Instrument-free Thermal Cycler for Simple, Inexpensive Polymerase Chain Reaction

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Abstract

Polymerase chain reaction (PCR) is the most common technique for DNA amplification. The PCR cycling process involves three temperature stages (95ºC, 55ºC, and 75ºC), which are repeated in a thermal-cycler instrument to amplify DNA samples. However, this method is not applicable to settings that do not have access to state-of-the-art instruments and that only have intermittent access to electricity. For this reason, a proof-of-concept, instrument-free thermal cycler is developed to conduct PCR in low-resource settings. The thermal cycler is driven by chemical reactions and does not require the use of electricity. The aim of this work is to design, fabricate and test two prototypes to maintain the stable temperature regions for 45 minutes. Water-activated chemical fuel, phase change materials and insulation housing are used. A polydimethylsiloxane cartridge is fabricated using plastic mold and is a cheap and disposable option to contain liquid and amplify DNA samples. Thermocouples are used in the experiment to measure the temperature change inside the liquid. The result from the 1st prototype shows that the 95ºC heater is able to maintain the temperature range of 90-100ºC for 1008 seconds. Meanwhile, the sandwiched structure of 2nd prototype can sustain a stable 85-95ºC range for a period of 2576 seconds. The latter result is based on using magnesium iron powder as fuel and glutaric acid as phase change material. Compared to the 1st prototype, the 2nd prototype does not require manual cycling, thus reducing the reaction time by eliminating the need to relocate the DNA fluidic cartridge to another temperature zone. Also, phase change material is proven to have the ability of setting the temperature ceiling with proper insulations to reduce heat loss. The findings have directed future work towards creating a steady temperature gradient within the liquid so that the sample can be cycled naturally by convection current.

Abrégé

La réaction en chaîne de la polymérase (PCR) est la technique la plus courante pour l'amplification de l'ADN. Le processus de cycle PCR implique trois étapes de température (95°C, 55°C et 75°C), qui sont répétées dans un instrument thermocycleur pour amplifier un fragment d'ADN. Cependant, cette méthode n'est pas applicable aux environnements n'ayant pas accès aux instruments les plus avancés et disposant uniquement d'un accès intermittent à l'électricité. Pour cette raison, un thermocycleur que n'utilise pas d'énergie électrique est établi pour prouver le concept et permettre la PCR dans des environnements à faibles ressources. Le thermocycleur est entraîné par des réactions chimiques et ne nécessite pas d'électricité. Le but de ce travail est de concevoir, fabriquer et tester deux prototypes afin de maintenir les régions à température stable pendant 45 minutes. Un combustible chimique activé par l'eau, des matériaux à changement de phase et un boîtier isolant sont utilisés. Un récipient en polydiméthylsiloxane est fabriqué à l'aide d'un moule en plastique. Il s'agit d'une option peu coûteuse et jetable pour contenir des échantillons d'ADN. Les thermocouples sont utilisés dans l'expérience pour mesurer le changement de température à l'intérieur du liquide. Le résultat du premier prototype montre que le réchauffeur à 95°C est capable de maintenir la plage de température de 90 à 100°C pendant 1008 secondes. Pendant ce temps, la structure en sandwich du 2^{e} prototype peut maintenir une plage stable de 85 à 95°C pendant une période de 2576 secondes. Ce dernier résultat est basé sur l'utilisation de la poudre de fer magnésium comme carburant et de l'acide glutarique comme matériau à changement de phase. Par rapport au 1er prototype, le 2^{e} prototype ne nécessite pas de cycle manuel, ce qui réduit le temps de reaction en éliminant le déplacement du cartouche fluidique dans une autre zone de température. En outre, il a été prouvé que les matériaux à changement de phase ont la capacité de fixer le plafond de température avec des isolations

appropriées pour réduire les pertes de chaleur. Les résultats ont orienté les travaux futurs vers la création d'un gradient de température stable dans le liquide, de sorte que l'échantillon puisse être soumis à un cycle naturel par le courant de convection.

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Acknowledgement

I would like to give special thanks to Professor David Juncker for inviting me to his Micro and Nano Bioengineering lab and the opportunity to take on this exciting project. Thank you to my two intermediate supervisors, Frédéric Normandeau and Oriol Ymbern Llorens for introducing me into this biomedical engineering project and guiding me through the development. I would also like to thank the students I have worked along with, namely Stephen Thangathurai and the members of the Capstone Engineering Design Team. They have assisted with the development of the instrument-free thermal cycler throughout the years.

Chapter 1: Introduction

1.1 Polymerase Chain Reaction

In the field of biomedical engineering, a nucleic acid amplification test (NAAT) is a technique used to detect a particular nucleic acid which functions as a pathogen in the human body. It is often used for early diagnosis of infectious disease [1]. In recent years, this test is becoming more popular due to its rapidness and accuracy. However, since the amount of the target genetic material is usually very small, the test often includes an amplification process to substantially increase the amount of DNA samples. Polymerase chain reaction (PCR) is one of the amplification techniques [2] and is used in molecular biology to amplify a few copies of a target DNA across several orders of magnitude. By generating thousands to millions of copies of a particular DNA sequence, it is an easy and reliable way to replicate DNA for disease diagnosis.

Most of the polymerase chain reaction are carried out by thermal cycling. Thermal cycling exposes the DNA samples to repeated heating and cooling to allow specific reactions to take place in a temperature-sensitive environment. The DNA polymerase are the original DNA to be replicated and DNA primers are short single fragments that are the complementary sequence to the target DNA region. The first part of the process occurs at a high-temperature environment where the two strands of the DNA double helix are physically separated. This process is called DNA melting. In the second part, the primers bind to the separated DNA strands under a lower temperature. The two DNA strands can now act as templates for DNA polymerase to assemble a new DNA strand with the building blocks of DNA, known as free nucleotides. The new DNA created can be used for replication in the same setting again, starting a chain reaction and amplifying the original DNA template exponentially.

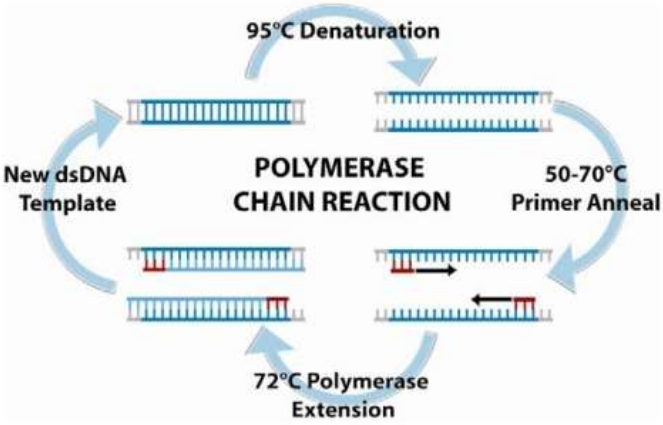

Figure 1: Polymerase Chain Reaction- Denaturation, Annealing, Elongation [3]

The three reaction steps form a cycle and each cycle consists of 3 discrete temperature stages. Stage 1 (denaturation) occurs at 95°C for 20-30 seconds, stage 2 (annealing) lowers the temperature to 55°C for 20-40 seconds and stage 3 (elongation) is optimal at 75°C.

1.2 Thermal Cycler and its Limitation

As thermal cycling demands an isothermal environment, it requires an energy-intensive precision thermal cycler, shown in figure 2:

Figure 2: Thermal Cycler [4]

The device has a thermal block with holes where tubes holding the reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. The tolerance for these 3 discrete temperature stages is +/- 5ºC. If the temperatures are not within the desired range, the specificity and sensitivity of PCR will be negatively affected. Therefore, it is crucial for the temperature to be as accurate and stable as possible.

A thermal cycler costs thousands of dollars (around USD$8000), requires infrastructure and capital equipment. Also, it can only be operated by users with extensive training. These requirements are often not available in low-resource settings. So researchers have come up with an unproven idea of an electricity-free thermal cycler [5]. This is very beneficial for several reasons.

1. If the device is used to test extremely virulent pathogens, it is desirable for immediate incineration or decontamination of entire devices.
2. There is no electricity supply to support the electricity-powered device.
3. It is not possible or environmental friendly to dispose used batteries (for battery-powered device).
4. There might an emergency where the instrument maintenance cannot be supported quickly.
5. Natural disaster might occur and electricity is cut off.

1.3 Magnesium Iron with Water Reaction

Magnesium is one of the alkaline earth metals and it is positioned between beryllium and calcium. Generally, magnesium reacts with water at room temperature, but the extent of the reaction is insignificant due to the magnesium hydroxide coating forming quickly at the beginning [6]. This behavior is in contrast with calcium, the element below magnesium in the periodic table, which has a slow but steady reaction with cold water. However, magnesium will react with water vapor to produce magnesium hydroxide and hydrogen gas.

$$\mathrm{Mg} + 2\mathrm{H_2O} \rightarrow \mathrm{Mg(OH)_2} + \mathrm{H_2} + \mathrm{heat}$$

Although magnesium is a slow-reacting element, it can be mixed with iron powder and sodium chloride to form a "Supercorroding Galvanic Cells". This exothermic reaction can generate a large amount of heat at a significantly fast rate. The theory behind the galvanic cell will be discussed in later session. Magnesium iron is chosen as the final chemical fuel in the experiment.

1.4 Phase Change Material (PCM)

When heat is added to a solid substance, the temperature increases until its phase changes from solid to liquid (or from liquid to gas). During each phase change, the temperature remains constant as more heat is given. Instead of raising the temperature, the heat input is used to change the phase. In the case of melting/freezing, this required energy is called latent heat of fusion. In the case of vaporizing/condensing, it is called latent heat of vaporization. This isothermal phase-change behavior of substance leads to the use of phase change materials.

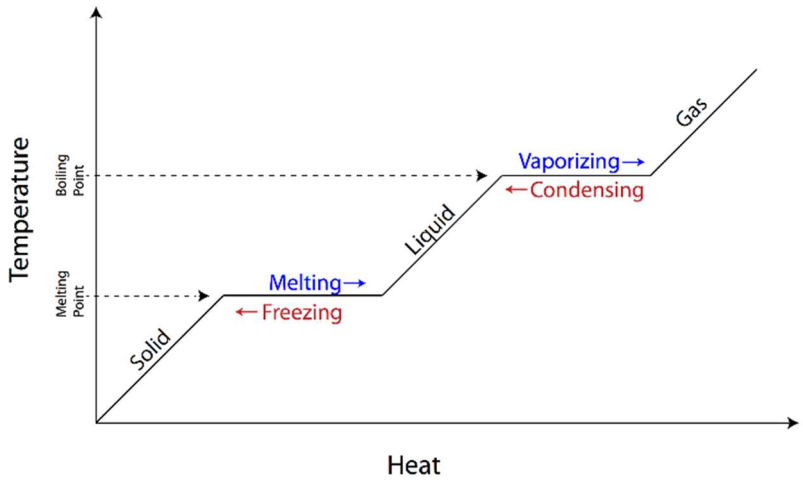

Figure 3: Phase Change Diagram [7]

A phase change material (PCM) refers to the particular substance which has a high latent heat of fusion. This means that when it is undergoing a phase change at the melting point, it can store and release a large amount of energy. Since PCMs have the characteristic of absorbing/releasing heat during solid to liquid transition, they are also known as latent heat storage units [8].

Although PCM can store and release energy in both phase change- between solid to liquid and between liquid to gas, only the changes between solid to liquid are used because they are more practical in the lab. Liquid to gas phase change are not feasible in storing energy because the material in gas phase requires a large volume or high pressure for storage. PCM is a very desirable material because it can maintain the isothermal environment required for thermal cycling without the use of electricity.

1.5 Convection Current of Boiling Water

Convection is the transfer of heat energy between particles when they circulates inside a fluid in a liquid or gas state. In order to distribute thermal energy equally to the whole system of the fluid, convection has to take place. Thermal energy can be transferred in one of the three ways- conduction, convection and radiation. And convection is the primary method of heat transfer in a liquid. In general, heat is always transferred from a higher temperature region to a lower temperature region.

When water is boiled, convection occurs to transfer heat to every water particle inside the system. As the heat source is located at the bottom of the pot, particles near the bottom of the pot gain more heat energy and move more quickly. Therefore, they will collide more easily and travel further apart. The region has a lower density and rises to the top of the pot. After the heated particles reach the top of the pot, they transfer heat to the surrounding cooler particles. As a result, their temperature will once again decrease. The particles lose some thermal energy and move more slowly and closer together. With a higher density now, they will sink to the bottom again. The cycle repeats when they gain energy back from the bottom of the pot. This example of moving particles is called a convection current, demonstrated in figure 4:

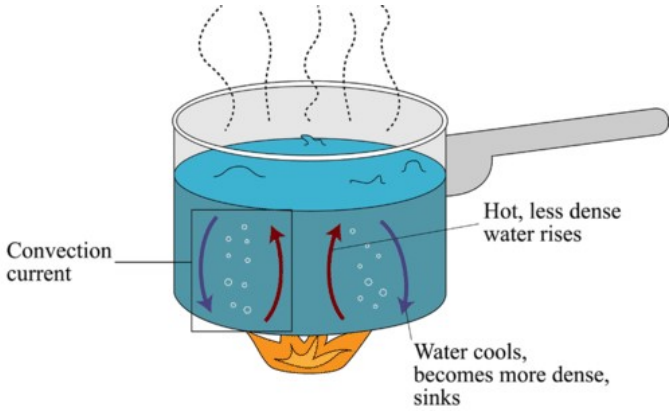

Figure 4: Convection Diagram of Boiling Water [9]

The behavior of the convection current is useful for the instrument-free thermal cycler because it allows the cycling of DNA sample in a test fluid similar to water. The temperature difference between the top and bottom region can create a convection current in the PCR liquid containing the DNA. Thus, the DNA sample can rotate through different temperature regions and undergo the polymerase chain reaction. The convection cycling mechanism is driven by nature and does not require any electricity and human intervention.

