Thermal Regulation In a PCR Chamber

[Final Project Milestone III]

Group 3:
Dimas & Dave

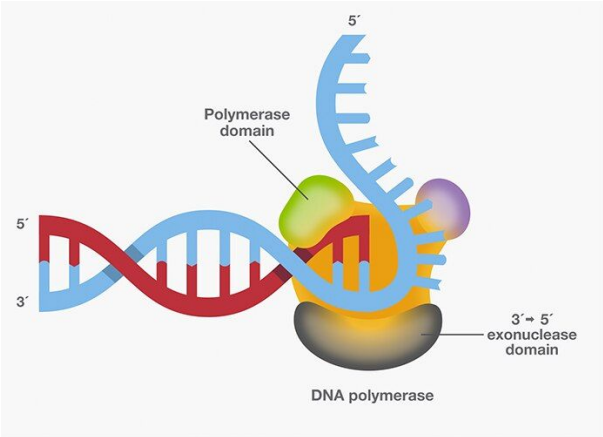
THEORETICAL OVERVIEW

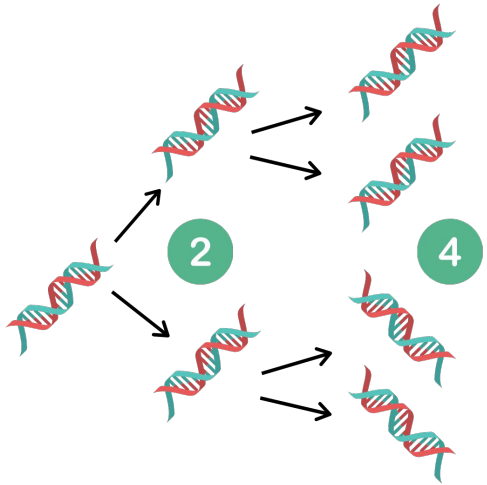
PCR Definition

PCR

Polymerase

Chain Reaction

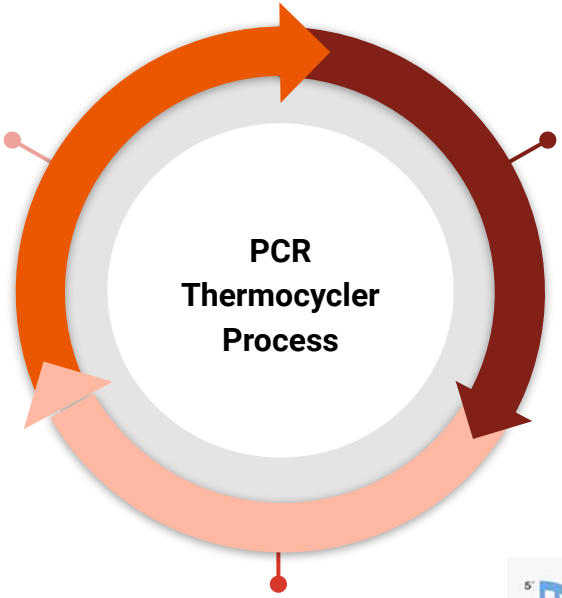




PCR Thermocycler Process

EXTENSION (72 Dg C)

Letting the polymerase extend the primers and synthesize new DNA



DENATURATION (95 Dg C)

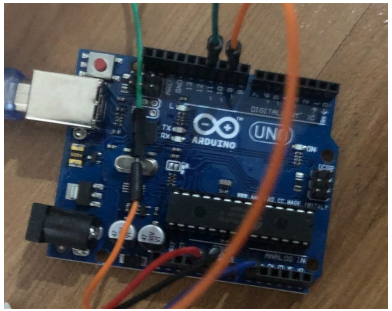
To separate the two strands of the target DNA

ANNEALING (55-65 Dg C)

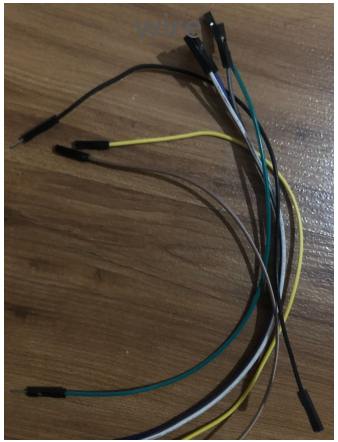
Letting primers to bind with the target sequence of the single stranded DNA

Material Used

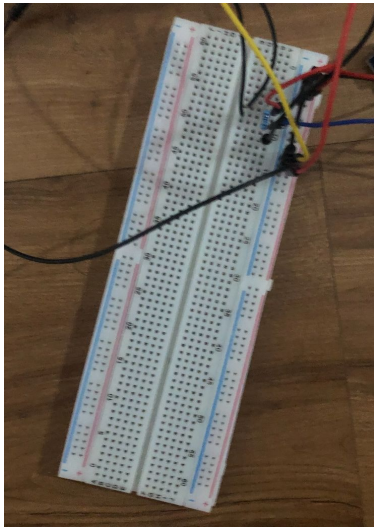
Arduino Uno
(Microcontroller)



