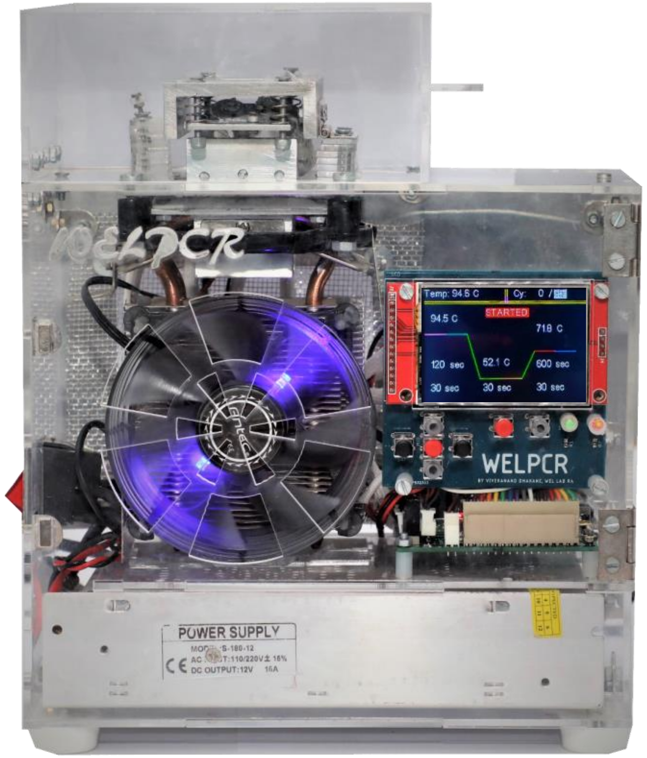
# Low-cost electrochemical sensing platform for DNA analysis



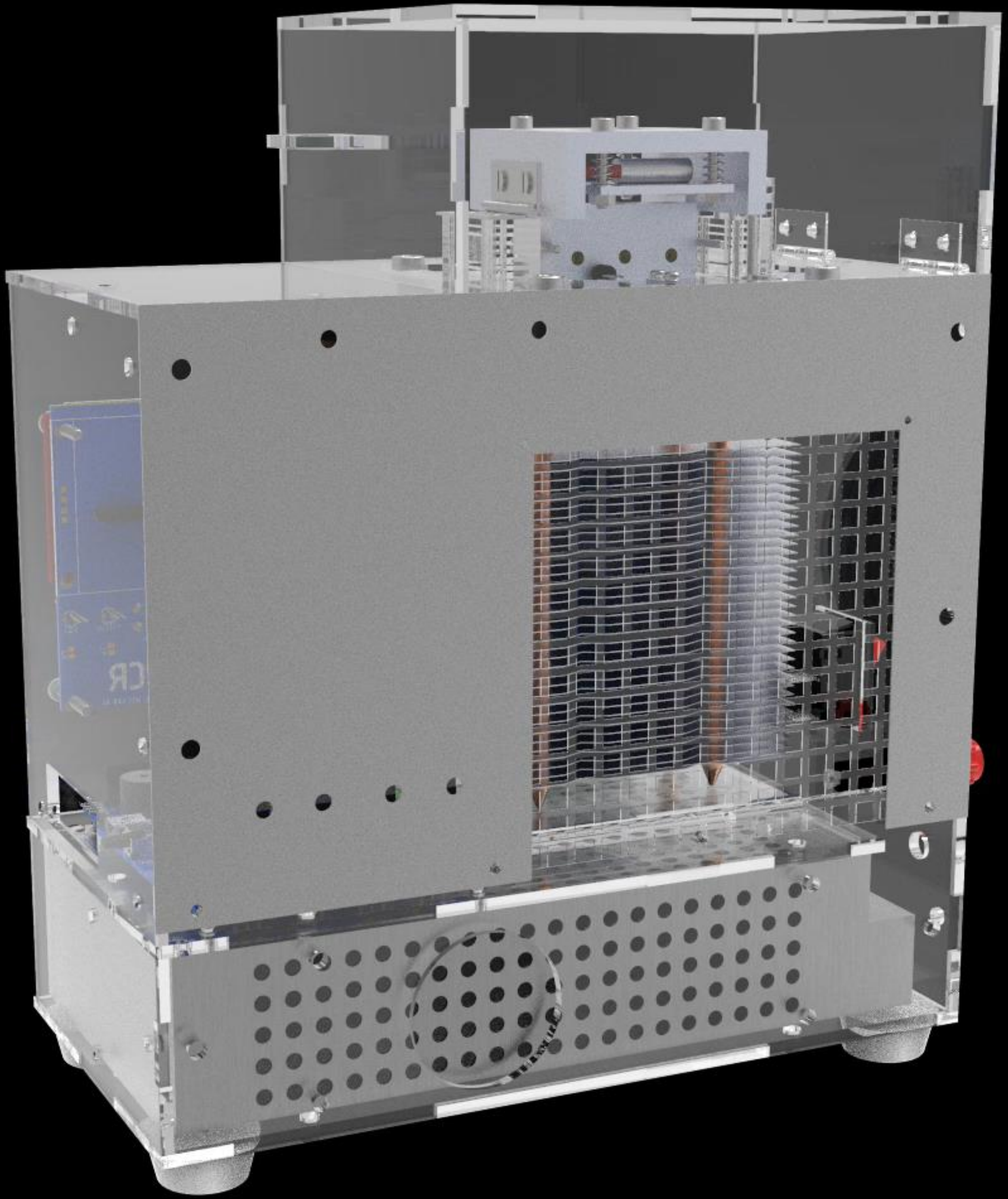

***WELPCR***


***WELSTAT***


Supervisor:
**Prof. Siddharth Tallur**
EE, IIT Bombay

Student:
**Vivekanand Ramrao Dhakane**
193079041, *Solid State Devices* (EE-7)
EE, IIT Bombay

# WELPCR



# Outline

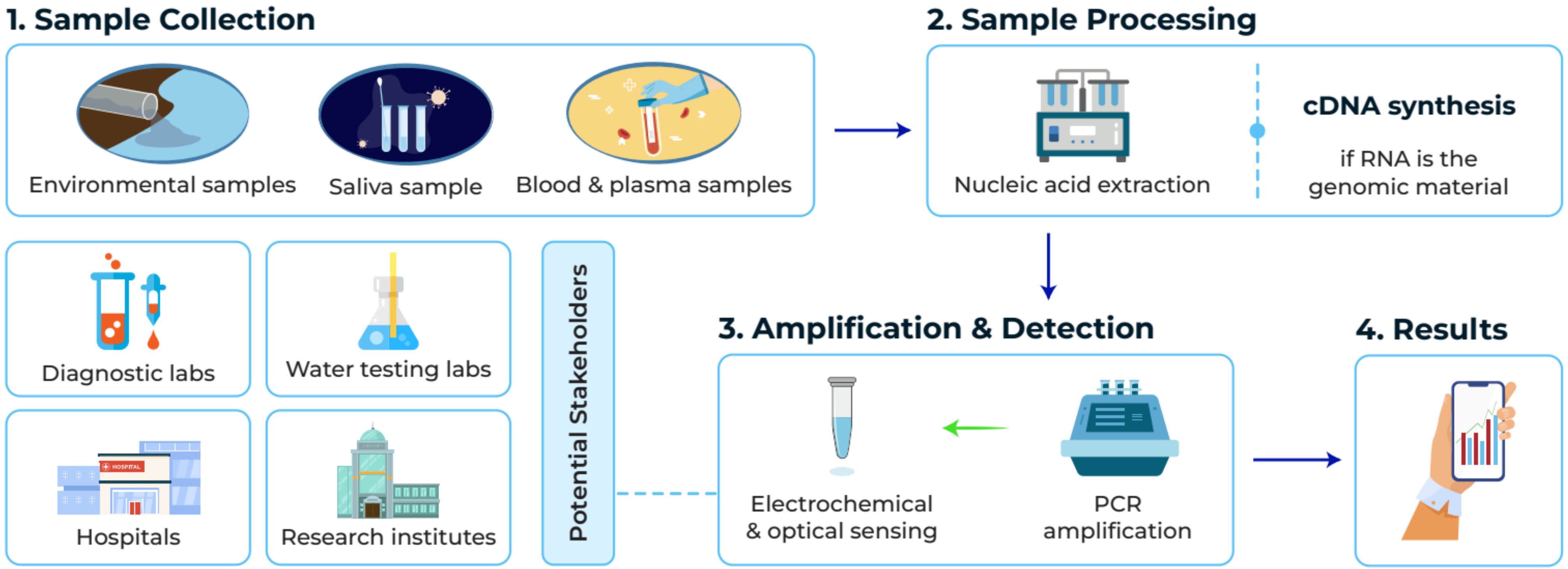
- Rationale
- Objectives
- **WELPCR**
  - Mechanical Assembly
    - Manufacturing of 2D (milling) and 3D (laser cutting) metal parts
  - Electronic Circuit
    - PID design
  - Challenges and Solutions
  - Testing samples using WELPCR
  - Comparison with commercial PCR devices
- **WELSTAT**
  - What is a Potentiostat and Why do we need it?
  - WELSTAT design
    - Selecting AFE for WELSTAT
  - Challenges and Solutions
  - End point PCR using WELPCR+WELSTAT
- Future Improvements

# Rationale

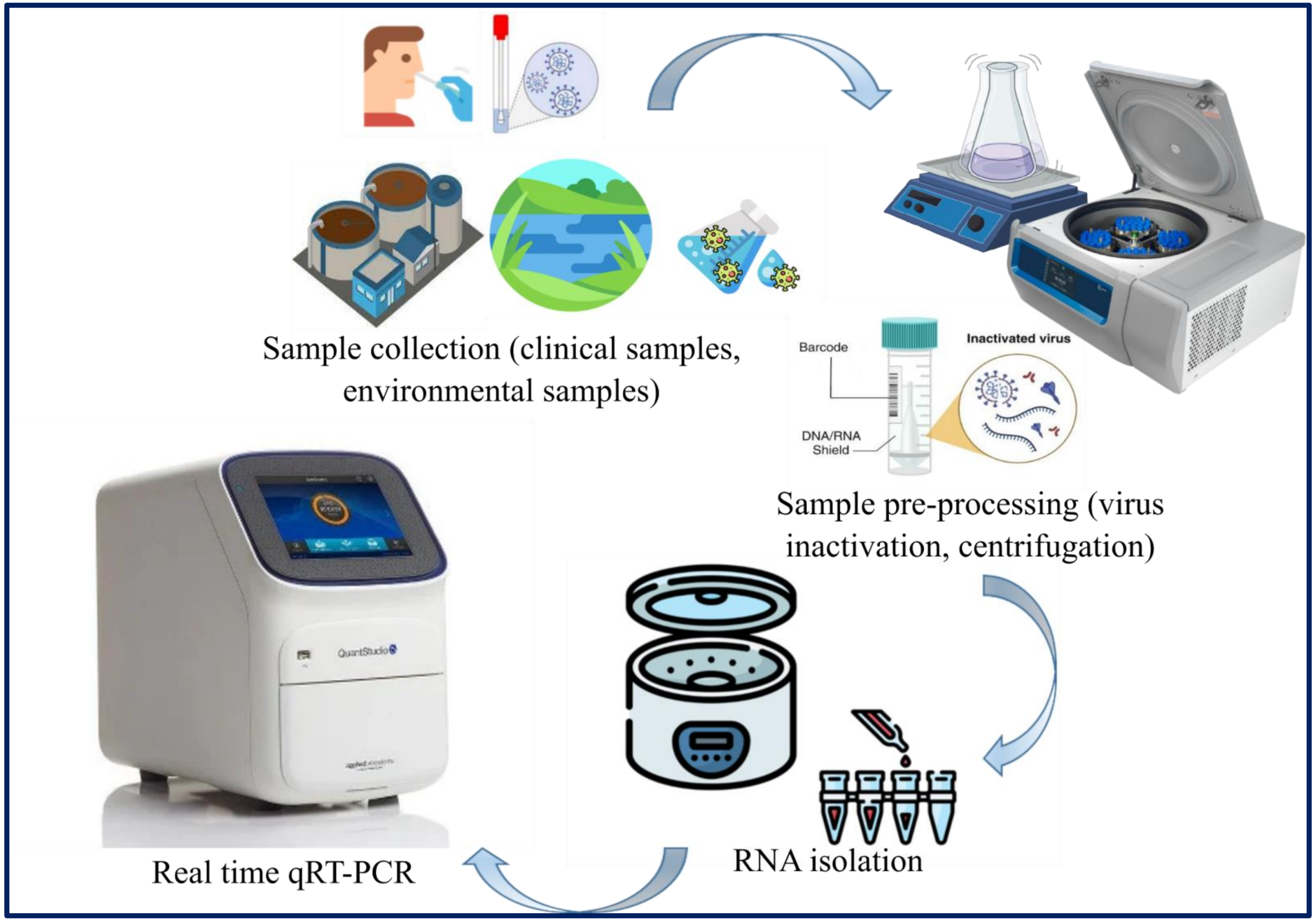
The COVID-19 pandemic has highlighted the need of diagnosis especially using the gold standard qRT-PCR

PCR (Polymerase Chain reaction) is a test to tell you if you have an infection

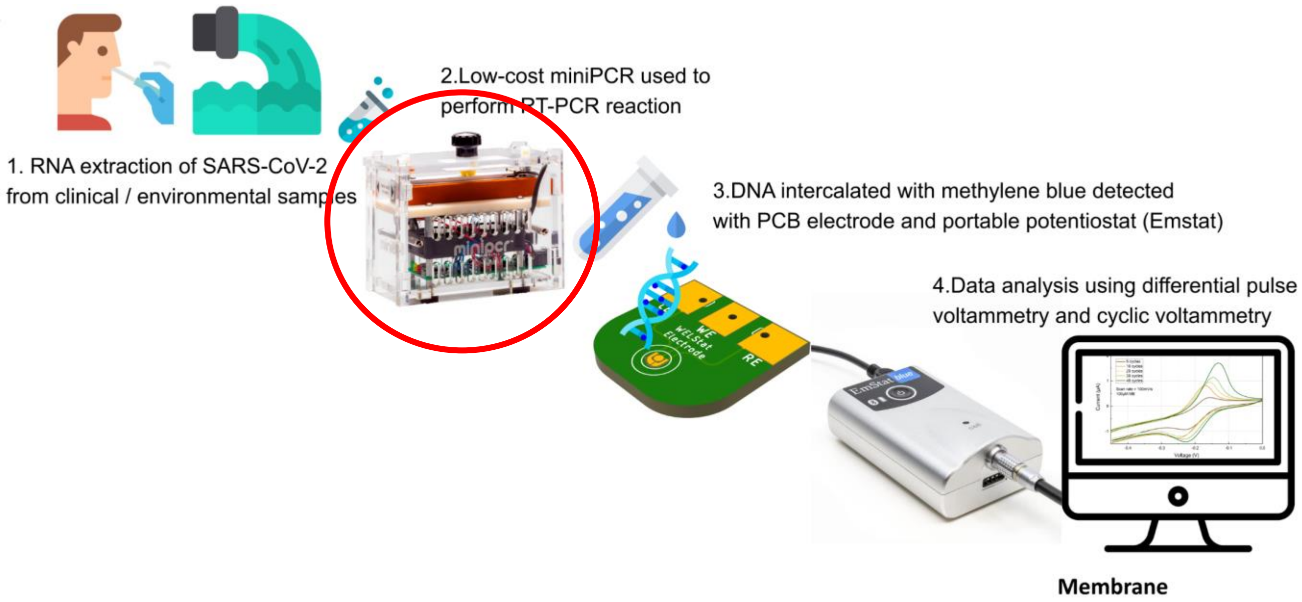
Apart from SARS-CoV-2 (virus that cause COVID-19), PCR can be used to detect other infectious pathogens from environmental samples; blood, saliva and other body fluid samples