1.6 Heat Transfer Theories

Heat transfer explains how heat energy can be transferred and predicts the heat exchange rate under different specified conditions. The three modes of heat transfer are conduction, convection, and radiation. From heat transfer theories the temperature can be expressed as a function of position and predict the temperature at a specified region.

When there is a temperature gradient in a body, energy is transferred from the high-temperature region to the low-temperature region. This energy transfer is done by conduction and the heat transfer rate is related to the temperature gradient along the position:

$$q_x = -kA\frac{\partial T}{\partial x} \tag{1}$$

where q_x is the heat transfer rate, k is the thermal conductivity, A is the cross-sectional area, $\frac{\partial T}{\partial x}$ is the temperature gradient of the heat flow. This is called Fourier's law of heat conduction [10].

Consider a 1-D system shown in figure 5:

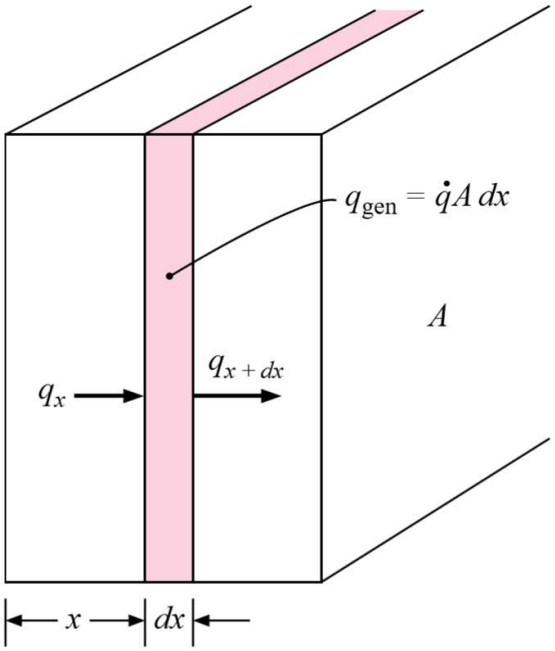

Figure 5: Elemental volume for one-dimensional heat conduction analysis

For the element of thickness dx, the energy balance equation is:

Heat transfer rate on left face + heat generation within dx = rate of change in internal energy + heat transfer rate on right face

$$\text{Heat transfer rate on the left face, } q_x = -kA\frac{\partial T}{\partial x} \tag{2}$$

$$\text{Heat generation within dx} = \dot{q}A\,dx \tag{3}$$

$$\text{Change in internal energy} = \rho cA\frac{\partial T}{\partial \tau}\,dx \tag{4}$$

$$\text{Heat transfer rate on the right face} = q_{x+dx} = -kA\frac{\partial T}{\partial x}\Big|_{x+dx} = -A\left[k\frac{\partial T}{\partial x} + \frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)dx\right] \tag{5}$$

Where $\dot{q}$ is the energy generation, c is the specific heat of material and ρ is the density.

The final 1-D heat-conduction equation becomes:

$$-kA\frac{\partial T}{\partial x} + \dot{q}A\,dx = \rho cA\frac{\partial T}{\partial \tau}\,dx = -A\left[k\frac{\partial T}{\partial x} + \frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)dx\right] \tag{6}$$

$$\frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right) + \dot{q} = \rho c\frac{\partial T}{\partial \tau} \tag{7}$$

The thermal resistance is defined as:

$$Heat\ transfer\ rate = \frac{thermal\ potential\ difference}{thermal\ resistance} \tag{8}$$

$$q = \frac{\Delta T_{overall}}{\sum R_{th}} \tag{9}$$

The thermal resistance for 1-D plane wall conduction is:

$$R_{th,wall} = \frac{L}{kA_{c.s.}} \tag{10}$$

where L is the thickness of the wall, $A_{c.s.}$ is the cross-sectional area

The thermal contact resistance is the thermal resistance as a result of the imperfect joint between two bodies [11]. This is due to the entrapped gases in the void spaces between the contact surfaces. The thermal contact resistance is:

$$R_{th,contact} = \frac{1}{h_{contact}A_{contact}} \tag{11}$$

where $h_{contact}$ is the contact coefficient and $A_{contact}$ is the contact area

1.7 Thesis Objective and Overview

Previous studies on an instrument-free thermal cycler have shown a feasible method of using phase change material to maintain a stable temperature for polymerase chain reaction [12]. The phase change material is capable of controlling the temperature for a specified duration of time for the reaction to take place. Therefore, the objective of the thesis is to investigate on the accuracy and repeatability of the temperature control inside the thermal cycler, with a focus on developing an efficient method to optimize the heat transfer condition.

The motivation behind the instrument-free thermal cycler has been discussed in Chapter 1. The theory behind every application used in this project have also been introduced. Chapter 2 details the fabrication of experimental apparatus to develop the thermal cycling prototype. Chapter 3 provides a theoretical prediction using 1D heat transfer analysis and thermal simulation on the software SolidWorks. Chapter 4 then outlines the development of experimental methods to record data and their accuracy and possible errors. Chapter 5 presents and discusses the preliminary results obtained from the 2 prototypes developed in the thesis project. Lastly, Chapter 6 summarizes the proven concept of an instrument-free thermal cycler for PCR and discusses limitations and future work.

Chapter 2: Fabrication of Experimental Apparatus

2.1 Overview of the Thermal Cycler

Since the project is divided into 2 parts, with a year in between, a lot of progress has been made by other researchers and students after the first part is completed. My work in both parts would be discussed in detail and the changes made in the second part would be provided with explanations. Two prototypes are created during the project with the help of the capstone team (for the housing of the second prototype).

The first prototype consists of 3 heaters situated in layers of foam boards for heat insulation. The set-up is shown in figure 6:

Figure 6: Overview of the first prototype

The cross sectional view of the heater is shown in the figure 7 below. In each heater, the main fuel - calcium oxide (bought off-the-shelf as “Barocook” pad [13]) is put inside an isothermal metal bottle. Water is added to the calcium oxide to start the reaction and produce heat. A hole is cut on the top of the metal bottle and a tin canister [14] is installed. The tin canister contains the phase change material (PCM), which helps to keep the temperature constant at all times. An additional ring of calcium oxide powder is also put around the canister for additional heat

transfer. Two water reservoirs are fixed beside this ring to supply water through filter papers. For the heater producing the highest temperature (95°C), a spring-assisted gas exhaust valve is installed to let the excess water vapor escape. This can prevent the pressure build up in the metal container. The PCR liquid sample sits directly on top of the tin canister to receive heat at a constant temperature.

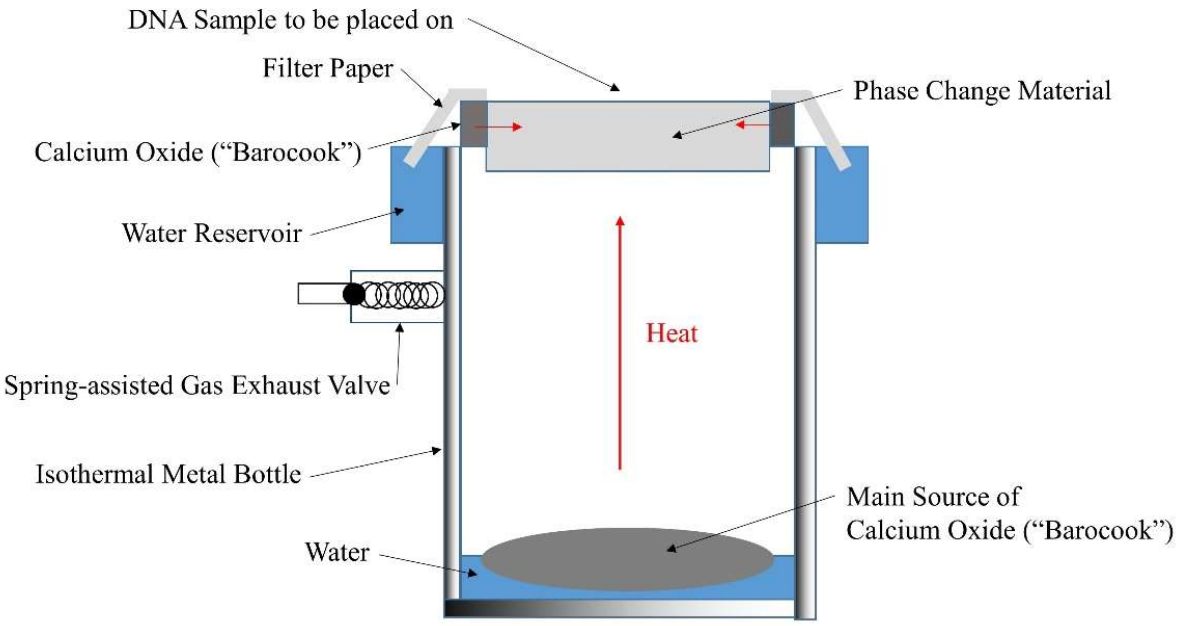

Figure 7: 1st Prototype Heater Layout

The first prototype lays the foundation of the operational structure for an instrument-free thermal cycler. However, the PCR liquid sample needs to be placed manually on different heaters and it requires intensive labor, such as timing each stage of the PCR reaction, pouring an accurate amount of water into the metal bottle, setting up the filter paper etc. A trained personnel might not be available in a low resource setting and therefore the device is not user-friendly. Furthermore, the size of the thermal cycler is not compact compared to a standard thermal cycler. All of the reasons above leads to the advanced development of the second prototype.

The second prototype combines the 55ºC heater and 95ºC heater together to create a gradient of temperature in between. The housing is 3D-printed by the capstone team and it is made to fit with the same canister from the first prototype. The overview is shown in figure 8:

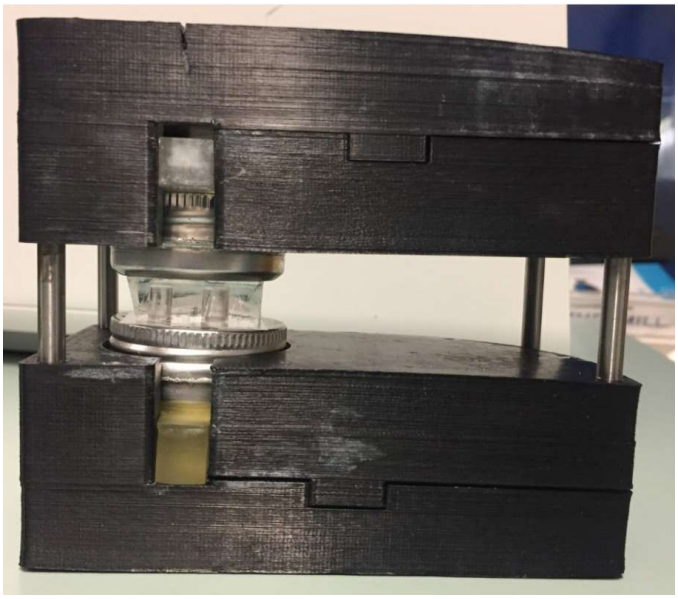

Figure 8: Overview of the second prototype

The layout of the 2nd prototype is demonstrated in the figure 9 below. The 55ºC heater assembly is located at the top of the system and the 95ºC heater is at the bottom. Similar to the first prototype, a water reservoir is used to supply water to the fuel through a filter paper. The fuel then heats up the canister containing the PCM, which then transfers heat energy at a constant temperature to the PCR liquid (in red). The cycling mechanism and fuel choice will be discussed in the later sections. By producing a temperature difference at the top and bottom, the DNA sample inside the PCR liquid will experience different temperature at different regions. Theoretically, there will be a temperature region at 75ºC.

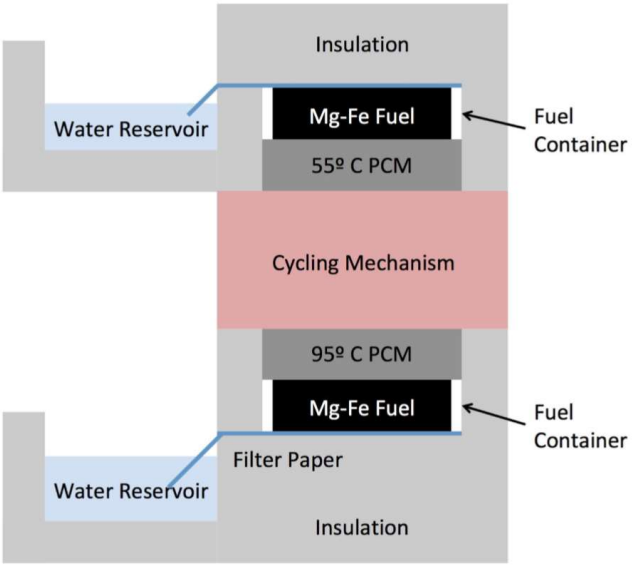

Figure 9: 2nd Prototype Design Layout

This second design eliminates the need to cycle the PCR liquid to different heaters and simplifies the steps in initiating 3 heaters at once. With the development of this second concept, the thesis emphasizes on improving the heat transfer process and evaluating the reproducibility of the steady-state temperature regions.

2.2 Cycling Mechanism

One of the major changes to the thermal cycler between the two parts is the cycling mechanism. The DNA sample has to be cycled through 3 stages because each reaction occurs at a specific temperature.

