THERMAL REGULATION IN A PCR CHAMBER

Elisha S. (12006007)
Katherine K. (12006010)

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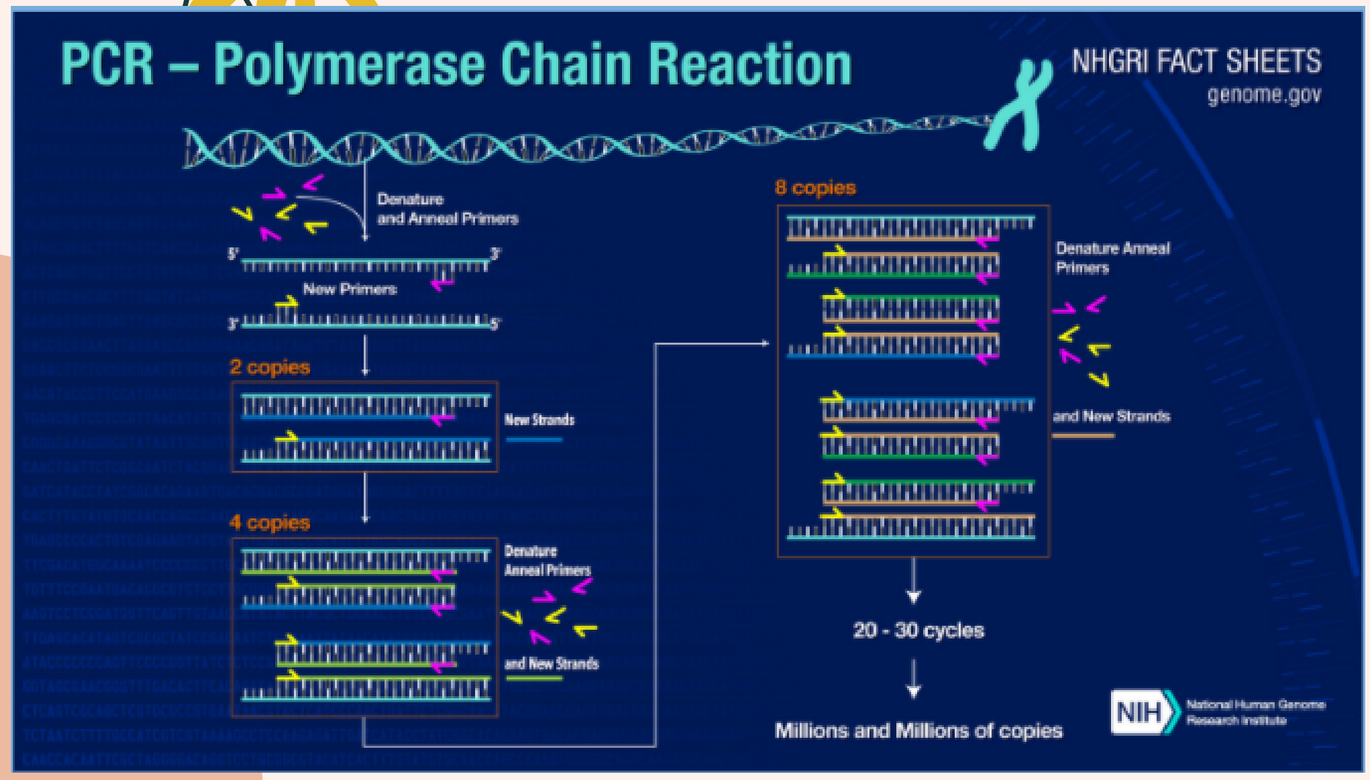
Objective

TO UNDERSTAND THE PRINCIPLE OF PCR CHAMBER

TO COMPREHEND THE PRINCIPLE PID CONTROLLER

TO KNOW HOW TO BUILD SIMPLE PCR CHAMBER

PCR



PCR IS A METHOD USED TO MAKE MILLIONS, EVEN BILLIONS OF A SPECIFIC DNA SAMPLE RAPIDLY. THIS METHOD IS DEVELOPED IN THE 1980S BY KARY B. MULLIS. THIS FAST AND RELATIVELY INEXPENSIVE TECHNIQUE SOMETIMES CALLED "MOLECULAR PHOTOCOPYING".

