LOW COST BIOSENSOR PLATFORM

THE DEVELOPMENT OF A LOW-COST INCUBATOR FOR BIOSENSOR APPLICATION

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ABSTRACT

Extensive research has been conducted in the development of biosensors for the developing world in recent years. Despite the plethora of novel sensing systems and an ever increasing library of testable analytes, the implementation and use of biosensors as a commercial product has been limited thus far. This limitation is mainly economic, due to the high cost of construction of complex biosensors and short shelf lives. This research summarizes a step in the development of an ultra-low cost biosensor platform which will allow for a greatly increased flexibility in the application of biosensors throughout the developing world by allowing for complex systems to be simplified, satisfying the ASSURED criteria for an ideal biosensor.

Demonstrated in this work is the creation, characterization and testing of an ultra-low cost incubator for the purposes of PCR, bacterial cell culturing and cell lysis as an initial example of the great promise of the development of this low-cost, versatile and reliable biosensor platform.

ACKNOWLEDGEMENTS

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I am also greatly indebted to both Mr. Vincent Leung and Dr. Sana Jahanshahi-Anbuhi for their mentorship and friendship over the years as well as their continued support, advice and valuable insights throughout the development of this project.

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TABLE OF CONTENTS

I: Introduction ..... - 0 -
Biosensor Classification ..... - 0 -
Biosensor Platforms ..... - 1 -
ASSURED Criteria ..... - 2 -
Research in Novel Biosensor Platforms ..... - 3 -
Affordable ..... - 3 -
Sensitive & Specific ..... - 4 -
User-Friendly ..... - 5 -
Rapid & Robust ..... - 6 -
Equipment Free ..... - 6 -
Deliverable to End User ..... - 7 -
Control of Experimental Conditions ..... - 7 -
II: Proposed Solution ..... - 10 -
III: Experimental Methods ..... - 12 -
Incubator Design & Preparation ..... - 12 -
Part Acquisition and Construction ..... - 15 -
Temperature Profile & Repeatability ..... - 20 -
Bacterial Culturing ..... - 23 -
Rolling Circle Amplification ..... - 24 -
IVI: Experimental Results ..... - 25 -
Part Printing ..... - 25 -
Temperature Profile and Heating Rate ..... - 26 -
Temperature Profile Repeatability ..... - 28 -
Bacterial Culturing ..... - 29 -
Rolling Circle Amplification ..... - 31 -
V: Economic Summary & Scale-Up ..... - 33 -
VI: Future Directions ..... - 35 -
VII: Conclusions ..... - 36 -
VIII: References ..... - 38 -
IX: Appendix A: CAD Drawings ..... - 44 -
X: Appendix B – Arduino IDE Code ..... - 50 -

LIST OF FIGURES

Figure 1: Schematic of how the programmable electronic component developed here can be adapted with different connections. Possible adaptations includes an isothermal RCA device, a device to culture, lyse and filter bacterial cells for subsequent detection and an on-paper PCR device presently under construction ……. -11-

Figure 2: CAD file images of the first generation of the constant temperature incubator. From top left, clockwise: (1) The reservoir design wherein the inner enclosure is the heated zone and the outer ring is where the heating elements lie within. (2) The lid overtop of the reservoir, secured with four screws to minimize heat loss. (3) The base, with a space beneath the reservoir for the circular ceramic heater, and four support pegs for the temperature control programmable board. (4) The complete assembly of the device, with all parts attached as designed. Note that a cover to fit overtop all underlying pieces was also designed and printed. …… -14-

Figure 3: CAD files of the second generation of the incubator (variable temperature and programmable profiles). Within the left panel, from top left, clockwise: (1) Base for the electronic component of the incubator. (2) Bridge to connect the electronic control portion to the reservoir portion of the incubator. (3) Cover for the incubator reservoir component of the device. (4) Reservoir model with imprints on the side for ceramic heaters, holes on corners for agitation. (5) Lid which fits overtop of the incubator reservoir cover. (6) Cover for the electronic control components of the incubator, including the Arduino, relay set, LCD display, user interface and associated wiring. The right panel depicts the complete unit assembly, including models for the electronic boards and assembly. ……-15-

Figure 4: Electronic components required for the first generation of the incubator. From left to right: (1) RioRand digital programmable temperature controller with single relay switch. (2) Metal ceramic heater (MCH) 12V. (3) 12V polyimide high temperature heating element …… -16-

Figure 5: Arduino Uno R3 programmable circuit board (PCB). This is the board onto which the entire operating sequence is loaded, and which controls the incubator's behaviour …… -17-

Figure 6: SainSmart LCD2004 4x20 LCD display. This is controlled via the SDA/SCL pins on the Arduino, and is responsible for communicating the incubator status to the operator ..…. -17-

Figure 7: Cell phone vibrator motor, four of which allows for some mixing of the incubator chamber. Signal is input through Button #3 and processed by the Arduino Uno R3 ……... -18-

Figure 8: Four relay control panel. Three heater elements are designated: HT1 = bottom heater, HT2 = front and back heaters, HT3 = left and right heaters. Control input is 5 V from Arduino, and the relay channels 12V to the heaters and agitators …….. -18-

Figure 9: Sunfounder thermistor and amplifier set for Arduino. Temperature sensitivity is +/- 0.01oC, and is delivered to the Arduino to modulate temperature and display on the LCD.... -19-

Figure 10: Ceramic 12V heating elements. Four 10x20 mm units for the front, back and sides of the reservoir, and a single 10x40 mm heater for the base of the incubator reservoir ……. -19-

Figure 11: Plot of the temperature profile over a steady temperature increase from 26°C to 50°C via three different temperature measurement devices. Onboard thermistor data are represented by , thermometer data by and thermistor data by ……... -21-

Figure 12: Final, assembled version of the first generation of the incubator. Note that this is solely an isothermal incubator, not suited to adaptable temperature profiles or different functions. Final voltage input ranges from 9V – 12V …….. -24-

Figure 13: Final, assembled version of the second generation of the incubator. Note the programmable core of the incubator to the left of the photo with the LCD display on top. This programmable unit can be programmed to interface with a wide range of attachments, thereby greatly increasing the potential function of this device. The final incubator is suited to programmable temperature profiles, steps, holds and stages. Final input voltage accepted ranges from 8V – 15V, or USB …….. -25-

Figure 14: Temperature profile upon heating of the second generation incubator from ambient conditions (T < 20°C) to a setpoint of 50°C. A slight overshoot of 1.5°C is observed at the peak, and a rapid stabilization (with slight oscillations) to 50 +/- 0.3 °C …… -26-

Figure 15: Temperature profile over a repeated heating and cooling cycle to verify repeatability of data. Note that the initial temperature is ambient (20°C) and the setpoint for each heating peak is 50°C which is held for four minutes at each peak before cooling begins. The heating rate for the first peak is 6.18 °C/min, and the heating rate for the second peak is 5.70 °C/min ..…... -28-

Figure 16: Optical density at 600 nm (OD600) of each bacterial culture tested. ● corresponds to the control culture grown in the laboratory incubator/shaker, ■ corresponds to the average value of the two test cultures grown in the battery powered incubator and ◆ corresponds to the blank buffer to ensure no contamination occurred ……... – 29-

Figure 17: 1wt% agarose gel run with a 1kb ladder demonstrating the efficacy of the incubator in performing isothermal rolling circle amplification (RCA) of T2 circle DNA at 30°C. From left to right, the occupied lanes are identified as: (i) 1 kb ladder for identification of sequence size, (ii, iii) duplicate results of the aforementioned reaction mixture following 1 hour at 30°C within the incubator described, and (iv, v) duplicate repeats of the aforementioned reaction mixture following 1 hour at 30°C within the conventional laboratory incubator …….. -31-

LIST OF TABLES

Table 1: The ASSURED criteria for the ideal biosensor, as developed by the World Health Organization. [19] …….. -2-

Table 2: Properties of VeroWhitePlus RGD835, as defined by the manufacturer Objet. Note that these properties are applicable only to the VeroWhitePlus material, used for this incubator project in conjunction with FullCure 705 support material …….....……. -13-

Table 3: Concentration of viable E. coli K12 cells in each of the samples collected. For experimental details, please see protocols listed above. Notable results is the complete absence of viable cells in any lysed samples, and the same order of magnitude of viable cell concentration in the laboratory incubator/shaker and the battery powered incubator …… -30-

Table 4: Unit cost of components of first generation incubator, along with references and an estimated scaled price. Note a total unit cost of $68.94 is obtained for the low-volume branded unit, and a unit cost of $8.95 for the high volume estimate ……. -32-

Table 5: Unit cost of components of the second generation incubator, along with references and an estimated scaled price. Note a total unit cost of $77.18 is obtained for the low-volume branded unit, and a unit cost of $9.75 for the high volume estimate ……. -33-

Table 6: Estimated costs of the production of all plastic and structural components for the second generation incubator via 3D printing and injection moulding approaches. It is noted that injection moulding is only suitable for much higher volume applications …….…… -34-

LIST OF ABBREVIATIONS & SYMBOLS

POCT	Point of Care Testing
PDMS	Polydimethylsiloxane
PCR	Polymerase Chain Reaction
PCB	Programmable Circuit Board
RCA	Rolling Circle Amplification
CAD	Computer Aided Design
TSB	Tryptic Soy Broth
MOSFET	Metal Oxide Semiconductor Field-Effect Transistor

DECLARATION OF ACADEMIC ACHIEVEMENT

The following is a declaration that the complete contents of the research summarized in this document has been completed by Kevin Pennings, and recognizes all the contributions of Dr. Carlos Filipe, Mr. Vincent Leung, Dr. Sana Jahanshahi-Anbuhi, Ms. Sophie Emerson and Mr. George Padeigis in the completion of the associated experimental work and research process. Kevin Pennings contributed to the experimental design, construction of components, data analysis and manuscript preparation. Dr. Carlos Filipe assisted and advised at each step throughout the process. Mr. Vincent Leung and Dr. Sana Jahanshahi-Anbuhi also offered insightful and constructive advice throughout the research process, and both Sophie Emerson and George Padeigis were invaluable in the development and construction of the incubator device described herein.

I: Introduction

The field of biosensors holds immense promise and potential in the development of low-cost, highly specific and user-friendly sensing devices which can be used to great advantage in low-resource environments. (Martinez, et al., 2010) (Dungchai, et al., 2009) Particularly in recent years, biosensors have become increasingly commonly demonstrated as commercially viable products in a range of fields such as point-of-care testing (POCT), disease diagnosis and monitoring, environmental monitoring and food safety. (Yetisen, et al., 2013) (Yu, et al., 2011) (Kerry, et al., 2006)

Biosensor Classification

Biosensors can be broadly classified as any sensing platform or system which incorporates some element of biological detection, amplification or significance. (Scheller, 2007) A biosensing system consists of a receptor, or method of detection, and a transducer to interpret the response to the analyte of interest. (Scheller, 2007) Several novel devices which further incorporate an amplification method to increase the limit of detection have been proposed in recent years as well. (Wang, et al., 2013)

The detection component of a biosensor can be composed of many different receptors. Biosensors have been demonstrated using enzymes, antibodies, cellular receptors, nucleic acids, full cells, aptamers and molecularly imprinted polymers (MIPs) to recognize or detect a specific analyte. (Jahanshahi-Anbuhi, et al., 2013) (Parolo & Merkoci, 2013) (Choi, et al., 2011) (Kriz & Mosbach, 1995) The transducer, or the means by which the signal caused by receptor recognition is conveyed to the user, is either electrochemical,

fluorescent, colorimetric or thermal. (Gerard, et al., 2002) (Pu & Liu, 2009) (Jahanshahi-Anbuhi, et al., 2013) Although there is a wide range of possibilities for the development of novel biosensors, the limitation in real-world use is nearly always in the sensor platform rather than the receptor or transducer itself.

Biosensor Platforms

Biosensor platforms exist in a wide range of materials and designs. One of the most common examples is traditional polydimethylsiloxane (PDMS) microfluidic platforms which have been adapted for use as biosensors. (Sang & Witte, 2010) The advantage of a PDMS based device is the readily available literature on microfluidic systems which allows for the rapid development of functional biosensors using novel biologically based receptors or transduction pathways. Furthermore, pre-developed methods of patterned PDMS for microfluidic applications means that these biosensors can be designed to utilize exceedingly small sample volumes. (Pantoja, et al., 2004) Furthermore, a PDMS system is well-suited for the inclusion of electrical and mechanical components, which greatly improves the flexibility of the biosensor platform. (Xu, 2012)

With the development of a host of novel methods of quantifying analytes in tiny concentrations, a significant amount of research has shifted to the real-world problems in commercializing and producing a biosensor. The existence of a highly advanced and well-developed network of laboratories, diagnostic facilities and clinics exists for the rapid detection, treatment and monitoring of illness and disease in developed countries. (Jelinek, et al., 2002) Therefore, the development of alternative sensors for many analytes which are already measureable in a laboratory setting has limited application in a developed country.

Biosensors are perfectly suited for low-resource areas, however, as the World Health Organization has recognized the need for low-cost diagnostic devices, environmental monitoring sensors and disease state monitoring devices for the developing world, where central laboratories and trained personnel are often in short supply. (Palchetti, et al., 1997) Biosensors are uniquely suited for application in the developing world due to their potential for low-cost, user-friendly operation and a rapidly growing range of analytes which may be detected.

