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Optimization of Simple Genomic DNA Quality Improvement Using Modified Chelex and Proteinase K-Based Boiling Methods

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Abstract

DNA extraction is a crucial step in molecular analysis. The boiling method is known as a simple and fast extraction technique, but it often produces DNA with low purity and stability due to protein and metal ion contamination. This study aims to compare the effectiveness of four DNA extraction methods, namely boiling (B), boiling with Chelex, boiling with Proteinase K, and boiling with a combination of Chelex and Proteinase K. The results of concentration measurements showed that the B+PK method produced the highest DNA concentration (320.40 ng/µL), followed by Boiling + Chelex + Proteinase K (268.50 ng/µL), B (179.15 ng/µL), and Boiling + Chelex (151.00 ng/µL). The purity value (A260/A280) was in the range of 1.78–1.92, indicating relatively pure DNA. Visual analysis through electrophoresis showed that all methods successfully extracted DNA, but the clearest and most distinct DNA bands were obtained through the Boiling + Chelex + Proteinase K method. These results indicate that the combination of Chelex and Proteinase K in the boiling method is able to improve the visual quality and stability of DNA, so it has the potential to be a simple, cheap, and effective alternative method for PCR-based DNA analysis purposes.

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Introduction

Deoxyribonucleic Acid (DNA) isolation is a method used to obtain pure DNA by physically or chemically disrupting cells in a sample to separate DNA from contaminants such as cell membranes, proteins, RNA, and other components (Gupta, 2019). DNA isolation methods generally have the same stages: lysis, separation, and precipitation. Cell lysis is the process of breaking down the cell to release the DNA. Separation separates the DNA from contaminants such as protein and RNA. Precipitation is used to isolate and purify the DNA (Hardianto, Indarto, and Sasongko, 2015). Cell lysis can be carried out using various techniques, such as SDS and Tween, phenol-chloroform freezing-thawing, sonication, glass bead beating, and boiling (Shehata & Hershan, 2016; Dimitrakopoulou et al., 2020).

The boiling method is one of the simplest, cheapest, and fastest DNA isolation techniques, making it widely used in laboratories with limited resources. The basic principle is to heat the sample at a high temperature (±95–100°C) in a buffer solution, thereby damaging the cell membrane, denaturing proteins, and releasing DNA into the solution. The advantage of this method is that it does not require hazardous chemical reagents such as phenol-chloroform or relatively expensive commercial kits, and can be performed with basic laboratory equipment such as a water bath or

heat block (Ribeiro Junior et al., 2016; Sarma et al., 2014). Several studies have shown that this method can produce PCR-amplifiable DNA, although its purity is relatively lower than that of commercial kit methods (Fihiruddin, Ilmi, & Khusuma, 2022). There is still high protein or RNA contamination, so that the A260/A280 ratio is often below 1.8, indicating less pure DNA. To overcome this, Proteinase-K and RNase enzymes can be added in the isolation stage (Lesiani et al., 2023).

Proteinase K is a proteolytic enzyme capable of degrading various types of proteins, including nucleases that can potentially damage DNA during the isolation process. This enzyme can work effectively even without the addition of detergent, thus playing a crucial role in increasing DNA purity by removing protein contaminants that can interfere with the PCR amplification process (Lesiani et al., 2023). In addition, RNase functions not only to eliminate RNA contaminants but also absorbs ultraviolet light at a wavelength of 260 nm, so its presence can affect DNA concentration readings. Therefore, the addition of RNase will increase the purity of isolated DNA and produce more accurate concentration measurements. The Chelex method works through cell lysis due to the heating, followed by the binding of divalent ions by the Chelex resin, so that DNA can be obtained in the form of a supernatant. However, the resulting DNA is generally smaller or fragmented because it does not go through further purification steps, making it more suitable for simple applications such as PCR rather than advanced applications such as sequencing or large-scale genome analysis (Humaryanto & Hanina, 2022).