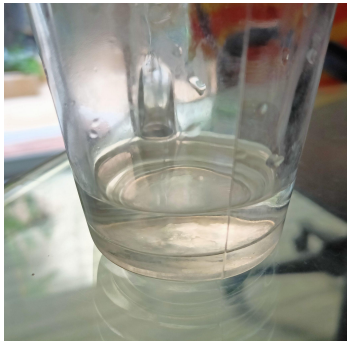
Jumper



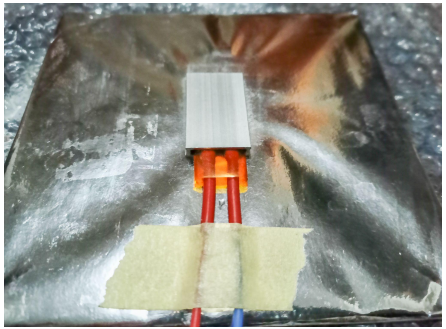
Breadboard MB-102 830P



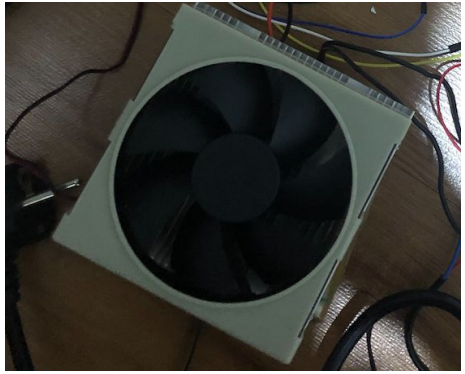
Water Sample



Heater Plate 220V AC 40W

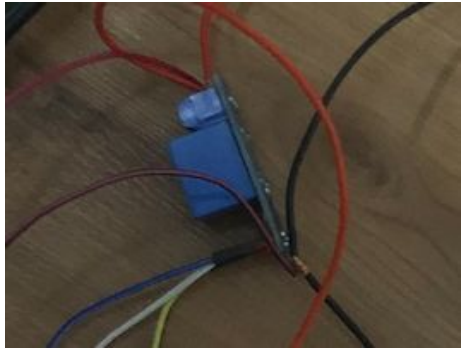


Fan for Peltier



Peltier TEC1-12706 12V 6A +

Relay 5V (STM32 PIC AVR ARM uC) [2 Pieces]



