



Electrical Engineering Department  
California Polytechnic State University

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Senior Project  
Report

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DIY Cell Incubator

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# Table of Contents

Table of Contents.....	ii
List of Tables and Figures.....	iv
Abstract.....	v
1. Background.....	1
1.1. Project Description.....	3
1.2. Product/Technology Research.....	4
2. Specifications.....	5
2.1. Engineering Requirements.....	5
2.2. Functionality.....	6
3. Design Analysis.....	7
3.1. System Hardware.....	7
3.1.1. Enclosure.....	7
3.1.2. Microprocessor.....	8
3.1.3. Power Distribution.....	8
3.1.4. Temperature Control Hardware.....	8
3.1.5. CO2 Control System.....	9
3.1.6. Overall Schematic.....	10
3.2. Control Algorithm Design.....	11
3.2.1. Heating Control System.....	12
3.2.2. CO2 Control System.....	14
4. System Firmware.....	17
4.1. Overall architecture.....	17
4.2. CO2 Sensor Calibration.....	17
4.3. Temperature filtering.....	18
5. Testing and Verification.....	19
5.1. Discussion of Results.....	21
6. Market Research.....	22
6.1. Customer Archetype.....	23
6.2. Market Description.....	24
7. Business Model.....	27
8. Marketing Requirements.....	28

9.	Schedule Of Completion.....	31
10.	Cost.....	32
11.	Impact Analysis of Senior Project .....	32
11.1.	Summary of Functional Requirements.....	32
11.2.	Primary Constraints.....	32
11.3.	Economic.....	32
11.4.	If Manufactured on a Large Scale .....	33
11.5.	Manufacturability .....	33
11.6.	Environment and Sustainability .....	33
11.7.	Ethical.....	34
11.8.	Health and Safety.....	34
11.9.	Social and Political .....	34
	References.....	35
	Appendix A: FMEA.....	37
	Appendix B: Bill of Materials.....	38
	Appendix C: Logic Flow of CO2 Digital Control Algorithm.....	39
	Appendix D: Arduino Uno Incubator Program.....	40

## List of Tables and Figures

Figure 1: Petri Dishes of Cell Culture [12].....	1
Figure 2: Cell Incubator Housing Cell Culture [14] .....	3
Figure 3: Poliovirus Cells [13].....	4
Figure 4: Shaking Incubator [15].....	4
Figure 5: U.S. Clinical Trials Market Size, by phase, 2016-2028 (USD Billion) [8].....	22
Figure 6: Synchronized Video Microscope from Lab Smith [16] .....	25
Figure 7: Fluorescent Microscope Capture [11] .....	26
Figure 8: Business Model Canvas.....	27
Figure 9: Marketing Datasheet.....	28
Figure 10: Marketing Needs Hierarchy with Weighted Characteristics .....	30
Figure 11: Level 0 Block Diagram .....	6
Figure 12: Level 1 Block Diagram .....	7
Figure 13: DIY Incubator Enclosure.....	7
Figure 14: Arduino Uno Microprocessor Board [18] .....	8
Figure 15: Scope Capture of SPI Signal Sent from Thermocouple A/D converter .....	9
Figure 16: CO2 Pneumatic System with Soda Stream Bottle as CO2 Source .....	9
Figure 17: Electrical Schematic of Cell Incubator.....	10
Figure 18: Bang-Bang Digital Control Logic Flow for Heating System.....	11
Figure 19: Closed Loop Control System Architecture .....	11
Figure 20: Transient Response of Incubation Bay Temp with Heating Element on Full Power..	12
Figure 21: Closed Loop Incubator Heating System Modeled in MATLAB .....	14
Figure 22: Simulation of Heating System MATLAB model.....	14
Figure 23: CO2 Concentration Within the Incubation Bay After CO2 is Turned on(left) and Turned on (right).....	15
Figure 24: Closed Loop Incubator CO2 System Modeled in MATLAB.....	16
Figure 25: Simulation of CO2 System MATLAB model.....	16
Figure 26: Relationship Between CO2 Sensor Voltage and Ambient CO2 concentration.....	18
Figure 27: Temperature Cycle of Closed Loop Heating System Compared with Simulation.....	20
Figure 28: CO2 Concentration Cycle of Closed Loop System Compared with Simulation .....	21
Figure 29: Gantt Chart of Entire Project.....	31
Table 1: Target Customer Archetype.....	23
Table 2: Market Leaders in the Cell Culture Industry .....	24
Table 3: Advantages and Disadvantages of DIY Incubator Compared to "run-of-the-mill" Incubators.....	25
Table 4:Different Marketing Design Features and their Level of Importance .....	29
Table 5: Pairwise Comparison of Major Needs to Fulfill.....	29
Table 6: Engineering Requirements.....	5

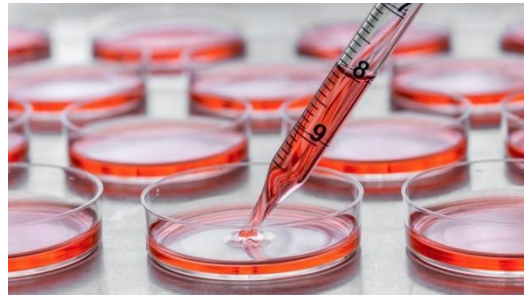
## Abstract

The purpose of creating a cell Incubator is for the development of cell and tissue production in laboratory settings. Large scale research projects and the medical community grow cells for various reasons, including experiments and creating tissue for patients. However, they cannot simply depend on growing cells in a petri dish that sit on a rack at room temperature. To grow healthy cells in the fastest way possible, they use cell incubators. Cell incubators create an atmosphere within the incubation bay that is designed to promote cell growth. The three main components that need to be constantly regulated, using a feedback process, are temperature, humidity, and pressure of carbon dioxide. For optimal cell growth, the incubation bay needs to create an environment with controllable and accurate temperature, humidity, and a CO<sub>2</sub> concentration.

Budget is a very large factor with any biomedical company or research university. Being able to purchase extremely expensive lab equipment oftentimes presents many obstacles. Most large and expensive incubators that can house a moderate amount of cell samples can exceed the price range of \$5000 [1]. In addition, the incubator is completely enclosed which often advantageous, but this means that there cannot be any extra measuring equipment placed into the incubator with it. However, this project creates a cell incubator that is for the smaller scale use. This would have a smaller incubating bay, but would, in turn, be cheaper to produce and have the capabilities powering an electronic microscope to observe the cells while in the incubation bay. Thus, the incubator would be more accessible to a larger audience that would not otherwise have access to a large-scale cell incubation. Also, the DIY-nature of the incubator allows for more flexibility with how the cells are incubated during the experiment.

# 1. Background

From growing viral cells used in vaccine testing to assisting the growth of human tissue, cell culture provides one of the largest tools for the study of biology and modern-day medicine. Cell culture refers to the process of removing cells from their natural environment and growing them in an artificial



*Figure 1: Petri Dishes of Cell Culture [12]*

environment [2] as shown in Figure 1. In the instance of growing tissue, the natural environment would be a on a human and the artificial environment would be the controlled environment of a laboratory. When treating cells in the lab, many lengths are gone to promote and optimize cell growth. To accomplish this task, scientists must know how cells grow, what nutrients needs to be given to the cells while growing, and the type of environment to keep the cells in while growing.

In analyzing the cell growth process there are two types of cells to define when considering how to grow them: primary cells and established cell lines [3]. Primary culture begins with taking cell samples directly from the natural environment like a living organism. When growing these cells, there is only a certain number of times that the cells can divide before they enter a stage called senescence, where they can no longer divide [4]. Unlike primary cells, established cell lines can reproduce indefinitely because they have either been chemically altered or have gone “through a process called transformation” [2]. Despite how long each of the types grow, both require ideal conditions to grow at a fast and healthy rate.

Next in the formula for cell reproduction, the cells need to be given the nutrients they would normally receive while in their natural habitat. To provide continuous vitamins, inorganic

salts, amino acids, and pH regulation [3], cells are kept in flasks of natural medium which mimic the properties of natural body fluids [5]. Not only do the cells have to think they are in their natural habitat, but they also need to be fed like they are. Serums provide sustenance for cell growth. Oftentimes derived from bovine(cattle) fetuses [5], serums contain macromolecules, lipids, growth factors, etc. for the cells to feed from. Finally, the last piece of the cell growth puzzle requires this solution to be held at an ambient temperature identical to that of its natural environment.

Once chemical treatment of the cells is completed, they are ready to be placed somewhere where ambient conditions match that of their natural environment. There are three factors to consider: temperature, carbon dioxide concentration, and humidity. Each of the three factors will vary depending upon what type of cells are trying to be grown, but temperature is the most intuitive. Mammalian and human cells need to be maintained at around 36°C to 37°C for optimal growth while cold blooded animal cells need to be regulated between 15°C and 26°C [4]. Carbon dioxide concentrations are a little more uncharacteristic between type of cells. Instead, ideal CO<sub>2</sub> concentration varies randomly from cell to cell, but all within the range of 4–10% CO<sub>2</sub> [4]. Slightly less important to regulate, but important to monitor is the humidity of the environment, which should be around 85-95% humidity [6].

## 1.1. Project Description

To provide an environment where cells can have these specific atmospheric conditions, lab technicians use specialized cell incubators. Cell incubators house samples of cell culture



*Figure 2: Cell Incubator Housing Cell Culture [14]*

where scientists can adjust the three previously discussed environmental conditions via user input. As shown in Figure 2, cell incubators hold a limited amount of cell samples within a largely insulated and tightly enclosed incubating bay. This is very important in keeping the environment within the bay accurate to the tolerances of the user input, but it can present two large issues for

customers. With so much bulk and high-quality materials, cell incubators are extremely expensive. The other issue originates from the cell incubator being *too* well enclosed. Because incubators completely seal the incubation bay off from the outside environment, no measurement equipment can be placed within the incubator to examine the cell culture during incubation.

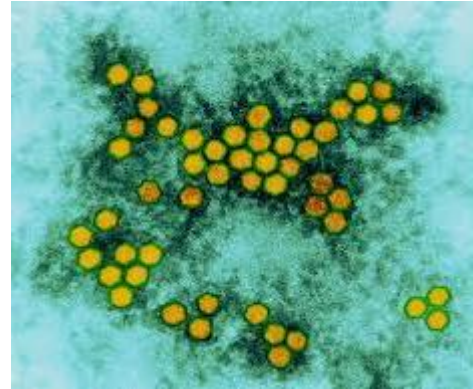
The DIY cell incubator project aims to solve both problems. Being constructed at the California Polytechnic State University Biomedical Department, the custom cell incubator can be tailored to meet budget and specific project requirements. The experiment currently being ran by Professor Ben Hawkins requires a large piece of lab equipment to be placed within the incubation bay so measurements can be taken while the cells are growing. The piece of equipment being an electronic microscope that must be plugged in during use. This means the DIY incubator will have a wired port to allow for equipment functionality within the incubation bay.



## 1.2. Product/Technology Research

Cell culture research first successfully started using cell incubators during the 1930's to grow poliovirus cells as shown under a microscope in Figure 3. Before this, researchers used live animals to grow the virus for scientific research [3].

Eventually, the cell incubator was created to emulate the natural characteristics of living within organisms. This led to the ability to produce massive amounts of cell culture and has helped scientists create almost all the vaccines we still use today, including the polioviral vaccine.



*Figure 3: Poliovirus Cells [13]*

Today, cell incubators are widely used everywhere in industry with many different features from the first ones produced back in the 1900's. Today, there are incubators that incorporate functions like shaking in the incubation bay to promote mixing, circulation of air to ensure even temperature distribution, and having an alternative power source during a power outage [7]. Each of these vary in size from a benchtop incubator to incubating rooms allowing different niches to be filled. The DIY cell incubator aims to combine one of these new technologies along with affordability and integrating measuring equipment in the tabletop size.



*Figure 4: Shaking Incubator [15]*

## 2. Specifications

### 2.1. Engineering Requirements

To meet marketing requirements, the project needs constraints for the engineering design to adhere to. Engineering requirements are created to address the connection between the marketing requirements and the engineering design process. The main engineering requirements are tabulated below in Table 6. Each of the requirements have a target, tolerance, and risk.

