Development of Portable Thermal Cycling Device UsingStationary Block and Rotary Heaters

By

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Development of Portable Thermal Cycling Device Using Stationary Block and Rotary Heaters

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DEDICATION

"In the name of Allah, the Most Gracious, the Most Merciful"

This thesis is dedicated to the One and Only God, who gave me the strength and guidance to complete this work. I am grateful for the blessings and opportunities He has bestowed upon me throughout my academic journey.

This thesis is dedicated to my loving and supportive parents who have always supported me throughout my academic journey with unwavering love, encouragement, and sacrifice. Without their guidance, I would not have been able to reach this milestone. Their unwavering support and guidance have been the pillars of my success.

I also dedicate this thesis to my supervisor, who have been instrumental in shaping my academic and intellectual growth. His guidance, wisdom, and expertise have been invaluable, and I owe him a debt of gratitude. He is a real mentor and I am blessed to have one Alhamdullilah.

I would also like to dedicate this thesis to my Lab fellows, whose commitment and teamwork have made this journey successful.

DECLARATION

I hereby declare that this thesis entitled "Development of Portable Thermal Cycling Device Using Stationary Block and Rotary Heaters"is the result of my original research and has not been previously submitted for any academic qualification.

Dated: _____

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Iqra Iftikhar

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Abstract

The Polymerase Chain Reaction (PCR) is an important molecular biology technique that is used for DNA amplification in diagnostics, research, and healthcare. Conventional PCR machines are often expensive, bulky, and power-intensive, limiting their use in low-resource settings, rural health units, educational labs, and field diagnostics. In this thesis the design, fabrication, and validation of a portable and low-cost PCR thermal cycling device has been done that uses a stationary PCR block and rotary heaters for thermal cycling. The main goal was to create a simplified and affordable alternative to commercial thermocyclers using accessible technologies such as 3D printing, CNC machining, and microcontroller-based automation.

The structural design of the device was carried out using Onshape CAD software. Mechanical components were fabricated using PLA-based Fused Deposition Modeling (FDM) 3D printing and CNC machining of aluminum parts. Key components included the PCR device base, rotary heater frame, stepper motor holder, heat sink slider and gears. Aluminum was selected for parts that required high thermal conductivity, such as the PCR tube block and heat sinks, while PLA was used for lightweight structural parts to maintain portability. The thermal cycling system was based on a rotary heater assembly that could rotate under the fixed PCR block.

The electronic control system was built using Arduino Uno microcontroller and simulated using Proteus. It is a Professional software before hardware implementation. The control system managed the heater's temperature using a PID-controlled feedback loop and thermistors. A stepper motor, driven through an L298N motor driver, controlled the rotation of the heater. A transistor-based circuit regulated a 12V fan for cooling. The power supply and sensor feedback circuits were designed for thermal stability and reliability during long-term operation.

Thermal performance tests confirmed that the device could reach 95°C for denaturation within 90 seconds and cool down to 72°C and 52°C for extension and annealing, respectively, within 30–40 seconds. The temperature range was maintained within a ±1.5°C tolerance, which is suitable for effective DNA amplification. The system functioned reliably over multiple cycles without structural or electronic failure.

To validate its biological functionality, DNA amplification of Plasmodium vivax was carried out using standard PCR reagents and a 30-cycle protocol. DNA was extracted using a commercial GF-1 kit, and the resulting PCR products were analyzed through agarose gel electrophoresis. The gel images showed clear DNA bands at the expected size in all samples, confirming that the device successfully executed denaturation, annealing, and extension steps. Although the bands were slightly less intense than those produced using a commercial thermocyclers but even then the results confirmed that the prototype could perform basic molecular diagnostics.

Despite its success, the device had a few limitations. It is not a real-time fluorescence-based qPCR. Small misalignments in heater-block contact could slightly affect performance. Nevertheless, the device met its goals of affordability, portability, and reliability for conventional PCR applications.

In conclusion, the developed system demonstrates a promising step toward decentralized molecular diagnostics. It offers a practical solution for DNA amplification where conventional thermocyclers are not accessible. Its ease of fabrication, low cost, and stable operation make it suitable for use in academic laboratories, rural healthcare units, field-based research, and emergency diagnostic setups. Future improvements may include integrating real-time fluorescence detection, wireless monitoring, and further miniaturization. This project and shows how accessible technologies can meet essential scientific needs.

.

Abstract

CHAPTER: 01

INTRODUCTION

Introduction

1.1 Background

Polymerase Chain Reaction (PCR) is a molecular biology technique used to amplify specific DNA sequences through enzymatic replication. It was first introduced by Dr. Kary B. Mullis in 1983 while he was working at the Cetus Corporation, a biotechnology company based in California, USA. Dr. Mullis was awarded with the Nobel Prize in Chemistry in 1993 for this invention.Molecular diagnostics has revolutionized healthcare by enabling early detection of infectious and genetic diseases, such as malaria, sickle cell anemia, and viral infections. This is done by amplifying trace amounts of pathogen-specific DNA or RNA with high sensitivity and specificity.(Niemz et al., 2011). PCR is a gold-standard technique for amplifying trace amounts of DNA/RNA with high sensitivity and specificity (Debnath et al., 2010).It is widely used in biology, medicine, and research to study small amounts of DNA. PCR allows scientists to target and multiply a selected region of DNA through a series of repeated steps. The process begins with heating the DNA to a high temperature (around 94–95°C), which causes the double-stranded DNA to separate into two single strands. This step is called denaturation. Next, the temperature is lowered (around 50–65°C) to allow short DNA pieces called primers to bind to the specific starting points on the single strands. This is known as the annealing step. Then, the temperature is increased to about 72°C, which allows a heat-stable enzyme called DNA polymerase to start building new strands of DNA by adding nucleotides. This stage is called extension. These three steps are repeated many times, usually between 20 and 40 cycles. As a result, the amount of target DNA doubles with each cycle, leading to millions of copies in a short time.

Despite its critical role, conventional PCR technology faces limitations in portability, cost, and operational complexity that hinders its accessibility in resource-limited settings. It rely on bulky thermoelectric modules, high energy

consumption, and limited portability which makes them impractical for point-of-care (POC) applications in resource-limited settings(Luo et al., 2025).

1.2 Traditional DNA Amplification Techniques

The Polymerase Chain Reaction (PCR) remains one of the most widely used techniques for DNA amplification (Fornells E, 2020).Studies have shown that the standard PCR process typically takes 1–2 hours to complete, depending on the number of cycles and the length of the DNA fragment being amplified. The temperature ramping speed of most economical thermal cyclers are relatively slow. And these thermal cyclers are still too expensive for low-resource setting uses (Wong et al., 2015).

A classic type of PCR device uses a solid metal block, usually made of aluminum that contains several small holes. These holes are made to fit standard plastic PCR tubes where the DNA samples are placed. The metal block helps in transferring heat evenly to all the tubes, so each sample receives the same temperature. Heating and cooling of the metal block are done using special components. One of the most common module used in PCR devices is the Peltier module. It is a solid-state device that has no moving parts and works by using electric current to produce heating or cooling. When the current direction changes, the same module can either heat or cool the block. This allows the PCR machine to quickly switch between the high and low temperatures needed for the PCR steps. A temperature sensor is also attached to the block to monitor the exact temperature and ensure it stays accurate during each cycle(Sidstedt, 2018).

The use of microfluidic technology has also made conventional molecular diagnostic methods more viable. Microfluidic platforms are also called Lab-on-Chip systems. They enable the miniaturization of diagnostic procedures, saving time and resources (Zubia, 2023).The small volume of fluid in the microfluidic channels reduces reagent consumption which makes the process more cost-

effective and they could achieve faster thermal cycling due to the small size of the fluid volumes (Si H, 2020).

PCR has a wide range of applications in various fields of science, medicine, and research. One of the most common uses of PCR is in medical diagnostics, where it is used to detect the presence of viruses, bacteria, or genetic mutations in patient samples. For example, PCR is used to identify infections such as COVID-19, tuberculosis, and hepatitis by amplifying the genetic material of the relavent pathogen. In genetic testing, PCR helps in identifying inherited diseases by detecting mutations in specific genes. It is also used in cancer research to detect oncogenes or monitor gene expression levels (Valones et al., 2009). In forensic science, PCR is used to analyze small DNA samples collected from crime scenes, making it possible to match DNA profiles even from a tiny trace of evidence (Budowle et al., 1994). In the field of agriculture, PCR is applied to detect genetically modified organisms (GMOs) in crops and to identify plant diseases (Levin, 2004). Environmental scientists use PCR to monitor water and soil for the presence of harmful microorganisms (Bej & Mahbubani, 1992). In research laboratories, PCR is a basic tool for cloning genes, studying gene functions, and preparing DNA for sequencing. Its speed, sensitivity, and accuracy make PCR an essential technique in both basic and applied biological sciences (Dieffenbach & Dveksler, 1993).

1.3 Research Objectives and Research Questions

This study aims to design and fabricate a portable thermal cycler that integrates a stationary aluminum block for sample holding and temperature stability and rotary heaters for rapid heating and cooling, optimizing thermal performance and operational efficiency.

Key objectives of this study are:

- To design and fabricate a Stationary Block rotary heater thermal cycling device
- Optimize thermal performance to achieve temperature uniformity across denaturation (95°C), annealing (55°C), and extension (72°C).

- Validate diagnostic accuracy using *Plasmodium vivax* DNA targets.

1.4 Problem Statement

The speed at which samples reach target temperatures is directly influenced by a thermal cycler's ramp rate; slower rates (2–3°C/sec) result in longer PCR durations and decreased efficiency. These delays restrict the use of PCR for quick diagnosis and have an impact on the quality of the amplification. This study is designed to achieve quicker ramp rate by effective temperature management using stationary block and rotary heaters with integrated heat sinks and cooling fans.

1.5 Significance of the Study

Polymerase chain reaction (PCR)-based techniques have revolutionized the detection and monitoring of infectious diseases. However, their widespread implementation remains limited by factors such as high costs, infrastructure dependency, and energy demands. This research contributes to the development of a compact, cost-effective, and energy-efficient thermal cycling device aimed at addressing these limitations. The design supports decentralized testing and aligns with global health priorities, especially in low-resource environments.

This work addresses three critical gaps in the field of molecular diagnostics:

- The development of a low-cost and portable thermal cycling device has the potential to significantly expand access to PCR-based diagnostics.
- By enabling rapid DNA amplification and on-site detection, the device aims to minimize diagnostic turnaround times.

