OPTIMIZATION OF A GLASS FIBER MEMBRANE BASED COLUMN FOR IMPROVED IN-HOUSE PCR DNA PURIFICATION

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by

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

(NH4)2SO4	Ammonium sulfate
°C	Degree Celsius
µl	Microlitre
A	Adenine
A260/A230	Absorbance at 260 nanometers/Absorbance at 230 nanometers
A260/A280	Absorbance at 260 nanometers/Absorbance at 280 nanometers
bp	Base pair
C	Cytosine
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
Eh-lectin	*Entamoeba histolytica*-lectin
G	Guanine
g	Gram
GC	Guanine cytosine
GMP	Good Manufacturing Practice
GRADE	Grading of Recommendations, Assessment, Development, and Evaluations
Gu-HCl	Guanidine hydrochloride
HCl	Hydrochloride
i.e.	*id est* - 'that is'
K+	Potassium ion

kb	Kilo base pair
M	Molar
Mg2+	Magnesium ion
MgCl2	Magnesium chloride
min	Minutes
ml	Millilitre
mM	Millimolar
NH4+	Ammonium ion
nm	Nanometre
PCR	Polymerase chain reaction
pET-14b-Eh-lectin	pET-14b-*Entamoeba histolytica*-lectin
pH	Potential of hydrogen
RNA	Ribonucleic acid
rpm	Revolutions per minute
s	Seconds
SPSS	Statistical Package for the Social Sciences
T	Thymine
TAE	Tris-acetate EDTA
Taq	*Thermus aquaticus*
Tris-HCl	Tris hydrochloride
USM	Universiti Sains Malaysia
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
V	Volts
VS	Versus

μg	Microgram
μg/ml	Microgram per millilitre
μM	Micromolar

PENGOPTIMUMAN LAJUR MEMBRAN GENTIAN KACA UNTUK MENINGKATKAN PENULENAN DNA PCR SECARA IN-HOUSE

ABSTRAK

Penulenan PCR berasaskan kolum sering digunakan di makmal untuk menulenkan amplicon PCR bagi pelbagai aplikasi hiliran seperti penjujukan dan kloning. Produk komersial seperti Qiagen PCR DNA purification kit yang mematuhi GMP direka khusus untuk kegunaan penyelidikan. Untuk latihan tenaga kerja atau pendidikan akademik, kit yang lebih murah tetapi mampu memberikan prestasi yang setara mungkin menjadi pilihan yang lebih baik. Dalam kajian ini, kit penulenan PCR buatan sendiri yang menggunakan membran gentian kaca telah dibangunkan, manakala kit QIAGEN digunakan sebagai rujukan. Untuk pengoptimuman, kolum dengan saiz liang membran yang berbeza (0.22 μm, 0.45 μm, dan 0.80 μm) dan lapisan membran (1, 2, dan 6) telah dipasang dan diuji. Hasil DNA dan ketulenannya dianalisis menggunakan biofotometer dan dibandingkan. Bagi keputusan, kolum yang menggunakan membran gentian kaca dengan saiz liang lebih kecil, iaitu 0.22 μm, menunjukkan hasil DNA tertinggi. Membran DNA dengan 6 lapisan juga memberikan hasil DNA yang tertinggi. Dalam kajian perbandingan, kolum berasaskan gentian kaca menunjukkan prestasi lebih tinggi berbanding kolum dalaman berasaskan membran silika, dan hasil DNA yang setara dengan kit QIAGEN. Semua kit menunjukkan ketulenan DNA yang baik bagi nisbah A260/A280, tetapi tidak untuk nisbah A260/A230. Walau bagaimanapun, jalur DNA yang dianalisis melalui elektroforesis gel agarosa menunjukkan hasil yang baik. Kajian ini mencadangkan bahawa membran gentian kaca dalaman yang dioptimumkan menawarkan alternatif yang berdaya maju dan berpatutan untuk penulenan DNA PCR,

sekali gus merapatkan jurang kos dan kecekapan bagi makmal yang mempunyai sumber terhad.