Thermistor **(NTC 3950) 10k Ohm +- 1% (-20 ~ 150 C)**

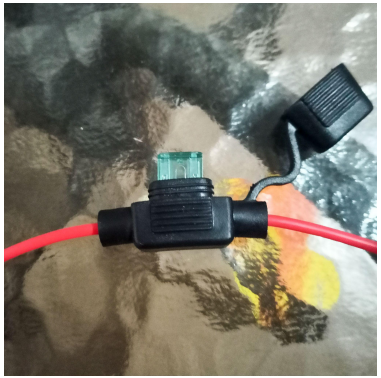
Cardboard

Adaptor 12V 10A DC

100k Ohm Resistor

AC Cable

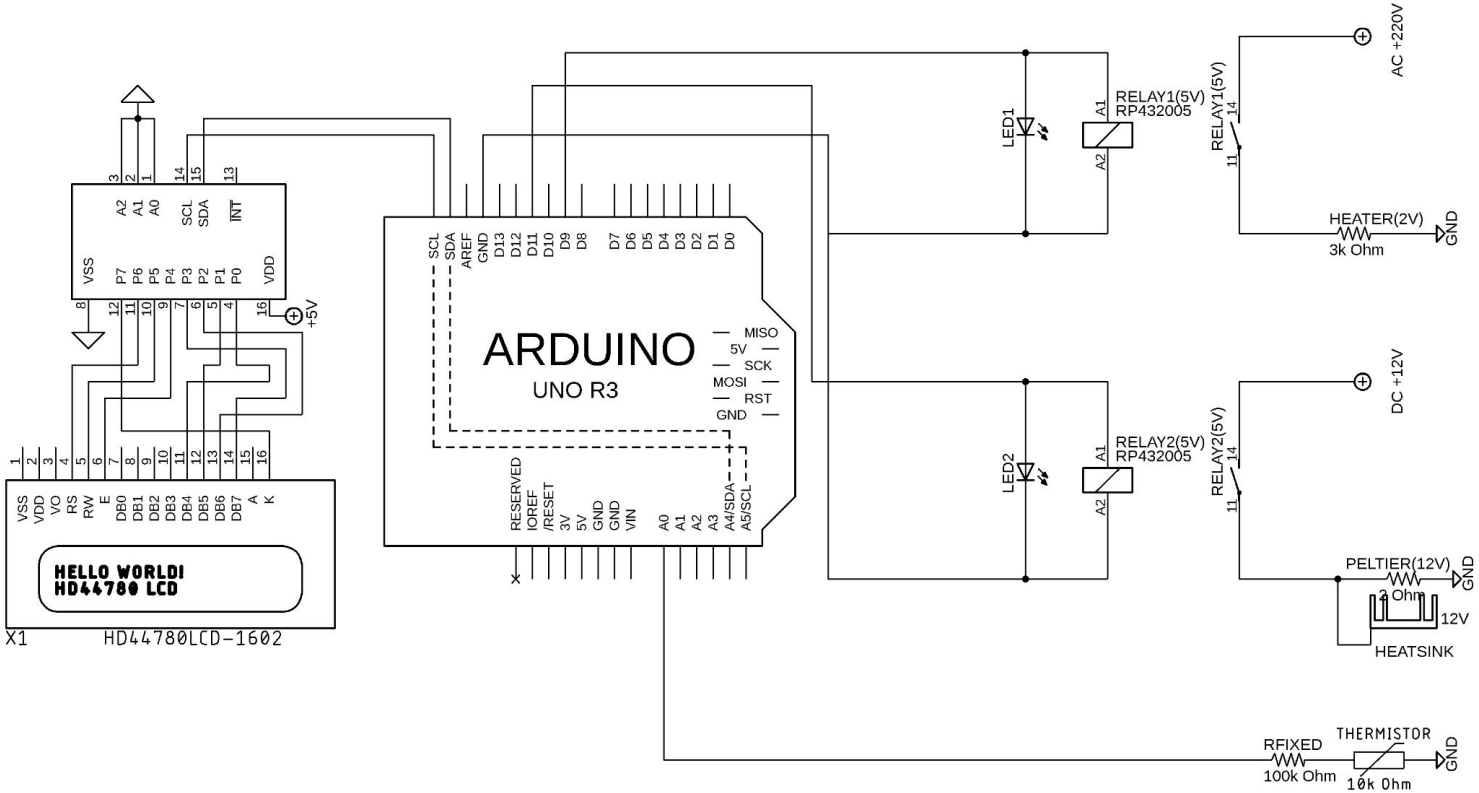
Fuse



Alumunium foil

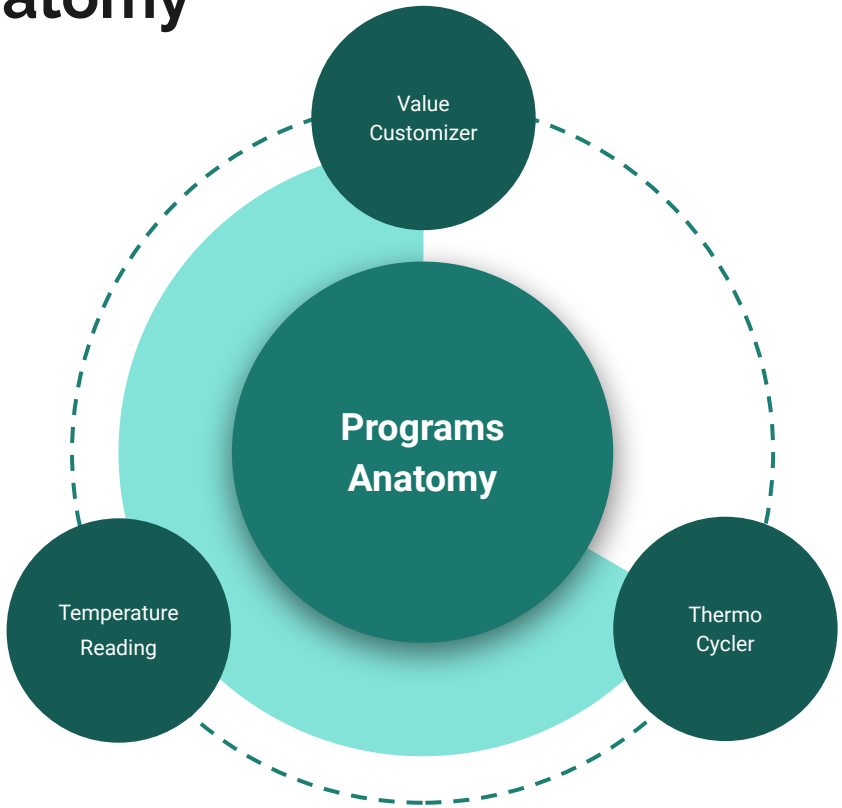
PROJECT LAYOUT

Circuit Schematic



Programming

Programs Anatomy



Value Customizer

```
//LCD Library
#include <Wire.h>
#include <LiquidCrystal_I2C.h>

//LCD Address
LiquidCrystal_I2C lcd(0x27,16,2);

//Temperature Reading
const int    SAMPLE_NUMBER      = 15;
const double BALANCE_RESISTOR   = 100800.0;
const double MAX_ADC            = 1023.0;
const double BETA               = 3997.0;
const double ROOM_TEMP          = 298.15;
const double RESISTOR_ROOM_TEMP = 10000.0;

//Save Current Temperature
double currentTemperature = 0;

//Inputs:
int thermistorPin = 0;

//Outputs:
int RelayHeaterPin = 9;
int RelayPeltierPin = 11;

//Number of Desired Cycle:
int cycle = 2;

//Thermocycle Process Time:
int Dentime = 5;
int Anntime = 5;
int Extentime = 5;
```

Temperature Reading

$$R = \frac{10k \times ADC}{1023 - ADC}$$

$$\frac{1}{T} = \frac{1}{T0} + \frac{1}{\beta}\ln\left(\frac{R}{R0}\right)$$

$$\beta = \frac{\ln\left(\frac{R0}{R}\right)}{\left(\frac{1}{T0} - \frac{1}{T}\right)}$$

$$°C = K - 273.15$$

$$\beta = \frac{\ln\left(\frac{10k}{0.7816k}\right)}{\left(\frac{1}{298.15} - \frac{1}{368.15}\right)}$$

$$\beta = 3997$$

$$\frac{1}{T} = \frac{1}{298.15} + \frac{1}{3997}\ln\left(\frac{0.7816k}{10k}\right)$$

