B.E PROJECT ON

Open Source Scientific Instrumentation

Submitted By

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CERTIFICATE

This is to certify that the report entitled “**OPEN SOURCE SCIENTIFIC INSTRUMENTATION**” being submitted by PRADEEP KUMAR, SRIJAN PABBI, to the Department of Electronics and Communication Engineering, NSIT, (now upgraded to Netaji Subhas University of Technology), for the award of bachelor’s degree of engineering, is the record of the bonafide work carried out by them under our supervision and guidance. The results contained in this report have not been submitted either in part or in full to any other university or institute for the award of any degree or diploma.

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We also thank our family for relentless encouragement and support. We will also place our sense of gratitude to all our friends and the members of Centre for Electronics Design and Technology, who helped us in transforming this project from an idea into a reality.

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Abstract

For many years PCR process has been improved upon creating new state of the art technologies in the process. Today PCR machines can perform the process in less than 20 minutes. Even if this technology exists, it is not easily accessible to many people and even to most educational institutions that have to potential to conduct microbiology and gene research due to costs ranging from upwards of 2 lakhs for descent PCRs. The problem we are trying to solve is to create a PCR thermo cycler which can perform satisfactorily in a reasonable price, and as the name of the project says is open source which can be easily replicated by anyone either for their own use or some institution's. We discuss 2 approaches for PCR, one uses peltiers and nichrome heaters and the other one uses halogen lamp and a DC fan.\newline

The next device we targeted in this project is the IOT Reaction multimeter and specifically 2 components involved, the pH meter and temperature meter which can perform all the functions necessary to implement such meter.

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1 Chapter 1: Introduction

Open source scientific instrumentation as the name suggests is about redesigning scientific instruments in a way that the design is openly available, easily replicable and cheap to make by science enthusiasts. Not everyone works at high end labs and gets to use high end scientific equipment, for example most schools don't have equipment that may be necessary for education but too expensive to own or be used by students. The aim of this project is to design and implement one such instrument, in a manner that it follows the aforementioned constraints.

1.1 Motivation Behind This Project

Not everyone works at high end labs and gets to use high end scientific equipment, for example most schools don't have equipment that may be necessary for education but too expensive to own or be used by students. The aim of this project is to design and implement one such instrument, in a manner that it follows the aforementioned constraints.

1.2 Background And Research

A major area of research is the human genome project which employs the Polymerase chain reaction process in it. the process of replication of DNA is very important for the sequencing of the human DNA
The various steps that are involved in the process of DNA replication are

- Extraction : involves the separation of the DNA from the cell constituents.
- Quantification : it is the step which determines the amount of DNA yield.
- Gel Electrophoresis: it is basically a process in which the positively and negatively charged particles like the DNA, RNA and the Protein particles are separated from the agar gel which is present as a

base constituent on which the charged particles travel due to the application of electromagnetic field. and the distance travelled by the the DNA segments is proportional to the weight of the DNA fragment.

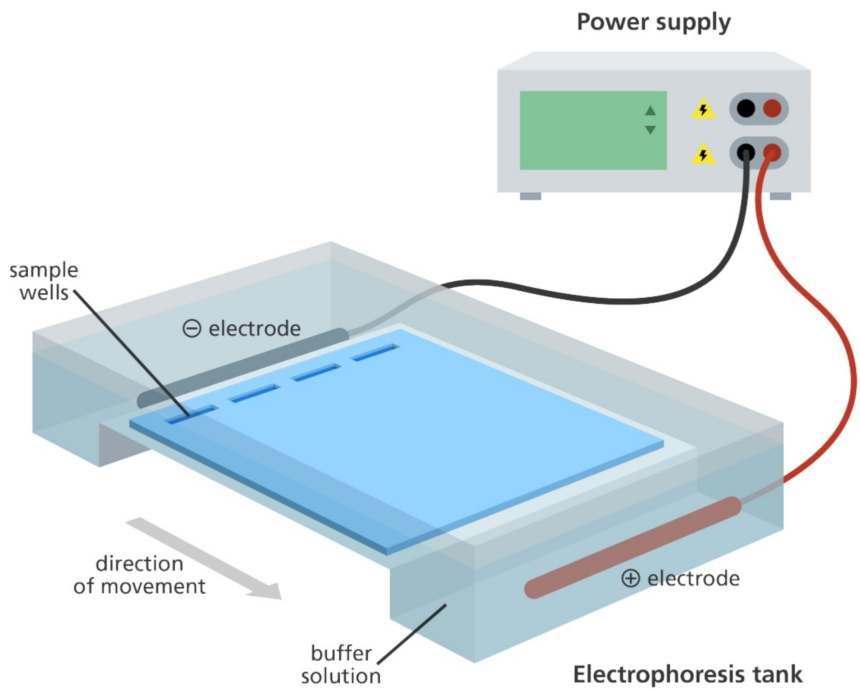


Figure 1: GEL electrophoresis setup.

- Results : the agar gel is cut into strips and then it is placed below a UV light source and the DNA fragment are observed under the light following is an illustration that shows the typical DNA bands observed

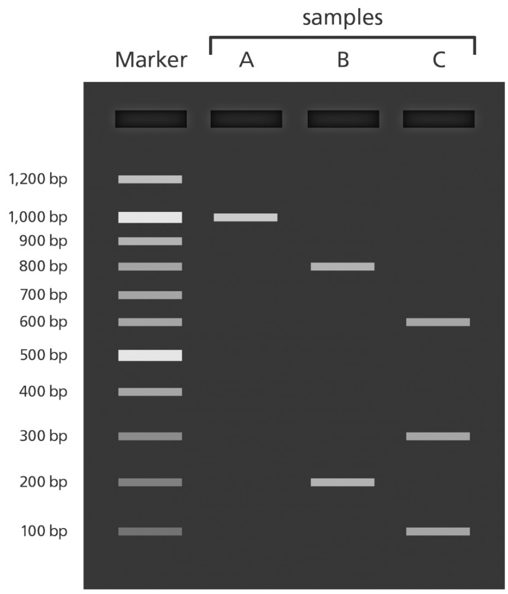


Figure 2: DNA Bands

1.3 PCR process

The Polymerase Chain Reaction mainly has the follwing steps that are critical to the process of DNA amplification and thus have to be carefully determined and implemented in the machine we have developed. The three basic steps which are important to the PCR reaction:

- Denaturation: it is the process during which the PCR tube having the solution is heated upto a temperature of about 94-96 °C. Prolonged maintenance of the solution at this temperature breaks the Hydrogen bond between the base pairs Adenine Guanine Cytosine and Thymine (A T G C) which are present on either strands of the DNA.
- Annealing: At this temperature specific RNA primers that are put in the PCR reaction mixture intentionally get attached to their complimentary base pairs on the now separated single strand DNA.This is the point where the replication of the DNA starts.

- Elongation or Extension: this is the temperature at which the base pairs from the reaction solution start to polymerise on the single strands of DNA and two copies of DNA are produced.
- **Several such cycles are performed to make millions of copies of a single strand of DNA** because each cycle that is performed makes two copies of DNA and the so the output of DNA content is basically expressed as 2^n (where 'n' is the no. of cycles performed).

given below is an illustration of the PCR process.

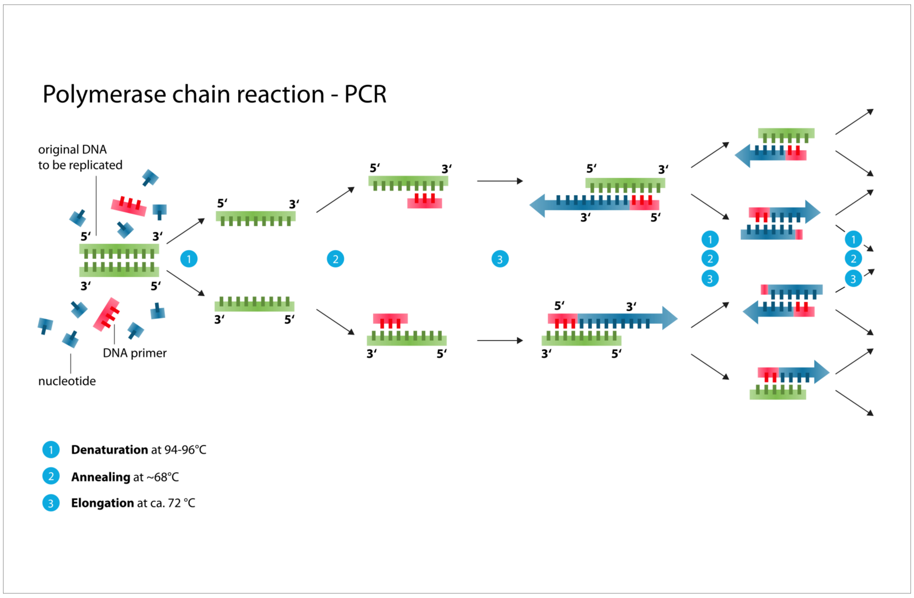


Figure 3: PCR reaction

2 Chapter 2: Developing The PCR Machine

2.1 Design Overview or Motivation

The primary function of a PCR machine is to perform the PCR biochemical reaction (as explained in **??**) which essentially makes multiple copies of the DNA sample present in the PCR solution. PCR machines are an essential part of the DNA sequencing and replication process and are critical for every research facility aiming to perform research on living organisms and improving medical facilities or diagnosing genetic disorders. The main aim for this project dictates that the prototype design should be as simple as possible and widely reproducible. The motivation behind the PCR machine is to make it available for schools, hobbyists and to anyone who wants to get involved in designing of scientific instruments, providing an open source platform which can be improved upon over time.

2.2 Design Process

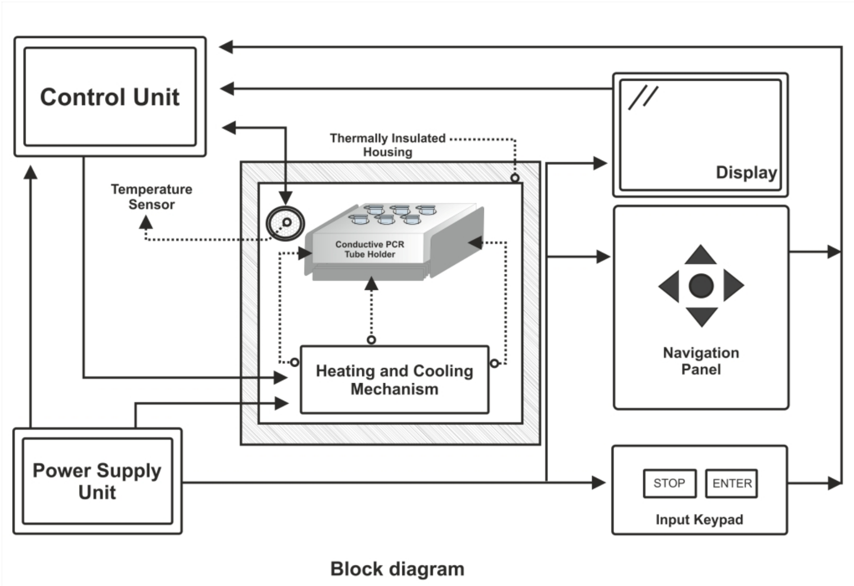


Figure 4: Block Diagram of the PCR Machine

PCR machine has a few major functional components. To decide on these components, analysis is needed so that specifications can be determined.

- **The Control Unit:**The control unit is the brain of this instrument and will perform the job of controlling different types of PCR cycles, taking user input and processing it,It will read all the sensors and make sense of the data, output meaningful information about the process status to the user and handle the device such that the instrument doesn't accidentally exceed critical temperatures.
  The controller that will be used in this application is ATMEGA328P, which will be responsible for controlling the H-bridge current drivers, reading the temperature from the PCR block, applying control algorithms to maintain a temperature, and handling the user interface for better interaction with the instrument.

- **Heating Mechanism:** Heating mechanism is a crucial part of the whole experimental setup and must conform to some standards which include but are not limited to maximum achievable temperature of at least 98°C (required for denaturation), rate of rise and fall of temperature that allows the reaction to finish in a timely manner and along with that provide a reasonable yield, the heating element must have a low inertia of heat retention with a reasonable efficiency for heat transfer.

- textbfCooling Mechanism : Similar to the heating mechanism the cooling mechanism is equally crucial and must be able to make the block reach as low as 4°C, reduce the temperature of the setup from about 100°C to about 60°C with a reasonable rate.

- textbfConductive PCR Tube Holder : This part is the one that will do the job of transferring heat to the PCR tubes and hold them in place. It should be a good conductor of heat which can be heated and cooled quickly.

- User Interface : User interface is a crucial part since it the one that will be taking user inputs and must be carefully designed so as to not break simplicity but also provide full functionality. The user interface will comprise of a 20x4 character LCD display with navigation keys along with a keypad for easy input of the parameters for the PCR cycle.

2.3 Finalizing Heating and Cooling Mechanisms

2.3.1 Heating

various methods can be employed to produce heat electrically.
After analysing the various method that can be employed to heat the PCR block the options were narrowed down to Nichrome Heaters because of the feasibility of use and the ease of installation. Also, the heaters could be driven easily by using the ac mains and are properly insulated, moreover after experimental analysis it was identified

that the rate of heating is considerably fast and these heaters could be employed in the machine.

2.3.2 Cooling

Peltier Modules were used for providing cooling to the thermocycler block. Peltier Modules were finalized because they have some features which make them standout among all other methods of cooling, some of these features are listed below :

- no refrigerant and no Chloroflurocarbon emission
- Low to negligible maintenance
- long lifetime
- Easily of control by DC sources
- Robust
- Capability to provide efficient cooling even far below ambient temperatures
- Performance is totally independent of orientation

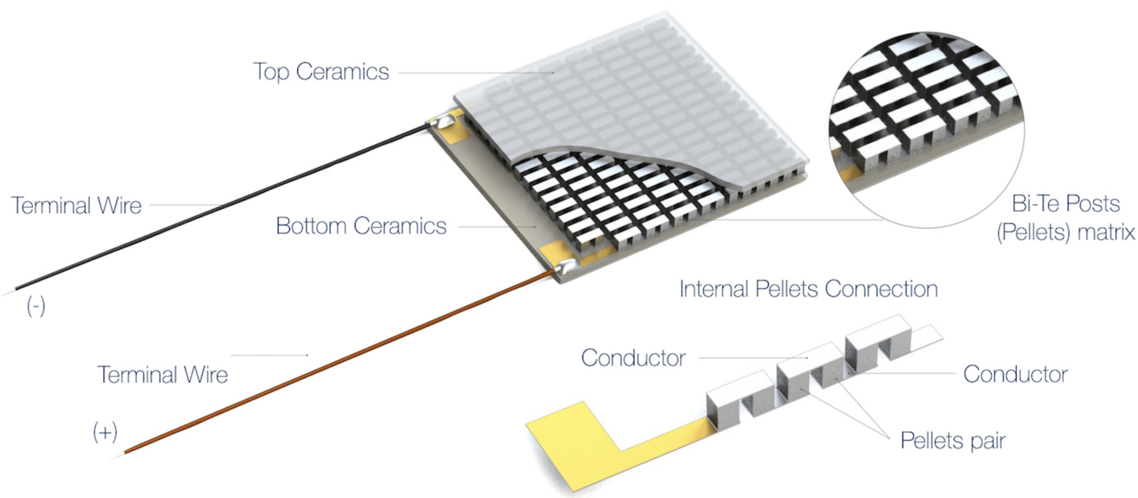


Figure 5: view of the Peltier Module

Technical Specification:

Hot Side Temperature (°C)	25°C	50°C
Q_{max}(Watts)	50	57
Delta T_{max}(°C)	66°C	75°C
I_{max} (Amps)	6.4A	6.4A
V_{max} (Volts)	14.4V	16.4V
Module Resistance(Ω)	1.98Ω	2.30Ω

The Peltier module work basically by utilizing the **Peltier effect**. It works by generating a temperature difference between the faces of the peltier module. whenever we apply potential difference across a peltier module one of the junctions becomes cool and the other junction becomes hot and vice versa is also true

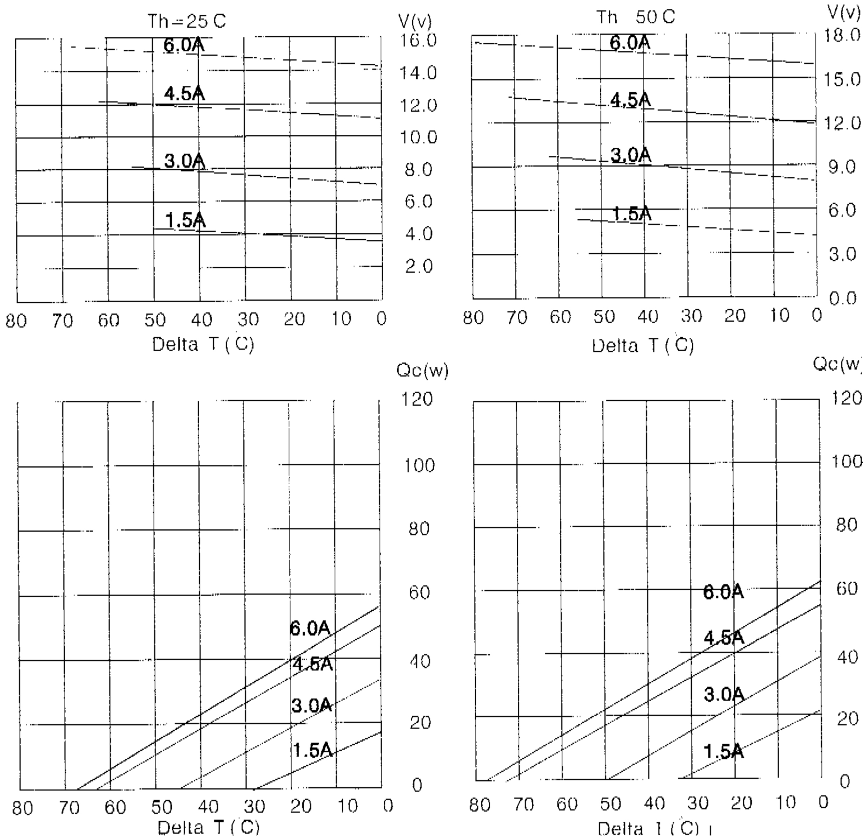


Figure 6: Performance of peltier Module

Most commonly the peltier Module is used for cooling applications but it can also be used for the heating and temperature regulation of devices in which the peltier module is used.