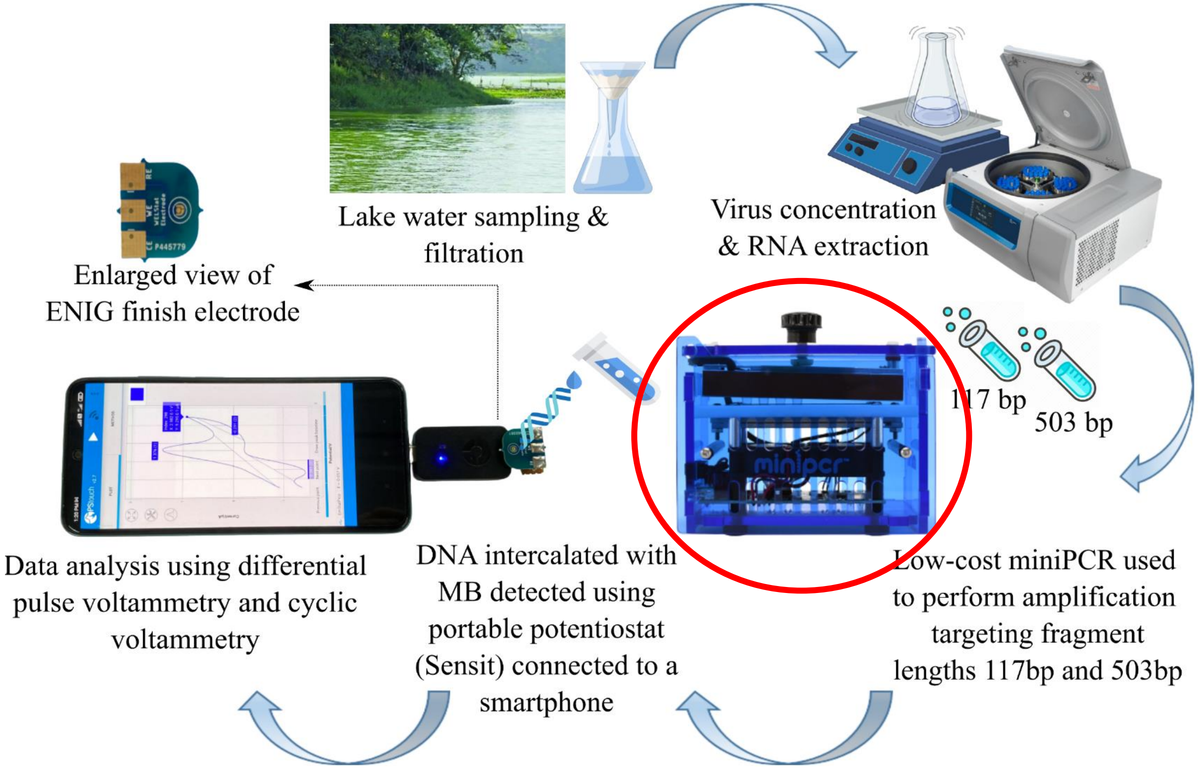
# Process-flow for virus detection



# Process flow for virus detection using electrochemical sensing




*Source: Kumar, M. S., et al. "Electrochemical sensing of SARS-CoV-2 amplicons with PCB electrodes." Sensors and Actuators B: Chemical 343 (2021): 130169*




*Source:* Ahuja S, et al. "Longer amplicons provide better sensitivity for electrochemical sensing of viral nucleic acid in water samples using PCB electrodes." *Scientific reports* 12.1 (2022): 1-10.

# Virus detection using electrochemical sensor

- The group I am associated with at AIMS lab, EE, IITB have successfully detected SARS-CoV-2 and Phi-6 from water samples using PCB based electrochemical sensing

- While testing the samples, amplification was performed using MiniPCR® thermocycler

- The device costs around $800-1000+shipping charges (imported from US)

- We faced several maintenance and service issues on the PCR device while performing experiments

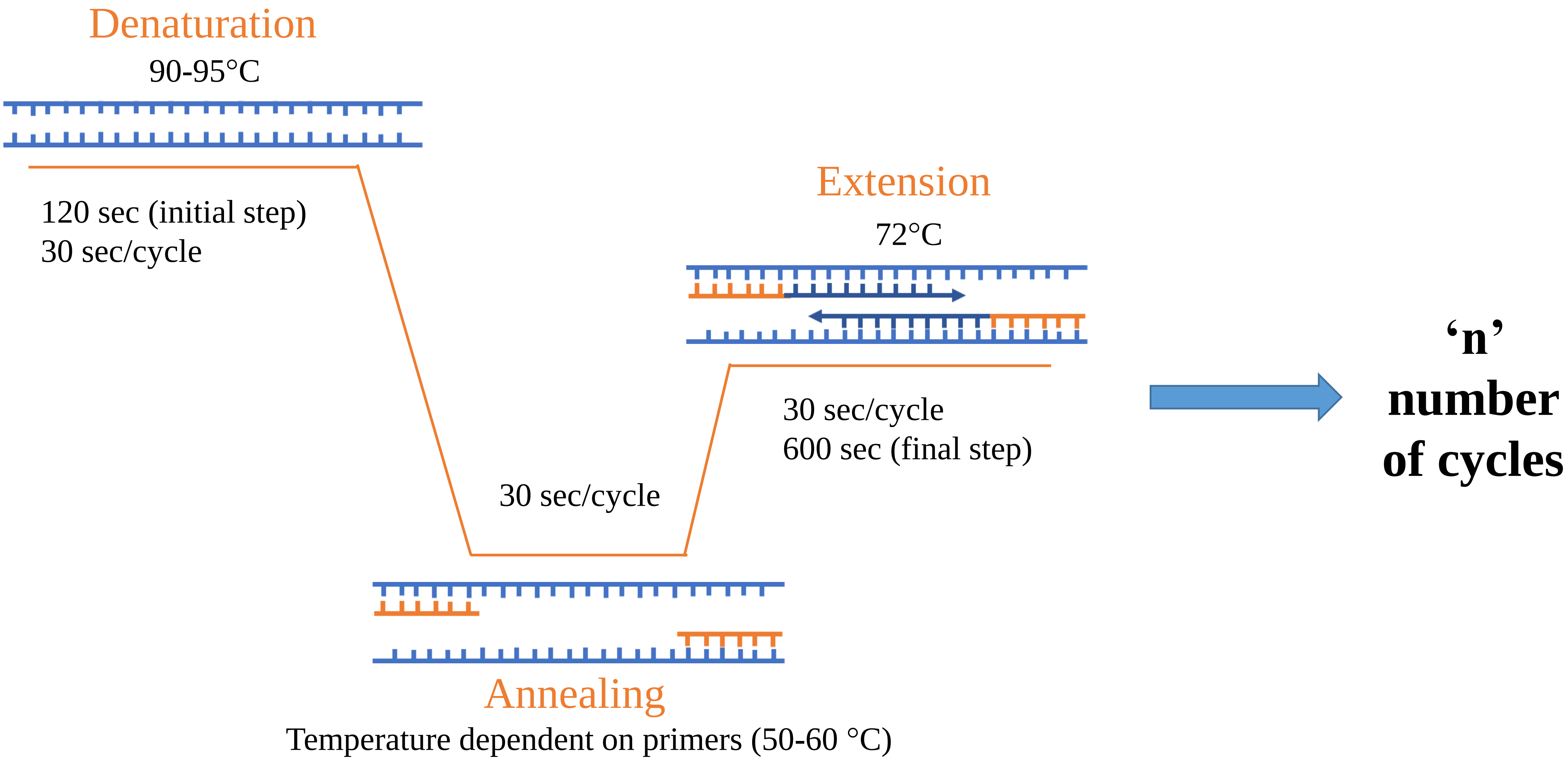
- This led to the motivation of designing a PCR device that can be used for virus (or any pathogen) detection using electrochemical sensing

# Objectives

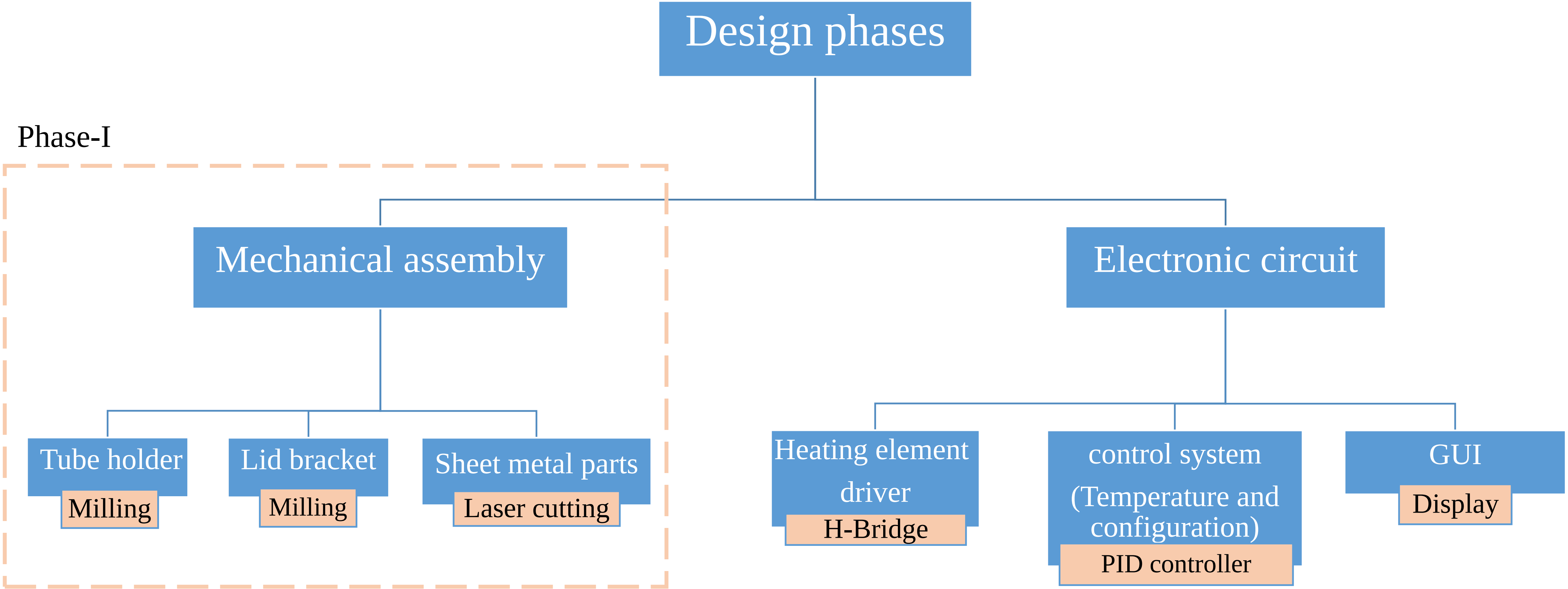
- Develop a low-cost, portable, user-friendly PCR device
- Provisions for simultaneous testing of multiple samples ($\geq$ 8 wells)
- Ramp rate ($\geq$ 1.5°C/s) similar to commercially available PCR device
- Develop a low-cost Potentiostat to perform electrochemical measurements (Cyclic Voltammetry)

# WELPCR

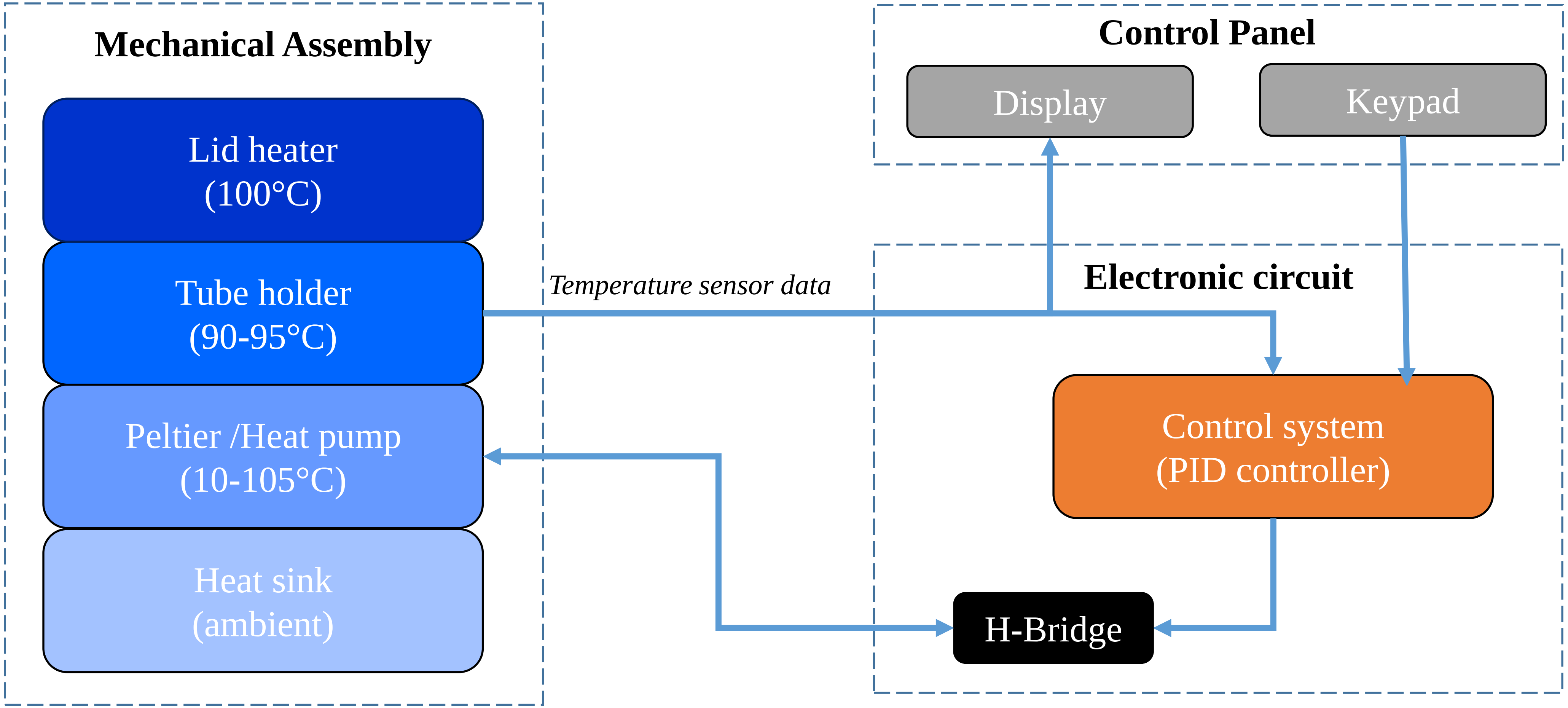
# Temperature profile of PCR



# WELPCR

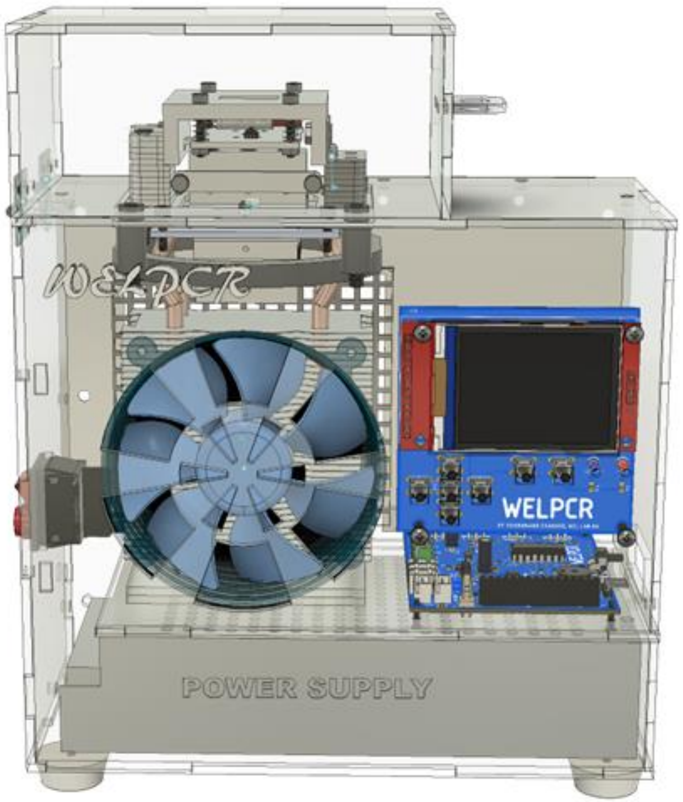

Design phases
Phase-I
Mechanical assembly
Electronic circuit
Tube holder
Milling
Lid bracket
Milling
Sheet metal parts
Laser cutting
Heating element driver
H-Bridge
control system (Temperature and configuration)
PID controller
GUI
Display