ONCE AMPLIFIED, THE DNA PRODUCED BY PCR CAN BE USED IN MANY DIFFERENT LABORATORY PROCEDURES. FOR EXAMPLE, MOST MAPPING TECHNIQUES IN THE HUMAN GENOME PROJECT (HGP) RELIED ON PCR.

PCR IS ALSO VALUABLE IN A NUMBER OF LABORATORY AND CLINICAL TECHNIQUES, INCLUDING DNA FINGERPRINTING, DETECTION OF BACTERIA OR VIRUSES (PARTICULARLY AIDS), AND DIAGNOSIS OF GENETIC DISORDERS.

PCR IS BASED ON USING THE ABILITY OF DNA POLYMERASE TO SYNTHESIZE NEW STRAND OF DNA COMPLEMENTARY TO THE OFFERED TEMPLATE STRAND. BECAUSE DNA POLYMERASE CAN ADD A NUCLEOTIDE ONLY ONTO A PREEXISTING 3'-OH GROUP, IT NEEDS A PRIMER TO WHICH IT CAN ADD THE FIRST NUCLEOTIDE. THIS REQUIREMENT MAKES IT POSSIBLE TO DELINEATE A SPECIFIC REGION OF TEMPLATE SEQUENCE THAT THE RESEARCHER WANTS TO AMPLIFY. AT THE END OF THE PCR REACTION, THE SPECIFIC SEQUENCE WILL BE ACCUMULATED IN BILLIONS OF COPIES (AMPLICONS).

How it Works?