CHAPTER: 02
LITERATURE REVIEW

Literature Review

2.1 Introduction

Polymerase Chain Reaction (PCR) is a cornerstone of molecular biology through which exponential amplification of DNA can be done for applications in diagnostics (e.g., pathogen detection), forensics (DNA profiling), and biotechnology (gene cloning). Thermal cycling involves repeatedly changing the temperature to allow DNA denaturation (95°C), primer annealing (54°C), and strand extension (72°C), enabling DNA replication using heat-stable polymerases.Recent advancements like quantitative PCR (qPCR) and digital PCR have broadened the applications of thermal cycling, but they depend on accurate temperature control(Higuchi et al., 1993; Vogelstein & Kinzler, 1999).

2.2 Conventional Thermal Cyclers

Traditional bench top PCR systems typically utilize Peltier elements for bidirectional temperature control, offering high thermal precision(Innis et al., 1990). However, these systems present several limitations that restrict their use in field-based or low-resource settings. One major drawback is their bulk and immobility. As most commercial PCR machines weigh more than 10 kilograms, making them impractical for portable applications. Additionally, they suffer from slow thermal cycling, with cooling phases often exceeding 10 seconds due to reliance on passive heat sinks. Furthermore, Peltier devices are highly energy-intensive, requiring between 100 to 1,500 watts of power and wasting over 50% of the energy as heat. These challenges collectively hinder the deployment of traditional PCR systems in decentralized andLow resource settings.

2.3 Limitations of Conventional PCR systems

Thermal cyclers have improved over time to make PCR faster (Farrar & Wittwer, 2015), but many of these machines are not useful in low-resource settings (LRS). This is because they are expensive, complex, and need a lot of

power. Traditional PCR machines heat and cool the reaction chamber, but their heavy parts heat up and cool down slowly (Xiang et al., 2005). Some new methods like shuttle PCR and continuous-flow PCR (CF-PCR) try to fix this by moving the liquid through areas with different temperatures (Obeid et al., 2003). However, these methods have their own problems, like difficulty in moving the fluids and reagents sticking to surfaces. Other ideas, like using computer heat sinks for heating also do not work well in low-resource places because they are too hard to use and the chemicals are not stable(Farrar & Wittwer, 2015).

2.4 Global Health Perspective on Infectious Diseases

Infectious diseases continue to be a major threat to global health, especially in low- and middle-income countries. Illnesses such as malaria, tuberculosis, HIV, and viral infections like COVID-19 spread quickly and cause many deaths each year(Nguyen et al., 2020)(Smyrlaki et al., 2020). Poor sanitation, lack of clean water, limited healthcare facilities, and slow diagnosis make the situation worse. Many people in remote areas do not have access to labs or hospitals. This makes early detection and treatment difficult. To control these diseases, there is a strong need for portable and affordable diagnostic tools. PCR technology, when made small and easy to use, can help detect infections quickly and accurately, even in areas with limited resources(Foudeh et al., 2012). This can greatly improve health outcomes and reduce the global burden of infectious diseases.

2.5 Technological Innovations in Nucleic Acid Testing

2.5.1 Nucleic Acid Extraction Techniques

Nucleic acid extraction is a fundamental step in molecular biology used to isolate DNA or RNA from biological samples such as blood, tissues, cells, or microorganisms. The quality and purity of the extracted nucleic acids directly affect the success of downstream applications like PCR, sequencing, or cloning. The goal of nucleic acid extraction is to break and open the cells, remove proteins and other contaminants, and collect pure DNA or RNA (Chen et al.,

2019),.

The extraction process typically involves three main steps: cell lysis, removal of proteins and contaminants, and purification of nucleic acids. Cell lysis is usually performed using chemical lysis buffers containing detergents and enzymes like proteinase K. These break open the cell membrane and nuclear envelope, releasing nucleic acids into solution. In some methods, mechanical disruption or heat may also be used to improve lysis (Li et al., 2021).

After lysis, proteins and other unwanted cellular components are removed using organic solvents such as phenol-chloroform or by using salt precipitation methods. Then the nucleic acids are purified using alcohol precipitation, silica column-based kits, or magnetic bead-based systems. Commercial kits have simplified this process and are commonly used due to their speed, safety, and reproducibility (Tang et al., 2020).

The choice of extraction method depends on the sample type, the type of nucleic acid required (DNA or RNA), and the intended application. High-purity nucleic acids are critical for accurate and reliable PCR results, making the extraction step one of the most important parts of any molecular workflow.

2.5.2 Nucleic Acid Amplification Methods

Nucleic acid amplification methods are techniques used to increase the quantity of a specific DNA or RNA sequence in a sample. These methods are essential in molecular biology, diagnostics, forensics, and genetic research, especially when the target nucleic acid is present in very small amounts. Amplification enables accurate detection, identification, and analysis of genetic material (El Wahed et al., 2021),.

The most widely used method is Polymerase Chain Reaction (PCR), which amplifies specific DNA sequences through repeated cycles of denaturation, annealing, and extension. PCR is highly specific, sensitive, and can produce millions of copies of a target sequence within a few hours. Various types of PCR have been developed, including conventional PCR, real-time PCR (qPCR),

and reverse transcription PCR (RT-PCR), which is used to amplify RNA by first converting it to complementary DNA (cDNA)) (Fire & Xu, 1995).

In addition to PCR, isothermal amplification methods have gained attention due to their ability to amplify nucleic acids at a constant temperature, eliminating the need for thermal cycling equipment. Examples include Loop-Mediated Isothermal Amplification (LAMP), Recombinase Polymerase Amplification (RPA), and Nucleic Acid Sequence-Based Amplification (NASBA). These methods are particularly useful for point-of-care diagnostics and field applications due to their simplicity and speed (Xie et al., 2024)..

2.5.3 Detection Techniques

Detection techniques are used to visualize and confirm the successful amplification of nucleic acids following PCR or other amplification methods. These techniques help determine the presence, quantity, and quality of the target DNA or RNA. The choice of detection method depends on the type of experiment, required sensitivity, and available resources (Sayad et al., 2018).

The most commonly used method for post-PCR detection is gel electrophoresis. In this technique, the amplified DNA is loaded into wells of an agarose gel and separated based on size using an electric current. After electrophoresis, the DNA bands are stained with a dye such as ethidium bromide or SYBR Safe, which binds to DNA and fluoresces under UV light. The appearance of a band at the expected position confirms successful amplification (Abi et al., 2018).

For more sensitive and quantitative detection, real-time PCR (qPCR) is used. It involves fluorescent dyes or probes that emit signals during the amplification process. The fluorescence intensity is measured in real-time and correlates with the amount of DNA produced, allowing both detection and quantification in a single run (Kulkarni & Goel, 2020),.

Other detection methods include lateral flow assays, which are simple paper-based strips used especially in field diagnostics, and fluorescent signal detection

systems coupled with isothermal techniques for point-of-care applications. Some advanced techniques also use microfluidic chips, electrochemical sensors, or colorimetric detection, depending on the application.

2.6 Point-of-Care Testing (POCT): The Future of Diagnostics

2.6.1 Emergence and Advantages of POCT

Point-of-Care Testing (POCT) refers to medical diagnostic testing performed at or near the site of patient care, rather than in a centralized laboratory. The emergence of POCT has transformed healthcare by enabling rapid, on-site decision-making and timely treatment, especially in emergency settings, rural areas, and resource-limited environments. Advances in microfluidics, biosensors, and portable devices have made it possible to carry out complex tests, such as nucleic acid amplification, outside traditional laboratory setups (Ming et al., 2024). POCT offers several advantages, including faster turnaround times, reduced sample handling, minimal infrastructure requirements, and improved patient management. It also plays a crucial role during outbreaks and public health emergencies by allowing rapid screening and monitoring at the community level (Xie et al., 2022). As a result, POCT is becoming an essential tool in modern diagnostics, bringing convenience, speed, and accessibility to various fields, including infectious disease detection, chronic disease monitoring, and personalized medicine(Tian et al., 2020).

2.6.2 Commercial POCT Systems and Their Limitations

Several commercial Point-of-Care Testing (POCT) systems are currently available and widely used in clinical and field settings. Devices such as the Cepheid GeneXpert, Abbott ID NOW(Dugas et al., 2014),, and Roche Cobas Liat offer rapid and accurate detection of various pathogens, including those responsible for respiratory and sexually transmitted infections (Smithgall et al., 2020). These systems typically integrate sample preparation, nucleic acid amplification, and detection into compact, automated platforms. While they provide high sensitivity and specificity, there are notable limitations. Many

commercial POCT devices are expensive, limiting their accessibility in low-resource settings. They often require proprietary cartridges or reagents, which adds to the operational cost and can lead to supply chain issues. Additionally, their dependence on electricity and specialized hardware makes them less suitable for remote or off-grid locations. Some systems are also limited in throughput, processing only one or a few samples at a time. Despite these challenges, ongoing innovations aim to improve affordability, portability, and scalability, making POCT more accessible and practical for broader use in global healthcare.

2.6.3 An Open, Rotating Cartridge System for Affordable POCT

The incompatibility between different instruments and cartridges could also lead to the waste of medical resources. Moreover, most closed cartridges had high transportation costs, limited flexibility, and difficulty adapting to both liquid and lyophilized reagents. These factors, such as high research and development costs, expensive manufacturing due to complex molds, and high storage and transportation costs, led to the high cost of individual tests for most commercial point-of-care nucleic acid testing products which remained prohibitively expensive for underdeveloped regions.

2.7 Innovations and Challenges in Miniaturized Thermal Cycling Systems

Recent advancements in thermal cycler miniaturization have yielded several promising alternatives to conventional bench top PCR instruments. Microfluidic chips and infrared-based systems have addressed many challenges including large size and high power consumption associated with traditional designs. However, these innovations have introduced new complexities that demand further optimization.

Microfluidic thermal cyclers reduce reagent consumption and enable compact designs, but they introduce significant fluidic complexity, posing a higher risk of cross-contamination(Ahrberg et al., 2016). Miniaturization often worsen the

thermal management issues, as reduced device size limits heat dissipation capacity. Materials with high thermal conductivity, such as aluminum nitride ceramics, have been proposed to enhance heat transfer(Negm et al., 2022). Additionally, power consumption remains a barrier for battery-operated devices. Solar-powered systems and energy recovery circuits show promise but require further optimization(He et al., 2020).

