. Q. (2008). Dual effects of Tween 80 on protein stability. International Journal of Pharmaceutics, 347(1–2), 31–38. https://doi.org/10.1016/j.ijpharm.2007.06.042
50. Wohlgemuth, R. (2019). Product recovery. In Comprehensive Biotechnology (pp. 681–691). Elsevier. https://doi.org/10.1016/B978-0-444-64046-8.00096-3
51. Zainabadi, K., Dhayabaran, V., Moideen, K., & Krishnaswamy, P. (2019). An efficient and cost-effective method for purification of small sized DNAs and RNAs from human urine. PLoS ONE, 14(2), 1–15. https://doi.org/10.1371/journal.pone.0210813