OPTIMIZATION OF A GLASS FIBER MEMBRANE-BASED COLUMN FOR IMPROVED IN-HOUSE PCR DNA PURIFICATION

ABTSRACT

Column-based PCR purification is commonly used in laboratories to purify PCR amplicons for various downstream applications including sequencing and cloning. The commercially available products such as the Qiagen PCR DNA purification kit, which comes with GMP are designed intended for research usage. For manpower training or academic education, a less expensive kit with comparable performance could be a better option. An in-house self-assembled PCR purification kit using a glass fiber membrane was developed in the present study, while the QIAGEN kit was used as the reference kit. For optimization, columns with different membrane pore sizes (0.22 μm, 0.45 μm, and 0.80 μm) and membrane layers (1, 2, and 6) were assembled and tested. The yield and DNA purities were analyzed via bio-photometer and compared. For results, columns utilizing GF membrane of a smaller pore size i.e. 0.22 um showed the highest DNA yield. DNA membrane with 6 layers also retains the highest DNA yield. In the comparative study, GF based column showed higher performance than the in-house silica membrane and a comparable DNA yield to the QIAGEN kits. All kits showed good DNA purities for A260/A280 purities, but not for A260/A230 purities. Nonetheless, the DNA bands showed to be good in agarose gel DNA electrophoresis analysis. This study suggests that optimized in-house glass fiber membranes offer a viable and affordable alternative for PCR DNA purification, bridging the cost-efficiency gap for resource-limited laboratories.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The polymerase chain reaction (PCR) is a precise and efficient technology utilized by laboratories to amplify specific DNA sequences into billions of copies from a template. A typical PCR has three major steps: denaturation, annealing, and elongation or extension (Shah, 2019). A typical PCR requires several components, including water (dH20), buffer, magnesium chloride (a cofactor), deoxynucleotide triphosphates (dNTPs), forward and reverse primers, DNA polymerase, and DNA template. These various components can be combined to make the PCR master mix, which is then used to execute PCR in thermocycler equipment (Barudin et al., 2019). These procedures rely on a thermocycler, which automates cycling through the distinct temperatures and incubation durations required for each step (Lischer et al., 2021). PCR allows for rapid DNA replication, making it crucial in molecular biology research and diagnostics. However, the DNA products produced frequently contain impurities such as residual Taq polymerase, primers, and salts, which can disrupt downstream applications. This demands using effective purification procedures to isolate high-quality DNA (Berdimuratova et al., 2020).

DNA purification from PCR results must meet several criteria, including efficient DNA isolation, sufficient elution for downstream applications, contamination removal, and high purity and quality (Abdel-Latif & Osman, 2017). There are several methods for PCR purification, each with its working principles, advantages, and limitations. Chemical-based methods, which include phenol-chloroform extraction and spin column-based DNA purification, are commonly utilized. Spin column-based methods, in

particular, use silica membranes and are valued for their simplicity, cost-effectiveness, and rapid procedure (Shi et al., 2018). On the other hand, physical methods such as magnetic bead-based DNA extraction offer an alternate mechanism for isolating DNA. Regardless of their utility, these solutions may involve cost, time, and technological trade-offs (Berdimuratova et al., 2020). Thus, there is a need to investigate and optimize economical yet efficient purification methods for greater accessibility and applicability.

In response to these challenges, the purpose of this research was to optimize an in-house alternative for PCR DNA purification employing glass fiber membranes with various pore sizes. Glass fiber membranes provide a low-cost, self-assembled option for DNA purification, making them a potential alternative to costly commercial kits. By manipulating pore sizes, researchers may be able to achieve DNA yields and purities comparable to or greater than those obtained using current methods (Abraham et al., 2017). This technique shows promise for improving academic and industrial applications.

Furthermore, assessing DNA purity and yield after purification is vital for evaluating the protocol's effectiveness. Ultraviolet-visible (UV-Vis) spectrophotometry and agarose gel electrophoresis are effective methods for testing DNA quality. UV-Vis spectrophotometry measures absorbance at particular wavelengths to measure concentration and purity (Viljoen et al., 2022). Whereas agarose gel electrophoresis visualizes DNA quality using band patterns (Bogiel et al., 2022). By combining various analytical tools, this research aimed to provide complete insights into the performance of glass fiber membrane-based DNA purification protocols.

1.2 Problem Statement

Impurities in DNA samples can lead to unfavorable outcomes in downstream applications, making efficient purification critical (Berdimuratova et al., 2020). The type

of PCR purification columns employed has a direct impact on the quality and quantity of extracted DNA. The high cost of commercially available DNA purification kits presents a significant challenge for laboratories, especially those in resource-constrained environments like teaching and training institutes (Abdel-Latif & Osman, 2017). While in-house techniques have been developed, complete data on their efficacy, particularly in terms of DNA yield and purity, is still limited (Shi et al., 2018). Furthermore, the specific role of glass fiber membrane pore sizes in DNA purification has not been adequately studied. This scarcity of empirical evidence impedes the creation of affordable, reliable alternatives to commercial kits. Hence, bridging these gaps is critical to providing laboratories with cost-effective and efficient DNA purification techniques that fulfill the needs of modern molecular biology applications (Ye & Lei, 2023).

1.3 Rationale of Study

This study addresses the need for cost-effective and efficient DNA purification methods that can overcome the high cost and limited accessibility of commercial kits. This study suggests an innovative approach to optimizing DNA purification for teaching, training, and research by utilizing low-cost, self-assembled glass fiber membranes (Abraham et al., 2017). Evaluating the influence of different pore sizes on DNA yield and purity will produce empirical data that will aid in the development of practical alternatives, improving molecular biology's relevance in resource-limited settings. Furthermore, this research helps to build skills and hands-on experience in laboratory techniques, which benefits both educational and industrial applications (Lázaro-Silva et al., 2015).

1.4 Significance of Study

This research has important implications for optimizing PCR DNA purification procedures by focussing on glass fiber membrane-based columns with varying pore sizes. The findings are expected to provide laboratories with low-cost alternatives to commercial purification kits, allowing resources to be directed toward other vital research topics (Shi et al., 2018). Furthermore, this study improves decision-making for researchers operating on a limited budget by providing proven DNA purification procedures. The study additionally promotes innovation and competition in the development of cost-effective purification tools, which benefits the molecular biology community (Fatima et al., 2024). Furthermore, developing best practices and guidelines through this research will result in more efficient DNA purification processes, assuring consistent and reproducible results across various studies. These advancements are anticipated to have wide-ranging applications, including improving teaching methodologies and assisting industrial-scale research initiatives (Lázaro-Silva et al., 2015).