2.4 Tube Holder Design and Machining

The block was designed in a CAD software from Autodesk called Tinkercad. The block was designed keeping the following in mind:

- At least 3 Peltier modules could fit below it.
- Nichrome-Mica heaters could fit into the space provided on the sides.
- Temperature sensors could be inserted into the sides of the block.
- A good number (16) of PCR tubes could be cycled simultaneously.
- PCR tubes would fit without airgaps between them and the block.
- The block and plate are mountable on standard CPU heatsink mounts.

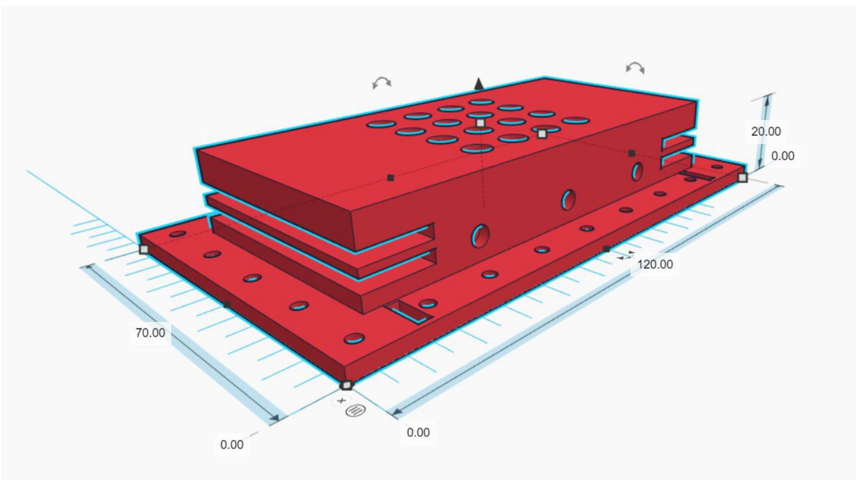


Figure 7: CAD Diagram

After developing the CAD diagram in order to better visualize it the CAD diagram was rendered using 3D builder (Windows Application). And the rendered block was visualized.

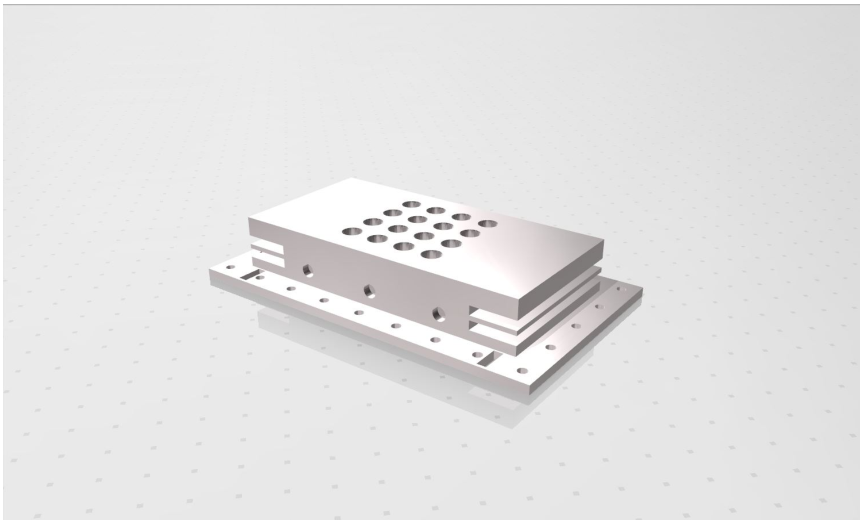


Figure 8: Software-rendered 3D Diagram

The block was then manufactured by a machine shop in Anand Parbat Industrial Area, New Delhi. The block is made from Al 6063 typically used for heatsinks and hence is on the higher side of heat conductivity.

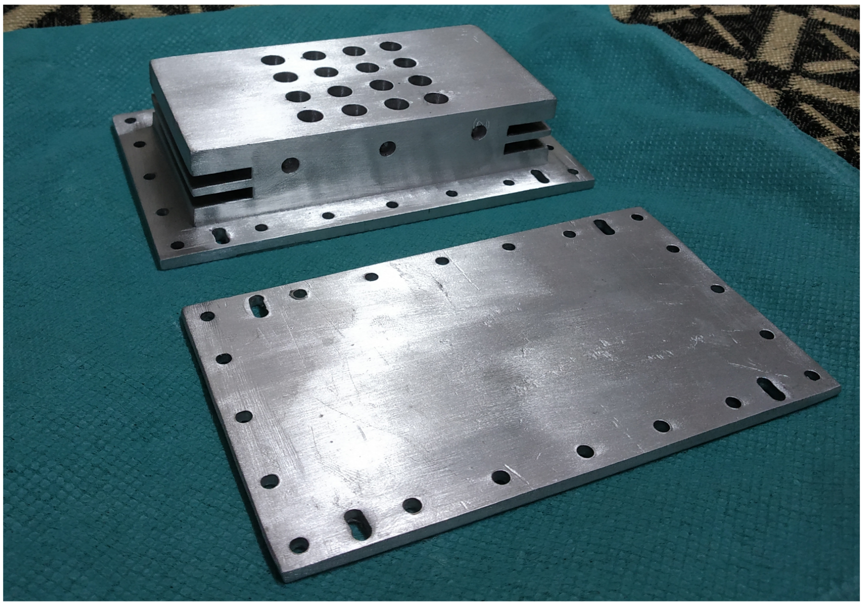


Figure 9: The machined Thermocycler Block

Provisions were made to provide space on the side to insert the Mica Heaters in the slots provided on the side of the Block.
Provision were also made to hold the PCR tube in top of the block along with the several cylindrical slots that were made on the front and back side of the PCR block to provide space to insert the temperature sensors and the value of temperature from all the sensors were averaged out to provide an accurate value of the temperature reading.

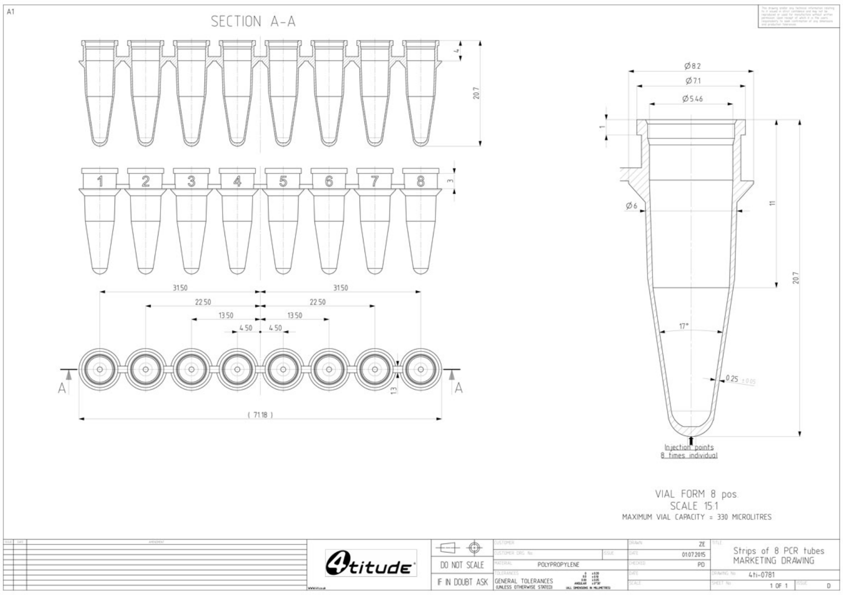


Figure 10: Dimensions of the PCR tube

Figure 11: PCR tube

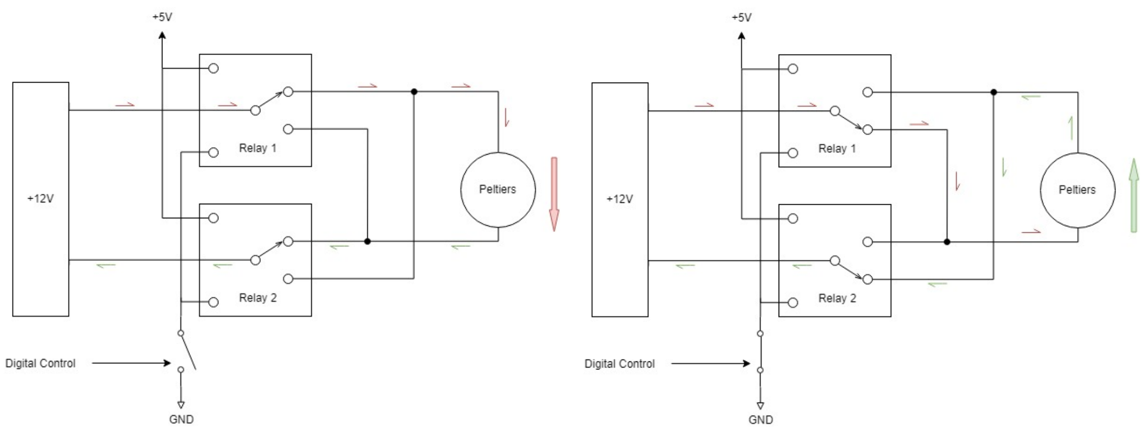
2.5 Circuit Design and Testing

2.5.1 Peltier Drivers and Mica Heater

The requirement this circuit needs to meet is that heaters and coolers can be switched on and off at will. Peltier thermo-electric modules can be used as both heating and cooling elements, depending of polarity of the voltage applied to the module. Mica heaters work when they are powered with direct AC mains, whereas the Peltier modules require DC electricity to operate.

First let us describe the Peltier driver circuit. Peltiers as we saw before draw current as per the voltage value applied on them, and hence the decided voltage being 12V will make them draw about 2-3A per peltier, having at max 4 peltiers we can control this with a 12A relay or 4 H-bridge drivers with capability of handling 3A each (or 1 H-bridge driver capable of handling over 12A). The following are the possible configurations with explanation on how they work.

- The first configuration involves usage of SPDT relays for creating two possible current paths from a load. In Figure 12a we can see that the switch on the low side is disconnected and hence the relay is in its default position, examining further reveals that the current is going through the peltier load in a downward fashion. Connecting the switch on the bottom side as in Figure 12b now causes the relays to switch positions and the new path of current is exactly opposite for the peltier but the polarity remains same for the +12V supply which is driving the peltiers.



(a) Relay Position 1 (b) Relay Position 2

Figure 12: Peltier driver successfully reversing the polarity of input voltage and current to the peltiers

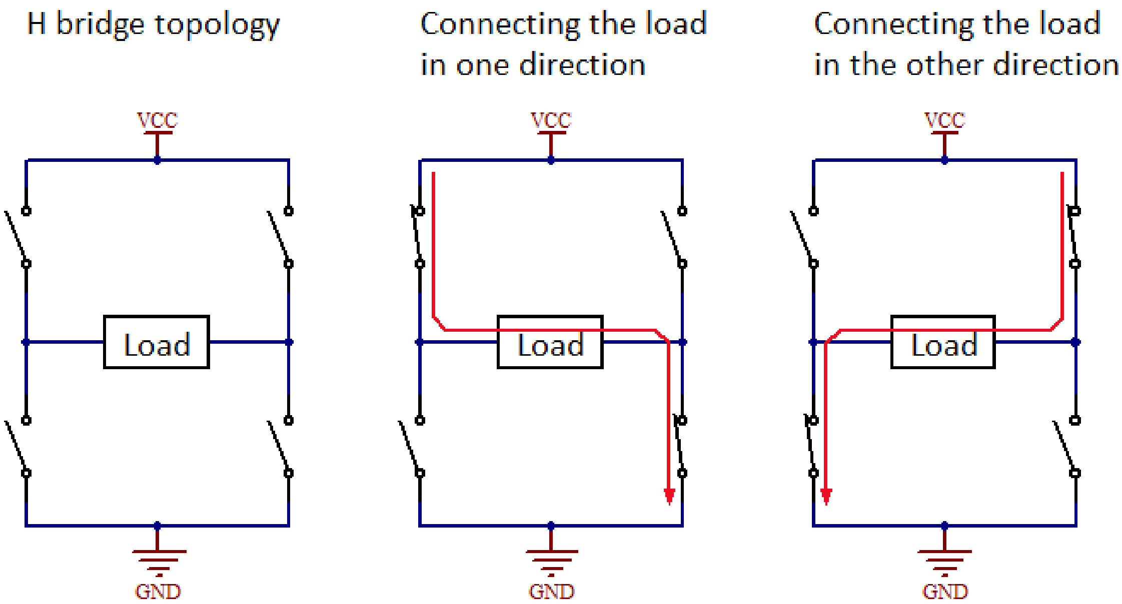


Figure 13: Basic circuit for the concept of h-bridge topology

- The Second configuration involves the use of H-Bridge driver. A H-bridge driver has a design which involves two high side switches and two low side switches with the driven load placed in between the junctions of the two switches, the switches are transistor based and are electrically controllable. The high and low side switches are controlled in such as fashion that only two are closed at any given time, a pair of high-low side switches and then the other pair when turned on reverses the flow of current as shown in Figure 13. This allows us to work with the peltier modules with a dual polarity which in this case causes the heat transfer to reverse its direction when the direction of current is reversed. This ultimately allows finer and better control of temperature because the flow can be controlled electronically as the switches are turned on and off.
  The H-bridge driver for this case needs to be high current capable since controlling 3 peltier devices simultaneously, each running at 3A requires the use of a higher end device which could handle at least 3-4A of current.

Now we move to the second type of heaters that are used in this project.