In the first part of the project, the main objective is to prove the concept of performing a polymerase chain reaction (PCR) using a chemical heat source without electricity. Therefore,

three separate heaters, shown in the figure 10 below, are created to satisfy the three different temperature stages.

Figure 10: Three heaters for PCR reactions at different temperatures

Each heater is responsible to supply heat to the DNA sample on top at a constant temperature. At the end of each stage, the DNA sample is placed on top of another heater by a rotational platform shown in figure 11:

Figure 11: Rotational Platform (on the right)

The rotational platform is not powered by electricity due to the electricity-free objective. Therefore, it must be rotated by hand multiple times in a cycle of around 1 minute. The manual procedure is tedious and requires training for those having little or no knowledge of thermal cycling.

In the second part of the project, a new method is developed to cycle the DNA sample. By heating the PCR liquid at two different temperatures from the top and bottom, a temperature gradient can be created inside the liquid. With convection (similar to that in boiling water), the convection current inside the PCR liquid can move the DNA sample in a circular motion, shown in the figure 12. Consequently, the DNA sample will go through different temperature regions for the PCR reaction to take place. This closed-loop buoyancy-driven flow is fully automated and user-friendly, so it is the most suitable for use in a low-resource setting. However, special care must be put in controlling the residence time in each temperature zone. If there is not enough residence time, the reaction cannot be completed. If the DNA sample stays for too long, it will reduce the efficiency of the PCR reaction.

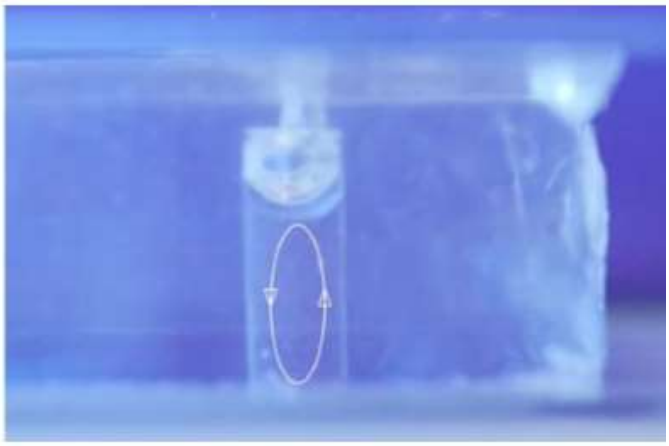

Figure 12: Convection Current inside the PCR liquid

2.3 Phase Change Material Selection

Polymerase chain reaction is very sensitive and dependent on the temperature. With electricity, thermal cyclers can easily maintain a temperature for a long time with the use of thermostat [15]. However, without electricity, phase change material (PCM) is used between the heat source and the DNA sample. When the PCM changes phase from solid to liquid, the temperature of the

material remains constant so it can heat up the DNA sample under a constant temperature. In order to select the best PCM for the instrument-free thermal cycler, their thermodynamic properties need to be taken into consideration. The phase change material should possess:

1. Melting temperature in the desired operating temperature (55ºC, 75ºC, 95ºC)
2. High latent heat of fusion per unit volume
3. Congruent melting
4. High rate of crystal growth, so that the system can meet demands of heat recovery from the storage system
5. High specific heat, high density and high thermal conductivity
6. Small volume changes on phase transformation and small vapor pressure at operating temperatures to reduce the containment problem
7. Chemical stability
8. Complete reversible freeze/melt cycle
9. No degradation after a large number of freeze/melt cycle
10. Non-corrosiveness, non-toxic, non-flammable and non-explosive materials
11. Low cost
12. Availability

For the cycling mechanism in the first part of the project, there are three heaters for each temperature stage. Thus, we need 3 different phase change material to maintain a stable temperature at 55ºC, 75ºC, 95ºC. After careful consideration of the thermodynamic properties mentioned above, paraffin wax is used to maintain the temperature at 40ºC, stearic acid is used to maintain the temperature at 70ºC and glutaric acid is used to maintain the temperature at 95ºC.

Since the melting points of these phase materials match with the target temperatures, they will have an endothermic phase transition over a narrow range centered at those temperatures [16, 17, 18].

For the cycling mechanism in the second part, only two heaters are needed because the top and bottom heater (at 55ºC and 95ºC respectively) will produce a gradient of temperature. In the final prototype, 10.22g of paraffin wax is placed in a 15ml aluminum tin canister to be used in the top heater, while 10.32g of glutaric acid is placed in another canister of the bottom heater. Additionally, fine steel wool is added into the canister to improve the heat transfer through these phase change materials.

Figure 13: Glutaric acid in a tin canister (with steel wool)

2.4 Fuel

In the first part of the project, calcium oxide is used as a fuel to heat up the phase change material. Without the need of electricity, calcium oxide can provide sufficient heat energy in a commercially available thermos. When water is added to the calcium oxide, it forms calcium

hydroxide. Then, the calcium hydroxide will react with carbon dioxide to produce calcium carbonate and water vapor [19]. The chemical reaction is:

$$CaO(s) + H_2O(l) \longrightarrow Ca(OH)_2(s)$$

$$Ca(OH)_2(s) + CO_2(g) \longrightarrow CaCO_3(s) + H_2O(l)$$

There is an off-the-shelf camping heat pack called “Barocook Flameless Cooking Heating Pad”. The calcium oxide comes in a paper bag, each bag weighing 50g. Due to its non-toxic products from the reaction, it is first chosen to be the suitable fuel for the thermal cycler. However, during the supply of heat at the highest temperature, there is a problem of a pressure buildup due to by-product.

Water vapor is produced from the chain reaction. For the 95°C heater, 25g of calcium oxide is used to supply enough heat. Since the heat pad is put inside an airtight metal bottle, the pressure inside builds up to a significant degree. A customized pressure valve is needed to let the water vapor escape. The pressure valve is designed to trap enough water vapor inside to transfer heat to the tin canister, while it should also prevent the pressure buildup to a point of explosion or leakage at anywhere else.

The pressure valve is built with a spring, rubber ball and some plastic pipes. The spring is rubber sprayed to prevent rusting after repeated use. The disassembled value is shown in figure 14 and figure 15:

Figure 14: Disassembled Valve

Figure 15: Assembled Valve

The initial compression of the spring inside the valve is adjusted by adding a plastic ring to the pipe. The thicker the ring, the more compressed the spring will initially be. This allows a higher pressure inside the bottle for more heat transfer. After several failed attempts to balance the pressure and heat supplied, it can be concluded that calcium oxide is not the best choice as a fuel.

In the second part of the project, magnesium iron powder is used as the fuel replacement. This decision is based on the careful evaluation of different heating source criteria. Our goal is to generate heat in a way that is repeatable, has been proven to work in prior literature, and will consistently deliver similar amounts of energy across trials. And magnesium iron, packaged as a “flameless ration heater”, satisfies most of these requirements.

A flameless ration heater is a chemical heater which can initiate exothermic heating by just adding water. In US military, these heaters are often used to heat up food pack known as the MREs (Meals, Ready-to-Eat). According to the patent specifications, the heater is able to raise the temperature of a 226.8g food by 56°C in 12 minutes. In the process, there is no visible flame and only hydrogen gas is produced as a by-product. The materials used to make a flameless ration heater are fine magnesium power mixed with fine iron powder and sodium chloride [20].

The principle behind a flameless ration heaters depends on the heat generation in an oxidation-reduction reaction. The added water oxidizes magnesium, then magnesium hydroxide and hydrogen gas are produced with heat [21]. The reaction formula is:

$$\mathrm{Mg} + 2\mathrm{H_2O} \rightarrow \mathrm{Mg(OH)_2} + \mathrm{H_2} + \mathrm{heat}$$

Since the rate of this chemical reaction is a very slow due to passivation (magnesium hydroxide forms a coating preventing further reaction) and cannot generate enough heat, the reaction is further accelerated by adding iron powder and sodium chloride (table salt). After water is added, the table salt will dissolve in the water and act as an electrolyte. The magnesium and iron particles then act as short-circuited batteries. Therefore, the process produces a sufficient amount of heat. This system is called "Supercorroding Galvanic Cells" [22].

The flameless ration heater is commercially available. The heater pack purchased contains 29.1 grams of a powdered magnesium-iron, consisting of 95% magnesium and 5% iron by weight, mixed with 0.5 grams of salt. In the experiment, the powder is taken out of the heater pack for weighing. Then they are added to the fuel holder.

Figure 16: Magnesium-iron powder in a fuel holder

2.5 PDMS Cartridge and Mold

The cycling mechanism relies on the convection current inside PCR liquid to circulate DNA strands. The convection rate is highly dependent on the dimension of the cylindrical PCR cartridge which contains the PCR liquid. Prior to experiments, there is no information concerning which height-to-diameter aspect ratio matches the target convection rate. Therefore, it is crucial to choose a material that can easily be molded to the desired geometry. Polydimethylsiloxane (PDMS) can be found in the Micro and Nanobioengineering Lab and it can be manufactured by molding.

Polydimethylsiloxane (PDMS) is a common silicon-based organic polymer, due to its unusual flow characteristic. When the PDMS is solidified in the mold after 24 hours, the smallest of details is left imprinted on the surface [23]. Based on its dimensional accuracy, low cost, easy fabrication, optical transparency and availability, PDMS is chosen to be the cartridge material.

To fabricate the PDMS cartridge, a plastic mold is designed to fill the PDMS in. The mold used by the last project team has an opening on only 1 side, so it is not suitable for heat transfer on both sides during the experiment. The mold made by the last project team is shown in figure 17 and 18:

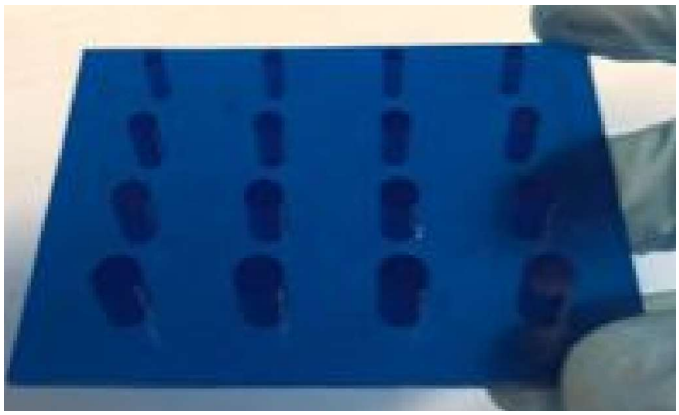

Figure 17: Mold made by the last project team

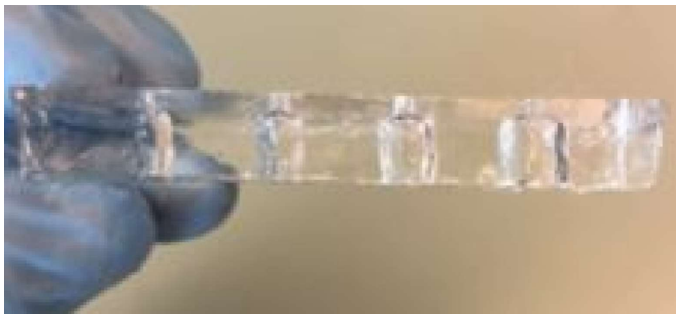

Figure 18: PDMS Cartridge with opening on only 1 side (bottom)

The project objective is to develop a fully-functional thermal cycler with an accurate temperature control. Therefore, the PDMS cartridge must be tested in the actual setting with both chemically-driven heater on the top and bottom side. A cylindrical cavity with 2 openings is needed for the PCR liquid so a new plastic mold is needed. The plastic mold is 3D-printed in the lab, illustrated in figure 19:

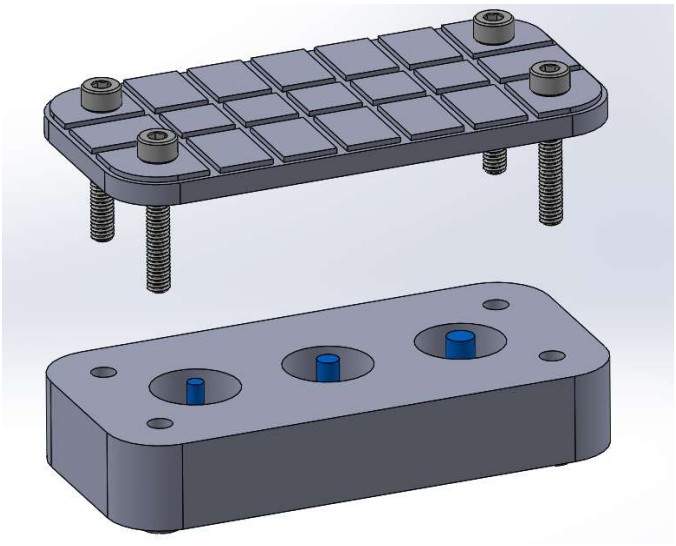

Figure 19: CAD design of the plastic mold

Note that the slot patterns on the top plate are only used for removing the 3D-printed part easily. It serves no other purpose in molding PDMS because the PDMS will be poured inside the mold.

