



## TEMPERATURE GRADIENT BASED WIRE-GUIDED RAPID PCR SYSTEM

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### ABSTRACT

Polymerase chain reaction (PCR) is an integral part of modern day biological research and medical care. PCR has applications in areas such as pathogen detection, medical diagnosis, forensics and genetic research. This process of amplifying DNA relies on the repeated heating and cooling of an enzyme-containing solution. Typical PCR machines, especially thermocyclers, are costly and take 1-3 hours to complete 25-50 cycles of DNA amplification. This lengthy duration is due to the slow conductive heating mechanism employed by the thermocycler. New developments have been able to greatly reduce the amplification time to under 10 minutes by working with a smaller sample size and employing more effective heat transfer methods [6]. One interesting and effective method involves a series of oil baths heated to different temperatures. A tiny droplet of fluid is moved between the baths, allowing for quick convective heat transfer [6]. However, this process requires multiple heaters and an advanced control strategy to maintain the three temperatures required for PCR and to move the droplet between them. This paper describes a new method under development, based off of the one just described, that will simplify the system by utilizing a single oil bath with a heat gradient ranging from  $>95^{\circ}\text{C}$  to  $<60^{\circ}\text{C}$ . Within this gradient are all of the required temperatures needed for PCR, and by guiding the droplet back and forth through the oil we can quickly change the temperature of the sample. A single temperature transducer alongside the droplet will provide constant sample-temperature feedback to the controller, thus simplifying the current control strategy. This device has the potential to be smaller, cheaper and faster than the current wire-guided droplet PCR device, which has accomplished 30 cycles in 7 minutes [6].

### INTRODUCTION

Polymerase Chain Reaction is a method of DNA amplification that was developed in the 1980's and has since become commonplace and essential in many areas of biological research. Fields of study that rely on PCR amplification include genome mapping, forensics, genetics, medical diagnosis and pathogen detection [2]. PCR has led to many advancements in modern biological sciences, however the technology is relatively old and the current standard methods have much room for improvement.

The major drawback of PCR is the amount of time it takes to complete the necessary number of DNA replications. The process involves cycling a sample solution through three different temperature stages to complete a

single replication of DNA (doubling the amount present at best) [3]. Current methods utilize a test-tube holding heat-sink with a heating and cooling element (often a Peltier heat pump), called a thermocycler, to raise and lower the temperature in the solution using conductive heat transfer. This means the user must wait for the entire heat sink to be heated, then cooled, by about  $30^{\circ}$  Celsius. To achieve the desired amount of sample DNA, this cycle is usually run 30 to 50 times, which can take an hour or longer to complete [5].

This paper outlines a design for a rapid PCR system that will utilize an oil reservoir with a temperature gradient to submerge an automated, needle guided droplet of reagent. The goal of this design is to achieve 30 temperature cycles in under 15 minutes. This report will outline the PCR process, the design concept, then describe our team's methods and progress towards development.

### PCR BACKGROUND

Polymerase Chain Reaction works by causing the enzyme DNA polymerase to generate copies of a target strand of sample DNA. DNA polymerase is a natural enzyme within a cell that assembles nucleic acids into a complementary strand of a given single stranded DNA, forming a double helix. This process occurs naturally during cell division, but can be exploited to generate billions of replicates if given the right environment [2]. Therefore, nucleic acids, or dNTPs, must also be provided to the PCR "recipe", as this is the raw material for building DNA. This also means that the double helix of the sample DNA must first be separated (denatured), as DNA polymerase only makes a complement of a single strand. Additionally, DNA polymerase can only build off of an already present 3' end of a partially complete double helix. Therefore a primer must be added. The primer is a short strand (about 20 nucleotides long) of DNA, specially formulated for the target DNA sequence, which binds to the separated target single strand of DNA to act as a starting point for DNA polymerase. Primers must be complementary to the target strand, and their sequence must end at the starting point of the segment to be copied.