1.5 Research Objective

1.5.1 General Objective

To develop a high-efficiency in-house glass fiber membrane-based PCR for DNA purification

1.5.2 Specific Objectives

1. To mass produce of plasmid DNA for purification by PCR
2. To determine the optimum membrane pore size and layer for DNA purification by PCR method

3. To compare the DNA purification efficiency with commercial and in-house silica membrane kits

1.6 Hypothesis

1.6.1 Null Hypothesis

The in-house glass fiber membrane-based PCR for DNA purification method shows no significant difference from the commercial columns in terms of yield and purities.

1.6.2 Alternative Hypothesis

The in-house glass fiber membrane-based PCR for DNA purification method shows a significant difference with the commercial columns in terms of yield and purities.

1.7 Conceptual Framework

Figure 1.1 depicts the relationship between the independent, dependent, and constant variables in this study. The independent variable was the pore size of the glass fiber membrane employed for PCR DNA purification (0.8 μm, 0.45 μm, and 0.22 μm). The dependent variables were DNA yield and purity, which were measured using UV-Vis spectrophotometry and agarose gel electrophoresis (Lucena-Aguilar et al., 2016). The constant variables include the type of plasmid, PCR conditions, and DNA purification techniques, ensuring that any observed alterations in the dependent variables are purely the result of changes in the independent variable. This conceptual framework emphasizes the methodical approach to understanding how differences in purification material parameters influence DNA quality and yield.

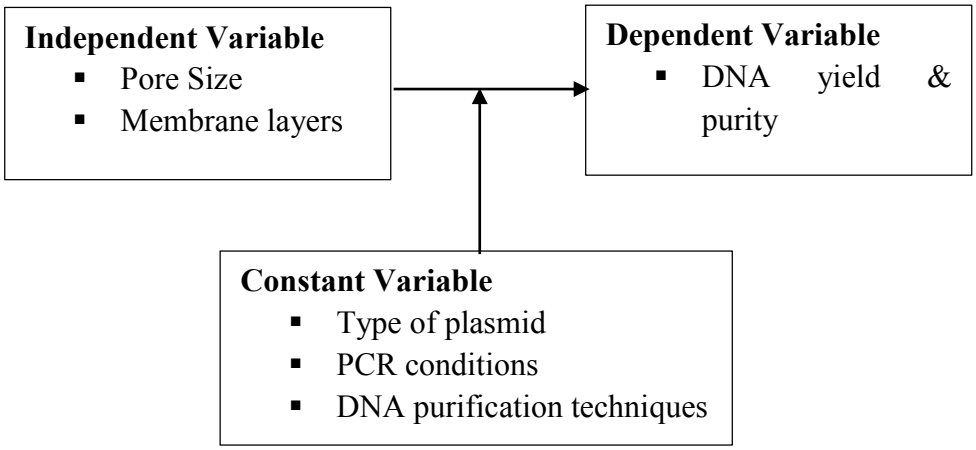

Figure 1.1 Conceptual framework of this study by correlating the relationship between independent variable, dependent variable, and constant variable.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to PCR and DNA Purification

The polymerase chain reaction (PCR) is a fundamental molecular biology technique for amplifying small and specific segments of DNA, allowing millions of copies to be generated from minute starting material (Shah, 2019). Kary Mullis, a technician at Cetus Corporation tasked with developing oligonucleotide synthesis methods, devised a polymerase chain reaction (PCR) in 1983 (Zhu et al., 2020). Mullis developed PCR as a method of synthesizing and amplifying specific DNA sequences, inspired by Sanger's DNA sequencing approach. Mullis and Michael Smith were awarded the Nobel Prize in Chemistry in 1993 for their revolutionary invention (Khehra et al., 2023). Initially, the approach was labor-intensive, needing manual sample transfers between water baths of varying temperatures for each phase of the process. DNA polymerases have to be replenished after each cycle since they are denatured by the high temperatures required for DNA denaturation (Zhu et al., 2020).

Two crucial advances made PCR automation possible: the isolation of the thermostable *Taq* polymerase from *Thermus aquaticus* and the creation of programmable thermal cyclers (Zhu et al., 2020). *Taq* polymerase is heat-resistant and can tolerate denaturation temperatures without becoming inactive, eliminating the need for manual additions after each cycle (Khehra et al., 2023). Programmable thermal cyclers streamlined the process by automating the denaturation, annealing, and extension cycles and considerably lowering the time and effort necessary for amplification (Qiu et al., 2010).

PCR has three key steps: denaturation, annealing, and extension. Denaturation occurs when the double-stranded DNA is heated to around 95°C, causing the hydrogen bonds between complementary base pairs to dissolve and the DNA strands to separate (Khehra et al., 2023). The annealing phase occurs when the temperature ranges from 37°C to 72°C, enabling the primers to bind or anneal to their specific complementary sequences on the single-stranded DNA template. The accuracy and specificity of primer annealing are crucial for effective amplification because they ensure that only the target area of DNA is amplified (Khehra et al., 2023). The final step, extension, occurs at around 72°C, which is the ideal operating temperature for *Taq* polymerase. During this phase, the polymerase extends the primers by incorporating deoxynucleotide triphosphates (dNTPs) to synthesize the complementary strand of DNA. These three processes are repeated 20-40 times, with each cycle doubling the amount of target DNA, resulting in exponential amplification and the formation of billions of copies of the desired DNA fragment (Sathyanarayana & Wainman, 2023).

