



UNDER UBIO BIOTECHNOLOGY SYSTEMS PVT LTD

INTERNSHIP PROJECT REPORT

4 CHANNEL REAL-TIME PCR BASED ON ARM CORTEX

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ABSTRACT

Polymerase chain reaction (PCR) is a technique that has revolutionized the world of molecular biology and beyond. In this article, we will discuss a brief history of PCR and its principles, highlighting the different types of PCR and the specific purposes to which they are being applied. Nucleic acid amplification and detection are among the most valuable techniques used in biological research and life science and have become an essential method in many aspects of science, including clinical diagnostics and genetics engineering. In traditional PCR, detection and quantification of the multiplied DNA sequences are performed after the last PCR cycle is carried out. To deal with these challenges, a Reverse Transcription PCR method was introduced. In traditional PCR, detection and quantification of the multiplied DNA sequences are performed after the last PCR cycle is carried out. To overcome these limitations, a real-time PCR method was introduced. The goal of this project is to develop a multichannel based real-time PCR, in order to analyze the amplified DNA sequences over real-time. A GUI was developed to monitor the PCR block temperature. GUI allows the end-user to define PCR protocol and record the data for further processing. The addition of multimode features helps the user to target multiple samples at a time within a single process.

CHAPTER 1

INTRODUCTION

Over the years, the advent of chemical novelties and instrumentation platforms that allow for the occasional discovery of PCR products has led to the acquisition of longer RT-PCR due to the selective method of measuring changes in organic phenomenon. In addition, the timing of RT-PCR has become a popular method of dynamic results found in a number of different analyses of various biological variables worldwide. To truly appreciate the benefits of PCR time, reviewing the basics of PCR is important. At the beginning of the PCR response, the excess square measure, for example and products square to a scale low enough that the product adjustment does not contradict the initial binding, as well as the incremental revenue at a continuous, definite value. The purpose for which the reaction rate ceases to be visible and enters a specific magnification phase varies greatly, even among recurring samples, however, it seems to be primarily thanks to a product adjustment competition with basic binding (since adding more reagents or accelerator has a much smaller effect). In one recent cycle, the rate of growth decreased to zero (plains), creating a much smaller product. For the purpose of accuracy and homosexuality, it is necessary to collect measurement information to a certain extent where each sample is within the emerging phase of breeding (because it is only at this stage when the breeding is most reproduced). Response analysis for the entire adjective phase in a given cycle range should provide more instructions for magnitude of the potential difference. Unusual targets may be below the acquisition limit, and the intentions of the streams will exceed the descriptive category. Upon application, 2-3 strong logs are usually measured throughout the final point associated with RT-PCR. To increase this variability, repetitive feedback can also be performed in large or small cycles, so that all samples are usually analyzed in the descriptive section. Real-time PCR automatically performs this complex procedure by measuring the response product {each | for each sample} throughout the cycle. The result of the Associate in Nursing is a staggering 107 range of variations, with no user or repetitive interventions required. Information analysis, as well as the development of a curve of culture and the calculation of repetitive distance, is done mechanically. As an increasing number of labs and key locations receive the tools needed for time analysis, this approach is changing into a RT-PCR-based specification method.

Real-time PCR requires a hardware platform consisting of a hot cycler, a computer, light optics and output emissions, as well as a system for obtaining information and software analysis. These machines, from where most manufacturers have come, are opposed to sample strength (another 96-standard format, some use fewer samples or need capillary glass tubes), a fun way (some use lasers, other lightweight filters are -tunable), and complete sensitivity. There is also a platform-specific variation on how the software system processes information. PCR machine time does not seem cheap, currently about \$ 25K - \$ 95K, however, it is between accessing key facilities or labs that require high ratings. We are building a 4 RT-PCR channel machine that can be used for 4 sources.

CHAPTER 2

REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

Real-time polymerase chain reaction (real-time PCR) is a laboratory method of molecular biology based on polymerase chain reaction (PCR). It monitors the enlargement of the DNA molecule targeted during PCR not at its end, as in conventional PCR. Real-time PCR can be used in quantity (maximum real-time PCR) and approximate number.

The two most common methods for the detection of PCR products in real-time PCR (1) are an unspecified fluorescent dye that binds to any double-stranded DNA and (2) DNA-specific sequences containing oligonucleotides labeled with a fluorescent reporter label which allows detection only after probe assembly in its corresponding sequence.

2.1 Basic principle

Real-time PCR is performed on a hot bike with the ability to illuminate each sample with a light beam of at least one specified measurement and detect fluorescence emitted by a happy fluorophore. The hot cycler is also able to heat rapidly and cool samples, thus utilizing the physicochemical power of nucleic acid and DNA polymerase.

The PCR process usually consists of a series of temperature changes repeated 25-50 times. The Real-Time PCR operating process can be divided into two steps:

Amplification and Enhancement Different steps in the process are as follows

1. Denaturation: High temperature incubation is used to “melt” DNA with double strands into a single strand and release a second structure into a single DNA. Denaturation time can be increased if the GC template content (or guanine-cytosine content) is high.
2. Annealing: During insertion, the corresponding sequence has the potential for mixing, so using the appropriate temperature based on the calculated melted temperature (T_m) of the primers (5°C below the T_m primer).
3. Extension: At $70\text{-}72^{\circ}\text{C}$, DNA polymerase activity is good, and the first expansion is done at speeds of up to 100 bases per second. When the amplicon in real-time PCR is small, this step is usually combined with the melting point using 60°C as heat.

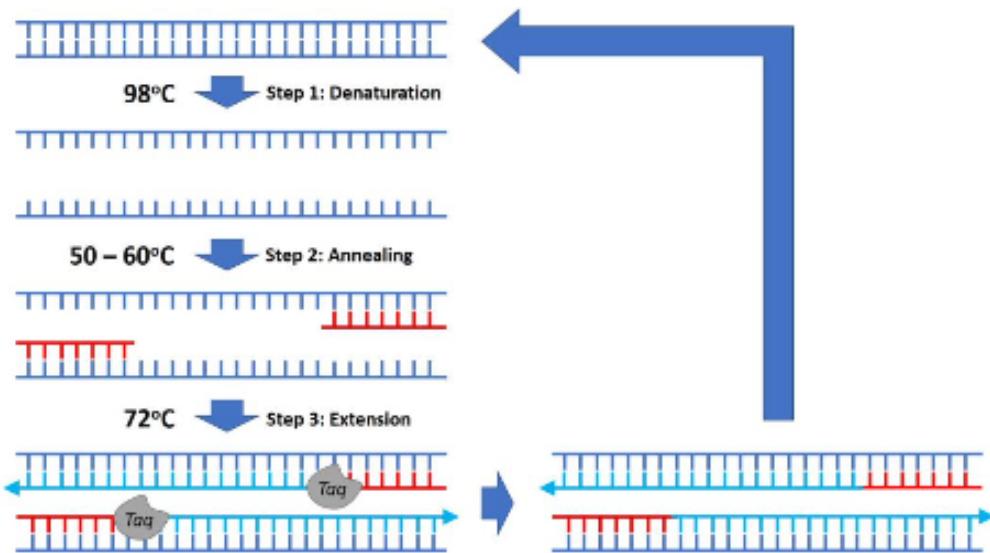


Figure 1: RT PCR Steps

Detection: The detection is based on fluorescence technology. The specimen is first kept in proper well and subjected to thermal cycle like normal PCR but at this machine it is subjected to tungsten or halogen source that leads to fluorescence the marker added to the sample and the signal is amplified with the amplification of copy number of sample DNA. The emitted signal is detected by a detector and sent to the computer after conversion into a digital signal that is displayed on the screen. The signal can be detected when it comes up to the threshold level (lower detection level of the detector), this causes the elimination of background noise. There are many different markers used as the marker of Real-Time PCR. There are mainly two types of markers used for this purpose.

1 .Taqman probe.

2. SYBR Green

Taqman Probe: This is a hydrolysis probe, it bears a reporter dye, often fluorescein (FAM) at its 5' end and a quencher tetramethylrhodamine (TAMRA), attached to the 3' end of an oligonucleotide. In normal condition, the probe remains coiled on itself bringing the fluorescence dye near the quencher causing quenching of the fluorescent signal of the dye

SYBR Green: This is a dye that emits a prominent fluorescent signal when it binds at the minor groove of DNA, non-specifically. Other fluorescent dyes like Ethidium Bromide or Acridine Orange can also be used but SYBR Green is better used for its higher signal

intensity. SYBR Green is more preferred than the Taqman Probe as it can provide information about each cycle of amplification as well as about the melting temperature which is not obtained from the Taqman probe. However, its disadvantage is the lack of specificity as compared to the Taqman Probe.