Figure 14: Nichrome Mica heaters

The nichrome-mica heater, which are generally used in household cloth irons, heat plates etc... is shown in Figure 14, These type of heaters work on the basic principle of resistive heating, nichrome is a material which is used to create high resistance wires on a further note:
Resistance wire: Metallic resistance heating elements are produced in many forms and may be wire or ribbon, straight or coiled. These are most commonly use in heating devices like soldering irons, hair dryers, furnaces for industrial heating, floor heating, roof heating, pathway heating to melt snow, dryers, etc.
The most common class of material which is used is Nichrome which has a composition of 80% Nickle and 20% Chromium. it is avaialble in various forms like strip ribbon or wire form. Nichrome 80/20 is ideal, because it has relatively high resistance and forms an adherent layer of chromium oxide when it is heated for the first time. Material beneath this layer will not oxidize, preventing the wire from breaking or burning out, thus making it reliable for re-heating again and again.
The Mica heater used, has a very simple construction it mainly comprises of 4 things:

- Nichrome Wire: thin Nichrome Wires

- Mica sheets : Mica is an electrical and thermal insulator and is used in electrical components, electronics,
- Ceramic Beads : These are the beads that are present on the nichrome wires coming out of the heater element they have a special purpose i,e, to prevent the contact of heated nichrome wire on the body of the device.
- Metallic strip : use for tightly packing the assembly of the sheets

the several interconnected straight sections of nichrome wire are sandwiched between the layers of mica sheets and is enclosed in a folded metal sheet to encase the assembly.

2.5.2 User Interaction

For user interaction we must provide a visual and tactile feedback so that a person can operate the device. For visual feedback a 20x4 character LCD seems more than enough to compensate for the various complicated PCR processes that will take place as a part of the PCR DNA amplification cycle. For tactile input a simple set of 6 push buttons will suffice any input that is required in relation to movement of the selector and increment and decrement in values.

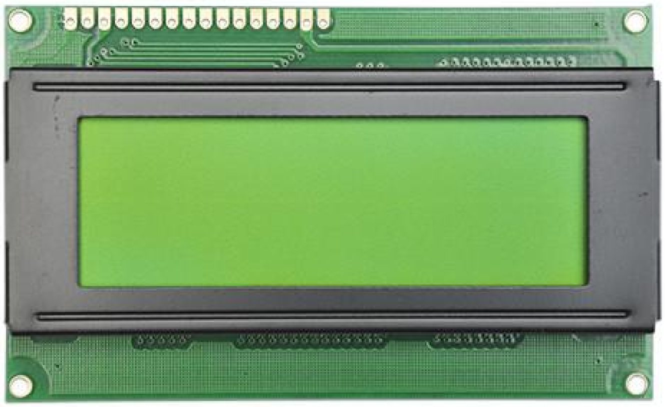


Figure 15: 20x4 LCD

The user will be given the freedom to choose the length of the cycle, the length of time each temperature value will be held at, and the values of the temperature levels. Each of these features will be controllable through 6 set of button arranged as shown in Figure 16.

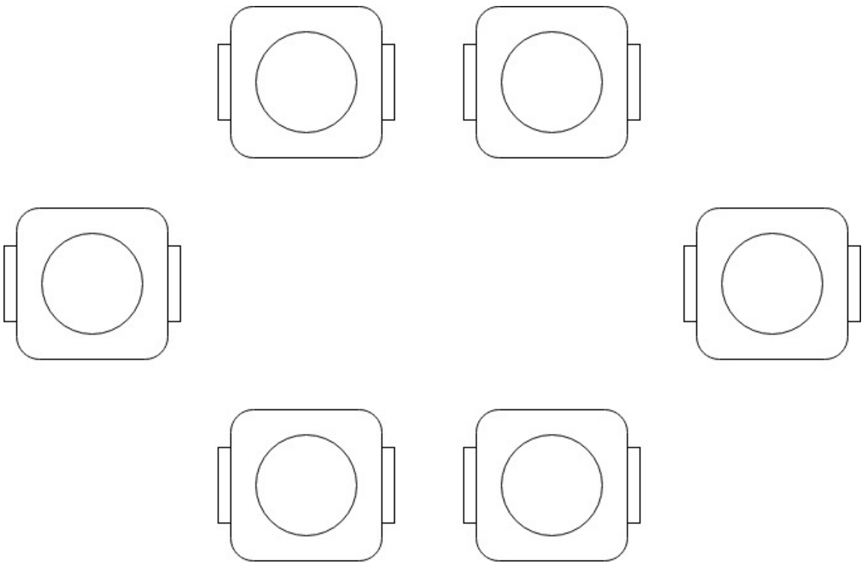


Figure 16: User input buttons configuration for left/right selector movement and up/down for increment and decrement

The user will be able to move around selecting parameters with the left and right button and change the value of the parameters with up and down buttons. This functionality eventhough can be implemented by using only one set of up and down buttons, the other set can be used for other purposes.

2.5.3 Controller

The controller of choice is Arduino Nano due to several reasons. The main reason for choice of this microcontroller is that it is easiest to code and use, along with minimal connectivity issues. The background developer support is strong and libraries for general problems are easily available. Arduino being based on the ATMEL328P microcontroller platform is chap such that it can be easily obtained thus adding value to the open source nature of this project.

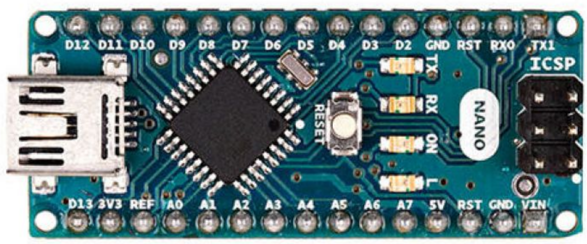


Figure 17: Arduino Nano with ATMEGA328P

Arduino nano offers a 10bit SAR ADC, 16MHz operating frequency, and a lot of I/O ports to implement various functionality which is required on the PCR machine. Having a Crystal oscillator, it can keep time fairly accurately and hence RTC is not really a necessity

Figure 18: 12V 30A DC Power Supply

for tracking the time intervals in the amplification process. Having an internal EEPROM, it can retain values between power ups and hence any information crucial to the amplification process can be retained incase of a power loss, although the recommended step would always be to reperform the process.

2.5.4 Power Supply And Powering Circuit

A power supply is the heart of any electronic circuit because without it no matter what the circuit is won't work. A power supply must be chosen carefully such that it provides constant power throughout the operating range of the circuit. In this case many components exist and one of the major ones is the peltier modules. As discussed previously the peltiers will draw about 3A of current per module and thus the supply needs to provide at-least 10A of current just for the peltiers. Peltier modules of various brands vary in their specifications for drawing current and hence as per data sheets this current can go to 16A total, and of more than 3 peltiers are used in a parallel configuration the current drawn will be even more, hence the power supply we are going with is a little overkill but will suffice any need for the foreseeable future.

2.6 Embedded Software

2.6.1 Operating The LCD

The LCD being the primary display component of the circuit is coded to display various crucial parameters involved in the process, such as temperature, cycle number, various temperature levels, heating and cooling status, etc... the following are few steps involved in coding for a character LCD.

Initialize

LCD can work in 2 modes one is with the full 8 bit bus and the other is with 4 bits at a time to save pins, following is the connections made in the circuit from LCD to controller pins.

```
/*
 * LCD RS pin to digital pin 12
 * LCD Enable pin to digital pin 11
 * LCD D4 pin to digital pin 5
 * LCD D5 pin to digital pin 4
 * LCD D6 pin to digital pin 3
 * LCD D7 pin to digital pin 2
 * LCD R/W pin to ground
 * LCD VSS pin to ground
 * LCD VCC pin to 5V
 */
```

The code to initialize is as follows :-

```
// include the library code:
#include <LiquidCrystal.h>

// initialize the library by associating any needed LCD interface pin
// with the arduino pin number it is connected to
const int rs = 12, en = 11, d4 = 5, d5 = 4, d6 = 3, d7 = 2;
LiquidCrystal lcd(rs, en, d4, d5, d6, d7);
//lcd: a variable of type LiquidCrystal

void setup() {
  // set up the LCD's number of columns and rows:
```

```
  lcd.begin(20, 4);
}
```

Display
To display text there are two main functions :-

```
//1. Set cursor position

//col: the column at which to position the cursor (with 0 being the
    first column)
//row: the row at which to position the cursor (with 0 being the first
    row)

lcd.setCursor(col, row)
```

```
//2. Print ascii text

//data: the data to print (char, byte, int, long, or string)
//BASE (optional): the base in which to print numbers: BIN for binary (
    base 2), DEC for decimal (base 10), OCT for octal (base 8), HEX for
    hexadecimal (base 16).

lcd.print(data)
lcd.print(data, BASE)
```

2.6.2 Reading Input from Navigation Buttons

To use the navigation keys, first we need to understand how they are providing the values. The navigation are connected such that only one button can be pressed at any given time since the input is taken through an analog value depending on which button is pressed. The way in which buttons are connected is shown in Figure 19

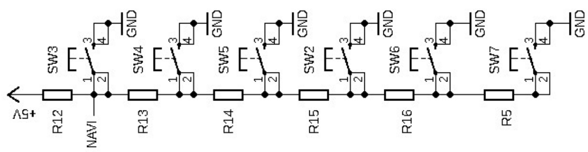


Figure 19: Navigation Keys Circuit

The code for reading is as simple as the circuit, the function which returns the button number is as follows :-

```
//ri are the ranges of the navigation switches
int button_poll(int navi_pin) {
  int ana_val = analogRead(navi_pin);
  if(ana_val >= 1000){
    return 0;
  }else if(ana_val >= r1){
    return 1;
  }else if(ana_val >= r2){
    return 2;
  }else if(ana_val >= r3){
    return 3;
  }else if(ana_val >= r4){
    return 4;
  }else if(ana_val >= r5){
    return 5;
  }else{
    return 6;
  }
}
```

2.6.3 Controlling the PCR cycle

Temperature control cycles can be implemented by keping in mind the difference remaining between the desired value and current value of the temperature. For heating the block is first heated until the temperature has a certain delta in relation to the final temperature and then the heating is regulated by turning the heater on and off.

```
void heat(int Th, int time_ip) {
  time_ip = time_ip * 20;
  analogWrite(heater, pwmduty);
  fan(false);
  int controlst = abs(Th - currtemp) / 2;
  while (currtemp < (Th + 1 - controlst)) {
    currtemp = tempdisp();
  }
  int controlfact = (255 / controlst);
  int delta = Th + 1 - currtemp;
  int ctr = 0;
  int temp = Th;
  while (ctr < time_ip) {
    currtemp = tempdisp();
    delta = temp + 1 - currtemp;
    Serial.print("DELTA=");
    Serial.println(delta);
    if (delta < -5) {
```

```
      temp = Th + 10;
      fan(true);
    } else {
      temp = Th;
      fan(false);
    }
    if (delta >= 0) {
      analogWrite(heater, pwmduty + (255 -  controlfact * delta));
    } else {
      analogWrite(heater, pwmduty + 255);
    }
    ctr++;
  }
}
```

2.6.4 Main Function

2.7 PCB Design

2.7.1 Schematic Design

The schematic is a representation of the actual required circuit with real component symbols. For example in theoretical circuit design we generally don't consider practical electrical constraints like current limit, voltage limit, operating temperature, package, and other specifications that matter. But for practical circuit design each component must be decided after theoretical analysis various circuit parameters, these parameters must fit in with the specs of the desired component. While creating a schematic of the circuit all the nuances and features of the final required output must be kept in mind and put into the schematic.

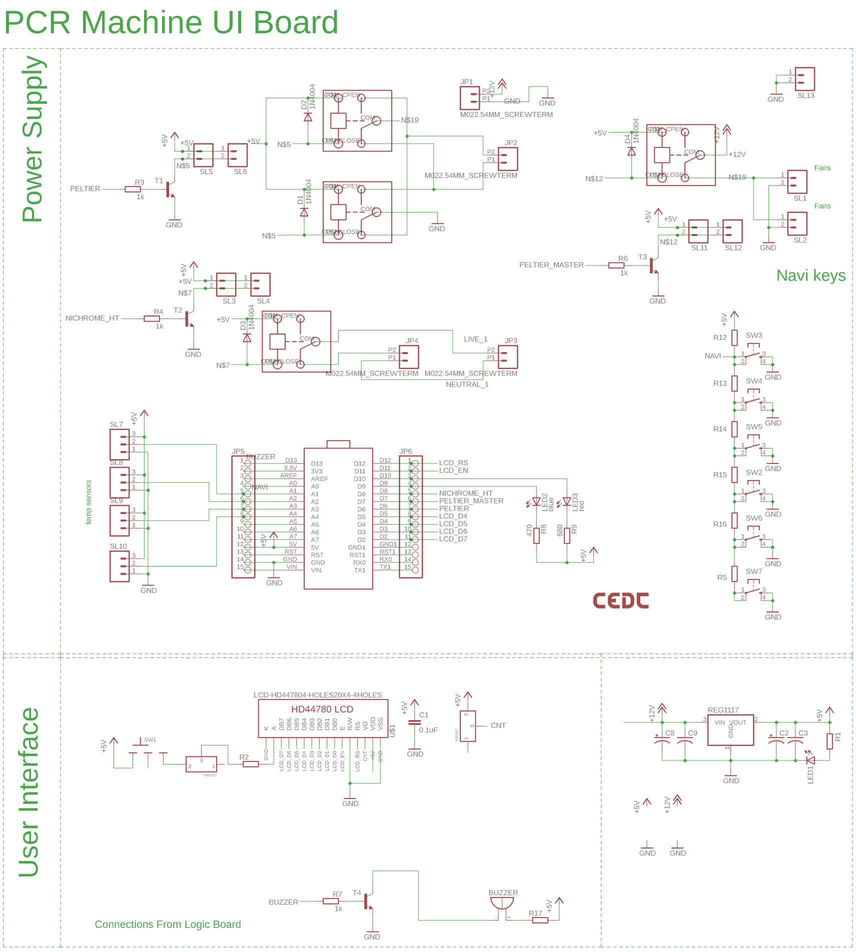


Figure 20: PCR UI BOARD

2.8 Prototype Working, Observations and Results

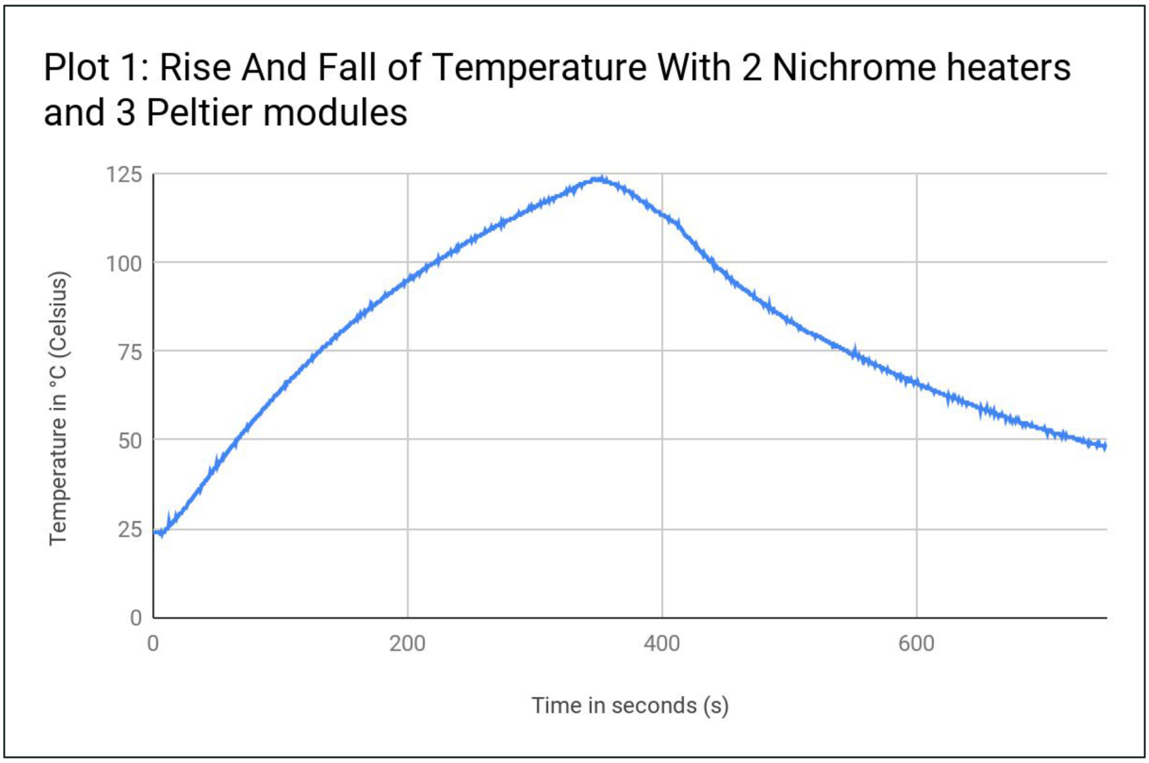


Figure 21: Sensor Readings: Temp vs time graph

Observations :