PCR has numerous applications in research, diagnostics, and forensics. In medical diagnosis, PCR is employed to detect genetic mutations, infectious illnesses, and cancer biomarkers (Nazir, 2023). It is important in forensic research because it allows for DNA fingerprinting and identification of trace biological materials (McDonald et al., 2024). PCR is used in agriculture to improve crops, identify pathogens, and analyze genetic alterations (Venbrux et al., 2023). Additionally, PCR has revolutionized evolutionary biology by making it easier to analyze ancient DNA and conduct phylogenetic research (Duchêne et al., 2020).

Recent advances, such as real-time PCR (qPCR) and reverse transcription PCR (RT-PCR), have extended PCR's potential. qPCR enables real-time monitoring of DNA amplification, resulting in exact quantification and mutation analysis (Artika et al., 2022).

RT-PCR, on the other hand, converts RNA into complementary DNA (cDNA), which is an important step in investigating gene expression and detecting RNA viruses (Mo et al., 2012). These advancements further strengthen PCR's relevance in molecular biology and its applications in a variety of fields.

2.2 Components and Reagents in PCR

2.2.1 Key Components of PCR

Performing PCR requires several essential components, including a thermal cycler, nuclease-free water, *Taq* buffer, magnesium chloride, deoxynucleotide triphosphates (dNTPs), forward and reverse primers, *Taq* polymerase, and a DNA template (Barudin et al., 2019). The thermal cycler is an important piece of equipment that controls the temperature changes that occur throughout the PCR process. Modern thermocyclers have heated lids to avoid condensation and evaporation within the reaction tubes, resulting in stable reaction conditions and reproducibility. Rapid temperature cycling in the thermocycler is required for the three primary PCR steps: denaturation, annealing, and extension, all of which require precise temperature control to maximize efficiency and accuracy (Wu et al., 2020).

Nuclease-free water is a crucial component of the PCR master mix, that is utilized to dilute reagents to their optimal concentrations. It prevents DNA degradation by eliminating any trace nucleases found in regular distilled water. The use of nuclease-free water ensures that the DNA template is intact during the amplification phase, preserving the PCR reaction's integrity (Karunanathie et al., 2022). Minimizing contamination reduces variability in results, which is especially crucial for sensitive downstream applications like sequencing or quantitative analysis. Nuclease-free water is

manufactured under rigorous conditions to ensure that it is free of enzymatic activity, making it a reliable diluent for PCR procedures (Mo et al., 2012).

Taq buffer is an important reagent in PCR because it keeps the pH stable of the reaction mixture and creates an optimal environment for enzyme activity. It provides critical ions such as potassium (K^+) and ammonium (NH_4^+), which increase primer annealing to the DNA template while destabilizing non-specific binds, enhancing the reaction's specificity (Karunanathie et al., 2022). The buffer also maintains the stability of the DNA polymerase by preventing it from denaturing during thermal cycling. Variations in buffer composition, such as the inclusion of co-factors or stabilizing agents, can considerably alter the effectiveness of the PCR, emphasizing the significance of accurate formulation for consistent and reproducible results (Sathyanarayana & Wainman, 2023).

Magnesium chloride ($MgCl_2$) is essential in PCR as a cofactor for DNA polymerase. It aids in the alignment of primers and DNA templates, stabilizing the interaction essential for DNA synthesis. Magnesium ions also help the polymerase generate phosphodiester bonds, which allows it to efficiently extend the DNA strand (Lorenz, 2012). Optimizing the $MgCl_2$ concentration is crucial, as minimal levels might impair enzyme activity and excessive concentrations can cause non-specific amplification or reaction inhibition. Magnesium ions also interact with dNTPs, helping to align their phosphate groups with the 3'-end of the DNA strand, which improves polymerase performance (Gao & Yang, 2016). Proper magnesium levels are thus essential for achieving excellent specificity and yield in PCR.

Deoxynucleotide triphosphates (dNTPs) are DNA's building components and are required for strand synthesis during the extension phase of PCR. dNTPs provide the adenine (A), thymine (T), guanine (G), and cytosine (C) units required to extend primers

and amplify the target DNA sequences (Mubarak et al., 2020). The concentration of dNTPs must be optimized since too little could decrease specificity, whilst too many can reduce PCR efficiency and result in non-specific amplification (Mubarak et al., 2020). Generally, 50 μM of each dNTP is employed, however changes may be required depending on amplicon size and magnesium content (Mubarak et al., 2020).

Primers are short, single-stranded oligonucleotides that bind to complementary sequences on the target DNA region's boundaries. Forward and reverse primers are required for DNA synthesis in both orientations (Remmers et al., 2015). The primer design has significant effects on PCR specificity, as poorly designed primers can cause non-specific amplification, secondary structures, or lower yields (Bustin & Huggett, 2017). Effective primers have an optimal length (18-25 bp), adequate melting temperatures, balanced GC content, and minimal secondary structure formation (Remmers et al., 2015).