# WELPCR Block Diagram



# Mechanical Assembly

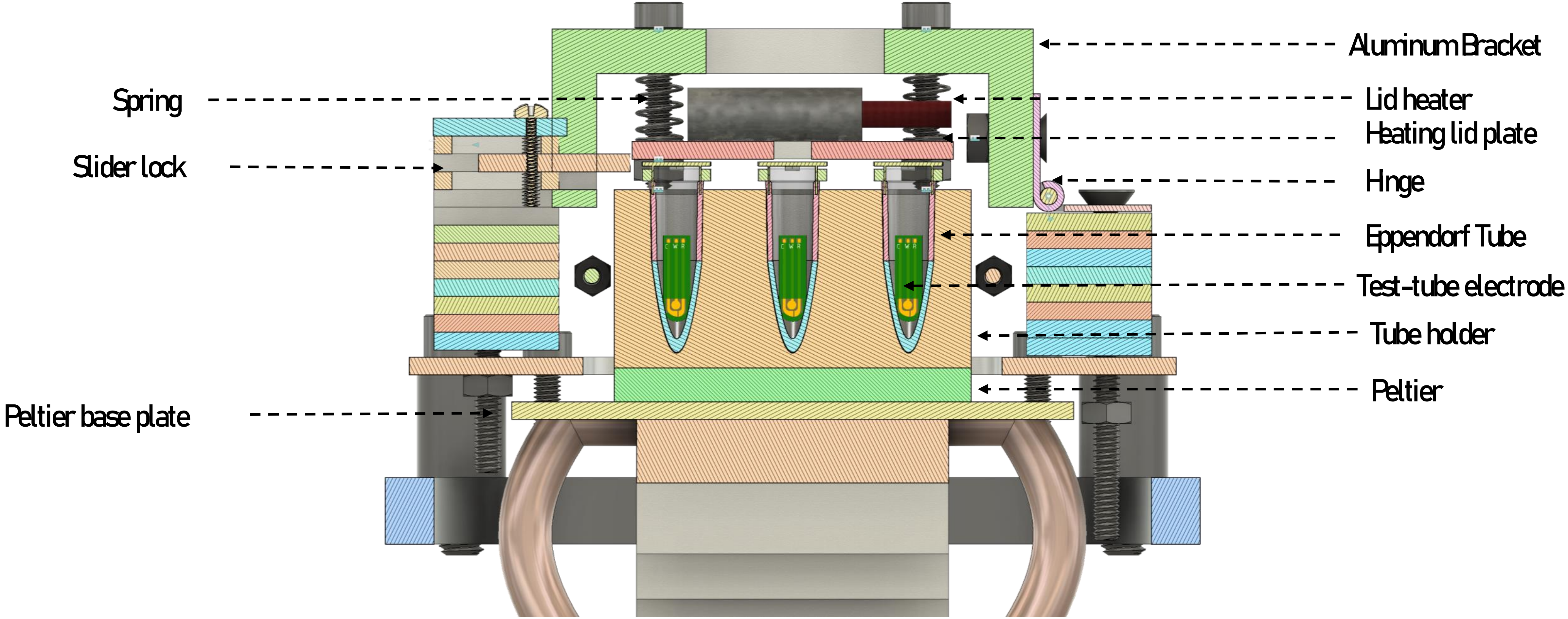
# WELPCR: designed using CAD



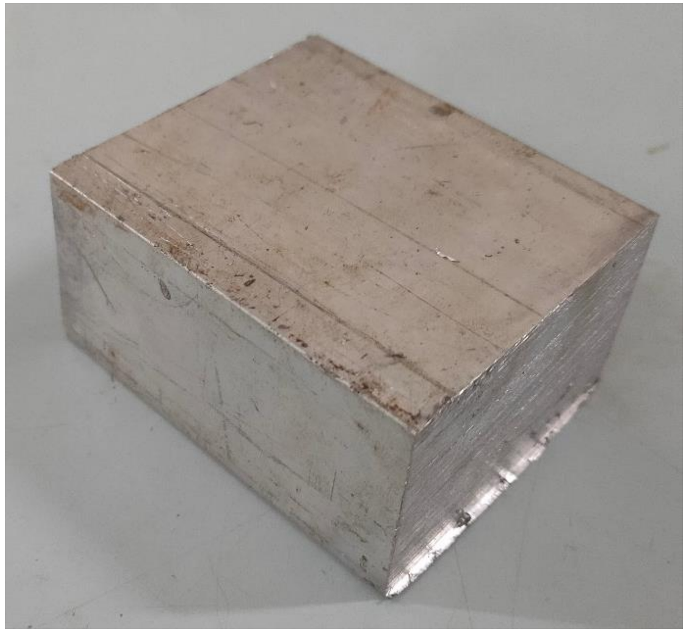

WELPCR designed using a Computer aided design tool Autodesk Fusion 360


WELPCR after manufacturing

# Cross-section view of WELPCR

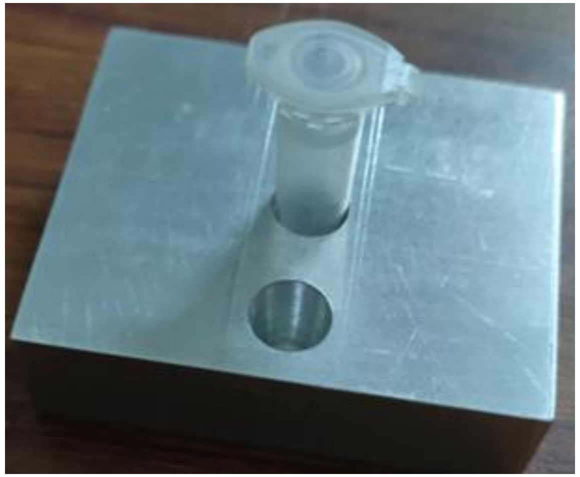


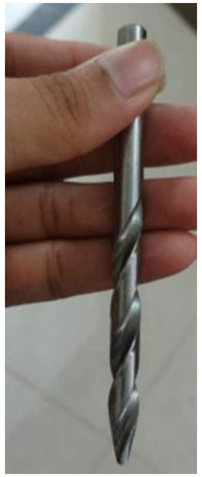
# Manufacturing 3D metal parts



Aluminum block

*Milling*

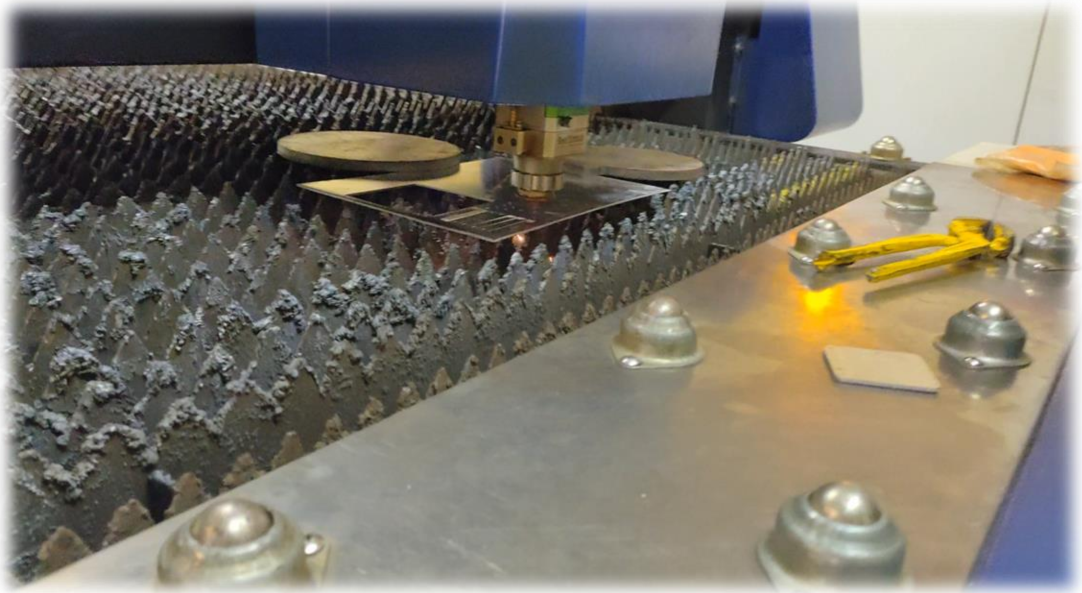




Tube holder

Lid bracket

# Manufacturing Sheet metal parts




Aluminium sheet after laser cutting

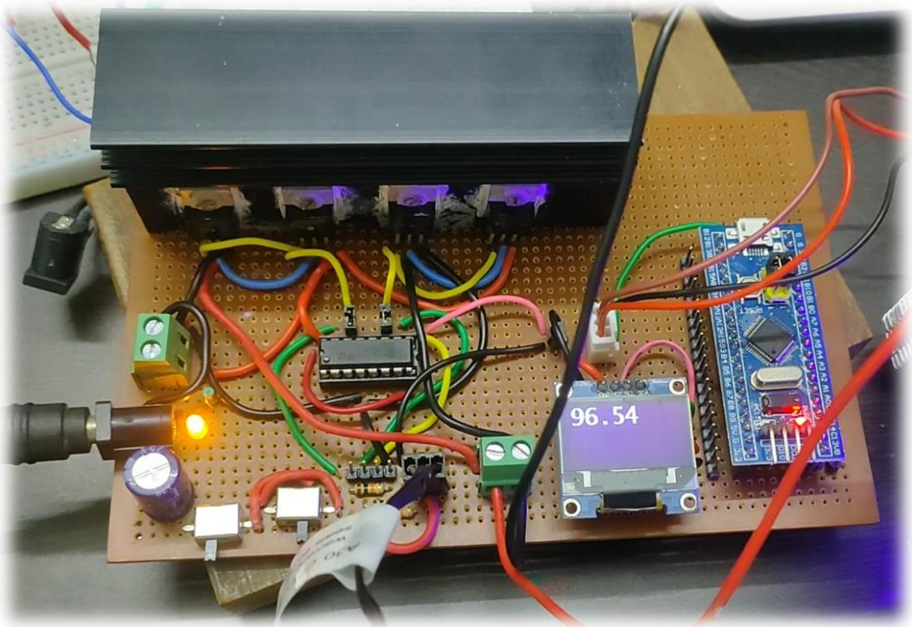
## **MILESTONE achieved during MTP Phase-I**

***Mechanical Assembly of WELPCR***

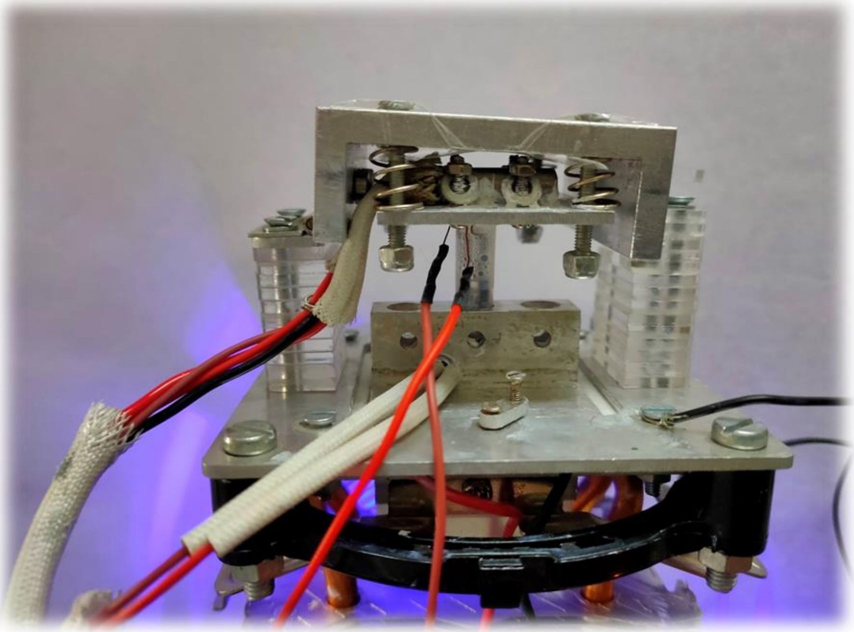
# Electronic circuit

# Electronic circuit

Circuit assembled on zero PCB to check required components and working of the circuit




H-bridge + micro-controller
+ logic level shifter + display



WELPCR mechanical assembly with sensors

# PID Design

*(Toughest part of the project)*

$$PWM = k_p \times e(t) + k_d \times \frac{de(t)}{dt} + k_i \times \int e(t)dt + \beta$$

$\beta$= PWM duty cycle required to maintain constant set-point temperature

- **Trial and error method to tune the PID**

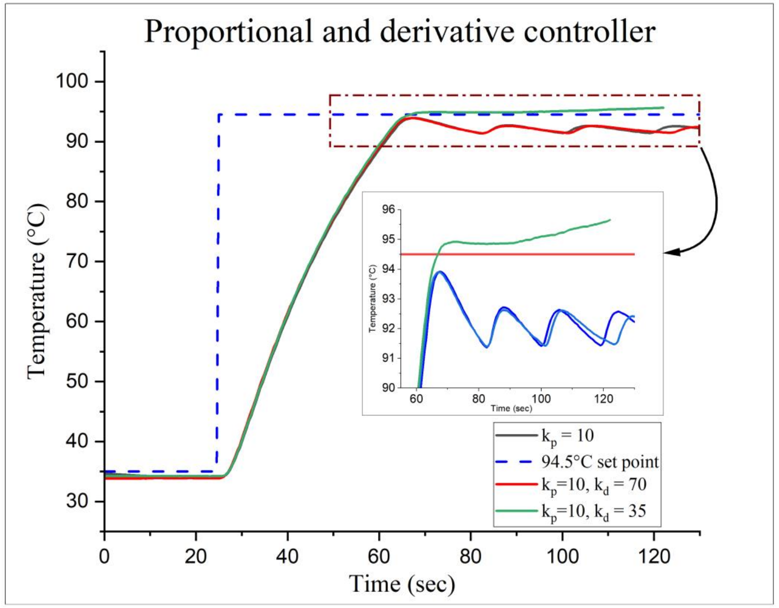
✓ Increase the $k_p$ till we get constant oscillations in steady state
✓ Increase the $k_d$ till the oscillation amplitude is within acceptable range