- Heating : 2 x 35W Nichrome heaters
- Cooling : 3 Peltier Modules

Calcualted Rate :

- Rate of Temp rise: 0.317 °C/s
- Rate of Temp fall: 0.24 °C/s

Another configuration which was tested yielded the following results :

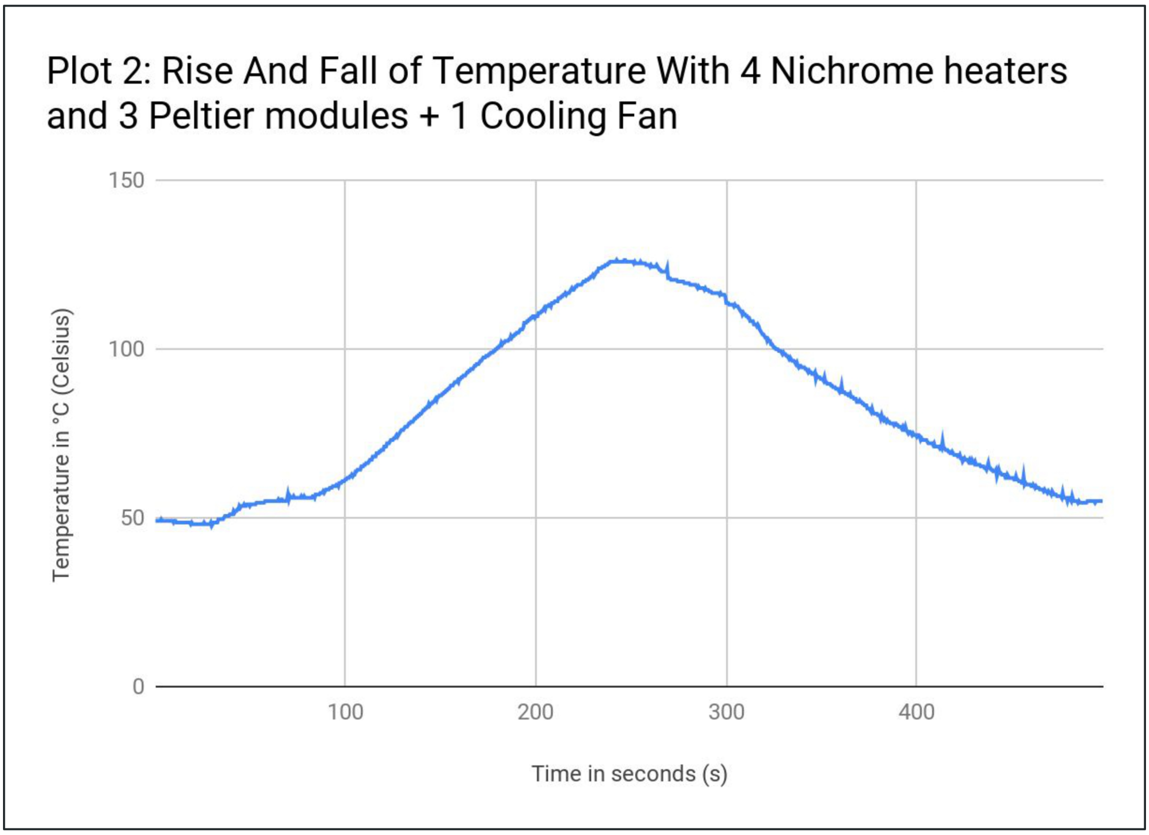


Figure 22: Sensor Readings: Temp vs time graph

Observations :

- Heating : 4 x 35W Nichrome heaters
- Cooling : 3 Peltier Modules + 1 Cooling Fan
- Rate of Temp rise: 0.502 °C/s
- Rate of Temp fall: 0.624 °C/s

2.9 Shortcomings of the current Prototype

- Rate of heating and cooling is not as good, so as to obtain a desirable performance, although the rate is still acceptable, it is not up to the mark with today's industry standards.
- The Aluminium block is way to bulky for it to be quickly heated and cooled at the desired rate.
- Copper may have been a better choice for constructing the whole block as its heat conducting capacity is more than that of aluminium by a significant magnitude.

2.10 Casing and Touchup

Every electronic design needs to have a proper housing to prevent the device getting damaged and prevent the machine getting affected from outside influences such as wind heat water etc therefore we also developed the device and alongside it developed the casing in which the entire machine is housed. following are the various steps taken in the construction of the casing of the PCR

- Develop a CAD Diagram: A CAD diagram was developed to better visualise the final casing of the PCR machine.

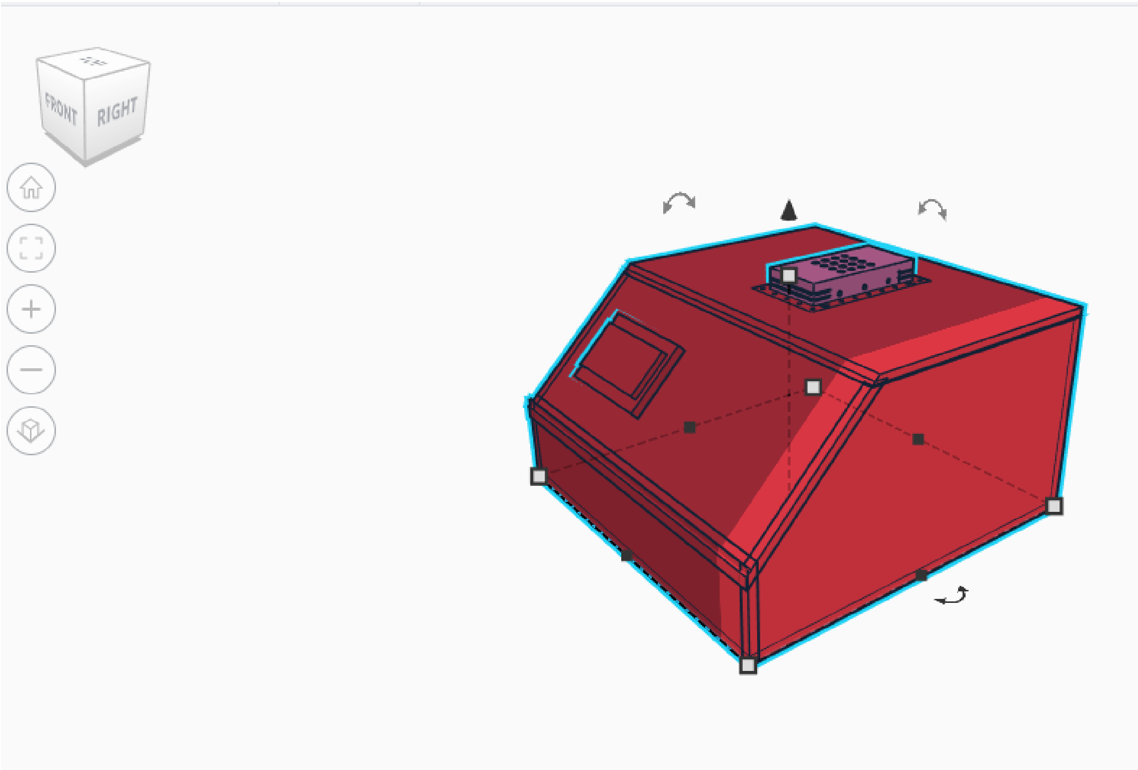


Figure 23: Casing CAD Diagram

- Gather the Resources : Bought the resources such as wood, Iron nails, wood screw hinges for flaps , wood glue etc.
- Dimensioning : dimensioned the wood and cutting of the elements of the housing.

Figure 24: Measurement and Cutting

- Assembly of the Casing: The casing was assembled using wood screws Iron nails and the Fevicol

Figure 25: Assembly of the casing

Figure 26: After Assembly

- Installation of the machine components : the machine components power supply, heat sink and the UI PCB was installed. after checking the orientation and other things the components were removed.

Figure 27: Installation of circuit components

- Painting and polishing the wood : The Painting was done using a brown enamel.

Figure 28: Painted casing

- Wiring : Wiring of the entire machine was done. given below is the image of the finished and assembled machine.

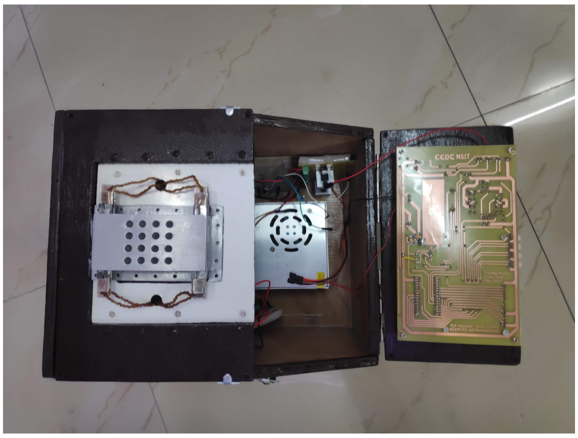


Figure 29: Wiring of the machine

- Final Assembly

Figure 30: final assembly

3 Chapter 3: PCR Machine A Different Approach

3.1 Concept and Inspiration

A basic PCR machine as discussed has 3 features, heating mechanism, cooling mechanism, and a control for both. The approach we are going for here is to use a Halogen lamp as a heater and a fan as cooler. The catch here is that, this setup will hold pcr tubes in thin electroformed mould of copper covered with black paint. This approach experimented by Dr. Pramod Upadhyay of NII Delhi. The experiment he conducted showed a high rate of temperature rise and thus perform the reaction at a fater rate than conventional PCRs. This setup being so straight forward that it could be made in less than 3000 rupees.

3.2 Heating and Cooling Mechanisms

Heating mechanism used here is a 500W halogen lamp. An electric bulb has a very fast response compared to nichrome wire heaters used in other design. Faster ramp rate with practically zero 'dead time' was attained by heating the copper foil by the light of a 500 W halogen lamp. PWM into a triac circuit of AC mains is used to control the light intensity of the bulb, and thus the temperature of the sample holder. For cooling mechanism a DC 12V fan is used to blow wind on top of the electroformed copper PCR tube holder. Being so light and thin, it looses heat quickly and thus this cooling method is enough to cater to our needs.

Figure 31: Halogen Lamp

3.3 Apparatus Design and Construction

The apparatus used is constructed fully in lab and has the three components placed in a vertical ordering fashion. As shown in Figure **??** the heating element(lamp) is placed at the bottom and PCR holder just above it, and finally the fan is placed on the top to cool the holder as required. The construction is shown in Figure 33.

3.4 Control Circuit

3.4.1 Halogen Lamp

Halogen lamp is powered via direct AC mains and hence doesn't need any additional circuitry to operate, although it will need some kind of control mechanism to be able to switch and turn on as per requirement. The circuit shown in Figure 34 uses a triac which is controlled by an opto coupled zero crossing trigerred triac which connects the gate and the MT2 terminals of the triac. The opto isolator is trigerred with the microcontroller Dc voltage and hence PWM can control this setup.

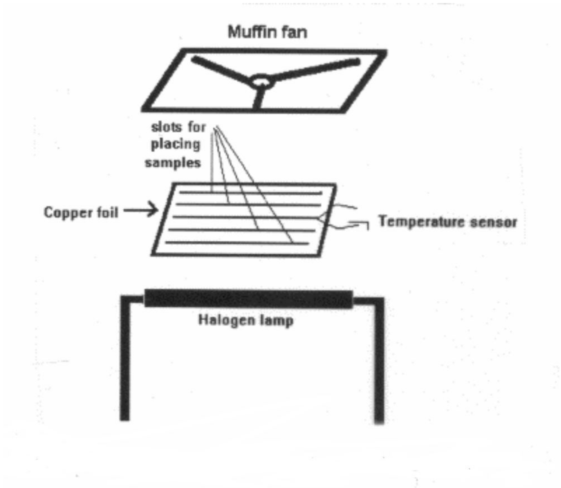


Figure 32: Illustriation of the assembly

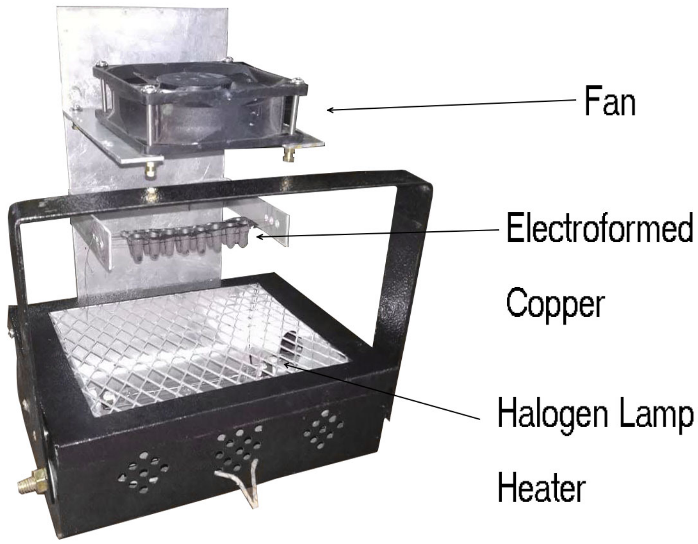


Figure 33: Our construction of the Halogen based PCR

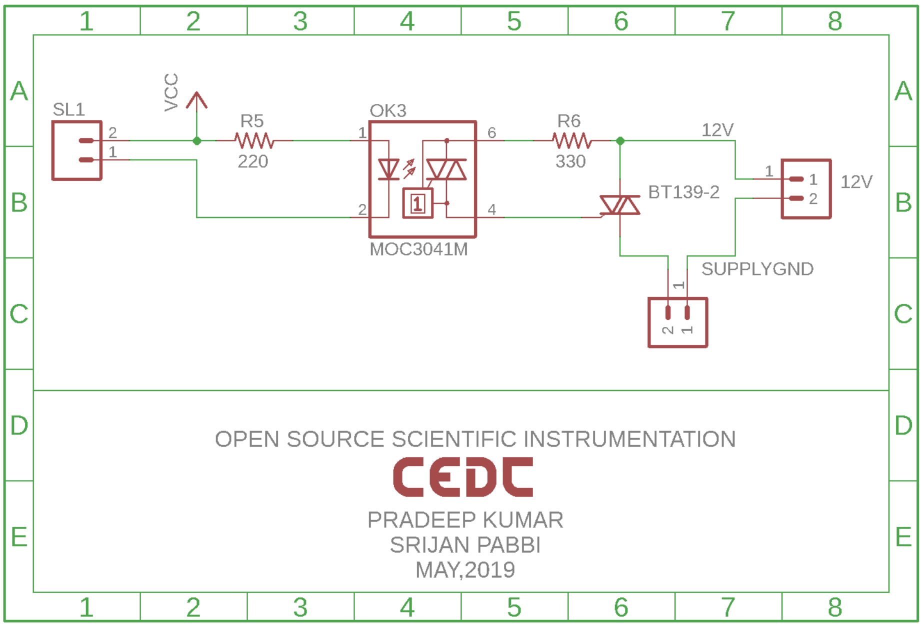


Figure 34: Triac Driving Circuit

3.4.2 Fan Control

Fan having to job to cool is just turned on and off using a simple relay circuit which is controlled by the microcontroller with the help of a transistor.