Taq polymerase, a thermostable DNA polymerase developed from *Thermus aquaticus*, is essential for synthesizing new DNA strands during PCR. Its thermostability allows it to remain active at high temperatures, including denaturation at ~95°C, lowering the need for replenishing after each cycle (Zhu et al., 2020). Taq polymerase adds dNTPs to primers bound to the DNA template, with just a minimal amount required for each reaction due to its reusability in successive cycles (Remmers et al., 2015).

The final component is the DNA template, that contains the specific region that will be amplified. Templates may include genomic DNA, plasmid DNA, or complementary DNA (cDNA). The quality of the template has a considerable impact on PCR efficiency, as impurities like proteins or RNA can block the reaction. High-quality, purified DNA ensures reliable and reproducible amplification results (Jalali et al., 2017)

2.2.2 Role of Binding, Washing, and Elution Buffers in DNA Purification.

DNA purification utilizes specific buffers for binding, washing, and elution to isolate high-quality DNA. Binding buffers, which often contain chaotropic agents such as guanidine hydrochloride, disrupt hydrogen bonds and denature proteins, allowing DNA to bind to silica-based membranes (S. M. Lee et al., 2023). Washing buffers, which are commonly made up of ethanol and low-salt solutions, effectively eliminate residual impurities such as proteins and salts while preserving the DNA bound to the silica membrane (Weng et al., 2020). Elution buffers, such as Tris-HCl, release DNA from the membrane at low ionic strength, to guarantee the purified DNA is appropriate for downstream applications (Weng et al., 2020).

2.3 DNA Purification Methods

2.3.1 Overview of Chemical and Physical Purification Methods

PCR purification is an essential step in molecular biology workflows to isolate and purify DNA fragments produced during PCR amplification (Gupta, 2019). PCR produces millions of copies of the target DNA sequence (Zhu et al., 2020). PCR products may contain unincorporated primers, nucleotides, polymerase enzymes, and other impurities that can disrupt downstream applications including sequencing, cloning, or restriction enzyme digestion (Berdimuratova et al., 2020). Thus, to ensure accurate and reliable downstream analyses, PCR purification removes unwanted components such as DNA polymerase, primers, dNTPs, magnesium chloride, and buffer (Berdimuratova et al., 2020).

2.3.1.(a) Phenol-Chloroform Extraction Method

The phenol-chloroform extraction method is one of the oldest chemical procedures for DNA purification, and it is based on nucleic acid solubility differences in organic solvents (Liu et al., 2022). This method requires mixing a sample with an equal volume of phenol-chloroform-isoamyl alcohol, which separates into two different phases during centrifugation: an aqueous phase containing DNA and an organic phase comprising proteins and lipids. The DNA in the aqueous phase can precipitate using ethanol or isopropanol to produce high-purity DNA suitable for downstream applications like sequencing and cloning (Sharma et al., 2023).

Despite its effectiveness, the phenol-chloroform method has certain disadvantages. The usage of phenol, a highly toxic and corrosive chemical, creates health and environmental concerns that necessitate strict safety precautions during handling and disposal (Da Silva et al., 2023). Furthermore, because this process involves multiple steps, it is labor-intensive and time-consuming, making it unsuitable for high-throughput applications. It is extremely difficult to automate, which limits its usefulness in modern laboratories with enormous sample volumes (Dandare et al., 2022).

Recent advances have sought to overcome some of the limitations of the phenol-chloroform method by using automation and decreasing toxic waste. Modified procedures with fewer hazardous substances have been devised, however they frequently reduce DNA yield or purity. Despite its drawbacks, the phenol-chloroform approach is nevertheless a useful tool in situations when maximum DNA purity is required, especially in research settings where DNA must be isolated from complex or degraded samples (Gautam, 2022).

2.3.1.(b) Spin Column-Based Purification

Spin column purification is a common physical method that uses silica-membrane technology to isolate DNA. This technology uses chaotropic salts, such as guanidine hydrochloride, to disrupt the hydrogen bonding between DNA and water molecules, allowing the DNA to bind to the silica membrane (Shi et al., 2018). Following binding, impurities such as proteins, salts, and other pollutants are removed by a series of washing procedures, leaving the DNA bonded to the membrane (Khehra et al., 2023).

Spin column-based purification has the benefit of being simple, fast, and compatible with a wide range of sample types, including blood, tissues, and cultured cells. The method is scalable for high-throughput operations, as it may be performed in minutes with centrifugation or vacuum systems (H. Lee et al., 2018). Furthermore, spin column kits are commercially accessible, with standardized protocols that guarantee consistent results (Diefenbach et al., 2018).

However, commercial spin column kits can be too expensive for some laboratories, particularly those with limited funding (Shi et al., 2018). In response, researchers created in-house spin column technologies that use alternate materials, such as glass fiber membranes, to cut costs while preserving comparable efficiency and DNA purity (Hoorzook & Barnard, 2022). This advancement emphasizes the adaptability and significance of spin column-based purification in contemporary molecular biology (Shi et al., 2018).

2.3.1.(c) Magnetic Bead-Based Purification

Another physical method that has grown in favor is magnetic bead purification, which is versatile and easy to automate. This method captures nucleic acids using magnetic beads coated with DNA-binding compounds like silica or carboxyl groups. In the presence of

binding buffers, DNA binds to the beads, and contaminants are eliminated by washing them repeatedly. A magnetic field is used to separate the beads, simplifying the purifying procedure (Berdimuratova et al., 2020).