$$T = \left(\frac{1}{T}\right)^{-1}$$

$$T = 368.14\ K$$

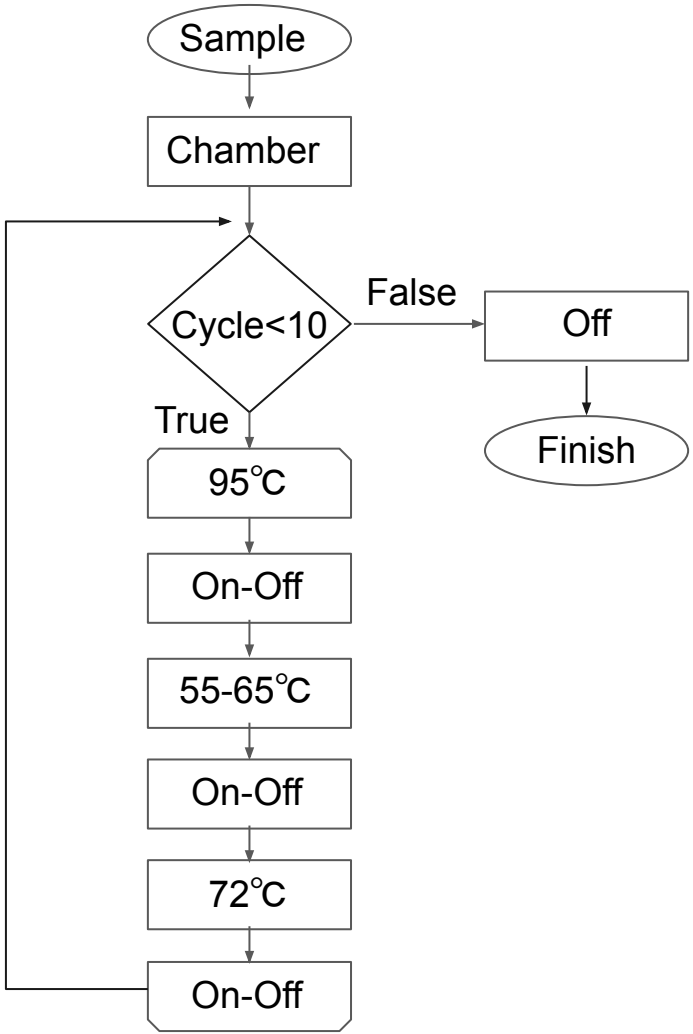
$$T = 368.14 - 273.15 = 94.99\ °C$$

Temperature Reading Code

```
//Read Thermistor
double readThermistor()
{
  // variables that live in this function
  double rThermistor = 0;            // Holds thermistor resistance value
  double tKelvin     = 0;            // Holds calculated temperature
  double tCelsius    = 0;            // Hold temperature in celsius
  double adcAverage  = 0;            // Holds the average voltage measurement
  int    adcSamples[SAMPLE_NUMBER];  // Array to hold each voltage measurement

  //Calculate thermistore average resistance
  for (int i = 0; i < SAMPLE_NUMBER; i++)
  {
    adcSamples[i] = analogRead(thermistorPin);  // read from pin and store
    delay(10);        // wait 10 milliseconds
  }
```

```
//average the sample
for (int i = 0; i < SAMPLE_NUMBER; i++)
{
  adcAverage += adcSamples[i];      // add all samples up
}
adcAverage /= SAMPLE_NUMBER;        // average it w/ divide

// Thermistor resistance
rThermistor = BALANCE_RESISTOR * ( (MAX_ADC / adcAverage) - 1);

//Temperature reading (conversion)[Kelvin]
tKelvin = (BETA * ROOM_TEMP) /
          (BETA + (ROOM_TEMP * log(rThermistor / RESISTOR_ROOM_TEMP)));

//Convert Temperature to Celsius
tCelsius = tKelvin - 273.15;  // convert kelvin to celsius

return tCelsius;    // Return the temperature in Celsius
```

Thermocycler Algorithm Flowchart



Thermocycler Code [Intro]

```
void loop()
{
    currentTemperature = readThermistor();

 //BIG THERMO CYCLER LOOP

  //Will automatically break after desired cycle achieved

  for (int cyclecount=0 ;cyclecount < cycle; cyclecount += 1){
    int DentimeCount = 0;
    int AnntimeCount = 0;
    int ExtentimeCount = 0;
    currentTemperature = readThermistor();
    Serial.print("We start at ");
    Serial.print(currentTemperature);
    Serial.println("C.");
```

Thermocycler Code [Denaturation]

```
//DENATURATION
  // OTW to Denaturation Temp
  Serial.println("Attaining Denaturation");
   while(currentTemperature < 95.5){
       digitalWrite(RelayHeaterPin, HIGH);
       currentTemperature = readThermistor();
       if (currentTemperature > 0){
         Serial.print("it's ");
         Serial.print(currentTemperature);
         Serial.println("C.");
         lcd.clear();
         lcd.setCursor(0,0);
         lcd.print("Denaturation [^]");
         lcd.setCursor(0,1);
         lcd.print("Cycle: ");
         lcd.println(cyclecount);
         delay(2000);
       }
     // On until temp > 95.5
     }
 currentTemperature = readThermistor();
 Serial.print("it's ");
 Serial.print(currentTemperature);
 Serial.println("C.");
```

```
 // Sustain Denaturation Temp
Serial.println("Sustaining Denaturation");
  currentTemperature = readThermistor();
  while (currentTemperature > 60 && DentimeCount < Dentime){
     currentTemperature = readThermistor();
     if (currentTemperature < 95){

          digitalWrite(RelayHeaterPin, HIGH);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Denaturation [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);

       }
     }
```

```
     else (currentTemperature >= 95);{

          digitalWrite(RelayHeaterPin, LOW);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Denaturation [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);
       }
     }
    DentimeCount += 1;
    delay(1000);
    //Break when reach Dentime
  }

Serial.println("Denaturation Complete");
```

Thermocycler Code [Annealing]

```
//Annealing
  // OTW to Denaturation Temp
  Serial.println("Attaining Annealing");
   while(currentTemperature > 66){
       digitalWrite(RelayPeltierPin, HIGH);
       currentTemperature = readThermistor();
       if (currentTemperature > 0){
         Serial.print("it's ");
         Serial.print(currentTemperature);
         Serial.println("C.");
         lcd.clear();
         lcd.setCursor(0,0);
         lcd.print("Annealing [^]");
         lcd.setCursor(0,1);
         lcd.print("Cycle: ");
         lcd.println(cyclecount);
         delay(2000);
       }
    // On until temp > 95.5
    }

 digitalWrite(RelayHeaterPin, LOW);
 digitalWrite(RelayPeltierPin, LOW);
 currentTemperature = readThermistor();
 Serial.print("it's ");
 Serial.print(currentTemperature);
 Serial.println("C.");
```

```
 // Sustain Annealing Temp
Serial.println("Sustaining Annealing");
  currentTemperature = readThermistor();
  while (currentTemperature < 150 && AnntimeCount < Anntime){
     currentTemperature = readThermistor();
     if (currentTemperature < 64){

          digitalWrite(RelayHeaterPin, HIGH);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Annealing [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);


       }
     }
```

```
     else (currentTemperature >= 64);{
          digitalWrite(RelayHeaterPin, LOW);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Annealing [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);
       }
     }
    AnntimeCount += 1;
    delay(1000);
    //Break when reach Anntime
  }

Serial.println("Annealing Complete");
```