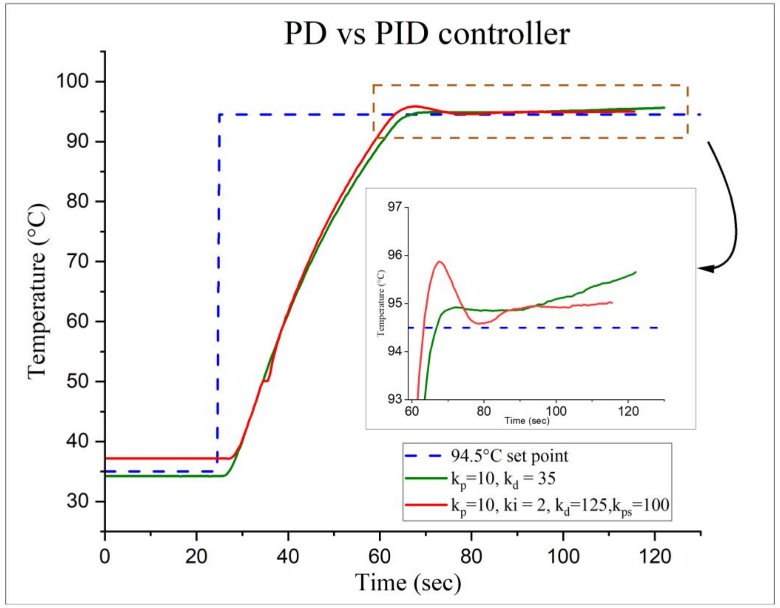
- **Introducing Integral Controller:** Reduced steady state error but **adds overshoot**

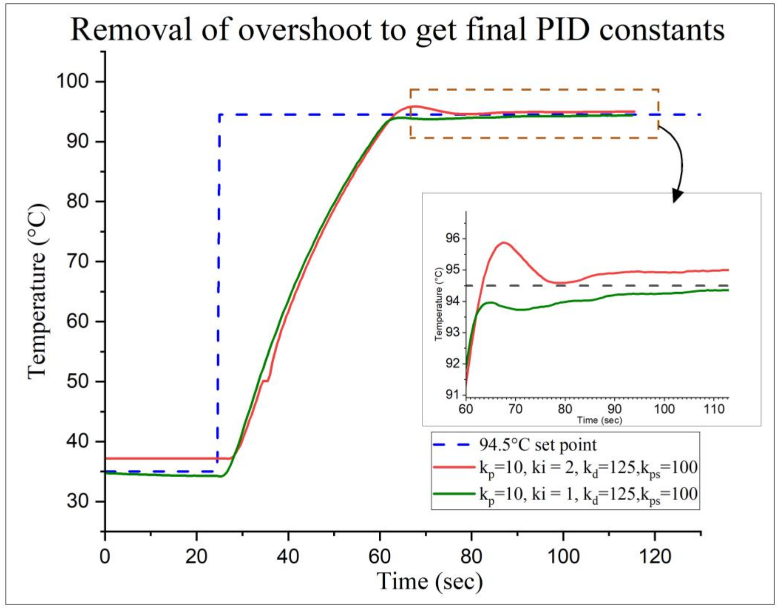
✓ Increase $k_i$ till you get steady state error in acceptable range

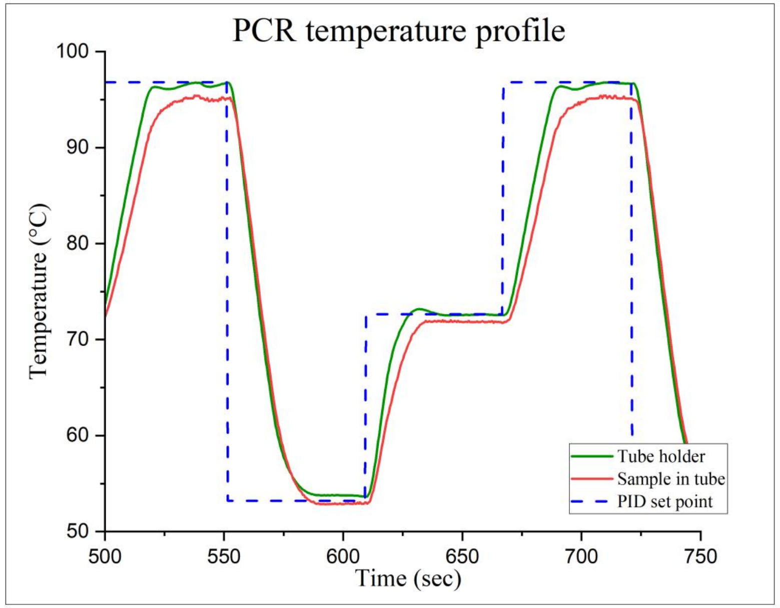
- **Reduced ki to get critically damped system**

# PID Tuning


Proportional and derivative controller
Temperature (°C)
Time (sec)
$k_p$ = 10
94.5°C set point
$k_p$=10, $k_d$ = 70
$k_p$=10, $k_d$ = 35


PD vs PID controller
Temperature (°C)
Time (sec)
94.5°C set point
$k_p$=10, $k_d$ = 35
$k_p$=10, ki = 2, $k_d$=125,$k_{ps}$=100


Removal of overshoot to get final PID constants
Temperature (°C)
Time (sec)
94.5°C set point
$k_p$=10, ki = 2, $k_d$=125,$k_{ps}$=100
$k_p$=10, ki = 1, $k_d$=125,$k_{ps}$=100


PCR temperature profile
Temperature (°C)
Time (sec)
Tube holder
Sample in tube
PID set point

# Challenges

| | |
|---|---|
| 1 | Poor heat transfer between tube holder and sample |
| 2 | Condensation on side walls of tube |
| 3 | Noise in temperature sensor |
| 4 | Micro-controller out of stock due to chip shortage |
| 5 | Accurate temperature sensor calibrations |
| 6 | Time required for self-heating of temperature sensor |
| 7 | Size of temperature sensor larger than sample size |
| 8 | SI2302 MOSFET damage exceeding $P_{max}$ of MOSFET |
| 9 | Damage of power supply due to short circuit |
| 10 | Spike in output voltage of supply damaging the controller |

# Challenge: Poor heat transfer

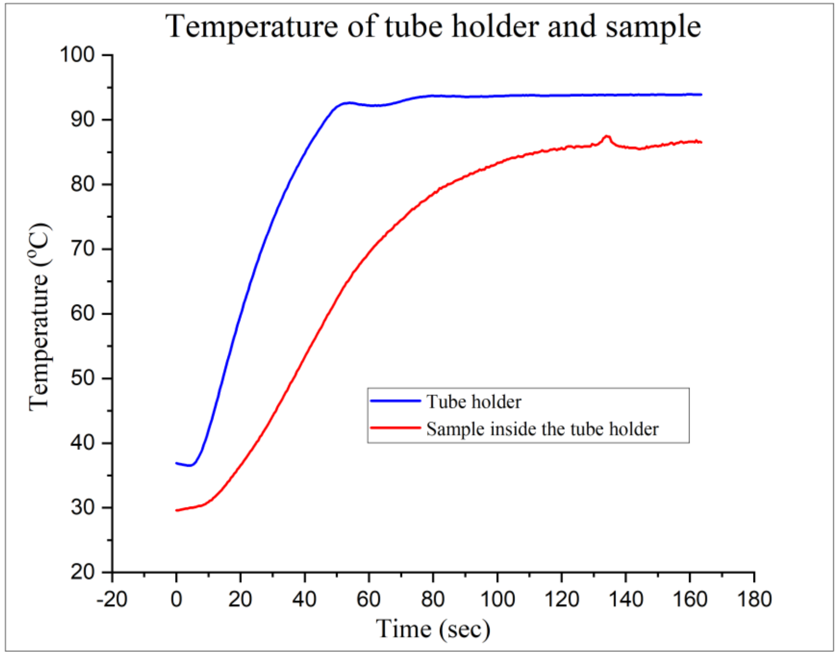
We tried amplifying 503bp fragments of bacteriophage Phi-6 (surrogate for SARS-CoV-2) and failed in getting a band in agarose gel electrophoresis

**Reason**

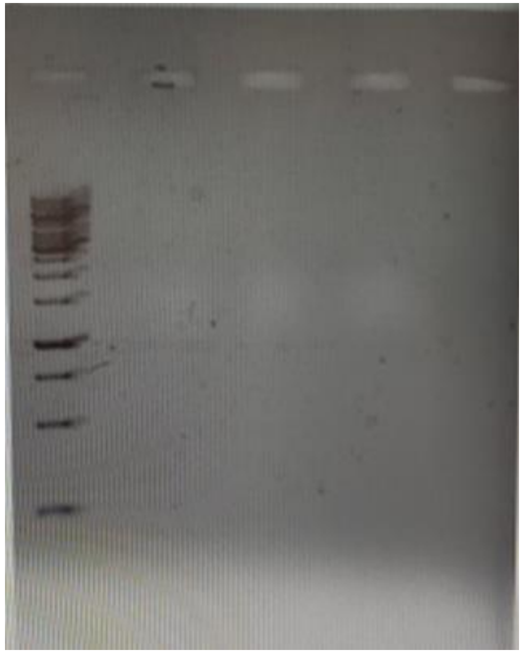
Sample inside the test tube could not reach the temperature of tube holder

* Use of 0.5ml tubes

* Tube not covered fully by tube holder




ladder NTC PC



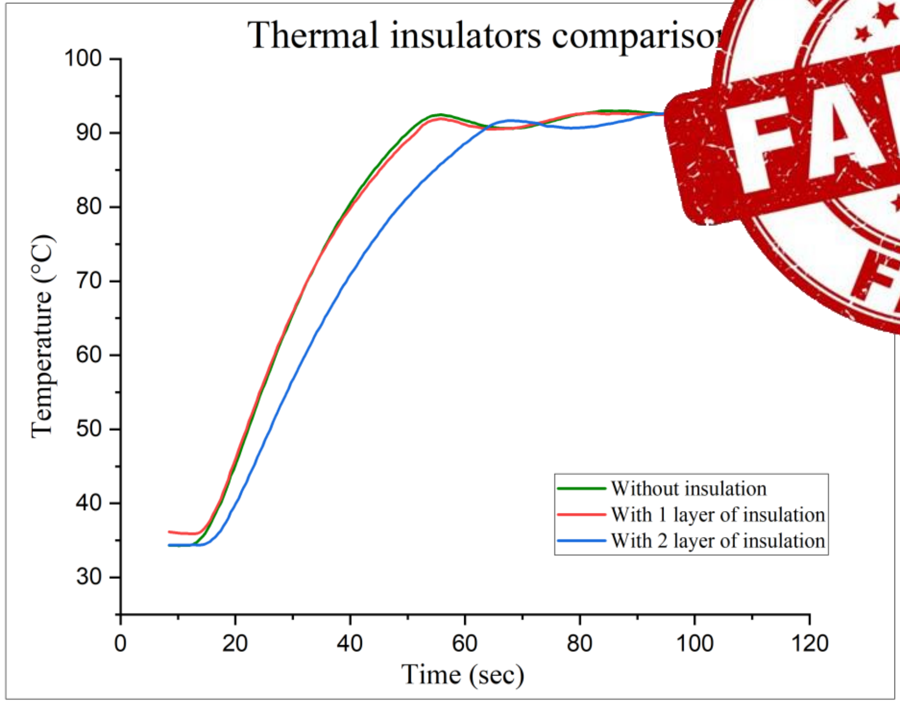
First test using 503bp fragment of Phi6

Tube side walls not fully covered by tube holder

# Solutions

## Solution 1: Insulation layer

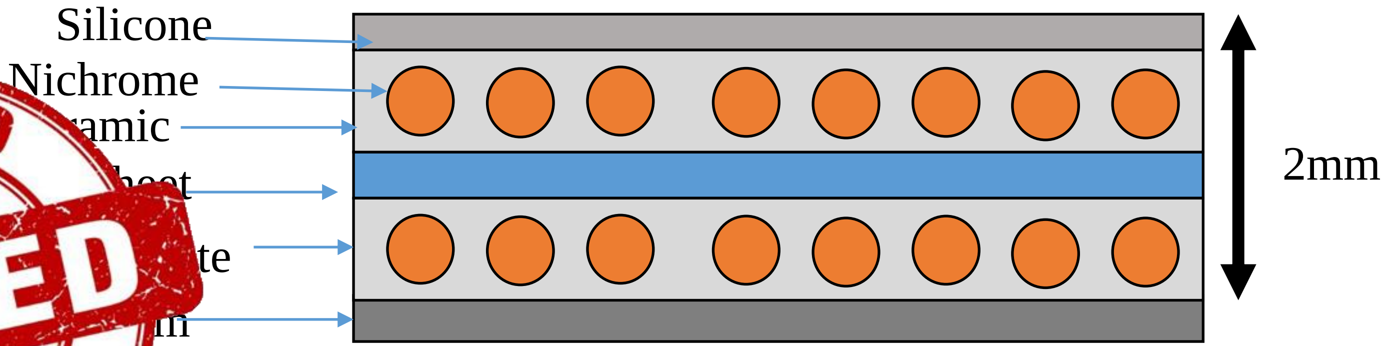
Applied thermal insulation tape covering full body of tube holder

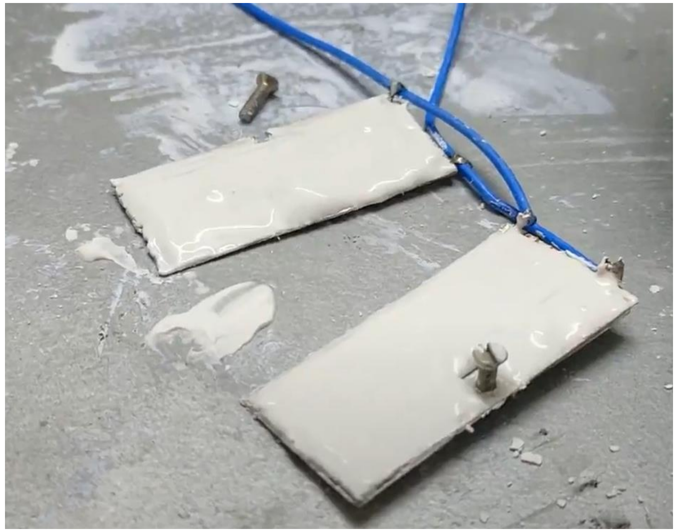




## Solution 2: Nichrome heater

Connected nichrome heater to tube holder to heat at higher temperature



# Challenge: Condensation

- While performing PCR we observed that no sample was present at the bottom of the tube where actual thermal cycling was being performed