Thermal cycling mechanisms are broadly categorized into contact and non-contact heating systems. **Contact heating**—where heaters are directly attached to or integrated into the chip—or **Non-contact heating**, where heat is applied externally without physical contact. Contact heating systems often employ MEMS-based thin film heaters, known for their low thermal mass and high heating rates (up to 10 °C/s) (Pawlak & Lebioda, 2018). For instance, (Kim et al., 2012)introduced a system with thin film heating and rapid cooling via a fan, while similar approaches were discussed in (Bu et al., 2013)(Sun et al., 2015).

2.8 Strategies for Cost-Efficient Heating and Temperature Control in PCR Systems

To address the cost and complexity of integrated heater fabrication, researchers have proposed physically decoupling the heating element from the PCR reaction chamber, thereby enabling reuse of heating modules across multiple disposable chips (Zhu et al., 2014)(Neuzil et al., 2006). Flexible thin film heaters are a promising low-cost option, delivering heating rates above 6.5 °C/s. Peltier devices are also frequently used for contact-based heating and cooling, achieving temperature changes of 2–3 °C/s with commercial controllers (Hatch et al., 2011).

While these separated heating systems offer the advantages of cost-effectiveness and reusability, they may inadvertently introduce additional thermal mass and contact resistance, which can delay heating or cooling transitions and impact PCR efficiency. Therefore, precise temperature regulation becomes critical in such systems.

2.8.1 Temperature Control Algorithms

Maintaining tight temperature control during PCR cycles is essential to avoid issues such as overshooting, undershooting, or delayed transitions—all of which can affect DNA amplification fidelity. Over time, control strategies have progressed from basic on/off switching circuits to more sophisticated digital systems. Analog temperature controllers were once the norm(Bolognesi et al., 2015), while modern systems incorporate digital electronics and data acquisition (Gou et al., 2018).

Among the various control approaches, **Proportional–Integral–Derivative (PID) controllers** are most commonly employed due to their adaptability and accuracy(Dinca et al., 2009). However, conventional PID systems often struggle with rapid thermal cycling, leading to temperature overshoot and sluggish stabilization, especially in high-speed PCR protocols(Niu et al., 2006). This limitation has prompted exploration into adaptive and model-predictive control algorithms, which better handle nonlinear system dynamics.

2.9 Thermal Challenges in Rotational Platforms and LOD Integration

The **lab-on-a-disc (LOD)** concept has garnered considerable attention as an effective and viable approach for point-of-care testing (POCT), primarily due to its ability to exploit centrifugal forces via simple rotational motion. Centrifugation plays a critical role in various molecular diagnostics—for instance, in DNA purification post-cell lysis, where separation of reagents into a DNA-containing supernatant and cellular debris is essential(Choi & Yoo, 2015). LOD platforms can utilize centrifugal force to drive fluid propulsion, mixing, and aliquoting without external pumps(Mark et al., 2011)(Park et al., 2012).

One of the primary challenges in integrating polymerase chain reaction (PCR) into an LOD platform lies in achieving non-contact thermal regulation that accommodates disc rotation. Commonly used LOD materials, such as polydimethylsiloxane (PDMS) and polycarbonate, are typically over 0.6 mm

thick and possess inherently low thermal conductivity. This hinders precise external temperature monitoring and control of the PCR reagents within the disc. Due to this limitation, performing PCR on an LOD remains technically challenging, despite PCR's reputation for being a sensitive and specific method of DNA amplification.

Several research efforts over the past decade have attempted to overcome these hurdles with promising outcomes. For instance, (Roy et al., 2015)employed Peltier elements for heating and cooling the disc surface and used wired thermocouples for temperature measurement. However, this setup necessitated a vacuum to connect the Peltier to the disc, requiring bulky auxiliary equipment such as air pumps, which increases the system's size and power consumption. Additionally, the use of wired thermocouples disrupts disc rotation. Cooling technologies are equally critical in determining PCR efficiency. Peltier-based cooling continues to offer high precision, but it is also among the most energy-intensive approaches (Rajput et al., 2023).

2.10 The Need for Hybrid PCR Systems

The problems mentioned above show that there is a need for a better thermal cycling system. This new system should be simple to build, use less power, and give correct results. In the future, designs may use small heating blocks, wireless sensors to check temperature, and smart control systems. These features can help make the device smaller, easy to carry, and still work well. A good system would mix the simple design of LOD (lab-on-a-disc) platforms with strong heating control. This would help move from big lab machines to small, useful tools in the field.

2.11 Addressing the Global Burden of Infectious Diseases with Portable PCR Systems

In many parts of the world, especially in poor areas, people suffer more from infections because testing takes too long. This makes it very important to

develop portable PCR systems. One big problem is that most people who need better testing live in places with very few lab facilities . (Yager et al., 2006). For example, in sub-Saharan Africa, 45% of malaria deaths happen because the disease is not diagnosed in time (Castillo-Fernández et al., 2024. Normal PCR machines need special lab setups, so they cannot be used in such places. Some new portable PCR devices are available, but they often do not work as well or give reliable results (Ameenudeen & Hemalatha, 2024).

There is still need for a thermal cycler that can work fast, use less power, and give accurate results. The goal is to complete each cycle in less than 30 seconds, use less than 50 watts of power, and keep the temperature very even (within ±0.5°C). Some rotary systems, like those that use joule heating, can heat and cool quickly. But they often do not keep the temperature stable when running many samples at the same time (Gui & Ren, 2008). Another problem is cost. Many portable PCR machines, like Biomeme™, are too expensive for regular use in all places. To solve these problems, we need to think of new ways to heat the samples and use better materials.

2.12 Implications for Global Health

The development of portable and reliable PCR systems make it easier to detect diseases quickly, especially in places where hospitals and labs are not available. Faster diagnosis helps start treatment early, which can save lives and stop the spread of infections. This is very important during outbreaks and in areas where diseases like malaria, tuberculosis, or COVID-19 are common. Low-cost and easy-to-use PCR systems can improve healthcare in poor regions, reduce the burden of infectious diseases, and support public health goals around the world.

CHAPTER: 03
MATERIALS AND METHOD

MATERIALS AND METHODS

3.1 Materials

Components used in the development ofStationary Block and Rotary Heaters (SBRH) are aluminum PCR block, two heat sinks, two cooling fans, To provide accurate temperature control and stable device functioning, PID controller, Arduino Mega 2560, Threethermistors, two heaters, MOSFETs, and other electrical components are carefully integrated.

3.2 Experimental Setup:

3.2.1 Thermal Cycling Technologies in PCR

The efficiency and accuracy of PCR largely depend on thermal cycling technology, particularly on how quickly and evenly temperature changes occur. Resistive heaters can heat faster over 5°C per second and they need currents of 12 Volts to work well.

This study used a rotary heater-based thermal cycling system in which a fixed PCR block with rotating heaters are used. To lower the temperature of PCR block quicky for the annealing step, two aluminum heat sinks were used. They were positioned beside the block.This setup enables fast temperature changes.

3.3 Design of the Apparatus:

3.3.1 Computer-Aided Design (CAD) Using Onshape

Onshape, a cloud-based computer-aided design (CAD) software, was used to design and model the primary components of the thermal cycling device, including the rotary heater, stationary block, and sample holder. The platform enabled accurate 3D modeling, real-time design modifications, and assembly simulations to ensure dimensional compatibility and functionality. Collaborative tools within Onshape facilitated timely feedback and design refinement. Finalized models were exported in standard file formats for fabrication using 3D printing and conventional machining techniques. A metal tube block was designed with a size sufficient to accommodate 16 PCR tubes. PCR tubes can be firmly fixed inside the tube block, while Heaters frameis adjusted below through Rotary Door Heater Mechanism (RDHM). This 3D designing was done

on CAD OnShape.

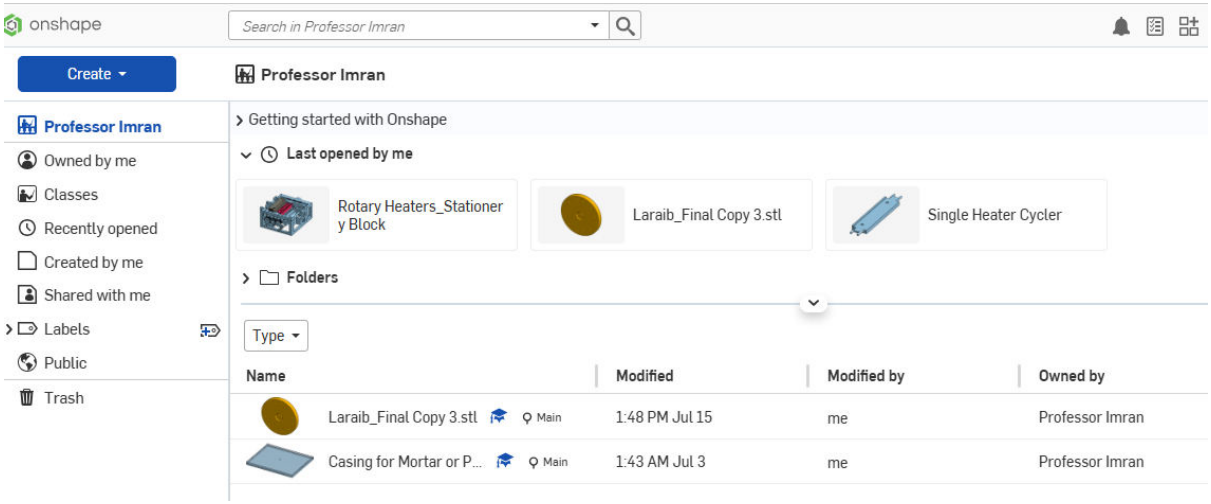

Figure 1 CAD OnShape interface

3.4 Fused Deposition Modeling (FDM)

Fused Deposition Modeling (FDM) was utilized to fabricate the custom-designed components of the thermal cycling device. A Geeetech A10T 3D printer was used for printing, which operates by extruding thermoplastic filament through a heated nozzle to build objects layer by layer. The CAD models designed in Onshape were exported in STL format and sliced using appropriate software to generate G-code for printing. Polylactic acid (PLA) filament was selected due to its biodegradability, ease of handling, and good dimensional stability. The nozzle temperature was set to 210 °C, and the print bed was maintained at 50 °C to ensure proper adhesion and minimize warping. This method allowed for the rapid prototyping of complex geometries, including rotary holders, component supports, and alignment structures, with high precision and repeatability.