ASSURED Criteria

To the end of furthering the development of biosensors for application in resource limited settings and providing more tools for disease diagnosis and monitoring, the World Health Organization has defined a list of criteria determining the ideal characteristics of a biosensor, termed the ASSURED criteria. (Peeling, et al., 2006)

Table 1: *The ASSURED criteria for the ideal biosensor, as developed by the World Health Organization. (Peeling, et al., 2006)*

Affordable by those at risk
Sensitive (low false negatives)
Specific (low false positives)
User-friendly (simple to perform and requires minimal training)
Rapid (to enable treatment at first visit) and **R**obust (does not require refrigerated storage)
Equipment Free
Deliverable to the end user

The ASSURED criteria, thereby, defines the characteristics a biosensor created for the real world should possess. The aforementioned PDMS based biosensor platforms are often excluded on the basis of cost, as micropatterning and material costs are often

prohibitive for high-volume production and distribution to a resource limited setting. (Dungchai, et al., 2009) Similarly, most electromechanically based disposable sensors are prohibitively expensive on a per unit basis.

Though extensive research has been performed on the development of novel antibody-antigen complexes, enzyme-substrate systems and other methods of analyte detection in recent years, and continues to be a focal point of research for many groups, limited work has been performed in the development of biosensor platforms. Advancing the development of platforms which satisfy the ASSURED criteria will allow for the application of the plethora of novel sensing and reporting systems to real-world situations. At the present time, the application of biosensors is not limited by the ability to detect analytes of interest in sufficiently low concentrations, but rather by logistical concerns regarding the implementation of biosensors: reagent stability, unit cost, required equipment and user training are the primary delays in the implementation of advanced biosensors around the world.

Research in Novel Biosensor Platforms

Affordable

As biosensors are primarily intended for use in resource-limited, developing areas, economics are of paramount importance. The development of low-cost biosensors is necessary, as prohibitively costly sensors are not possible to be adopted on a large scale. To this end, complex electromechanical systems which are constructed for a single use, as well as micro-patterned PDMS and advanced microfluidic systems are not well-suited for the development of a low-cost system.

The area of paper-based biosensors holds immense promise in the development of extremely low-cost, single use and disposable sensors. Significant research has been performed in this area, notably by the group led by Dr. Pelton at McMaster and the Whitesides group at Harvard. (Pelton, 2009) (Martinez, et al., 2010) (Jahanshahi-Anbuhi, et al., 2013) (Martinez, et al., 2010) (Pelton, 2009) Paper-based devices are further advantageous in their readily and easily scalable production which can be performed in resource limited areas, not requiring the presence of a central laboratory or manufacturing facility.

However, paper-based sensors are limited in their application. Significantly, early sensors were limited to single step reactions with no time or rate control. Advances in this area includes three dimensional flow control, fast flow channels, microfluidic valves and sequential release of different reagents over time. (Ge, et al., 2012) (Jahanshahi-Anbuhi, et al., 2012) (Jahanshahi-Anbuhi, et al., 2014)

While paper-based systems provide a means to combine several reagents, and tools now exist for flow control and shutoff, as well as sequential release of components and timed reactions, paper-based biosensors are not able to perform several required procedures. Namely, solution chemistry is impossible via a paper-based device, and temperature control has not been demonstrated in a low-cost and reproducible manner.

Sensitive & Specific

The sensitivity and specificity of a biosensor is dependent on the underlying system which is used for detection and transduction. As aforementioned, extensive research has

been conducted in the development of novel enzyme-substrate systems, antibody-antigen interactions and amplification methods to increase both the sensitivity and specificity of assays for specific analytes. This has allows for the detection of many novel analytes leading to previously inconceivable disease diagnosis and monitoring, water quality testing, and even proposed methods of monitoring spoilable goods via active packaging.

While the value of research in the pathways associated with both detection and transduction cannot be understated in the development of commercializable biosensors, the vast library of known systems which can be adapted for a detection method means that the difficulty in producing a sensitive and specific biosensor is once again dependent on the biosensor platform. For instance, the use of a paper-based biosensor means that either immobilized biomolecules must be used, or a much slower reaction rate is observed on the paper due to limited molecular mobility. Consequently, significant losses in sensitivity is commonly noted when a paper-based device is created based on a solution phase system. (Sicard, et al., 2015)

User-Friendly

As biosensors are intended primarily for application in resource limited settings where few trained professionals are available, the user-friendliness of the biosensor is of great importance. To this end, a biosensor should require minimal user intervention, and be as automated as possible, while still satisfying the low-cost requirement. This is a difficult balance to strike, but has been accomplished well with the paper-based biosensors developed at McMaster which require the user only to immerse one portion of the sensor

into the sample solution and wait several minutes to read the result. (Jahanshahi-Anbuhi, et al., 2016)

However, with more advanced and flexible biosensors, this aspect of user-friendliness, and the requirement for minimal user training and intervention must be maintained if a biosensor is intended to be used in a widespread manner.

Rapid & Robust

A biosensor which is intended for use by the general populace, with minimal training and limited resources for repairs, must be designed to be robust, and not require continuous user intervention and repairs. While an intuitive requirement for a biosensor, this is nonetheless a difficult requirement to satisfy due to the innate complexity of most biosensors, incorporating multiple reactions and reagents which must be combined at specific conditions and timing.

Similarly, the speed with which the sensor returns results is critical as well. For use with minimally trained personnel, rapid results which are easily interpreted are required. For instance, if a biosensor is to be used to test a water sample for contaminants, a turnaround time of several days is far too long to be of practical use for users who require immediate results.

Equipment Free

The equipment free criteria is due to the lack of availability of a centralized laboratory or healthcare system, as well as related to the requirement of minimal training and low cost. Complex tests which require costly equipment and highly trained staff

members is logically incompatible with a limited resource area with no laboratory facilities and a shortage of trained healthcare professionals.

Deliverable to End User

Paper-based biosensors, and biosensors in general, are often compromised in their performance by the use of labile biomaterials or oxygen sensitive reagents. Therefore, the biosensors have a defined shelf life which is often much too short to be viable for worldwide distribution. (Jahanshahi-Anbuhi, et al., 2014) To circumvent this shortcoming, extensive research has been performed in the evaluation of silk fibroin, sol-gel and polysaccharides in the stabilization of biomolecules. (Lu, et al., 2010) (Wang, et al., 2011) (Jahanshahi-Anbuhi, et al., 2016) Research in this area has resulted in the development of biomolecule containing sensors capable of being stored at ambient conditions for months at a time, thereby marking a significant advance in the ability of producing a biosensor which is deliverable to the end user.

Control of Experimental Conditions

A biosensor is dependent on one or more chemical or biochemical reactions proceeding at calibrated rates in order to return a meaningful result. Therefore, it is important that the reaction conditions which may significantly affect the reaction rate, or determine whether the reaction will proceed, are controlled carefully. Within a laboratory setting, this is not a difficult requirement: pH, temperature and humidity can all be carefully and precisely controlled. However, in the field, particularly when addressing the economic constraint associated with biosensors, this is a much more difficult problem to address.

Research in the control of the reaction pH has been addressed by Sana Jahanshahi-Anbuhi et al via the use of water-soluble polysaccharide films which have been demonstrated to allow for pH control in a time-dependent manner. (Jahanshahi-Anbuhi, et al., 2013) Conversely, the issue of variable humidity which is experienced in the field has not been addressed explicitly. However, when humidity is foreseen to be a concern with a biosensor, for example in an application where a hygroscopic material is to be used, ambient humidity is investigated as a potential confounding factor, and is thus accounted for in experimental design and calibration. (Jahanshahi-Anbuhi, et al., 2014)

The third factor, temperature, is intuitively an extremely important variable to control when addressing chemical reactions which are calibrated to calculate concentrations. (Jahanshahi-Anbuhi, et al., 2016) However, the issue of temperature control is a difficult problem to solve. This problem is further complicated when considering temperature limits of thermally labile biomolecules which are often used to construct biosensors, thereby meaning that an effective maximum temperature exists above which a biosensor will not be functional following exposure to that temperature.

Within a laboratory setting, the concern of temperature control is easily dealt with. A cold chain exists to transport labile biomolecules and even completed biosensors throughout developed countries, and many options of temperature control within a laboratory ranging from cryogenic storage to high temperatures are readily available. However, a biosensor must be deliverable to the end user, equipment free and requiring minimal user training. Therefore, present methods of temperature control available within a laboratory are unsuitable for a biosensor in the developing world.

A recently proposed mechanism of controlling the temperature of a biosensor is the use of an exothermic chemical reaction to keep a reaction chamber at a constant temperature. (Buser, et al., 2015) Although an ingenious solution to this problem, chemical heating has several issues. Namely, reagents must be loaded in such a manner as to allow for a constant temperature to result. However, this temperature is not customizable or programmable by the user. Furthermore, each loading of chemicals is suitable for a single operation following which is must be replaced. (Buser, et al., 2015) Finally, the accompanying storage, loading and disposal of chemicals requires significant user training and user intervention throughout the testing procedure, thereby limiting the applicability of the biosensor.

The use of electricity from an established network, or grid power, is not a suitable option for the widespread use of a biosensor throughout a developing country, as this limits the use of the biosensor to areas which have grid power, thereby excluding a large portion of the world. However, the concept of electrical heating is well-suited to the development of a portable temperature control unit. Present technology of battery powered incubators is well-developed, but economic considerations are not typically primary objectives in development. For example, currently available polymerase chain reaction (PCR) incubators cost approximately $1,000 - $2,500 USD. (Hach, 2015)

The difficulties associated with the production of a robust, reliable, low-cost and user-friendly biosensors which are capable of controlling critical experimental variables such as temperature are easily recognizable. However, present technologies have advanced

suitably to allow for a novel and flexible device to be created to further the application of biosensors.

II: Proposed Solution

The proposed solution is the development of a biosensor platform which is a combination of presently available technologies, designed with the ASSURED criteria in mind. The inspiration for this avenue of development is the extreme drop in electronic component prices which has been recently observed, the increasing ease with which programmable circuit boards (PCBs) may be acquired and customized, and the ready availability of rapid prototyping (3D-printing) technologies.

We propose the development of a system which is constructed out of readily available materials, which could be replicated at any point around the world with minimal training and very little equipment. This method relies on the development of a low-cost electronic control module which may be customized to suit various biosensor requirements. We demonstrated here the application of this control module to a portable, low-cost incubator device.

This project was developed with world-wide access to materials as a priority. Therefore, all materials are readily available to the end user and the unit operates on a flexible voltage battery power supply which may be charged with a solar panel between uses thereby removing the requirement of grid power. Furthermore, all structural components are made available through a publicly accessible Google Drive folder for 3D

printing at any location, which allows the end user to choose the modules they require for their specific device.

The overarching goal for this project is the creation of a small programmable module which may be connected to a wide range of devices which it can power and control, as demonstrated in Figure 1 below.

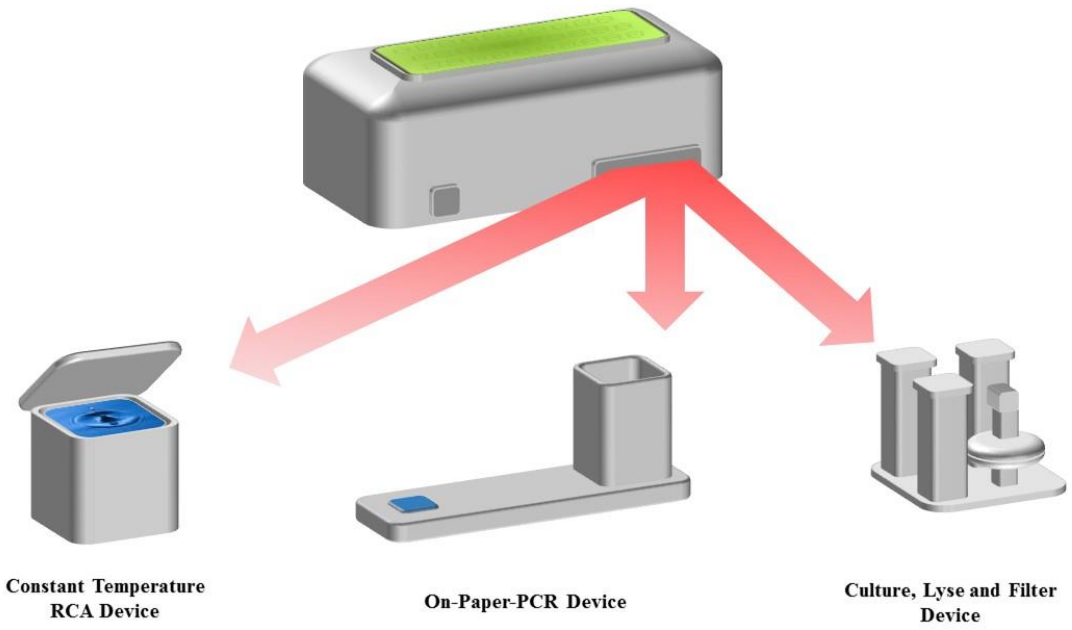

Figure 1: *Schematic of how the programmable electronic component developed here can be adapted with different connections. Possible adaptations includes an isothermal RCA device, a device to culture, lyse and filter bacterial cells for subsequent detection and an on-paper PCR device presently under consideration.*

We envision a device which can be obtained from a website: all required CAD figures, programming and links to purchasing electronic components will be included, and the end user selects what their specific goal for the platform is. For instance, the control

device seen in Figure 1 above may be printed, along with an isothermal reservoir. The programmable electronic unit has the required code already loaded to operate the incubator.

Herein developed is a single demonstration of this novel, flexible and versatile device. The central programmable unit is designed and constructed, and a variable temperature incubator reservoir is constructed and demonstrated. It is further noted that this approach holds great possibility for subsequent research into other devices which may be attached to this electronic core module.

III: Experimental Methods

All experimental methods are detailed in the sections below, corresponding to each procedure followed.

Incubator Design & Preparation

Each printed component was designed using a student licensed copy of Autodesk Inventor 2014 via McMaster University's Technology Services. Each part was then exported as STL files which were printed on an Objet 24 3D printer in the Biointerfaces Institute with the assistance of Dr. Mehdi Keramane. The Objet 24 printer has a resolution of 0.1 mm and a max build volume of 7.2 L (200 mm x 240 mm x 150 mm). Each object is printed of VeroWhite Plus, with the physical properties contained in the table below.