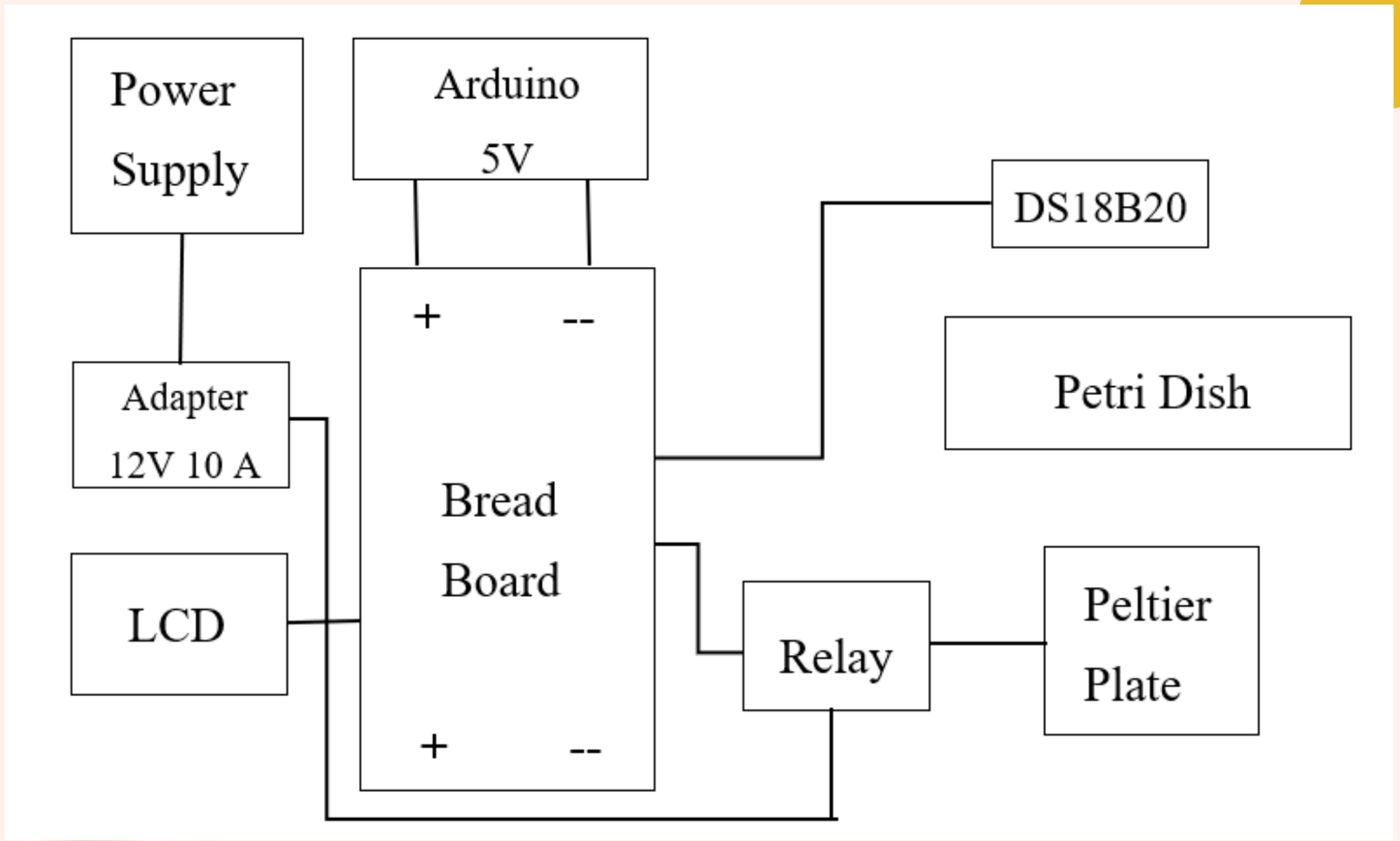
- INITIALIZATION: THIS STEP IS ONLY REQUIRED FOR DNA POLYMERASES THAT REQUIRE HEAT ACTIVATION BY HOT-START PCR. IT CONSISTS OF HEATING THE REACTION CHAMBER TO A TEMPERATURE OF 94–96 °C (201–205 °F), OR 98 °C (208 °F) IF EXTREMELY THERMOSTABLE POLYMERASES ARE USED, WHICH IS THEN HELD FOR 1–10 MINUTES.
- DENATURATION: THIS STEP IS THE FIRST REGULAR CYCLING EVENT AND CONSISTS OF HEATING THE REACTION CHAMBER TO 94–98 °C (201–208 °F) FOR 20–30 SECONDS. THIS CAUSES DNA MELTING, OR DENATURATION, OF THE DOUBLE-STRANDED DNA TEMPLATE BY BREAKING THE HYDROGEN BONDS BETWEEN COMPLEMENTARY BASES, YIELDING TWO SINGLE-STRANDED DNA MOLECULES.
- ANNEALING: IN THE NEXT STEP, THE REACTION TEMPERATURE IS LOWERED TO 50–65 °C (122–149 °F) FOR 20–40 SECONDS, ALLOWING ANNEALING OF THE PRIMERS TO EACH OF THE SINGLE-STRANDED DNA TEMPLATES.
- EXTENSION/ELONGATION: THE TEMPERATURE AT THIS STEP DEPENDS ON THE DNA POLYMERASE USED; THE OPTIMUM ACTIVITY TEMPERATURE FOR THE THERMOSTABLE DNA POLYMERASE OF TAQ POLYMERASE IS APPROXIMATELY 75–80 °C (167–176 °F), THOUGH A TEMPERATURE OF 72 °C (162 °F) IS COMMONLY USED WITH THIS ENZYME. IN THIS STEP, THE DNA POLYMERASE SYNTHESIZES A NEW DNA STRAND COMPLEMENTARY TO THE DNA TEMPLATE STRAND BY ADDING FREE DNTPS FROM THE REACTION MIXTURE THAT IS COMPLEMENTARY TO THE TEMPLATE IN THE 5′-TO-3′ DIRECTION, CONDENSING THE 5′-PHOSPHATE GROUP OF THE DNTPS WITH THE 3′-HYDROXY GROUP AT THE END OF THE NASCENT (ELONGATING) DNA STRAND.
- FINAL ELONGATION: THIS SINGLE STEP IS OPTIONAL, BUT IS PERFORMED AT A TEMPERATURE OF 70–74 °C (158–165 °F) (THE TEMPERATURE RANGE REQUIRED FOR OPTIMAL ACTIVITY OF MOST POLYMERASES USED IN PCR) FOR 5–15 MINUTES AFTER THE LAST PCR CYCLE TO ENSURE THAT ANY REMAINING SINGLE-STRANDED DNA IS FULLY ELONGATED.
- FINAL HOLD: THE FINAL STEP COOLS THE REACTION CHAMBER TO 4–15 °C (39–59 °F) FOR AN INDEFINITE TIME, AND MAY BE EMPLOYED FOR SHORT-TERM STORAGE OF THE PCR PRODUCTS.