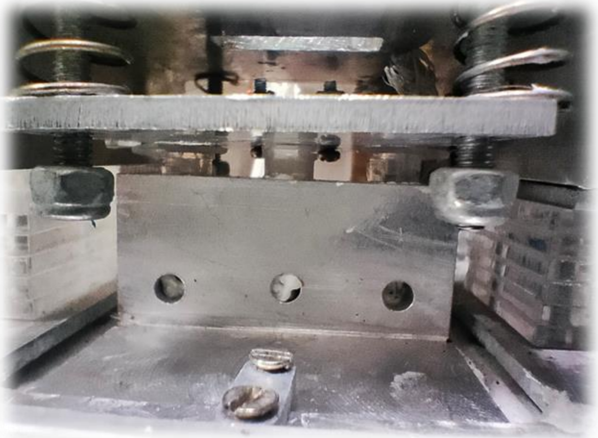
**Reason:**

- Uncovered part of the tube remains at low temperature causing condensation

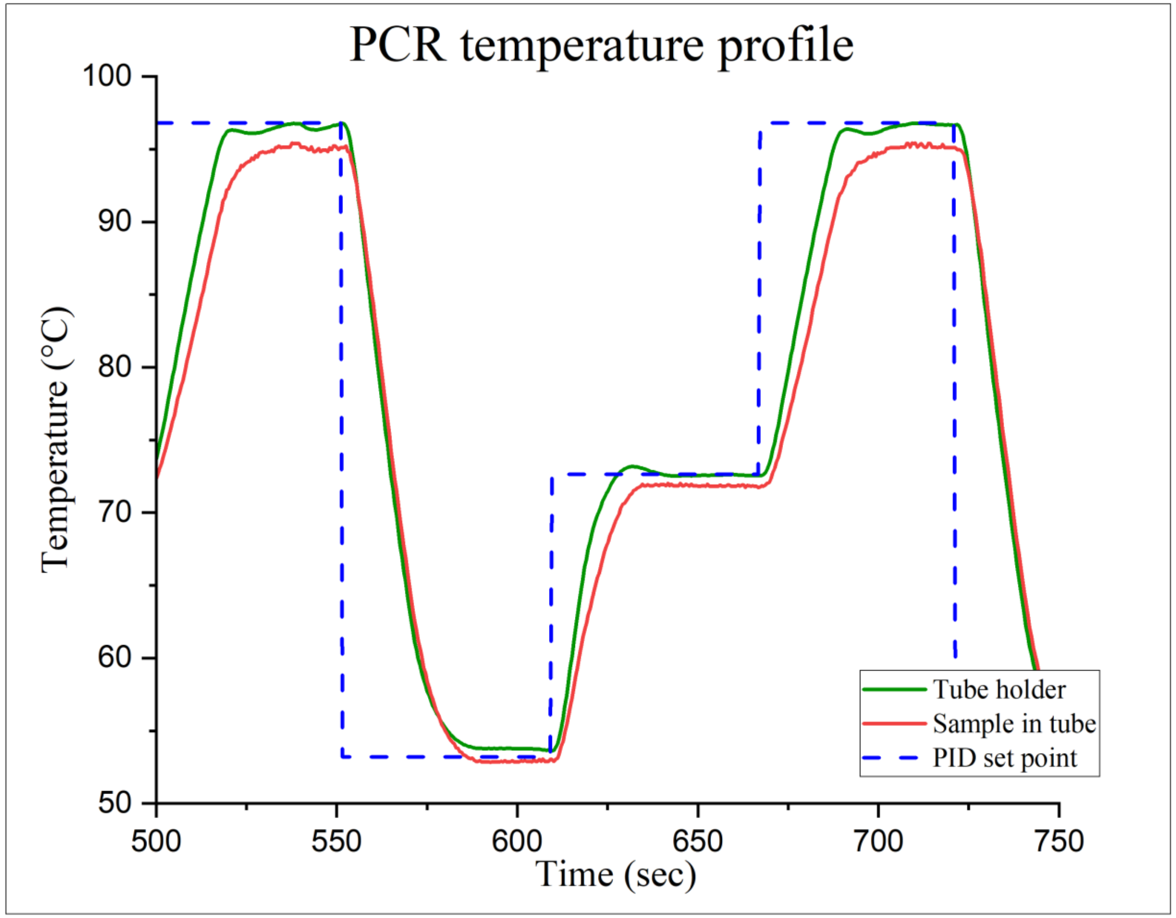
# Solution

***Re-designed tube holder for 0.2ml tubes***

Before



After



# Challenge: Noise in temperature sensors

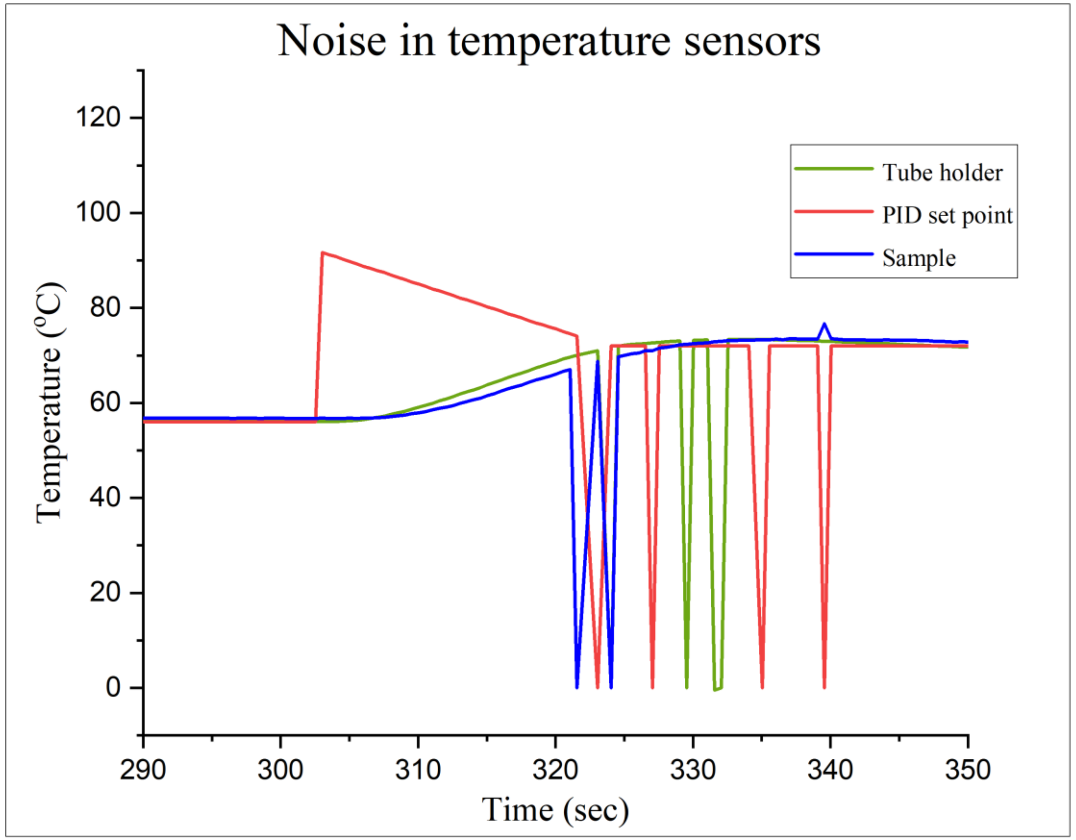
- Wrong feedback by sensors to the control system (PID controller)

**Reason:**

- Peltier is driven by PWM of 12V amplitude, creating Electro Magnetic Interference (EMI) in nearby sensors

**Solution:**

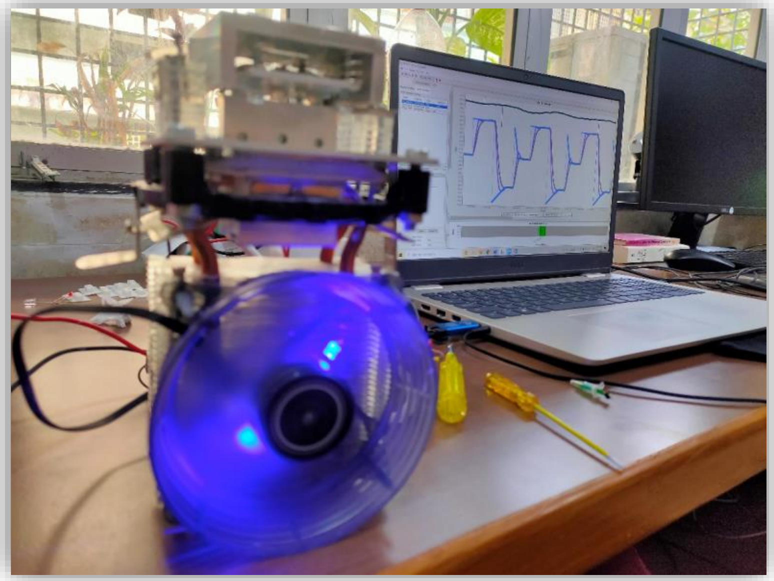
- Connecting full mechanical assembly to ground of the circuit



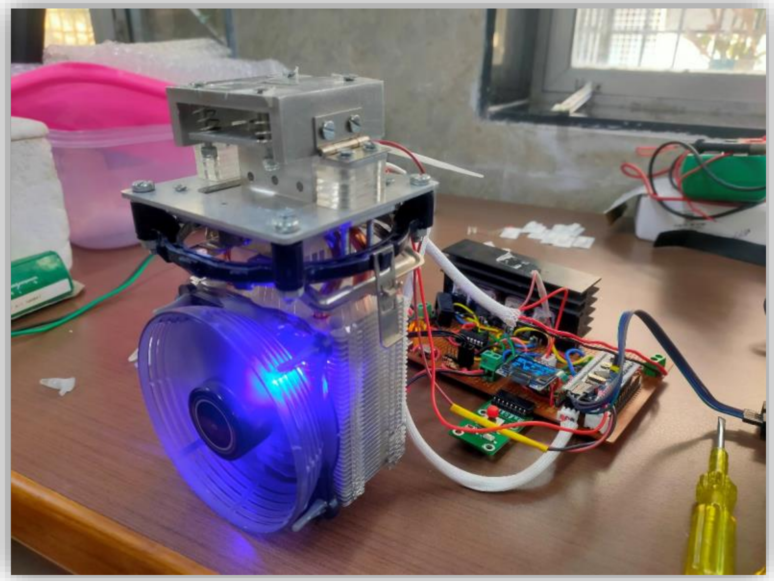
| Challenges | Solution | Remark |
|---|---|---|
| Poor heat transfer between tube holder and sample | Insulation of tube holder | Failed |
| | Additional nichrome coil heater along with Peltier | Failed |
| Condensation on side walls of tube | Tube holder designed for smaller tubes covering entire tube sidewalls | Successful (Solved poor heat transfer problem too) |
| Noise in temperature sensor | Connecting full mechanical assembly to ground of the circuit | Successful |
| Micro-controller out of stock due to chip shortage | De-soldered microcontroller from old board | Successful |
| Accurate temperature sensor calibrations | Calculated different equations for each temperature sensor | Successful |
| Time required for self heating of temperature sensor | Use of smaller 1mm sensor | Successful |
| Size of temperature sensor larger than sample size | Use of smaller 1mm sensor | Successful |
| SI2302 MOSFET damage exceeding $P_{max}$ of MOSFET | Use of ON/OFF switch instead of PID controller which operates MOSFET in cutoff or linear region | Successful |
| Damage of power supply due to short circuit | Use of fuse | Successful |
| Spike in output voltage of supply damaging the controller | Use of Transient Voltage Suppression(TVS) diode | Successful |