Table 2: *Properties of VeroWhitePlus RGD835, as defined by the manufacturer Objet. Note that these properties are applicable only to the VeroWhitePlus material, used for this incubator project in conjunction with FullCure 705 support material.*

Properties	ASTM	Units	Value
Tensile strength	D-638-03	MPa	50-65
Elongation at break	D-638-05	%	10-25
Modulus of elasticity	D-638-04	MPa	2000-3000
Flexural strength	D-790-03	MPa	75-110
Flexural modulus	D-790-04	MPa	2200-3200
HDT, ºC @ 0.45 MPa	D-648-06	ºC	45-50
HDT, ºC @ 1.82 MPa	D-648-07	ºC	45-50
Izod notched impact	D-256-06	J/m	20-30
Water absorption	D-570-98 (24 h)	%	1.1-1.5
Glass transition temperature	DMA, E>>	ºC	52-54
Shore hardness (D)	Scale D	Scale D	83-86
Rockwell hardness	Scale M	Scale M	73-76
Polymerized density	ASTM D792	g/cm^3	1.17-1.18
Ash content	USP281	%	0.23-0.26

The Objet 24 printer is capable of printing a temporary support material (FullCure 705) about the permanent plastic structures which must be removed. Therefore, each part is washed via pressurized water and sanded with 400 grit paper to remove surface roughness from the layered deposition. The parts were verified for size and physical features and assembled as designed. All electronic and mechanical components were fitted for size, and assembled. Adhesive was used to secure all parts not intended to be moveable. A brief summary of the printed parts for each incubator generation is included in the figures below.

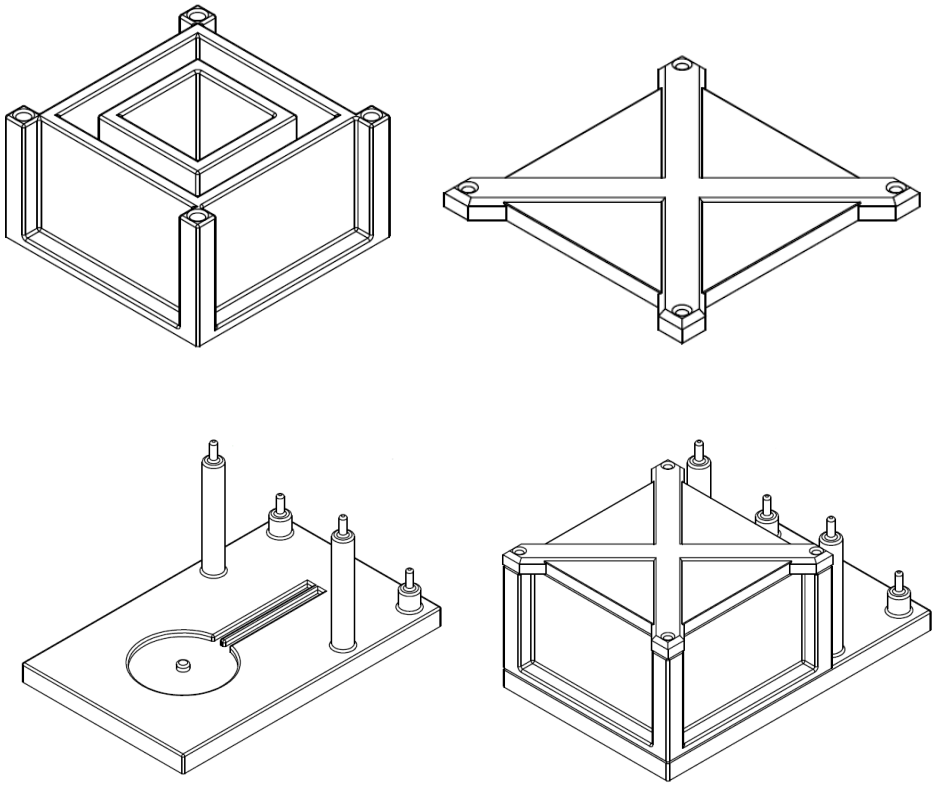

Figure 2: *CAD file images of the first generation of the constant temperature incubator. From top left, clockwise: (1) The reservoir design wherein the inner enclosure is the heated zone and the outer ring is where the heating elements lie within. (2) The lid overtop of the reservoir, secured with four screws to minimize heat loss. (3) The base, with a space beneath the reservoir for the circular ceramic heater, and four support pegs for the temperature control programmable board. (4) The complete assembly of the device, with all parts attached as designed. Note that a cover to fit overtop all underlying pieces was also designed and printed.*

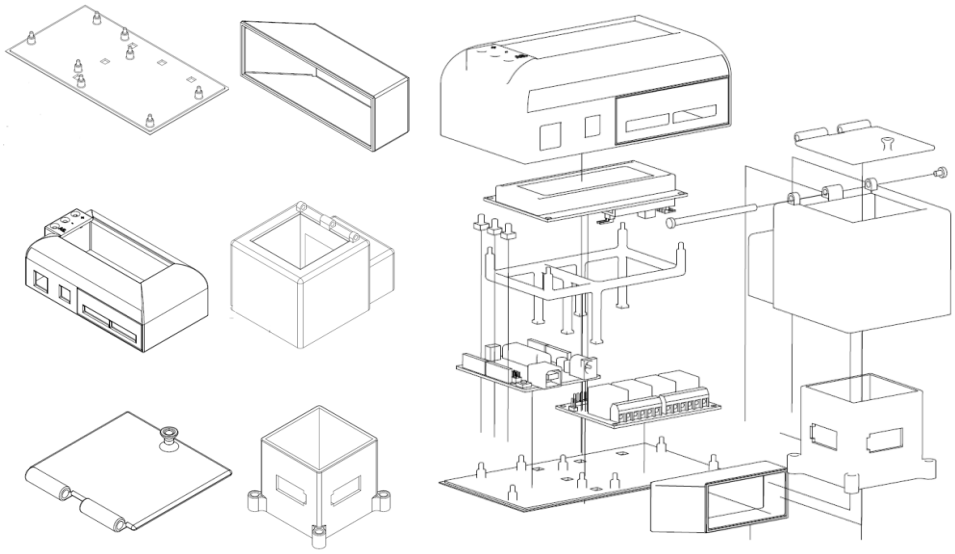

Figure 3: *CAD files of the second generation of the incubator (variable temperature and programmable profiles). Within the left panel, from top left, clockwise: (1) Base for the electronic component of the incubator. (2) Bridge to connect the electronic control portion to the reservoir portion of the incubator. (3) Cover for the incubator reservoir component of the device. (4) Reservoir model with imprints on the side for ceramic heaters, holes on corners for agitation. (5) Lid which fits overtop of the incubator reservoir cover. (6) Cover for the electronic control components of the incubator, including the Arduino, relay set, LCD display, user interface and associated wiring. The right panel depicts the complete unit assembly, including models for the electronic boards and assembly.*

Part Acquisition and Construction

As the primary focus of this incubator project is the ready availability of these parts around the world, parts were selected for ease of access and user-friendliness. At each point throughout the development cycle, parts were obtained from regular consumer websites such as eBay™ and Amazon™. While this guaranteed that the parts are readily available to any individual who would wish to repeat the work, it is not the most financially sensible option. It is noted that much less costly options are available for each component. For instance, rather than purchasing an Arduino Uno R3 for the PCB at the heart of the

incubator, it is entirely viable to purchase off-brand PCBs in bulk at a savings of greater than 90%. This concern is further addressed in the economic evaluation section to follow. The photos below depict the electronic components used to build each respective generation of incubator.

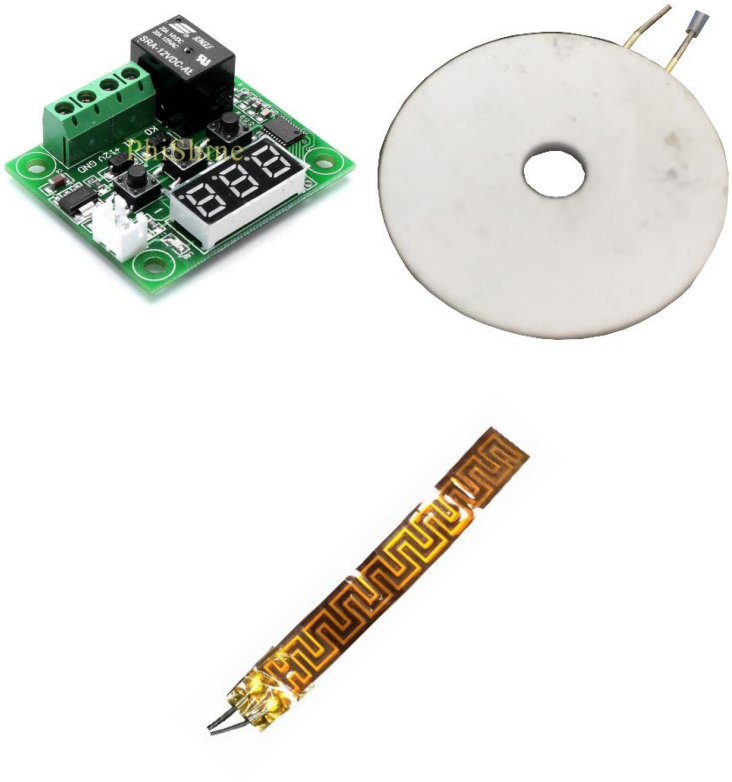

Figure 4: *Electronic components required for the first generation of the incubator. From left to right: (1) RioRand digital programmable temperature controller with single relay switch. (2) Metal ceramic heater (MCH) 12V. (3) 12V polyimide high temperature heating element.*

Figure 5: *Arduino Uno R3 programmable circuit board (PCB). This is the board onto which the entire operating sequence is loaded, and which controls the incubator's behaviour.*

Figure 6: *SainSmart LCD2004 4x20 LCD display. This is controlled via the SDA/SCL pins on the Arduino, and is responsible for communicating the incubator status to the operator.*

Figure 7: *Cell phone vibrator motor, four of which allows for some mixing of the incubator chamber. Signal is input through Button #3 and processed by the Arduino Uno R3.*

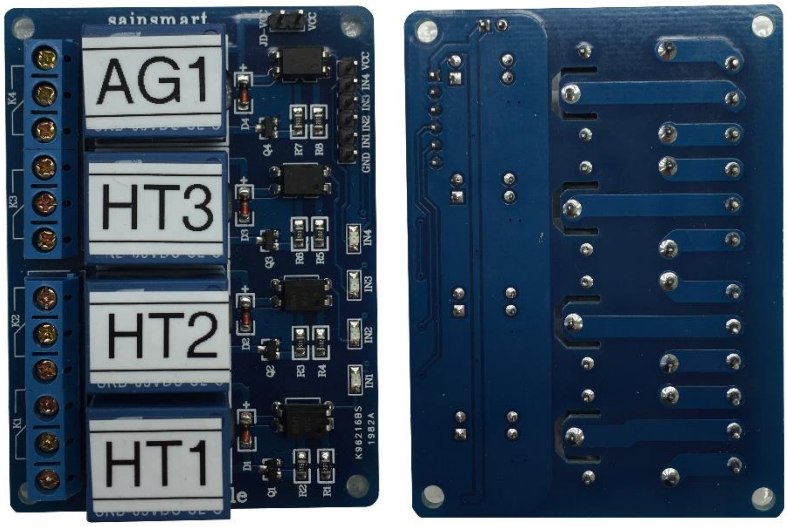

Figure 8: *Four relay control panel. Three heater elements are designated: HT1 = bottom heater, HT2 = front and back heaters, HT3 = left and right heaters. Control input is 5 V from Arduino, and the relay channels 12V to the heaters and agitators.*

Figure 9: *Sunfounder thermistor and amplifier set for Arduino. Temperature sensitivity is +/- 0.01°C, and is delivered to the Arduino to modulate temperature and display on the LCD.*

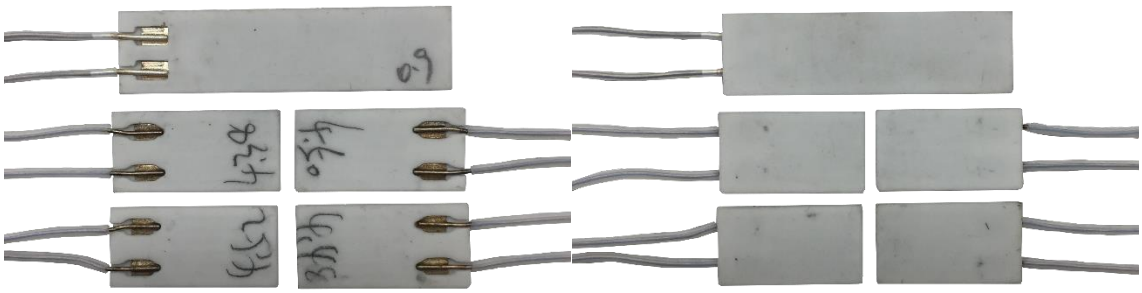

Figure 10: *Ceramic 12V heating elements. Four 10x20 mm units for the front, back and sides of the reservoir, and a single 10x40 mm heater for the base of the incubator reservoir.*

Once the electronic parts were obtained and fitted to the plastic printed components, all connections are soldered and prepared for connection to the Arduino. Programming is accomplished with Arduino IDE, an open source program designed to upload programming to the Arduino Uno R3 via a USB connection. Note that the finalized Arduino code for the incubator is contained in Appendix B at the conclusion of this document. All code used for this project is developed independently.