2.2 Components used in the real-time PCR:

As like the conventional PCR, the real-time PCR reaction contains almost the same components except for the fluorescent dye or fluorescent labelled probe

dNTPs: dNTPs are added during the synthesis of the growing DNA strand by the Taq DNA polymerase. The dNTPs remain the same as the conventional PCR.

Taq DNA polymerase: Normal Taq cannot work efficiently for the real-time PCR, instead always using the hot-start Taq DNA polymerase. The hot start Taq DNA polymerase is the best choice for the quantification.

MgCl₂: Magnesium ion also plays a crucial role in the amplification during real-time PCR. However, the concentration of the Mg²⁺ ions is different from the conventional PCR. Use 3 to 5mM of MgCl₂ in the real-time PCR.

Template: 100pg to 1 microgram template DNA is sufficient for real-time PCR. We required only 100 copies of genomic DNA/RNA fragments for the amplification and to start the reaction. The template DNA or RNA must be pure and free from any contaminants.

Primers: The primer should be shorter, it can amplify only 100 to 160bp fragments, and avoid longer amplicons. It must be unique and contains 50% GC with the GC sequences at the end of the 3' end. It should be 18 to 20 nucleotides long. The primer containing all these criteria are the best for a real-time PCR assay.

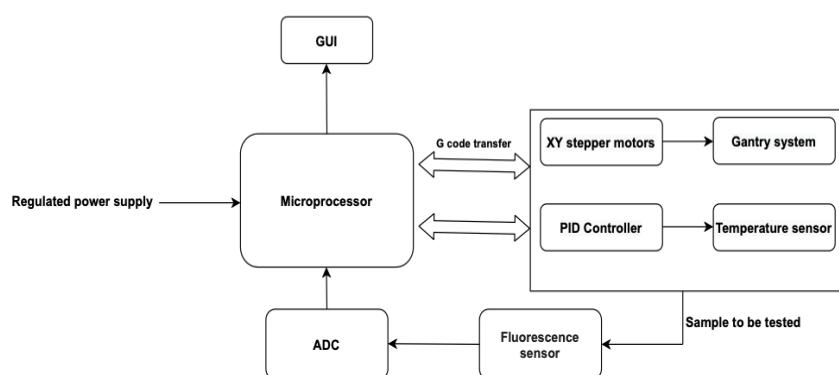
DNA polymerases: DNA polymerases are critical components in PCR, since they synthesize the new complementary strands from the single-stranded DNA templates. All DNA polymerases possess 5' → 3' polymerase activity, which is the incorporation of nucleotides to extend primers at their 3' ends in the 5' to 3' direction

CHAPTER 3

DESIGN OF A MULTICHANNEL RT-PCR

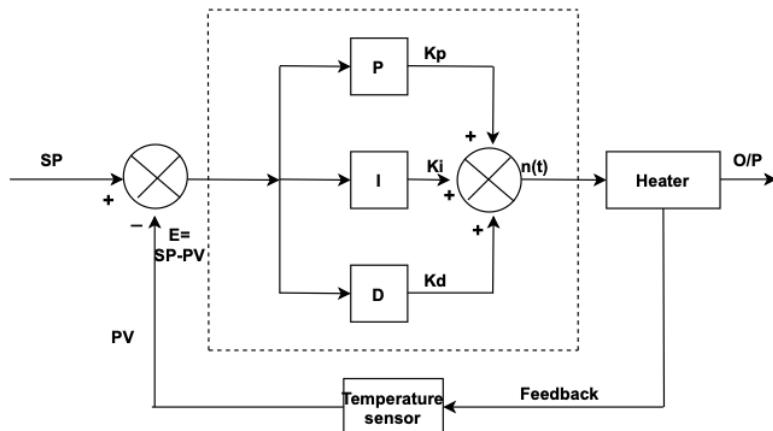
System consists of a thermal cycler unit, temperature control unit, movement unit, detection unit and a GUI Interface. In order to detect the current block temperature, a thermistor along the circuit is used. PID is implemented using a microcontroller. The exact same microcontroller is used for the movement system and a 5 inch display is also used. Detection is implemented using an AS7341 Spectral Color Sensor. For implementing Multichannel RT-PCR we use different LEDs in a single source.

For the temperature control unit, the main components include an ATmega-2560 microcontroller with a PID control algorithm implemented inside it. The microcontroller outputs control signals based on the current temperature detected from the thermistor and the desired temperature from the user which we get from a microprocessor. The control signal is then output through the cartridge heater in order to increase or decrease the temperature of the sample blocks. Since, The three steps (denaturation, annealing, and extension) take place at different heating blocks, a motor movement system is added to carry the samples between heat blocks. It consists of two stepper (NEMA 17) motors with its driving circuit. The two axis movement system provides a speed sample transition between heating and detection blocks. For the GUI interface and detection part we use Broadcom BCM2711 quad-core Cortex-A72 (ARM v8) 64-bit SoC.



3.1 PID Temperature System

The term PID stands for derivative derivatives and is another type of device used for flexible control of different processes such as pressure, flow, temperature, and speed in industrial applications. In this controller, a loop response device is used to control all the changing processes. This type of control is used to drive the system toward a purpose in another direction. Almost the whole area is controlled by temperatures and is used for scientific processes, automation and many chemicals. In this control, the closed response is used to store the actual output from the path closest to the target, otherwise, it is removed from the corrected position if possible.



To install modern microcontrollers like Arduino, PID must be used digitally. The PID number can be written digitally as

$$u(n) = k_p * e(n) + k_i \sum e(n) T + k_d * (e(n) - e(n-1)) / T$$

When T is sample time or time limit. The approximate term is obtained by multiplying the profit by a different error signal. The compound word is derived from the abbreviation of the sigma and the derivative word is calculated as the difference between the current error and the previous error.

The error signal is treated as an incorrect timing and the result is calculated accordingly. Applications are used to detect PID activity on the Atmega processor. PID

Library is available for calling as open-source for ATmega2560. The programmer has to specify the kd, ki, kp and setpoint values alone.

PID controllers receive many programs such as Furnace Temperature Control, Neutralization pH Control by chemical process, MPPT Design (Maximum power point tracking) solar PV charging controller, Power electronics converter, and much more. The reliability and stability of PID controllers makes it the most sought after control in the industry.

Automatic Tuning PID: Automatic tuning can eliminate many of the tests and errors of the manual method, especially if you do not have a lot of loop tuning experience. Performing the Autotuning process will help Tuning Parameters get closer to their correct values, but additional power settings may be required to get Tuning Parameters to their correct values.

A popular way to adjust the P, PI, and PID controls is the Ziegler - Nichols method. This approach starts by subtracting the combined benefits and variances and then proposes the equal gain until the system is unstable. The value of KP in an unstable environment is called KMAX; oscillation frequency is f0. This method also reverses a profit equal to the predetermined value and sets the combined benefits and variations as the f0 function.

The Ziegler-Nichols method is one of the most well-known tuning methods, based on the function of the open-step response. The generated response function is approached by the first order transfer function in a deadline.

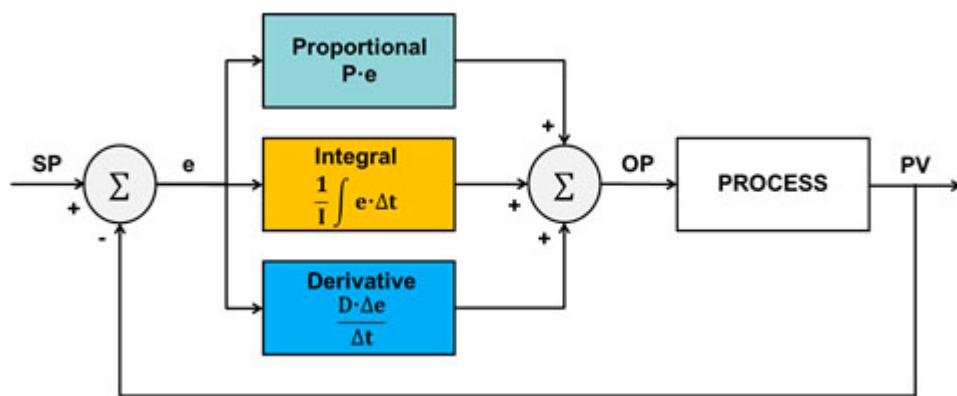


Figure 2: PID Diagram

3.2 Microprocessor- ARM Cortex-A72

BCM2711 is a quad-core Cortex-A72 64-bit CPU with Videocore IV Graphics Processing Unit (GPU) that handles all image capture / output. Designed to handle 4K resolution and H.265 video, as well as video ratings, camera inserts and all HDMI video output and integration. 2711 also has ‘appropriate’ USB3.0 and Gigabit ethernet connectors. The direct chip is used inside the raspberry pi and we can design the board using the Pi computing Module.