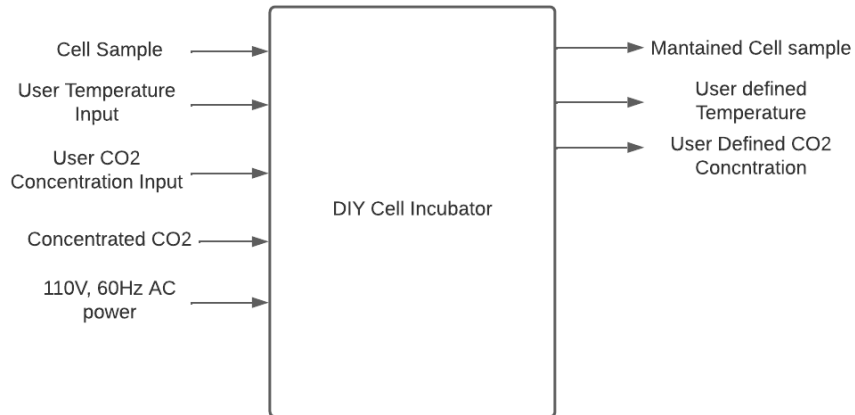
Tolerances were created by using the research data from the background selection that allows for prosperous cell growth. Risk factors (H=high, M=medium, L=low) represents the importance that each of the requirements are built to target within tolerance. These were created using the marketing requirements that appeal to the customer and are outlined in depth in Appendix A in the FEMA.

*Table 1: Engineering Requirements*

Requirement #	Parameter Description	Requirement or Target (units)	Tolerance	Risk
1	Temperature Control	20-45°C	± 2°C	H
2	Correction Period	15 Min	Max	L
3	Production Cost	\$1000	Max	M
4	Size	24" x 18" x 18"	Max	L
5	Incubation Space	1 cubic foot	Min	M
6	AC Voltage Power Source	120Vrms	± 10Vrms	H
7	Power Source Freq	60Hz	± 1Hz	H
8	CO2 Control	0-20% CO2 Concentration	± 0.25%	H

## 2.2. Functionality

With the engineering requirements in mind, the high-level design of the project is created using block diagrams. Figure 11 shows the highest-level block diagram that shows the various inputs and outputs of the system.



*Figure 5: Level 0 Block Diagram*

A more in depth look at how the incubator will work is illustrated by the level 1 block diagram. This shows the main internal components implemented and the interconnection between them. The microprocessor is taking in user inputs and communicating with the heating and CO2 control system to output the user defined CO2 concentration and temperature. The CO2 and heating control systems uses sensors within the incubation bay to read current temperature and CO2 readings. The control system blocks are powered by the power relay system that rectifies the wall outlet AC signal into DC voltages the components can handle.

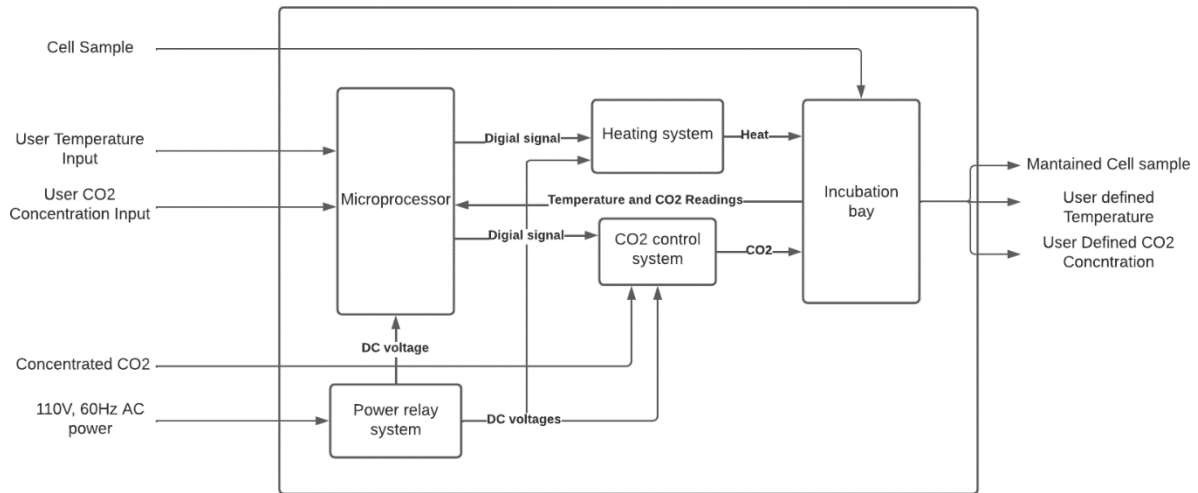


Figure 6: Level 1 Block Diagram

## 3. Design Analysis

### 3.1. System Hardware

#### 3.1.1. Enclosure

The incubator encloses the incubating bay in two main layers: plastic HDPE sheets for structural support and bubble wrap reflective thermal insulation to prevent heat from escaping and wasting energy. The door is gasketed with weather stripping and secured with a locking, gasket-specific latch. The design of the door was to create a seal within the incubation bay, preventing heat or CO<sub>2</sub> from escaping and providing a more stable incubating environment.

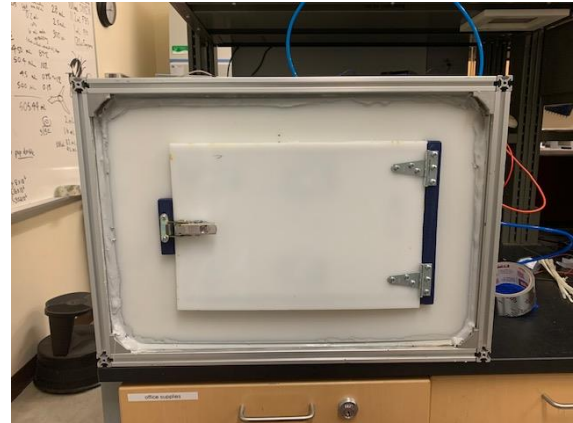


Figure 7: DIY Incubator Enclosure

### 3.1.2. Microprocessor

The microprocessor accomplishes a multitude of tasks including reading inputs and data, computing control voltages, and iterating through the control algorithm. Due to the simplicity of the software needed to operate a simple closed loop control system and GPIO pins, the project uses a common Arduino uno as a microprocessor.



*Figure 8: Arduino Uno Microprocessor Board [18]*

### 3.1.3. Power Distribution

The power distribution system steps down the high voltage AC power to low voltage DC power for the other systems to using a simple off-the-shelf 12V power supply. Relays are used to convert the 5V control signals to the 12V actuating signals read by the heating element and CO2 solenoid valve.

### 3.1.4. Temperature Control Hardware

The temperature in the incubating bay is read from a thermocouple. The small thermocouple voltage ( $\sim 1\text{-}20\text{mV}$ ) is then converted to an SPI signal by a thermocouple interface board with a cold-junction-Compensated K-Thermocouple digital converter (MAX6675). An important note about this type of analog-to-digital converter is that it reports the exact reading the thermocouple outputs, without any filtering. Because the thermocouple is a little too sensitive to temperature changes, filtering will be implemented in the microprocessor's software. A scope capture of the interface board sending a temperature reading to the microprocessor is shown below. To heat the incubating bay, a heating element with two fans were placed at the corners of the incubating bay to circulate the air within and evenly disperse temperature throughout the bay.

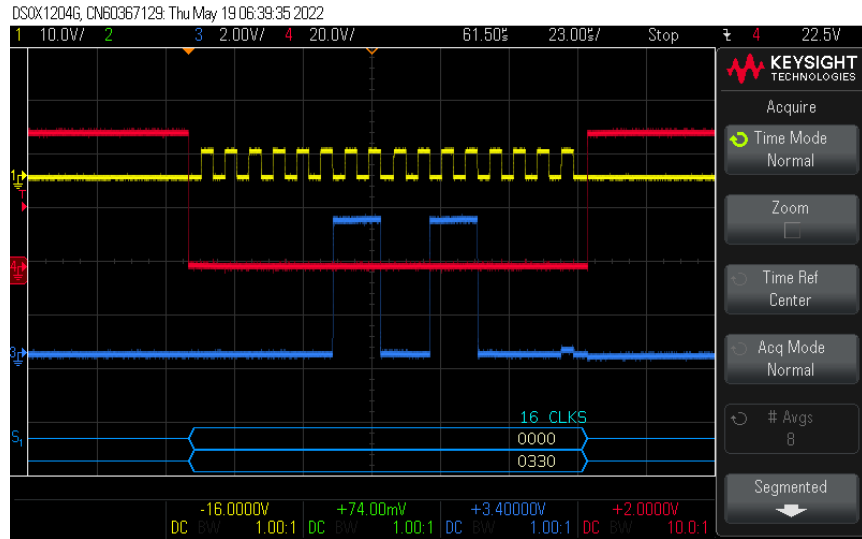


Figure 9: Scope Capture of SPI Signal Sent from Thermocouple A/D converter

### 3.1.5. CO2 Control System

The CO2 system uses an IR optical based sensor to provide CO2 concentration feedback to the processor. The sensor, being costly, has multiple settings that were programmed with an Arduino uno communicating through UART. The settings chosen outputs an analog signal on a range of 0-3.3V that is read from the analog GPIO port on the microprocessor – the relationship between the CO2 reading and output voltage is described in the sensor calibration section later.

To provide controllable CO2 concentration, a precise system provides a stream CO2 delivery actuated by a

digital signal from the microprocessor. As a source of CO2, a soda stream bottle with a pressure

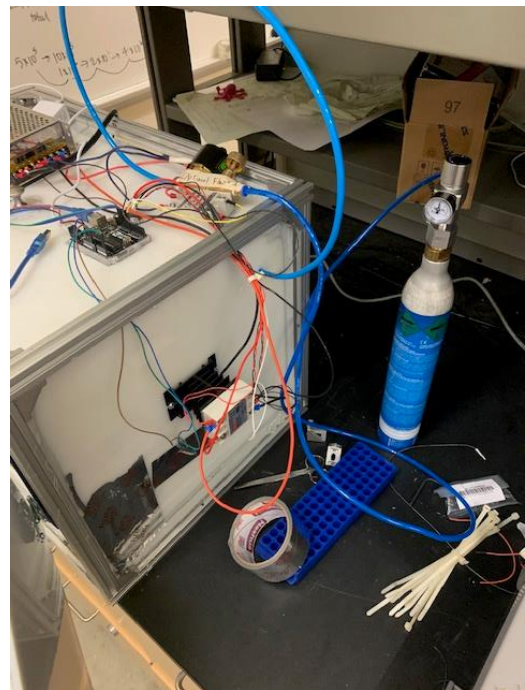


Figure 10: CO2 Pneumatic System with Soda Stream Bottle as CO2 Source

regulator can be connected to the input of the CO2 flow control. Conversely, any source of CO2 (below the pneumatic tubing rated 60psi) can be connected to the input hose, like the large CO2 supply in the Cal Poly biomedical engineering lab. The CO2 flow control module consists of a solenoid valve and needle valve. The first, controlled by the microprocessor, actuates the flow of CO2 (on/off), and the ladder controls the flow rate of CO2 through the system.

### 3.1.6. Overall Schematic

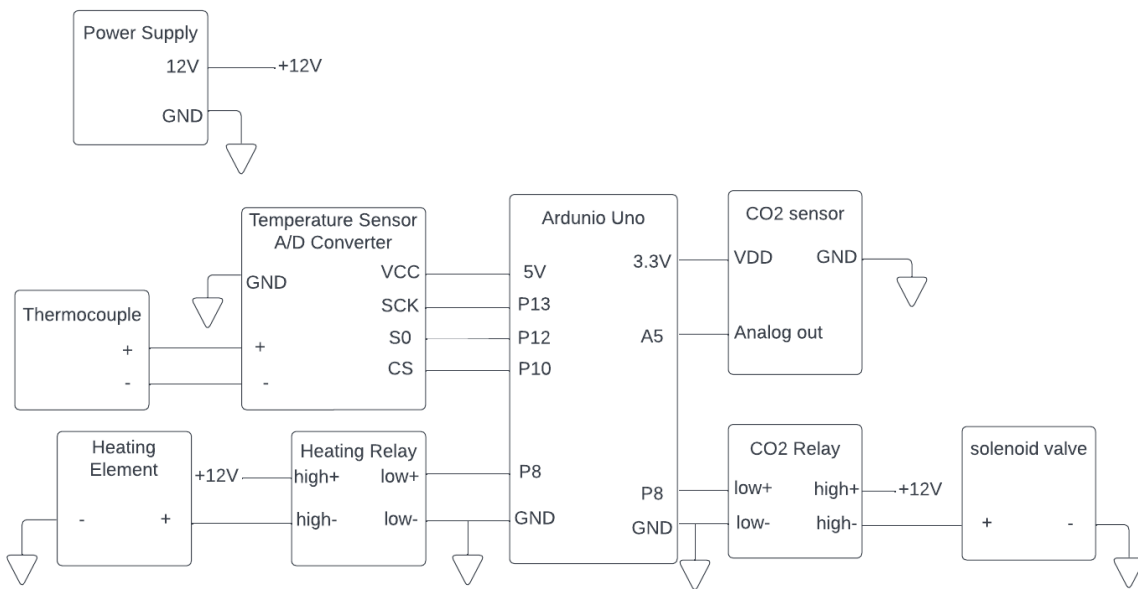


Figure 11: Electrical Schematic of Cell Incubator

### 3.2. Control Algorithm Design

The cell incubator uses a bang-bang (aka binary or on/off) digital controller architecture. The logic flow of the algorithm is illustrated in Figure shown to the right.