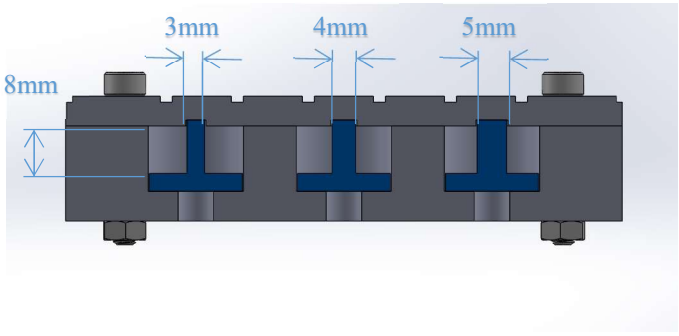

Figure 20: Sectional view of the plastic mold

The whole structure is 3D-printed. As seen in the cross-sectional view, the 3 parts combined create a cylindrical cavity for the PDMS. There are 3 slots for different geometries, which are

- 8mm in height and 3mm in diameter
- 8mm in height and 4mm in diameter
- 8mm in height and 5mm in diameter

Four standard screws are used to fasten the parts together once the PDMS has been poured into the mold cavity.

Figure 21: 3D-printed mold

After the body is 3D-printed, the mold surface is found to be too rough to produce a smooth cylindrical cartridge, so the final mold is made of acrylic board instead. The mold is fabricated by laser cutting 3 layers of acrylic. The 4 dowel pins at the corners are for aligning the 3 parts. And 3 dowel pins of 3mm, 4mm and 5mm diameter are inserted to create the hole in the PDMS cartridge. The layout is shown in figure 22:

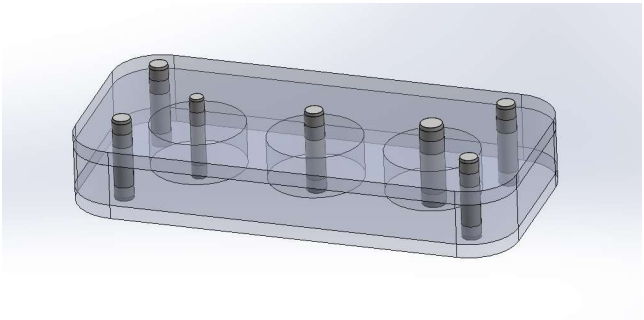

Figure 22: Final CAD design of acrylic mold

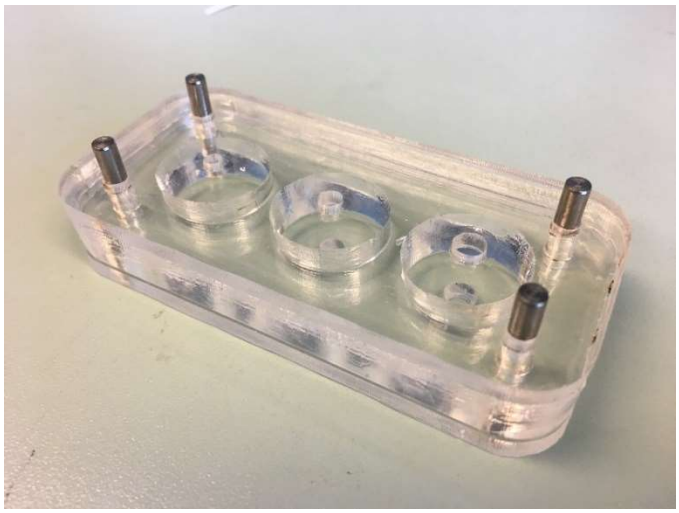

Figure 23: Acrylic mold (Made by laser cutting)

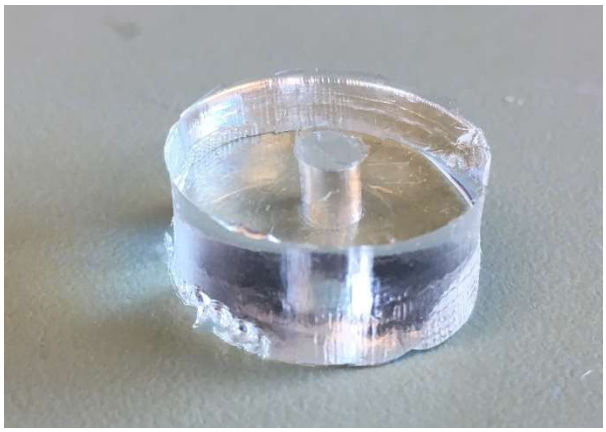

Figure 24: PCR cartridge made by acrylic mold

After the PDMS main body is fabricated, PCR liquid as phosphate-buffered saline solution [24] is placed inside and sealing has to be done to the top and bottom hole. Glass slides can be bonded to the PDMS using plasma etching technique. Plasma treatment disrupts surface silicon-oxygen bonds on the PDMS, making the side hydrophilic with imprints. Then a plasma-treated glass slide is placed on the activated side of the PDMS. After the activation wears off, silicon-oxygen bonds are formed between the atoms on the glass surface and those on the PDMS surface. Finally, the glass slide becomes permanently sealed to the PDMS, creating a waterproof airtight

channel [25]. This treatment is applied to both the top and bottom surface of the PDMS body.

Figure 25 shows the PDMS and glass slide under plasma condition:

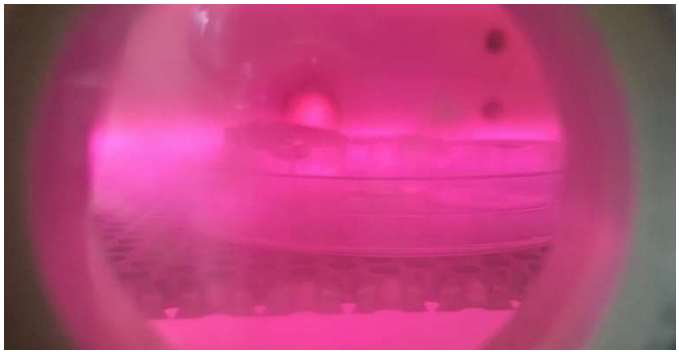

Figure 25: PDMS and glass slide under plasma condition

The final product containing the PCR liquid is demonstrated in figure 26:

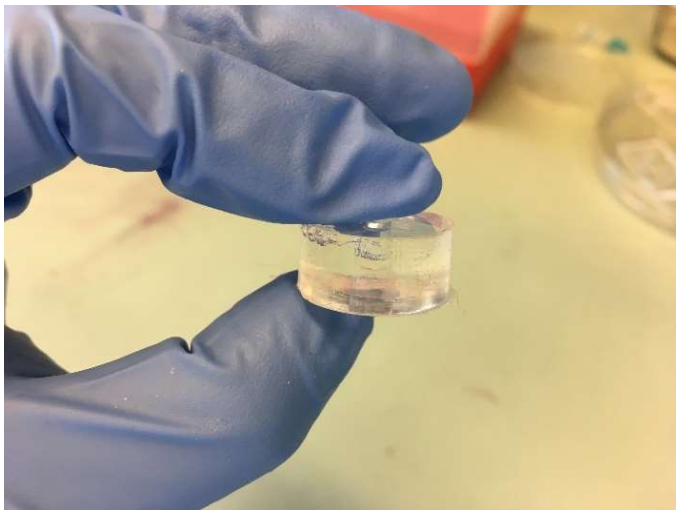

Figure 26: Plasma-bonded Glass (Top and Bottom) on PDMS

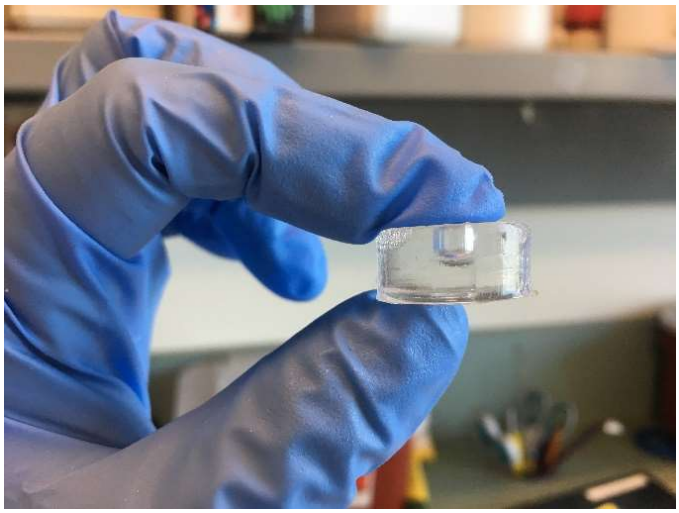

Figure 27: PCR Liquid inside PDMS

A second method is developed to insert the PCR liquid after the cavity has been sealed with glass slides. One 5mL syringe with a 0.4mm needle contains the PCR liquid and is inserted into the bottom of the cavity. And 1 more syringe is empty and is inserted into the top of the cavity. The empty syringe is used to let the air escape when the liquid is pumped in. Without the additional syringe, the air pressure inside will increase and break the glass slide. This method allows more PCR liquid to be filled in the cavity. The final solution contains 70 µL of phosphate-buffered saline with 0.1% tweet and 10 µL of oil. The solution is situated in a cylindrical PDMS container that is 5mm in internal diameter and 8mm in height.

Figure 28: Adding PCR liquid into PDMS cartridge by inserting two syringes

2.6 Insulation Housing

In order to minimize heat loss to the surrounding, an excellent insulation system should be considered. At this stage, the 3D-printed housing that contains the fuel, PCM canister and water will provide some heat insulation. However, the thermal cycler might be used in extreme environment. Therefore, better insulation is needed to fully justify the device's feasibility.

The material chosen to make the insulation housing is expanded polystyrene. The material can be bought off-the-shelf as "Isolofoam vapor barrier insulation panel" with a laminated aluminum reflective membrane. It is generally used for interior insulation for foundation walls due to its large thermal resistance. The extremely low thermal conductivity (0.03 $W/m \cdot K$) and the low density of expanded polystyrene make the material an excellent choice for insulation [26].

Size is still an important consideration when determining how thick the insulation layer should be. The objective is to provide sufficient insulation while keeping the device as portable as possible. Thus, the overall thickness is chosen to be 1mm on all three sides. Layers of 1mm polystyrene panels are then cut by hand using an auto-retracting knife and assembled together by Scotch tape. The housing is shown in figure 29 and 30:

Figure 29: Insulation housing (1mm thick polystyrene panel)

Figure 30: Gap in the middle layer

On the top layer, a hatch is made to add the water into the water reservoir at the beginning. There is a gap in the middle layer, where the PCR cartridge is located. With the insulation, the whole system of apparatus is set up for testing.

Chapter 3: Heat Transfer 1D Model and Simulation

3.1 1D Analysis of Heat Transfer Model

The system of the experiment is described in figure 31:

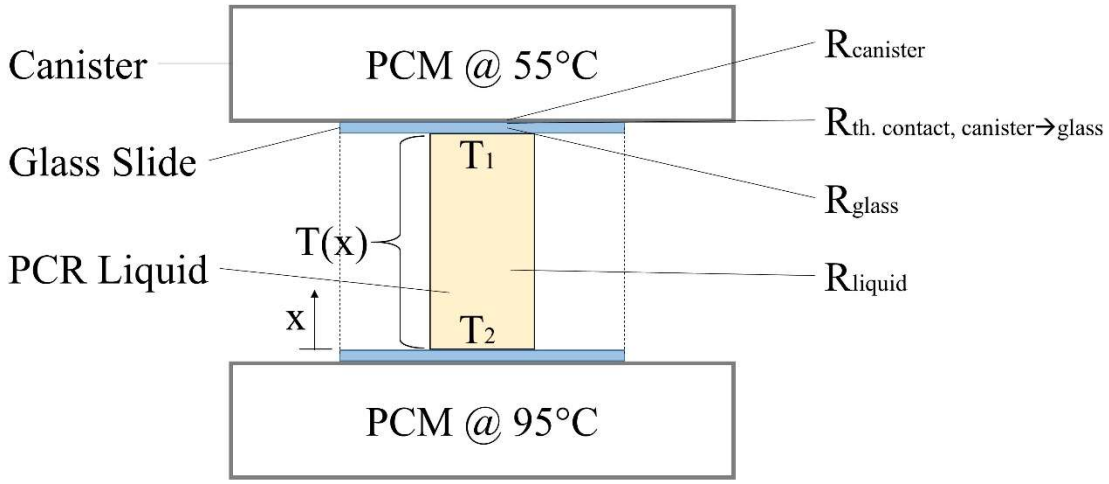

Figure 31: Heat transfer diagram of thermal cycler

The PCR liquid is situated in a PDMS cartridge sandwiched by 2 glass slides and 2 canisters. Inside each canister is a phase change material which maintains the temperature at 55ºC or 95ºC.