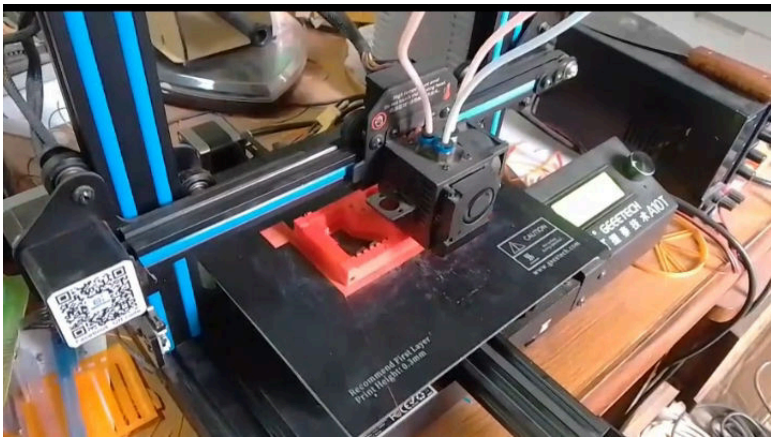

Figure 2 FDM Printer

3.5 Heating Mechanism:

Two heaters are controlled through an Arduino-based looped heating cycle. A PID (Proportional-Integral-Derivative) controller is integrated to ensure precise temperature regulation and stability. The heating sequence follows a continuous loop as follows:

Step 1: Heater 1 → 95°C for 25 sec (15–30 seconds) | Heater 2 → 54°C for 45 sec (30–60 seconds)

Step 2: Heater 1 → 72°C for 1 min(60 sec to several minutes)| Heater 2 → 95°C for 25 sec

Step 3: Heater 1 → 54°C | Heater 2 → 72°C (Simultaneously).

Step	Heater 1 Target (°C)	Duration	Heater 2 Target (°C)	Duration
1	95	30 sec	54	40 sec
2	72	100 sec	95	40 sec
3	54	30 sec	72	100 sec
→ Repeat from Step 1				

The cycle repeats in a loop, ensuring consistent thermal cycling for DNA amplification.

Although first cycle takes longer to completely denature the DNA strands before starting the cycles. After that cycle repeats in a loop. And final extension also take longer about 4-5 minutes to ensure that all PCR products are fully

extended. The PID controller maintains each temperature precisely, compensating for heat losses and fluctuations to optimize the efficiency of the amplification process.

3.6 Cooling System:

To ensure efficient heat dissipation during the annealing step, a cooling system is integrated with the PCR block. This system consists of:

1. **Heat Sink**
   - To lower the temperature from 95 to 54, aheat sink (made of aluminum) is positioned at the side of PCR Block to come in direct contact with the PCR block before the annealing step.
   - A rack and pinion system moves the heat sink into place, ensuring efficient heat transfer.
2. **Active Cooling with Fans**
   - Cooling fans are positioned alongside the PCR tube block to dissipate excess heat when required.
   - These fans are controlled by the PID system, ensuring precise temperature managementand reducing ramp time.

This combined approach ensures rapid cooling, enabling a smooth transition to the annealing temperature while maintaining system efficiency.

3.7 Electrical Components

3.7.1 Heaters (12V, 6.7A)

Two 100k resistive heaters were used to provide the required temperatures for the PCR process. These heaters helped in reaching the specific temperatures needed for denaturation, annealing, and extension. A PID controller was used to manage the temperature, allowing it to change smoothly and accurately whenever needed during the thermal cycling steps.

Figure 3PTC Heaters

3.7.2 Thermistors (100kΩ)

A thermistor is a type of temperature sensor that tells us a predictable change in resistance with variations in temperature. It is widely used in thermal control systems due to its high sensitivity, fast response time, and compact size. Thermistors are typically classified into two categories: Negative Temperature Coefficient (NTC) thermistors, where resistance decreases with increasing temperature, and Positive Temperature Coefficient (PTC) thermistors, where resistance increases with temperature. In thermal cycling devices, NTC thermistors are often preferred for precise temperature monitoring and feedback control that enables accurate regulation of heating elements during amplification processes. 100 k thermistor.Thermistors were chosen for their precise temperature sensitivity and ease of integration with microcontroller-based systems (Elangovan et al., 2022). For this study 100 kΩ NTC thermistor (100 kΩ at 25 °C) fixed resistor equal to 100 kΩ are used. The 100kΩ thermistors are widely used in thermal management applications due to their reliability and compatibility with the Steinhart Hart equation for temperature calculations (Steinhart & Hart, 1968)

Figure 4 Thermistor

3.7.3 MOSFETs (IRF)

The Metal-Oxide-Semiconductor Field-Effect Transistor (MOSFET) is a vital component in electronic circuits, especially for switching and amplification purposes. In thermal cycling devices, MOSFETs efficiently regulate the power supplied to heaters by acting as high-speed electronic switches controlled by input from temperature sensors like thermistors. For this project, IRF MOSFETs were chosen due to their high current-handling capability which ensures minimal heat loss and efficient operation during heater switching. Additionally, their logic-level gate operation allows direct control through the Arduino's PWM pins without the need for additional driver circuitry. This simplified the design while maintaining precise thermal regulation (GANESH et al.)(Chowdhury et al., 2021). The high switching speed, low gate drive requirements, and robust performance of the MOSFETs contribute significantly to the stability and responsiveness of the temperature cycling process, which is critical for successful DNA amplification. For this study logic-level N-channel MOSFET (IRLF and IRLZ) are selected that fully switched on at 5 V. The MOSFET's source goes to ground and the drain to the heating plate (low-side switch). The Arduino PWM pin drives the MOSFET gate through a small resistor (100Ω) to limit surge current. A gate-to-source pull-down (e.g. 100 kΩ) ensures the MOSFET is off on Arduino reset.

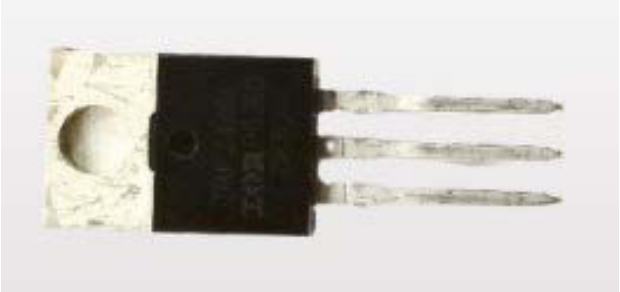

Figure 5 MOSFET

3.7.4 Stepper Motor (28 BYJ)

A 28 BYJ Stepper Motorwas used to ensure precise movement and positioning of the heaters under PCR tube block within the thermal cycling device. Stepper motors are known for their ability to divide a full rotation into a large number of discrete steps, allowing accurate and repeatable positioning without the need for external feedback. The 28 BYJ model offers a good balance between torque, size, and control simplicity which makes it suitable for laboratory automation. Its compatibility with the A4988 stepper motor driver allows smooth and accurate control through microcontrollers. This combination makes it ideal for the rotational requirements of the device, enabling consistent thermal contact and enhancing the reliability of the amplification process (Saravanan et al., 2024).

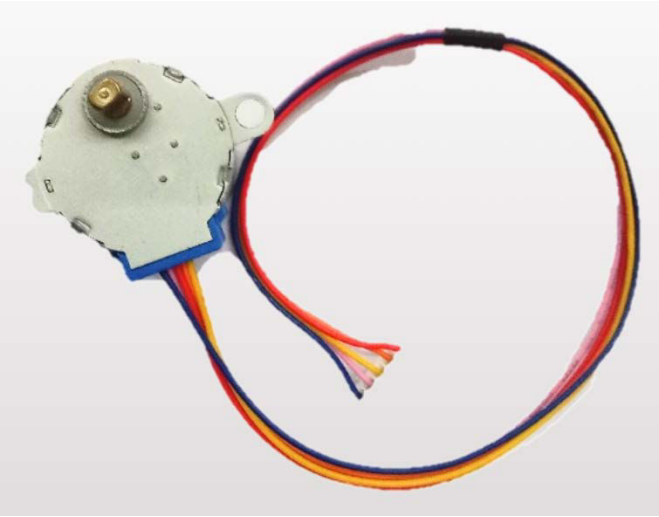

Figure 6Stepper Motor (28 BYJ)

3.7.5 Resistors (100kΩ and 220Ω)

In the thermal cycling device, 100 kΩ resistors were used as pull-down resistors

in the thermistor voltage divider circuits. They helped keep the signal stable and avoided floating voltages, which ensured accurate temperature readings. The 220 Ω resistors were used to limit the current in the LED circuits and to control the gate voltage of the MOSFETs. This helped protect the components and allowed the switching system to work properly.

Figure 7 100K resistor

3.7.6 Arduino Mega 2560

The Arduino Mega 2560 was used as the main microcontroller in the thermal cycling device. It was chosen for its large number of digital and analog input/output pins, which made it suitable for handling multiple sensors, heaters, fans, LEDs, and MOSFETs. Its built-in timers and serial communication ports also supported smooth data handling and reliable control of different components in the system. The open-source environment made it easy to program and integrate with other hardware.

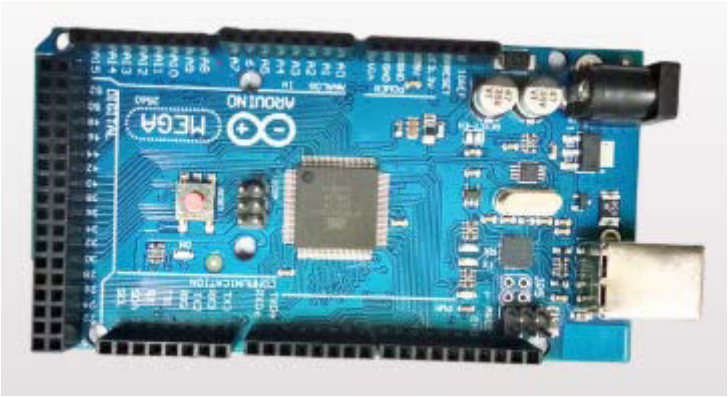

Figure 8Arduino Mega 2560

3.7.7 16x4 LCD with I2C Module (PCF8574)

A 16x4 LCD display with an I2C module (PCF8574) was used to show real-time data such as temperature, time, and device status. The I2C module allowed communication with the Arduino using only two pins (SDA and SCL), which helped save digital pins for other components. This setup made the display easy to connect, reduced wiring complexity, and improved overall efficiency in monitoring the device operation.