The Arduino Uno R3 was connected to the LCD display using a modified I2C library, which allows for the connection and control of an LCD display using only two pins on the Arduino (SCL and SDA). The relay board was connected with isolated 12V power to avoid power fluctuations to the Arduino as a result of relay and heater operation, and pins 9-12 are connected as control pins for relays 1-4, respectively. The thermistor and amplifier board was connected to the Arduino via an analog pin to allow for continuous temperature monitoring. A function was defined to calculate the temperature from thermistor input within the operating sketch.

All structural components were hydro-washed following printing to remove support material. A 600-grit wet sandpaper was used to remove any residual ridges and artifacts from the printing process, and electronic components and heaters added to the plastic frames. When the plastic components are expected to be exposed directly to high temperatures, an aluminum foil tape is used for shielding to prevent material degradation. Printed covers were placed overtop each component to improve aesthetics. Components were then assembled as demonstrated in Appendix Figure *"Generation 2: Incubator Assembly"*. When required, adhesive (LePage gel glue) was utilized to secure plastic components together. A single 12V power supply was set up from the battery to the Arduino board, as accessible from the outside of the incubator. All relevant incubator control sketches and programming was uploaded via USB from a laptop directly to the incubator.

Temperature Profile & Repeatability

The profile of temperature increase and subsequent decrease is an important characteristic of a device designed to control temperature, as is the steady state behaviour

of the incubator. Therefore, the rate of heating, rate of cooling and the reliability with which the incubator is able to maintain a set temperature point is to be evaluated. In the present form of this device, the maximum temperature attainable is 95^{o}C, beyond which the printed plastic material will begin to melt. This limitation is overcome by the use of injection moulded plastic components, however.

The incubator was constructed in two distinct generations. The first generation was designed as an easy-to-use, inexpensive isothermal incubator suitable for cell culturing. Although the incubator proved to be effective at maintaining a constant temperature, as evidenced by Figure A1 in Appendix A, it was desired to create a device which is more adaptable to changing requirements – a true biosensor platform, while maintaining the low-cost and equipment free requirements. Therefore, a second generation of the incubator which is programmable for temperature profiles, timed periods of isothermal operation and allows for user input to alter and adjust the programming was developed.

The trials herein described were performed with the second generation of the incubator, which is the device used in all subsequent sections of this document. Initially, to verify the accuracy of the onboard thermistor in displaying the actual temperature of the water within the reservoir, a verification trial was conducted in which 7 mL of distilled water was placed into the reservoir and the setpoint increased from 22^{o}C to 50^{o}C. Three temperatures were monitored: (i) the temperature displayed by the Arduino via the onboard thermistor, (ii) the analog temperature as read from a conventional thermometer immersed in the reservoir and (iii) a second thermistor immersed within the incubator's reservoir.

From the results indicated in *Figure 11* below, each of the three measurement devices determines the same temperature over time, thereby verifying the calibration on the Arduino temperature sensor. Furthermore, this verifies that the selected location of the thermistor in the incubator reservoir allows for an accurate measurement of the actual reservoir temperature. This is a critical requirement, as the operation of the incubator device relies on accurate, timely and reliable temperature sensing.

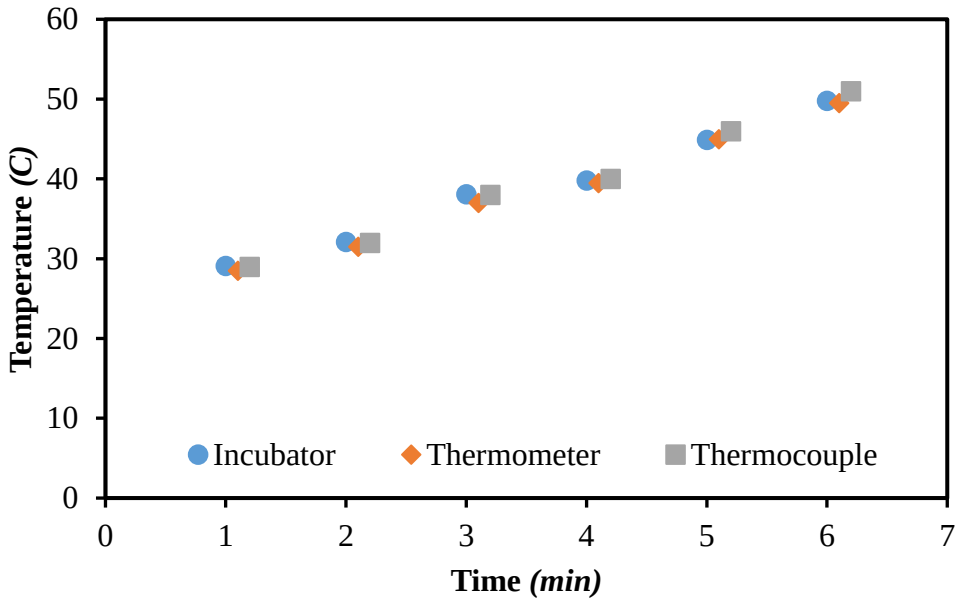

Figure 11: *Plot of the temperature profile over a steady temperature increase from 26°C to 50°C via three different temperature measurement devices. Incubator is prepared with 7 mL of distilled water within the reservoir. Onboard thermistor data are represented by ●, thermometer data by ◆ and thermistor data by ■.*

To establish the incubator's temperature profile upon heating and cooling, the incubator's battery was charged, temperature set to below ambient temperature (< 20 °C), and then raised to a setpoint of 50°C. Temperature displayed was manually logged every

20 seconds to ensure a complete data set was obtained. In Figure 11 above, every fifth data point is plotted, to ensure readability of the figure.

To establish the repeatability of the incubator's heating and cooling profile, the incubator was returned to ambient conditions, initialized with a setpoint below ambient conditions (< 20^{o}C), and the setpoint raised to 50^{o}C. Once the incubator stabilized at the setpoint, the setpoint was once again lowered to ambient conditions, and the cycle repeated again. This test was completed for four cycles, with results for the first two cycles presented in Figure 15 below for readability.

Bacterial Culturing

The incubator is prepared and programmed to maintain a constant temperature of 37^{o}C, with 7 mL of distilled water contained in the incubator chamber as a heat transfer medium. A master solution of Tryptic Soy Broth (TSB) was prepared, and inoculated with a culture of *E. coli* K12 at a very small starting cell concentration obtained by transfer from the colony to the medium via a wooden stick. This solution was divided into three components, two of which were cultured in Eppendorf tubes placed inside of the incubator, and one of which was cultured in a cell culture incubator and shaker to compare against currently accepted methods. Finally, one sample of TSB was kept as a blank to ensure no experimental contamination.

Cells were cultured for four hours at a constant 37^{o}C, and a 100 μL sample was taken at thirty minute intervals, scanned for absorbance in a 96 well plate at 600 nm via a Tecan M1000 plate reader, and then returned to the culture at temperature to monitor cell

growth. Following cell growth, half of each sample was lysed by heating to 90ºC for 5 minutes. The incubator cultures were heated to 90ºC via the incubator and a setpoint change, and the culture grown in the laboratory incubator/shaker was lysed at 90ºC by a laboratory hotplate.

Following lysis, samples of both un-lysed cells and lysed cells were subjected to a six-fold serial dilution in Phosphate Buffered Saline (PBS), and 100 μL of each sample plated onto half a culture plate. The cultures were incubated for 12 hours at 37ºC, and colony forming units counted and recorded. The colony forming unit concentration per millilitre was then back calculated out when the colonies were within a countable range to obtain a value for the concentration of viable cells in each sample.

Rolling Circle Amplification

Rolling circle amplification (RCA) was performed isothermally on a circle ligation template (CLT) DNA sequence at 30ºC. For the purposes of comparison with currently accepted methods, a control was run in a conventional heater element set at the target temperature of 30ºC.

Each reaction mixture contained 1 μL of T2 circle DNA (1 μM) was obtained from Integrated DNA Technologies (IDT), 5 μL of Phi29 buffer (10x), 2.5 μL of dNTP's (10 mM), 1 μL Phi29 DNA polymerase obtained from ThermoFisher, 1 μL circle ligation template (CLT, 1 μM) obtained from IDT and 39.5 μL sterile water. Each reaction was run for a total of 60 minutes, and duplicates were performed for the isothermal incubator and duplicate control experiments via the currently accepted laboratory heater.

The incubator was filled with water as a heat transfer medium, and 600 μL Eppendorf tubes were used to mix reagents to form the reaction mixture. Similarly, the laboratory heater was brought to temperature prior to preparing the reaction mixture. A single master mix was prepared, and aliquoted into the four tubes to ensure the experiment is identical.

Following the 60 minute run time, each sample was run in a 1wt% agarose gel prepared in Tris buffer, acetic acid and EDTA (TAE) buffer prepared within the lab. The gel was polymerized following complete dissolution within a microwave, and an eight channel comb placed into the mold. A 1 kb DNA ladder was used along with the amplified samples to serve as a reference.

IVI: Experimental Results

Part Printing

In order to ensure that the CAD designed parts are printed at the appropriate scales, each dimension is measured prior to assembly. Figures 12 and 13 below illustrates the final product for each of the incubator generations.

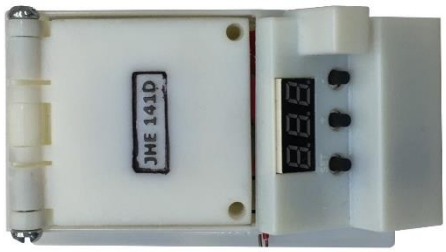

Figure 12: *Final, assembled version of the first generation of the incubator. Note that this is solely an isothermal incubator, not suited to adaptable temperature profiles or different functions. Final voltage input ranges from 9V – 12V.*

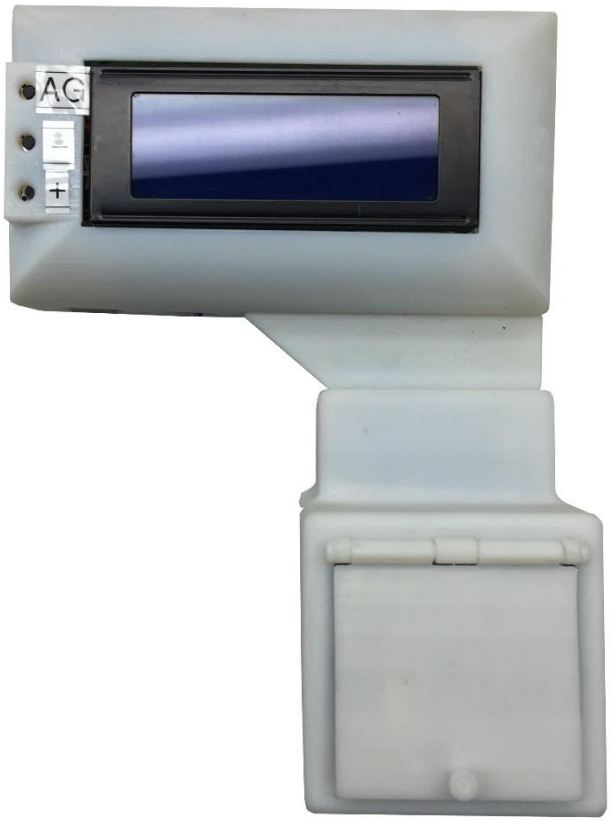

Figure 13: *Final, assembled version of the second generation of the incubator. Note the programmable core of the incubator to the left of the photo with the LCD display on top. This programmable unit can be programmed to interface with a wide range of attachments, thereby greatly increasing the potential function of this device. The final incubator is suited to programmable temperature profiles, steps, holds and stages. Final input voltage accepted ranges from 8V – 15V, or USB.*

Temperature Profile and Heating Rate

Figure 14 below presents the temperature profile of the second generation incubator upon heating from ambient (~25^{o}C) to 50^{o}C. Note that this temperature profile increases nearly linearly over the first 400 seconds (6 minutes) as the temperature approaches 50^{o}C. A slight overshoot occurs, due to the binary nature of the controlled heating elements, but the incubator rapidly stabilizes to 50^{o}C +/- 1.5^{o}C with a slight oscillation. The maximum

temperature overshoot at 50°C is 51.5°C, which is an acceptable margin of temperature range for the vast majority of biosensor requirements.

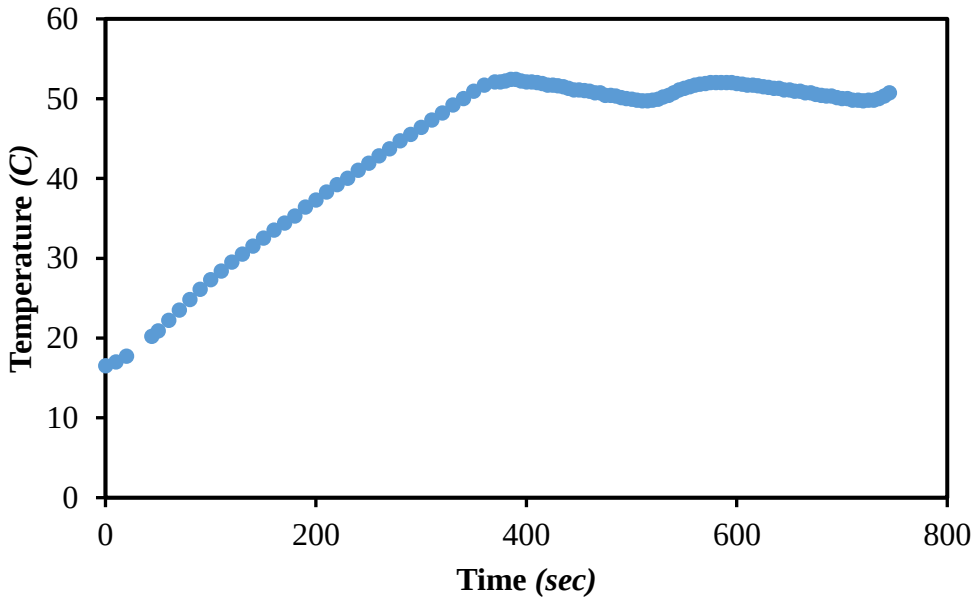

Figure 14: *Temperature profile upon heating of the second generation incubator from ambient conditions (T < 20°C) to a setpoint of 50°C. A slight overshoot of 1.5°C is observed at the peak, and a rapid stabilization (with slight oscillations) to 50 +/- 1.5 °C.*

The initial linear heating profile demonstrated in Figure 14 above represents the maximum rate of heating of the incubator with a reservoir containing 10 mL of distilled water and a fully charged battery. At these conditions, a heating rate of 0.1022 °C/s, or 6.13 °C/min. This heating rate allows the incubator to reach its maximum temperature of 95°C from ambient conditions within 11 minutes, which is an excellent response time for a 70°C temperature change using power from a portable battery. This response time is a characteristic of the battery capacity, the number of heating elements, and the volume of the incubator reservoir. The maximum temperature this incubator as presented is capable

of maintaining is 95°C due to the material melting point as discussed previously. Furthermore, it is noted that 11 minutes for the expected maximum temperature increase is not a prohibitive length of time, particularly when compared to laboratory testing. As the battery drains over time, the rate of heating reduces slowly, with a heating rate of 0.1022 °C/s representing the maximum heating rate with a fully charged battery.