Consider the PCR liquid. Recall the 1-D heat-conduction equation from section 1.6,

$$\frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)+\dot{q}=\rho c\frac{\partial T}{\partial \tau} \tag{12}$$

There is no heat generation and change in internal energy inside the PCR liquid, the equation becomes

$$\frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)=0 \tag{13}$$

Assuming thermal conductivity k is a constant,

$$k\frac{\partial^2 T}{\partial x^2}=0 \tag{14}$$

Integrating,

$$\frac{\partial^2 T}{\partial x^2}=0 \tag{15}$$

$$T(x) = c_1 x + c_2 \tag{16}$$

Denote T_1 and T_2 as the temperature of the liquid at the top and bottom respectively. Denote L as the total height of the liquid. When x = 0, T = T_2. When x = L, T = T_1. Applying these boundary conditions,

$$T(0) = c_1(0) + c_2 = T_2 \rightarrow \ c_2 = T_2 \tag{17}$$

$$T(L) = c_1(L) + T_2 = T_1 \rightarrow c_1 = \frac{T_1 - T_2}{L} \tag{18}$$

Therefore, the temperature equation becomes

$$T(x) = \frac{T_1 - T_2}{L}\ x + T_2 \tag{19}$$

This expresses the temperature of the liquid at position x in terms of the two end temperatures T_1 and T_2. Now the two end temperatures T_1 and T_2 needs to be determined. Applying the Fourier law on the PCR liquid,

$$q_{liquid} = -k\frac{dT}{dx}A_{c.s.} = k\frac{T_2 - T_1}{L}A_{c.s.} = \frac{T_2 - T_1}{R_{liquid}} \tag{20}$$

Where

$$R_{liquid} = \frac{L_{liquid}}{k_{liquid}A_{c.s.}} \tag{21}$$

In the top heater, the heat generated by the phase change material in the canister has to go through 3 thermal resistance before reaching the PCR liquid. The first layer is the aluminum canister thermal resistance, the second layer is the thermal contact resistance between the canister and the glass slide, and the third layer is the glass slide thermal resistance.

$$R_{canister} = \frac{L_{canister}}{k_{canister}A_{c.s.}} \tag{22}$$

$$R_{th.\ contact,canister\rightarrow glass} = \frac{1}{h_{contact}A_{c.s.}} \tag{23}$$

$$R_{glass\ slide} = \frac{L_{glass\ slide}}{k_{glass\ slide} A_{c.s.}} \tag{24}$$

The sum of the top part of the heater is:

$$\sum R_{top} = R_{canister} + R_{th.\ contact,canister \rightarrow glass} + R_{glass\ slide} \tag{25}$$

Therefore, the heat transfer rate of the top heater is:

$$q_{top} = \frac{T_1 - 55^o C}{\sum R_{top}} \tag{26}$$

Similarly, the heat transfer rate of the bottom heater is:

$$q_{bottom} = \frac{95^o C - T_2}{\sum R_{bottom}} \tag{27}$$

With the bottom heater having the same 3 layers of thermal resistance,

$$\sum R_{bottom} = \sum R_{top} \tag{28}$$

Equating all the heat transfer rate in the system, the governing equation is:

$$q_{liquid} = q_{top} = q_{bottom} \tag{29}$$

$$\frac{T_2 - T_1}{R_{liquid}} = \frac{T_1 - 55^o C}{\sum R_{top}} = \frac{95^o C - T_2}{\sum R_{bottom}} \tag{30}$$

Neglecting the heat transfer from the PDMS side wall into the liquid, the heat transfer cross-sectional area is assumed to be the same for all layers. The values for the material thickness and thermal conductivity [27, 28, 29] used for estimation are listed in appendix A1.

Substituting the values into the governing equation (30),

$$\frac{T_2 - T_1}{R_{liquid}} = \frac{T_1 - 55^o C}{\sum R_{top}} = \frac{95^o C - T_2}{\sum R_{bottom}}$$

$$\frac{T_2 - T_1}{\left(\frac{0.0155518}{A}\right)} = \frac{T_1 - 55^o C}{\left(\frac{0.000206759}{A}\right)} = \frac{95^o C - T_2}{\left(\frac{0.000206759}{A}\right)}$$

$$\frac{T_2 - T_1}{0.0155518} = \frac{T_1 - 55^o C}{0.000206759} = \frac{95^o C - T_2}{0.000206759}$$

Solving the simultaneous equation for T_1 and T_2, the estimated temperatures of the PCR liquid at the top and bottom region are:

$$T_1 = 55.518$$
$$T_2 = 94.482$$

Using the result in the temperature equation (19),

$$T(x) = \frac{T_1 - T_2}{L}\, x + T_2$$

$$75 = \frac{55.518 - 94.482}{0.0093}\, x + 94.482$$

$$x = 0.00465\ m$$

When T = 75°C, x = 0.00465 m. This indicates that the temperature at the center of the liquid is 75°C, which is the region designed for the third step of the thermal cycling process at 75°C.

The steady-state one-dimensional model has its limitation. For example, the thermal conductivity of materials varies with temperature. The PDMS cartridge is also heated up in the process and additional heat is transferred into the PCR liquid. Another error would be the slight fluctuation of the temperature maintained by the phase change material. One should keep in mind that the convection heat loss by the air is insignificant because PDMS has a low thermal conductivity (~0.18 $W/m \cdot K$) [30] and the cylindrical PDMS wall is relatively thick.

3.2 Thermal Simulation

In order to further evaluate the thermal distribution of the thermal cycler, SolidWorks Thermal Simulation is used to model the system in 3D. The finite element analysis is believed to provide

a more sophisticated study because it takes the air convection, component shape and thermal contact resistance into account. The 3D model of the critical elements is created in figure 32:

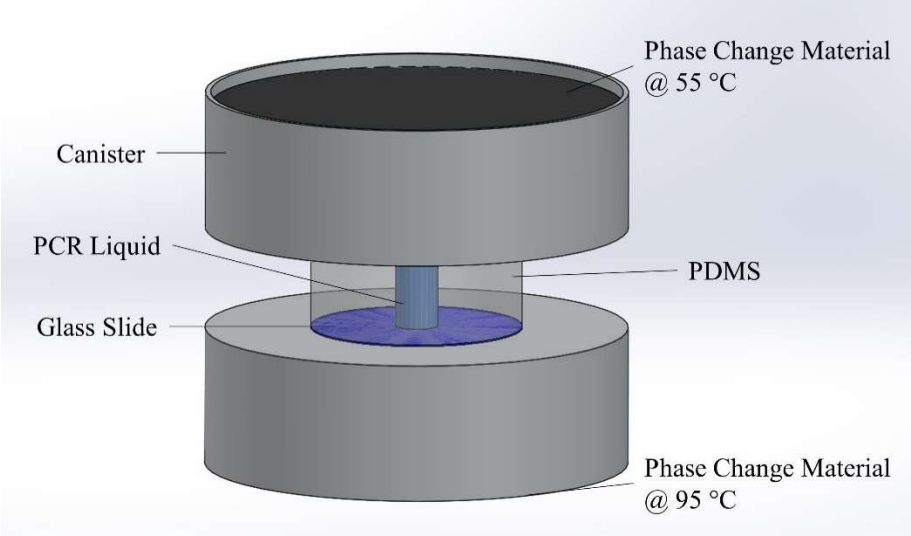

Figure 32: SolidWorks CAD model for thermal simulation

In the model, the PCR liquid is contained in PDMS. The container is sandwiched between two glass slides and two aluminum tin canister. The phase change material is created as a block sitting inside the canister as an isothermal heat source.

The material settings for the parts are: 1. Paraffin wax (top phase change material) 2. Glutaric acid (bottom phase change material) 3. Aluminum (canister) 4. Glass (glass slide) 5. Rubber (PDMS) 6. Salt water (PCR liquid). Some parts are chosen to be a different material, but it would not contribute to errors as long as the chosen material has the same thermal conductivity as the real part.

The thermal contact coefficient settings are: 1. Perfect contact surface between phase change material and canister 2. $h = 16{,}667\ W/m^2 \cdot K$ between canister and glass slide 3. Perfect

contact surface between glass slide and PCR liquid. Some contact surfaces have been assumed to be perfect because one of the materials is in liquid form.

The thermal load settings are: 1. Isothermal temperature (55°C) on top phase change material 2. Isothermal temperature (95°C) on bottom phase change material 3. Free convection by still air ($5\ W/m^2 \cdot K$) [31]. The mesh density is set to fine for a more accurate result while the mesh element size is set to 0.80071046mm with a 1.5 ratio. The result is presented in figure 33:

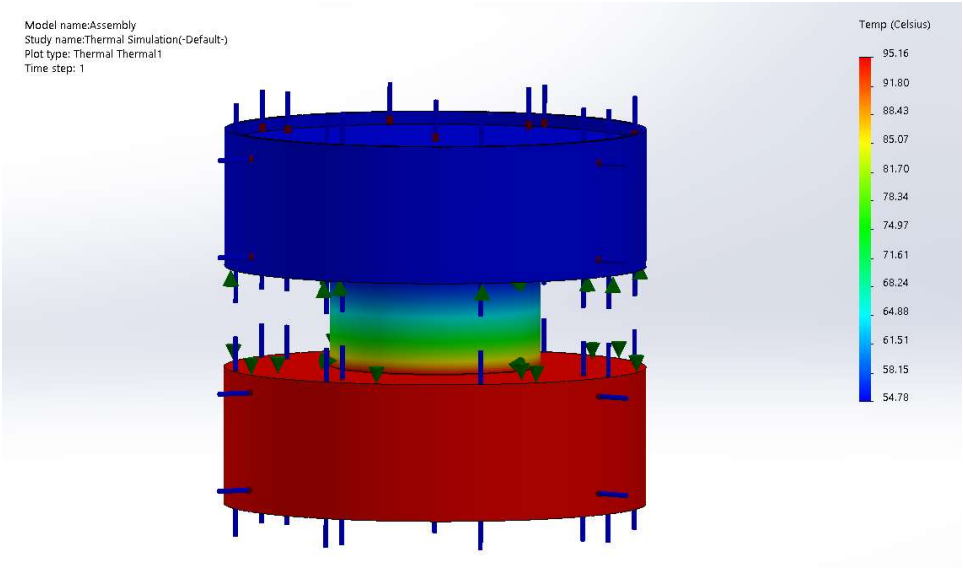

Figure 33: Thermal simulation result of thermal cycler

As expected, the highest and lowest temperatures are maintained at 95.16°C and 54.78°C respectively. High temperature regions are denoted as red while low temperature regions are denoted as blue. There is a smooth temperature gradient along the vertical axis of the PDMS container in the middle. In the figure above, the PCR liquid temperature distribution is blocked by the PDMS container. Therefore, the PDMS container is removed from the display in figure 34:

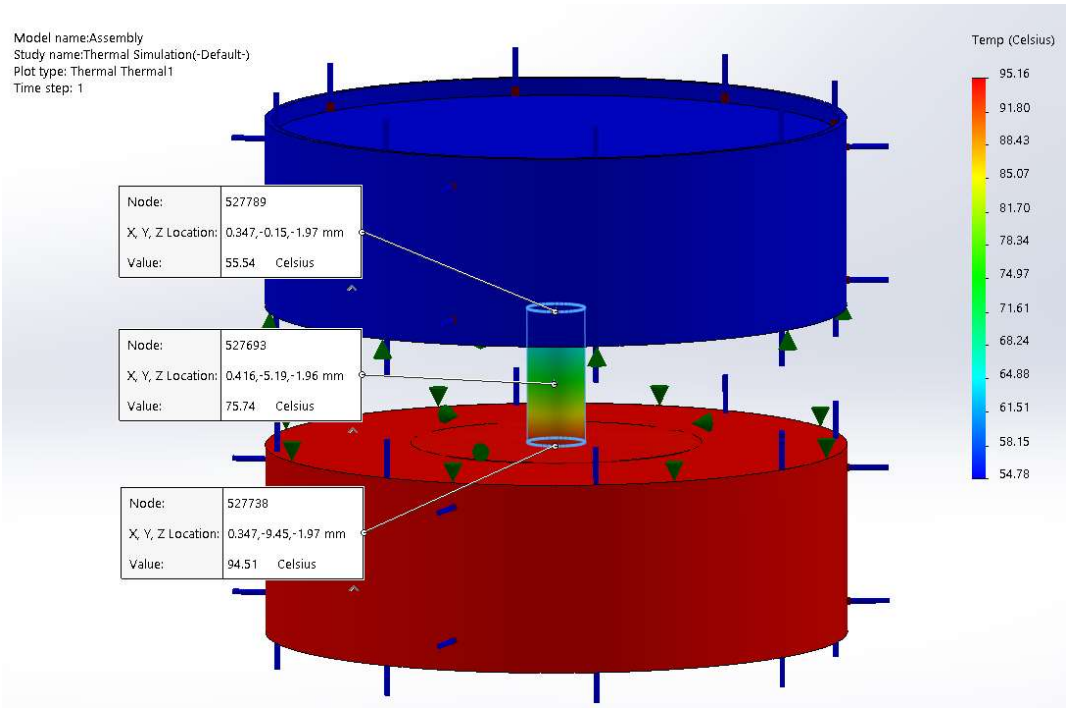

Figure 34: Thermal simulation result of thermal cycler (PDMS container removed)

The temperature distribution of the PCR liquid is very similar to that of the PDMS container. Within the PCR liquid, the bottom region has the highest temperature (94.51°C) while the top region has the lowest temperature (55.54°C). The temperature is 75.74°C when the position "x" is 4.26 mm from the bottom. Recall the result from the 1D analysis,

$$T_1 = 55.518\,℃$$

$$T_2 = 94.482℃$$

$$T = 75℃\ when\ x = 0.00465\ m$$

Therefore, the simulation results are very close to the analytical calculations. This proves that air convection and other factors are insignificant to the heat distribution. Due to the overall heat transfer, the maximum temperature will be slightly lower and the minimum temperature will be slightly higher. But they are still within our desired temperature range for polymerase chain reaction (PCR) to take place. These theoretical results will be compared and verified with the experimental results later.

Chapter 4: Development of Method for Data Measurements

4.1 Measurement with Thermocouples on 1st Prototype

For the 1st prototype, the project is at a very beginning stage of testing the feasibility of calcium oxide and phase change material to sustain a stable temperature. Focus has been put on recording the temperature output from the PCM canister instead of the actual temperature inside the PCR liquid. Therefore, thermocouples are used to directly measure the temperature on top of the tin canister containing the phase change material. Our goal is to achieve reproducibility of the system (same steady state temperature each time) and heat isolation between the three temperature regions. It is not desirable for the temperature of one heater to be affected by the heat source from another heater. So the temperatures of the other inactivated heaters (shown in red) are also monitored to make sure the operating heater (in blue) does not affect them. In the set-up, 3 separate thermocouples are taped onto the 3 heaters' canisters.