The nearly identical logic flow for the CO2 system can be found in Appendix C. In short, the algorithm uses a nested if conditional with a sampling period of 1s to actuate the heating element in a non-linear fashion between the set thresholds.

From this initial logic flow design and discovering system parameters, the system can be modeled in

MATLAB to predict behavior. Both heating and CO2 control systems flow the same closed loop model in

MATLAB where there is a controller, plant, and

feedback sensor. The goal of the design is to derive equations and coefficients to quantify the physical properties of each system. Once accurately modeled, the response can be predicted and potentially optimized.

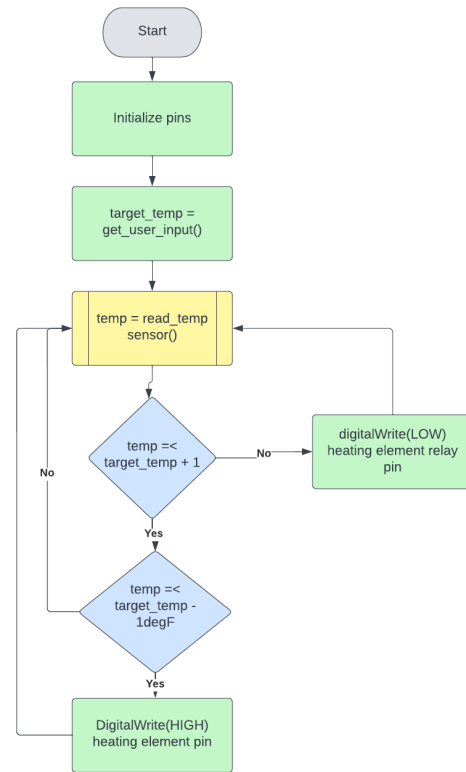


Figure 12: Bang-Bang Digital Control Logic Flow for Heating System

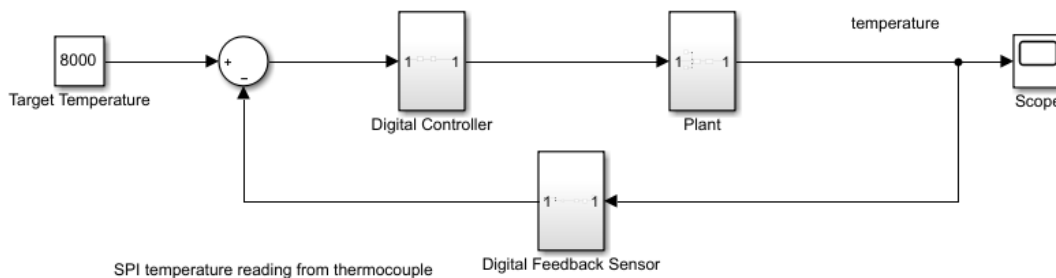


Figure 13: Closed Loop Control System Architecture



### 3.2.1. Heating Control System

Knowing that a temperature transient response is a first-order system, we can write the plant transfer function in the form of [11]

$$G_p(s) = \frac{\theta(s)}{Q_i(s)} = \frac{R}{sRC + 1} = \frac{R}{RC \left( s + \frac{1}{RC} \right)} = \frac{\frac{1}{C}}{s + \frac{1}{RC}}$$

Where

$$R = \text{Thermal Resistance } \left( ^\circ C \frac{\text{sec}}{\text{Kcal}} \right)$$

$$C = \text{Thermal Capacitance } \left( \frac{\text{Kcal}}{^\circ C} \right)$$

$$\tau = RC = \text{time const}$$

To find these coefficients, I analyzed a transient response of running the heating element within the incubation bay and recording the temperature via the thermocouple. The temperature response is shown below in Figure 20. Some data was lost in the recording – the temperature settled at 110degF.

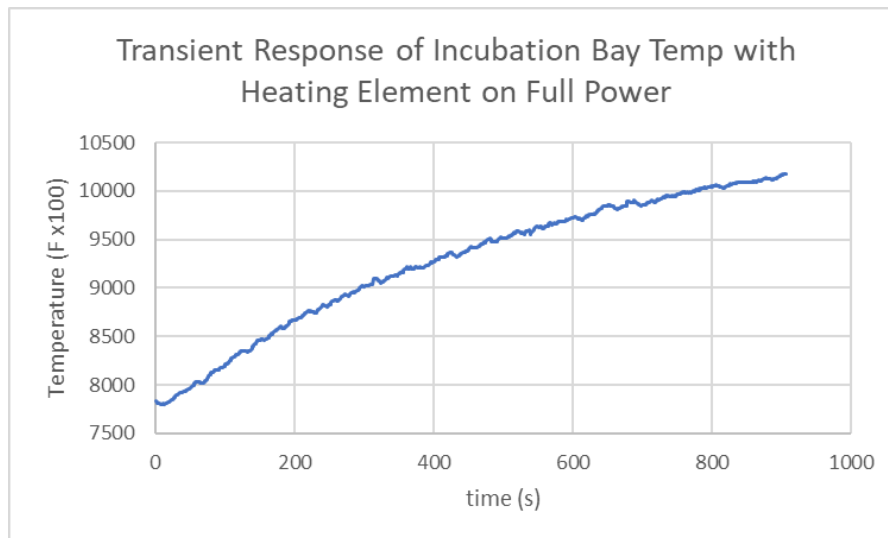


Figure 14: Transient Response of Incubation Bay Temp with Heating Element on Full Power

Using the above response,

$$\tau = 621s$$

$$\begin{aligned} \text{settling temp(heating)} &\approx 110^\circ F \\ \text{settling temp(cooling)} &= \text{room temperature} \approx 73^\circ F \end{aligned}$$

Because we know the final and temperature of cooling and heating, we can ignore the numerator and model the plant transfer function as

$$\begin{aligned} G_{p(\text{cooling})}(s) &= \frac{70}{s + .00161} \\ G_{p(\text{heating})}(s) &= \frac{110}{s + .00161} \end{aligned}$$

The entire system can be modeled as shown below in Figure 21 below. The digital controller was modeled using a relay block with hysteresis that takes a switch off and switch on point as arguments. The switch on and switch off point being the +/-1 degF thresholds. In addition, a zero-order hold device was placed afterwards to sample the input at a period of 1s. Finally, the feedback sensor has a gain of 100 because the processor processes temperature values multiplied by 100 to prevent floating point arithmetic.

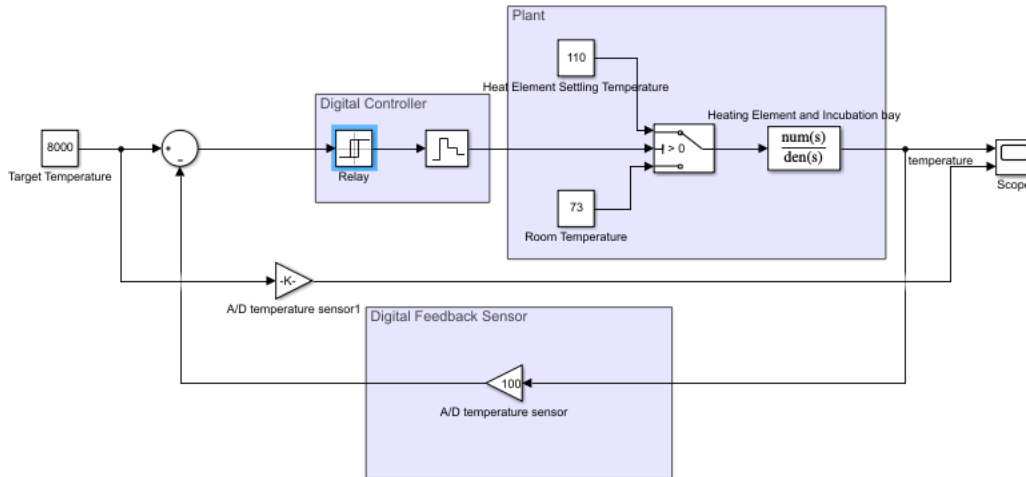


Figure 15: Closed Loop Incubator Heating System Modeled in MATLAB

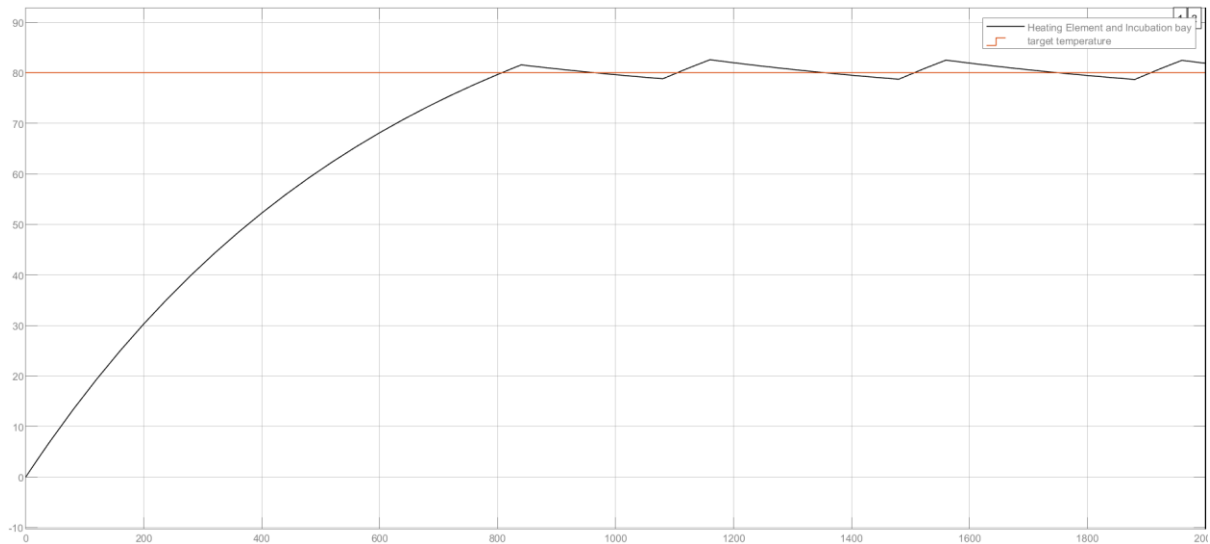


Figure 16: Simulation of Heating System MATLAB model

### 3.2.2. CO<sub>2</sub> Control System

Using the same approach as the heating control system, the CO<sub>2</sub> control system only has a few differences. The main differences being that the transient response of CO<sub>2</sub> injected into the incubating bay is linear can be represented by a simple integrator with a gain represented by the slope of the transient response.

The gains for the transfer function were found experimentally by running the CO<sub>2</sub> at a constant rate, and by letting the incubating bay settle and measuring the gains of each.