Thermocycler Code [Extension]

```
//EXTENSION
  // OTW to Extension Temp
  Serial.println("Attaining Extension");
   while(currentTemperature < 72.5){
       digitalWrite(RelayHeaterPin, HIGH);
       currentTemperature = readThermistor();
       if (currentTemperature > 0){
         Serial.print("it's ");
         Serial.print(currentTemperature);
         Serial.println("C.");
         lcd.clear();
         lcd.setCursor(0,0);
         lcd.print("Extension [^]");
         lcd.setCursor(0,1);
         lcd.print("Cycle: ");
         lcd.println(cyclecount);
         delay(2000);
       }
     // On until temp > 72.5
     }

 digitalWrite(RelayHeaterPin, LOW);
 digitalWrite(RelayPeltierPin, LOW);
 currentTemperature = readThermistor();
 Serial.print("it's ");
 Serial.print(currentTemperature);
 Serial.println("C.");
```

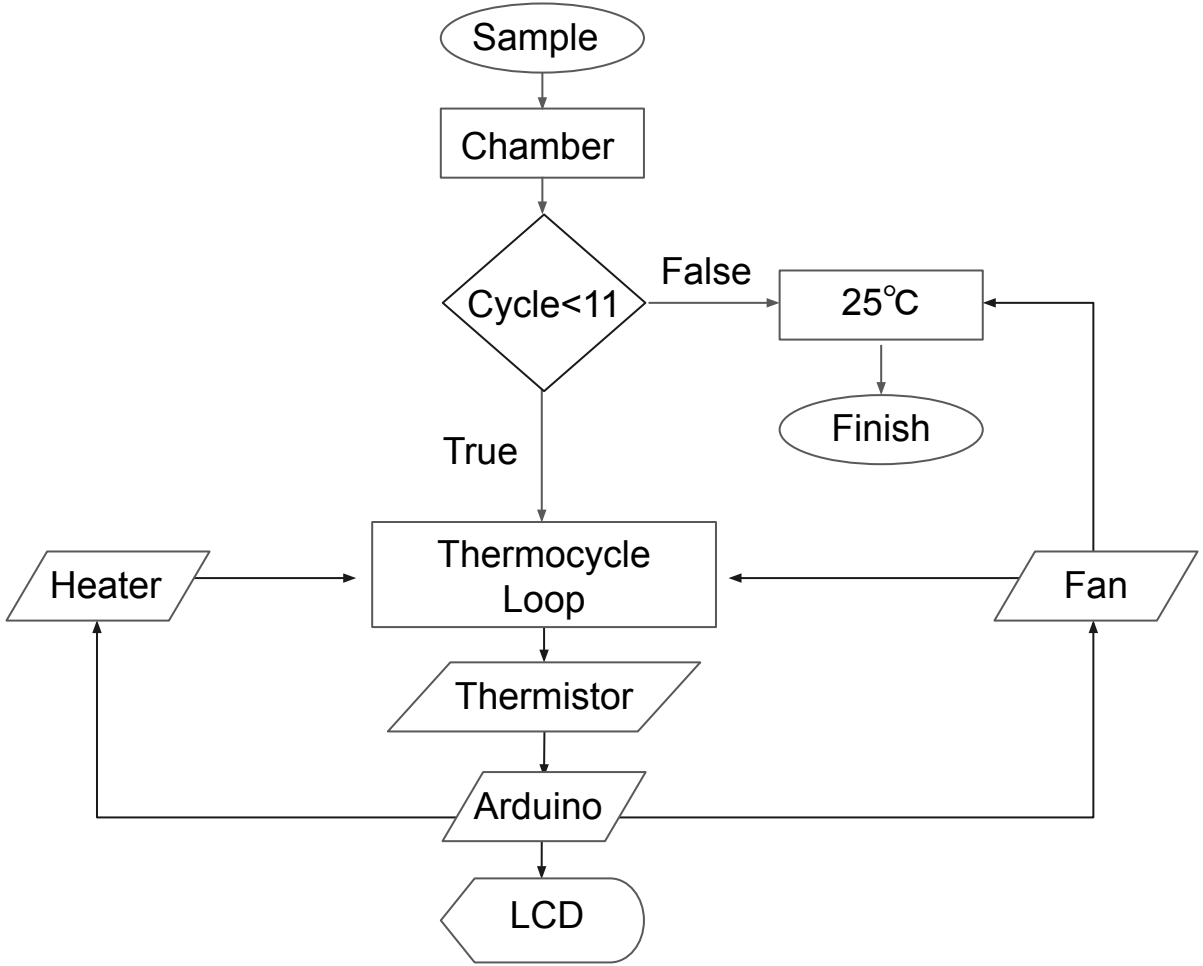
```
 // Sustain Extension Temp
Serial.println("Sustaining Extension");
  currentTemperature = readThermistor();
  while (currentTemperature > 30 && ExtentimeCount < Extentime){
     currentTemperature = readThermistor();
     if (currentTemperature < 72){

          digitalWrite(RelayHeaterPin, HIGH);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Extension [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);

       }
     }
```

```
     else (currentTemperature >= 72);{

          digitalWrite(RelayHeaterPin, LOW);
          currentTemperature = readThermistor();
          if (currentTemperature > 0){
           Serial.print("it's ");
           Serial.print(currentTemperature);
           Serial.println("C.");
           lcd.clear();
           lcd.setCursor(0,0);
           lcd.print("Extension [-]");
           lcd.setCursor(0,1);
           lcd.print("Cycle: ");
           lcd.println(cyclecount);
           delay(2000);
       }
     }
    ExtentimeCount += 1;
    delay(1000);
    //Break when reach Dentime
  }

Serial.println("Extension Complete");
```

Thermocycler Code [Outro]