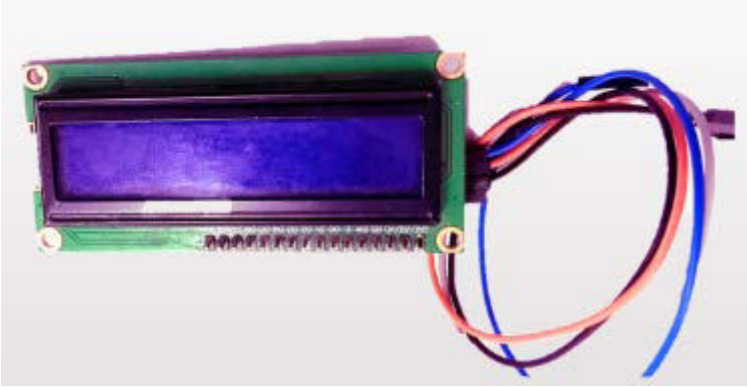

Figure 916x4 LCD with I2C Module (PCF8574)

3.8 Circuit Design and Connections

3.8.1 Thermistor Connections

Thermistors were connected in a voltage divider configuration to measure temperature. Each thermistor was paired with a 100 kΩ pull-down resistor to ensure stable voltage output. The analog output from the voltage divider was connected to the analog input pins of the Arduino Mega 2560. This allowed the microcontroller to read the temperature changes and adjust the heating or cooling accordingly. Proper placement of thermistors ensured accurate monitoring of the sample block temperature during thermal cycling.

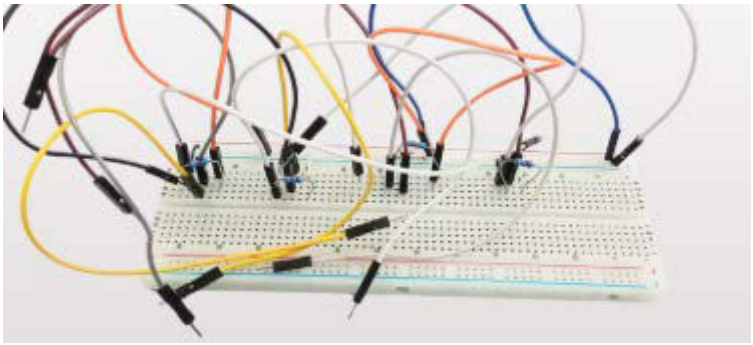

Figure 10Thermistor Connections

3.8.2 Heater Control

The heaters were controlled using N-channel MOSFETs, which acted as electronic switches. The gate of each MOSFET was connected to a PWM pin of the Arduino Mega 2560 through a 220 Ω resistor to limit the current. When the Arduino sent a HIGH signal to the gate, the MOSFET allowed current to flow through the heater, turning it on. When the signal was LOW, the heater turned off. This method allowed precise control of heating based on the temperature readings from the thermistors.

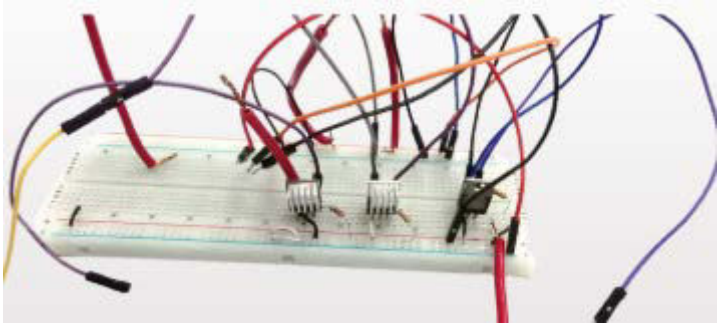

Figure 11Heater connection

3.8.3 Stepper Motor Control

A stepper motor was used to operate a rotary door mechanism that brings the heaters undern the stationary PCR block, which is placed or fixed on a rotary heating frame. The motor was controlled using a motor driver module, which received step and direction signals from the Arduino Mega 2560. These signals controlled the rotation angle and direction of the rotary door. The motor driver was powered by an external power supply to provide sufficient current. This arrangement allowed accurate positioning of the heaters beneath the PCR block during each stage of the thermal cycling process.

3.9 Software Implementation

The software for the thermal cycling device was developed using the Arduino IDE. It controlled the timing and temperature of the heaters by reading data from the thermistors. Based on the temperature readings, the program switched the heaters on or off through the MOSFETs to maintain the desired temperature cycles. The software also controlled the stepper motor to move the rotary door

mechanism at the correct times. Heat sinks and cooling fans were used to lower the temperature of the PCR block, which helped reduce the ramp time between temperature cycles. Status information and temperature data were displayed on the 16x4 LCD with the I2C module for easy monitoring. The program was designed to run automatically once started, ensuring reliable and repeatable thermal cycling.

3.10 Assembly and Testing

The thermal cycling device was carefully assembled by connecting all electronic components, including the Arduino Mega 2560, thermistors, heaters, stepper motor, LEDs, and display. Wiring was done following the circuit design to ensure correct connections and functionality. The circuit was first assembled on a breadboard to allow initial testing and troubleshooting. After successful verification, it was transferred to a soldered prototype board to improve reliability and durability. The system was tested for several key functions:

- **Temperature Accuracy:** The PID control algorithm's ability to maintain target temperatures within a tolerance of ±1°C was evaluated.
- **Thermistor Readings:** The consistency of temperature measurements from the three thermistors assigned to each heater was checked.
- **Stepper Motor Functionality:** The precision of the rotary door mechanism in positioning the heaters during thermal cycling was tested.
- **User Interface:** The responsiveness and accuracy of the rotary encoder and the LCD display were verified.

These tests ensured the overall reliability and suitability of the system for droplet-in-oil PCR applications.

3.11 Malaria as Model for testing

3.11.1 Ethical Approval and Clinical Sampling

All procedures conducted in this study were reviewed and approved by the Institutional Bioethics and Biosafety Committee (IBBC) of the International

Islamic University Islamabad (IIUI). Blood samples were collected from patients clinically diagnosed with *Plasmodium vivax* malaria at the pathology laboratory of Pakistan Institute of Medical Sciences, Islamabad.

3.11.2 DNA Extraction Using Commercial Kit

DNA extraction was performed using the GF-1 nucleic acid purification kit, following the manufacturer's standard protocol. The kit is designed for the isolation of nucleic acids from various biological specimens such as serum, saliva, body fluids, plasma, and virus-infected cell cultures, providing high efficiency and rapid processing.

Cell lysis was done through the application of denaturing agents, which facilitated the breakdown of proteins and the subsequent release of nucleic acids. The extracted DNA was purified using specialized buffers provided with the kit. Furthermore, DNA binding efficiency was improved through the use of specially treated glass filter membranes included in the kit.

Table 1 Components of GF 1 DNA extraction Kit

Components	Quantity
GF-1 columns	100
Collection tubes	100
Blood lysis Buffer	24ml
Wash Buffer-1	30ml
Wash Buffer-2	34ml
Elution Buffer	20ml
Proteinase K	2x1.5ml

Figure 12GF-1 nucleic acid purification kit

Preparation of Solutions:

For the nucleic acid extraction from 10 samples following steps were performed for solution preparation:

- 3ml of washing Buffer-1 was mixed with 3ml of absolute ethanol.
- 3.4ml of washing Buffer-2 was mixed with 8ml of absolute ethanol.

Procedures:

All steps were performed at room temperature.

Washing buffers were stored at room temperature in caped tight bottles.

Water bath was set at 65°C for cell lysis process.

Protocol for DNA extraction:

1. **Sample Lysis:**
   First, 200µl of Buffer BB was added into a 200µl blood sample in a micro centrifuge tube. 50ul of Proteinase K was added into 200ul of sample and was mixed thoroughly; and mixed consistently by pulsed-vortexing. Then it was incubated for 10 min at 65 C^0.

2. **Addition of Ethanol:**
   After that 200ul of absolute ethanol was added. It was mixed thoroughly and immediately so that any irregular precipitation of nucleic acid can be prevented due to high local ethanol concentrations.

3. **Loading to Column:**
   Sample was transferred into a column which was assembled in the collection tube provided by kit. It was centrifuged for 1min at 5,000 x g. flow through was discarded.

4. **Column washing 1:**
   Column was washed with 500ul of Wash Buffer 1 and centrifuged for 1 min at 5,000 x g. Flow through was discarded.

5. **Column washing 2:**
   Column was washed with 500ul of Wash Buffer 2 and centrifuged for 1 min at 5,000x g. Flow through was discarded. Column was again washed with 500ul of Wash Buffer 2 and centrifuged for 3 min at 14,000 x g. Centrifugation was performed for 3 min so that ethanol can be removed completely.

6. DNA Elution:

Elution Buffer was poured directly on spin column membrane to avoid the buffer contamination. After 2 minutes spin column was settled into the centrifuge machine tube. After completing this procedure centrifuge run for 1 minute at 5,000x g so that DNA can be eluted, DNA was stored at -20 C^0 so that it can be used to perform PCR reaction.

3.11.3 PCR Primers used

Table 2PCR Primers used

For detecting P.*vivax* primers sequences				
	Primer	Strand	Sequence	
2	rVIV1	Sense	F	CGCTTCTAGCTTAATCCACATAACTGATAC
			R	GTATCAGTTATGTGGATTAAGCTAGAAGC
3	rVIV2	Antisense	F	ACTTCCAAGCCGAAGCAAAGAAAGTCCTA
			R	TAAGGACTTTCTTTGCTTCGGCTTGGAAGT

3.11.3.1 PCR Mix Components

The extracted DNA was subjected to PCR amplification using primers specific for the *Plasmodium vivax* 18S rRNA gene, targeting the small subunit of the ribosome. For each PCR reaction, a total volume of 35 µL was prepared, consisting of 5 µL of purified DNA, 25 µL of HotStarTaq Master Mix (Catalog No. 203443; Qiagen), 5 µL of PCR-grade water, and 0.2 µL each of forward and reverse primers. The PCR reactions were carried out according to the manufacturer's guidelines.

3.11.3.2 Additional Equipment:

In addition to the custom-built thermal cycling device, various laboratory

instruments were utilized throughout the experiment. These included a precision micropipette set (Eppendorf), a digital weighing balance (Sartorius), a centrifuge (Eppendorf MiniSpin Plus), a UV-Vis spectrophotometer for nucleic acid quantification (NanoDrop 2000, Thermo Fisher Scientific), and a gel electrophoresis unit (Bio-Rad) for PCR product analysis. A biosafety cabinet (Class II, Thermo Scientific) was also used for all sample handling to ensure contamination-free processing.