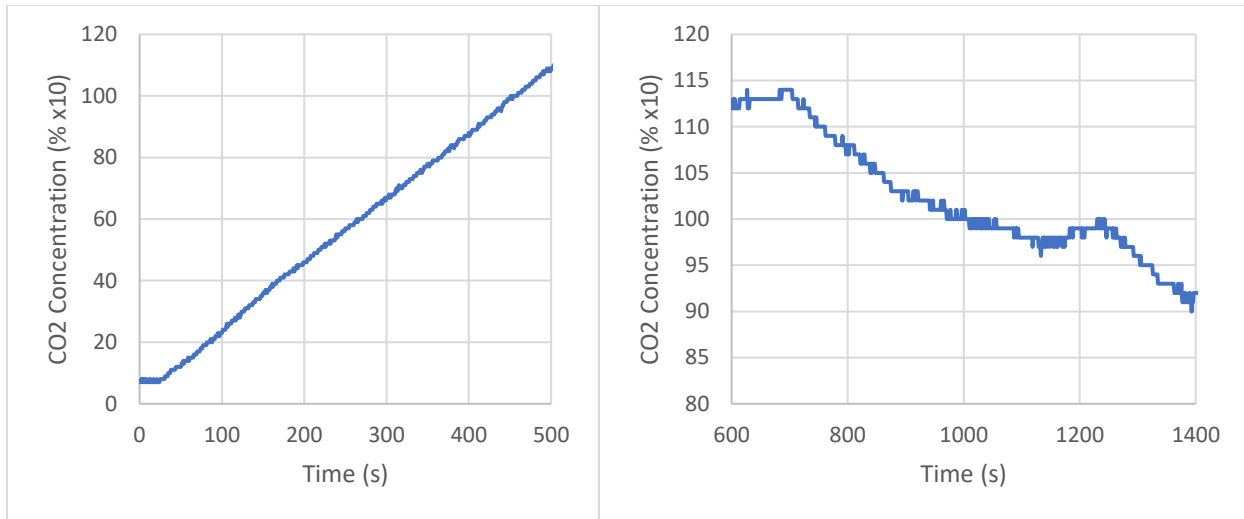


Figure 17: CO2 Concentration Within the Incubation Bay After CO2 is Turned on(left) and Turned on (right)

From the figures the slopes were calculated

$$\text{slope} = \frac{\Delta \text{CO2 concentration}}{\Delta t}$$

$$\text{CO2 injecting in gain} = \frac{(10.8 - 0.8)\%}{(493 - 28)s} = 0.0215 \%/s$$

$$\text{CO2 leaking out gain} = \frac{(9.2 - 11.3)\%}{(1383 - 588)s} = -0.00264 \%/s$$

Therefore, the following transfer functions can be used to describe the plant for the CO2 control system model:

$$G_{p(\text{CO2 injecting})}(s) = \frac{0.0215}{s}$$

$$G_{p(\text{CO2 leaking})}(s) = \frac{-0.00264}{s}$$

Plugging the coefficients into the model yields the following results.

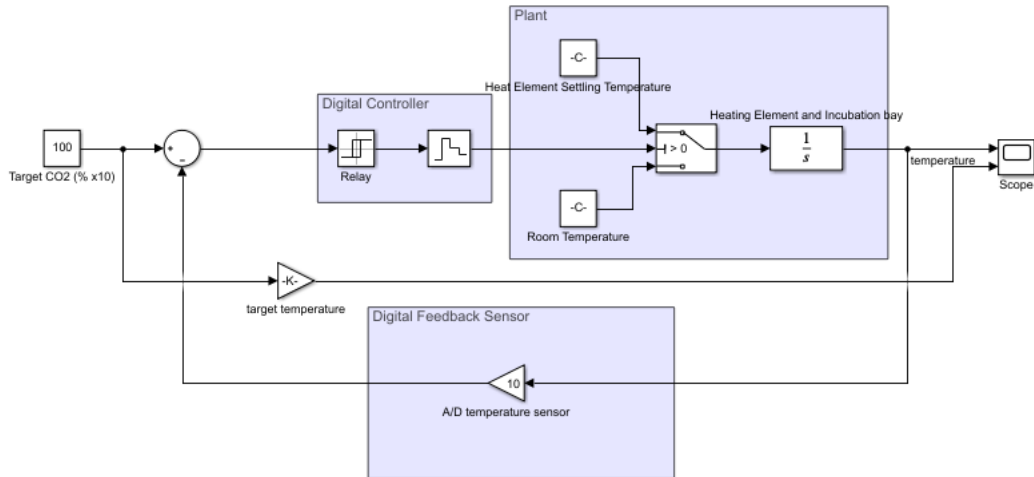


Figure 18: Closed Loop Incubator CO<sub>2</sub> System Modeled in MATLAB

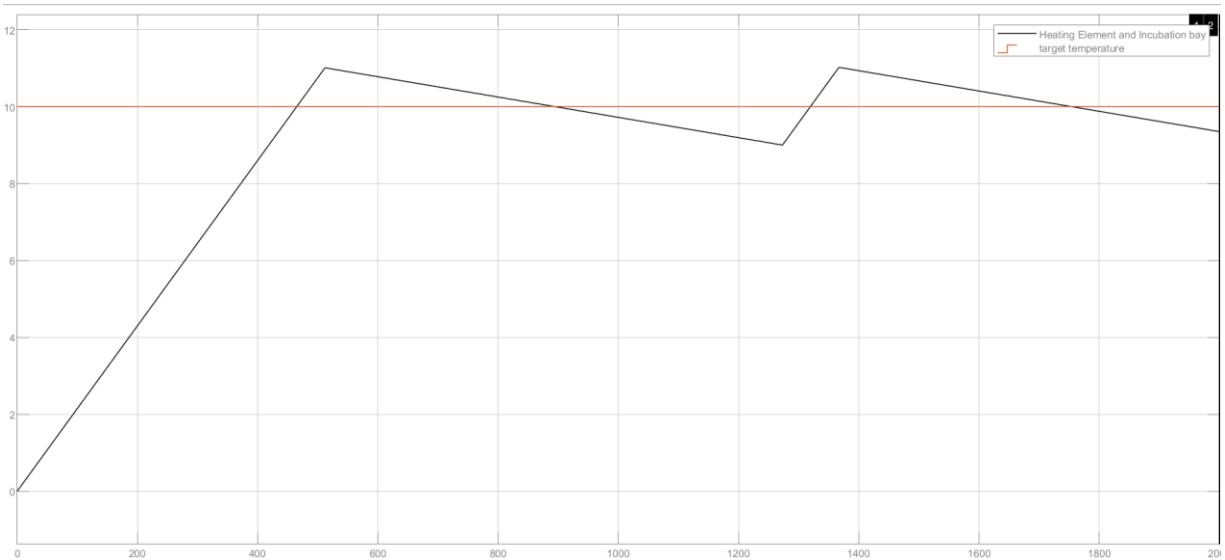


Figure 19: Simulation of CO<sub>2</sub> System MATLAB model

The flow rate of the CO<sub>2</sub> inject was chosen to prevent too much overshoot, but also provide a fast enough response time that it would not overheat the solenoid valve when it is active.

## 4. System Firmware

### 4.1. Overall architecture

The software architecture is rather simple in this project because the processor is reading from two sensors and performing two primary tasks on. The program runs in an infinite loop after it has run through its initialization. In the initialization, the program sets up the GPIO pins for their associated tasks, calibrates the SPI settings according to the CO2 sensor datasheet, and fills the temperature filtering queue (this is described later). The while loop executes 3 main instructions: read both the sensors, map the values accordingly, and actuate peripheral relays based upon the digital control logic flow diagrams explained in the digital control design section. A note about the temperature and CO2 concentration values – temperature values are multiplied by 100 after they are read from the sensor and CO2 concentration values are multiplied by 10 after they are read to prevent floating point arithmetic. The whole program can be found in Appendix D.

### 4.2. CO2 Sensor Calibration

To determine how to map the analog output voltage from the CO2 sensor to the correct CO2 concentration reading, experimental analysis was performed. The sensor was placed inside an industrial cell incubator, an environment where I could know and control the CO2. Then, I plotted the relationship between ambient CO2 concentration and output voltage of the sensor. Figure 26 illustrates the linear relationship and shows the equation used by the algorithm to convert the sensor analog output voltage to CO2 concentration used by the program.

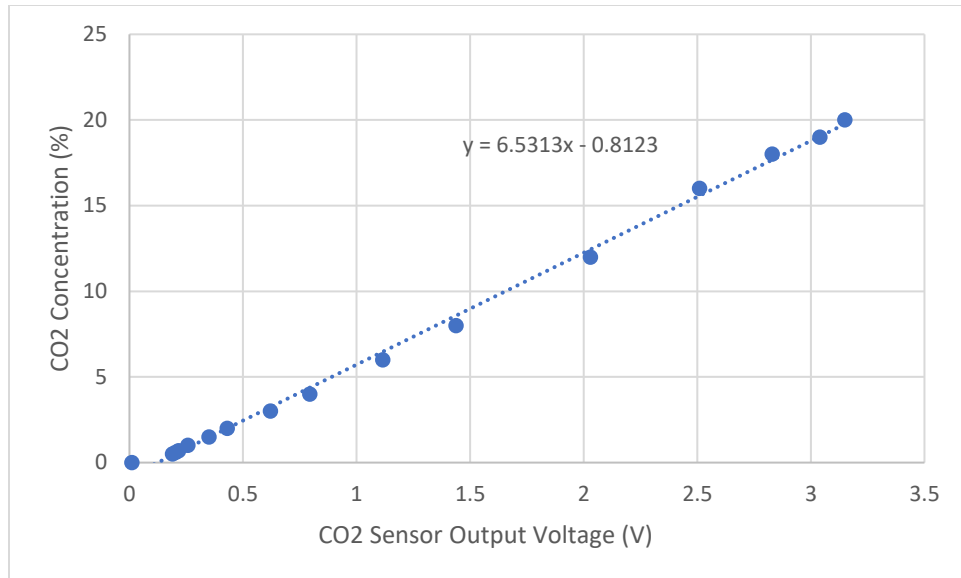


Figure 20: Relationship Between CO2 Sensor Voltage and Ambient CO2 concentration

### 4.3. Temperature filtering

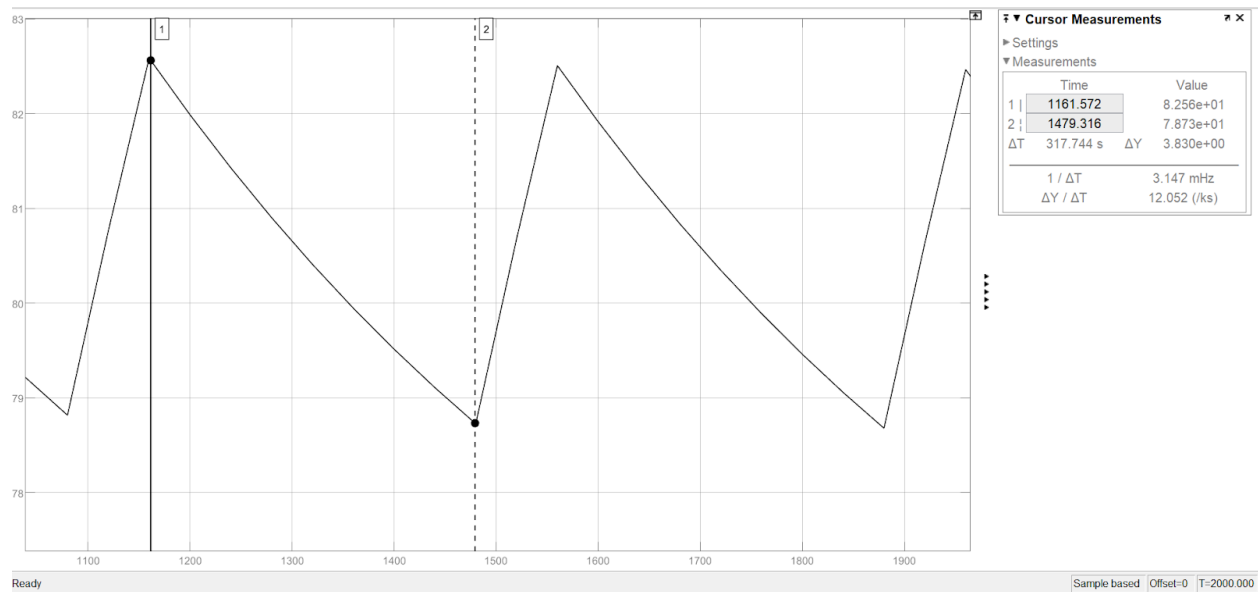
As previously mentioned, the temperature sensing hardware does not have any sort of output filtering embedded in the A/D converter, as opposed to the CO2 sensor which does. To prevent erratic readings from the temperature sensor, potentially unnecessarily actuating the heating element, the program filters the input read from the temperature sensor. To filter the readings from the temperature sensor, the program uses a walking average. Each instance a temperature is read from the sensor:

1. The reading is divided by the queue length (10) and added to the walking average
2. The walking average subtracts the dequeued reading(divided by 10) from the end of the queue
3. The current reading is enqueued

```
reading = 10 * read_temp() / sample_size;
AVG = AVG - avgq.dequeue() + reading;    //takes a walking average
avgq.enqueue(reading);
```

## 5. Testing and Verification

To test the performance of the system, experimental tests were performed to compare with the simulated results. In the simulation and test, target temperature was set to 80F and CO2 concentration was set to 10%.