Denaturation of the double helix can be achieved with thermal energy. The denaturation temperature of DNA is around  $90^{\circ}\text{C}$ , depending on the strand length and composition [1]. Thus, the first step in the PCR process is to raise the temperature of the solution to  $90-95^{\circ}\text{C}$ . Most DNA polymerase cannot withstand these temperatures and will denature themselves. Therefore a special polymerase discovered in a thermophilic bacteria called TAQ

polymerase is used, which can withstand these high temperatures.

Once separated, the solution is then cooled to allow the primers to bind to the single strands, called the annealing stage. The primers will bind to the corresponding location and act as a starting point for the polymerase to build a new strand. This occurs at 60°C. Once the primers are bound, the solution is heated to 75°C to promote and optimize polymerase function. The single strand is completed and the amount of DNA in the sample is approximately doubled (some denatured single strands may rejoin during the annealing stage before being replicated). One complete cycle of temperatures is called a thermocycle. Generally about 30 cycles are required to achieve the desired amplification.

Conventional thermocyclers are in essence a heatsink block that heats up then cools a test tube containing the sample solution using conductive heat transfer. Conduction is the slowest form of heat transfer, and the test tubes usually contain a larger-than-necessary sample volume, leading to a slow completion time of over an hour for 30 cycles. Current research has provided many ways to improve this outdated system and complete the desired amplification in a fraction of that time. Several methods have already been developed that can finish 30 thermocycles in under 10 minutes [6].

A rapid PCR system is highly desirable in both research and industry. It would improve point of care medical diagnostics, enabling a DNA test to be conducted while the patient waits. It could also lead to in-field pathogen detection within food, water or soil. Forensics, tissue identification, and genetic research would also benefit by becoming faster and cheaper.

## DESIGN CONCEPT

To complete 30 thermocycles in under 15 minutes, we must greatly reduce the time it takes to change the temperature of the sample solution containing the PCR ingredients. Our goal is to achieve a 30°C temperature change in as little as 15 seconds. Thus, the first step in our design process was to identify ways we could speed up heat transfer to and from our solution.

Temperature change is affected by mass, specific heat and absorbed energy. We can increase the rate of temperature change by decreasing the mass and increasing the rate at which heat can enter and leave the solution. The simplest form of heat transfer, and also usually the slowest, is conduction [5]. This is the effective heat transfer mechanism utilized when conventional thermocyclers heat the block and then the test tube in order to heat the sample. These intermediate materials that must also be heated and cooled substantially increase the time required. Our proposed design utilizes a bath of silicone oil to completely engulf a 10  $\mu$ L droplet of water containing the reagents. The hydrophobicity of the oil allows the water droplet to retain its form without any material insulation. The oil will fill a small reservoir and have a temperature gradient from one end to the other, spanning all of the required temperatures. The hot end will be greater than 90°C and the cold end will be less than 60°C, and it will contain all the temperatures in between. By moving the droplet back and forth through the reservoir, we can completely engulf the droplet in different temperature environments without having to wait for anything other than the sample to heat and cool. A syringe will inject the droplet directly into the oil bath. Due again to

the hydrophobicity of the oil and the surface tension of the water, the needle will be able to drag the droplet through the oil. Additionally, the tiny volume of the droplet will heat up and cool down much quicker than the larger volumes of sample used in conventional methods. This droplet is still large enough to contain the required sample volume and provide the required reaction. We also plan to have the needle vibrate the droplet which would cause quicker convective heat transfer throughout. Similar experiments and modeling regarding wire guided PCR and droplet temperature manipulation, such as those performed by Dr. David You in Dr. Jeong-Yeol Yoon's laboratory at the University of Arizona, have already proven effective [6]. This design expands on those efforts by integrating a temperature gradient which will simplify their system.