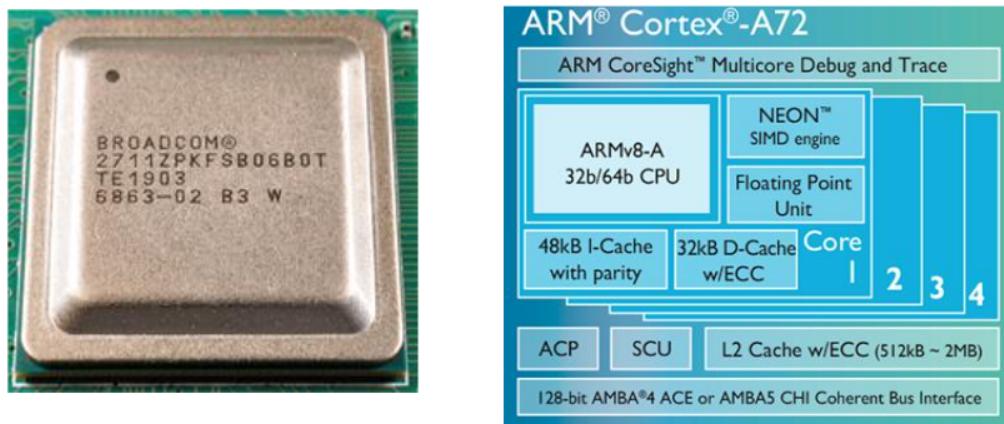


Figure 3 : BCM2711 is a quad-core Cortex-A72 64-bit CPU

Cortex-A72 processor details can be paired with a larger Cortex-A53 processor. Minor configurations for many different programs include mobile, embedded and automotive. The Cortex-A72 processor cache has one to four cores, each with its own L1 instructions and data repositories, as well as one L2 shared cache.

Key features are Triple-Issue Out-of-Order Dispatch pipelines and computational bandwidth over the Cortex-A57 to increase the performance of the triple-output pipeline to remove the dependence of the codes to achieve higher heights and more stable installation of higher frequencies of 3GHz in 16FF + process technology. And Advanced Branch Predictor, a new sophisticated algorithm, greatly enhances predictive accuracy, reducing the power consumption used in the wrong code system.

3.3 Microcontroller ATmega-2560

The ATmega2560 is a powerful CMOS 8-bit microcontroller based on RISC enhanced AVR architecture. By performing dynamic commands in a single clock cycle, the ATmega2560 gains input up to 1 MIPS per MHz which allows the system designer to increase power consumption compared to the speed of repair.

This device is made using Atmel's special non-functional memory technology. By combining 8-bit RISC CPU and In-System Self-Programmable Flash into a monolithic chip, the Atmel ATmega2560 is a powerful microcontroller that provides a flexible and inexpensive solution for many embedded applications. The ATmega2560 AVR is supported by a complete list of system and system development tools including C compilers, macro assemblers, program debugger / simulators, in-circuit emulators, and test kits.

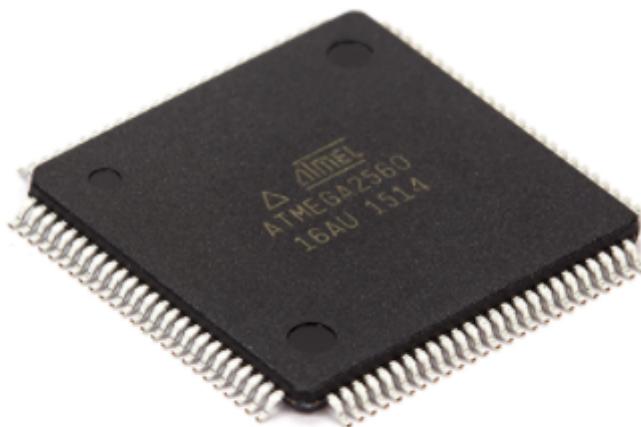


Figure 4 : ATMega 2560

3.4 Stepper motors and drivers

NEMA 17 Stepper Motor : NEMA 17 is a hybrid stepping motor with a 1.8° step angle (200 steps/revolution). Each phase draws 1.2 A at 4 V, allowing for a holding torque of 3.2 kg-cm. NEMA 17 Stepper motor is generally used in Printers, CNC machines and Laser Cutters. Rated Voltage is 12V DC and current is 1.2A at 4V. The Step Angle is 1.8 deg and no. of Phases are 4. Motor Length Is 1.54 inches 4-wire, 8-inch lead 200 steps per revolution, 1.8 degrees Operating Temperature: -10 to 40 °C and Unipolar Holding Torque: 22.2 oz-in.

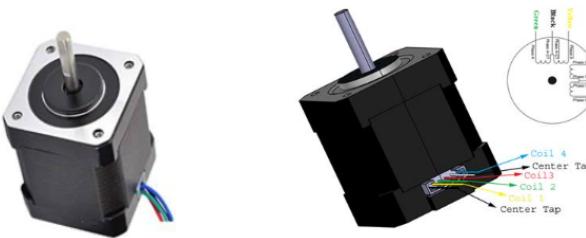


Fig 5: Nema 17 Stepper Motor

A4988 Stepper Motor Driver Module: A4988 is a complete Microstepping Motor Driver with a built-in translator for easy operation. The driver has a maximum output power of 35 V and ± 2 A. It can operate bipolar stepper motors in full, semi-, quarter-, eighth and sixth modes. Max. The effective voltage is 35V, Min. The operating voltage is 8V, Max. Per Phase 2A and Microstep resolution are full steps, 1/2 step, 1/4 step, 1/8 step and 1/16 step.

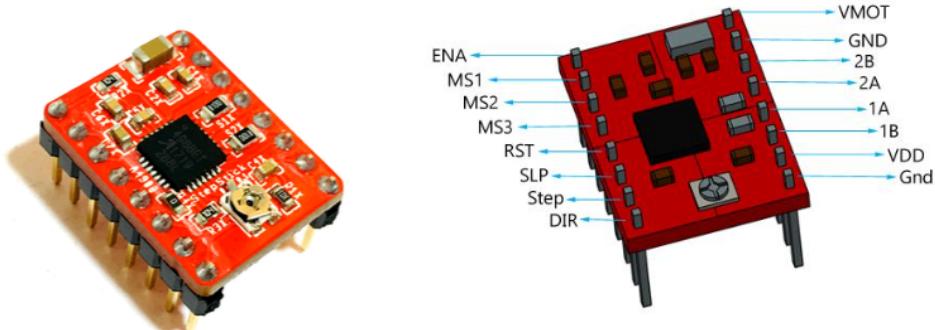


Fig 6: A4988 Stepper Motor Driver

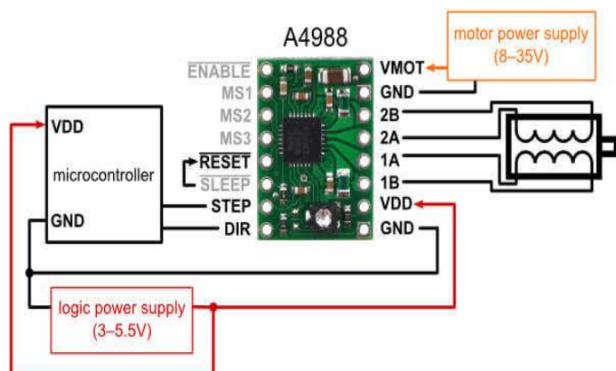


Fig 7: A4988 Motor Driver

3.5 Cartridge Heater And Thermistor

Increases the reliability of the extruder with this heater cartridge and also it heats up faster. It fits nicely in one's extruders, hence is an easy replacement. 12v 40W Ceramic Cartridge provides an excellent uniform distribution of heat. Cartridge heaters consist of a cylindrical stainless-steel tube 6mm dia *20mm L and a heating coil insulated inside the tube using ceramic. Wires are silicone Teflon insulated (1 meter length) withstands high temperature heat. We use a Thermistor 100k NTC with 1 Meter Cable Temperature Sensor for our device .The thermistor is a thermally sensitive resistor known for showing a large change in resistance with only a little change in temperature.



Fig 8: Cartridge heater



Fig 9: Thermistor NTC 100k

3.6 Display

15-inch Resistive Touch Screen LCD, HDMI interface. 800x480 hardware resolution. The display uses Resistive touch control. It is compatible and can be directly inserted in any version of Raspberry Pi (For the Raspberry Pi 1 B and Raspberry Pi Zero, an HDMI cable is required and should be purchased separately). Provide a driver. (support Raspbian\Ubuntu\Kali and Retropie system).