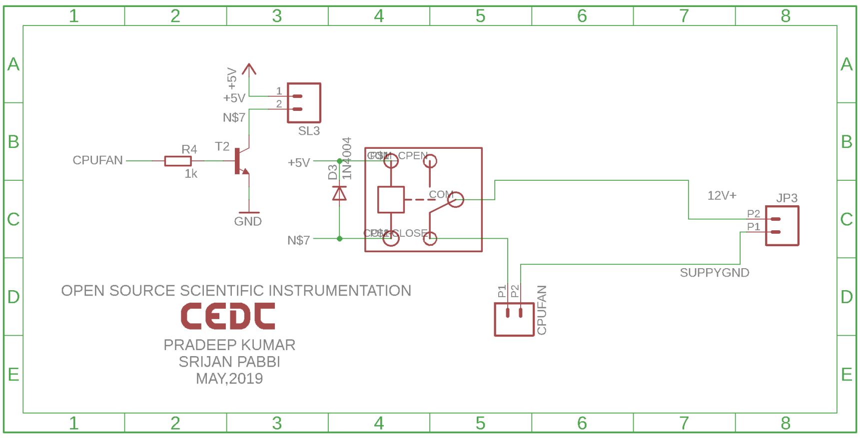


Figure 35: Relay Control for Fan

3.5 Basic Run: Heating and Cooling Rate

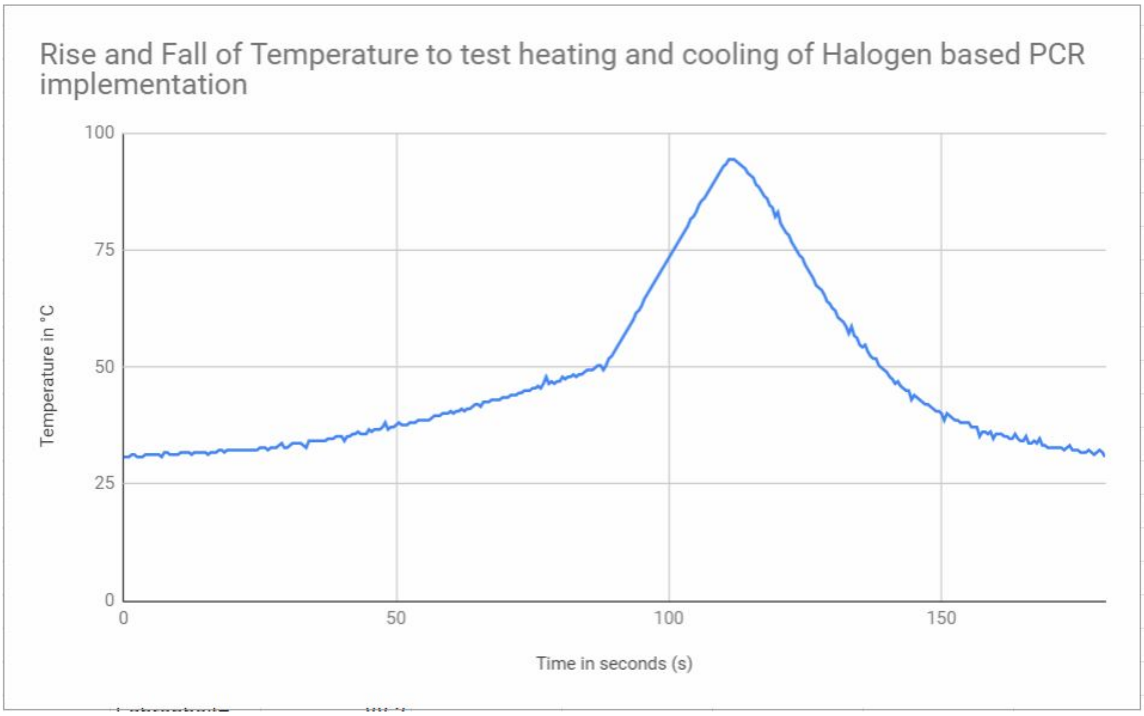


Figure 36: Rise and Fall cycle of the Halogen based PCR

Observations :

- Heating : 500W Halogen Lamp heater
- Cooling : 12V DC Fan
- Rate of Temp rise: 0.3 and then 1.96 °C/s
- Rate of Temp fall: 1.4 °C/s

3.6 Halogen PCR Mock Run; Sample Cycle

Parameters :

- Cycle Temperatures : 95, 40, 72 °C

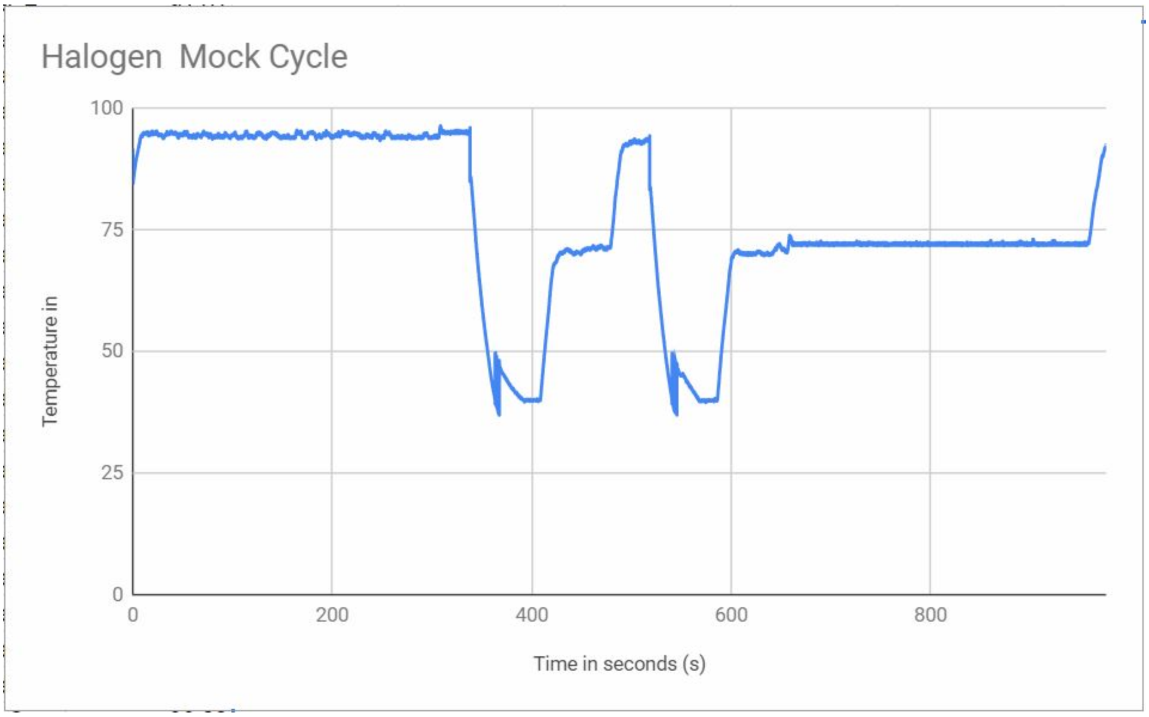


Figure 37: A Mock PCR cycle run for 3 level temperature variation

- Cycle Times :
  Initial 95°C for 300 seconds
  Ending 72°C for 300 seconds
  N Cycles 95°C for 30 seconds, N Cycles 40°C for 45 seconds N Cycles 72°C for 60 seconds

- Rate of Temp rise: 1.8 °C/s

- Rate of Temp fall: 1.7 °C/s

4 Chapter 4: Developing Wireless Biochemical Reaction Multimeter

4.1 Design Overview or Motivation

During various biotechnology procedures involving processing of different chemicals, reactions are involved. These reactions are pH, conductivity and temperature sensitive, so all parameters must be tracked throughout the duration of reaction. These parameters are then controlled by adding external agents and sometimes the data is what is required from the reaction. This collection of data can be automated and and also the whole instrument setup including pH meter and temperature meter can be made to interface on a mobile app and rest of the functionality remains same. This not only makes data gathering simpler but it also adds a automated component to it, one can always programs features that can control the reaction status hence removing the need for physical attention required.

4.2 Design Process

For Making a Wireless Biochemical Reactions Multimeter a few operations must be taken care of such as :-

- **Reading value from the pH electrode:** The values from the pH electrode come in the range of mV as shown in Figure 38 and hence need amplification. Here the simple concept of pH follows, which is the natural log of [H+] ion concentration in the solution and hence the name pH(power of H+). This amplification is done in conjunction with clamping the values above zero so that they can be read by an ADC of a microcontroller.

VOLTAGE (mV)	pH value	VOLTAGE (mV)	pH value
414.12	0.00	-414.12	14.00
354.96	1.00	-354.96	13.00
295.80	2.00	-295.80	12.00
236.64	3.00	-236.64	11.00
177.48	4.00	-177.48	10.00
118.32	5.00	-118.32	9.00
59.16	6.00	-59.16	8.00
0.00	7.00	0.00	7.00

Figure 38: pH to Voltage Table

- **Storing the value for future use:** The value obtained then needs to be stored somewhere to be accessed by the mobile app interface. For this utility an online database provided by google is use named ”firebase”. This service provides support for realtime database and hence the data can be uploaded in realtime and read by the app giving proper control over the data through the app.

- **Wireless Device:** The wireless medium used here is WiFi 2.4GHz because of is long range and widely available access, and for this a embedded device named esp8266 is used. ESP8266 connects to wifi of the choice and then using the firebase api and arduino json library the data can be pushed to the database.

- **Reading Temperature:** This is the easiest task and takes little to no resources. The temperature sensor used is directly connected to the ADC input pin.

4.3 Database Usage

The database used here is ”FireBase” provided by google as a service for data analytics. This database can be used to capture the reaction data and then compute on it as required, which can be plotting pH trends throughout the reaction, calibration of the pH probe etc... To use this database on has to follow some steps which are as follows:-

1. **Create a google account:** To use this database one must have access to a google account which is free to create and use, just visit gmail and create a account if you don't have one.

2. **Create a new firebase project:** The next and the logical step in this process is to create the a firebase project.

   - Go to https://firebase.google.com/ and log in with your google account.
   - Go to "Get Started" or click on "Go to console"
   - After that click on "Add Project" and enter a name.
   - Click the acceptance of TnC and proceed to the project page.

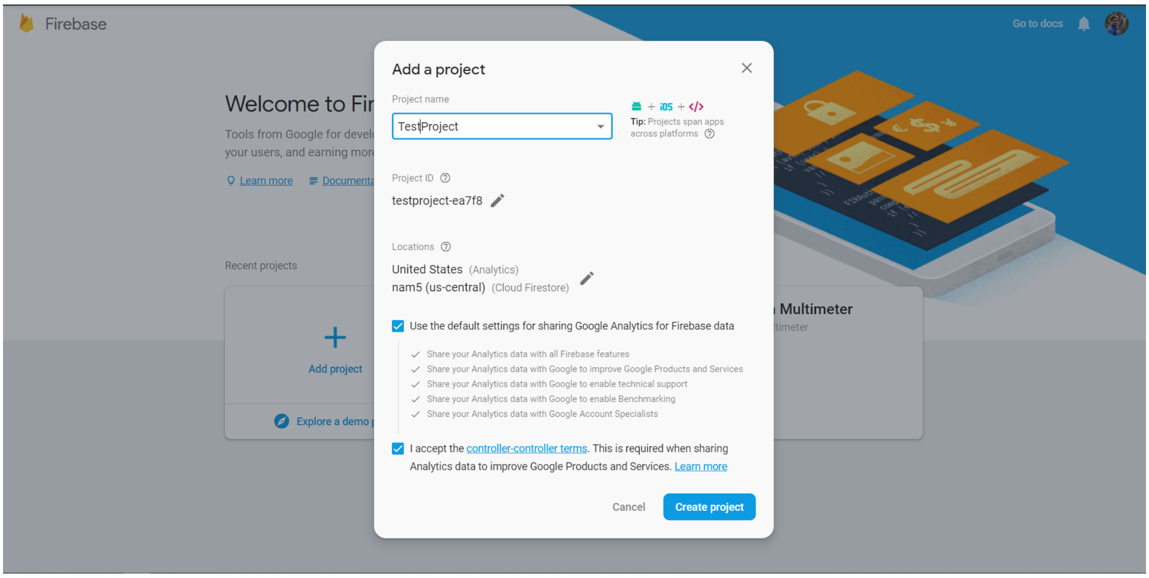


Figure 39: New Project Dialog

3. **Create a database:** For this specific project we need a real time database and the steps are as follows:-

   - Now in the project page go to the "Database" tab on the left under "Develop" menu.

- Scroll down a little and select the option to add a new real time database.

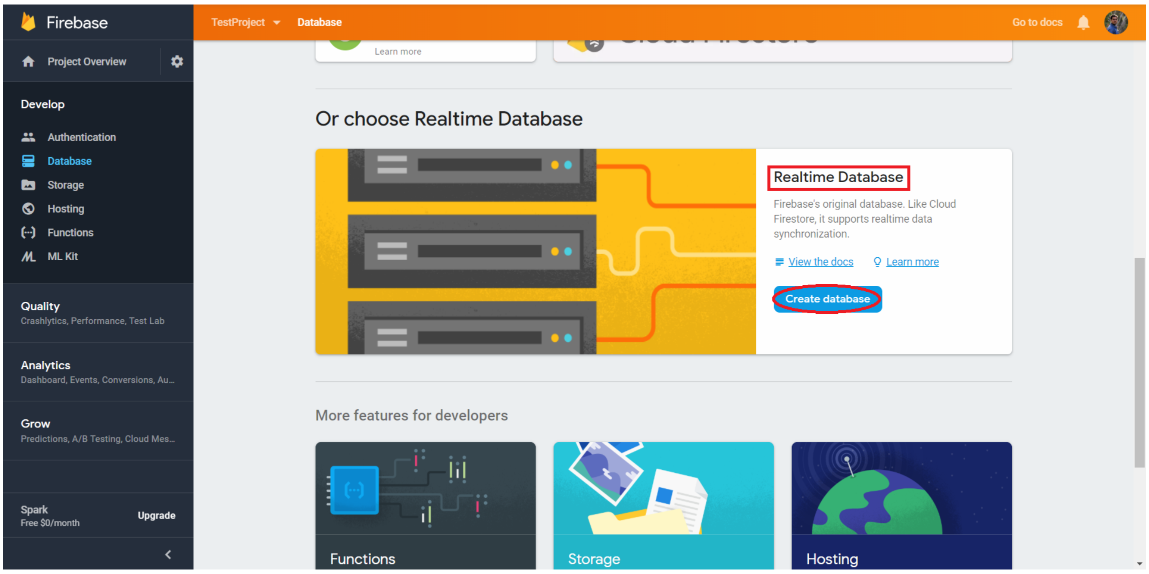


Figure 40: Select the real time DB option

- Now accept the rules with the "test mode" indicated in the following way:-

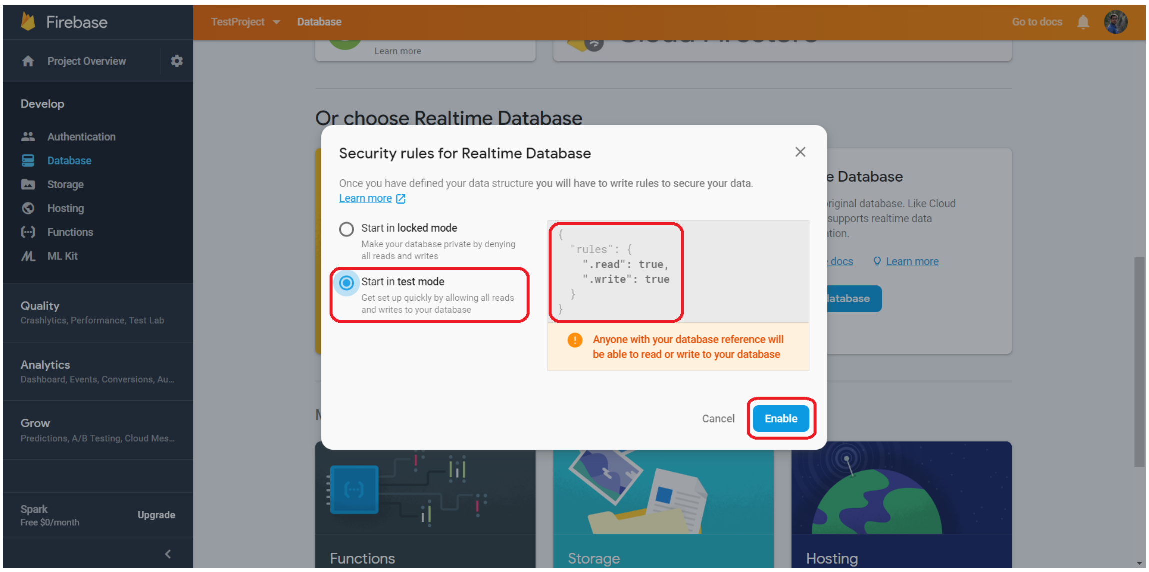


Figure 41: Dabase access mode

- Setting the rules in this way still doesn't give wide open access so its the method we are going to use in this prototype development.
- The created database will have a unique link for access as shown.