Temperature Profile Repeatability

Figure 15 below illustrates the data obtained from a heating and cooling cycle designed to demonstrate the repeatability of the temperature profile and the reliability of the incubator device itself. Each heating peak is held for four minute at 50°C, then the setpoint is lowered to ambient conditions to allow for the cooling. It is noted that the heating rates are 6.18 °C/min and 5.70 °C/min respectively. This slight difference in heating rate is due to the slight decrease in battery voltage due to the battery having a finite capacity. The number of cycles that is possible to evaluate using this method is entirely dependent on the battery capacity.

Furthermore, it is noted that the cooling rate is significantly slower than the heating rate in both instances. While this is a consistent trend, this is a source of concern for any design which requires rapid cycling of temperatures. This is a suggested area of further development.

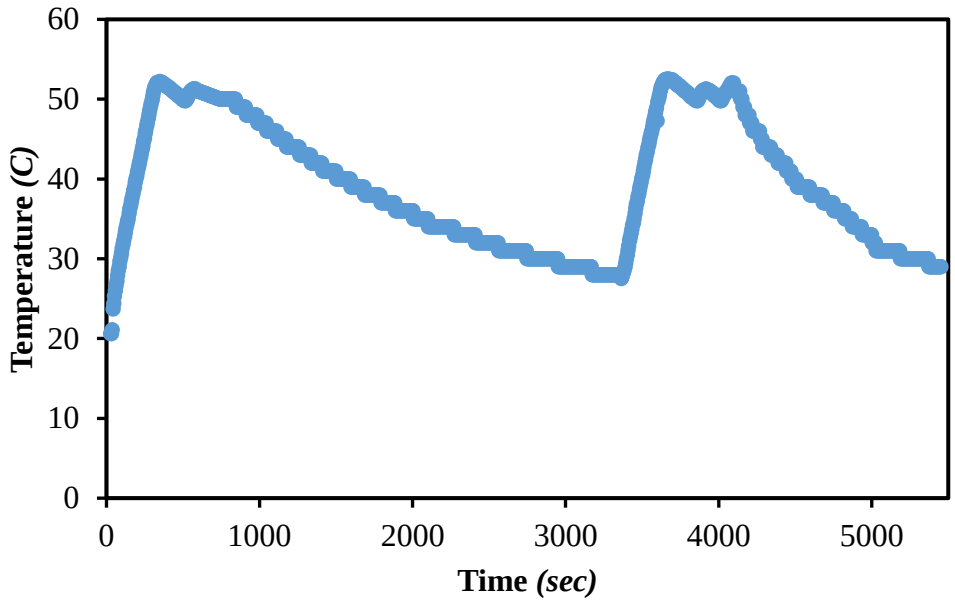

Figure 15: *Temperature profile over a repeated heating and cooling cycle to verify repeatability of data. Note that the initial temperature is ambient (20°C) and the setpoint for each heating peak is 50°C which is held for four minutes at each peak before cooling begins. The heating rate for the first peak is 6.18 °C/min, and the heating rate for the second peak is 5.70 °C/min.*

Bacterial Culturing

The results of the *E. coli* culturing are demonstrated in Figure 16, which demonstrates the growth rate (as reflected by OD_{600}) of *E. coli* K12 in TSB for the three conditions tested: an average of the two samples cultured in the battery powered incubator, the sample cultured in the laboratory incubator/shaker and the media blank. It is noted that for the first 150 minutes the growth rates of the control and test samples is virtually indistinguishable. Beyond 150 minutes, however, the laboratory incubator/shaker exhibits a higher growth rate than the battery powered incubator. The reason for this observed differential in growth rate is most likely due to the absence of shaking within the battery powered incubator. This hypothesis may be evaluated in future studies by placing the

incubator device within the laboratory shaker to quantify the effect of agitation. The inclusion of small vibrator motors on the battery powered incubator is insufficient to properly agitate the entire contents of the reservoir to match the agitation the laboratory incubator/plate shaker accomplishes.

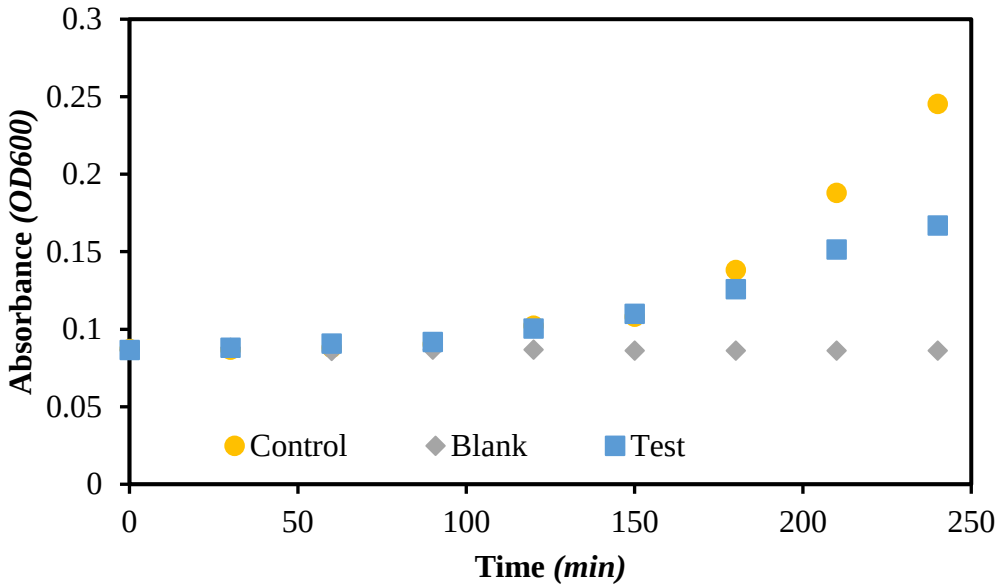

Figure 16: *Optical density at 600 nm (OD_{600}) of each bacterial culture tested. ● corresponds to the control culture grown in the laboratory incubator/shaker, ■ corresponds to the average value of the two test cultures grown in the battery powered incubator and ◆ corresponds to the blank buffer to ensure no contamination occurred.*

In addition to the OD_{600} measurements taken of the cell culture, half of each sample was lysed at 90°C. Table 3 below summarizes the calculated concentration of viable cells in each sample. It is immediately noted that the samples lysed within the battery-operated incubator had no viable cells remaining, identical to the laboratory incubator, as expected. Furthermore, it is noted that the concentration of viable cells in the un-lysed sample is on

the same order of magnitude as that of the culture grown in the laboratory incubator/shaker, further reinforcing the validity of the battery powered incubator.

Table 3: *Concentration of viable E. coli K12 cells in each of the samples collected. For experimental details, please see protocols listed above. Notable results is the complete absence of viable cells in any lysed samples, and the same order of magnitude of viable cell concentration in the laboratory incubator/shaker and the battery powered incubator. Initial cell concentration was very small, as cells were transferred by the touching of a colony in culture to a sample of pure TSB.*

Laboratory Incubator/Shaker	**Lysed**	0 cfu/ml
	Non-Lysed	$1.6x10^8$ cfu/ml
Battery Powered Incubator	**Lysed**	0 cfu/ml
	Non-Lysed	$1.2x10^8$ cfu/ml
	Lysed	0 cfu/ml
	Non-Lysed	1.1 cfu/ml

Rolling Circle Amplification

Figure 17 below depicts the agarose gel run with the duplicates of each RCA reaction mixture run. It is noted that the band thickness and location is equal for each of the four samples, thereby demonstrating that the RCA proceeded at the same rate in the battery powered incubator as it did in the laboratory incubator, confirming the operation of the battery powered incubator. This is an isothermal process, thereby is the simplest type of reaction the incubator is expected to encounter in its role as a biosensor platform.

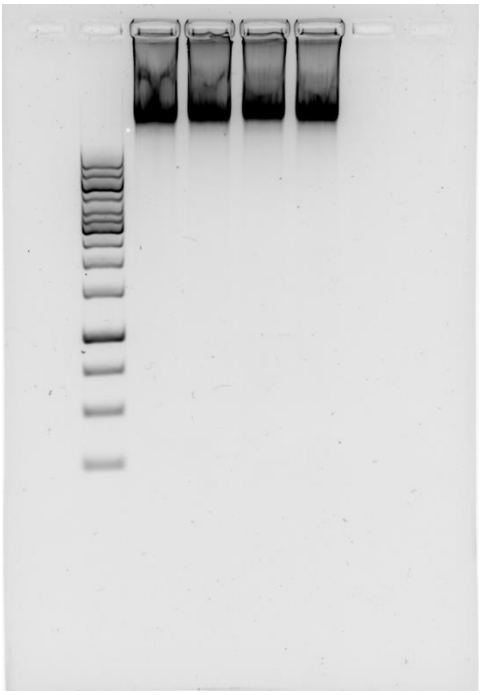

Figure 17: *1wt% agarose gel run with a 1kb ladder demonstrating the efficacy of the incubator in performing isothermal rolling circle amplification (RCA) of T2 circle DNA at 30°C. From left to right, the occupied lanes are identified as: (i) 1 kb ladder for identification of sequence size, (ii, iii) duplicate results of the aforementioned reaction mixture following 1 hour at 30°C within the incubator described, and (iv, v) duplicate repeats of the aforementioned reaction mixture following 1 hour at 30°C within the conventional laboratory incubator.*

The location of the amplified bands relative to the ladder indicates that the product fragments are larger than the components in the 1 kb ladder in the first lane. Due to this mismatch, it is difficult to obtain an estimate of the molecular weight of the product bands in each lane. Furthermore, a broad band is evidenced, which demonstrates that clear resolution of the components has not been achieved. Clearer separation may be obtained by the use of a lower concentration of agarose gel, or the use of a pulsed field gel-electrophoresis apparatus.

V: Economic Summary & Scale-Up

The development of an incubator in itself is not revolutionary nor ground breaking, but what sets this research apart is the practical approach to each component. The ready availability of the parts and the highly adaptable design with a centralized electronic controller and attachments that may be added perfectly suits this device as user-friendly, deliverable, (external) equipment free, rapid and robust. However, it is of key importance that this device be affordable as well.

As previously detailed, the parts selected for this particular example of the incubator are not the most cost effective options available. The use of branded items that are readily available was selected as it is a manner of guaranteeing that the components are in fact readily available. An economic summary of the total cost of the first and second generation incubators is thus included in Tables 4 and 5 below, respectively.

However, in addition to the cost of the branded parts purchased individually is an estimate of the price of each part if obtained in bulk and distributed as parts alone. These prices are very significantly lower than the low volume costs. Bulk prices are estimated by contact with high volume manufacturers, primarily in China, with minimum orders of several thousand units. (Jiangsu, n.d.), (Huilong, n.d.)

Table 4: *Unit cost of components of first generation incubator, along with references and an estimated scaled price. Note a total unit cost of $68.94 is obtained for the low-volume branded unit, and a unit cost of $8.95 for the high volume estimate.*

Component	Unit Price (USD)	Ref.	Scaled Price (USD)	Ref.
Ceramic disc heater	$10.17	(UXCell, n.d.)	$0.85	(4U, n.d.)
Polyimide heater (x2)	$8.90	(UXCell, n.d.)	$0.50	(Huilong, n.d.)
12 V control board	$14.50	(RioRand, n.d.)	$2.10	(Jiangsu, n.d.)
12 V power supply	$4.65	(Phoenix, n.d.)	$1.00	(HOPcell, n.d.)
9 V control board	$20.77	(Erus, n.d.)	$2.00	(DLO, n.d.)
9 V power supply	$9.95	(XVolt, n.d.)	$2.50	(OEM, n.d.)

Table 5: *Unit cost of components of the second generation incubator, along with references and an estimated scaled price. Note a total unit cost of $77.18 is obtained for the low-volume branded unit, and a unit cost of $9.75 for the high volume estimate.*

Component	Unit Price (USD)	Ref.	Scaled Price (USD)	Ref.
Arduino Uno R3	$35.79	(Arduino, n.d.)	$2.45	(Electronics, n.d.)
SainSmart 4-Relay Switch	$9.81	(SainSmart, n.d.)	$1.25	(Electronics, n.d.)
16x4 LCD Display	$17.99	(SainSmart, n.d.)	$4.40	(Electronics, n.d.)
Thermistor Sensor	$7.99	(SunFounder, n.d.)	$0.45	(Electronics, n.d.)
10x20 mm heater (x4)	$2.10	(UXcell, n.d.)	$0.45	(MCH, n.d.)
10x40 mm heater (x1)	$3.50	(UXcell, n.d.)	$0.75	(MCH, n.d.)