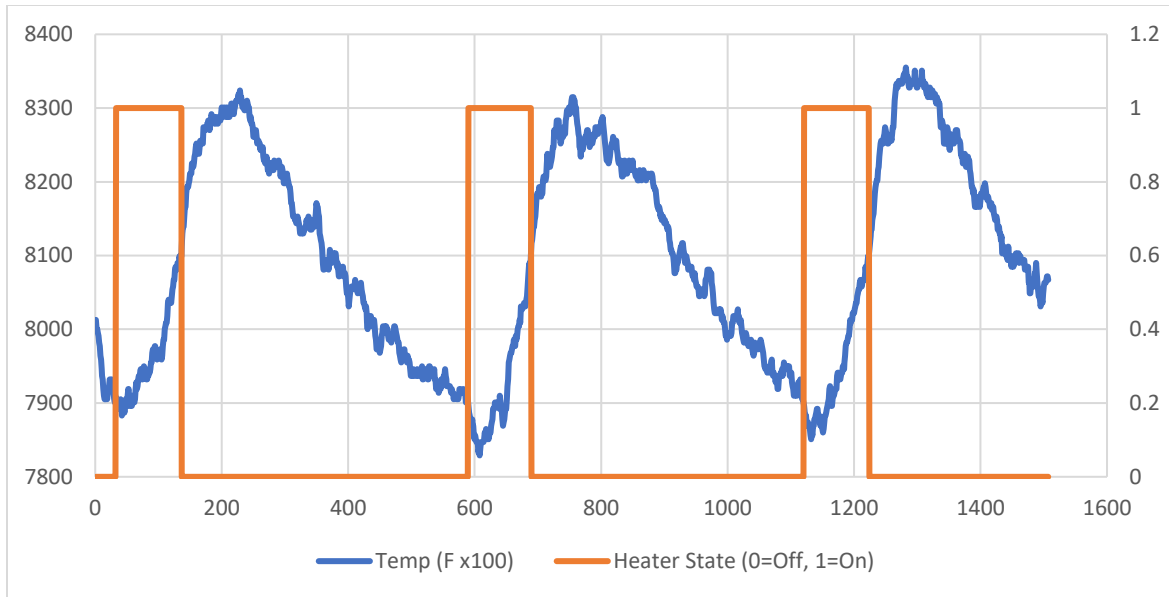
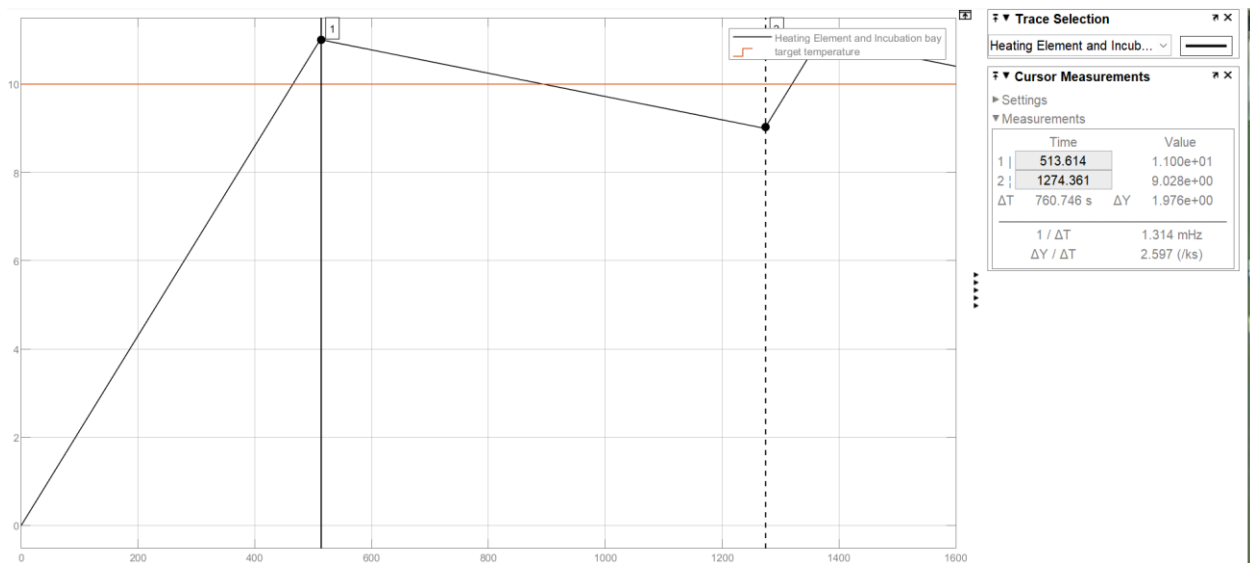


Figure 21: Temperature Cycle of Closed Loop Heating System Compared with Simulation

The rise time of both the simulation and experimental results approximates to 100s and the fall time 300s.



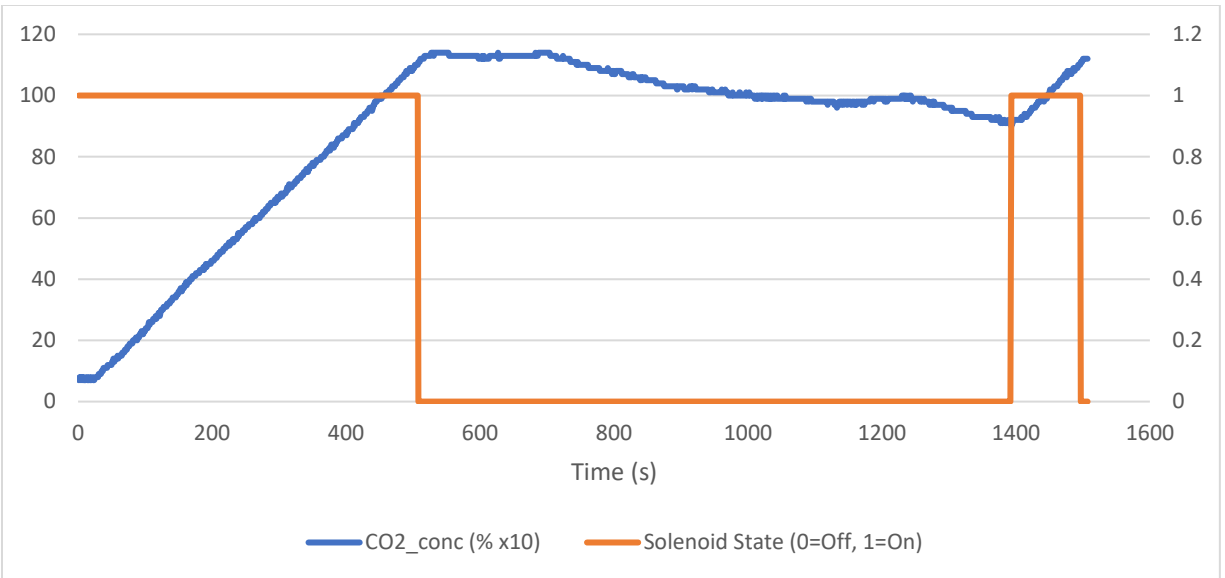


Figure 22: CO2 Concentration Cycle of Closed Loop System Compared with Simulation

The rise time of both the simulation and experimental results approximates to 100s and the fall time 750s.

## 5.1. Discussion of Results

The final result of system performance meets all the engineering requirements while keeping the system relatively simple. To further improve the system response would be to implement a PID controller in the digital control algorithm. Specifically, this would provide a potentially faster system response and would reduce or eliminate steady state error. However, the reason a bang-bang control architecture was initially chosen over the PID controller was because a PID controller would require more advanced and expensive actuating hardware. The PID controller would require a variably controlled solenoid valve and heating element. Because this project was designed to mitigate expenses, the bang-bang control architecture provided results that met the requirements while remaining economically feasible.

## 6. Market Research

As previously described, cell culture plays an extremely large role in medical research, so it goes without saying, the cell culture/incubation market trends alongside with the medical market. With how many medical conditions plague the world like autoimmune disease, cancer, a global pandemic just to name a few, the medical research market is in high demand [8]. The upward slope the market is currently experiencing as shown in Figure 5 will not stop anytime soon even if all the illnesses aforementioned are solved. The medical community is constantly evolving and developing new studying that will keep driving the market upward like stem cell research, embryonic cell research, and neuroscience [9].

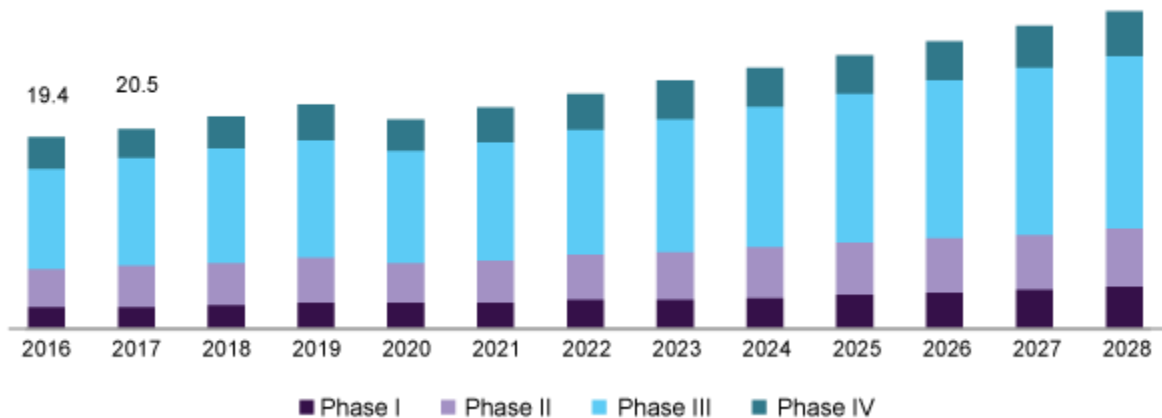


Figure 23: U.S. Clinical Trials Market Size, by phase, 2016-2028 (USD Billion) [8]

## 6.1. Customer Archetype




The DIY incubator fills the niche market where scientists are looking for an affordable incubator that are trying to measure cell experiments in the incubation bay. Researcher organizations use their large and expensive cell incubators for most of their applications, but those incubators do not have any accommodations for feeding wiring for measurement equipment into the incubation bay. The perfect product for these researchers would be a cheap, specialized cell incubator used to house single experiments that need a microscope during growth. Table 1 illustrates the two customers that would need the product.

*Table 2: Target Customer Archetype*

<b>Type of Customer</b>	<b>Description</b>	<b>Reason</b>
University Department Head	These are professors that lead the department of biomedical engineering at universities and are the last say in purchasing decisions	Department heads are constantly under scrutiny about staying under budget, so this product is perfect to aid their research
Research and medical companies	These are the industry leaders in developing medical advances	There is a large variety of experiments that these companies partake in which requires a large variety of type of lab equipment including the need of this project

With such a large market, there will be a lot of competition and a lot of top dogs desiring to squash the smaller companies trying to siphon off a portion of their sales. Table 2 illustrates the top competitors to the project and a description of their target market.

Table 3: Market Leaders in the Cell Culture Industry

Market Leader	Description
	<p><b>ThermoFisher:</b> an American company that produces and supplies scientific instrumentation, cell culture consumables, and software [10]. Founded in 2006, the company has quickly rose to the top of the market</p>
	<p><b>Corning:</b> A much older company that was founded in 1851, the company produces many specialty products such as display technologies, optical communications, and environmental technologies for life science research [10].</p>
	<p><b>Becton Dickson:</b> a company that is largely engaged in the development and manufacture of laboratory equipment. The company operates through two business segments: BD Medical and BD Life Sciences [10]</p>

## 6.2. Market Description

The DIY cell incubator will be able to fulfill a specific need for the customer by providing them the ability to have a separate cell incubator for specialized research projects. Ordinarily, this would be absurd to have a separate incubator for specific project, but the DIY incubator fills this need without burning a hole in the customer’s pocket. With an affordable price point and filling a niche that other companies have yet to fill, the DIY incubator can thrive in the market today.

Table 3 shows some advantages and disadvantages of the product comparing to that of a “normal” large cell incubator. As described before, the customer the product is aiming to appeal

to already has a large-scale cell incubator in the lab, so a lot of the disadvantages of the product become obsolete when there is another incubator present. However, since the other incubators do not have the advantages described, the consumer will still need the product.