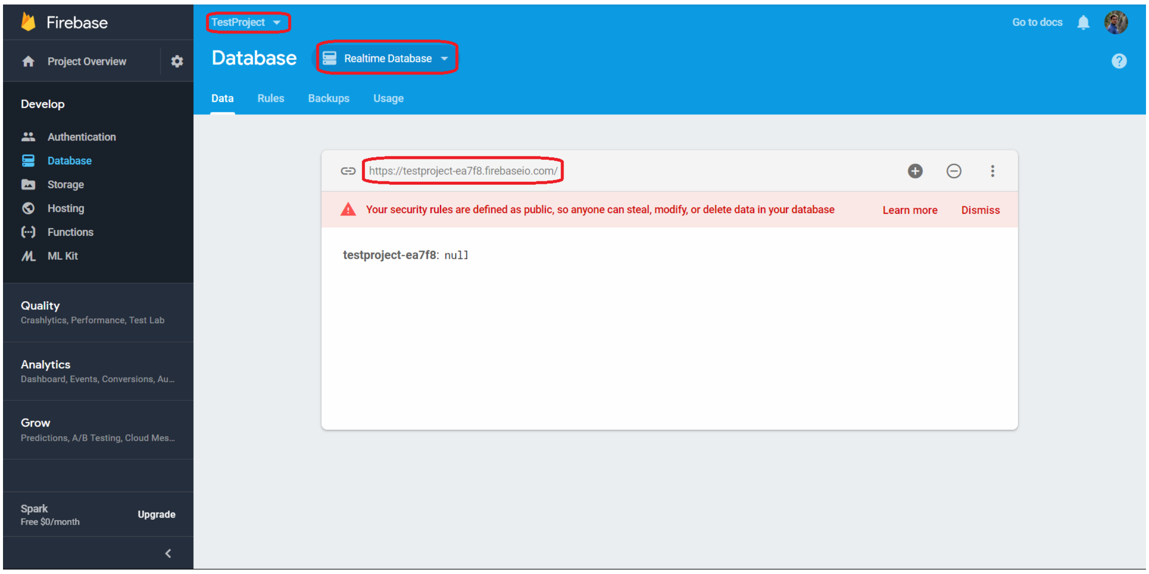


Figure 42: Database Created And Shown

4. **Giving access to your database:** Every app or device that is going to interact with your database is going to need an access key, which is available in a particular section of the "Project settings".

   - Go the the "Cog" i.e. "Settings" icon beside the Menu label "Develop".
   - Click on the option "Project Settings".

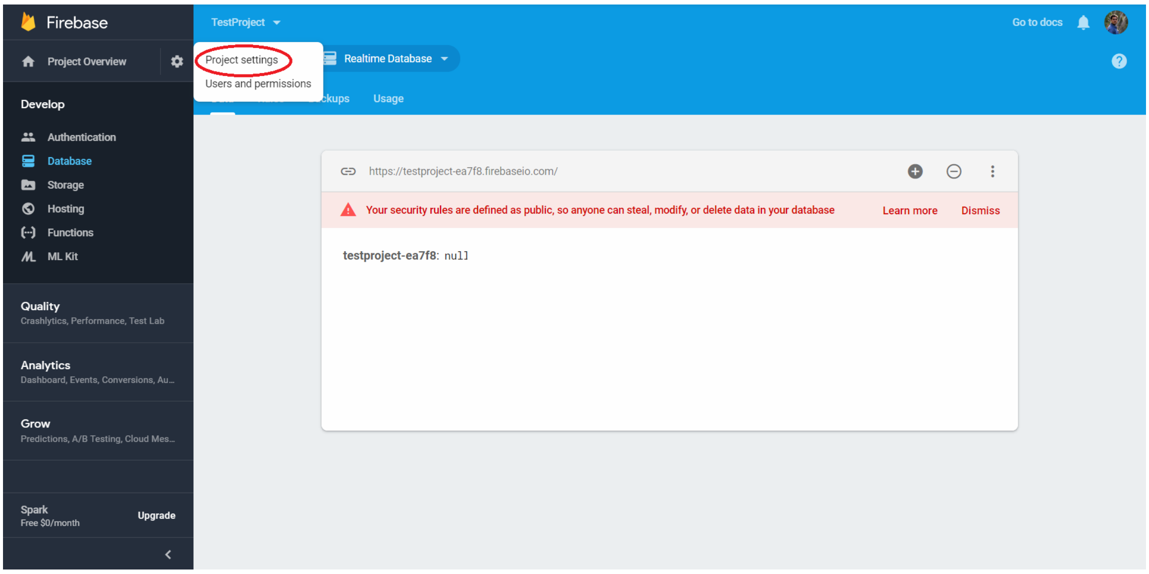


Figure 43: Go to Project Settings

- Go to the tab "Service Accounts" and here you will find an option named "Database Secret".
- Go to this option and reveal your authentication key, this key will be required whenever you will want to give access of your database to any software implementation without account authentication feature.

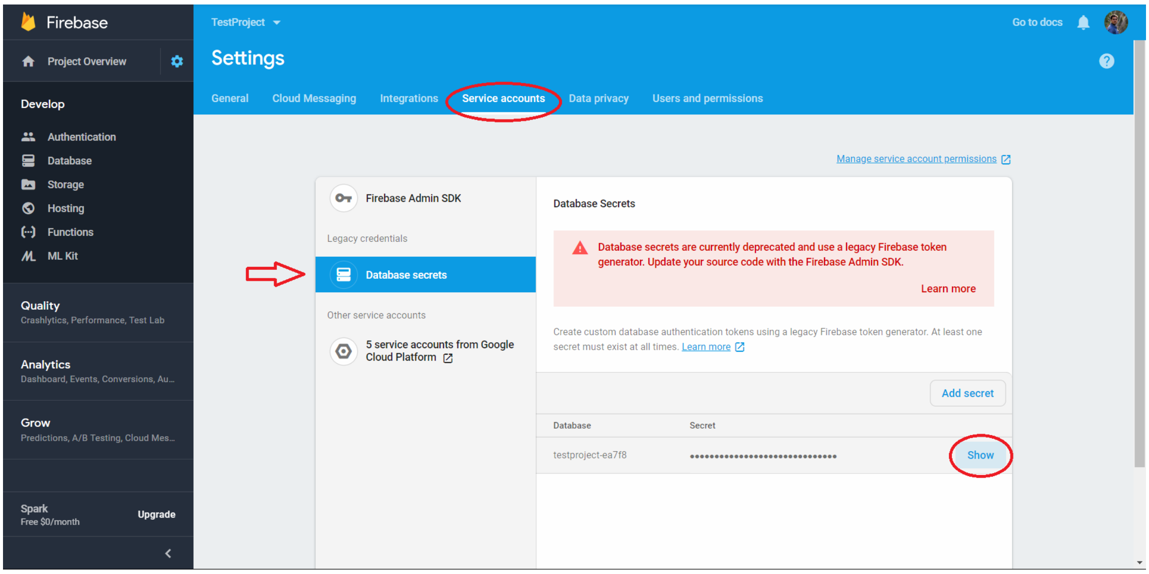


Figure 44: Database Secret

- Copy this key from here whenever required, and newer keys can be generated so that you can manage database access via different groups of keys.

4.4 App Design

To design an Android app an SDK is required. One can use google's android studio to code the app in java and create a package. Another approach than can be taken involves using MIT's app inventor tool. This tool makes it significantly easy to create an app since it is a drag and drop type software.

1. First we need to plan what features the Android app must have, for this we can broadly list a few functional features such as

   - pH probe calibration mechanism.
   - pH meter reading display
   - Temperature meter reading display

- Reset readings
- Record readings to a table

2. Next we design the UI layout of the App including all necessary features on the main screen, the app created is a single page app that can provide all the necessary functionality.

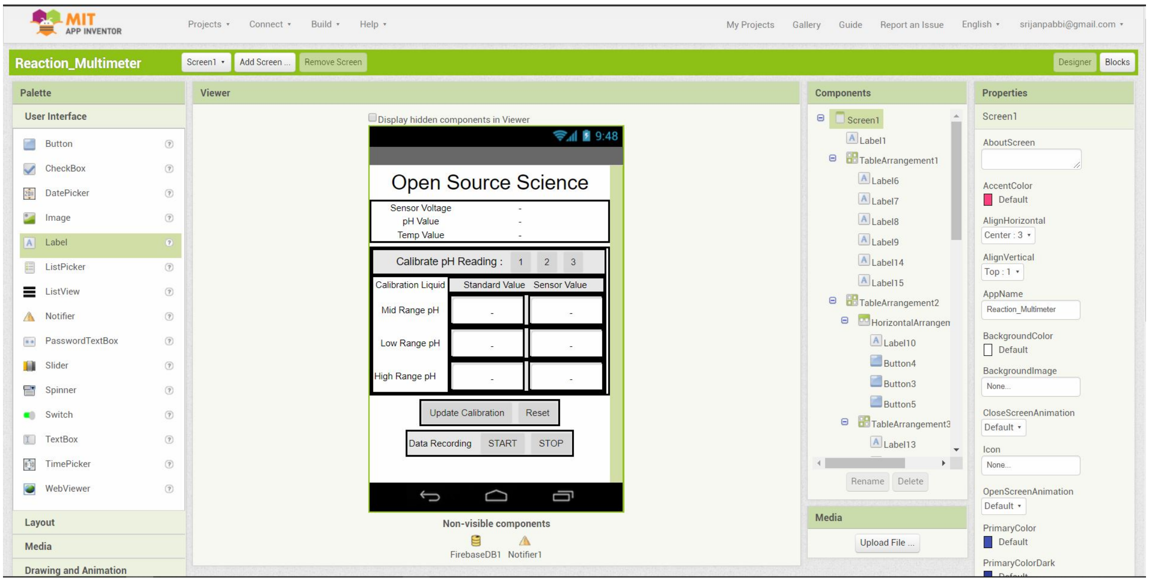


Figure 45: App Layout

3. Now we need to code the different components which are added in the layout and give them the functionality they are ment to have. Following are some active components in the app :-

   - Database: To setup database connection with firebase following properties need to be set. The Firebase Token is the "Database Secret", Firebase URL is the database unique URL, and finally the Project bucket is the prefix for the database tree.

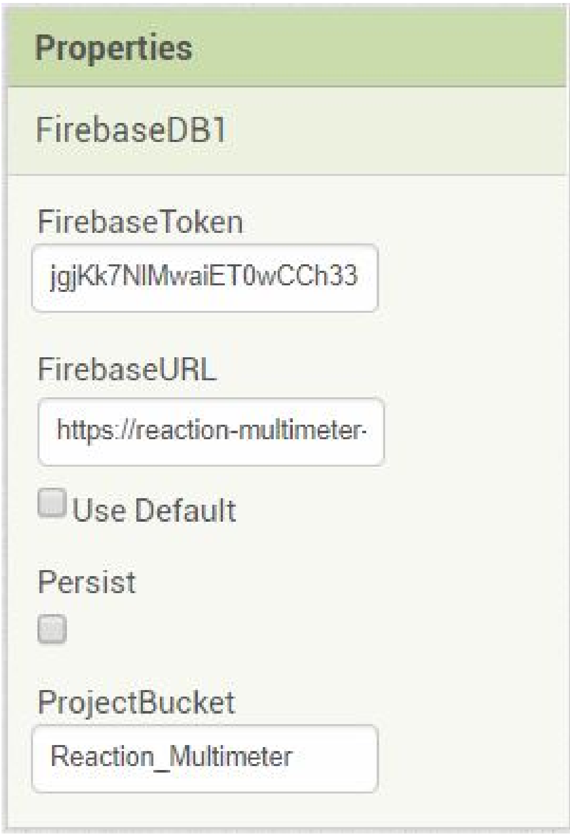


Figure 46: Properties to be set for firebase in MIT AppInventor

For coding the firebase data-flow, three functions are used as follows:-

– Data Changed: Perform retrieval action

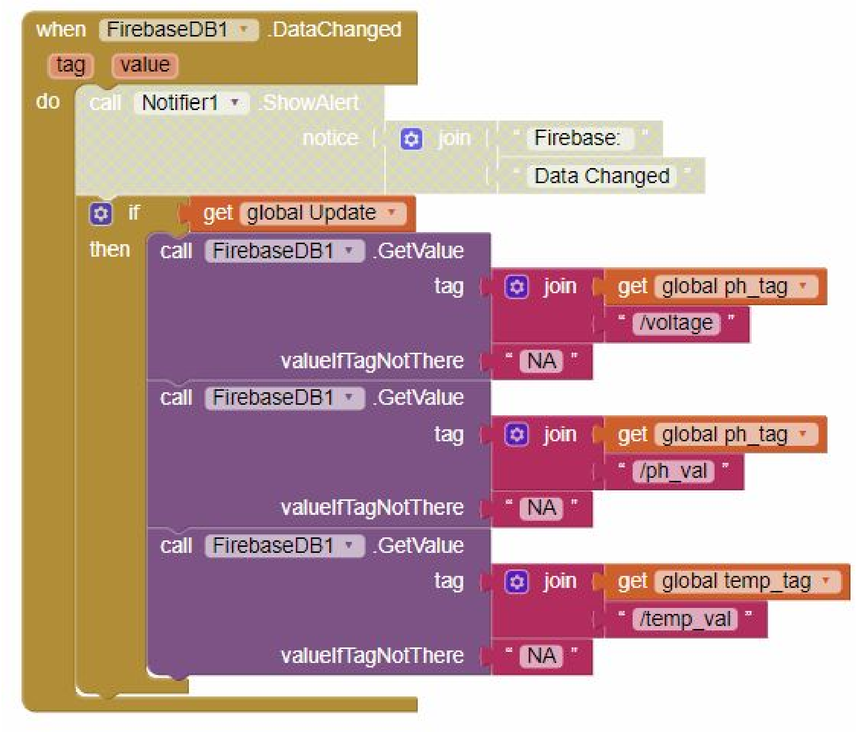


Figure 47: DataChanged Function

– Firebase GotValue: Store and sidplay values in the App

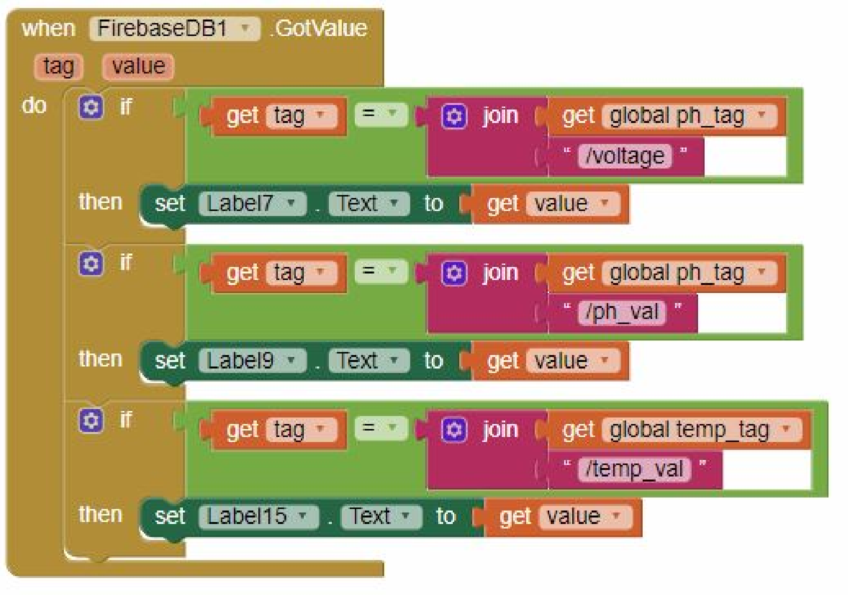


Figure 48: GotValue Function

– Firebase Error: For handling errors such as connection lost, retrieval failed etc...