# Results: Set-up for first successful test

*using 503bp fragment of bacteriophage Phi6 (surrogate for SARS-CoV-2)*

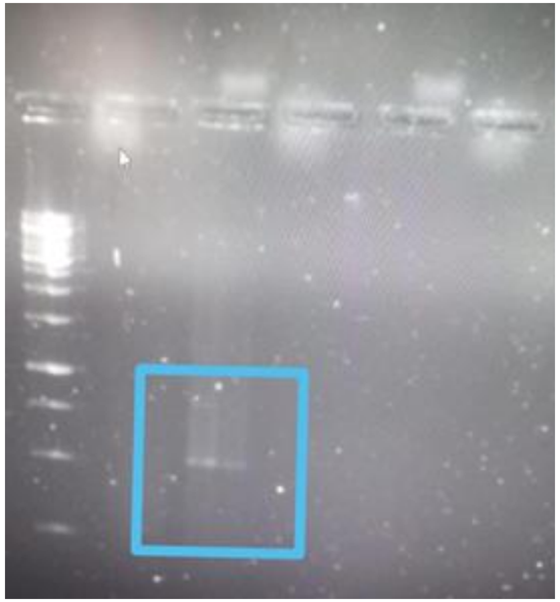


PCR with live temperature graph on laptop



Mechanical assembly connected to the circuit assembled on zero PCB

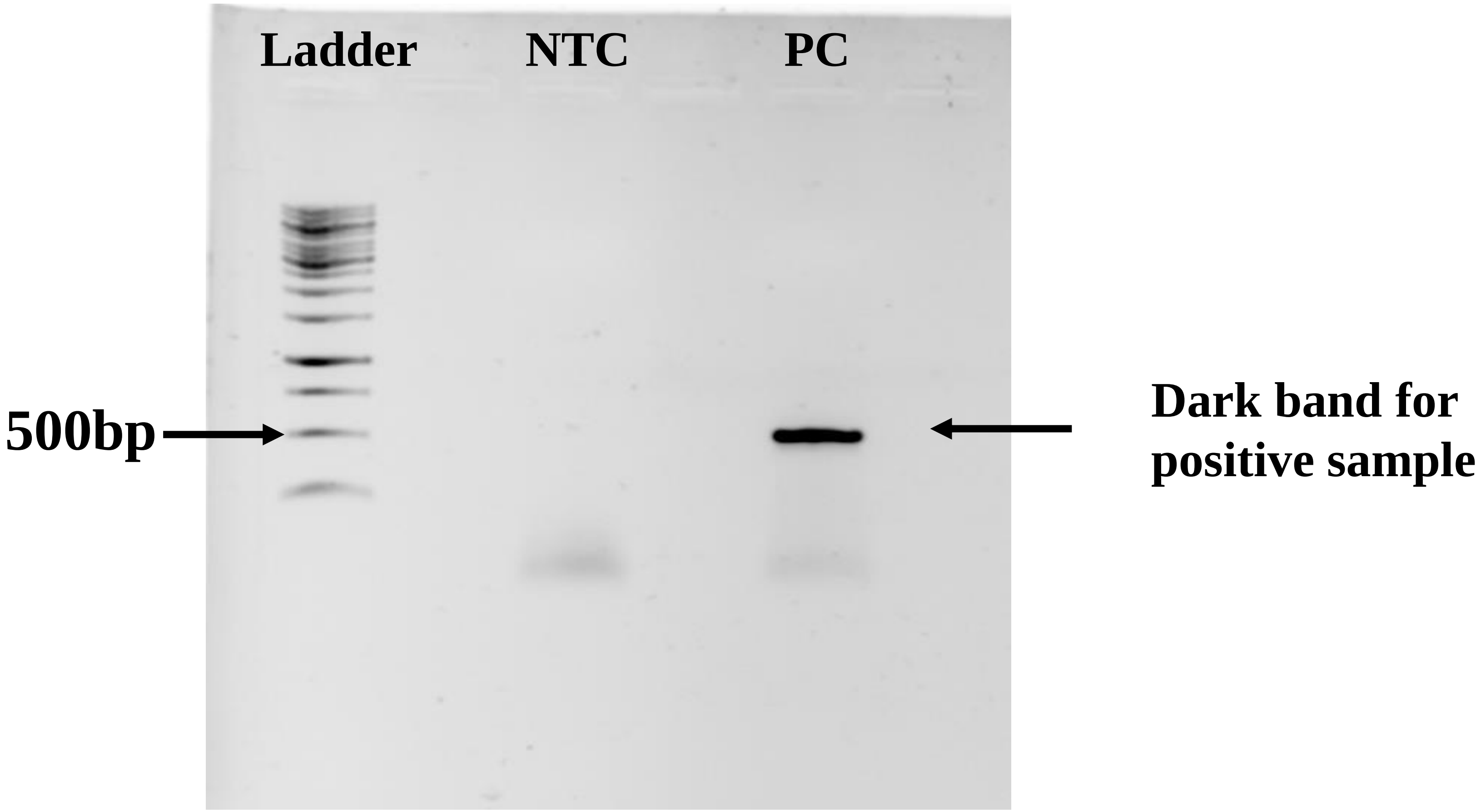
ladder NTC PC



Agarose gel electrophoresis result

# Results: Second successful test

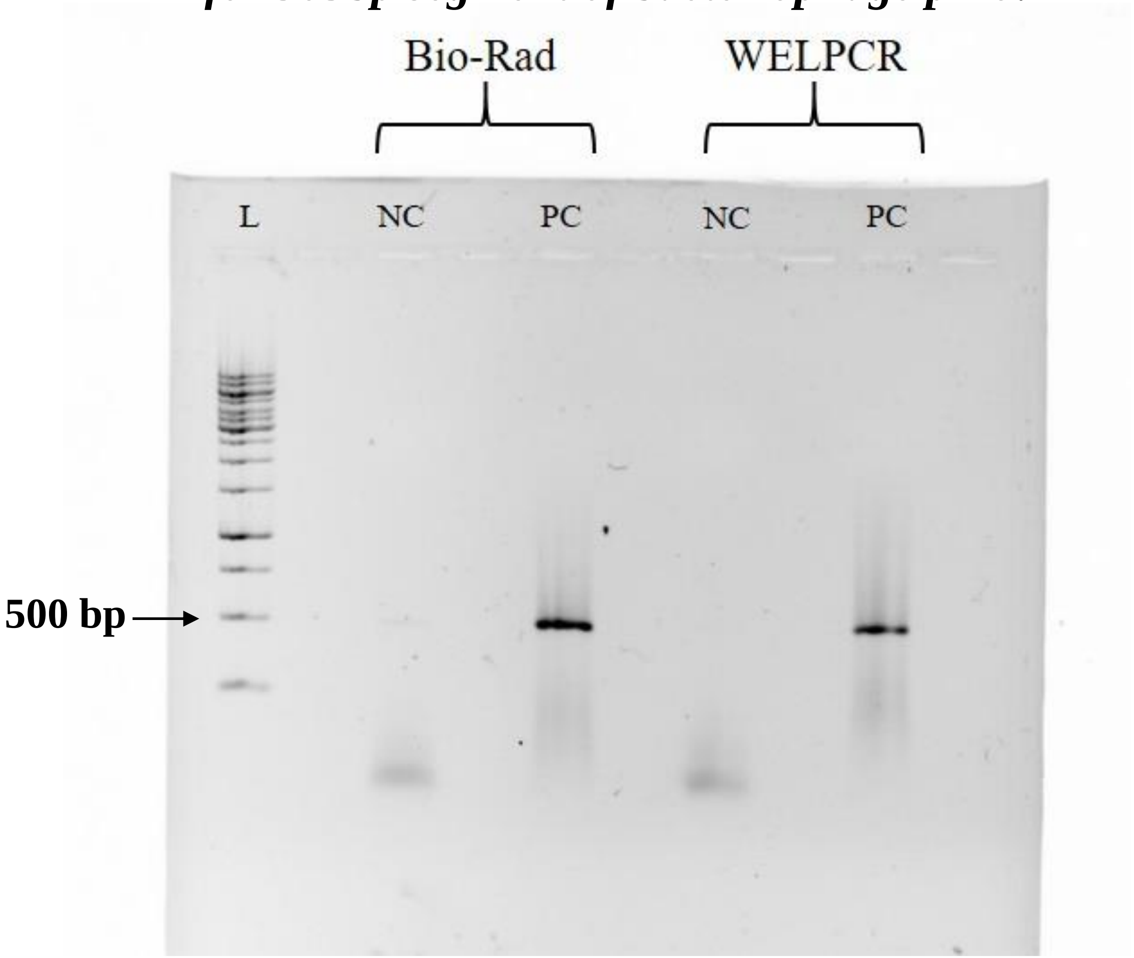
*using 503bp fragment of bacteriophage Phi6 (surrogate for SARS-CoV-2)*




Agarose gel electrophoresis result

# Comparison of WELPCR and Bio-Rad

***Comparative image of agarose gel electrophoresis for 503bp segment of bacteriophage phi6.***

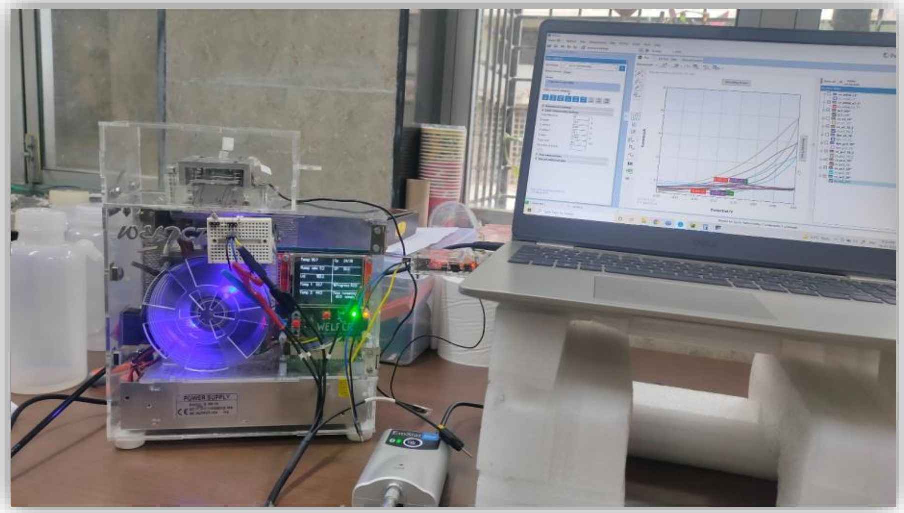



***Estimation of amplified DNA (Nanodrop Spectrophotometer at 260nm)***

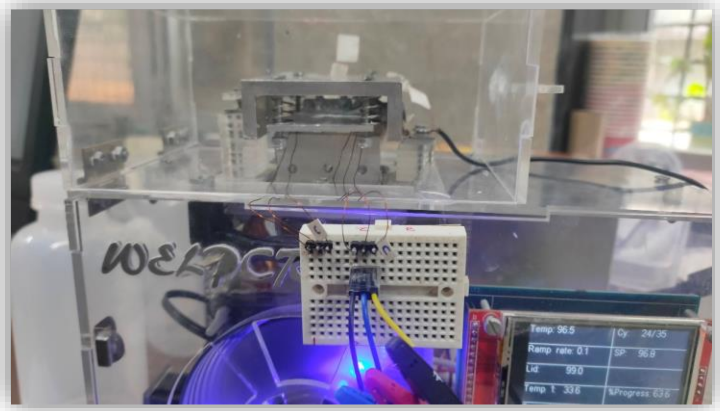
| | Thermal cycler | Concentration (ng/µl) |
|---|---|---|
| 1 | Bio-Rad MJ Mini$^{TM}$ Personal thermal Cycler | 20.1 |
| 2 | WELPCR | 16.5 |

# Real time sensing using EMStat Potentiostat

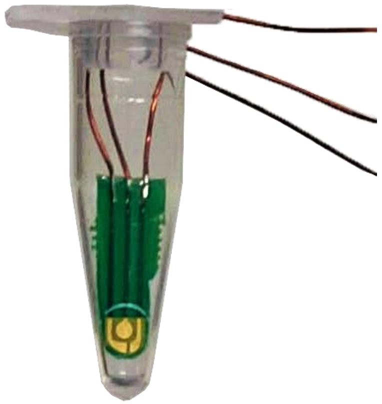
*Setup for real time testing*

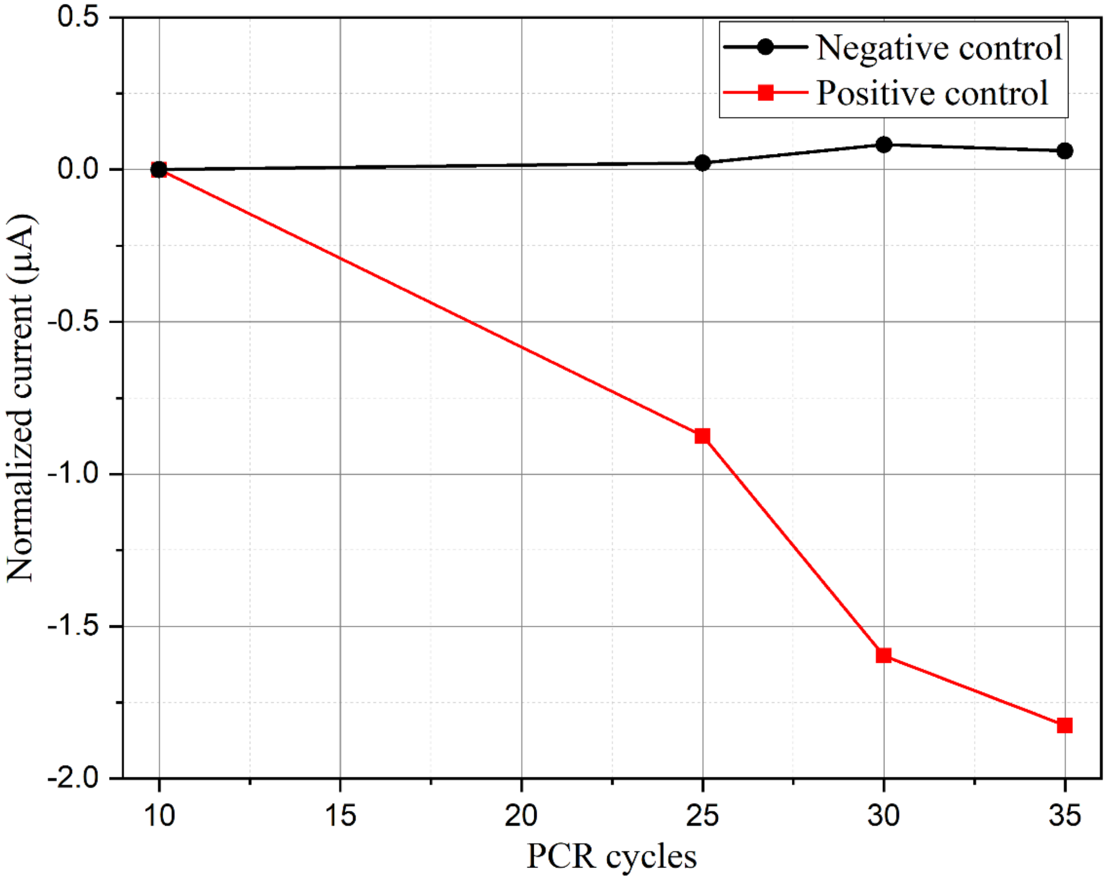


*Electrode wire connection*



*Micro-electrode inserted in tube*






***Real time PCR testing result***

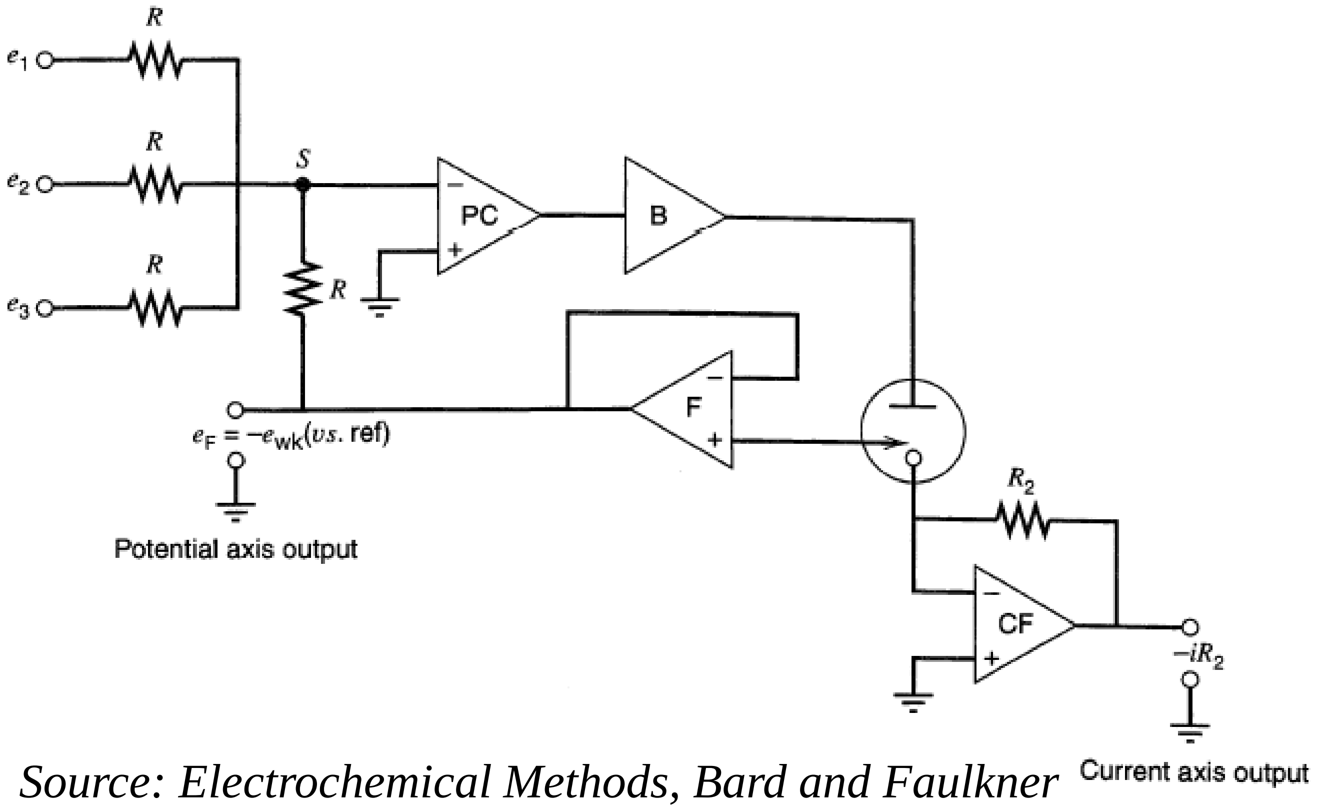
# Comparison

| | WELPCR, EE IITB | OpenPCR (open-source PCR machine design) | miniPCR® mini8 | Biorad CFX96 real time PCR | Himedia Wee-32™ |
|---|---|---|---|---|---|
| **Maximum ramp rate** | 2°C/s | 1°C/s | 2.4°C/s | 2.5°C/s | 5°C/s |
| **Reverse transcription (RT-PCR) possible?** | Yes | Yes | No | Yes | Yes |
| **Sample capacity** | 9 wells | 16 wells | 8 wells | 48 wells | 32 wells |
| **User interface** | Built-in touchscreen and control panel | Software controlled (requires computer) | Mobile app; Software controlled (requires computer) | Built-in control panel and screen | Built-in control panel and screen |
| **Weight** | 1.9 kg | - | 0.45 kg | 4.1 kg | 3.2 kg |
| **Price** | BOM < Rs. 10,000 | BOM ~ Rs. 38,500 (USD $500) | Rs. 50,000 (USD $650) + shipping and taxes | ~ Rs. 15,00,000 | ~ Rs. 1,30,000 |

# WELSTAT

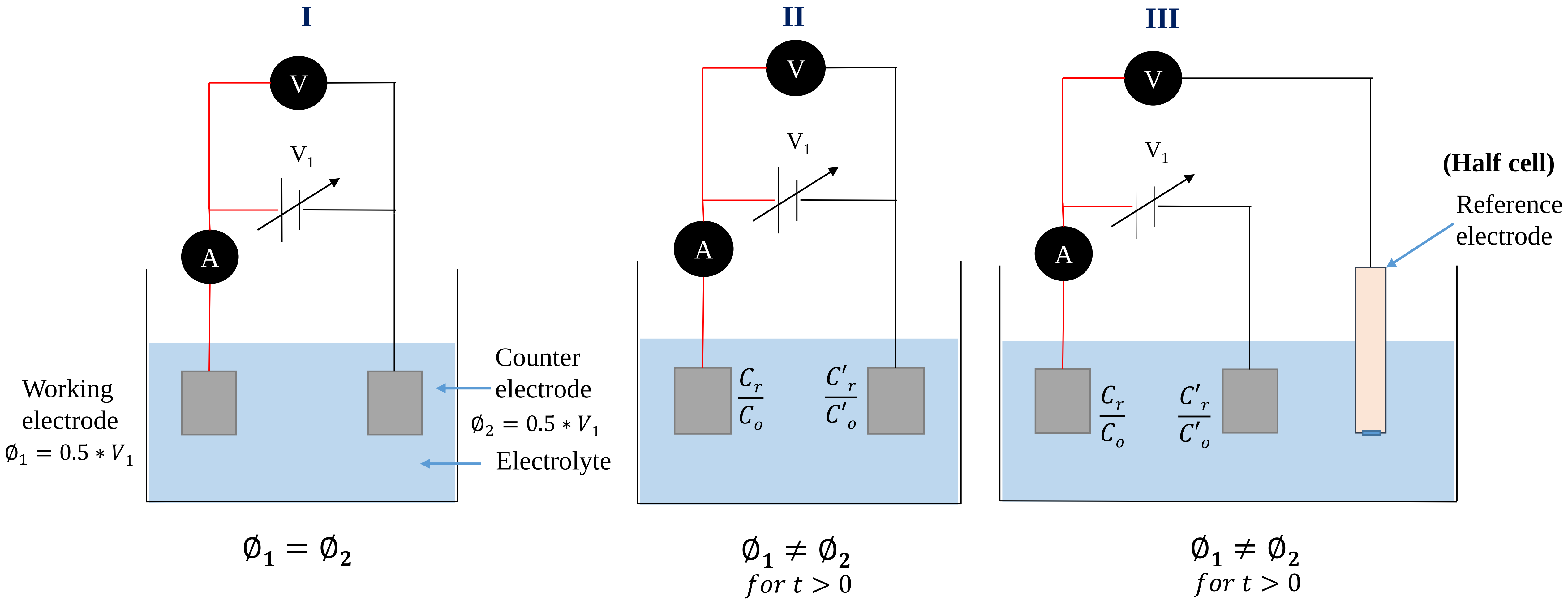
# What is Potentiostat?