*Table 4: Advantages and Disadvantages of DIY Incubator Compared to "run-of-the-mill" Incubators*

Advantages	Disadvantages
Affordable price point	smaller incubating bay
Ability to house measurement equipment and feed wires into the incubation bay	Cheaper materials used, so does not hold ambient temperature as well
Air circulation within the bay to disperse temperature	No humidity control, only monitoring

Some key partners that would be beneficial to partner with would be lab measurement companies as well as biomedical components producers. Companies like Lab Smith produce

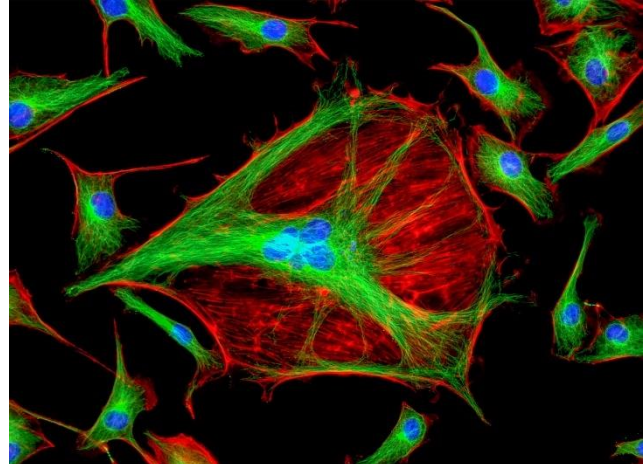


*Figure 24: Synchronized Video Microscope from Lab Smith [16]*

microscopes that are used for analyzing cellular growth via light and fluorescence light imaging. The microscope shown in Figure 6 on the left is a research-grade inverted fluorescence video microscope that captures high-quality imaging while storing it on a hard drive. This allows

researchers to see the different layers of cells in difference fluorescent colors as shown in Figure

7. Being able to partner with this company would provide the opportunity to ensure compatibility between the measuring equipment and the cell incubator. Another mutually beneficial partnership could be made with the manufacturer of the electronic and consumable products used to manufacture and operate the cell incubator.



*Figure 25: Fluorescent Microscope Capture [11]*

Companies like Texas instruments would be excellent partners by tailoring a microcontroller manufacturing process around the cell incubator's specifications. Also, an add on to the cell incubator that could be the CO<sub>2</sub> required for the cell incubator. Reselling this at a profit would only be beneficial as well.

In addition to key partners, there are multiple key customers that the DIY incubator would appeal to and should be addressed individually. For a start, the colleges and universities in California would be a prime target audience. Having the product originate for a California State University, and if the product successfully fills its niche, California Universities would be enamored by the product. Another key customer would be large medical research facilities like that of Massachusetts General Hospital. With the volume of testing ongoing in these facilities, they are another prime customer to aim the product towards.

## 7. Business Model

Below shows the project's business model canvas that outlines the main goals for how the business aims to take to structure itself. Some of the key take aways from the model are the value proposition, revenue streams, and key partners.

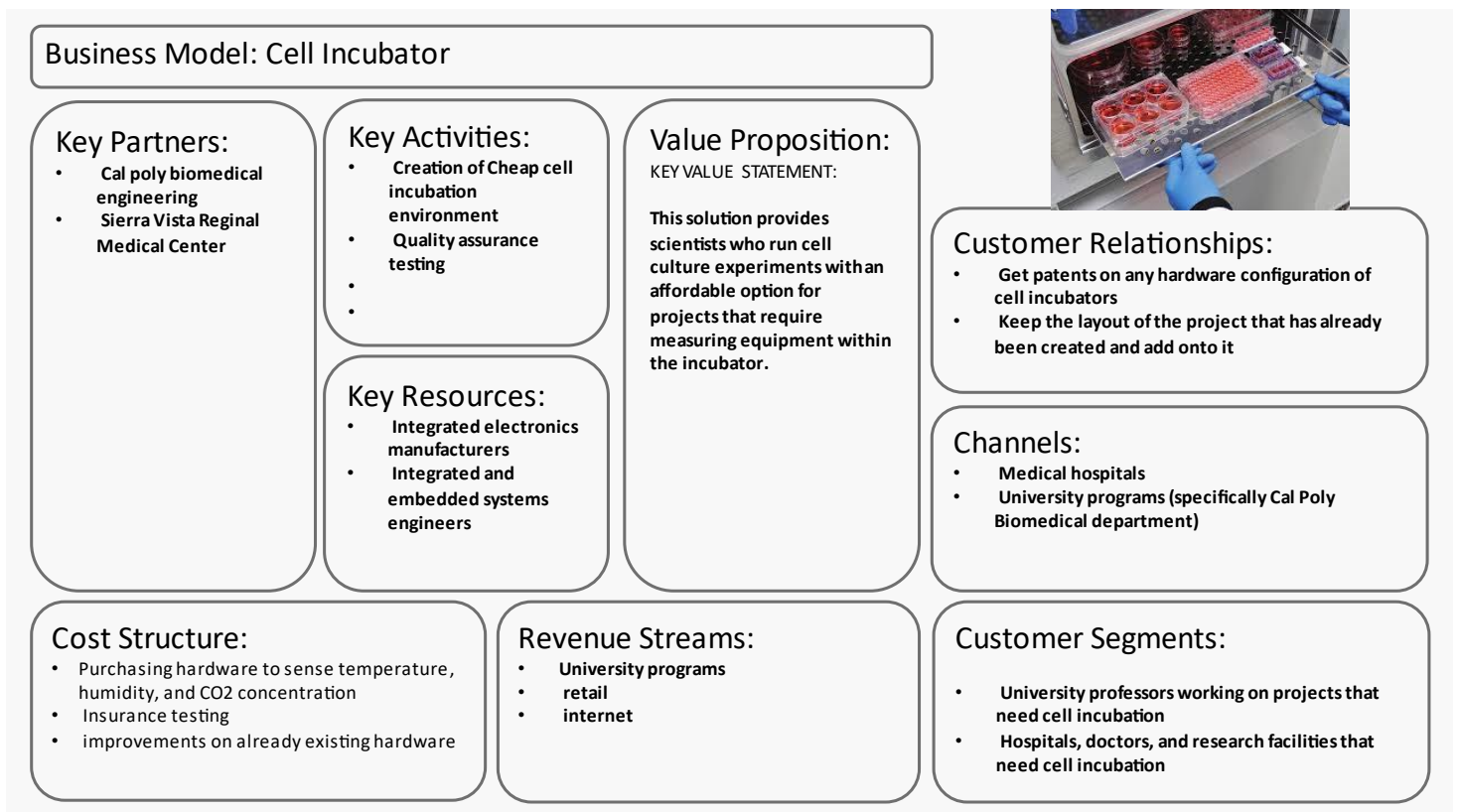
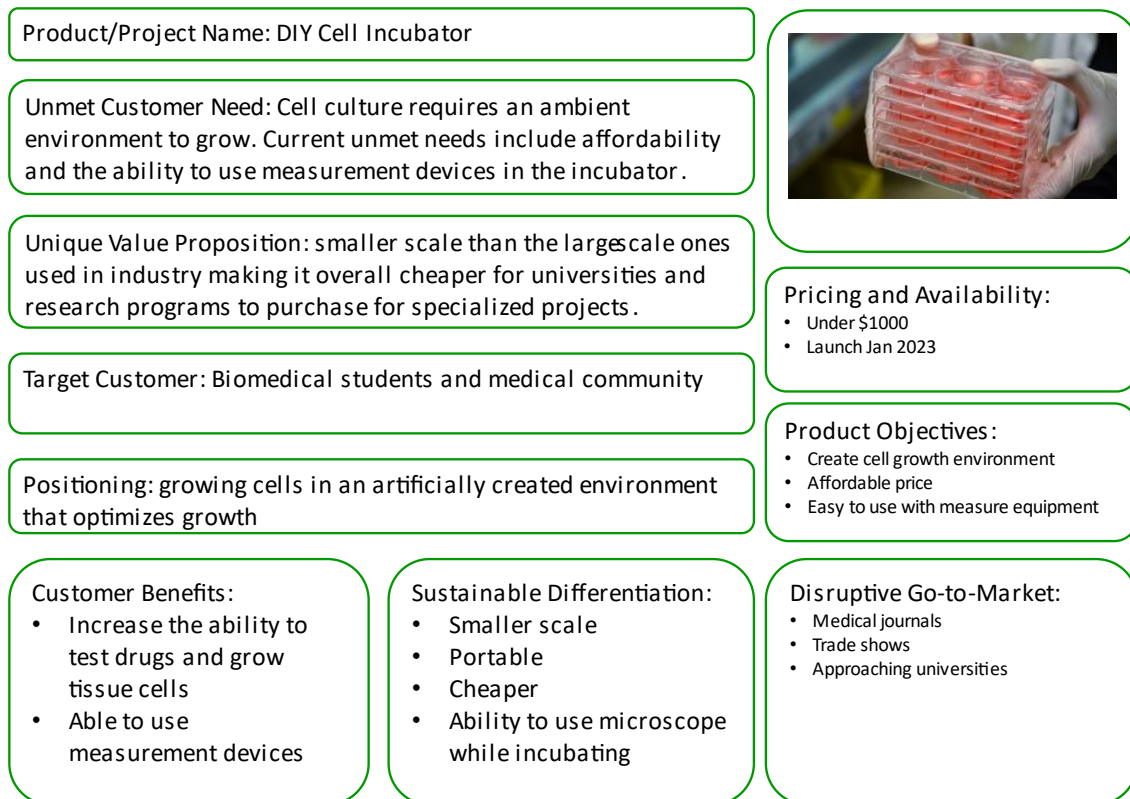


Figure 26: Business Model Canvas



## 8. Marketing Requirements

The DIY aims for a fairly niche audience, so the product must be carefully built around the need of that specific market. The marketing datasheet, shown below in Figure 9, covers the needs and requirements for the project to appeal to the market.



*Figure 27: Marketing Datasheet*

The product is made to satisfy the needs of the customer. Table 4 below shows the various needs that needs to be addressed and how important they are. Then, Table 5 shows a pairwise comparison between the three main components that need to be delivered to the customer in correspondence with the weight of importance of each of them. These are illustrated by Figure

10 that shows the marketing needs of the product in a marketing hierarchy tree along with the weightings of each need.

*Table 5: Different Marketing Design Features and their Level of Importance*

<b>Design Feature</b>	<b>Importance</b>
Temperature Control	High
Quick Correction Period	Low
Low Production Cost	High
CO2 Control	High
Humidity Monitoring	Low
Powered by Wall Outlet	Low
Ease of Use	High
Mechanically Enclosed	Medium

*Table 6: Pairwise Comparison of Major Needs to Fulfill*

<b>Parameter</b>	<b>Cost Effective</b>	<b>Creates Cell growth environment</b>	<b>Easy to Use</b>	<b>Geometric Mean</b>	<b>Weights</b>
Cost Effective	1	1/3	1/2	0.55	0.16
Creates Cell growth environment	3	1	2	1.82	0.54
Easy to Use	2	1/2	1	1.00	0.30