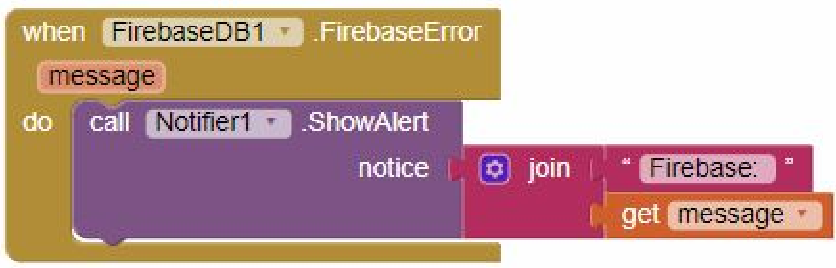


Figure 49: Error Handling

- For reading and updating values from Text box, labels, etc we can use the following code and then update those values on the cloud database as well.

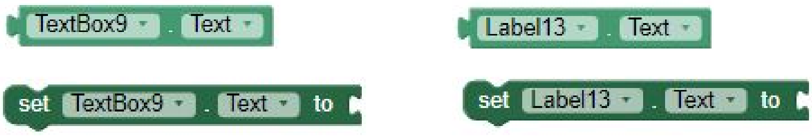


Figure 50: Snippets for editing and reading Textbox and Labels

- For using buttons there are easy action codes available which can track the type of press, eg- long press, click, release etc... The code snippets are shown in the figure below.

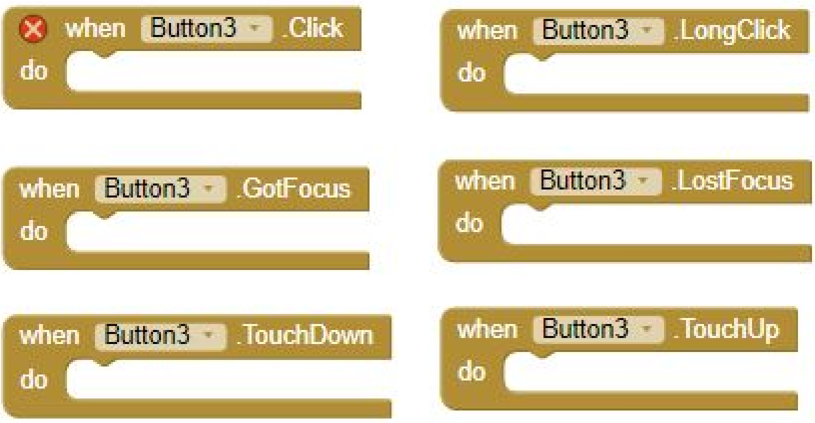


Figure 51: Button Action Functions

4. Now that we have coded the app we can use it. This app provides 3 main features.

   - Real time display of values crucial to reaction tracking as shown in the figure below

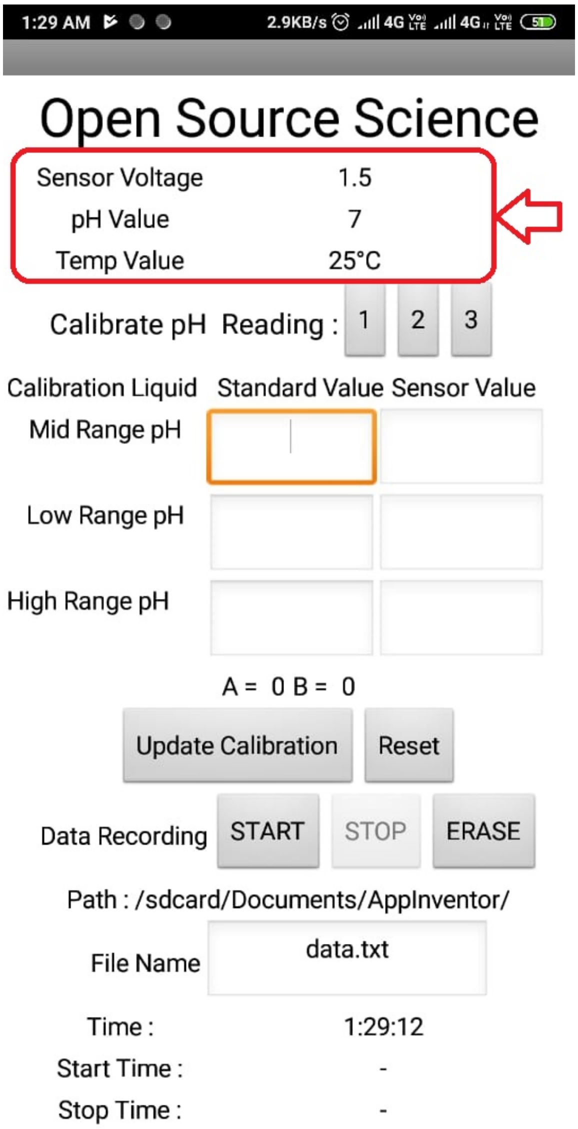


Figure 52: Real Time Value Display

- pH Probe value calibration. Pressing buttons 1,2,3 respectively for medium range, low range and high range pH buffer solutions after entering values into textbox and inserting probe into that solution. Pressing the "Update Calibration" Button will calculate new parameters and update their values in the database.

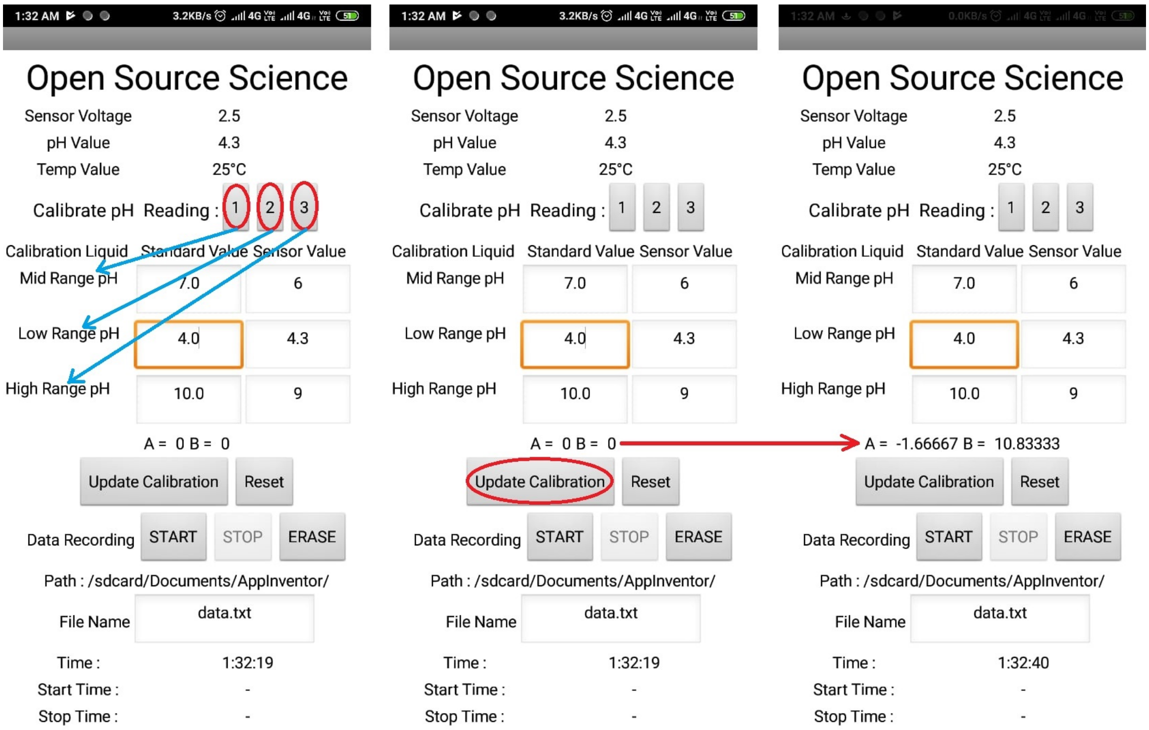


Figure 53: Calibration Steps

- The data received in realtime can be recorded on the device and then plotted as required. This is done in a custom datafile in the folder /sdcard/Documents/AppInventor/filename.txt. the filename and type can be changed at will by altering the name in textbox provided.

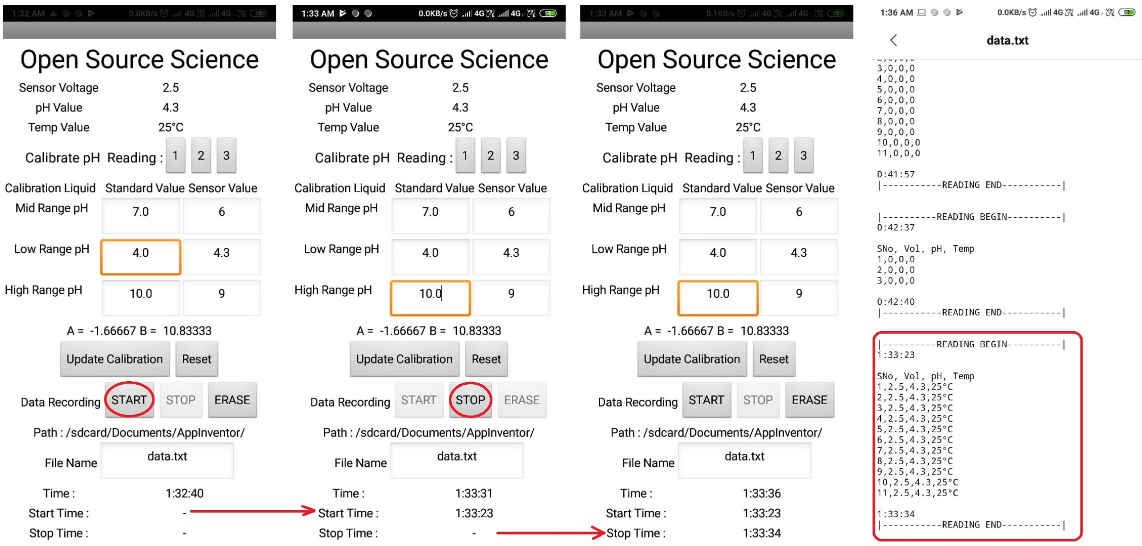


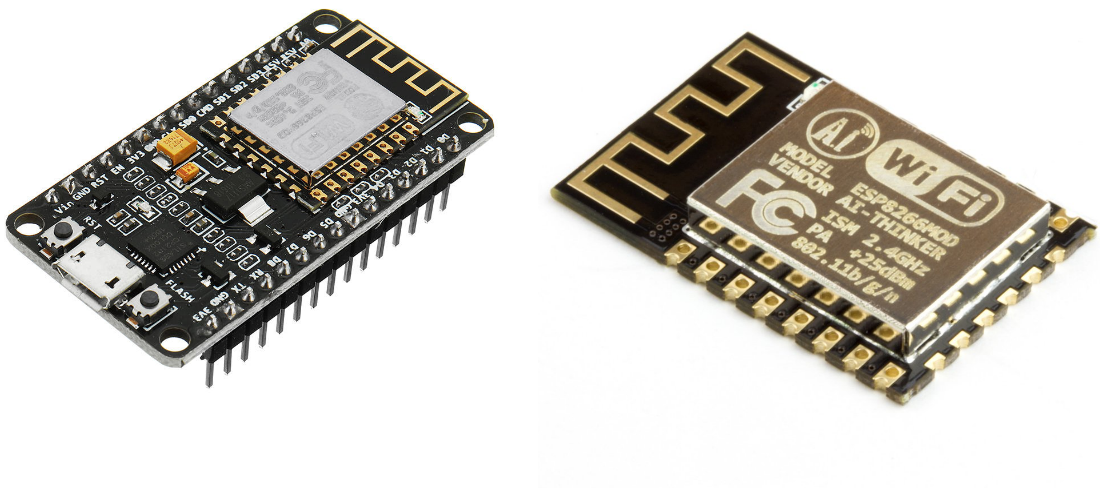
Figure 54: Data Recording for Manual Experimental Analysis

4.5 Node MCU and ESP

NodeMCU is an open source IoT platform. It includes firmware which runs on the ESP8266 Wi-Fi SoC from Espressif Systems, and hardware which is based on the ESP-12 module. The Open source community has developed arduino core for nodemcu which makes it very easy to code, and scale to large projects. Figure 55 The main concern here is to code this device to upload and download data along with reading from the sensor connected. The main steps to be followed in programming are as follows :-

1. **Setup NodeMCU:** To setup node mcu some the arduino IDE must be configured to work with the device.

   - Download Arduino IDE.
   - Open you IDE and click on "File - Preferences".
   - In "Aditional Boards Manager URLs" add line "http://arduino.esp8266.com/ and click on "OK"
   - Go to "Tools - Board - Boards Manager", type "ESP8266" and install it.



(a) Node MCU (b) ESP8266

Figure 55: (a) is the full standalone board which is plugged in directly whereas (b) is the entity responsible for processing and wifi connection

- Go again to "Tools - Board" and select "Generic ESP8266 Module".

2. **Initialize WiFi:** To get connected to the internet we must connect the Node MCU to WiFi of our choice. For this folow the following code snippet and use it in the setup sequence of the code.

```
#include <ESP8266WiFi.h>
#define WIFI_SSID "Wifi_Name"
#define WIFI_PASSWORD "Password@123"
void setup(){
    //computer serial
    Serial.begin(9600);


    // connect to wifi.
    WiFi.begin(WIFI_SSID, WIFI_PASSWORD);
    Serial.print("connecting");
    while (WiFi.status() != WL_CONNECTED) {
        Serial.print(".");
        delay(500);
    }
```

```
    Serial.println();
    Serial.print("connected: ");
    Serial.println(WiFi.localIP());
}

```

3. **Connect Succesfully to Database:** To have a successful database connection first a stable internet is required. To communicate with the database library "FirebaseArduino" is used. to setup this library follow the following steps

   - Download the .ZIP of the library from github: https://github.com/FirebaseExtended/firebase-arduino.
   - In Arduino IDE go to Sketch - Include Library - Add .ZIP Library and then select the downloaded library .ZIP.
   - Now after installing the library go to the installed location (The location may be different for your PC but this is the default path) C:/Users/Uname/Documents/Arduino/libraries/firebase-arduino-master/. In this directory enter the src folder and look for the file "FirebaseHttpClient.h".
   - Open this file in the text editor of your choice and make sure you don't change anything rightaway.
   - Go to this URL for website fingerprint tracking : grc.com/fingerprints.htm and enter "test.firebaseio.com" in the search bar and look for the website fingerprint as shown in Figure 56 (Note: The fingerprint in this image may become obsolete when by the time this document is read).
   - Now scroll down in the file previously opened in the text editor to the section where the constant character array of this fingerprint is defined and change the values in the fingerprint to the latest values if necessary as shown the previous obsolete values are commented out.

```
static const char kFirebaseFingerprint[] =
//"E2 34 53 7A 1E D9 7D B8 C5 02 36 0D B2 77 9E 5E 0F 32 71
    17"; // previous value
```

Figure 56: Original Website Unique Fingerprint Tracker

```
"B6 F5 80 C8 B1 DA 61 C1 07 9D 80 42 D8 A9 1F AF 9F C8 96 7D
  "; //updated value

```

Now that the library is setup the following few lines of code when used in setup initialize the connection.

```
#include <FirebaseArduino.h>
#define FIREBASE_HOST "reaction-multimeter-xxxx.firebaseio.com" //
    Link to the database
#define FIREBASE_AUTH "Databse Secret" //Paste the database secret
    as seen earlier

void setup(){
    Serial.begin(9600);
    //Begin Firebase Connection
    Firebase.begin(FIREBASE_HOST, FIREBASE_AUTH);
    if (Firebase.failed()) {
        Serial.print("Firebase Connection Failed");
        Serial.println(Firebase.error());
        return;
   }
}

```

Following are few functions that can be used to upload,set and download the database values.

```
    // set value setString, setInt, setFloat, etc...
    // get value getString, getInt, getFoat, etc...
    Firebase.setString("Reaction_Multimeter/pH_meter/voltage", String(pHVol));
    // handle error
    if (Firebase.failed()) {
        Serial.print("setting Reaction_Multimeter/pH_meter/voltage failed:");
        Serial.println(Firebase.error());
        return;
    }

```

4. Now that everything is setup one can easily use nodemcu to sync data from the internet. Although for every wifi change the code must be altered so once can take input from user or figure something out to remove this code change, for now we are at the first prototype stage so we wont bother with this quirk.