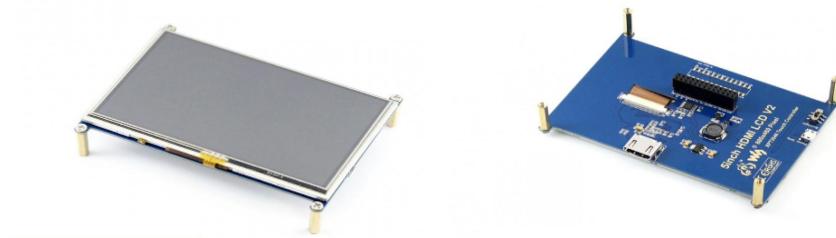


Fig 10: 5-inch Display

3.7 Regulated power supply

SMPS stands for switch-mode power supply. Its job is to convert wall-voltage AC power to lower voltage DC power. The term SMPS is defined as when the power supply is involved with the switching regulator to change the electrical power from one form to another form with required characteristics is called SMPS. This power supply is useful to achieve a regulated DC output voltage from the DC input voltage (or) unregulated AC.

- 1) 12V 10A SMPS - 120W - DC Metal Power Supply

Input Voltage is AC 100 - 264V 50 / 60Hz and Output Voltage: 12V DC, 10A

- 2) 12V 6A SMPS - 72W - DC Metal Power Supply

Input Voltage is AC 100 - 264V 50 / 60Hz Output Voltage is 12V DC, 6A



Fig 11: SMPS

3.8 Temperature Controller-UTC 421P

An external PID temperature controller is used to heat the lid of those sources. This temperature controller is set up once and is controlled using a transmission module. We use the process of heating the lid to keep the upper part of the well at a constant temperature of 110 ° C. The user does not have to adjust this temperature so that we can always set this temperature. We could easily attach it to the microcontroller, but reducing the number of microcontroller heater devices directly affected the external PID controller.

3.9 3-Volt Relay

It can control both AC and DC objects. This 1 Road / Channel Relay Module (with easy assembly) 12V module meets the safety level as control areas and loading areas have isolation areas. Separation module alone. The pull of the 1 Road / Channel Relay Module is more reliable, more stable. Dual circuit board design high SMT process. It has the power and commands to transfer operations. Terminal transmission (C, NC, NO) is accessible through online terminals which makes board cables much easier. Transfers are securely operated by transistor BC547 which is why your input device, such as Arduino, is secured to the transmission circuit. The input of the 1 Channel 12V Relay Module is set aside to protect any critical control cycles.

The use of such a high-power transmitter eliminates the risk of relay heating as electromechanical transmission limits current consumption in terms of power consumption.



Fig 12: Relay

3.10 AS7341 – 11-Channel Spectral Color Sensor

AS7341 is an 11-channel multi-spectral sensor for colour detection and spectral analysis applications. The spectral response is defined in the wavelengths from approximately

350nm to 1000nm. 6 channels can be processed in parallel by independent ADCs while the other channels are accessible via a multiplexer. 8 optical channels cover the visible spectrum, one channel can be used to measure near infra-red light and one channel is a photodiode without filter (“clear”). The device also integrates a dedicated channel to detect 50Hz or 60Hz ambient light flickers. The flicker detection engine can also buffer data for calculating other flicker frequencies externally. The NIR channel in combination with the other VIS channel may provide information of surrounding ambient light conditions (light source detection). The device can also be synchronized to external signals via pin GPIO. AS7341 integrates filters into standard CMOS silicon via Nano-optic deposited interference filter technology and its package provides a built-in aperture to control the light entering the sensor array.

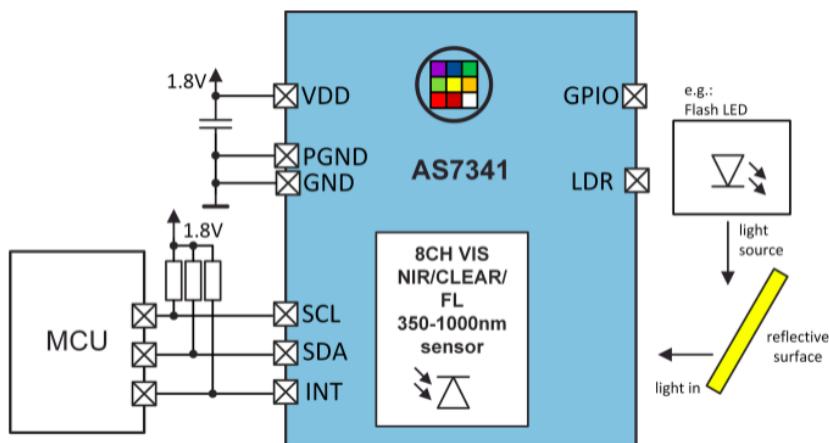


Fig 13: AS7341 schematics

3.11 TCA9548A I2C Multiplexer

The TCA9548A is an 8-channel, bidirectional translating I²C switch. The master SCL/SDA signal pair is directed to eight channels of slave devices, SC0/SD0-SC7/SD7. Any individual downstream channel can be selected as well as any combination of the eight channels. First, it was tested using the adafruit module. By using this we can detect from 4 wells easily.

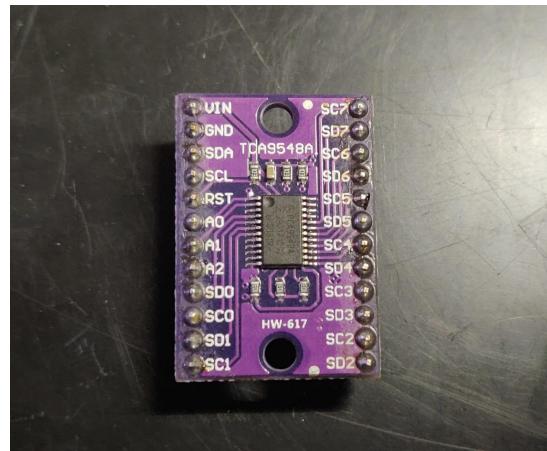


Fig 14 : I2C MUX

3.12 Real-Time PCR Tubes

PCR tubes are small tubes made of high-quality virgin polypropylene with a conical bottom and snap-cap lead. They have uniform thin walls to facilitate efficient heat transfer to the sample. These tubes are autoclavable and work well with most thermal cyclers.