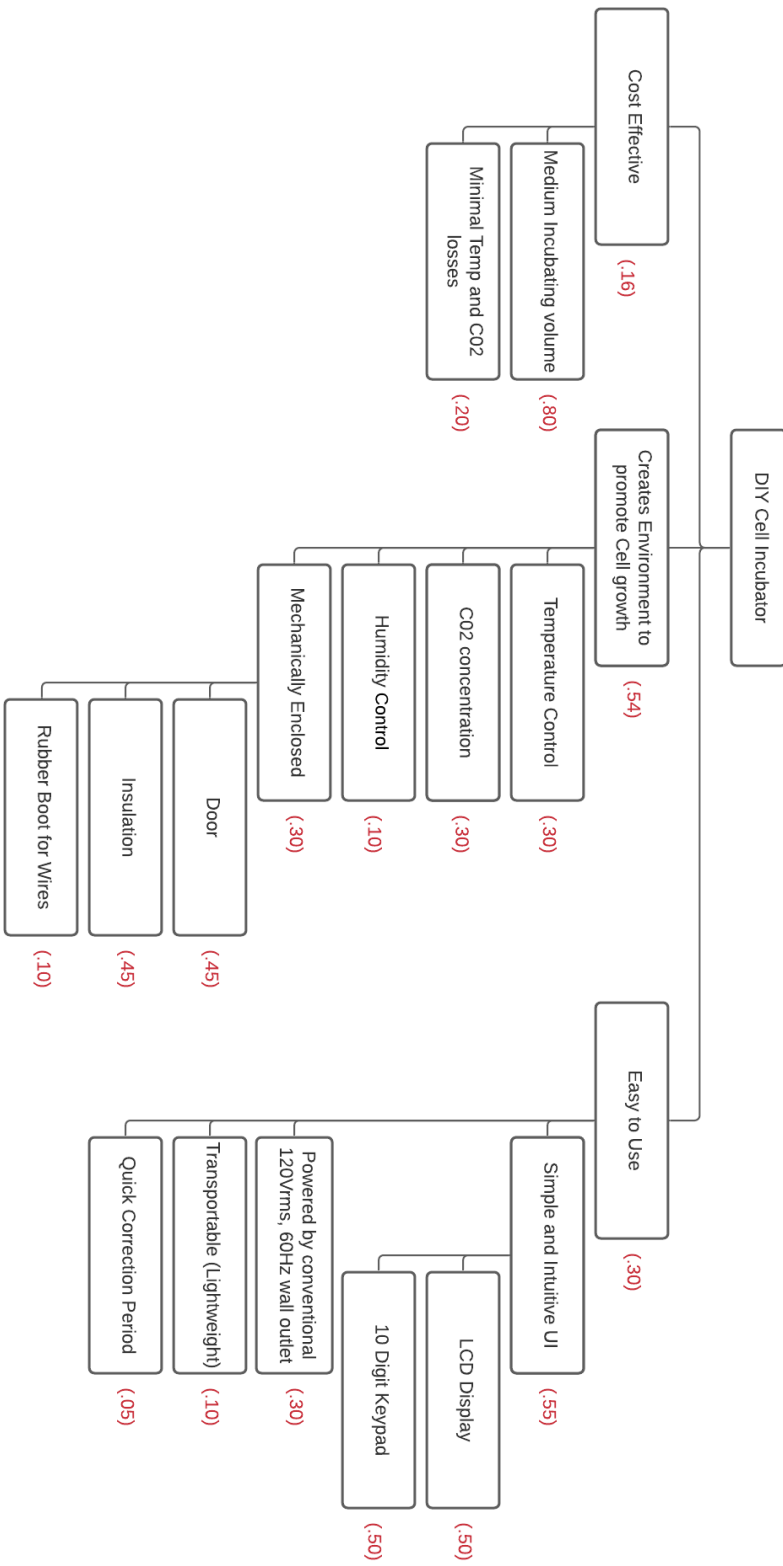


Figure 28: Marketing Needs Hierarchy with Weighted Characteristics

# 9. Schedule Of Completion

The Gantt Chart shown in Figure 13 illustrates the critical path (red) and the tasks that were taken before the project deadline in December.

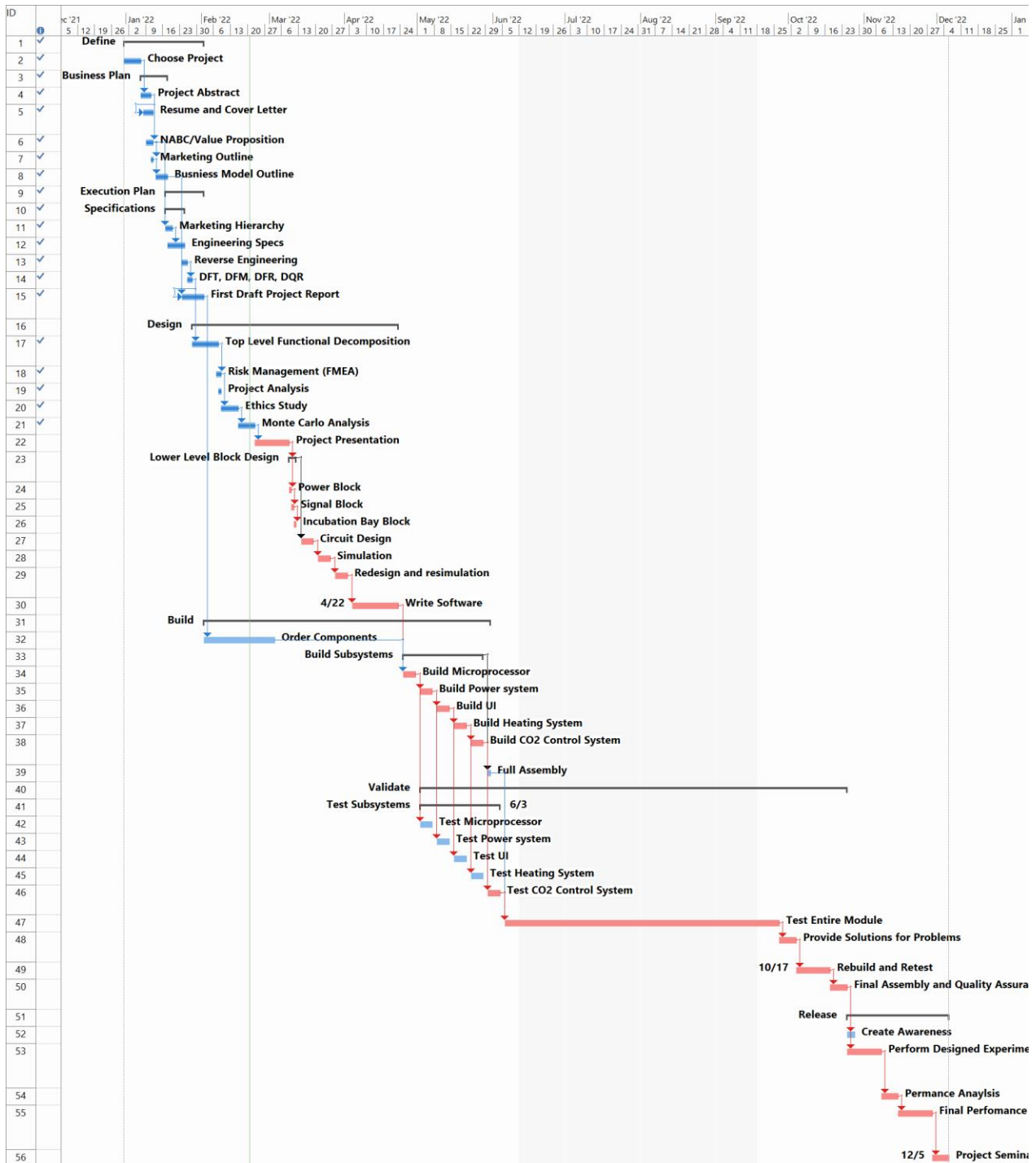


Figure 29: Gantt Chart of Entire Project

## 10. Cost

The cost of the project is outlined in the Bill of Materials (BOM) located in Appendix B.

## 11. Impact Analysis of Senior Project

### 11.1. Summary of Functional Requirements

The cell incubator maintains a user defined temperature and CO<sub>2</sub> concentration within a mechanically enclosed incubation bay. It receives this input via a user-friendly keypad and display. It then relays this information to a microcontroller that controlled how much heat or CO<sub>2</sub> is applied to the incubation bay to maintain the cell growth environment. The enclosure also has an opening for electrical wiring to be fed into the incubation bay, as to allow measurement equipment in the incubation bay.

### 11.2. Primary Constraints

The primary constraints of this project require it to be cheap, fully enclosed, and powered by a wall outlet. Allowing electrical equipment within the bay presents problems with controlling the environment within the bay, but the constraint still must be met. Also, for each of use, the project must be powered by a conventional wall outlet voltage.

### 11.3. Economic

- *Human Capital*: the project provides many jobs within the production, design, and selling of the product.
- *Financial Capital*: This project will require some investment from medical and biomedical investors to provide scaling.

- *Natural Capital*: The incubator will require many electrical components which is often derived from refinement of natural metals and other inorganic materials.
- *Cost and Timing*: The cost is supposed to be on the cheaper side compared to other cell incubators, thus being the favored option.

#### 11.4. If Manufactured on a Large Scale

- Estimated number of devices per year: 1000
- Estimated manufacturing cost for each device: \$700
- Estimated purchase price for each unit: \$1000
- Estimated profit per year: \$300,000
- Estimated cost for user to operate devices: \$40/year

#### 11.5. Manufacturability

The DIY incubator has no need for manufacturing because it is made to be assembled by the customer.

#### 11.6. Environment and Sustainability

The product will not have a large environmental impact while in use because it is powered by a wall outlet. As for the end-of-life cycle for the product, the electrical components of the device are not easily cycled back into the environment. Therefore, prolongation of the product life is a large goal during design.

## 11.7. Ethical

The cell incubator is designed around helping the medical community with as little negative side-effects as possible. Although the DIY incubator is supposed to provide a cheap solution to the target consumer, the product is still aimed to be designed so the overall device has high quality.

## 11.8. Health and Safety

The incubator does not present many health and safety concerns from the primary use perspective. However, like any electrical devices, there is always the worry of injuries from electric shock. This can be prevented by the operator practicing common sense while using electrical devices powered by wall outlets and by the incubator having properly insulated wiring.

## 11.9. Social and Political

The only social or political connections that arise from the project is the rise and fall of consumer purchasing of medical equipment which is often funded by government subsidies or non-profit organizations. This means the demand for the incubator will follow the trend of this funding.

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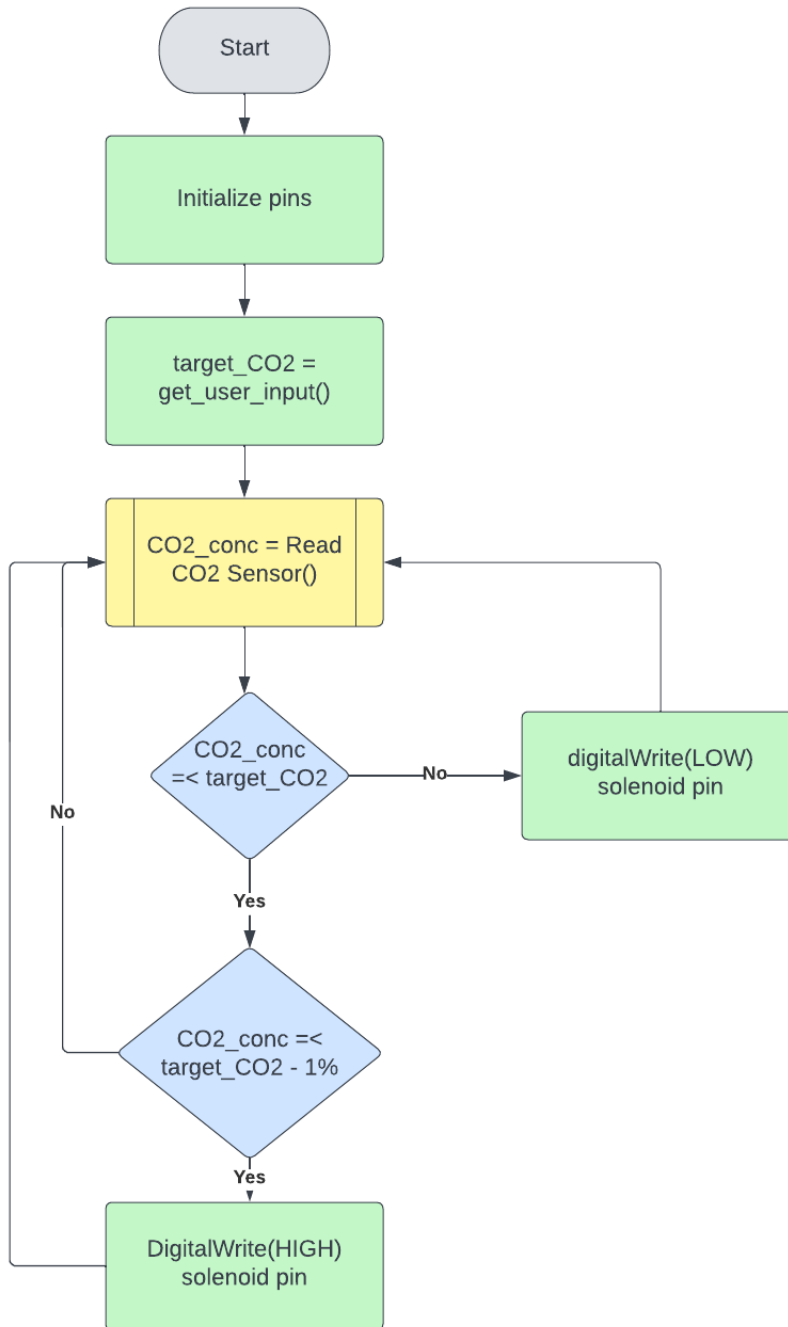
## Appendix A: FMEA