3.11.3.3 PCR Process

PCR amplification was performed using the developed portable thermal cycling device based on stationary block and rotary heaters. The system was programmed with cycling parameters optimized for the amplification of the 18S rRNA gene of Plasmodium vivax. The device maintained stable temperature transitions through controlled rotation of the heater beneath the PCR block and consistent thermal contact with the stationary sample wells. Each amplification run included a positive control (known P. vivax DNA) and a no-template negative control to validate the specificity and accuracy of the reaction.

The cycling protocol consisted of an initial denaturation step, followed by 15–35 cycles of denaturation, annealing, and extension, and concluded with a final extension phase. Temperature settings and dwell times were adjusted based on primer melting temperature and DNA input concentration. The system's thermal uniformity and repeatability were verified prior to amplification to ensure consistent performance.

3.11.3.4 Post-PCR Analysis

About 0.9g agarose was added in 60 ml 1x TBE buffer and boiled in oven for a 1 minute and then leave it for cooling so that it can be solidified. After that 3.0µl ethidium bromide was added in gel that help to visualize the DNA under ultraviolet light. Agarose was poured into gel caster with well comb placed in it, PCR product having quantity of 5ul was mixed with 2µl loading dye bromophenol blue and loaded into well combs placed in gel caster. Gel

electrophoresis was performed at 120 V for 30 minutes until dye line was approximately 80% of way down the gel, PCR amplicons were visualized with UV light. With 2µl loading dye bromophenol blue and loaded into well combs placed in gel caster. Gel electrophoresis was performed at 120 V for 30 minutes until dye line was approximately 80% of way down the gel, PCR amplicons were visualized with UV light

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CHAPTER: 04

RESULTS&

DISCUSSIONS

Results

4.1 Final Design

The final prototype of the PCR device was successfully developed with an emphasis on simplicity, low cost, and portability. The design integrated multiple mechanical and electronic components, fabricated using a combination of 3D printing with PLA and CNC machining of aluminum parts. The following subsections describe the physical construction and integration of the system.

4.1.1 3D Design Using Onshape

The entire structural framework was designed using Onshape, Designs were iteratively improved to achieve compactness and optimal alignment of the heater, PCR block, fans, and sensors. All files were exported in STL format for 3D printing.

4.1.2 PLA-Based 3D Printed Components

Several parts were printed using PLA (Polylactic Acid) filament on a standard FDM 3D printer. PLA was chosen due to its environmental friendliness, ease of use, and dimensional accuracy. The printed components including Heater movement frame, Stepper motor housing and base support, PCR block holder and guide rails, Fan brackets and mounts and Wiring support clips and structural covers. These parts provided the necessary mechanical structure and supported proper alignment of the moving and fixed components. The lightweight nature of PLA helped maintain the device's overall portability.

4.1.2.1 PCR Device Base

The base served as the foundational platform of the device, providing mounting support for all major components, including the sample block, rotary heater assembly, and electronics. It was designed with integrated cable channels and fastening points to ensure a clean and stable layout. During testing, the base demonstrated strong adhesion to the surface and vibration resistance.

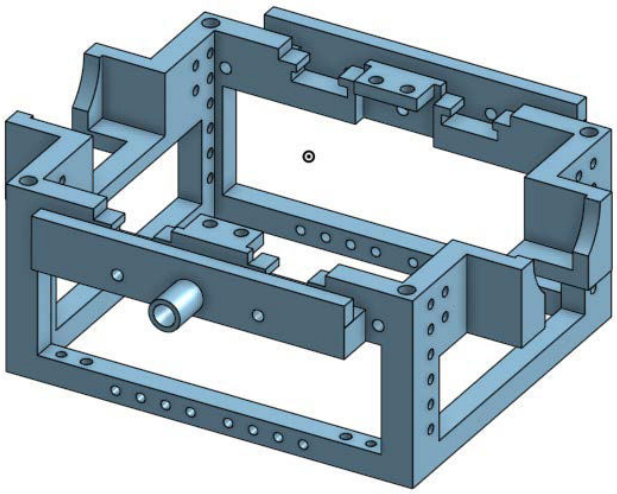


Figure 13CAD design of the PCR device base created using Onshape software.

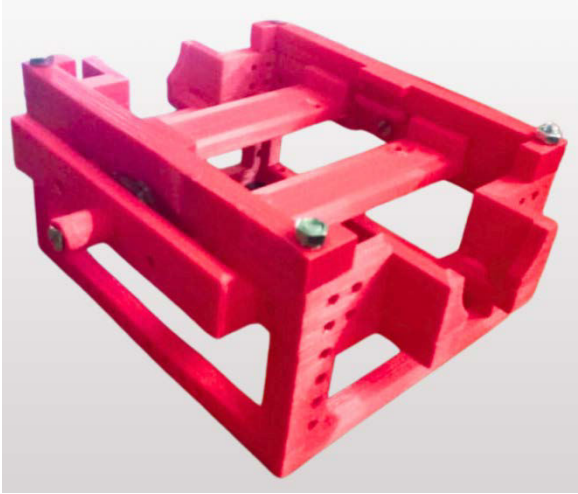


Figure 14FDM-printed PCR device base using PLA material on Greentech A10T printer.

4.1.2.2 Heater Frame

The heater frame housed the rotary heating elements and ensured accurate alignment with the sample block. It was designed to withstand repeated thermal contact and mechanical rotation. The printed frame exhibited high dimensional accuracy and no signs of thermal degradation during cycling.

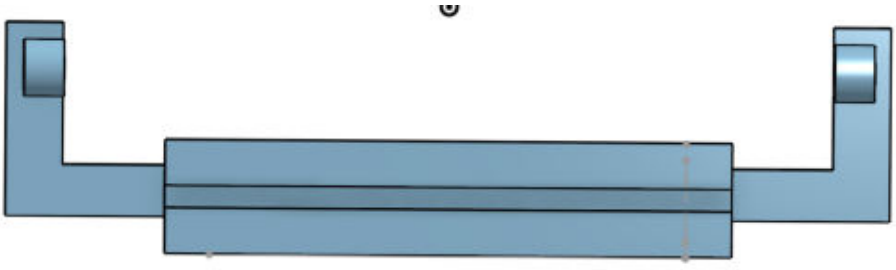


Figure 15CAD model of the rotary heater frame designed in Onshape.

Figure 163D printed rotary heater frame fabricated via FDM using PLA.

4.1.2.3 Heat Sink Slider

This slider mechanism enabled precise movement of heat sinks toward or away from the sample block to facilitate passive cooling. It operated smoothly along its track and allowed easy integration of cooling fans if required. Its movement was controlled manually and later adapted to automated actuation using gears and stepper motors.

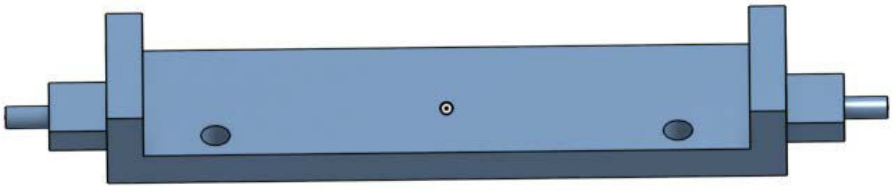


Figure 17CAD design of the heat sink slider for controlled passive cooling.

Figure 18FDM-printed heat sink slider integrated with PLA-based sliding mechanism.

4.1.2.4 Gears

Custom gears were designed to enable the controlled rotation of rotary heaters and to actuate the heat sink slider. The gear train was optimized for torque and synchronization, ensuring smooth transitions between temperature zones. These components functioned without slippage, validating their effectiveness in mechanical transfer.

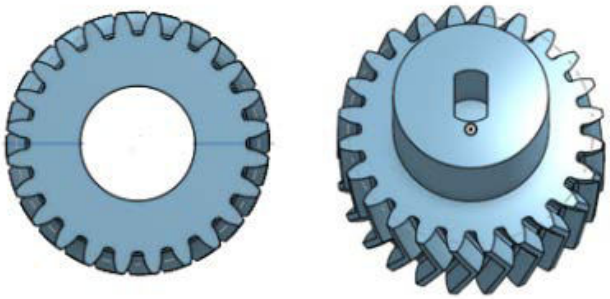


Figure 19Onshape CAD design of gear components used in the thermal cycling device.

Figure 20FDM-printed gear system optimized for torque and synchronization.

4.1.2.5 Motor Holder

The stepper motor holder was designed to secure the motor precisely in place to avoid misalignment during operation. It featured heat-resistant mounting and vibration-absorbing slots. During testing, the holder maintained firm fixation under rotational load without displacement.

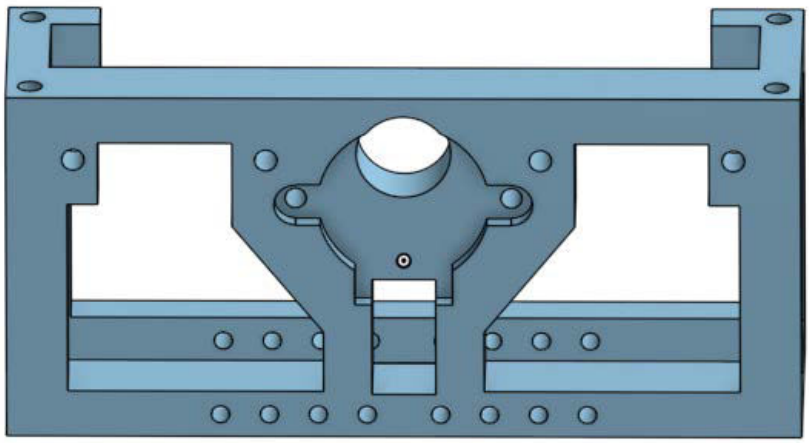
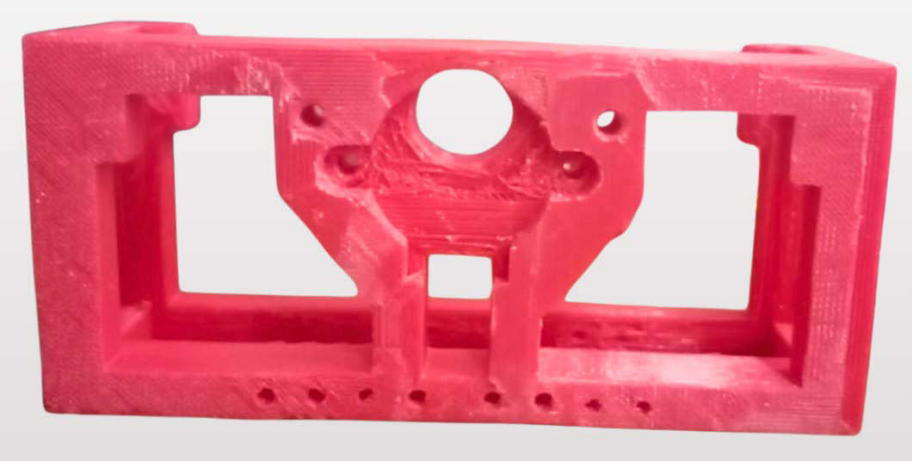


Figure 21CAD model of the stepper motor holder with thermal-resistant features.