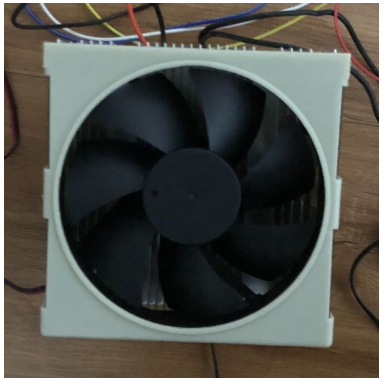
```
//AFTER EACH CYCLE PROCESS ====================

 digitalWrite(RelayHeaterPin, LOW);
 digitalWrite(RelayPeltierPin, LOW);

 //cycle count notification
 Serial.print(cyclecount + 1);
 Serial.println(" CYCLE HAVE BEEN ACHIEVED");
 lcd.clear();
 lcd.setCursor(0,0);
 lcd.print(cyclecount + 1);
 lcd.println(" CYCLE HAVE");
 lcd.setCursor(0,1);
 lcd.print("BEEN ACHIEVED");
 delay(3000);

}
 //After desired cycle had been achieved
 Serial.println("YOUR SAMPLE IS READY");
 delay(3000);
 digitalWrite(RelayHeaterPin, LOW);
 digitalWrite(RelayPeltierPin, LOW);

 for (;;){}

}
```

Hardware

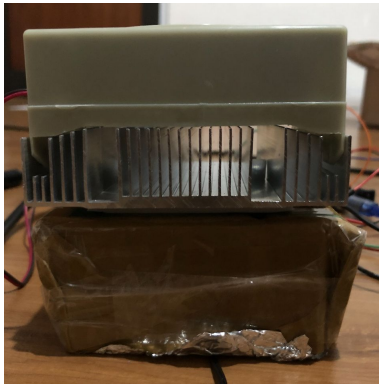

Sample
Chamber
Cycle<11
False
25°C
Finish
True
Heater
Thermocycle Loop
Fan
Thermistor
Arduino
LCD

Hardware Exterior Preview

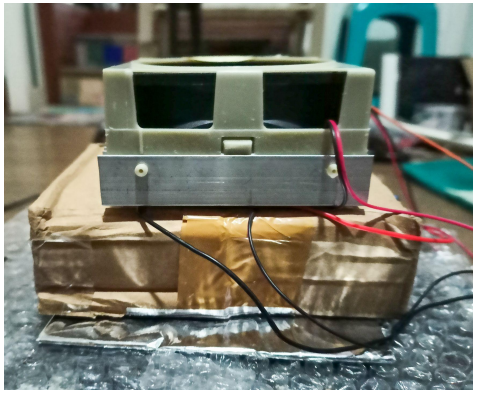
Back View

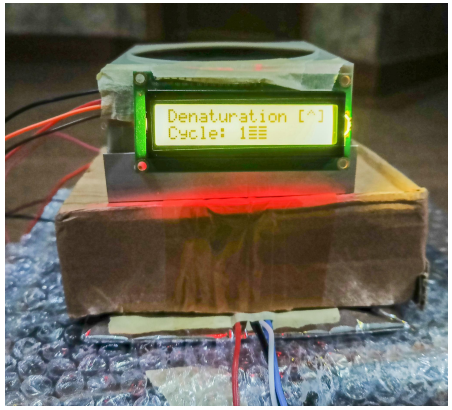


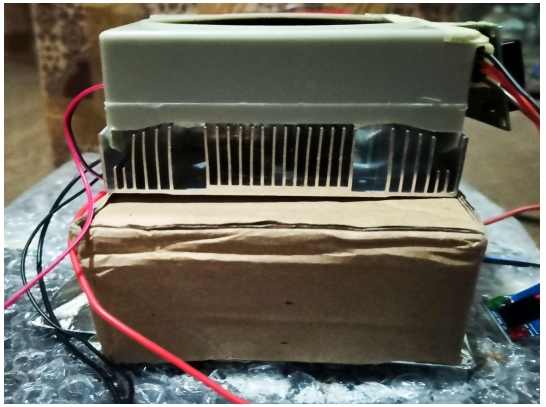
Front View



Side View

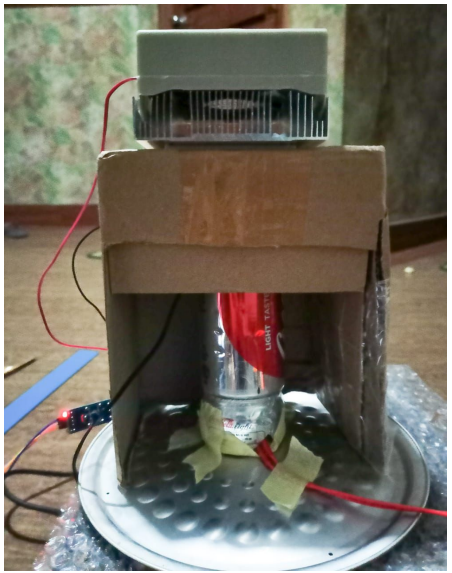




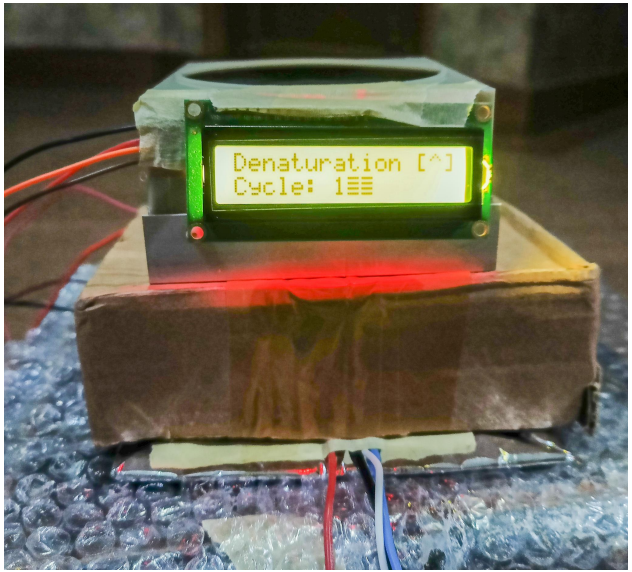


Hardware Exterior

Previously
[Milestone II]