Item / Function	Potential Failure Mode(s)	Potential Effect(s) of Failure	Sev	Potential Cause(s)/ Mechanism(s) of Failure	Prob	Current Design Controls	Det	RPN
CO2 solenoid	Blockage of CO2 system	loss of cell samples	7	solenoid failure/stuck	2	none	10	140
Wire boot/Main Door Seal	Main incubation bay leak	deviation from desired ambient temp/wasting CO2 and heating to counteract	3	inadequate sealing of bay	8	environment monitoring with display of parameters (no failure mode yet)	10	240
CO2 feeding system	leak in the hose/adaptor/ fitting	wasting CO2	3	loose fittings / moving CO2 feeding hose	6	threaded manufactured fittings	8	144
Heating system	incubation bay overheats past desired temp	Loss of cell sample	7	temperature outside of incubator is too high	5	none	10	350
Power system	loss of power	loss of cell samples	7	power relay failure/power outage	9	insulation to maintain incubation bay conditions/ power outages are noticeable	5	315
Chassis	item droppage	chassis damage	3	user error	6	metal chassis surrounds incubator	1	18
Feedback system	malfunctioning feedback process	loss of cell samples	7	bugs in software	3	none	10	210
circulating fan	air circulation blockage	unequal environments distribution	1	debris/fan failure	2	none	4	8
Temp sensor	inaccurate temperature readings	inaccurate temperature control	5	temp sensor fault or wire fault	2	none	10	100
CO2 Sensor	inaccurate CO2 readings	inaccurate CO2 control	5	CO2 sensor fault or wire fault	2	none	10	100
heating element	heating element overheating	inaccurate temperature control/cell loss	7	heating on for too long/too cold in room	4	heat sink for heating element	4	112
humidity sensor	in accurate humidity readings	not as vital as temp and CO2 but still not good	5	humidity sensor fault or wire fault	2	none	8	80

# Appendix B: Bill of Materials

Item Description	Product Number	Purpose	Associated Task	Unit	Quantity	Cost/Unit	Total Cost
SodaStream CO2 60L	B0092H2K6E	CO2 Source	CO2 Aspects		1	\$30.00	\$40.00
Sterile Syringe Filters, 0.22um dia. (10PK)	41000000	Filtration of CO2 Gas	CO2 Aspects	um	1	\$8.66	\$9.29
Air/Gas 12V Solenoid	B07D9JLQC9	Turn on/off CO2 flow	CO2 Aspects		1	\$15.43	\$16.55
Barbed Brass Fittings	40170000	Adapter for connecting a hose	CO2 Aspects		2	\$3.99	\$8.58
Pneumatic Tubing	APU1/4	Gas/Fluid Transfer	CO2 Aspects		1	\$10.48	\$11.24
ExplorIR 20% CO2 Sensor	N/A	CO2 Sensor	CO2 Aspects		1	\$109.00	\$123.49
Arduino UNO R3	A000066	Programming Aspects	Programable Controls and Electrical Aspects		1	\$18	\$19.31
SodaStream Adapter from Female Thread TR21x4 to Male Thread W21.8 for Soda Stream	B07D5SIBJ4	Electrical Components	Programmable/Electrical Controls		1	\$11.99	\$14.98
Arduino Power Supply	B07P6X87L6	DC to AC converter	Programmable/Electrical Controls	V	1	\$7.78	\$7.78
IRFZ44N Power Transistor MOSFET	60101700	Electrical Components	Programmable/Electrical Controls		1	\$6.75	\$7.24
S8050 NPN Transistors	26111800	Electrical Components	Programmable/Electrical Controls		1	\$4.99	\$5.35
Breadboard Kit	B01HRR7EBG	Electrical Components	Programmable/Electrical Controls		1	\$12.99	\$13.93
TIP120 Power Transistor	32110000	Electrical Components	Programmable/Electrical Controls		1	\$5.98	\$6.48
5V Relay SPDT	N/A	Electrical Components	Programmable/Electrical Controls		1	\$4.95	\$5.33
12V 20A 240W Power Supply Transformer Switch	B078RZ6C3N	Electrical Components	Programmable/Electrical Controls		1	\$18.99	\$23.58
120pcs Dupont Wire (Assorted 50 cm)	B07GD3KDG9	Electrical Components	Programmable/Electrical Controls		1	\$13.99	\$15.00
SSR-400D 40A Solid State Relay	B083TLPPKL	Electrical Components	Programmable/Electrical Controls		1	\$13.68	\$14.67
Pass & Seymour Switch, 6-Amp, 120-volt	B00826P0AO	Electrical Components	Programmable/Electrical Controls		1	\$7.99	\$8.57
BNTECHGO 16 Gauge Silicone wire	B00TG1TRL2	Electrical Components	Programmable/Electrical Controls		1	\$5.98	\$6.41
140 PCS Insulated Fork Spade U-Type	B07PG1NCQZ	Electrical Components	Programmable/Electrical Controls		1	\$7.99	\$8.57
6ft AC Power Cord	8011616157592	Electrical Components	Programmable/Electrical Controls		1	\$6.80	\$7.31
Hinges	31162403	Hinged-Door	Structural		2	\$1.48	\$2.96
2020 T-slot Aluminum Extrusion Combo Kit	EXT-2020-REG-COMBO	Physical build for enclosure	Structural	in	1	\$79.95	\$79.95
.1875" x 48" x 48" HPDE Plastic	8619K457	Structural Aspects	Structural	in^2	1	\$77.00	\$135.62
K-Type Thermocouple + MAX6675 Sensor	N/A	Probe Temp Sensor	Temperature Measuring	°C	1	\$5.90	\$6.36
Air Heater 100W 12V	B07JKNKX7J	Heating Element	Temperature Measuring		2	\$13.19	\$28.30
12V DC Fan (PWM, 4 Pins)	B07CG2PGY6	Cooling inside Incubator	Temperature Monitoring		1	\$13.90	\$14.91
12c LCD 16x2	B019K5X53O	LCD Screen	Temperature Monitoring		1	\$8.99	\$9.64
Bubble wrap Reflective Thermal Insulation	B000BPF22U	Temperature Control	Temperature Monitoring	ft^2	1	\$9.98	\$10.70
Foil Tape	119877	Structural Aspects	Structural		1	\$4.42	\$4.75
Air Filter	721404314127	Structural Aspects	Structural		1	\$4.75	\$5.10
LocTite							
Polyurethane Sealant	1002938768	Structural Aspects	Structural		1	\$11.99	\$12.89
Permatex 80638 Super Weatherstrip Adhesive, 2 oz.	80638	Door Sealing	Structural				\$8.09
Reflectix SPW0602508 6-Inch by 25-Foot Spiral							
Pipe Wrap	SPW0602508	Temperature Control	Temperature Monitoring				\$10.74
Fan 12V 60mmx10mm (Pack of 2Pcs)	43201619	Temperature Control	Temperature Monitoring				\$9.78
Polyurethane PU Air Hose Pipe Tube Kit	40180000	Gas/Fluid Transfer	CO2 Aspects				\$14.67
Brass Needle Valve	40141600	Gas/Fluid Transfer	CO2 Aspects				\$16.30
CO2 Gas Regulator Soda Pressure Gauge with Adapter	B082WVQ1CL	Gas/Fluid Transfer	CO2 Aspects				\$34.85
HDPE plastic sheet	8619K452	Door Structure	Structural				\$29.80
Rubber Seal	1142A39	Door Sealing	Structural				\$16.60
Gasket-Sealing draw latch	4567A14	Door Sealing	Structural				\$22.77
Misc Hardware		Fasteners	Structural				\$17.03
T-hinge		Door Structure	Structural				\$3.99
weather seal gasket		Door Structure	Structural				\$16.99
<b>TOTAL =</b>							<b>\$886.45</b>

## Appendix C: Logic Flow of CO2 Digital Control Algorithm



## Appendix D: Arduino Uno Incubator Program

```
#define analog_pin 5

#include <SPI.h>
#include <ArduinoQueue.h>

#define sample_size 10
#define heaterPin 9
#define slavePin 10
#define CO2_pin 8

ArduinoQueue<uint32_t> avgq(sample_size);

uint32_t reading = 0;
uint32_t AVG = 0;
uint32_t CO2_conc = 0;

//DONT MOTIFY CODE ABOVE HERE
//*****
*****
//set target CO2 and temp here

uint16_t target_temp = 2900; //target temp x100 (eg. to set target temp to 85.3,
target_temp = 8530)

bool toggle_celsius = 1 ; // 0: target_temp operates in degress F
                          // 1: target_temp operates in degress C

uint32_t target_CO2 = 50; // target CO2 concentration x10 (eg. to set target CO2
to 7.2%, target CO2 = 72)

//*****
*****
//DONT MOTIFY CODE BELOW HERE

uint16_t read_temp(void) { //increase to 32 bit output if shoving values into
ints is a problem
    digitalWrite (slavePin, LOW);
    // reading only, so data sent does not matter
    uint8_t val0 = SPI.transfer(0); //sending two packets of 8 0's
```

```

    uint8_t val1 = SPI.transfer(0);
    digitalWrite (slavePin, HIGH);
    SPI.endTransaction();

    return ((val0 << 8 | val1) & 0x7FF8) >> 3;
}

float mapfloat(float x, float in_min, float in_max, float out_min, float out_max)
{
    return (x - in_min) * (out_max - out_min) / (in_max - in_min) + out_min;
}

void setup() {

    Serial.begin(9600); //9600 baud rate

    // CO2 system
    pinMode(CO2_pin, OUTPUT);

    //heating system
    SPI.beginTransaction(SPISettings(20000, MSBFIRST, SPI_MODE0)); //20kHz SCLK,
    SPI mode : idles low and captures data on rising edge
    SPI.begin(); //sets pin 10 to CS
    pinMode(heaterPin, OUTPUT);

    if (toggle_celsius == 0){
        Serial.print("Temp (F x100)");
        Serial.print("\t");
    }
    else {
        Serial.print("Temp (C x100)");
        Serial.print("\t");
    }
}

Serial.print("Heater State (0=Off, 1=On)");
Serial.print("\t");
Serial.print("CO2_conc (% x10)");
Serial.print("\t");
Serial.print("Solenoid State (0=Off, 1=On)");
Serial.print("\n");
}

void loop() {

```

```

while (!avgq.isFull()){ //intitially fills the queue with readings to calc
average
    reading = 10 * read_temp();
    AVG = AVG + (reading / sample_size);
    //Serial.println(AVG);
    avgq.enqueue(reading / sample_size);
}

uint8_t solenoid_state = 0;
uint8_t heater_state = 0;
uint32_t temp = 0;
uint16_t temp_tolerance = 0;

while(1){
    delay(1000);

    reading = 10 * read_temp() / sample_size;
    AVG = AVG - avgq.dequeue() + reading; //takes a walking average
    avgq.enqueue(reading);
    //takes a Queue of the previous values and a new reading. Uses the Queue of
    //past readings to compute the walking average of all the readings. It then
removes the oldest
    //reading from the queue and adds the new reading.

    if (toggle_celsius == 1){
        uint32_t celsius = map(AVG, 0, 40960, 0, 102375); //accurate to .24 deg
        Serial.print(celsius);
        Serial.print("\t");
        temp = celsius;
        temp_tolerance = 50;
    }
    else{
        uint32_t farenhiet = map(AVG, 0, 40960, 3200, 187475); //accurate to .432
deg
        Serial.print(farenhiet);
        Serial.print("\t");
        temp = farenhiet;
        temp_tolerance = 100;
    }

    if (temp <= target_temp + temp_tolerance) {

        if (temp <= target_temp - temp_tolerance){ //bangs between +/-1 deg f(.5
degC) toleance within +/-4 deg f(2 deg C)

```

```

        digitalWrite(heaterPin, HIGH);
        heater_state = 1;

    }
}
else {
    digitalWrite(heaterPin, LOW);
    heater_state = 0;
}

Serial.print(heater_state);
Serial.print("\t");

float voltage = mapfloat(float(analogRead(analog_pin)), 0, 1023, 0, 5);
CO2_conc = (voltage * 65.3) - 8.1;

if (voltage < 0.19){
    CO2_conc = 0;
}

Serial.print(CO2_conc);
Serial.print("\t");

if (CO2_conc <= target_CO2) { //bangs between +/-1% tolerance is +/-4%

    if (CO2_conc <= target_CO2 - 10){
        digitalWrite(CO2_pin, HIGH);
        solenoid_state = 1;

    }
}
else {
    digitalWrite(CO2_pin, LOW);
    solenoid_state = 0;
}

Serial.print(solenoid_state);
Serial.print("\n");

}
}

```