The thermocouple reader used is the Omega TC-08 data acquisition module. With a built-in cold junction compensation (CJC), the TC-08 has an effective measuring range of -270 to 1820°C but the range also depends on the type of thermocouple used. When type K and T thermocouples are used, the temperature accuracy is the sum of ± 0.2% of the reading ± 0.5 degrees C and has a resolution of better than 0.1°C [32]. The PicoLog Recorder software [33] would be launched to record the temperature every 1 second.

Figure 35: Thermocouples on the 3 heaters from the first prototype

4.2 Measurement with Thermocouples on 2nd Prototype

For the second part of the project, emphasis has been put on investigating the actual temperature inside the PCR liquid. Type K and T thermocouples are again used. The goal is to verify if the temperature of the top and bottom of the PCR liquid is stable at the 55°C and 95°C respectively. Therefore, holes have been created by piercing through the PDMS with a 0.4 mm needle. Thermocouples are then inserted into the PDMS to reach the PCR liquid inside. Attempts have been made to make a hole as close to the top and bottom surface as possible. However, when the hole is made too close to the top and bottom glass slides, the stretching of PDMS caused by thermocouple often breaks the glass slide. So the thermocouple cannot be located at the ideal top and bottom region. As a result, the accuracy of the measurement location is limited.

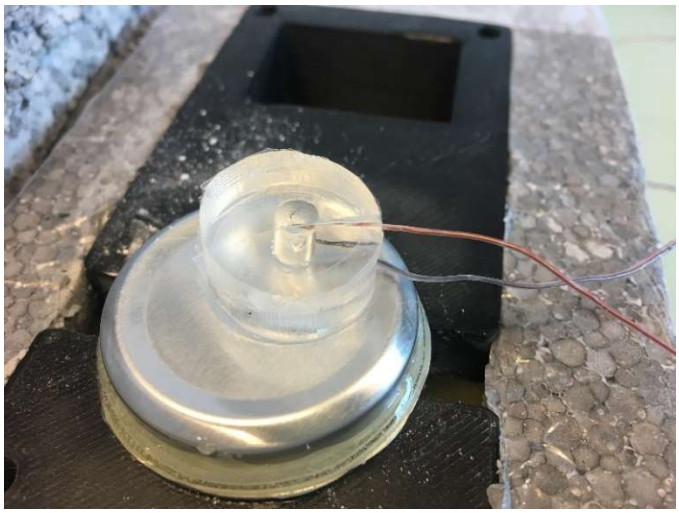

Figure 36: Thermocouples inserted into PDMS (Isometric view)

The two thermocouples are connected to the same thermocouple reader (Omega TC-08) used in the 1st prototype and data is recorded by the PicoLog Recorder on computer. The temperature sampling rate is set to 1 sample per second. The total sampling time has been set to 1 hour since the whole PCR reaction process takes around 45 minutes. 2 channels are used because only 2 temperature regions are needed for verifying the 2nd prototype. The complete experiment setup is illustrated in figure 37.

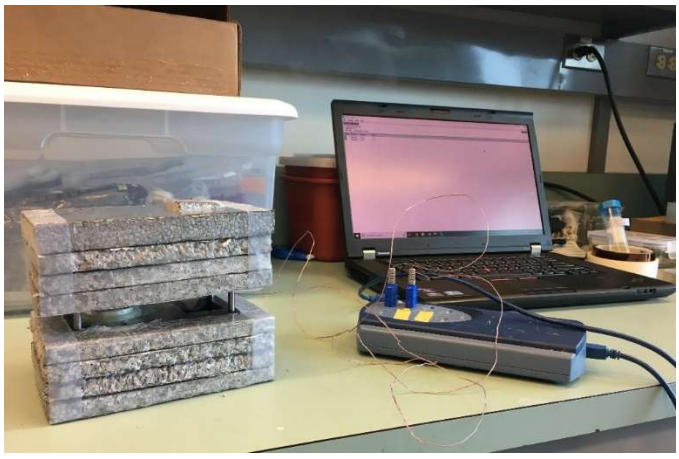

Figure 37: Setup with 2 thermocouples for data recording

Chapter 5: Preliminary Results and Observation

5.1 1st Prototype Result

The first part of the project is about evaluating the ability to maintain steady-state temperature for a prolonged period of time. Heater 1 (55ºC) and Heater 2 (75ºC) both show satisfying results in keeping the temperature constant. In heater 3 (95ºC), the increase in pressure inside the container has significantly affected the amount of heat supply to the PCM canister. Therefore, it is difficult to adjust the pressure so that the right amount of heat is supplied to stay within the temperature bandwidth. The spring-assisted gas exhaust valve has been adjusted for many trials (mentioned earlier in chapter 2.4) and one of the best results obtained in an experiment is presented in figure 38:

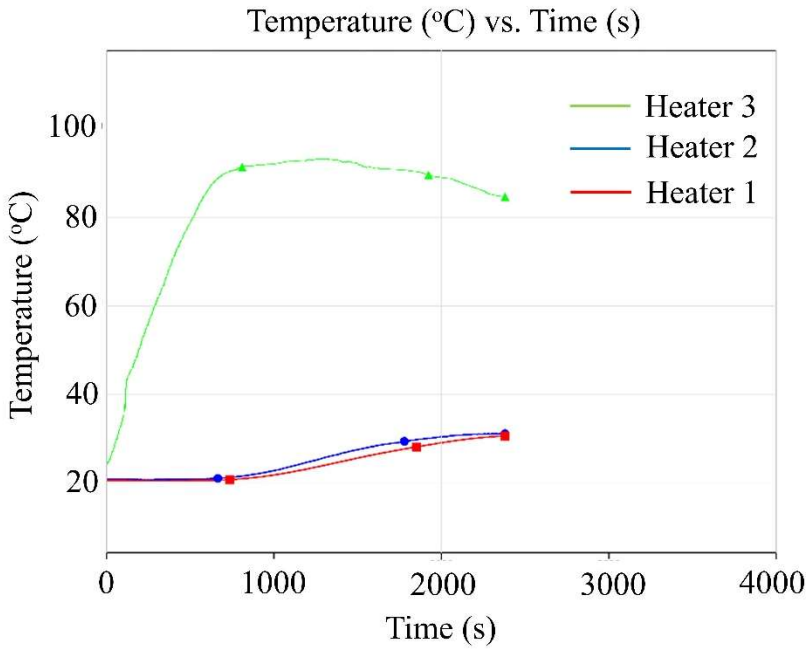

Figure 38: Temperature of the Canister for Heater 3 (95ºC) with Time

The channel 3 (green) refers to the temperature of the canister on heater 3 (95ºC) being tested. The thermocouple records the temperature of the top surface of that canister. The other 2

channels (blue and red) do not have activated chemical reaction and serve as controls. Since the thermocouple is directly in contact with the canister surface, the temperature accuracy can be assumed to be the sum of ± 0.2 % of the reading ± 0.5 ºC with a 0.5ºC resolution. The error bars are 0.68 – 0.7 ºC between 90 –100ºC and does not have a significant effect on the accuracy. As heater 3 is activated, the temperature rises to our desired temperature region (90 – 100ºC) in 912 seconds. It successfully maintains the temperature within +/- 5ºC for 1008 seconds before dropping below the critical range. Since the residence time is only around 17 minutes, the result does not satisfy the requirement for a 45-minute PCR reaction.

5.2 2nd Prototype Result

As mentioned previously, the second part of the project involves two heaters at 55ºC and 95ºC and two thermocouples are used to measure the temperature at the top and bottom region of the PCR liquid. Improvements have been made after each experiment. This section will discuss the observation and insights from some of the experiments.

In the first experiment, both the top and bottom heaters are activated at the same time to create a temperature gradient from 55ºC to 95ºC. The setting is shown in the table 1 below:

Activated Heaters	Top (55ºC) and bottom (95ºC) heater
Amount of Mg-Fe Fuel	5g for top heater 7g for bottom heater
Amount of Water Added	15mL for each heater
Steam Blockage	No
Outer Insulation	No

Table 1: Trial 1 setting without insulation on canisters

During the experiment, a significant amount of water vapor is produced from chemical reaction. The steam comes out of the gap between the housing and canister and surrounds the PDMS cartridge. Therefore, it transfers heat to the cartridge and the PCR liquid inside. The steam is shown in figure 39 and 40.

Figure 39: Steam observed during chemical reaction

Figure 40: PCR cartridge during experiment

Since both heaters give out an excess amount of steam, it leads to temperature fluctuation. Despite the fluctuation, there is a steep increase in temperature at the first 300 seconds. The temperature then reaches a maximum temperature and decreases at a slower rate back to room temperature. The result with minor fluctuations is illustrated in figure 41 below.

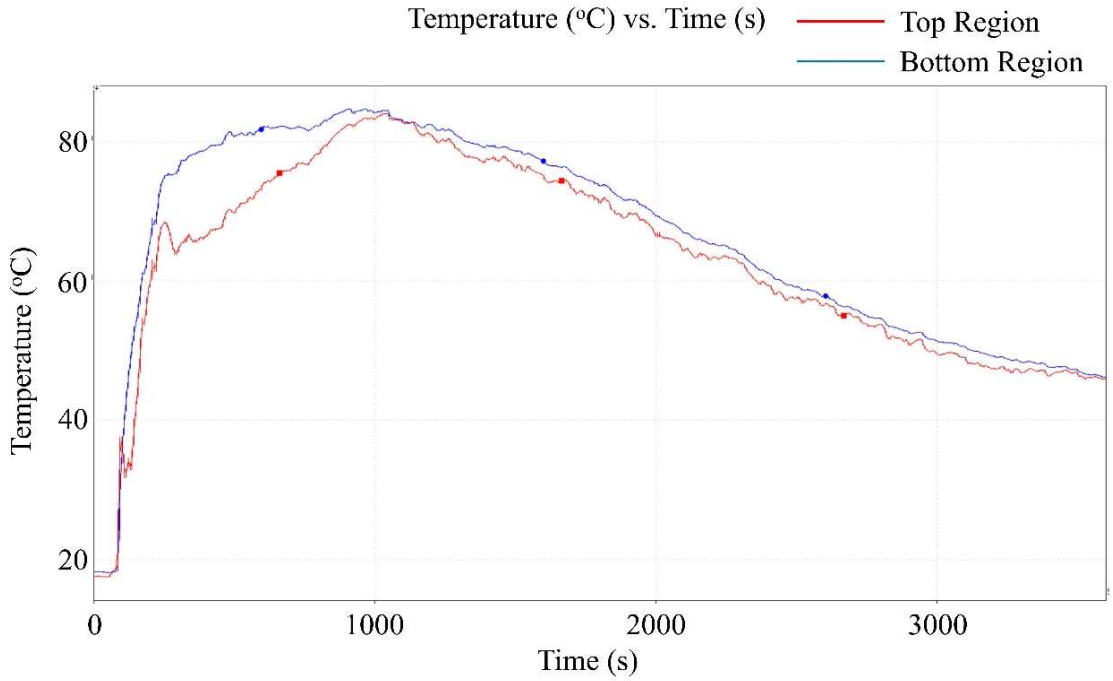

Figure 41: Trial 1 experiment result without insulation on canisters

The previous experiment suggests that a blockage is needed to prevent the water vapor from going around the canister to the PDMS cartridge on top. For the bottom heater, a polystyrene cover is fixed tightly on the canister. For the top heater producing fewer steam, a plastic paraffin film is wrapped around the canister to direct the steam to other directions. The steam blockages are shown in figure 42.

Figure 42: Steam blockage on each heater

Other settings in the experiment remains unchanged with only the additional steam blockage to test its effect. The setting is described in the table 2 below:

Activated Heaters	Top (55ºC) and bottom (95ºC) heater
Amount of Mg-Fe Fuel	5g for top heater 7g for bottom heater
Amount of Water Added	15mL for each heater
Steam Blockage	Yes
Outer Insulation	No

Table 2: Trial 2 setting with insulation cover on canisters

The result, given in figure 43, proves that the steam blockage on the canister can effectively reduce the steam from disturbing the PDMS cartridge, thus reducing the minor fluctuations in temperature. The new graph shows a stable and smooth temperature rise. The maximum temperature is 89.68ºC at time 1334 seconds. Then the temperature decreases slowly after the fuel is no longer giving out heat. Overall, the temperature maintains above 80ºC from time 445s

to 3014s. Channel 1 (in red) refers to the top region inside the PCR liquid while channel 2 (in blue) refers to the bottom region inside the PCR liquid. Although the bottom region succeeds to reach a temperature near 95°C, the top region is expected to stay at 55°C during the whole experiment. It can be observed that the top regional temperature follows closely behind the bottom regional temperature. Therefore, the top regional temperature is affected by the bottom heater as well. Excessive heat is transferred from the bottom heater through the PCR liquid to the top region.