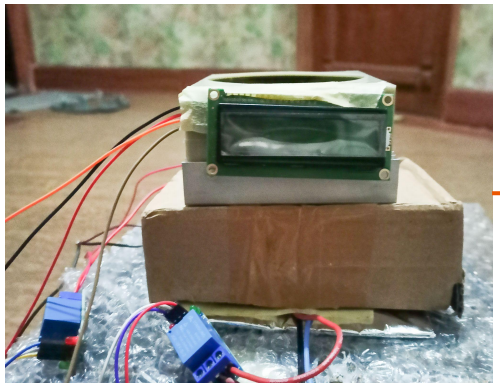


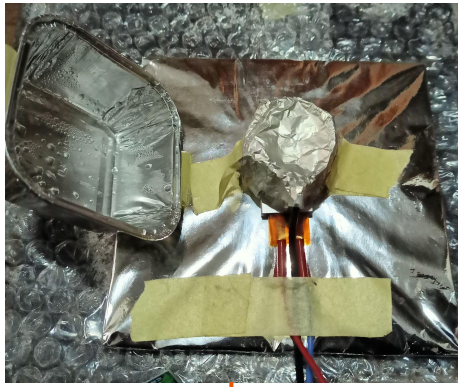
Now
[Milestone III]



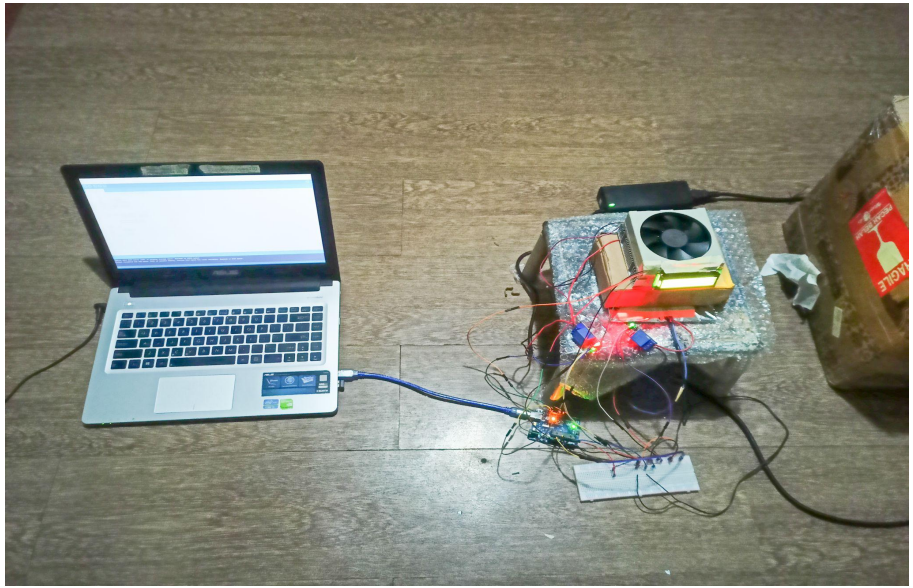
Hardware Interior Anatomy

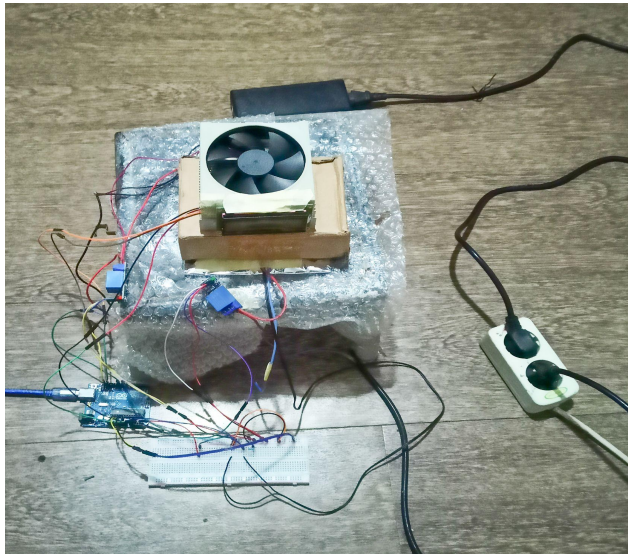
Let’s try to peel and see what’s inside...





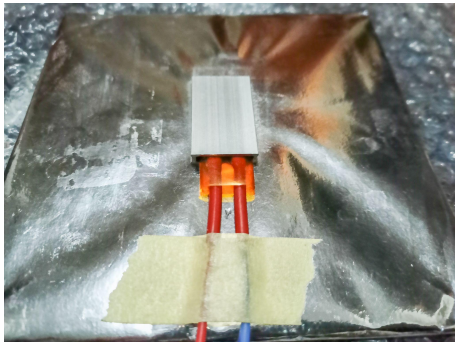
Connectivity Preview





Problem Solving

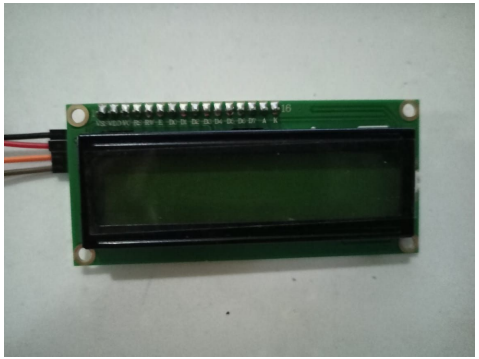
Temperature Limit Problem

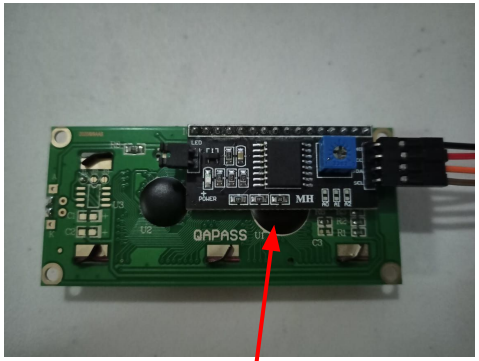
Previously [Milestone II]	Now [Milestone III]
	
Printer Heater [DC 12V, 40 Watt] Max 65 C	Heater Plate [AC 220V, 15 Watt] Max 120 C

Container Material Problem

Most Suitable !