- Essential tool in electrochemical research
- It allows the user to apply a potential to a system (i.e. an electrochemical cell) and measure the resulting current, or vice versa
- It can do so while keeping the path where the current flows separate from the path where the potential is measured



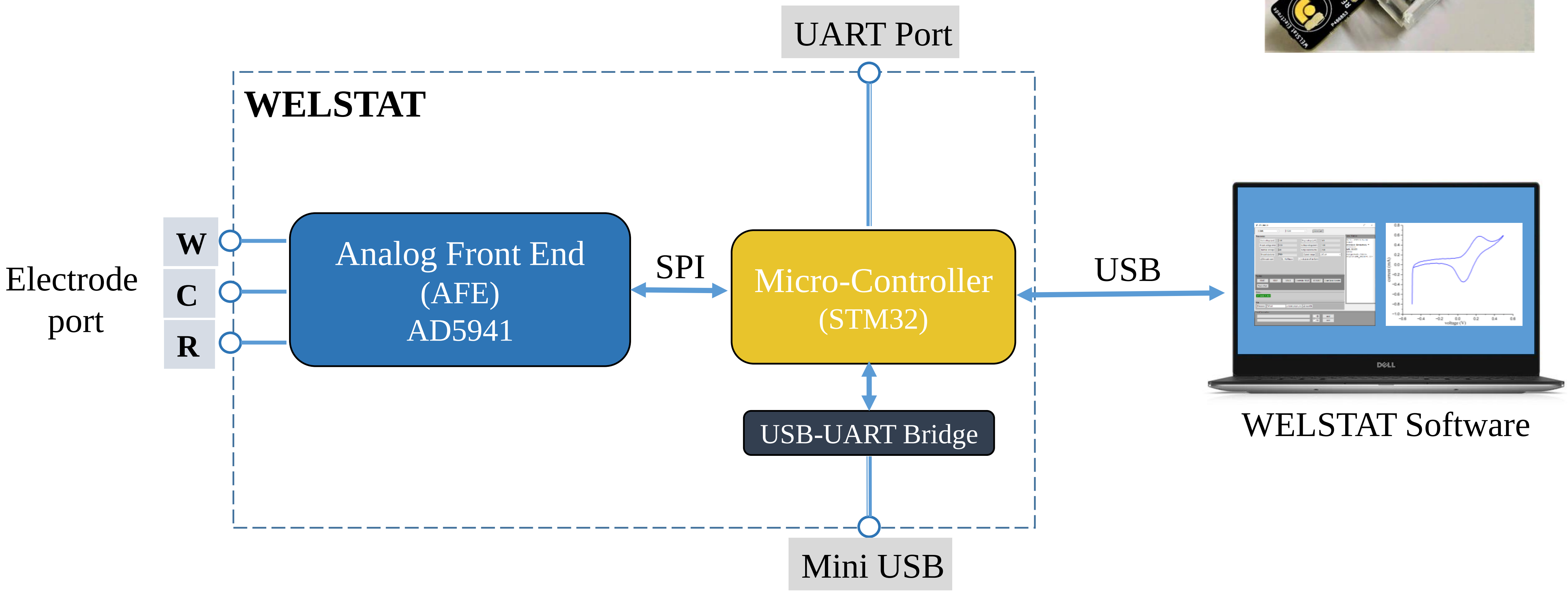

*Source: Electrochemical Methods, Bard and Faulkner*

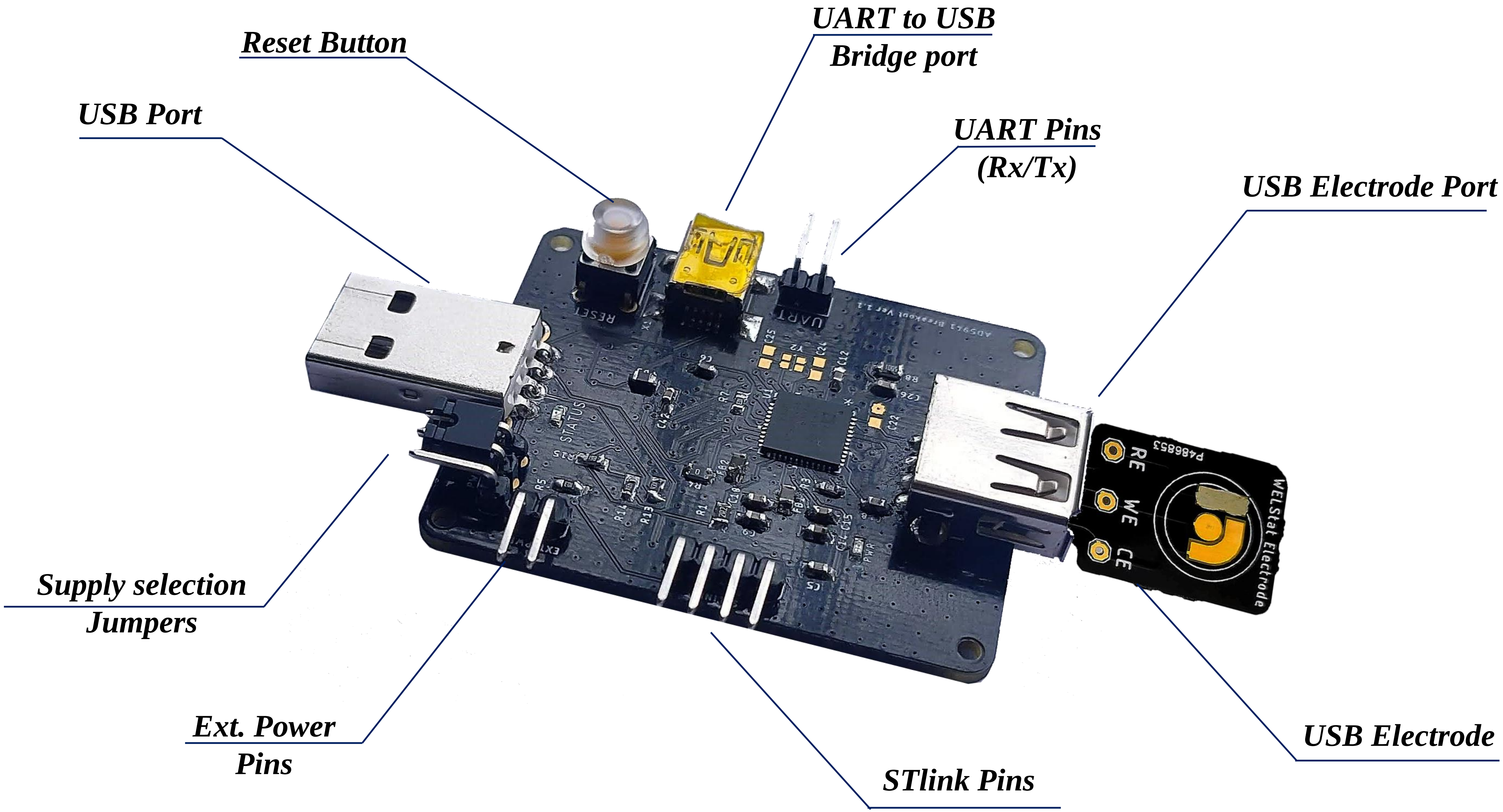
# Why do we need Potentiostat?



# WELSTAT

- WELStat is a low cost, portable potentiostat
- Currently it can perform Cyclic voltammetry (techniques like DPV can be added)
- Used for detecting target DNA ( amplified by our WELPCR)

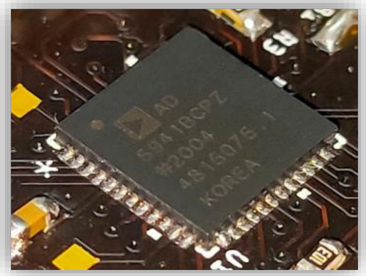



Reset Button
UART to USB Bridge port
USB Port
UART Pins (Rx/Tx)
USB Electrode Port
Supply selection Jumpers
Ext. Power Pins
STlink Pins
USB Electrode
RESET
UART
STATUS
RE
WE
CE
WELStat Electrode

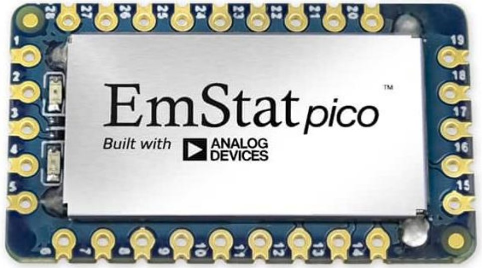
# Choosing Analog Front End IC (AFE)

We started with AD5941 to see whether it can used for our work instead of EMSTAT Pico

**AD5941**



**EMStat Pico**



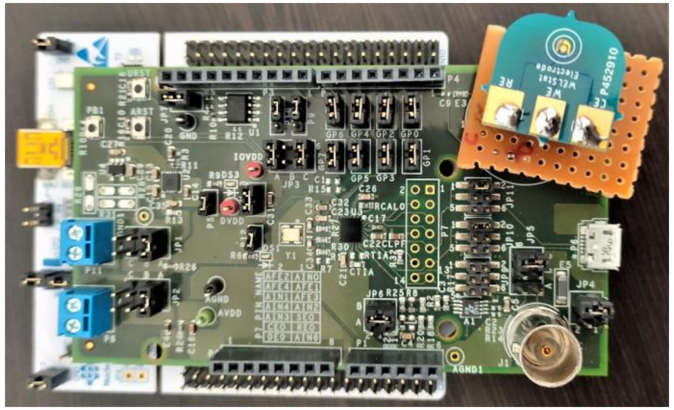

| AD5941 | EMStat Pico |
|---|---|
| **₹ 700** | **₹ 45,000** |
| Needs an external micro-controller | Micro-controller + Potentiostat |
| Not pre-programmed | Pre-programmed |
| Programing using embedded-C language | human readable scripting language for ease of programming |

| Parameter | Similarity |
|---|---|
| **Excitation voltage range** | -1.2 to +2 V |
| Max current | $\pm$3mA |
| ADC | 16 bit |
| DAC | 12bit |
| No. of Potentiostat | 2 |
| No. of channels | 2 |

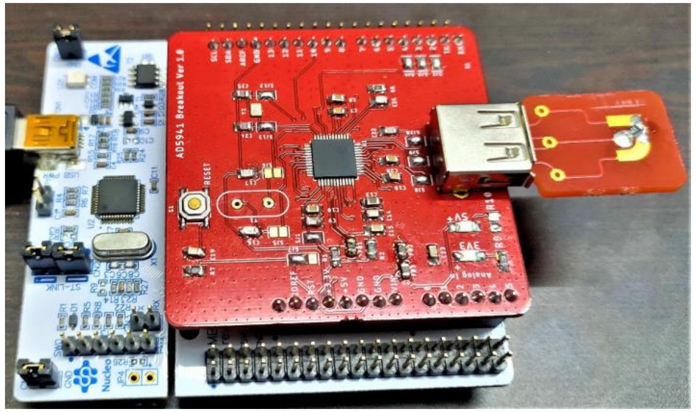
Although both EMSTAT Pico and AD5941 have almost similar specifications, the high-cost of EMSTAT Pico is mainly due to the well designed user-friendly software and accurately calibrated Potentiostat

# Testing AD5941 for WELSTAT

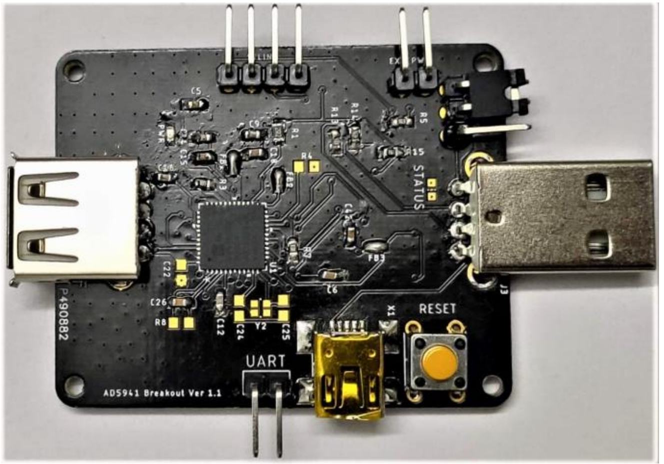
(a) Testing AD5941 for our application using readymade Evaluation board

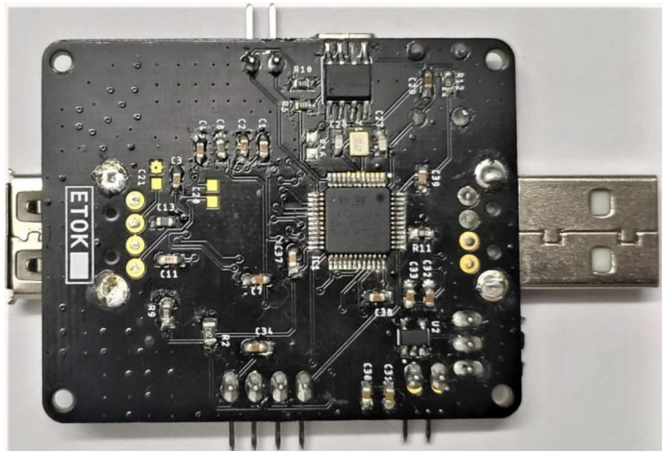


(b) Made PCB for AD5941 IC to check required minimal circuit for the IC



(c) Final PCB with AD5941 and micro-controller on same PCB



# WELSTAT 2.0

- Both AFE AD5941 and Micro-Controller STM32 on same PCB
- Data can be read using USB or UART
- Status LED to indicate status of current running task