Fig 15 : RT PCR Tubes

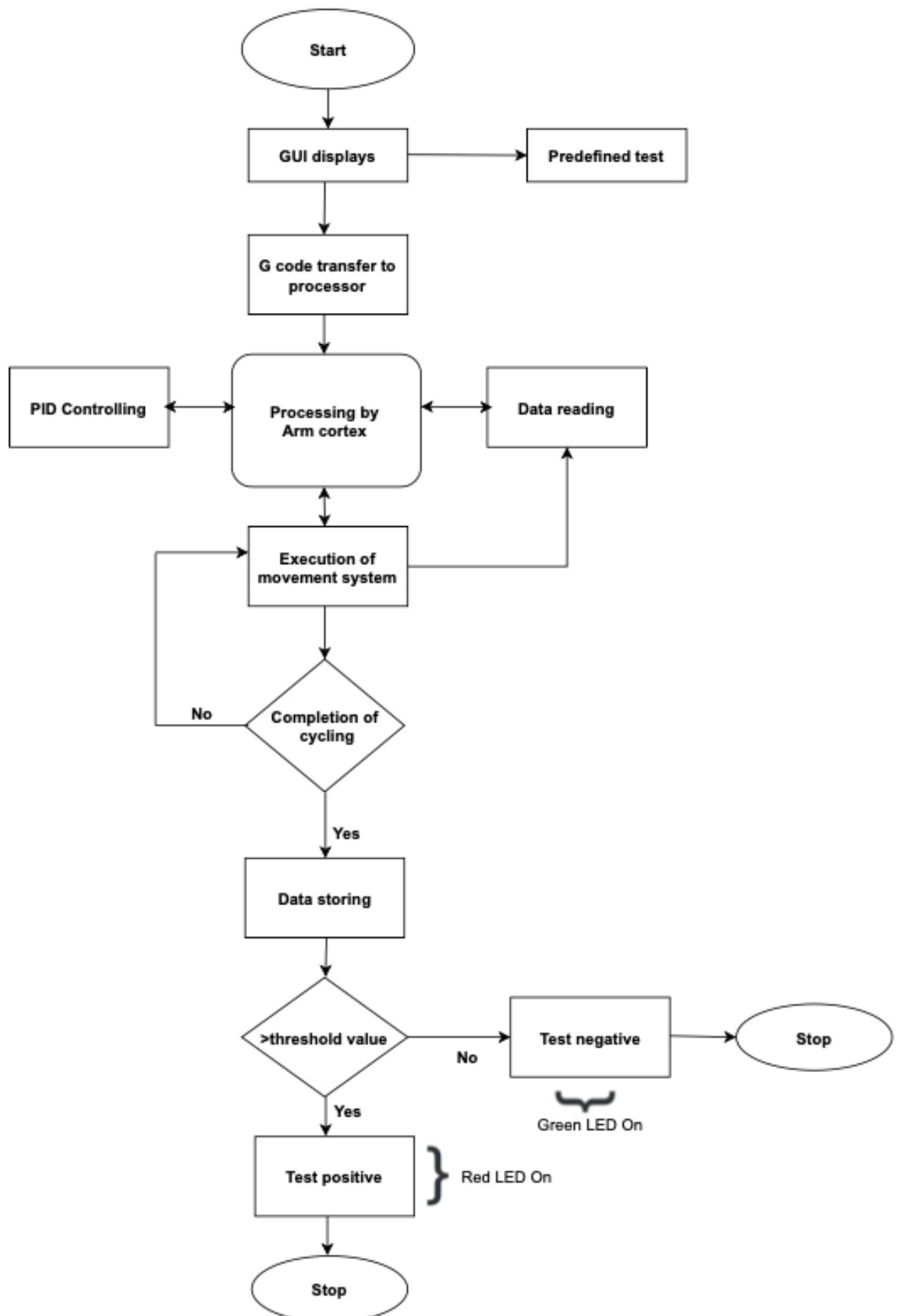
CHAPTER 4

SYSTEM WORKING

This RT-PCR device consists of three Heating metal blocks and a detection block unit. Each heating metal block has 4 micro-well fitted on a 9 mm thick aluminum holder and in tight contact with standardized 0.1 μ L PCR tubes. DNA samples are placed in the heating blocks to perform DNA amplification. The four blocks are built with aluminum alloy (1060) material, because Most of the PCR block in commercial thermal cyclers is using aluminum material and they have high thermal conductivity and low heat capacity. The PCR block was heated and cooled using a cartridge heater. It provides sufficient heat for thermal cycling. The heating/cooling rate of the sample depends on the results in increasing or decreasing the heater temperature. The amount of heat transfer was determined by the temperature of the resistance coil wound around a ceramic core inside the cartridge. The heater is inserted into drilled holes on the PCR blocks. All the components are fitted into metal using screws.

A PID controller algorithm is used to control the heating module and set the temperature of the PCR block. A NTC thermistor is used as a temperature sensor system to measure and maintain the temperature of a PCR block. Since, The three steps (denaturation, annealing, and extension) take place at different heating blocks, a motor movement system is added to carry the samples between these blocks. There is a PID control algorithm inside the microcontroller. A GUI was developed to monitor the entire PCR processes, such as block temperature, number of cycles, and the detection. GUI allows the user to define PCR parameters and record the data for further analysis. The PCR protocol used to amplify the DNA samples is shown below.

In order to analyze the DNA sequences The entire system is controlled externally via a Microprocessor with a GUI which is connected to a microcontroller unit (MCU) through serial communication and users can set various PCR parameters including the temperature, set point, duration, and the number of cycles. All these parameters are stored inside the microprocessor unit for future use.



4.1 Graphical User Interface

Graphical user interface design principles conform to the model–view–controller software pattern, which separates internal representations of information from the manner in which information is presented to the user, resulting in a platform where users are shown which functions are possible rather than requiring the input of command codes. Users interact with information by manipulating visual widgets, which are designed to respond in accordance with the type of data they hold and support the actions necessary to complete the user’s task. Graphical user interface testing refers to the systematic process of generating test cases in order to evaluate the functionality of the system and its design elements. Graphical user interface testing tools, which are either manual or automated and typically implemented by third-party operators, are available under a variety of licenses and are supported by a variety of platforms. Screenshots of GUI are shown below.

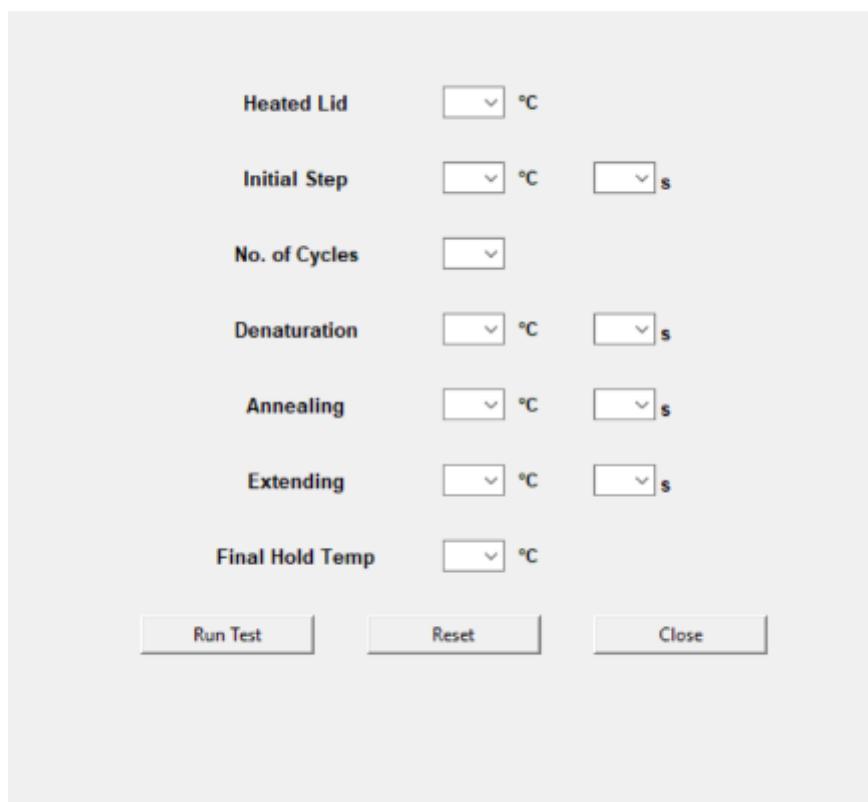


Fig 16: Graphical User Interface for Machine

4.2 Thermal Cycler

Movement 1 is from the Home position to the 3rd block. The process at 3rd block-Setting initial temperature for initial denaturation Initially the gantry motion takes place from

home position to third block where we set the required temperature for initial denaturation specific for respective tests. In a PCR reaction, an initial denaturation step is needed at the beginning of PCR, before the cycling of the three stages begins. This initial denaturation step usually occurs at around 95°C (or in the range of 320C-1100C), depending on the specific polymerase involved. To fully denature the DNA, initial denaturation can take up to 2 minutes which is represented by fig:1

Movement 2 is from the 3rd block to 1st block. The process at 1st block is DenaturationThe denaturation step is usually set at the same temperature as initial denaturation, ~95°C, and will require only 10-60 seconds. A higher GC content requires higher and longer denaturation temperatures. However, it's better to avoid extended incubations at this denaturation temperature to prevent inactivation of the polymerase or damage to the DNA template.

Movement 3 is from the 1st block to the 2nd block: Process at the 2nd block is Annealing. In this step, primers anneal, or bind, at complementary sequences of the denatured DNA strands. This will only occur successfully if temperature and primer pairs have been optimized. The annealing temperature (Ta) should be 5°C below the lowest Tm of either primer. Ideally, Ta falls between 52-58°C, and the annealing time is maintained for 15-60 seconds, with an optimum time at 30 seconds. If the annealing temperature is too high, primers do not anneal efficiently; if it's too low, primers may bind non-specifically to the template.

Movement 4 is from the 2nd block to the 3rd block. The process at 3rd block is Extension. The extension is the final stage of a cycle and occurs at a temperature that allows optimal polymerase activity of binding nucleotides to the annealed primer resulting in exponential amplification of the template strand. Usually, the extension step can be carried out at 72°C.

Movement 5 is from the 3rd block to the 4th block. The process at 4th block-Fluorescence detection. Fluorescent dyes or probes are included in PCR mixes to monitor the change in DNA amplification concentration as the reaction proceeds. Double-stranded DNA (dsDNA) binding dyes function as intercalating and/or minor groove binding agents and emit detectable fluorescence when bound to dsDNA but have a very low background when free in solution. Therefore, fluorescent signal intensity increases proportionately to the quantity of amplicon present. We are using SYBR Green dye which is the most popular dsDNA-binding dye and has a long history of use in molecular biology.

When free in solution, with only single-stranded DNA (ssDNA) present, SYBR Green dye emits a signal of low intensity. As the PCR progresses and the number of dsDNA increases, more dye binds to the amplicons and hence, signal intensity increases.