From the estimated costs of scale-up, it is clear that this proposed unit is significantly cheaper than the alternative units presently available, which typically cost upwards of $1,500 . In addition, this device is designed to be reusable for many tests,

thereby adding only a fractional expense to the reagent costs associated with a biosensor. As such, this device satisfies the Affordable criteria of the World Health Organization.

It is noted that in addition to the cost of the electronic components, there is a cost associated with the production of the plastic and structural components. By using the ShapeWays cost estimators for various three dimensional printer, it is demonstrated in Table 6 below that the approximate cost to produce all the plastic components required for this device is approximately $28 via 3D printing, or $0.70 via injection moulding. (Shapeways, n.d.), (CustomPart, n.d.) This being noted, however, 3D printing holds the additional advantage of the option of printing alternative units to add onto the electronic core of this device, thereby greatly increasing the flexibility.

Table 6: *Estimated costs of the production of all plastic and structural components for the second generation incubator via 3D printing and injection moulding approaches. It is noted that injection moulding is only suitable for much higher volume applications.*

Manufacturing Method	**Cost**	**Ref.**
Additive (3D printing)	$28.16	(Shapeways, n.d.)
Injection Moulding	$0.691	(CustomPart, n.d.)

VI: Future Directions

Herein demonstrated is a novel, low-cost, adaptable, deliverable and user-friendly electronic modular device which has been proven by the addition of a temperature controlled incubator unit capable of isothermal operation, temperature profiles and staged reactions. The central goal of this technology, however, is in adaptability, and the ready use of this electronic core for other biosensing applications.

As such, it is of interest to develop novel additions which can be controlled by the electronic module to extensively demonstrate the adaptability and usefulness of this technology. Some suggested routes of investigation includes a device which will automatically inject solutions or reagents at a specific time, a device to culture, lyse and filter cells without user intervention and an on-paper PCR device.

Furthermore, regarding the incubator design in specific, PID control may be implemented to further improve and fine-tune temperature control. Similarly, the use of a metal oxide semiconductor field-effect transistor (MOSFET) in place of static relays may be investigated to improve temperature control.

VII: Conclusions

The device presented herein holds promise in the development of commercializable biosensors by filling the need for an adaptable sensor platform which will allow for deliverable, low-cost, robust and user-friendly biosensors which has for so long slowed the implementation of biosensor systems in the developing world. Furthermore, the specific application of temperature control in a resource limited setting with a reuseable, low-cost approach is a novel application of this adaptable technology.

Presented here is a temperature control device capable of maintaining a set target temperature, or carrying out a sequence of timed temperature conditions, with a unit cost as low as $10. This cost, coupled with the readily available components and construction required ensures that this device satisfies the ASSURED criteria of the World Health Organization. The performance of this device has been thoroughly characterized by

examining heating profiles and temperature setpoint stability. Furthermore, the functionality of the device was demonstrated by cell culturing, RCA and cell lysis.

It is envisioned that the electronic core of this technology may be used with a host of biosensing tools and add-ons, which can be constructed on-site, around the world with minimal user training and immediately used. Though this represents the first step in the development of this system, it is an exciting research opportunity in a rapidly growing field.

VIII: References

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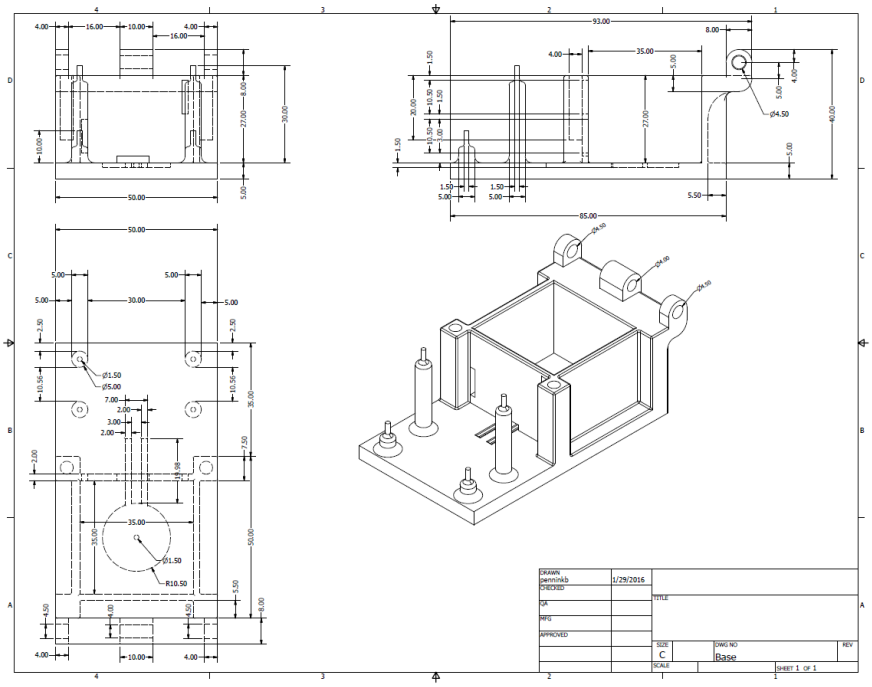
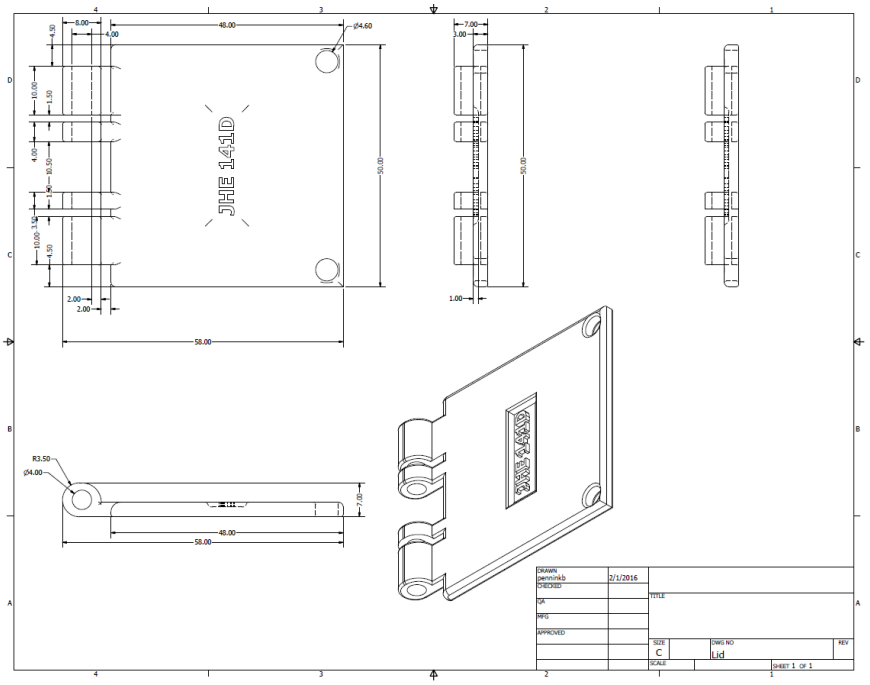
IX: Appendix A: CAD Drawings