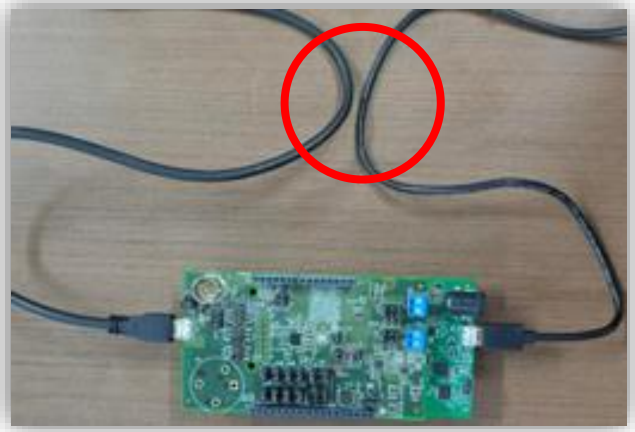

*WELStat connected to mobile and mobile showing raw data*

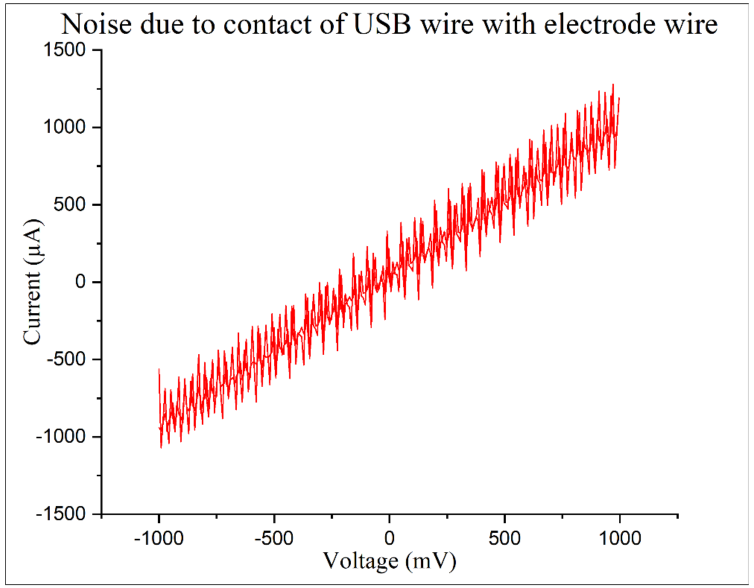
# Challenges

| | |
|---|---|
| 1 | Soldering QFN and QFP package ICs |
| 2 | Porting the Example program given by manufacture for STM32 controller |
| 3 | Electromagnetic interference (EMI) |
| 4 | Every time connector need to be soldered on electrode |
| 5 | Real time sensing while PCR reaction is in progress |

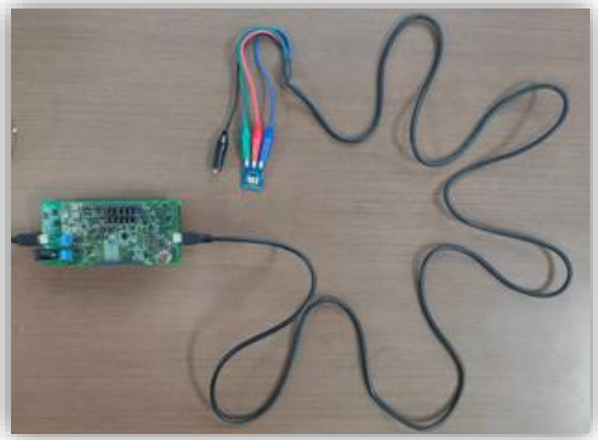
# Challenge: Electromagnetic Interference (EMI)

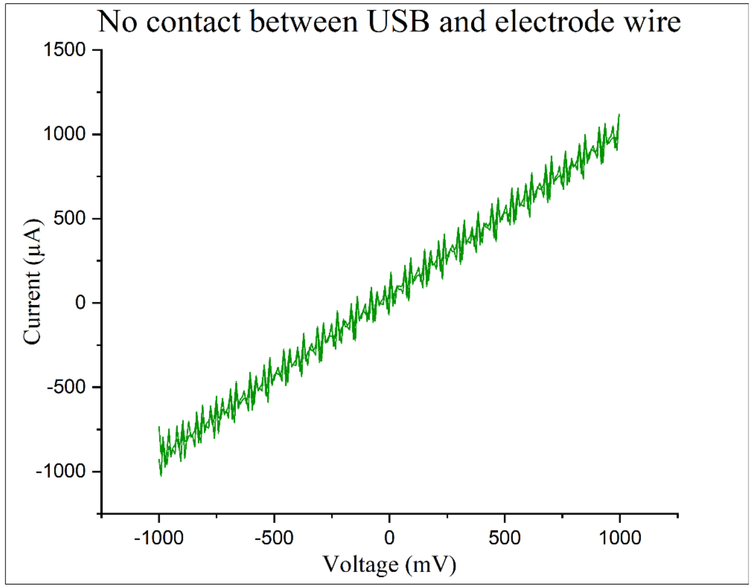
***Electrode wire in contact with signal wire***



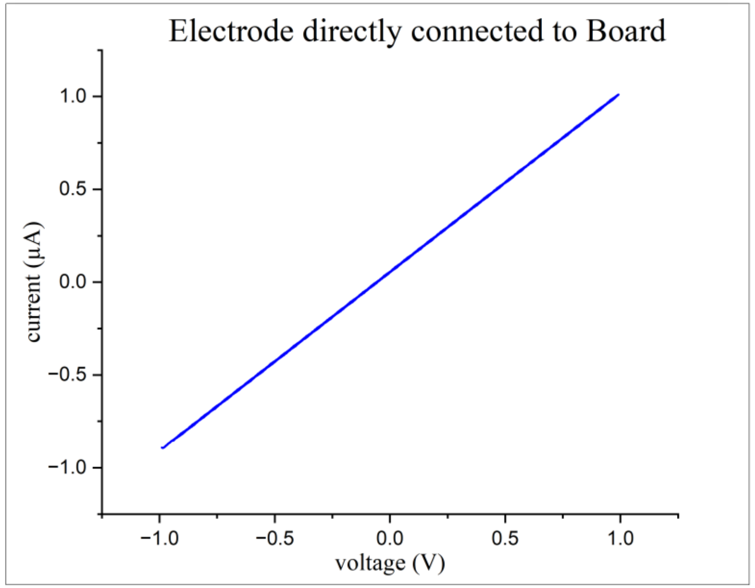



***Long wire captures signal of nearby digital devices***





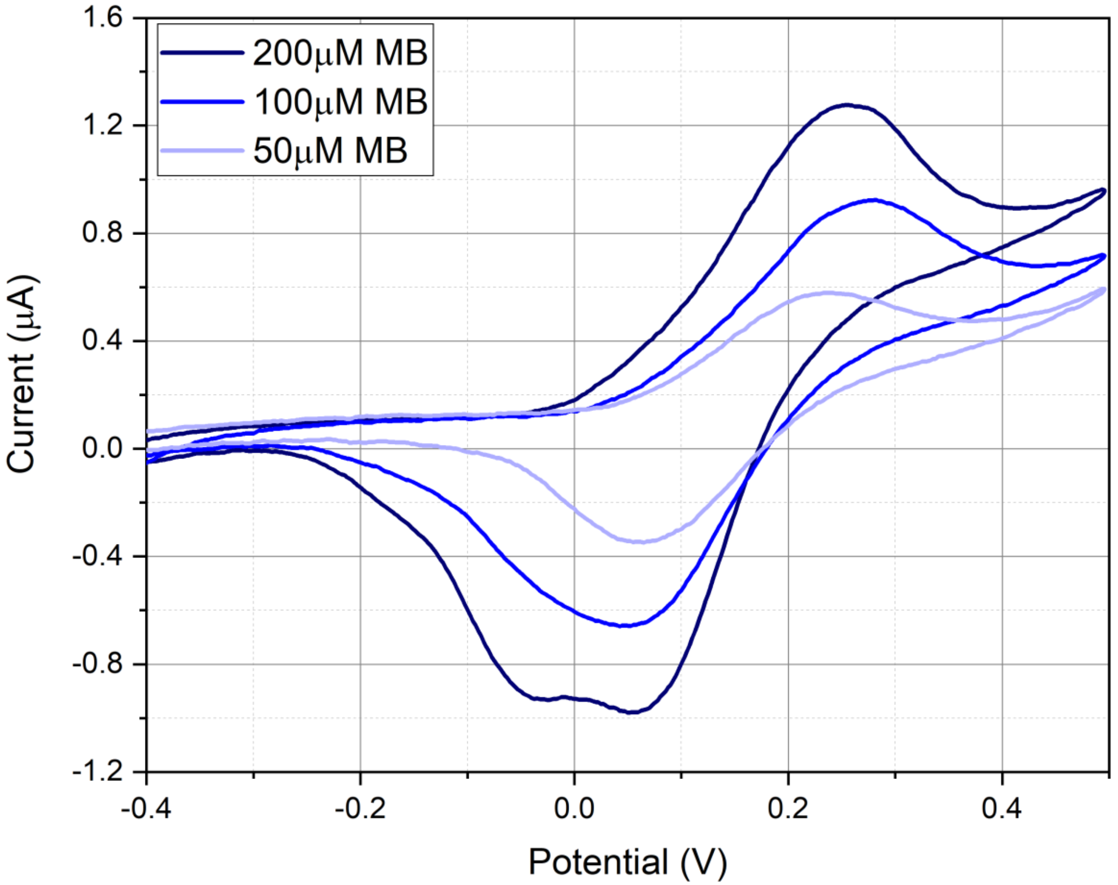

***No wire***



| Challenges | Solution | Remark |
|---|---|---|
| Soldering QFN and QFP package ICs | Self-learnt soldering QFN and QFP package ICs | Successful |
| Porting the example program given by manufacturer for STM32 controller | Converted the program given for STM32F411 to STM32F103 | Successful |
| Electromagnetic interference (EMI) | Care taken while PCB designing to minimize electrode track length as small as possible. No signal carrying track should be near the electrode tracks. | Successful |
| Every time connector needs to be soldered on electrode | USB male connector directly printed on electrode | Successful |
| Real-time electrochemical sensing of PCR amplicons | Need for further calibration of potentiostat and make it Noise-proof | Future scope |

# Electrochemical measurement using WELSTAT

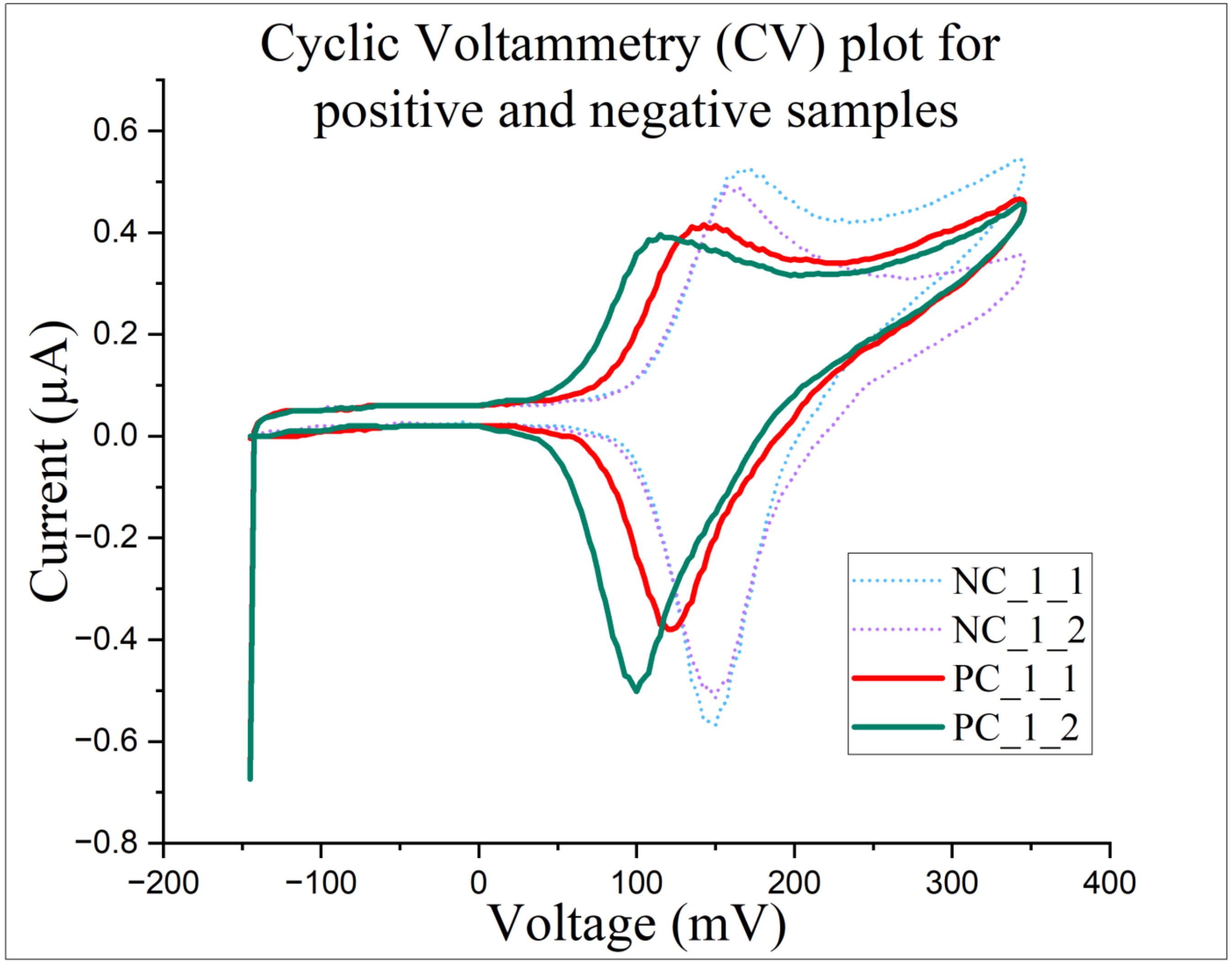
Electrochemical measurements using cyclic voltammetry (CV) for different concentrations of methylene blue (MB)




*Conclusion:*
increase in MB concentration results in an increase in current

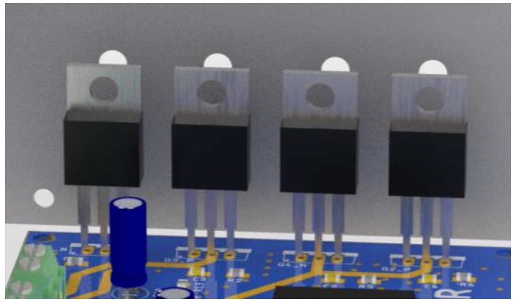
# End point PCR using WELPCR+WELSTAT

- 503bp fragment of bacteriophage Phi-6 was amplified using WELPCR
- The amplicons were incubated with 50µM methylene blue for 1 hour at 4°C
- Samples were dispensed on PCB electrode and electrochemical measurements (CV) was performed using WELSTAT



# Future Improvements

- Reduce the size of WELPCR device. The MOSFETs used in the current device require a big heat sink thereby occupying more space. These MOSFET, gate driver and heat sink can be replaced by VNH2SP30, which is an H-bridge motor driver IC to reduce the overall size of the device.



H-bridge using discrete MOSFET

VNH2SP30: H-bridge IC

- Reduce noise for WELSTAT to perform Real-time electrochemical PCR

# Acknowledgement


- Wadhwani Electronics Lab, EE, IIT Bombay
- Prof. Siddharth Tallur, EE, IIT Bombay
- WEL Lab Staff Maheshwar Mangat, Mahesh Bhagnagare, Amit Shetye, Sadanand Sawant, V V Shaheen, Suraj Sarfare
- Mechanical and Tools Lab Staff Vishwas Kewale
- WEL RA Poulami Mandal, Bhagyashree Singh
- AIMS lab members Ruchira Nandeshwar, Avani Kulkarni, Shruti Ahuja, Rutuja Chalke

# THANK YOU


WELPCR
LID_HEATER
+PELTIER-
GND
12V
FAN
EX2_TEMP
EX1_TEMP
LID_TEMP
BLOCK_TEMP
J3
J1
SUPPLY_OUT
D3
C12
PWR
R13
STATUS
USB
RESET
BUZ
IC2
U1
JP1
JP2
CLK
SWD
GND
3.3V
Q1_N
Q3_P
Q4_N
Q2_P