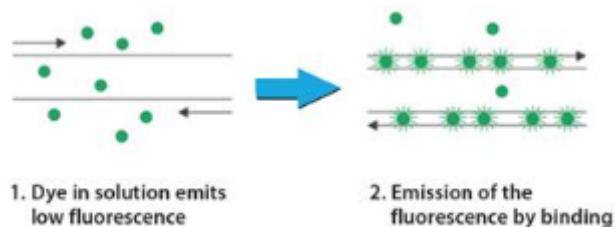


Fig 17: Emission due to Heating Cycles

The fluorescence emitted by the amplicon is detected by photodiode BPW34. Next 3 movements for Annealing, extension and fluorescence detection cycles repeats ‘n’ times as per instruction of the user. Movement 6 is from the 4th block to the 2nd block is Annealing. Movement 7 is from the 2nd block to the 3rd block is Extension. Movement 8 is from the 3rd block to the 4th block is Fluorescence detection. After completing the cycles gantry system return to position A.



Fig 18: Setting Initial Temperature and Denaturation



Fig 19: Annealing and Extension



Fig 20 : Fluorescence Detection and Annealing



Fig 21: Extension and Fluorescence Detection

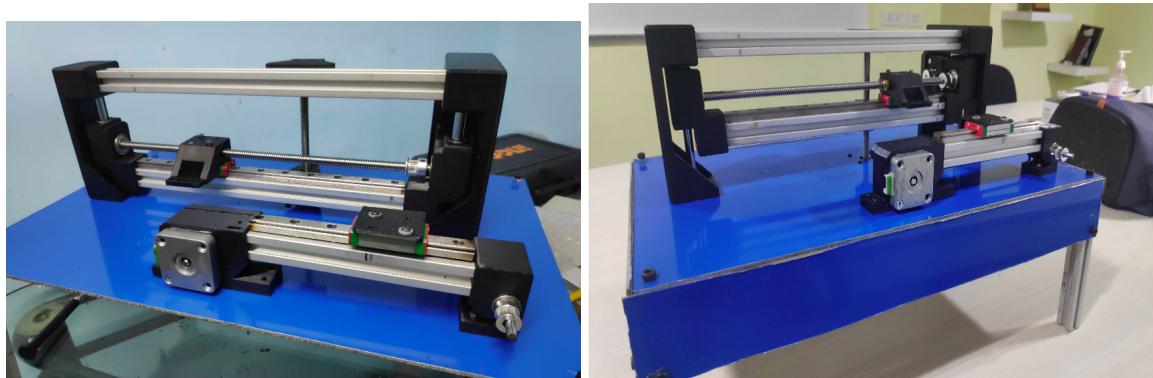


Fig 22,23: Thermal Cycler Part Of Machine which is controlled using the board made from this Project.Later Detection part and Display were

Thermal Block: This was made using Copper for high efficiency. Copper has many desirable properties for thermally efficient and durable heat exchangers. First and foremost, copper is an excellent conductor of heat. This means that copper's high thermal conductivity allows heat to pass through it quickly. Other desirable properties of copper in heat exchangers include its corrosion resistance, biofouling resistance, maximum allowable stress and internal

pressure, creep rupture strength, fatigue strength, hardness, thermal expansion, specific heat, antimicrobial properties, tensile strength, yield strength, high melting point, alloyability, ease of fabrication, and ease of joining. These wells were available at the company so we were able to use them easily.

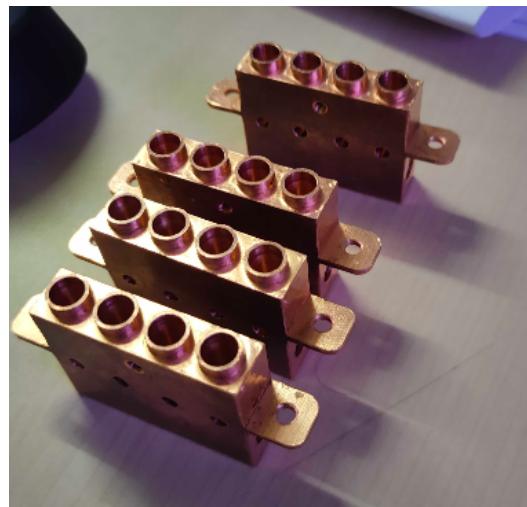


Fig 24 : Wells made from Copper Block

The thermal cycler was checked from outside too if our conditions are met. The test was successful. We were able to implement this in Machine.

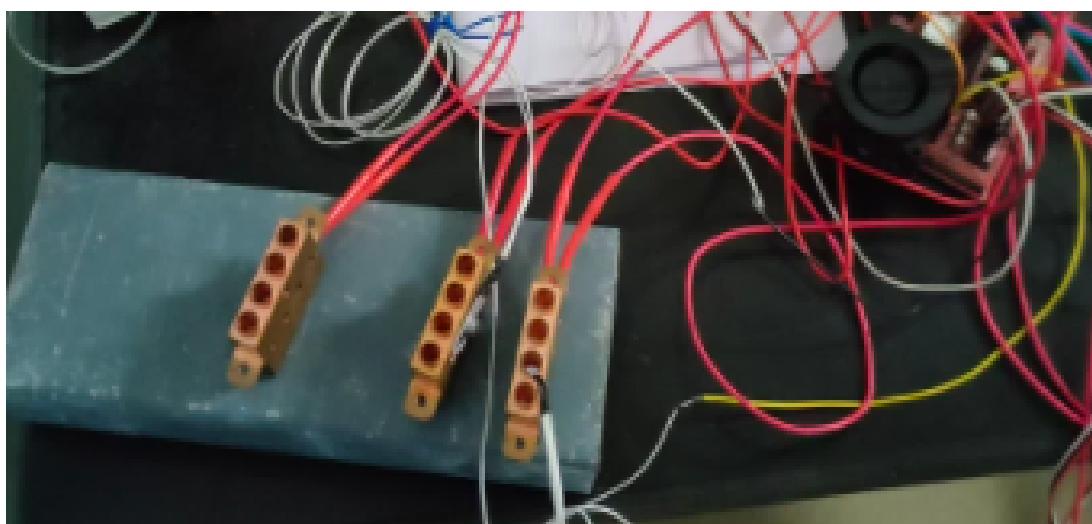


Fig 25 : Three blocks were heated. The 4th block is for the Detection part.

Heating was done perfectly.

4.3 Detection System

The detection and analysis of PCR products can be accomplished by a number of techniques. The most widely used method for PCR analysis is to simply run the PCR products on an agarose sizing gel, stain the gel with ethidium bromide, then observe and photograph the gel on a fluorescence transilluminator. Fluorescent methods of analysis have proven very useful in biomedical research and in clinical diagnostics (Taylor et al., 1986). Fluorescent analysis is frequently used in immunodiagnostics and other important areas of clinical diagnostics.

We are using the AS7341 spectral sensor. By using these sensors we can easily find the fluorescence emitted by each channel. We have to attach those sensors in a pair of two and attach to the thermal block by making the sensor face the hole.

However the usage of multiple filters can be avoided by using this single sensor for each well. The device features a 4x4-photodiode array. On top and below the photodiode array there are two photodiodes with dedicated functions such as flicker detection (“FLICKER”) and near-infrared response (“NIR”). A clear channel (“C”) – photodiode without filter – is provided at the left and right bottom corner. Each of the filter pairs can be mapped to one of the six internal ADCs (CH0 – CH5). This device communicated with the microprocessor using I2C communication protocol.

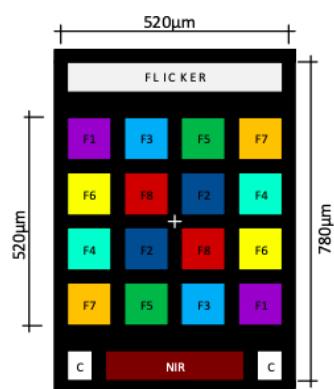


Fig 26 : Sensor array

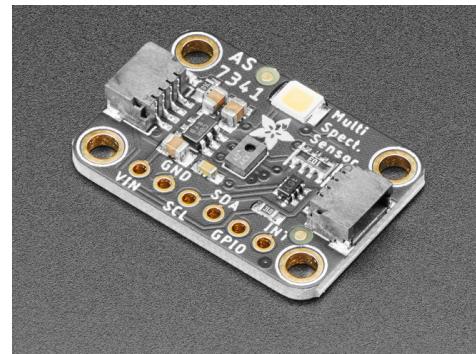


Fig 27 : Sensor Module for AS7341 used for Testing Purposes



Fig 28 : AS7341 used directly in PCB

Led Source: A led driver using spi communication is used. Multiple Channel Leds are used and those are connected to the driver. The benefit of using multiple channel leds is that we can use the same source for all the 4 channels. By making a common board for all the 4 wells we have a source board for the multichannel light emitting function. The Led driver we use is TLC9547.