CNC-Machined CompFigure 223D printed PLA motor holderonents

The PCR tube blockandheat sink components were fabricated from solid aluminum using CNC machining to ensure precise dimensions and thermal performance. Aluminum was selected for its high thermal conductivity and structural strength. The PCR block was designed with drilled holes to securely hold standard PCR tubes and ensure direct heat transfer during thermal cycling. The block surface was smoothed to ensure uniform contact with the heater.

4.1.2.6 PCR Block

The PCR block was designed to accommodate 0.2 mL PCR tubes in a high-density aluminum holder for efficient thermal conductivity. The block was mounted over the stationary region of the base and remained in fixed alignment with the rotary heaters. PLA brackets and insulators were used to support the block without compromising thermal performance.

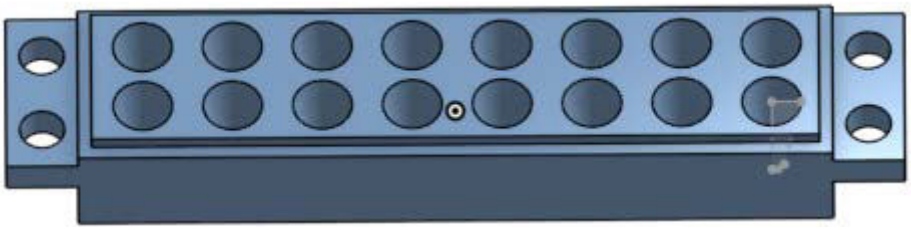


Figure 23 CAD designed PCR block

Figure 24CNC-machined PCR block made from aluminum, designed

4.1.2.7 Heat Sinks

Aluminum heat sinks were incorporated to facilitate rapid cooling during the thermal cycling process. Mounted on PLA sliders, they could be moved into contact with the PCR block during the cooling phase. Their effectiveness in dissipating residual heat was enhanced through passive airflow and optional fan integration. PLA mounts provided stability and smooth sliding motion without structural fatigue.

Figure 25 CAD designed heat sinks for passive cooling integration.

Figure 26Aluminum heat sinks machined via CNC for passive cooling integration.

4.1.3 Device assembly

Following the fabrication of individual parts, all components, both 3D printed (PLA) and CNC-machined, were assembled into a compact and functional thermal cycling system. The design emphasized modularity, alignment precision, and ease of maintenance.

The PCR device base, made from PLA, served as the central support structure. Onto this base, the CNC-machined aluminum PCR block was firmly mounted to ensure stable positioning and direct thermal contact with the rotary heaters. The PCR block was aligned precisely under the rotation path of the heating elements to maintain consistent temperature application across all sample wells.

The rotary heater frame, also PLA-based, was fixed adjacent to the PCR block and designed to rotate around it, delivering heating in discrete zones. A stepper motor, secured in a custom PLA motor holder, was connected to the gear mechanism which enabled controlled movement of the rotary heater assembly.

On the opposite side, the heat sink was mounted on a PLA slider which allowed it to move toward the PCR block during cooling phases and retract when inactive. This motion was also actuated through the gear system, allowing dual-function control—rotation of heaters and translation of the heat sink.

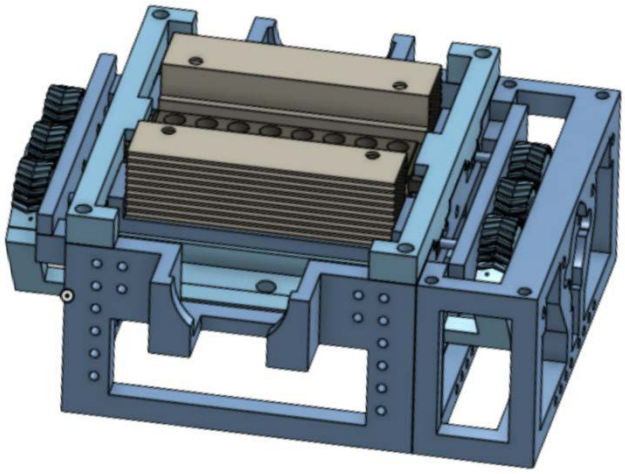


Figure 27Assembly of key components on the PCR device base showing block, heater frame, and motor holder.

Figure 28Assembly of key components on the PCR device base and CAN machined components and cooling fans mounted o above.

4.2 Circuit Design and Simulation Using Proteus

The complete electronic control system of the thermal cycling device was designed and simulated using Proteus Professional software. This platform allowed for virtual testing of circuit behavior, verifying component interaction and functional response before hardware implementation.

The control system was built around an Arduino Uno microcontroller, which served as the central processing unit responsible for managing temperature regulation, heater control, and stepper motor operation. An L298N motor driver was connected to the Arduino to enable bidirectional control of the stepper motor, which powered the rotary motion of the heaters and the translation of the heat sink slider.

Temperature monitoring was achieved using a DS18B20 digital temperature sensor. This sensor provided real-time temperature data from the PCR block to the Arduino, enabling precise feedback control of heating and cooling through a programmed temperature profile. For heater control, a MOSFET-based switching mechanism was used to regulate power supply to the resistive heating elements. Pulse-width modulation (PWM) signals generated by the microcontroller allowed fine control of heating rates and stable maintenance of

set temperatures.

In addition, a transistor-based switching circuit was used to control an optional 12V cooling fan for rapid heat dissipation during the cooling phase of the PCR cycle. A regulated 12V DC power supply was used to power the motor, heaters, sensors, and microcontroller, with necessary voltage division and filtering circuits integrated to ensure stable and safe operation.

The entire schematic was assembled and validated in Proteus. Logical and electrical simulations confirmed accurate signal transmission, temperature feedback response, synchronized motor operation, and reliable heater activation. The final simulated diagram is presented in Figure 4.2 as the complete representation of the circuit architecture used in the device.

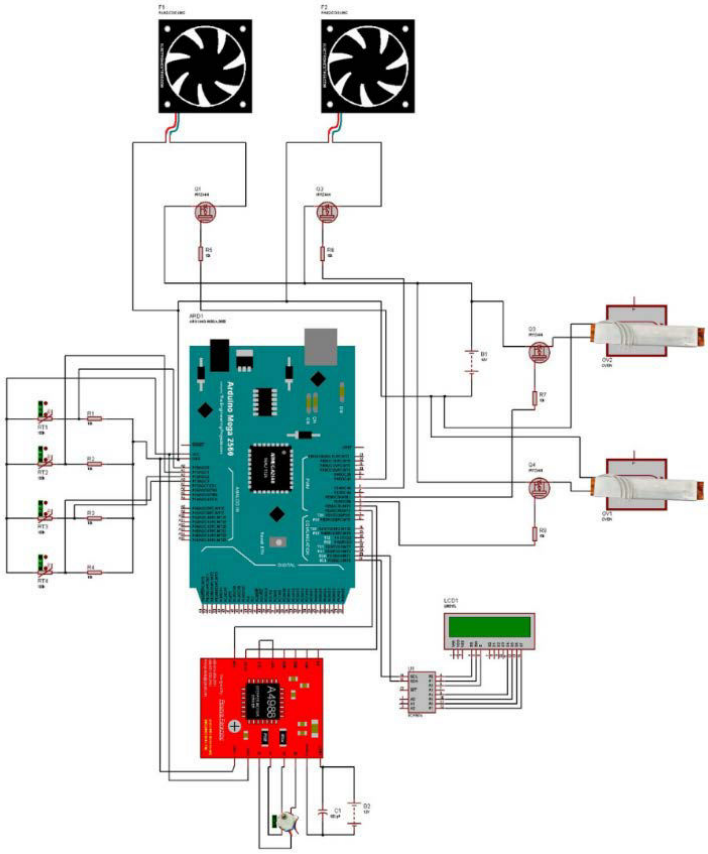


Figure 29Complete circuit schematic of the thermal cycling system designed and simulated in Proteus.

4.3 Fabricated Prototype of the PCR Device

The final prototype was assembled by combining the 3D printed structural

components, CNC-machined parts, and electronic control system. All mechanical elements were aligned to ensure precise movement of rotary heaters and heat sinks. The microcontroller, along with the temperature sensors and drivers, was integrated into the housing. The overall assembly was compact and functionally organized to support stable thermal cycling. Images of the assembled device are provided in the following figures.

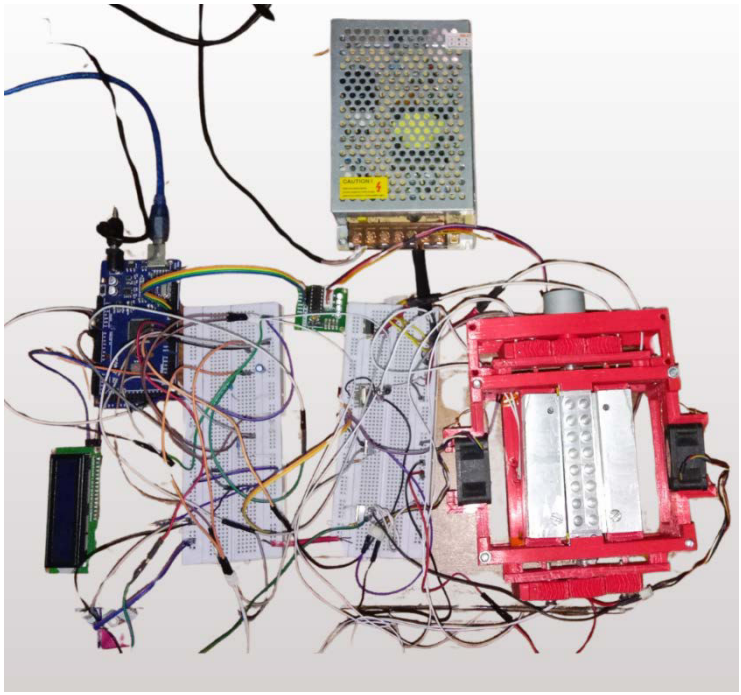


Figure 30Final fabricated prototype of the PCR thermal cycling device showing all assembled components.