Equipment

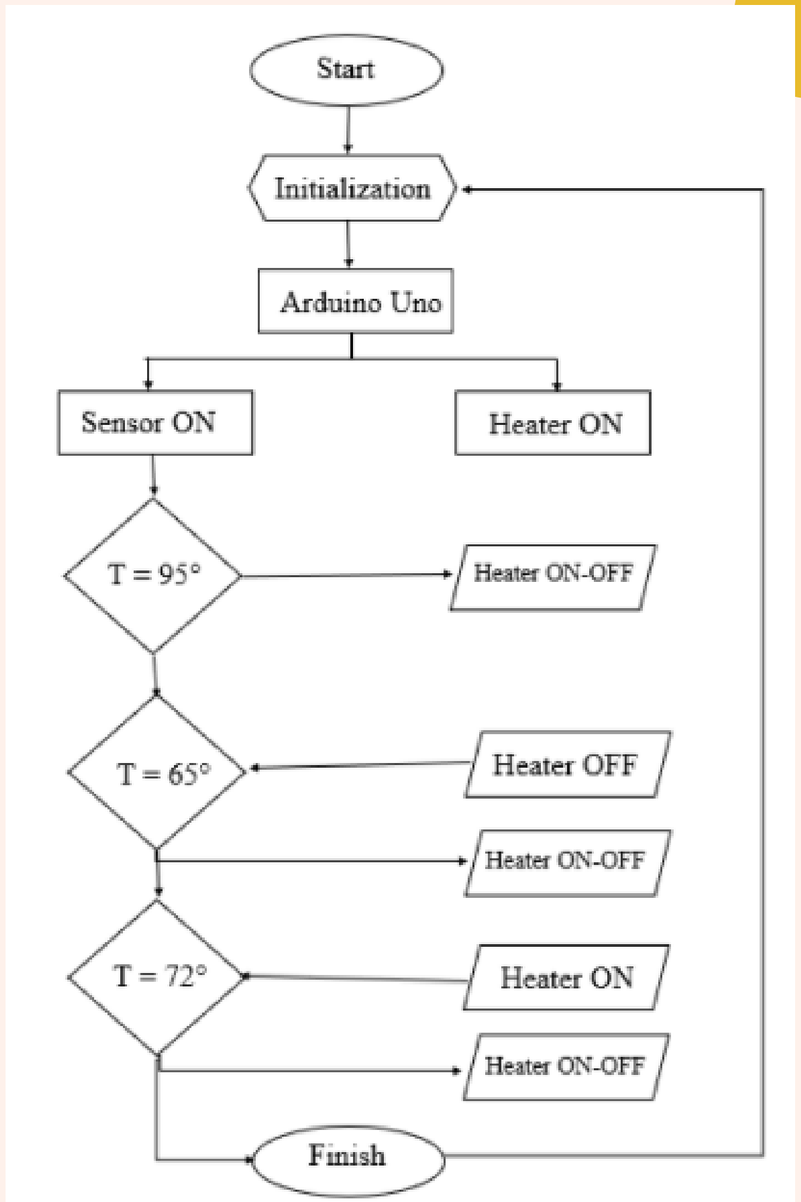
- Arduino Uno R3(5V)
- Breadboard
- Temperature Sensor (DS18B20)
- Jumper Wire
- Adapter (12V 10A)
- Peltier Plate (TEC1-12706)
- Arduino Relay
- LCD Monitor (16 x 2 I2C)
- Resistor 4.7k Ω