## DEVELOPMENT PROGRESS

The first step in the development of this design is to create the temperature gradient in a small reservoir. This has been the bulk of our team's work to date. We devised a series of tests to optimize the gradient in hopes of spanning all of the required temperatures. The first of our tests was to determine the appropriate material for our reservoirs. We decided to test two materials; aluminum, a very good thermal conductor, and plastic (acrylonitrile butadiene styrene), a very good insulator. We made two reservoirs with the same dimensions and attached a Peltier heat pump, supplying about 5 watts of power, to one side (Fig. 1). We took temperature measurements at the hot side and the cold side every few minutes for about half an hour. The tests showed that plastic facilitated a greater temperature difference from one end to the other, with the hot end at 63°C and the cool end at 52°C. This was good news for our team because plastic reservoirs could be designed using CAD software and printed with a rapid prototype, 3D printer.

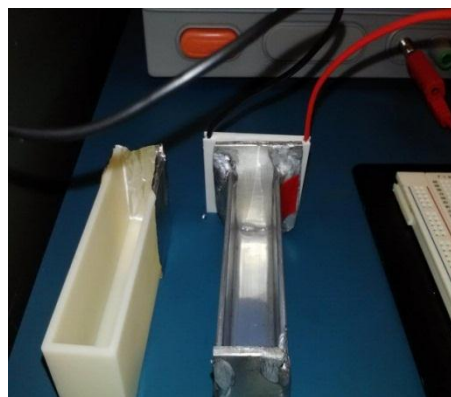


Figure 1: Material Test

We continued to experiment with different lengths, shapes, use of heat sinks, fans and different heater elements. We are currently still in the reservoir testing stage working toward the temperature gradient. Our most successful test achieved the entire gradient, but the extreme temperatures, 90°C and 60°C, were only present within a very close proximity to the heater and heat sink, respectively. The bulk of the fluid remained relatively uniform at about 70°C as illustrated in figure 2.

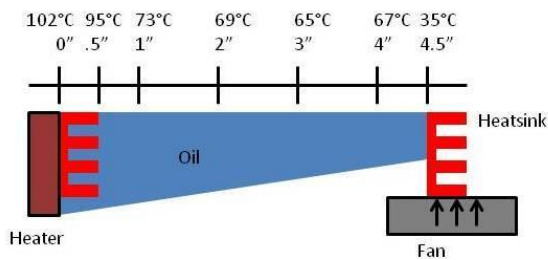


Figure 2: Temperatures in Reservoir

To address this issue we have several design ideas. The first is to boost the power of the input and output. We are currently designing a reservoir which will contain two heaters and a much bigger heat sink. The second idea is to change the shape of the reservoir. By making it longer and skinnier we can limit the amount of mixing between the hot and cold ends. Another idea is a vertical heat gradient with the heater at the top and the heat sink at the bottom. The difference in density between the hot and cold oil will naturally create a gradient. The problem with this design is the added complexity when guiding the droplet vertically as compared with horizontally.

#### FUTURE WORK

Once the heat gradient is achieved, the wire guiding mechanism can be addressed. We are currently using a thermistor and an Arduino microcontroller to control the heaters to prevent overheating. We plan to use the Arduino to control the droplet as well. The droplet will be guided by a needle that moves back and forth along a track. The needle will be moved with a motor assembly controlled by the Arduino. We plan to implement a feedback control from a temperature sensor located on the needle. The needle will move toward the hot end until the temperature sensor reads 90°C. At this point it will stop, wait several seconds (with the exact time to be determined by modeling), and then move toward the cold end until it reads 60°C and so on.

This control strategy eliminates the need to keep a precise control over the temperature gradient. Small fluctuations will not matter because the needle will find the correct temperature. This strategy will also allow us to overshoot the temperature with a small adjustment to the program. The idea here is that we can further increase the heat transfer rate by moving the droplet to 95°C, for example, before turning around. This would decrease the “wait” step in our program. Additionally, with this control scheme it will be possible to use a normal brush motor rather than a stepper motor, as precise motion will not be required. This will further decrease the cost relative to other wire guided designs. The device will be programmed to run through 30 thermocycles, and should be able to complete the process in under 15 minutes.

Our design has the potential to be faster and cheaper than previous wire guided PCR machines. This technology could be developed into a deployable, compact, easy-to-use device. With this design it is foreseeable that a lunch box-size device could be capable of amplifying DNA within minutes. We hope to complete our initial prototype by May 2013.

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