4.6 Working of the pH Sensor

Voltage, value finding, calibration Any solution is like a small battery. It can generate voltage depending on Hydrogen ion (H+) concentration. Acidic solution has more Hydrogen ion concentration than alkaline solution.
pH probes measure pH by measuring the voltage or potential difference of the solution in which it is dipped. By measuring potential difference, hydrogen ion concentration can be calculated using the *Nernst equation* which gives the relationship between Hydrogen ion concentration and Voltage or Potential.

$$E_{cell} = E^{\circ} - \frac{RT}{nF} ln(K_{eq})$$

Hence, a pH probe measures the potential difference generated in the solution by measuring the difference in hydrogen ion concentration using the *Nernst equation* and displays the pH as output.

1. **Electrodes:** To make electricity flow through the test solution, two electrodes (electrical terminals) have to be put into it. (I

case of the pH probe both of the two electrodes are built inside it for simplicity and convenience). The two electrodes used in a pH meter are :-

- First, A glass electrode which has a silver alloy electrical wire submerged in a neutral solution of potassium chloride all contained inside a thin bulb (or membrane) made from a special glass containing salts of metals, such as sodium and calcium.
- Second, the electrode named the reference electrode which also has a silver alloy electrical wire suspended in a solution of potassium chloride contained inside the same special glass bulb.

2. **Ion Exchange Process:** Suppose you dip the probe in a solution, for example, a solution of hydrochloric acid. HCl solution contains small hydrogen ions H+ and big chloride ions Cl- as shown in Figure 57

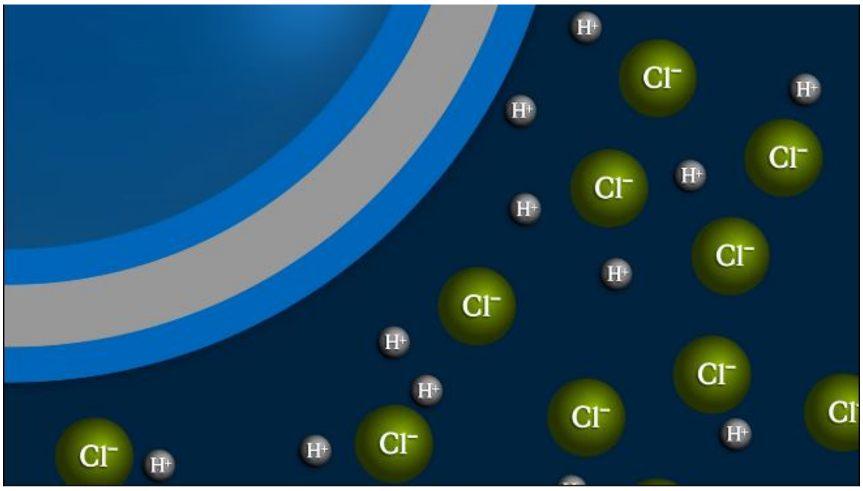


Figure 57: Large Cl- ions and small H+ ions

When the probe is immersed in the solution, the smaller hydrogen ions are able to penetrate the boundary area of the glass membrane and the larger chloride ions remain in the solution itself. Thus, the results is a separation of charge as shown in Figure 58.

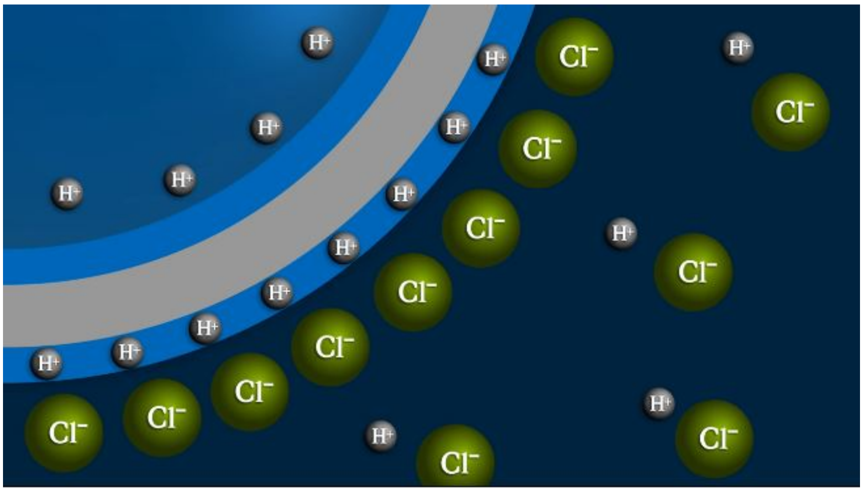


Figure 58: Small H+ ions can pass through the membrane the other side also has some concentration of H+ ions

The same process happens inside the probe which contains a neutral potassium chloride solution, that is, it has pH = 7 and has a constant concentration of hydrogen ions.

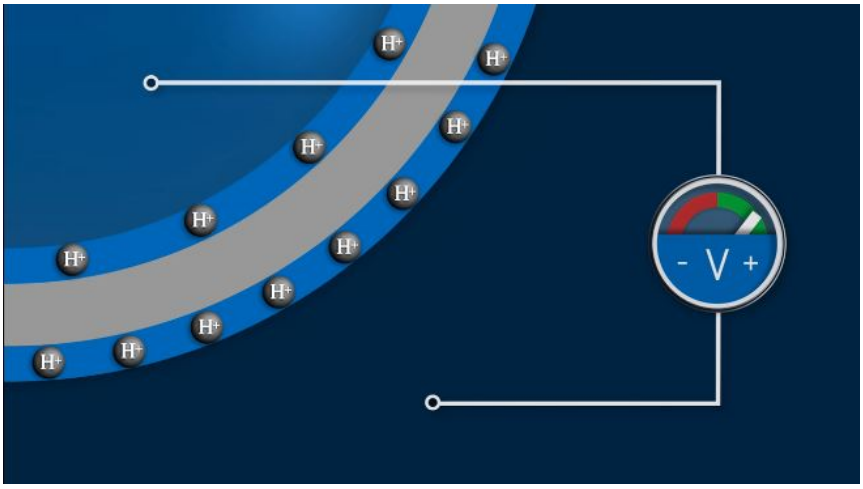


Figure 59: The difference in ion concentration causes a voltage difference

Now, if the hydrogen ion concentration, or pH, in the outer solution differs from that of the inside solution, then a measurable potential difference is detected and pH is simultaneously calculated as shown in Figure 59.

In our case, since HCl is acidic; it will contain more hydrogen ion concentration than the inside solution. Hence, the potential difference measured will give a pH¡7.

In case of a neutral solution, the inside and outside concentrations of hydrogen ions will be the same and no potential difference is formed and pH = 7 will be measured. In the case of an alkaline solution, the hydrogen ion concentration outside will be less than the solution inside. Hence, the potential difference measured will give a pH¿7

3. **Calibration:** For pH meters to be accurate, they have to be properly calibrated. So they usually need testing and adjusting before you start to use them. You calibrate a pH meter by dipping it into buffers (test solutions of known pH) and adjust the meter accordingly. Buffer solutions having a pH of 1, 2, 4, 7, 10, and 12 are all commonly used and commercially available.

4.7 Working of Temperature meter

In a biochemical reaction, temperature is also a crucial component and hence we have provided the functionality to track temperature along with pH.

The temperature sensor used in this project is a thermistor, a temperature dependent resistor as shown in Figure 60.

Figure 60: Temperature Sensor: Thermistor

This sensor is one of the easiest sensors to interface and since it is so straight forward, we can just connect it as a part of a resistor divider and read values into the ADC of a microcontroller as shown in the following Figure 61. The code for processing this temperature information through the ADC value is straight forward passing the value through the Steinhart-Hart equation , which lets us do a good approximation of converting values. Its not as exact as the thermistor table (it is an approximation) but its pretty good around the temperatures that this thermistor is used.
Steinhart-Hart equation: $\frac{1}{T} = A + Bln(R) + C(ln(R))^3$
Simplified equation : $\frac{1}{T} = \frac{1}{T_o} + \frac{1}{B}ln(\frac{R}{R_o})$ For simplified equation one we only need to know To (which is room temperature, 25°C = 298.15 K), B (in this case 3950, the coefficient of the thermistor), and Ro (the

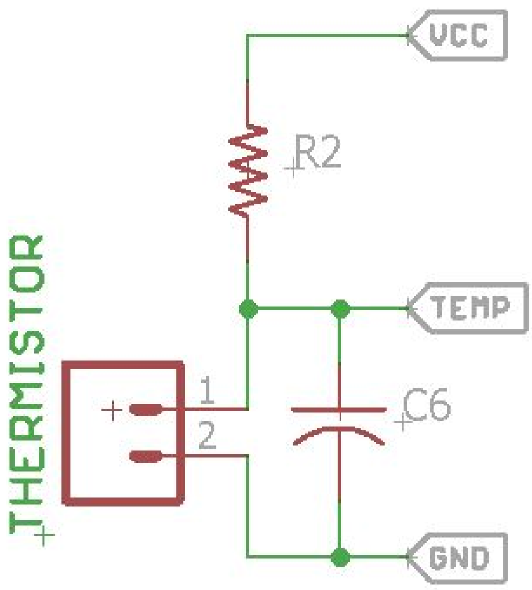


Figure 61: The input circuit for taking thermistor value into ADC

resistance at room temp, in this case 10Kohm). We plug in R (resistance measured) and get out T (temperature in Kelvin) which is easy to convert to °C.

```
// which analog pin to connect
#define THERMISTORPIN A0
// resistance at 25 degrees C
#define THERMISTORNOMINAL 10000
// temp. for nominal resistance (almost always 25 C)
#define TEMPERATURENOMINAL 25
// how many samples to take and average, more takes longer
// but is more 'smooth'
#define NUMSAMPLES 5
// The beta coefficient of the thermistor (usually 3000-4000)
#define BCOEFFICIENT 3950
// the value of the 'other' resistor
#define SERIESRESISTOR 10000

int samples[NUMSAMPLES];

void setup(void) {
  Serial.begin(9600);
}
```

```
void loop(void) {
  uint8_t i;
  float average;

  // take N samples in a row, with a slight delay
  for (i=0; i< NUMSAMPLES; i++) {
   samples[i] = analogRead(THERMISTORPIN);
   delay(10);
  }

  // average all the samples out
  average = 0;
  for (i=0; i< NUMSAMPLES; i++) {
     average += samples[i];
  }
  average /= NUMSAMPLES;

  Serial.print("Average analog reading ");
  Serial.println(average);

  // convert the value to resistance
  average = 1023 / average - 1;
  average = SERIESRESISTOR / average;
  Serial.print("Thermistor resistance ");
  Serial.println(average);

  float steinhart;
  steinhart = average / THERMISTORNOMINAL;     // (R/Ro)
  steinhart = log(steinhart);                  // ln(R/Ro)
  steinhart /= BCOEFFICIENT;                   // 1/B * ln(R/Ro)
  steinhart += 1.0 / (TEMPERATURENOMINAL + 273.15); // + (1/To)
  steinhart = 1.0 / steinhart;                 // Invert
  steinhart -= 273.15;                         // convert to C

  Serial.print("Temperature ");
  Serial.print(steinhart);
  Serial.println(" *C");

  delay(1000);
}
```

4.8 Prototype Working, Observations and Results

To show the working of the prototype pH meter the following data on the forebase will show voltage values corresponding to the standard pH readings.

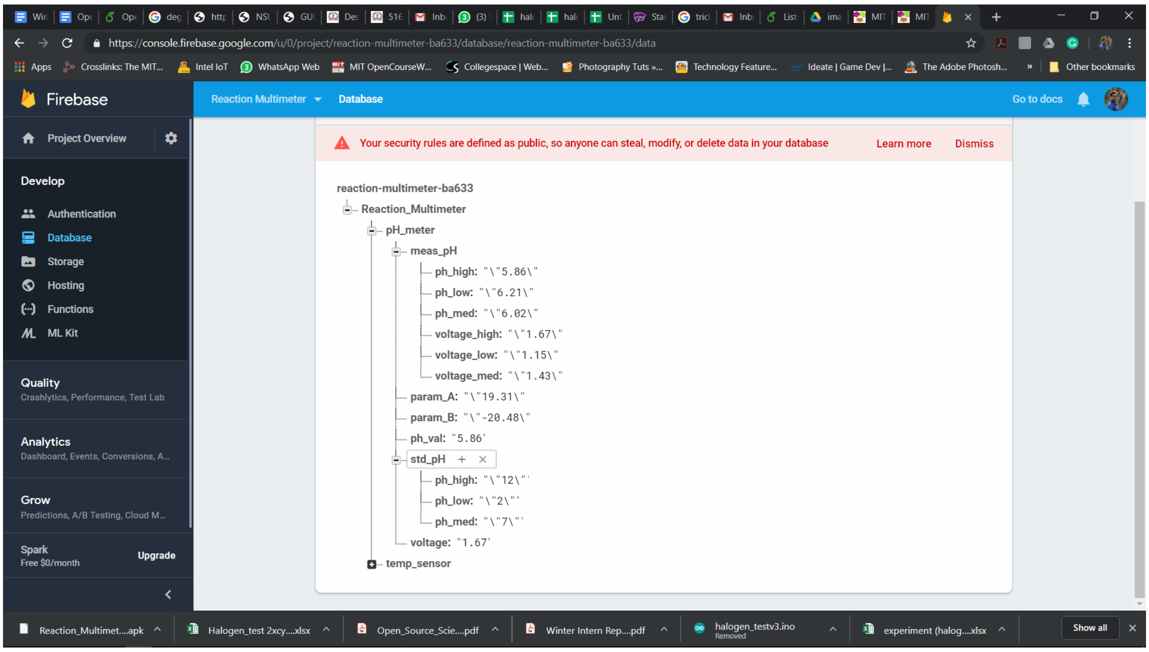


Figure 62: Parameter values after mapping the voltage values to standard pH values

5 Results and Conclusion

After the development of the various devices and performing analysis on them for their reliability, It was found that the results obtained were well within the error limits.
Doing this project gave us an insight to the domain of bio-medical sciences and we got to know how deep the link between electronics and biology is. We got to know the process of the Polymerase chain reaction which is very critical for the evolution of the human race beside other applications which have been discusse already. We were able to record post and retrieve the pH data of a solution remotely through wifi which provides a user to easily view the pH of the reaction and monitor the state of the biochemical reactions. Also many times it is critical to take note of the temperature of the reaction because the amount of energy released or taken up in a chemical reaction defines many parameters of the reaction (the type of reaction, the enthalpy of the reaction and the chemical kinetics. therefore we developed a temperature sensing device which can accurately measure the temperature of the reaction and transmit the temperature data over the internet to the mobile device (Android application). So the remote user can completely monitor the reaction that are time intensive yet require frequent monitoring.
The speed of the PCR machine the earlier Prototype which is robust is little slow as compared to the other model- Halogen PCR but the latter is fragile because of the delicate electroformed tube holder. It is easily observable that if we can combine both of the models then another PCR can be developed which has both speed and reliability.
The devices we developed can be used in educational institution to impart the knowledge of the PCR reactions. The importance of the temperature and pH monitors.
since the devices we have developed are very low in cost they can be easily afforded by institutions and hobbyist and also medical institutions.
usability as a product

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