System Plan

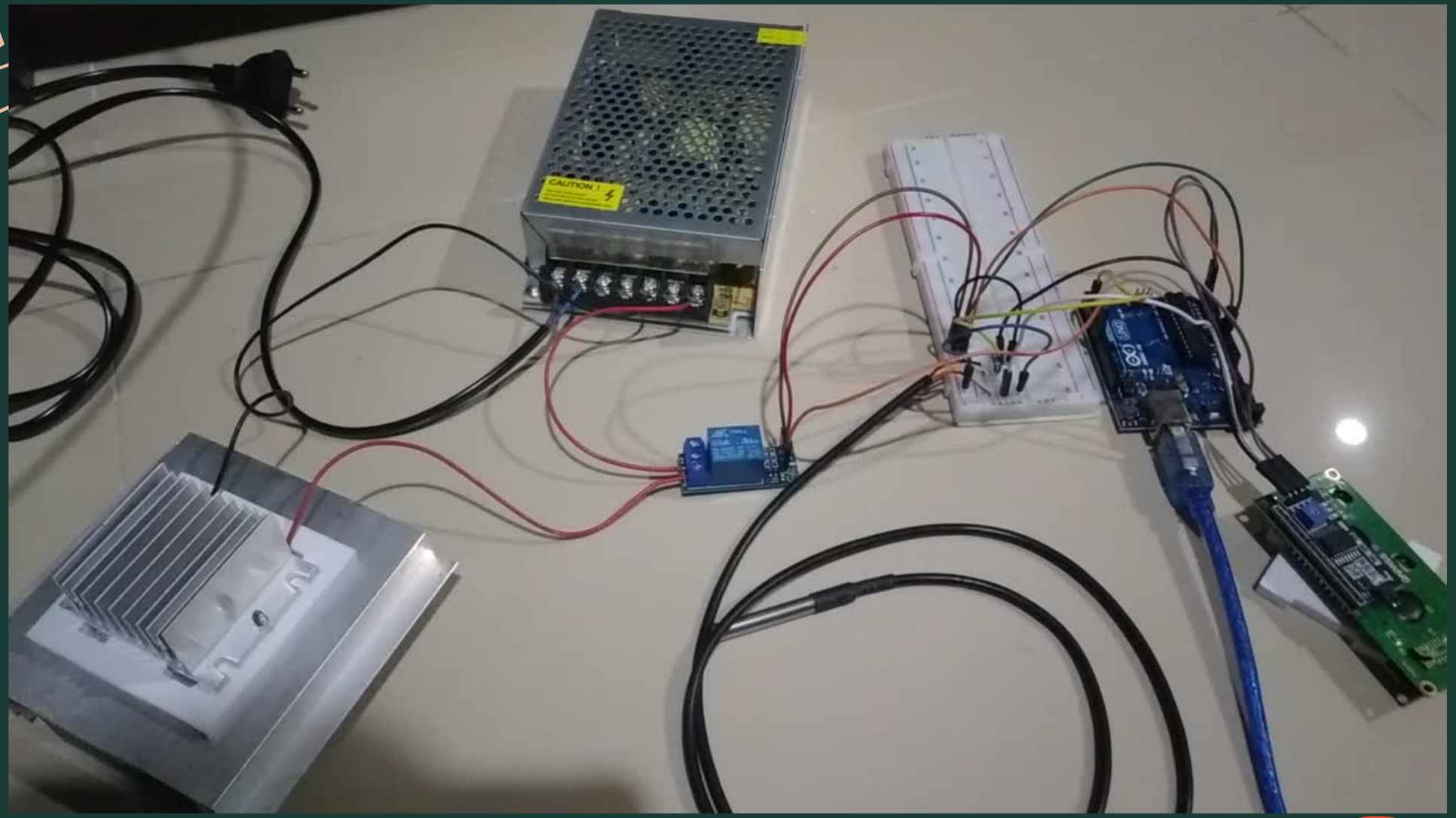
Schematic Circuit



System Flow Diagram



Circuit Building



Coding

sketch_jun29b

```
#include <LiquidCrystal_I2C.h>

#include "Wire.h"
#include <OneWire.h>
#include <Wire.h>
#include <LiquidCrystal_I2C.h>

LiquidCrystal_I2C lcd(0x27, 16, 2);

OneWire  ds(2);  // on pin 10 (a 4.7K resistor is necessary)

float kp = 2;
float ki = 0.5;
float kd = 2;

float p,ix,d,suhu,pid;
float error,errorx,sumerr;

float sp;
float spx = 65;  //set point
int pinpwm = 11;

 byte i;
  byte present = 0;
  byte type_s;
  byte data[12];
  byte addr[8];
```

sketch_jun29b

```
 byte i;
  byte present = 0;
  byte type_s;
  byte data[12];
  byte addr[8];
  float celsius, fahrenheit;


void setup() {

  pinMode(pinpwm,OUTPUT);
  Serial.begin(9600);
  lcd.init();
  lcd.clear();
  lcd.noCursor();

}

void loop() {

  if ( !ds.search(addr)) {
    ds.reset_search();
    delay(250);
    return;
  }
```

sketch_jun29b

```
for( i = 0; i < 8; i++) {

}

if (OneWire::crc8(addr, 7) != addr[7]) {
    return;
}

switch (addr[0]) {
  case 0x10:
    type_s = 1;
    break;
  case 0x28:
    type_s = 0;
    break;
  case 0x22:
    type_s = 0;
    break;
  default:
    return;
}

ds.reset();
ds.select(addr);
ds.write(0x44, 1);        // start conversion, with parasite power on at the end
```

sketch_jun29b

```
ds.reset();
ds.select(addr);
ds.write(0x44, 1);         // start conversion, with parasite power on at the end

delay(1000);     // maybe 750ms is enough, maybe not

present = ds.reset();