Research conducted by Yang et al. (2008) also showed that the Chelex method produces DNA concentration and purity that is almost equivalent to commercial kits in intestinal microflora, with an A260/A280 ratio of around 1.84. This method has several advantages, namely a relatively short processing time (less than 1 hour), lower costs than commercial kits, and a simpler procedure because it does not require complex washing steps. However, the weakness of this method is that the resulting DNA concentration can be lower than that of commercial kits, depending on the type of sample and the initial conditions of the biological material used. Therefore, this study aims to describe and compare the effectiveness of a simple boiling-based genomic DNA extraction method with the addition of Proteinase K, Chelex, and a combination of both, based on parameters of concentration, purity level (A260/A280), and DNA band quality and is expected to produce recommendations for the most optimal modified boiling method as an alternative DNA isolation method that is simple, fast, inexpensive, and applicable for molecular analysis, especially PCR.

Method

2.1. Materials

This descriptive study aims to describe and compare the effectiveness of a simple boiling-based genomic DNA extraction method with the addition of Proteinase K, Chelex, and a combination of both. Comparisons were made to determine the treatment that provides the most optimal results based on parameters such as DNA quantity, purity level, and visual quality of the DNA bands. Through this approach, the study is expected to identify the most effective and applicable modification of the boiling method as an alternative, simple, fast, and efficient DNA extraction method. The bacteria used were *Bacillus subtilis*, because the bacterial DNA banding pattern has been confirmed and consistently reported in previous studies. The use of *Bacillus subtilis* aims to serve as a biological control to ensure the accuracy of the DNA extraction results and to identify possible errors or deviations in the DNA banding pattern produced through various extraction method treatments used.

2.2. Methods

This study used four variations of boiling-based genomic DNA extraction methods, namely: (1) the boiling method, (2) the boiling method with the addition of Chelex, (3) the boiling method with the addition of Proteinase K, and (4) the boiling method with the addition of a combination of Chelex and Proteinase K. The four variations of the method were observed and compared descriptively based on the characteristics of the DNA produced, including concentration, purity ratio (A260/A280), and the quality of the DNA bands formed in agarose gel electrophoresis.

The bacterial culture was rejuvenated in identification medium (sodium broth), then 1 mL was taken and centrifuged to obtain a pellet. The pellet was then suspended in 500 µL of TE buffer 1/10 (Sirwati et al., 2024). The suspension was then heated at 100°C for 30 minutes to lyse the bacterial cells. In a variation of the treatment, proteinase K and Chelex were added before heating, as proteinase K was stored at –80°C before heating. After heating, the sample was centrifuged for 15 minutes, and the supernatant containing DNA was used as a template (Walsh et al., 1991).

The resulting supernatant was used as a DNA template in the PCR process. Amplification products were then separated using gel electrophoresis, and the results were visualized using the UVitec gel documentation system to observe the size and presence of DNA bands.

Results and Discussion

The results showed that variations in the boiling extraction method optimized with the addition of Chelex and Proteinase K resulted in significant differences in DNA characteristics, including concentration, purity, and band integrity (Table 1). The boiling method can generally lyse bacterial cells through high heat, damaging the cell walls and membranes, releasing DNA into the solution. However, this method has drawbacks, including protein contamination and the possibility of activating nuclease enzymes, which can cause DNA degradation (Vignoli et al., 1995; Sepp et al., 1994). Therefore, additional materials are needed to improve the quality of the resulting DNA.

Table 1. DNA concentration and purity

Isolate	Cocentration	Purity A260/A280
Boiling	179.15	1.927
Chelex Boiling	151.00	1.911
Chelex Boilling Proteinase K	268.50	1.786
Boiling Proteinase K	320.40	1.837

Research on the DNA extraction method using the boiling technique combined with Chelex and Proteinase K produced the strongest and clearest DNA bands in electrophoresis, while the Boiling + Proteinase K variation yielded a higher DNA concentration than the other methods (Figure 1). This suggests that the addition of chemical and enzymatic agents can increase the efficiency of the cell lysis process and the stability of the resulting genomic DNA (Walsh et al., 1991; Singh, Kumari & Iyengar, 2018; Francez et al., 2021).