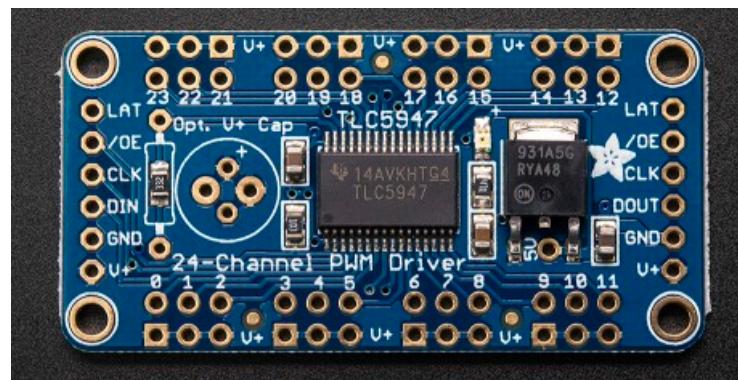


Fig 29: LED driver used to control Leds for equal Light distribution



Fig 30: Multiple Channel Led used for 4 different wavelength light emission

This is customised according to our use and our need of wavelengths

CHAPTER 5

PRINTED CIRCUIT BOARD DESIGN

We created a mainboard using the Raspberry pi computing module. Here the main processor is BCM 2711. We connect the ATmega 2560 chip to the main processor using serial communication. To reduce the heat of the total board we connect a 4 wire Tachometer fan to this board which depends on the PWM signals. We use multiple sub-boards to this mainboard by using connectors. The mainboard consists of MOSFETs, motor drivers, heater pins etc. The display is connected using a 22 pin ribbon cable. The boards are designed using KiCAD.

5.1 Detection Board

We designed two boards for a detection block. Each side consists of two AS7341 sensors on each side and from that board, we are giving a ribbon cable to the mainboard we have a thermally insulating material in between the block and board leaving a small hole to read the fluorescence.

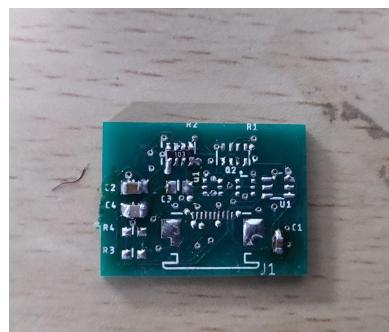


Fig 31: Detection board consisting of 2 Sensors AS7341. Similarly, Mainboard was designed. It is used inside the Machine. All external boards are connected with Ribbon cables

5.2 Source board

Our Source board consists of a led driver and multiple channel LEDs. The board is connected via a ribbon cable.

5.3 Relay Board

We have a device using AC current like an external PID controller which can be controlled using a relay so that we can optimise the heat loss and turn it on only when we

need it. Similarly, for using an external lock for ensuring safety we also need an IR sensor. However, we have to control the lock from the input from the sensor and output to the relay to control the lock. We can also add Fans for reducing cooling time. This board is also connected to the mainboard using a ribbon cable.

5.4 Raspberry PI Compute Module and IO Board :

The Compute Module is a Raspberry Pi in a more flexible form factor, intended for industrial application. The Compute Module contains the guts of a Raspberry Pi (the BCM2835 processor and 512MB RAM) as well as a 4GB eMMC Flash device (which is the equivalent of the SD card in the Pi). The Raspberry Pi Compute Module 4 was a bit of a surprise when it was announced, but it is fair to say that it was a pleasant surprise. Squeezing the power of a Raspberry Pi 4 into an even smaller package, the Compute Module 4 is a product designed for the industrial and embedded markets. We use the compute module inside the board that is designed. The IO board can be customised according to our needs

The Compute Module 4 IO Board is a companion board for Raspberry Pi Compute Module 4 (supplied separately). It is designed for use both as a development system for Compute Module 4 and as an embedded board integrated into end products.

The IO board is designed to allow you to create systems quickly using off-the-shelf parts such as HATs and PCIe cards, which might include NVMe, SATA, networking, or USB. The major user connectors are located along one side to make enclosures simple. This should be considered as the mainboard or the Master Processor. The user interacts with this processor and this processor controls other controllers.



Fig 32: Raspberry PI IO board and Compute Module 4

We designed a board using this idea, with mega 2560 integrated with it. We also added all the required ports and components into it like MOSFET VS3060AD for all the 3 heaters for controlling heat and voltage regulator for getting 5v 3 amps. Etc. However, a board was designed to keep each and every small thing. The first prototype was made using this compute module and an ATmega2560 board connected serially using a USB cable. Mosfets and drivers can be connected to the ATmega2560 board using Ramps1.4 for easy access too. This Ramp board helps in connecting the drivers easily.



Fig 33: ARM cortex A72 based board used for the first testing of this project

5.5 ATMega 2560 and RAMPs 1.4 Board

In RAMPS 1.4 the capacitors and resistors are now surface mount (SMD) which provides more space for other passive components and headers. In RAMPS 1.4, the resistors and capacitors are now surface mounted to fit more passive components. The Mega 2560 is a microcontroller board based on the ATmega2560. It has 54 digital input/output pins (of which 15 can be used as PWM outputs), 16 analogue inputs, 4 UARTs (hardware serial ports), a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It contains everything needed to support the microcontroller; simply connect it to a computer with a USB cable or power it with an AC-to-DC adapter or battery to get started. We use the PWM pins to control the heaters. Drivers thermistors are all controlled in a common way.

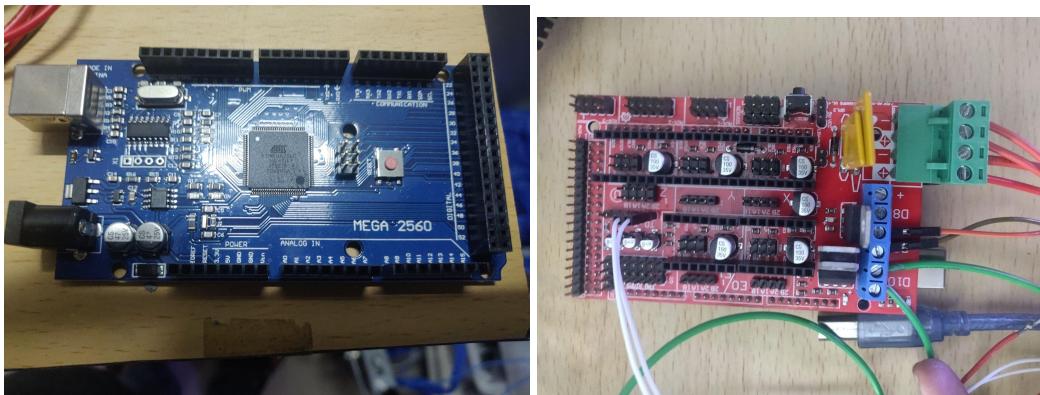


Fig 34: ATmega2560 and RAMP board that was used for first testing and prototype of the machine.

All of these boards work together to make maximum efficiency in the process. Combining all of these boards creates a new board. This board is the main circuit of our system. The motherboard of this machine is designed like this. Measurements were taken from the prototype and all of them were valid. Minute variations were able to be measured.

CHAPTER 6

MEASUREMENT OF SAMPLES

Samples were made from a BioTechnology lab. These samples were then run in the thermal cycler. After running a number of thermal cycles they gradually started showing fluorescence. This was measured using our detection system. Fluorescence increased gradually with a gradual increase in the number of cycles. When it was measured for one cell we got a similar result.