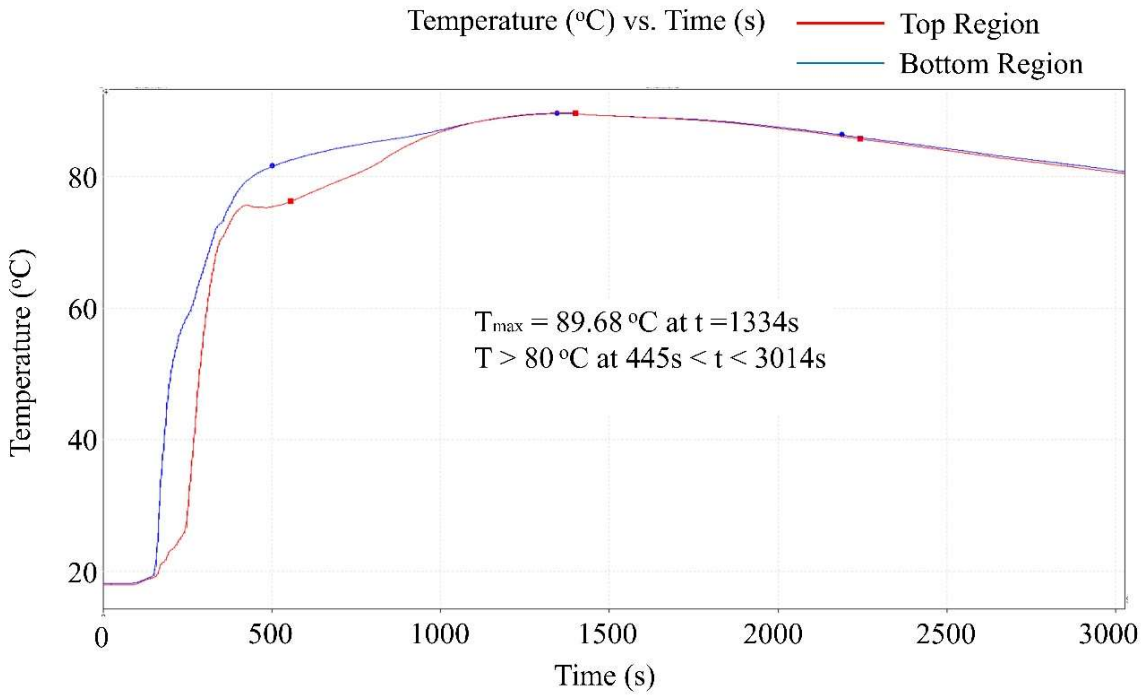

Figure 43: Trial 2 experiment result with insulation on canisters

Based on the previous experimental result, even when the top heater is maintained at 55°C, the bottom heater will heat up the entire PCR liquid above 80°C. Therefore, the following experiment discussed here is to investigate whether the top regional temperature will be lower when the top heater is not activated. Only the bottom heater is activated.

Activated Heaters	Bottom Heater (95°C) Only
Amount of Mg-Fe Fuel	7g for bottom heater
Amount of Water Added	15mL added at the beginning Continuous feed of water afterwards
Steam Blockage	Yes
Outer Insulation	No

Table 3: Trial 3 setting with only bottom heater activated

After the top heater is deactivated, the top regional temperature (in red) reaches its maximum temperature of around 60°C. It still receives heat from the bottom heater but convection cooling from the top lowers its temperature in general. An additional measurement (in green) is made on the top glass slide. Its purpose is to verify the effectiveness of the cooling air by convection. Since the glass slide temperature follows the behavior of the top regional temperature, heat can be effectively taken out of the top region of the PCR liquid through convection. Furthermore, the bottom regional temperature (in blue) rises steeply to a maximum temperature of 81.84°C, which is lower than that from the previous experiment. And then it has a higher decreasing rate than that from the previous experiment. This suggests that the bottom heater alone cannot provide the heat needed to maintain a 95°C temperature region.

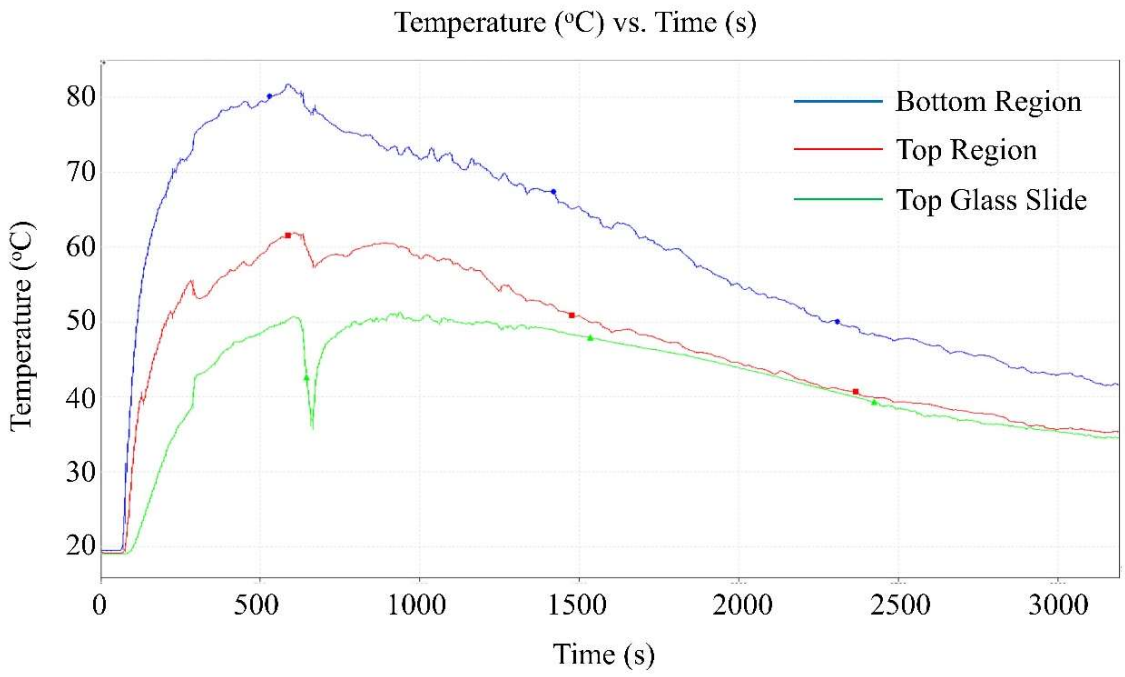

Figure 44: Trial 3 experiment result with 7g of Mg-Fe fuel in bottom heater only

Since the supply of heat is concerned, the amount of Mg-Fe fuel can be increased to supply more heat. This was proposed since the temperature drops too quickly after the initial increase. The previous experiments have all been performed with 7g of Mg-Fe fuel. So this experiment investigates the effect of doubling the amount of fuel to 14g. A larger fuel holder is replaced to hold more fuel and an additional 15mL of water is added after the first 15mL of water is consumed by the fuel. The time of adding the extra 15mL of water is 823 seconds after initial reaction.

Activated Heaters	Bottom Heater (95°C) Only
Amount of Mg-Fe Fuel	14g for bottom heater
Amount of Water Added	15mL added at the beginning 15mL added at time = 823 s

Steam Blockage	Yes
Outer Insulation	No

Table 4: Trial 4 setting with only bottom heater activated and 14g of fuel

Contrary to the expectation, the result shown in figure 45 suggests that more fuel does not increase the maximum temperature or slow down the cooling rate afterwards. Based on the temperature behavior, even fewer heat has been transferred to the canister and the PCR liquid. Some observations might explain the phenomenon. When the additional 15mL water is added at 823s, the decreasing temperature rises again. And the Mg-Fe fuel expands after undergoing the chemical reaction with water. Therefore, the canister is pushed upward by the reacted fuel. The layer of reacted fuel forms an insulation barrier for the reacting fuel at the bottom. As a result, it is difficult to transfer heat to the canister through this layer of reacted fuel. This behavior is promoted by increasing the amount of fuel to 14g and is less dominant when the fuel is kept at 7g. As seen in figure 45, the bottom regional temperature decreases steeply again after reaching the maximum temperature, indicating one heater is not sufficient to slow down the cooling rate.

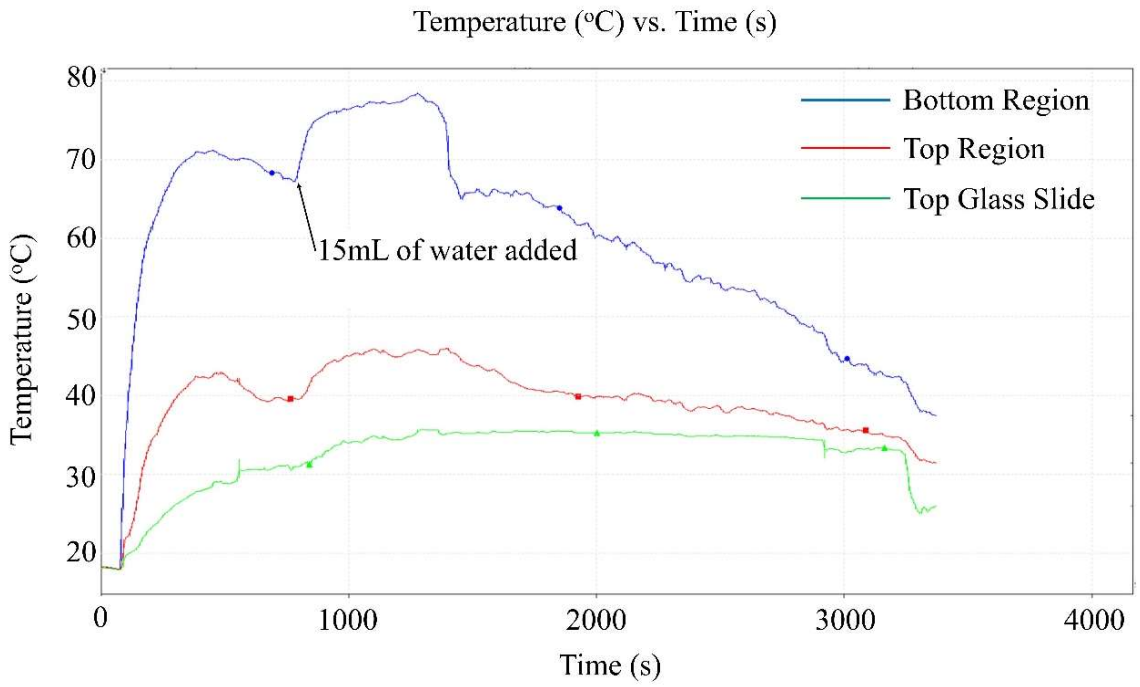

Figure 45: Trial 4 experiment result with 14g of Mg-Fe fuel in bottom heater only

Since the proposed solution of using the top and bottom heater to create a temperature gradient from 55°C to 95°C is proven to be impracticable, it is now important to test whether two identical 95°C heaters can maintain the desired temperature for the required time of around 45 minutes. The top and bottom heaters now contain the same phase change material (glutaric acid) designed to stabilize the temperature at 95°C. If this concept is feasible, three identical structures can be created as the whole thermal cycler. However, the PCR liquid is again needed to be manually cycled by hand during the reaction, similar to the first prototype.

Activated Heaters	Top and bottom heater both at 95°C
Amount of Mg-Fe Fuel	7g for both heater
Amount of Water Added	15mL in each heater
Steam Blockage	Yes

Outer Insulation	No

Table 5: Trial 5 setting with two 95°C heaters

When both heaters are activated at 95°C, the temperatures in the top and bottom regions are very similar. The maximum temperature is 91.63°C and the temperature range 80 – 90°C is maintained for 1521 seconds (around 25 minutes). However, the desired time period should be around 45 minutes. It is obvious that the two 95°C heaters are able to reach the target maximum temperature but there are too much heat loss afterwards. The average cooling slope is -0.0138 °C/s.

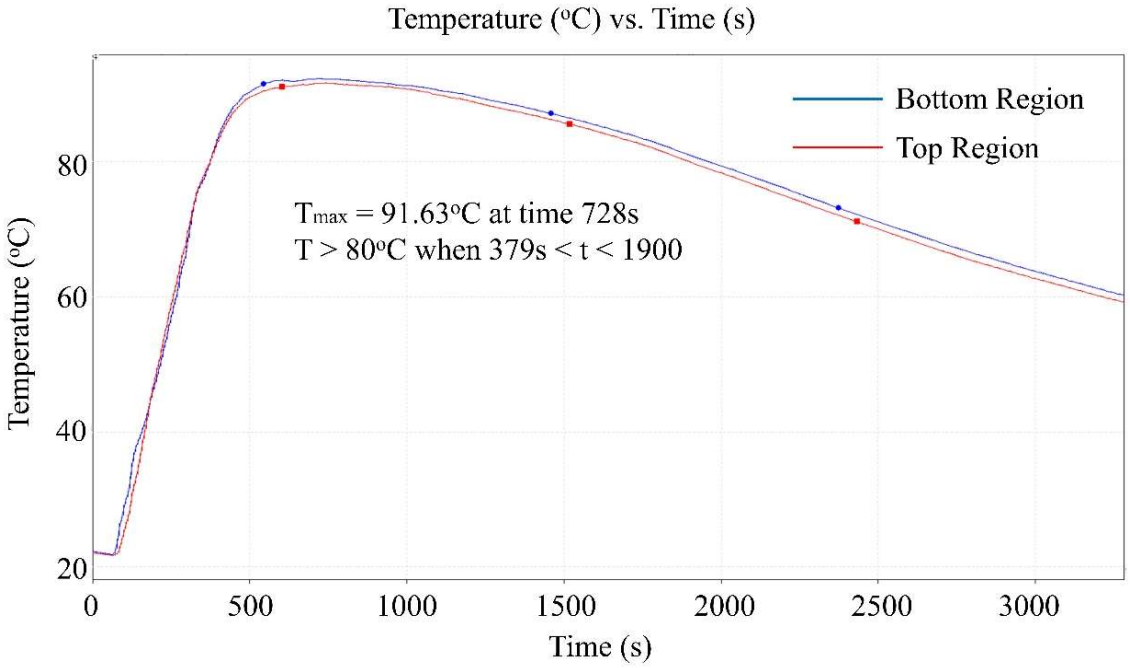

Figure 46: Trial 5 experiment result with both heaters activated at 95°C

Since the heat loss is a critical factor, outer insulations are added to the whole structure. Five polystyrene board are taped around the thermal cycler, providing an enclosed environment. Additional clothes are also wrapped around the boards to further minimize the heat loss.