ds.select(addr);
ds.write(0xBE);          // Read Scratchpad

for ( i = 0; i < 9; i++) {           // we need 9 bytes
  data[i] = ds.read();
}

int16_t raw = (data[1] << 8) | data[0];
if (type_s) {
  raw = raw << 3; // 9 bit resolution default
  if (data[7] == 0x10) {
    raw = (raw & 0xFFF0) + 12 - data[6];
  }
} else {
  byte cfg = (data[4] & 0x60);
  if (cfg == 0x00) raw = raw & ~7;  // 9 bit resolution, 93.75 ms
  else if (cfg == 0x20) raw = raw & ~3; // 10 bit res, 187.5 ms
```

sketch_jun29b

```
  } else {
    byte cfg = (data[4] & 0x60);
    if (cfg == 0x00) raw = raw & ~7;  // 9 bit resolution, 93.75 ms
    else if (cfg == 0x20) raw = raw & ~3; // 10 bit res, 187.5 ms


    else if (cfg == 0x40) raw = raw & ~1; // 11 bit res, 375 ms

  }

  celsius = (float)raw / 16.0;
  fahrenheit = celsius * 1.8 + 32.0;
  suhu = celsius;


  analogWrite(pinpwm,pid);

  sp = spx + 30;  //atur range overlap kalibrasi

  error = sp - suhu;
  p = error * kp;
  sumerr = error + errorx;
  ix = ki * sumerr;
  d = kd * (error - errorx);
  pid = p + ix + d;
//pid = 255.0 - pid;
```

sketch_jun29b

```
//pid = 255.0 - pid;

//  if(pid < 1){
//  pid = 0;
//  }

 lcd.setCursor(0,0);
 lcd.print("Suhu=");
 lcd.print(suhu);
 lcd.print("/");
 lcd.print(spx);
 lcd.print("   ");

 lcd.setCursor(0,1);
 lcd.print("PID=");
 lcd.print(pid);
 lcd.print("     ");

Serial.println("suhu= ");
Serial.println(suhu);
Serial.println("pid= ");
Serial.println(pid);

delay(1000);

errorx = error;

}
```

The Result

Conclusion

THANK YOU!!