The magnetic bead-based method is especially useful for high-throughput and automated operations, as it eliminates the need for centrifugation or vacuum systems. This makes it excellent for robotic liquid handling systems, which are widely utilized in clinical diagnostics and genomics research (Váradi et al., 2014). Furthermore, because the beads have a large surface area for binding, the approach works with a wide range of sample types, which includes those with low DNA concentrations (Berdimuratova et al., 2020).

However, the expense of beads and specialized equipment can make magnetic bead-based purification more expensive than other methods (Oberacker et al., 2019). Furthermore, optimizing the binding and washing conditions for specific applications may necessitate extensive trial and error, which can be time-consuming. Despite these obstacles, magnetic bead-based purification remains the preferred method for laboratories seeking flexibility and scalability in DNA isolation (Berdimuratova et al., 2020).

2.3.2 Mechanisms of Silica Membrane-Based Purification.

Silica membrane-based purification is one of the most widely used methods for DNA isolation due to its efficiency and reliability. The method is based on silica's ability to bind DNA in the presence of chaotropic agents like guanidine hydrochloride, that disrupt hydrogen bonds and enhance nucleic acid binding to the silica surface (S. M. Lee et al., 2023). After the binding step, washing buffers are employed to remove proteins, salts, and other contaminants while leaving the DNA on the membrane (Khehra et al., 2023). Finally, an elution buffer releases the DNA, resulting in high-purity nucleic acids suitable

for downstream applications such as PCR, sequencing, and cloning (Khehra et al., 2023). This approach is well regarded for its simplicity and reproducibility, making it widely utilized in both research and clinical laboratories.

2.3.3 Comparison between Commercial (QIAGEN) and In-house DNA Purification Methods

2.3.3.(a) QIAGEN Columns

Commercially available DNA purification kits, including QIAquick PCR purification kits from QIAGEN, are well-known for their convenience, reliability, and consistent performance. These kits use silica membrane-based technology, in which DNA fragments selectively bond to the column's silica membrane in the presence of chaotropic salts provided in a binding buffer, like the buffer PB (Quek et al., 2021). Chaotropic salts break up the hydrogen bonds between nucleic acids and water, enabling DNA to adhere to the silica membrane. Following the binding process, impurities are removed using a washing buffer (buffer PE), and DNA is eluted using an elution buffer (buffer EB), preparing it for downstream applications such as sequencing and cloning (Weng et al., 2020).

QIAGEN's extensive research and development efforts have resulted in these kits' consistent performance. Their patented formulas and manufacturing processes guarantee high DNA yield and purity, making them ideal for sensitive downstream applications including sequencing, cloning, and PCR (Siuta et al., 2023). Rigorous quality control measures assure reproducibility and reliability while adhering to Good Manufacturing Practices (GMP) and regulatory requirements (Hörner et al., 2021). Furthermore, QIAGEN provides substantial customer support, including troubleshooting guides and application protocols, which improve the user experience and justify their premium pricing (Quek et al., 2021).

Despite their benefits, QIAGEN kits can be prohibitively expensive for certain laboratories. Their high price reflects the quality assurance systems, proprietary materials, and research investments required to ensure the items satisfy stringent specifications. These qualities make QIAGEN a preferred alternative for high-stakes applications that require substantial reproducibility and reliability (Abdel-Latif & Osman, 2017).

2.3.3.(b) In-house Columns

In contrast, In-house DNA purification methods involve using self-assembled purification columns, and they are frequently modified to specific experimental requirements (Abraham et al., 2017). These methods often use locally sourced materials, such as glass fiber membranes, which act as an alternative to silica membranes for DNA binding (Jones & Huff, 2018). While cost-effective, in-house procedures require optimization to attain comparable DNA yield and purity as commercial kits (Shi et al., 2018). One of the key benefits of in-house methods is their flexibility. Researchers can tailor protocols to specific experimental settings, such as adjusting buffer compositions or changing column design to improve binding and elution efficiency requirements (Abraham et al., 2017). This flexibility makes in-house methods appealing for specialized research or applications that require non-standard settings.

However, in-house methods frequently lack the standardization and rigorous quality control found in commercial kits. Variability in materials, assembly procedures, and execution can result in inconsistencies in DNA yield and purity. Minor protocol changes might have a major impact on outcomes, making reproducibility across different experiments challenging (Goff et al., 2020). To address these difficulties, comprehensive validation experiments and quality control measures are required.

In terms of cost, in-house methods are much less expensive than commercial alternatives, making them accessible to laboratories with limited budgets. However, the time and effort necessary to optimize these methods may outweigh some of the financial savings. Thus, while in-house methods are useful for cost-sensitive or customized applications, they might not always be suitable for high-throughput or crucial research where standardization is paramount (Hoorzook & Barnard, 2022).

2.4 Glass Fiber Membranes in DNA Purification

2.4.1 Structure, Properties, and Applications of Glass Fibre Membranes.

Glass fiber membranes are frequently employed in molecular biology for nucleic acid purification because of their unique structural and chemical properties. These membranes are made up of finely woven glass fibers that form a porous matrix, allowing for effective DNA binding and separation under appropriate conditions. Glass fiber membranes exhibit high porosity, which improves the accessible surface area for DNA adsorption, as well as mechanical stability, making them appropriate for repeated usage in laboratory processes (Jangam et al., 2012). Furthermore, their surface is modified with silanol groups, which improve DNA binding in the presence of chaotropic salts by breaking the hydrogen bonds between nucleic acids and water molecules (Cao et al., 2016).