4.4 PCR program (for Plasmodium vivax testing)

Step	Temp (°C)	Hold time	Notes
Initial denaturation	95	2.5 min	Ensures complete denaturation
Denaturation (per cycle)	95	30 s	Standard melt step.
Annealing (per cycle)	52	30 s	Set to primer Tm.
Extension (per cycle)	72	30 s	For amplicons (18S rRNA gene) (200 bp)
Repeat cycles	—	30 cycles	30 cycles typical for diagnostic sensitivity
Final extension	72	5 min	Completes any partial products.

4.5 Thermal Cycling Performance

The system's thermal cycling capability was evaluated by monitoring temperatures during multiple PCR cycles. The heater successfully reached 95°C for denaturation when in full contact with the PCR block. The transition to lower temperatures (72°C and 52°C) was achieved by lowering the heater to create an air gap while activating the cooling fans. The measured temperature profiles showed that the system could reach 95°C in under 90 seconds and cool down to 72°C and 52°C within 30–40 seconds. The PID control algorithm maintained stable temperatures within a ±1.5°C range during each phase, ensuring consistent thermal conditions for DNA amplification.

4.6 System Stability and Automation

The Arduino-based control system successfully automated all phases of the thermal cycling process. The stepper motor responded accurately to phase transitions, and the heater movement was consistent across multiple cycles. The fans activated and deactivated according to predefined temperature conditions, effectively managing the cooling periods. Real-time monitoring on the LCD provided continuous feedback on temperatures and cycle progress, contributing to user-friendliness and clarity of operation. No system failures or errors were observed during testing, and the temperature readings remained stable with minimal fluctuation.

4.7 DNA Amplification Results

To check whether the thermal cycling device works properly, a DNA sample of *Plasmodium vivax* was used for testing. Standard PCR reagents and a 30-cycle amplification program were used. After the PCR was completed, the DNA samples were loaded on an agarose gel and run using electrophoresis. The purpose of this step was to check if the DNA was successfully amplified.

In the first gel image (Figure 4.4a), Lane 1 shows the result of DNA that was extracted using a commercially available GF-1 kit. This sample was amplified

using a standard thermocycler and served as a positive control. A clear and bright band is visible, which confirms that the DNA was successfully amplified. Lanes 2, 3, and 4 show the results of DNA samples that were extracted and amplified using the developed thermal cycling device. These samples also showed visible DNA bands at the expected size, indicating successful amplification of the *P. vivax* gene. Although the bands in lanes 2 to 4 are slightly lighter than the control in lane 1, they clearly confirm that the device was able to perform the PCR process. This means the device was able to carry out all three main steps of PCR: denaturation, annealing, and extension.

The second image (Figure 4.4b) shows the gel during the electrophoresis run. The bands can be seen even before staining, which also supports that the DNA was amplified properly.

These results confirm that the device is capable of amplifying DNA. It may not be as efficient as a commercial thermocycler, but it still performs all the basic functions needed for PCR.

Figure 31Agarose gel electrophoresis showing PCR amplification results of Plasmodium vivax DNA. Lane 1: Positive control amplified using commercial thermocycler; Lanes 2–4: DNA amplified using the developed thermal cycling device.

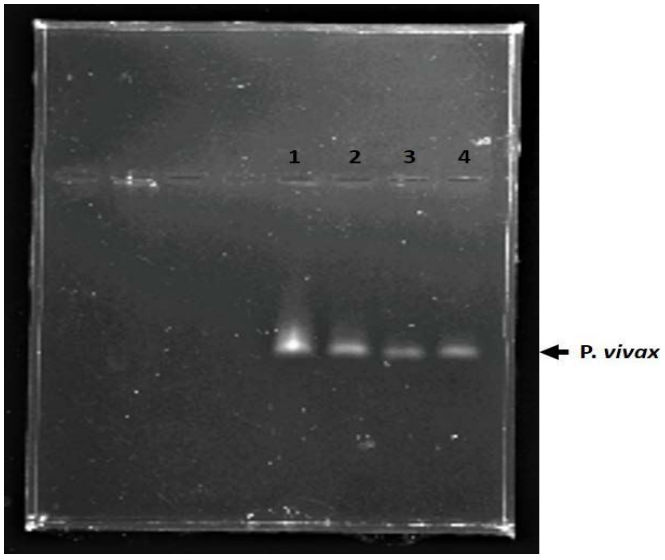


Figure 32Electrophoresis gel image during the run showing visible DNA bands even before staining, indicating successful amplification by the developed device.

The final version of the prototype was able to achieve its main goals. It worked as a low-cost and portable PCR device using basic components. The device was

able to maintain the required temperatures for each PCR step and continued working properly in repeated tests.

However, some challenges were noticed during testing. The thermistors needed to be calibrated manually, which took extra time. Also, if the heater or block was slightly moved or misaligned, the performance of the device was affected. Another limitation is that the device can only be used for endpoint detection. It does not have a system for real-time fluorescence detection, which is required for qPCR.

Overall, the prototype performed well in laboratory tests. It showed that simple and low-cost devices can be built for basic DNA amplification. This type of device can be useful in teaching labs, rural healthcare units, and emergency situations where access to commercial equipment is limited.

Discussions

This study focused on the development and validation of a portable, low-cost thermal cycling device for DNA amplification. The purpose was to create an alternative to commercial PCR thermocyclers that could be used in resource-limited settings. The results obtained from mechanical, electronic, and biological testing confirmed that the prototype worked successfully and fulfilled its basic functions.

The device was designed using a combination of 3D-printed PLA parts and CNC-machined aluminum components. PLA was used for its light weight and ease of printing, while aluminum was selected for parts where good thermal conductivity was essential, such as the PCR block and heat sinks. All components were assembled into a stable and compact structure. The rotary heater and heat sink movement system worked smoothly using gears and a stepper motor, and alignment remained consistent throughout testing.

The electronic control system was built using an Arduino Uno microcontroller, along with a motor driver, temperature sensors, and a cooling fan. The Proteus simulation of the circuit showed that all components functioned as expected before real hardware testing began. The final system operated reliably under programmed conditions and was able to regulate temperatures accurately using a PID control algorithm. This ensured that the device followed the required thermal profile for PCR.

The temperature response of the system showed that the heater could reach 95°C in under 90 seconds, and cooling to 72°C and 52°C was achieved within 30–40 seconds using passive and fan-assisted cooling. Temperature stability during each phase was maintained within a ±1.5°C range, which is acceptable for successful DNA amplification.

The final and most important test of the device was its ability to amplify DNA. The gel electrophoresis results confirmed that the device was able to amplify

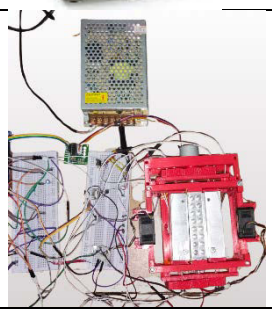
Plasmodium vivax DNA successfully. The DNA bands produced by the prototype device were visible and clear, although they were slightly less intense compared to the control sample amplified by a commercial thermocycler. This suggests that the amplification efficiency was slightly lower, possibly due to minor differences in heating uniformity or heat transfer. However, the essential steps of PCR—denaturation, annealing, and extension—were completed properly.

To evaluate the economic feasibility of the developed rotary heater–stationary block thermocycler, a cost comparison was conducted against commonly used commercial thermocyclers. Table 5.1 highlights selected models, covering both standard and low-cost options.

While commercial thermocyclers typically range between $3,500 and $35,000, the fabrication cost of the developed prototype remains within $300–$700, making it significantly more affordable. This low-cost advantage enhances its suitability for resource-constrained laboratories, educational institutions, and point-of-care diagnostics.

Table 3Cost Comparison of Commercial and Custom Thermocyclers

Device	Image	Sample Capacity	Key Features	Approx. Cost (USD)
Bio-Rad T100		96 wells	Basic PCR, reliable	$3,500 – $5,000
Eppendorf Mastercycler Nexus		96 wells	Gradient feature	$6,000 – $8,000

Thermo Scientific SimpliAmp		96 wells	Touchscreen, rapid ramping	$5,500 – $7,000
miniPCR mini16		16 wells	Portable, Bluetooth connectivity	$800 – $1,200
OpenPCR (DIY)		16 wells	Open-source, low-cost	$650 – $800
PCRMax Alpha Cycler 1		96 wells	Compact and robust	$3,000 – $4,500
Bio-Rad CFX96 Touch (Real-time PCR)		96 wells	Multiplex detection	$25,000 – $35,000
This Project: Custom Thermo cycler		16 samples	Rotary heating, low-cost build	**$50**

Some of the limitations were observed during testing. Slight misalignment of the heater or PCR block could affect performance. Another important limitation is that the current device only supports endpoint detection, meaning it does not monitor DNA amplification in real time. This limits its use for quantitative PCR (qPCR) applications, which require fluorescence detection systems.

Despite these challenges, the prototype demonstrated that simple, low-cost

devices can still achieve meaningful results. The system proved suitable for basic molecular diagnostics and teaching purposes, especially in settings where commercial equipment is too expensive or unavailable. With further improvements, such as adding automatic calibration, better insulation, and fluorescence-based detection, this device could be expanded for broader applications.

4.8 Conclusion

This study successfully developed a portable and low-cost thermal cycling device using a combination of stationary block and rotary heaters. The primary goal was to create a system that can perform DNA amplification without relying on expensive or bulky commercial thermo cyclers. The project focused on simplifying the heating mechanism, optimizing temperature control, and ensuring reliable thermal cycling required for PCR.

The device operated effectively for DNA amplification and demonstrated consistency and mechanical stability. Its performance confirms the potential for developing accessible PCR-based diagnostic tools, especially in low-resource settings. This approach also allows for greater flexibility in design and usage, offering a promising alternative for small labs, academic settings, and field diagnostics.

Although the device has some limitations, such as lack of real-time detection and need for further automation, it provides a strong foundation for future improvements. Integrating feedback control systems, expanding sample capacity, and enhancing user interface features can further improve its reliability and ease of use.

Overall, the study demonstrates that essential molecular biology techniques like

PCR can be made more accessible through engineering innovation. The developed prototype adds value to the field of portable diagnostics and opens possibilities for its adaptation in various scientific and practical applications.

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