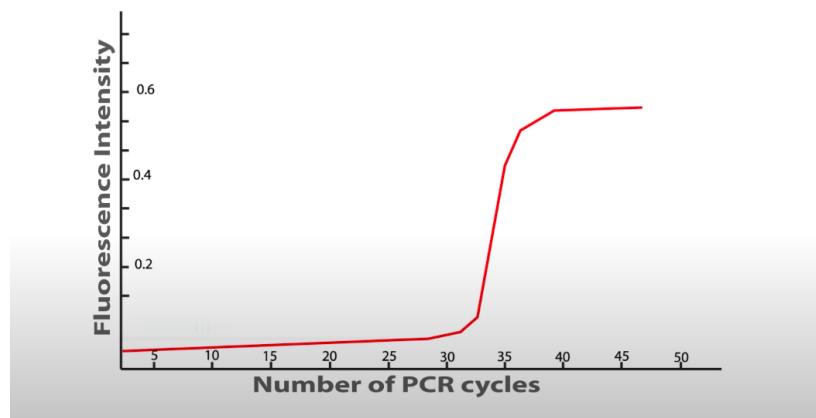


Fig 35: Fluorescence measured using AS7341 after the thermal cycler for a single channel and single well

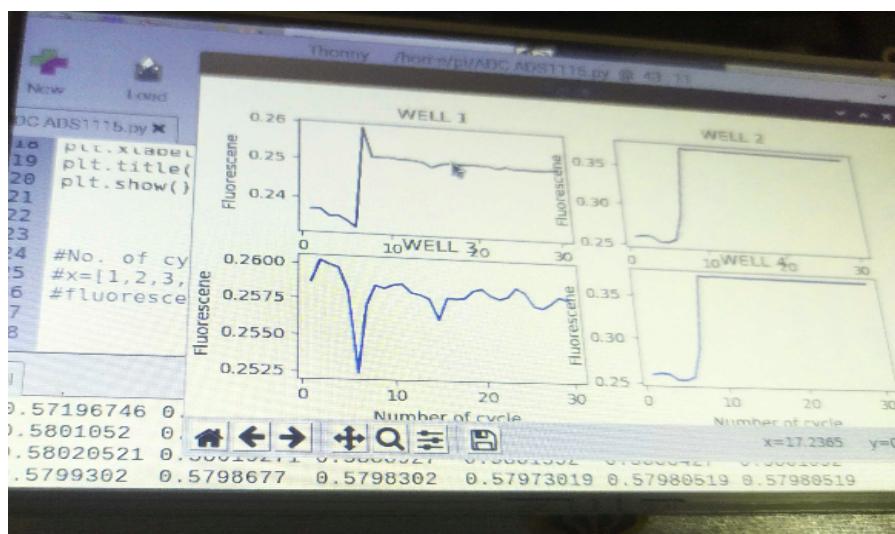


Fig 36: Fluorescence measured using AS7341 for all 4 wells without dye. To check if all the graphs are plotted. The graphs were then combined to one with different colours.

We used a fam dye of emission wavelength 520 nm and excitation wavelength 424 nm for the first channel, Hex dye of emission wavelength 550 nm and excitation wavelength 535 nm for the second channel, Rox dye of emission wavelength 586 nm and excitation wavelength 610 nm for the third channel and CY-5 dye of emission wavelength 520 nm and excitation wavelength 424 nm for the fourth channel.

On Testing the samples with the following dye we got a result like this:

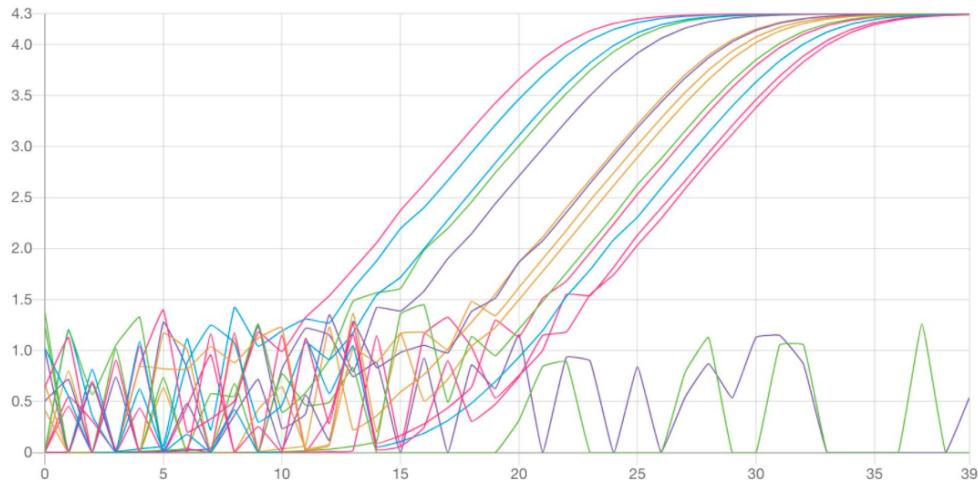


Fig 37: Final Result Graph the test was done successfully. All 4 channels were recorded after running through the thermal cycler and Multiple test values can be seen in this. The AS7341 detection part got all the values. Fluorescence after a certain number of cycles started gradually increasing. However, we were able to record 4 channel test values. After the GUI and the time required A graph similar to the above based on the test case is shown.

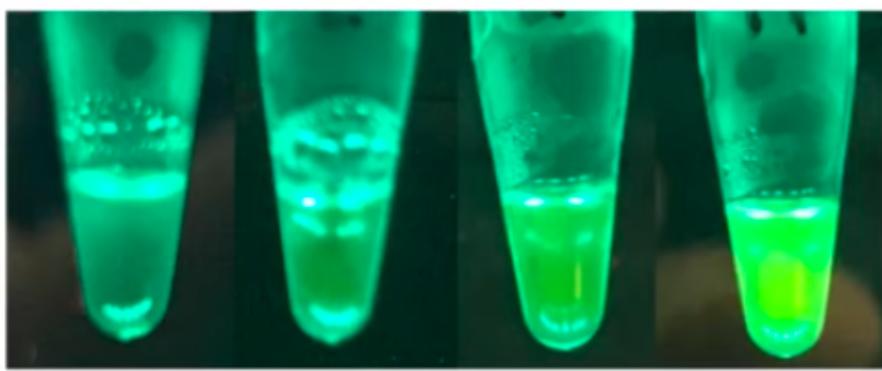


Fig 38: 4 Samples in the Tubes mixed with one of the 4 dyes. Similarly, other 3 were also used. While testing the solution we first preferred a dark room. Fluorescence was increasing after a number of cycles. If the test was positive the fluorescence increased a lot after many

cycles and if the test case is negative fluorescence emitted till a threshold value and then nothing

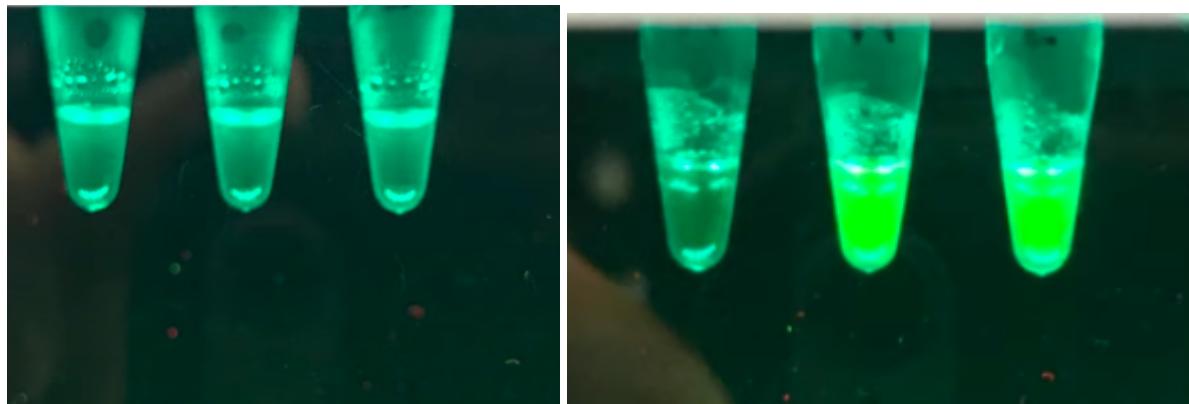


Fig 39 : Tubes with Samples before Cycling

Fig 40: Tubes with samples after a number of cycling

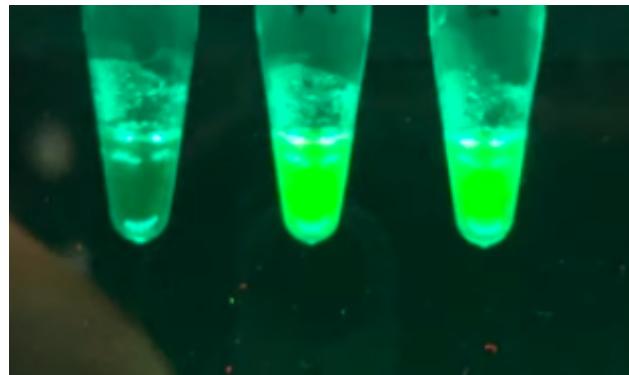


Fig 41: Tubes after testing with cycler

Similarly, many tests were conducted and fluorescence was measured. All the tests verified that a negative case fluorescence varied till a certain point, The threshold value and in positive cases, the fluorescence increased. The Gui showed the graph and if the test is positive or negative. LEDs are also added to the machine to indicate whether it is positive or negative.

CONCLUSION

We constructed a multichannel RT-PCR machine with a display, GUI and thermal cycler and detection system based on a spectral sensor. The system utilised a low power commercial cartridge heater that allows fast heating and temperature cycles. The use of the Spectral Sensor system provides fast and accurate detection over real-time for multiple channels. Further improvements are ongoing in this system.

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