Glass fiber membranes' chemical resistance enables them to withstand extreme conditions such as exposure to chaotropic agents and buffers used in DNA purification protocols (Vandeventer et al., 2013). Furthermore, these membranes have great thermal stability, which makes them suitable for high-temperature applications such as PCR-based workflows (Sherif et al., 2020). Glass fiber membranes are used for a variety of molecular biology methods, including protein separation, cellular debris filtration, and DNA and RNA purification. Their cost-effectiveness compared to silica membranes

makes them a preferred alternative for in-house DNA purification methods, especially in resource-constrained settings (Shi et al., 2018).

2.4.2 Influence of Pore Size and Membrane Layers on DNA Binding Efficiency

The pore size and number of layers in glass fiber membranes have a significant impact on DNA binding efficiency. Smaller pore sizes are better for capturing smaller DNA fragments, resulting in a tighter matrix for effective adsorption. Larger pore diameters, on the other hand, are more suited for binding larger nucleic acid molecules since they require greater access via the membrane structure (Wang et al., 2024). Multi-layered membranes increase total binding capacity by offering more surface area for DNA adsorption. However, excessive layering might cause flow resistance during the washing and elution processes, reducing the overall efficiency of the purifying process (Orr et al., 2013). Optimizing these parameters is critical for establishing a balance between yield, purity, and process efficiency.

2.5 Assessment of DNA Yield and Purity

2.5.1 Importance of A260/A280 and A260/A230 Ratios in Assessing DNA Quality.

DNA purity and yield are essential measures used to assess the quality and quantity of DNA after purification. The A260/A280 ratio is an established measure to detect protein contamination in DNA samples. Proteins absorb UV light around 280 nm, and a ratio of around 1.8 suggests low protein contamination and high DNA purity (Lucena-Aguilar et al., 2016). A lower ratio, such as 1.6 or lower, indicates the presence of proteins, phenols, or other contaminants, whereas a higher ratio may suggest RNA contamination (Lucena-Aguilar et al., 2016). The A260/A230 ratio, which detects contamination by organic solvents, salts, or carbohydrates, should be between 2.0 and 2.2. Values outside of this

range indicate impurities like guanidine hydrochloride, phenol, or EDTA (Lucena-Aguilar et al., 2016). These ratios are critical for determining if the DNA is suitable for sensitive downstream applications such as PCR, cloning, and sequencing.

2.5.2 Techniques for Measuring DNA Concentration and Purity.

UV-Vis spectrophotometry is one of the most common methods for determining DNA content and purity. This method measures DNA by determining absorbance at specified wavelengths: 260 nm for nucleic acids, 280 nm for proteins, and 230 nm for contaminants (Viljoen et al., 2022). Modern spectrophotometers, such as the NanoDrop, can perform quick and accurate measurements with minimal sample quantities, making them suitable for routine laboratory workflows (García-Alegría et al., 2020). Agarose gel electrophoresis offers a complementary method for visually assessing DNA integrity by separating fragments based on size (Bogiel et al., 2022). Intact DNA appears as sharp, distinct bands, whereas degraded DNA is smeared, allowing researchers to assess contamination and degradation (Bogiel et al., 2022). Combining spectrophotometry with gel electrophoresis provides a thorough evaluation of DNA quality and concentration.

DNA yield is the quantity of DNA obtained after purification, usually quantified in μg/mL or total yield (μg). High DNA yield is desired, but it may also imply contamination with RNA, proteins, or salts, which might have an impact on downstream applications (Lucena-Aguilar et al., 2016). Low yields can be caused by degraded DNA, insufficient lysis, or the presence of inhibitors such as heme or humic acid, which interfere with DNA extraction (Lucena-Aguilar et al., 2016). DNA purity, measured by A260/A280 and A260/A230 ratios, guarantees that impurities do not interfere with enzymatic reactions required for applications such as PCR or sequencing (Lucena-Aguilar et al., 2016). Finally, agarose gel electrophoresis is used to measure DNA

integrity; undamaged DNA enables consistent performance in molecular biology research, whereas degraded DNA affects results (Zonta et al., 2015).

2.6 Current Trends and Innovations in DNA Purification

Advancements in PCR and DNA purification technologies have transformed molecular biology workflows by increasing efficiency, accuracy, and accessibility. Modern PCR technologies currently incorporate high-speed thermocyclers, digital PCR systems, and multiplex PCR, which allows for the simultaneous amplification of multiple targets with greater precision and reduced reaction times (Zhu et al., 2020). Similarly, developments in DNA purification include magnetic bead-based methods, that reduce the need for centrifugation and enable automation, making them ideal for high-throughput applications (Li et al., 2023). Furthermore, silica and glass fiber membranes have been optimized to improve DNA binding efficiency, yield, and purity, overcoming the limitations of previous technology (Parizi et al., 2020). These improvements address the rising need for scalable and reproducible DNA purification technologies in both research and clinical settings.