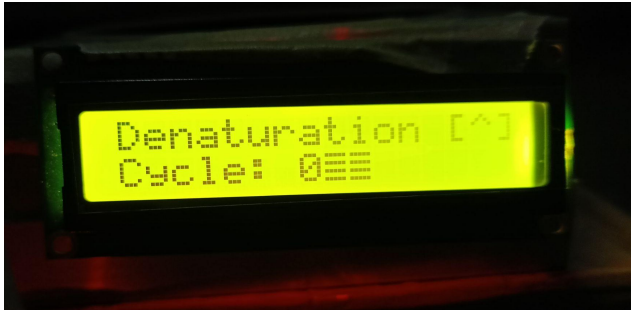
LCD Usage Problem





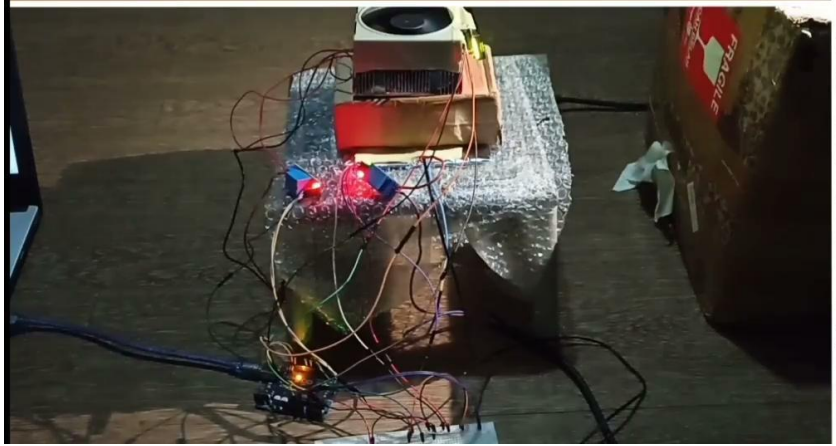
I2C Backpack

Problem Solved !



Results

Temperature Control Flow



```
it's 98.12C.
it's 98.50C.
it's 98.89C.
it's 98.70C.
it's 98.89C.
it's 98.70C.
it's 98.89C.
it's 98.89C.
it's 99.09C.
it's 98.89C.
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it's 98.89C.
it's 98.89C.
it's 98.89C.
it's 99.09C.
it's 98.89C.
it's 98.89C.
it's 98.70C.
it's 99.09C.
it's 98.70C.
it's 98.89C.
it's 99.09C.
it's 99.09C.
it's 99.09C.
it's 98.89C.
it's 98.89C.
it's 98.89C.
it's 99.09C.
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it's 98.89C.
it's 98.89C.
it's 98.89C.
it's 98.89C.
it's 98.89C.
it's 98.89C.
```
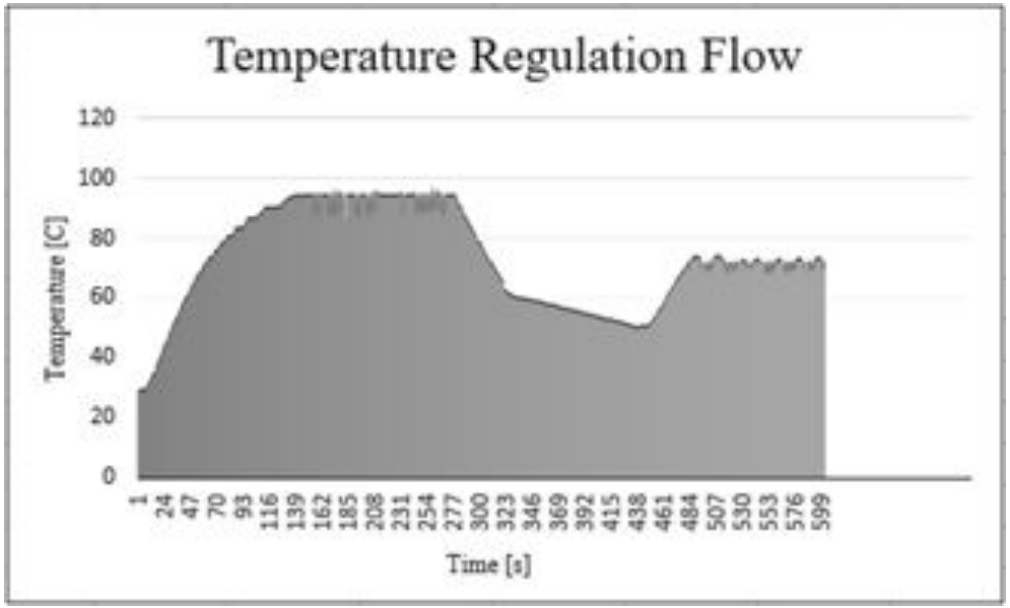

☑ Autoscroll ☐ Show timestamp

One Cycle Temperature Control Flow



Setted Sustaining Duration: 2 minutes

THANK YOU
ANY QUESTION ?