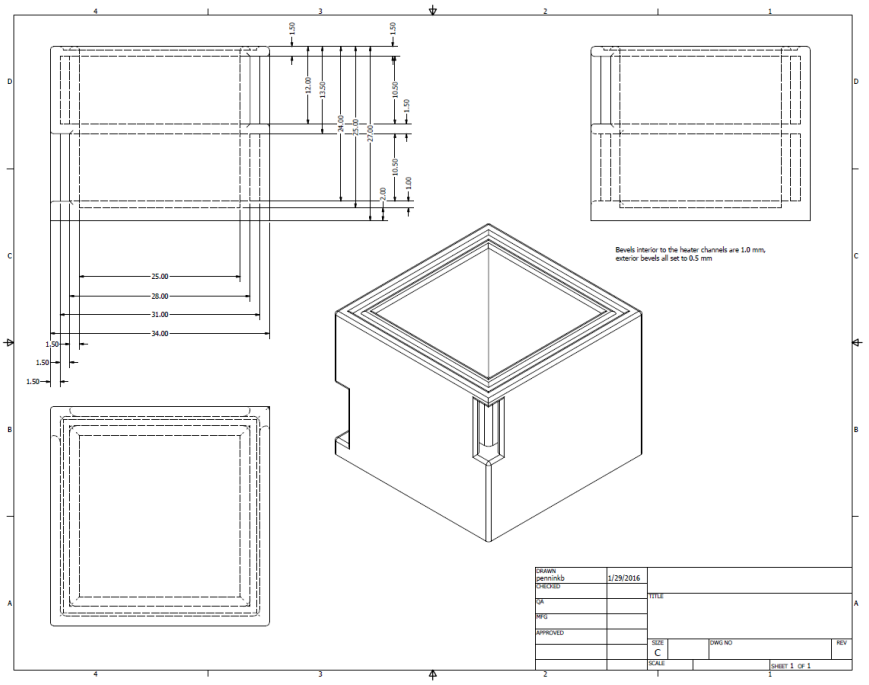
Generation 1: Base and External Wall



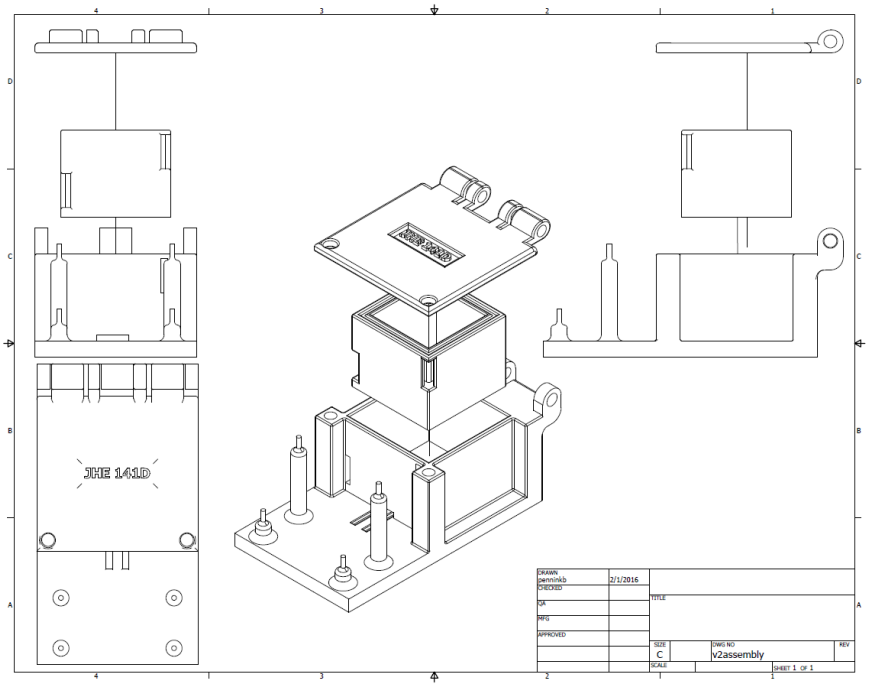
Generation 1: Lid Drawing



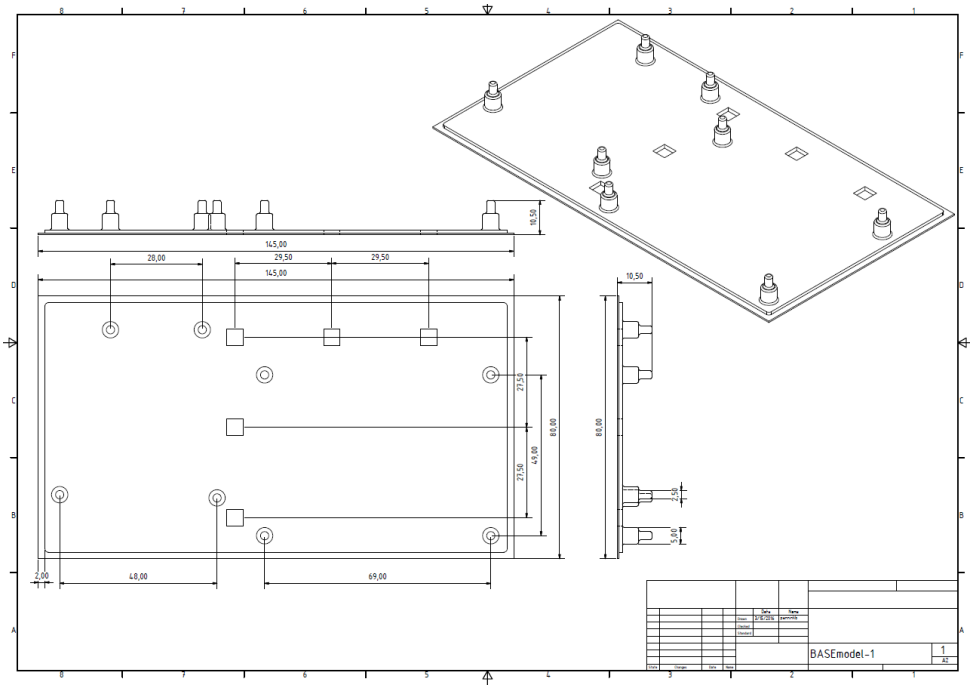
Generation 1: Reservoir Drawing



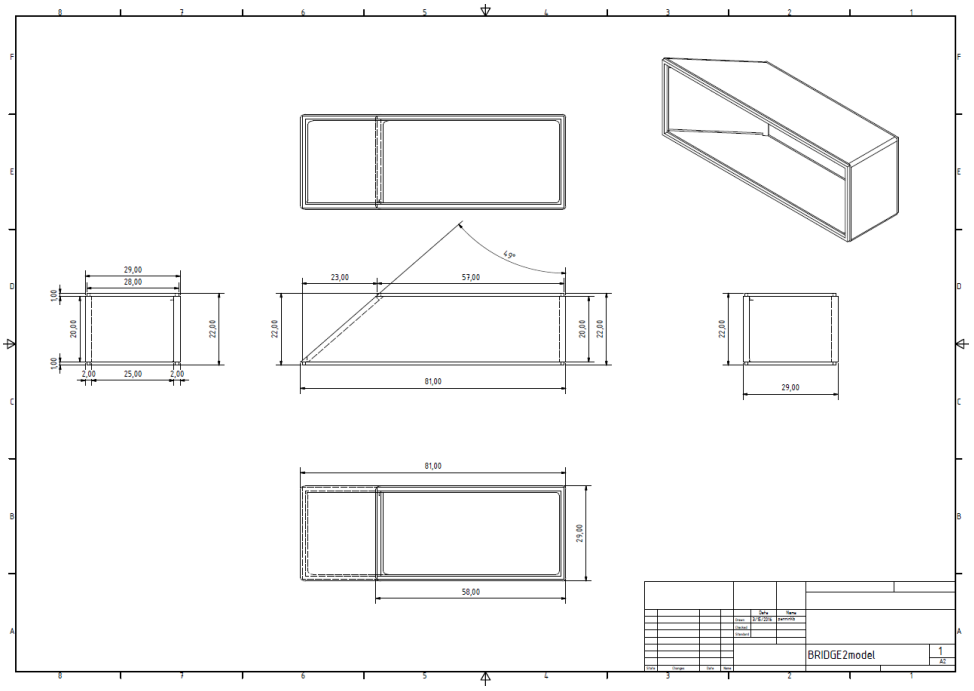
Generation 1: Assembly Drawing



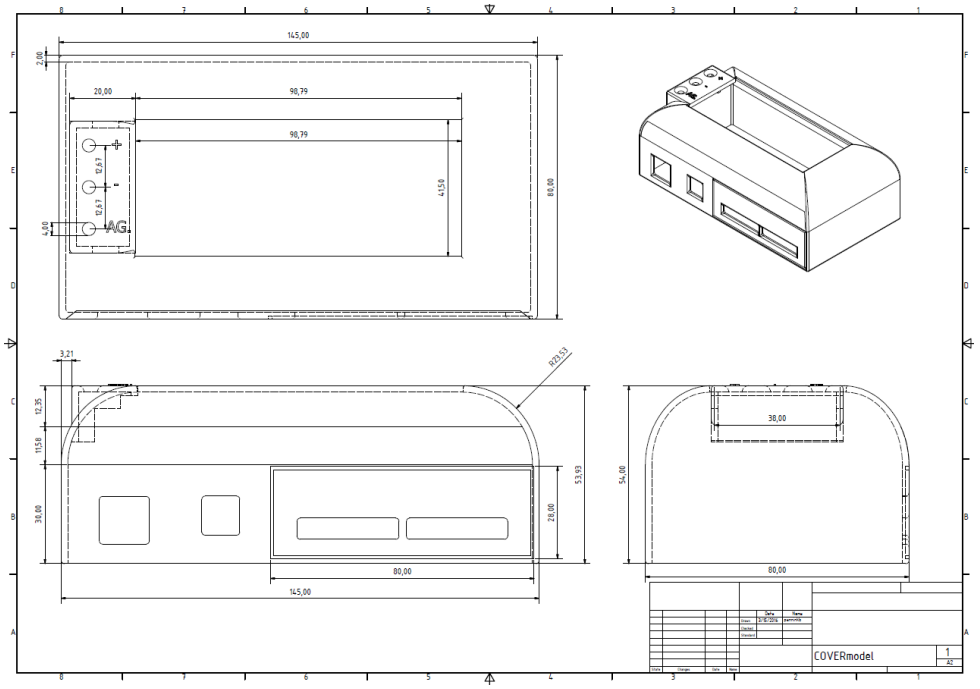
Generation 2: Central Base Drawing



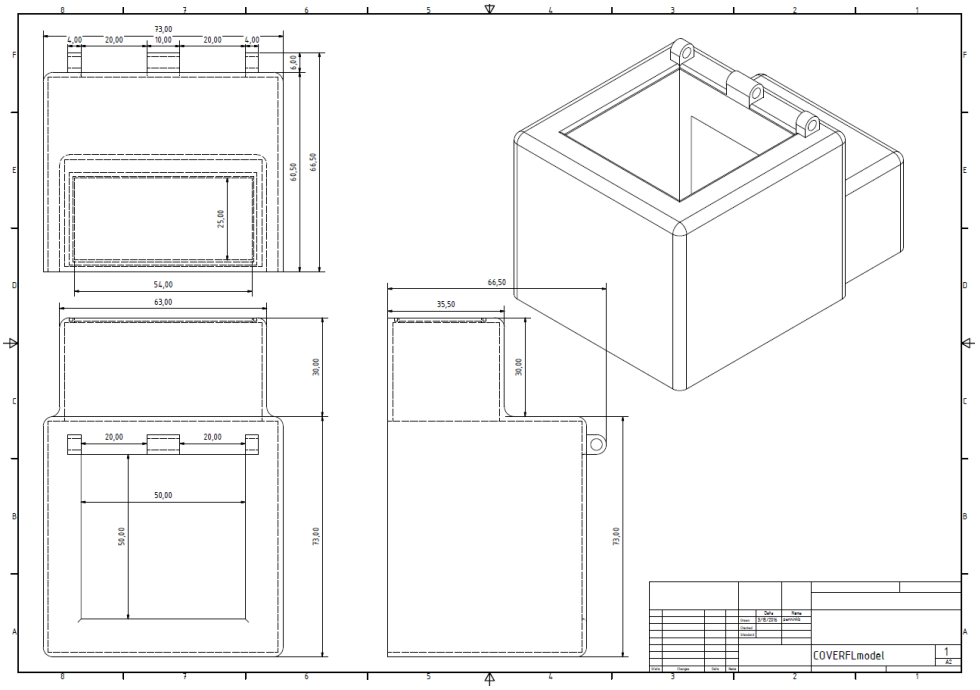
Generation 2: Connecting Bridge Drawing



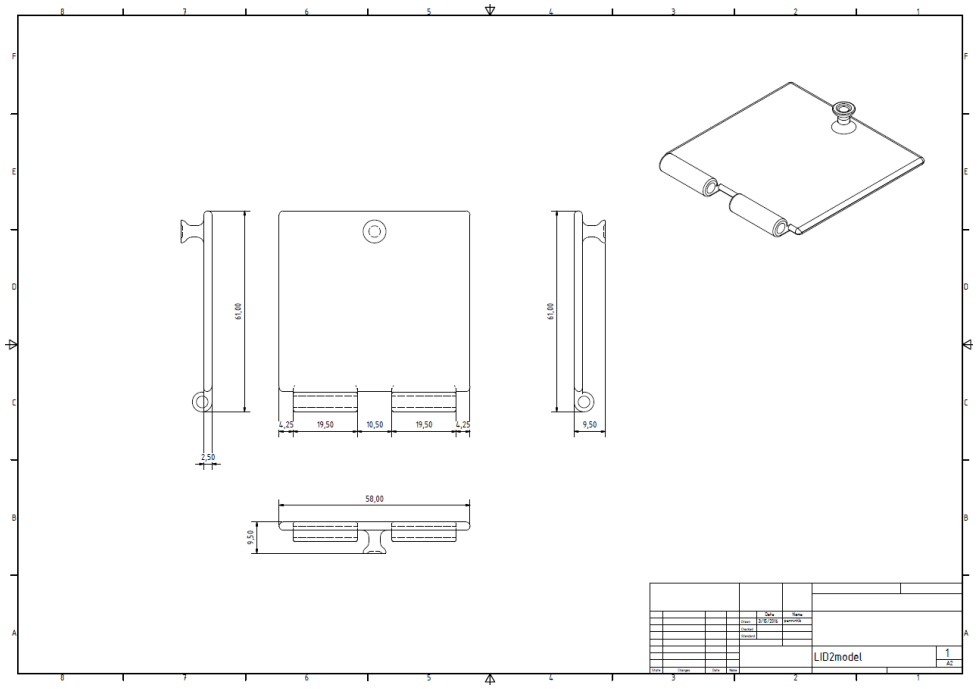
Generation 2: Central Cover Drawing



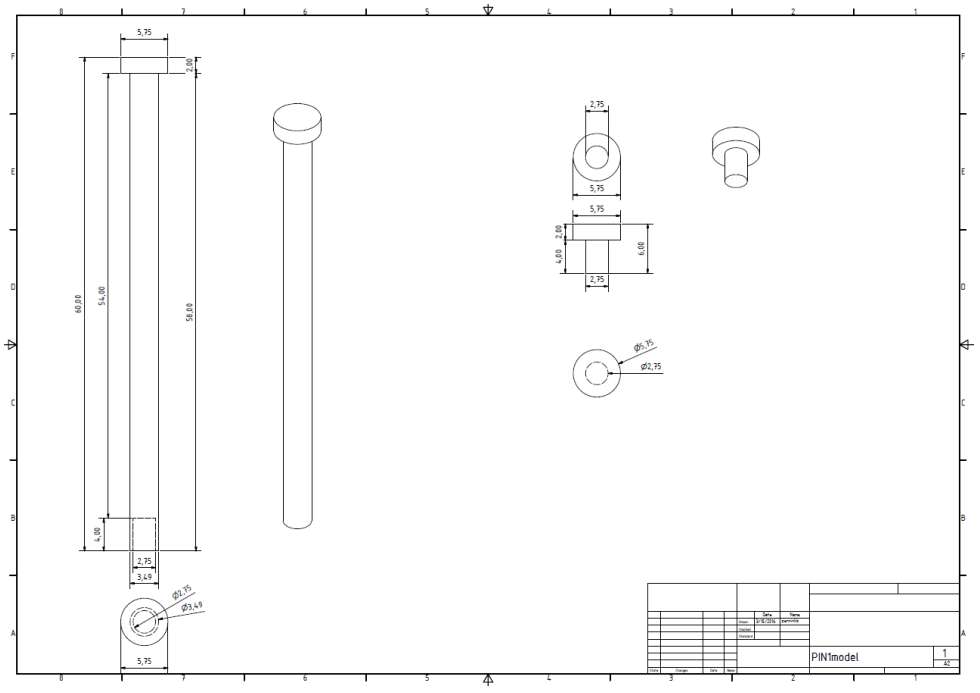
Generation 2: Reservoir Cover Drawing



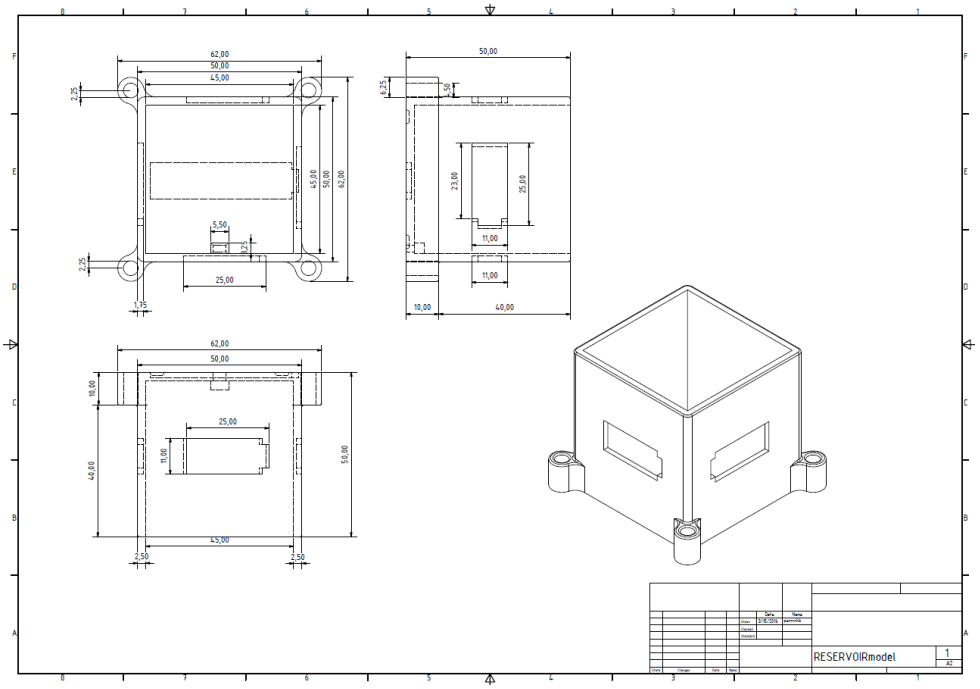
Generation 2: Reservoir Cover Drawing



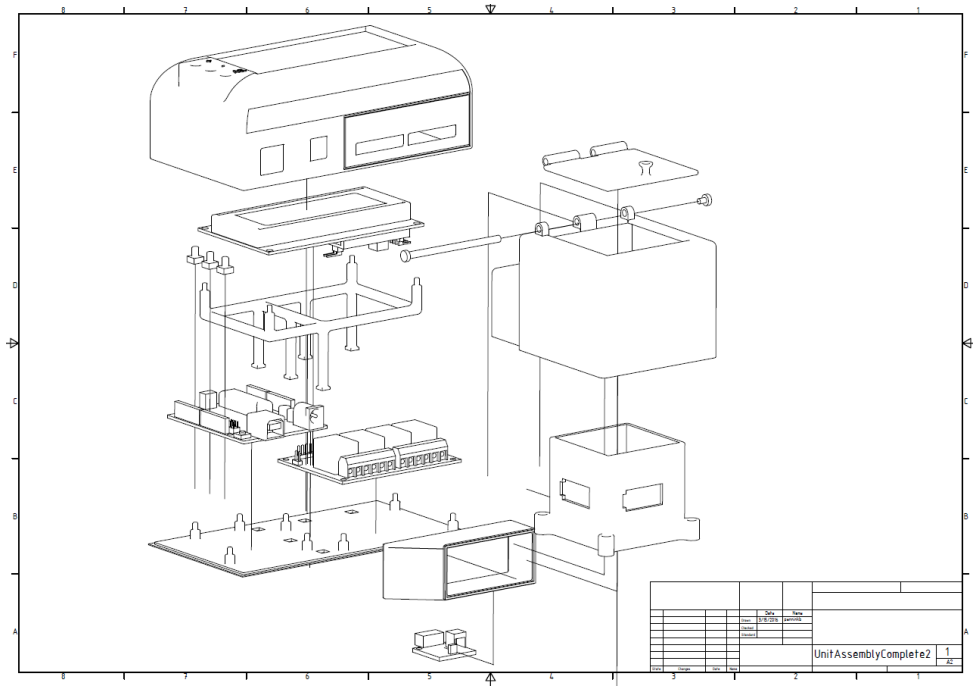
Generation 2: Reservoir Cover Pin Drawing