Activated Heaters	Top and bottom heater both at 95°C
Amount of Mg-Fe Fuel	7g for both heater
Amount of Water Added	15mL in each heater
Steam Blockage	Yes
Outer Insulation	Yes

Table 6: Trial 6 setting with two 95°C heaters and outer insulations

After outer insulations are added, the heat loss is reduced to a great extent, as shown in figure 47. The maximum temperature is also increased because of the trapped steam at the beginning of the chemical reaction. As both heaters are at 95°C, the top and bottom region inside the PCR liquid has nearly the same temperature throughout the experiment. The maximum temperature is 94.73°C at time 478s. The temperature stays within the range of 85 - 95°C during time 293s to 2869s (a period of 2576s ≈ 43 minutes). The average cooling slope is reduced to -0.00304 °C/s. Overall, the result satisfied the required function of a thermal cycler.

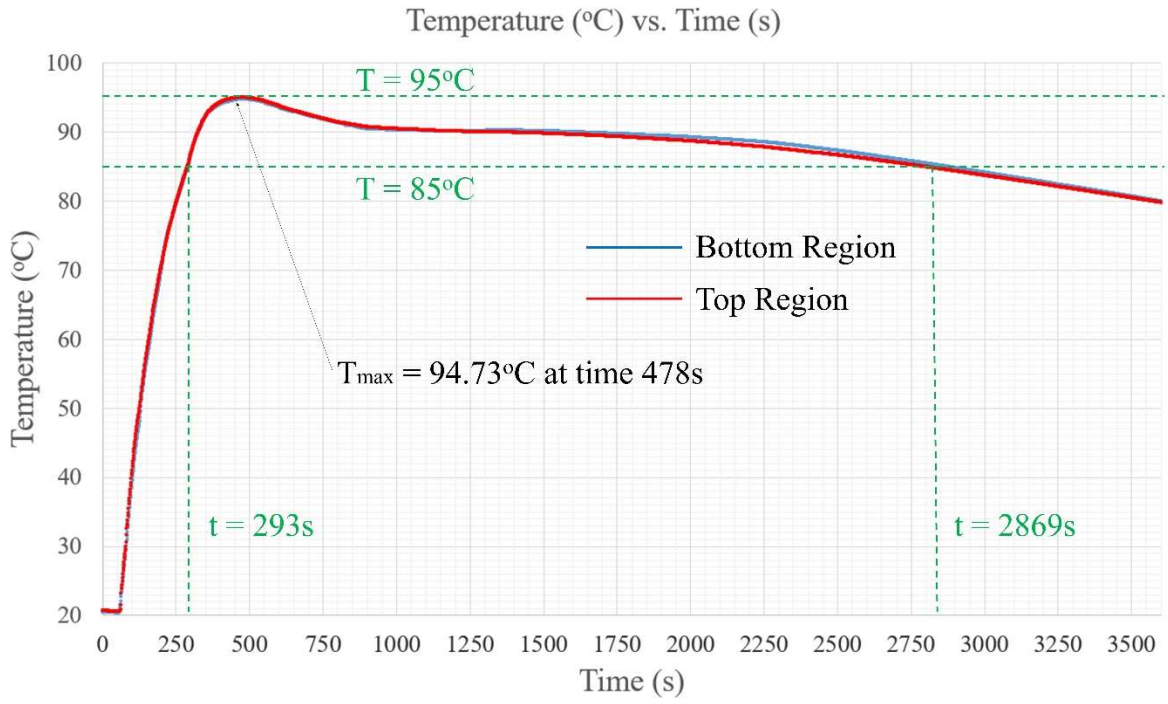

Figure 47: Trial 5 experiment result with both 95ºC heaters and outer insulations

5.3 Discussions from the 2nd Prototype Results

It is obvious that the ideal situation of maintaining a stable temperature gradient from 55ºC to 95ºC is not feasible in the actual setting. Activating the top and bottom heaters at 55ºC and 95ºC heats up the entire PCR liquid to a temperature range of 80 – 90ºC. This temperature range is too high for the 55ºC region and is too low for the 95ºC region. The temperature accuracy by the thermocouples can again be assumed to be the sum of ± 0.2 % of the reading ± 0.5 ºC with a 0.5ºC resolution. The error bars are 0.66 – 0.68 ºC between 80 – 90ºC and does not have a significant effect on the result.

The settings for all the experiments are summarized in appendix A2. After adding the insulation cover, changing the amount of fuel, using only one heater and activating both heaters at the same temperature, a final solution has been discovered. Activating both heaters at the same temperature can provide a steady and stable heat supply. The phase change material (PCM) sets the temperature ceiling for the PCR liquid. As heat transfer relies on the temperature difference between the two bodies, the temperature of the PCR liquid will not be higher than that of PCM. Using glutaric acid as the PCM, the temperature is sustained at 85-95ºC. With additional outer insulations, the temperature range is extended for around 43 minutes, which is suitable for the 45-minute PCR operation. As the PCM constrains the upper temperature limit at its melting point, a PCM with 100ºC melting point would be more suitable for a 90-100ºC range.

Chapter 6: Final Conclusion and Summary

6.1 Summary and Conclusion

The development of the instrument-free thermal cycler has been divided into two parts with different prototypes. The first prototype examines the use of chemical fuel, phase change material and insulated bottle to create an isothermal environment for PCR reaction. This prototype is not practical because the calcium oxide reaction is not able to supply enough heat to reach the 45-minute benchmark. It only kept the temperature within the 90ºC – 100ºC range for 1008 seconds in the most successful trial. Most importantly, the prototype require manual cycling- placing the DNA samples onto different heaters. This has greatly increased the time needed to change the sample temperature from one stage to another and to achieve thermal equilibrium for each temperature step.

In the second prototype, a compact 3D-printed housing with high-energy-density Mg-Fe fuel leads to a great improvement. The device is lightweight and portable while Mg-Fe fuel can supply the heat needed for the 45-minute reaction. Experimental results with different settings have offered insights to the performance under different amount of fuels, insulation and activated heaters. The final configuration uses two heaters with 95ºC phase change material to sandwich the PCR liquid. The temperature inside the liquid can be stabilized at 90ºC within +/- 5ºC for around 43 minutes. The phase change material in the heater is able to define a temperature ceiling so that the liquid temperature is always below the material's melting point. Outer insulation also plays an important role in concentrating heat during reaction and reducing heat loss afterwards. However, a temperature gradient cannot be created by combining the top and bottom heaters at 55ºC and 95ºC respectively. This is due to the excessive heat transfer inside the

liquid. Therefore, the preliminary findings have encouraged future work to focus on optimization of the two heaters to obtain a 55–95ºC gradient throughout the liquid. The objective is to cycle the samples to different regions naturally by a convection current, which relies on a temperature difference.

6.2 Limitations and Recommendations for Future Work

In the development of the two prototypes and experiment, there are limitations that lead to different sources of errors. These limitations caused a deviation from the ideal behavior and they could be reduced for future work. They are summarized as follow:

a) Temperature gradient inside liquid

   In the configuration of using a top 55ºC heater and bottom 95ºC heater, the temperature gradient cannot be produced. This is because the top region is heated up by both heaters. In the future, work can be done on reducing the top temperature to 55ºC while maintaining the bottom temperature at 95ºC. On top, cooling fluid can be introduced to lower the regional temperature. At the bottom, heat transfer between the phase change material and liquid can be improved to raise the temperature. The temperature gradient will then trigger the convection current required to cycle DNA samples.

b) Phase change material

   A phase change material with a 95ºC melting point was originally chosen because it is expected to heat the PCR liquid to a center temperature of 95ºC with a +/- 5ºC range. However, the experiment has suggested that the melting point corresponds to the

maximum temperature instead of a center temperature. Therefore, future work can focus on selecting another phase change material with a 100ºC melting point to target the 95ºC center temperature.

c) Air cavity in PDMS cartridge

During the fabrication of PCR cartridge, liquid PDMS is mixed with reacting agent and poured into a fabrication mold to solidify. Although the liquid PDMS had already been degassed in a vacuum chamber beforehand, there are still some air bubbles formed during the filling process. These bubbles resulted in air cavities in the solidified PDMS cartridge and the heat transfer process might be disturbed. With air trapped inside the cartridge, the thermal conductivity would not be constant throughout the entire body because the air has a different thermal resistance. Therefore, the system cannot be modelled using a 1 degree of freedom.

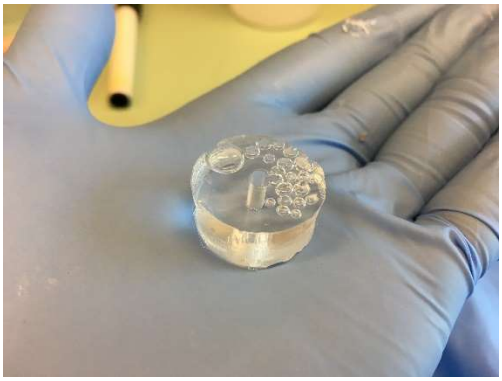

Figure 48: Air cavity formation inside PDMS cartridge (not used in experiment)

d) Air trapped in PCR liquid

Inside the PCR cartridge, PCR liquid was added into the cylindrical cavity before the cartridge was sealed. A proportionally small amount of air is intentionally trapped with

the liquid to compensate for the expansion of liquid under high temperature. If the liquid fills the whole cartridge, as the temperature rises, the liquid will expand and turn partly into gas. As a result, the pressure increases until the glass ceiling breaks. To avoid the high pressure build-up, a small amount of air is conserved in the cylindrical cavity. However, this poses a source of error disrupting the heat gradient inside the liquid. Future work should be focused on searching for a solution with a lower expansion coefficient to minimize pressure rise.

e) Sealing of PDMS cartridge with glass slides

After the PCR liquid added to the PDMS cartridge, the cartridge is sealed with glass slide using plasma bonding. The glass slide has a high surface flatness and low roughness but the PDMS cartridge does not [34]. Thus, when the two parts were bonded together, the two surfaces were not perfectly in contact. Little air channels existed and caused an insignificant leakage of PCR liquid from the inside. This leakage becomes an important factor when the temperature and pressure inside the liquid increases during the experiment. The leakage affected not only the heating, but also the liquid current.

f) Insulation housing gap

In designing the insulation housing, a small gap is made to observe the PCR liquid during heating. While it is the only solution to observe the liquid, the insulation gap makes the liquid vulnerable to the surrounding temperature. When the temperature of the environment is significantly higher or lower than the liquid, heat can be added or taken away from the system. In the future, researchers could fill the insulation gap with

insulation boards which is equipped with a camera hole and internal lightings. Heat exchange with the environment should then be minimized.

g) Advanced temperature measurement

Thermocouples have been used in the experiment because the cost is low and the size is small. However, thermocouples can only be used to measure temperature change in 1 location and the amount of thermocouples that can be inserted into the PCR liquid is limited. So it is difficult to get a full temperature distribution. A recommendation for future work would be using a heat camera. A heat camera [35] can monitor the entire region in real time and produce an accurate quantitative result. Apart from the heat camera, Thermochromic liquid crystal (TLC) is a substance with the property to change color due to a change in temperature [36]. It can be fabricated to change colors from a temperature range of -30 to 120ºC, and has the ability to detect temperature changes as small as 0.36ºC. The crystal can be placed inside the PCR liquid and their color changes due to temperatures can be observed by a camera.

h) Different temperature of the environment

All experiments had been carried out at room temperature so far. The main purpose of the instrument-free thermal cycler is to amplify DNA samples in a low-resource environment. Therefore, environment with extreme temperature (from -30ºC to 40ºC) should also be considered. For future work, the thermal cycler could be tested in temperature controlled room. The experiment results should prove that the device has a high reproducibility and repeatability under any room temperature.

i) Desired systems

The main problem observed for the first prototype is the fact of having a moving part (the fluidic cartridge to allocate the DNA sample), which thermally affects the temperature of the actuators and/or increases the time to achieve thermal equilibrium, increasing the residence time of the cartridge in each temperature position in order to achieve the desired reaction temperature for sufficient time. In this way, systems without moving parts such as the second prototype are preferred.

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Appendix

A1: Material thicknesses and thermal properties for 1D heat transfer analysis

Material	Thickness (m)	Thermal Conductivity k (W/m·K)	Thermal Contact Resistance $h_{th.\ contact}$ ($W/m^2 \cdot K$)
PCR liquid	0.0093	0.598	-
Tin Canister	0.0008	205	-
Glass Slide	0.00015	1.05	-
Canister to Glass	-	-	16,667

Table 7: Material thicknesses and thermal properties for 1D heat transfer analysis

A2: Summary of Experimental Settings for 2nd Prototype

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
No. of Activated Heaters	2 (Top and Bottom)	2 (Top and Bottom)	1 (Bottom)	1 (Bottom)	2 (Top and Bottom)	2 (Top and Bottom)
Amount of Mg-Fe Fuel (g)	7 (on bottom) 5 (on top)	7 (on bottom) 5 (on top)	7	14	7 (on top and bottom)	7 (on top and bottom)
Amount of Water Added (mL)	15 (on each heater)	15 (on each heater)	Continuous	15 (added 2 times)	15 (on each heater)	15 (on each heater)
Steam Blockage	No	Yes	Yes	Yes	Yes	Yes
Temperature of Activated Heater	55°C and 95°C	55°C and 95°C	95°C	95°C	95°C for both	95°C for both
Outer Insulations	No	No	No	No	No	Yes

Table 8: Summary of Experimental Settings for 2nd Prototype