The high cost of commercial DNA purification kits has prompted the development of cost-effective alternatives, especially among laboratories with limited resources. In-house DNA purification techniques using locally sourced materials, like glass fiber membranes, provide a viable alternative to the costly silica membrane-based commercial kits (Fatima et al., 2024). These protocols enable researchers to tailor buffer compositions and membrane properties to suit specific experimental requirements, resulting in comparable DNA yield and purity for a fraction of the cost. Furthermore, simple chemical-based methods, like ethanol precipitation and phenol-chloroform extraction, continue to be cost-effective alternatives for DNA purification, especially when high

throughput or automation is not required (Li et al., 2023). The adaptability of these alternatives guarantees their relevance across a wide range of molecular biology applications.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Study Design

This was an experimental study that compares the efficiency of DNA purification procedures employing QIAGEN columns to in-house spin columns using glass fiber membranes with varied pore sizes and layers. The presence of the target sequence was first confirmed in the plasmid utilized in this study, which was pET-14b-Eh-lectin, using an established PCR procedure. The procedure then proceeded to determine the optimal PCR cycle settings for the pET-14b-Eh-lectin plasmid. Once optimized, PCR amplicons were mass-produced for further purification. The study compared one- and six-layer glass fiber membranes with a 45 µm pore size. Additional tests evaluated numerous layers (e.g., one, two, or six) with varied pore sizes of 22 µm, 45 µm, and 80 µm to determine the ideal configuration. Finally, the chosen configuration was compared to a QIAGEN spin column to determine DNA yield and purity. The type of plasmid, chemicals, and methods were all constant variables, however, the pore size and layer number of the glass fiber membrane were independent variables. Dependent variables included the yield and purity of pure DNA. SPSS software was used for data analysis to ensure statistical accuracy and validation of findings.

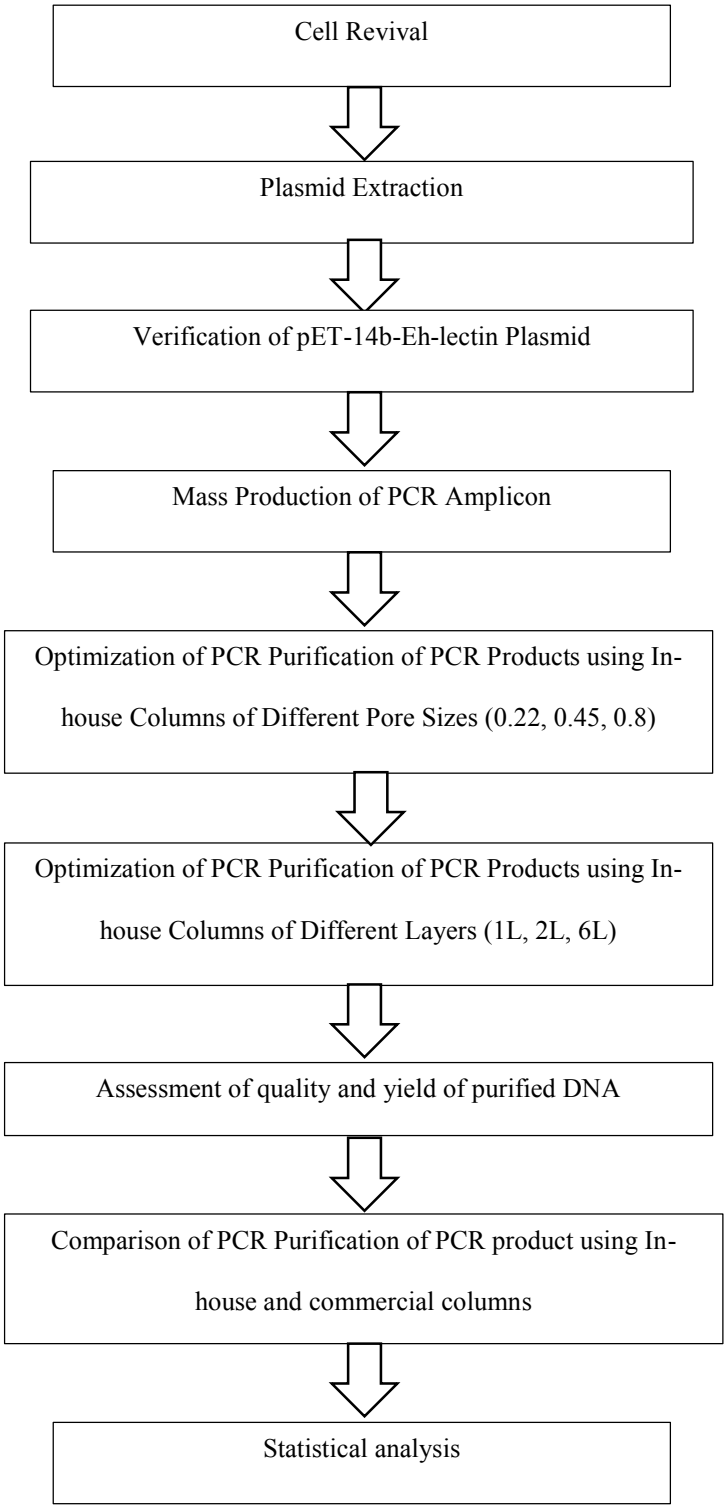

Figure 3.1 Flow chart of study