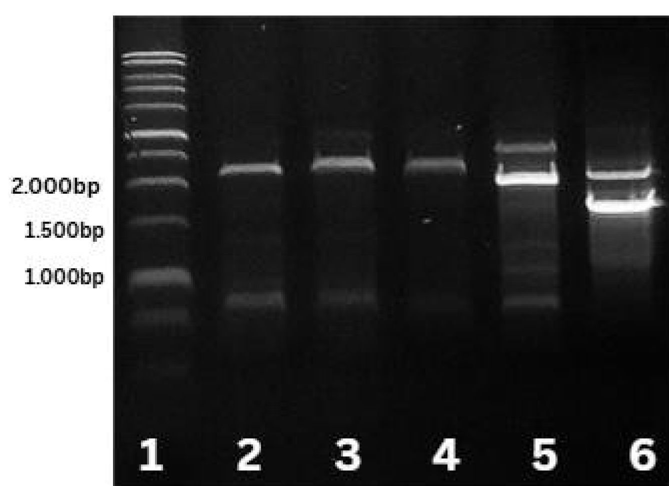

Figure 1. Amplification from Isolation DNA. 1) Leadder DNA, 2) Boiling, 3) Boiling+Chelex, 4) Boiling+Proteinase K, 5) Boiling+chelex+proteinase K, 6) Control+

Chelex-100 is an ion-exchange resin capable of binding divalent metal ions such as Mg^{2+}, which are key cofactors for nuclease enzymes. By binding these metal ions, nuclease activity can be

suppressed, thereby minimizing DNA degradation (Walsh et al., 1991; Francez et al., 2021). This is evident from the electrophoresis results of the Boiling + Chelex variation, which showed clearer DNA bands than those obtained with boiling without Chelex.

Proteinase K is a proteolytic enzyme that can degrade various types of proteins, including cellular structural proteins and nucleases (DNase and RNase) that can potentially damage DNA. The addition of Proteinase K helps release DNA from protein complexes and increases the purity of the resulting DNA (Singh, Kumari & Iyengar, 2018; Merck Millipore). This is evident from the increased DNA concentration in the Boiling + Proteinase K combination, demonstrating the effectiveness of Proteinase K in aiding DNA lysis and purification.

The extracted DNA in this study was then used as a template in the PCR process. Good DNA quality is crucial for successful amplification, as the presence of protein, fat, and heavy metal ions can inhibit DNA polymerase activity (Sepp et al., 1994; Roche Custom Biotech, 2024). The clarity of the DNA bands in the visualization results indicates that the Boiling + Chelex + Proteinase K combination method produces DNA compatible with advanced molecular applications such as PCR. This method has high practical value because it does not require hazardous chemicals such as phenol or chloroform and does not require expensive commercial kits. This makes the modified boiling method with Chelex and Proteinase K a simple, fast, inexpensive, and effective alternative for laboratories with limited facilities (Francez et al., 2021; Merck Millipore).

Conclusion

The boiling method combined with Chelex and Proteinase K produces bacterial genomic DNA that can be used as a template in PCR. Heating at 100°C for 30 minutes effectively lyses the cells, releasing the DNA into the supernatant solution. The addition of Chelex binds divalent metal ions that can inhibit PCR reactions, while Proteinase K helps degrade proteins, including nuclease enzymes, thereby increasing the purity of the resulting DNA. All treatment variations produced amplifiable DNA, but descriptively, the combination of Boiling + Chelex + Proteinase K produced relatively better DNA quality for amplification. The isolated DNA was then successfully used as a PCR template and visualized by agarose gel electrophoresis using UVitec, as indicated by the appearance of corresponding DNA bands. Therefore, this method can be recommended as a simple, rapid, and economical alternative for DNA isolation for PCR purposes, particularly in basic laboratory-scale research. However, this method still has limitations, particularly related to the potential for DNA fragmentation due to high heat and DNA stability during long-term storage. Therefore, further research is recommended to optimize the heating temperature, incubation time, and concentration of Chelex and Proteinase K to obtain more stable and consistent results.

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