Generation 2: Reservoir Drawing



Generation 2: Assembly Drawing



X: Appendix B – Arduino IDE Code

Initial Control Sketch – Isothermal Programming

```
/************************************************/
//          Incubator Control Sketch            /
//        Kevin Pennings & Dr. C. Filipe        /
//  McMaster University, Chemical & Bioengineering  /
//              March, 2016                     /
/************************************************/
/* Include libraries required for code */
#include <Wire.h>
#include <LiquidCrystal_I2C.h>
LiquidCrystal_I2C lcd(0x27,2,1,0,4,5,6,7,3,POSITIVE);  // Use the I2C Code with 0x27 Address
/* Define pin locations */
const int digitalPin = 7;          // Analog Temperature Sensor pin DO to pin7
int analogPin = A0;                // Analog Temperature Sensor pin A0 to pin A0
boolean Dstate = 0;                // Digital Temperature Sensor Output
int Astate = 0;                    // Analog Temperature Sensor Output
const int buttonOne = 6;           // Define Button 1: Temp SP Increase
const int buttonTwo = 5;           // Define Button 2: Temp SP Decrease
const int buttonThree = 4;         // Define Button 3: Agitation When Pressed
const int AG1 = 9;                 // Define Relay 1 Output: Agitation
const int HT3 = 10;                // Define Relay 3 Output: Heater 3
const int HT2 = 11;                // Define Relay 2 Output: Heater 2
const int HT1 = 12;                // Define Relay 1 Output: Heater 1
float sp = 30.0;                   // Define Temperature Setpoint (30.0 C default)
float tts = 0;                     // Define Time to Temperature Setpoint Variable "tts"
int agit = 0;                      // Set agitation to "off" to start
void setup()                       // BEGIN SETUP LOOP
{ Serial.begin(9600);              // Begin Serial Connection at 9600 bps
          lcd.begin(20,4);                // Initialize Display for 20 Columns, 4 Rows
          lcd.backlight();                // Turn on display backlight
          lcd.setCursor(0,0);             // Move Cursor to Top Left Position on LCD
           lcd.print("Initializing Program");// Print "Initializing Program"
           for (int i = 0; i< 20; i++)     // Counted Loop to Display Loading Tracker
                    { lcd.setCursor(i,1);
                     lcd.print("*");
                     delay(25);}
          pinMode(digitalPin, INPUT);          // Define Digital Pin as INPUT
          lcd.setCursor(2,2); lcd.print("Incubator Ready");
          int count = 0;
          while (count < 4)                    // Loop for a 4x Flash of Text
                    { lcd.setCursor(1,3); lcd.print("                ");
                    delay(500); lcd.setCursor(1,3); lcd.print("Hello, Dr. Filipe!!"); delay(500);
                    count++;}
          lcd.clear();                         // Clear the LCD Display of All Text
          lcd.setCursor(0,0);  lcd.print("SETP:"); lcd.setCursor(13,0); lcd.print("deg C"); delay(250);
           lcd.setCursor(0,1);  lcd.print("TEMP:"); lcd.setCursor(13,1); lcd.print("deg C"); delay(250);
          lcd.setCursor(0,2);  lcd.print("REFR:"); lcd.setCursor(13,2); lcd.print("ms   "); delay(250);
          lcd.setCursor(0,3);  lcd.print("TIME TO SP: ");
```

```
 // Define the Input Pins (Buttons 1 - 3)
        pinMode(buttonOne,INPUT); pinMode(buttonTwo,INPUT);
pinMode(buttonThree,INPUT);
 // Define the Output Pins (Relay Signals)and set Default Values to "OFF"
        pinMode(AG1,OUTPUT);digitalWrite(AG1,LOW);
        pinMode(HT1,OUTPUT);digitalWrite(HT1,LOW);
        pinMode(HT2,OUTPUT);digitalWrite(HT2,LOW);
        pinMode(HT3,OUTPUT);digitalWrite(HT3,LOW);}

void loop()   /*----( LOOP: RUNS CONSTANTLY )----*/
{  { float start = millis();
   int analogValue; int correctedAnalogValue; float T;
   analogValue = analogRead(analogPin);
   correctedAnalogValue = map(analogValue, 0, 1023.0, 1023.0, 0);
   T = Thermistor(correctedAnalogValue);       // Calculate temperature
   float Ttemp = (round(T*10)); T = Ttemp/10;
   lcd.setCursor(7,1); lcd.print(T);
   if (digitalRead(buttonOne) == HIGH)         // Check if button 1 is pressed
        {sp = sp + 0.1;}                              // If pressed, raise SP by 0.1 C
   if (digitalRead(buttonTwo) == HIGH)         // Check if button 2 is pressed
        {sp = sp - 0.1;}                              // If pressed, lower SP by 0.1 C
   if (digitalRead(buttonThree) == HIGH)       // Check if button 3 is pressed
        {if (agit == 1)                               // If agitation on, turn it off
                {agit=0;}
        else                                          // If agitation off, turn it on
                {agit=1;}}
   lcd.setCursor(7,0);                          // Refresh the temperature setpoint
   lcd.print(sp);
   if (T < sp)                                  // If temp is below SP
        {if (T < (sp - 10))                           // If temp is more than 10 C below SP
                {digitalWrite(HT1,HIGH); delay(50);    // Turn on all three heaters
                digitalWrite(HT2,HIGH); delay(50);
                digitalWrite(HT3,HIGH);
                 tts = (sp-T)/5.94;}                  // Calculate time to setpoint
        else
                 {digitalWrite(HT1,HIGH);             // If temp offset is less than 10 C
                tts = (sp-T)/5.94;}}                  // Calculate time to setpoint
   else                                         // If temperature is at or above SP
        { digitalWrite(HT1,LOW); delay(50);  // Turn off all three heaters
         digitalWrite(HT2,LOW);delay(50);
         digitalWrite(HT3,LOW);
          tts = 0.0;}                                 // Display time to SP as zero
   if (T < sp)                                  // If temp is below SP
        { int ttsmin = tts;                           // Define variables to print time to SP
         int ttssec = (tts-ttsmin)*60;
        if (ttsmin > 9)
                {lcd.setCursor(13,3); lcd.print(ttsmin);}
        else
                 { lcd.setCursor(13,3); lcd.print("0");
        lcd.setCursor(14,3); lcd.print(ttsmin); }
```

```
        lcd.setCursor(15,3); lcd.print(":");
        if (ttssec > 9)
                {lcd.setCursor(16,3); lcd.print(ttssec);}
        else
                { lcd.setCursor(16,3); lcd.print("0");
                  lcd.setCursor(17,3); lcd.print(ttssec);}}
  else
        {lcd.setCursor(13,3); lcd.print("N/A ");}
  float Time = 0; delay(Time);                 // To slow loop refresh, can use a time delay
  float end = millis();                        // Time of loop end
  float dely = end - start;                    // Calculate loop time
  lcd.setCursor(6,2);lcd.print(dely);}}       // Display loop time

// Declare Functions - the Thermistor Function calculates the temperature from ADC input
double Thermistor(int RawADC)
{ double Temp;  // Working variable
 Temp = log(((10240000.00 / RawADC) - 10000.00));
 Temp = 1 / (0.001129148 + (0.000234125 * Temp) + (0.0000000876741 * Temp * Temp * Temp));
 Temp = Temp - 273.15;
 return Temp;}
```

Second Control Sketch – Variable Time & Temperature with Periods

```
// Incubator Control Sketch Version 4
// Dr. C. Filipe, Kevin Pennings, Vince Leung, George Padeigis
// McMaster University, Chemical Engineering Department

#include<Wire.h>
#include<LiquidCrystal_I2C.h>
LiquidCrystal_I2C lcd(0x27,2,1,0,4,5,6,7,3,POSITIVE); // Use the I2C code with 0x27 address

const int HT1 = 10; // Heater 1
const int HT2 = 11; // Heater 2
const int HT3 = 12; // Heater 3

const int buttonPlus = 3; // Controls increasing
const int buttonMinus = 6; // Controls decreasing
const int buttonPause = 7; // Pause Start button

const int analogPin = A0; // For thermistor

int setupStage = 0; // Used to keep user in the setup process while loop
int instructionsStage = 0; // Used to keep user within Instructions Interface
int cyclesStage = 0; // Used to keep user in while loop to determine number of desired cycles
int cycleperiodsStage = 0; // Used to keep user in while loop to determine number of time/temperature
periods per cycles
int periodlengthStage = 0; // Used to keep user in while loop to determine length of each period in
minutes
```

```
int periodtemperatureStage = 0; // Used to keep user in while loop to determine temperature of each period in degrees Celsius


//Pause button is used to exit each of the stages once parameters have been determined

int buttonPresses = 0; // Counter for number of consecutive button presses, useful for increasing the magnitude of increment/decrement
int refreshTemp = 0; // Counter to refresh measured Temperature on screen after a given amount of loops
int cycles = 1; // Number of cycles
int cycleperiods = 1; // Number of time/temperature periods per cycle
int buffer1 = 250; // Time delay between button presses
int buffer2 = 1500; // Alternative time delay between button presses
int buffer3 = 5000; // Alternative time delay, for displaying text
int startpause = 0; // Assigned millis() time when a pause period is initiated
int runningpause = 0; // Assigned millis() time continuously through a pause period
int netpausedtime = 0; // The difference between the runningpause and startpause
int totalpausedtime = 0; // Assigned netpausedtime once the pause period has ended, before netpausedtime is reset
int timeelapsed = 0; // The amount of time that a given sample has been held at a particular temperature in any given cycle period, in minutes
int time1 = 0; // timeelapsed in milliseconds
int referencetime = 0; // References time at which sample has been raised to desired temperature in a given period
int periodtimelength = 0; // Assigned value of a given period's time length
int periodtemperature = 0; // Assigned value of a given period's temperature
int currentperiod = 0; // Corresponds to a period in a cycle; used to access that period's time length and temperature through indexing an array
int timesetpoint = 10000; // Assigned different time values depending on period, given arbitrary large value
int sp; // The Temperature Setpoint, assigned different temperature values depending on period
int cyclecount = 1; // Counts the number of cycles elapsed
int programfinished = 0; // Allows for program to run continuously, user allowed to enter and leave reaction conditions manipulating its value
float T = 0;
float refreshedT = 0;
int done = 0;

//Imposed abitrary maximum of 100 periods in a cycle, although this can be increased with consideration for arduino memory
int myPeriodLengths[100]; // Array for period time lengths
int myPeriodTemperatures[100]; // Array for period temperatures


// Declare Functions - the Thermistor Function calculates and rounds the temperature from ADC input
double Thermistor(int RawADC)
{ double Temp;  // Working variable
  Temp = log(((10240000.00 / RawADC) - 10000.00));
  Temp = 1 / (0.001129148 + (0.000234125 * Temp) + (0.0000000876741 * Temp * Temp * Temp));
  Temp = Temp - 273.15;
```

```
 //rounding
 float Temp1 = (round(Temp*10))/10;
 return Temp1;}

void setup() {

Serial.begin(9600);
lcd.begin(20,4);
lcd.backlight();

lcd.setCursor(0,0); // might not be necessary
lcd.clear();
//lcd.setCursor(1,0); lcd.print("DNA Amplification");
//lcd.setCursor(1,1); lcd.print("Bacteria Incubation");
//lcd.setCursor(2,3); lcd.print("FILIPE PENNINGS PADEIGIS");

lcd.setCursor(1,0); lcd.print("LOW COST INCUBATOR");
lcd.setCursor(1,2); lcd.print("      FILIPE");
lcd.setCursor(1,3); lcd.print("PENNINGS  PADEIGIS");
//lcd.setCursor(0,0); lcd.print();
delay(buffer3);

lcd.setCursor(0,0); // might not be necessary
lcd.clear();
lcd.setCursor(1,0); lcd.print("Welcome to");
lcd.setCursor(1,1); lcd.print("     MCMASTER");
lcd.setCursor(1,2); lcd.print("     CHEMICAL");
lcd.setCursor(1,3); lcd.print("     ENGINEERING");
delay(buffer3);

lcd.setCursor(0,0); // might not be necessary
lcd.clear();
lcd.setCursor(0,0); lcd.print("Set your parameters");
lcd.setCursor(0,1); lcd.print("Plus to increase");
lcd.setCursor(0,2); lcd.print("Minus to decrease");
lcd.setCursor(0,3); lcd.print("Pause to confirm");
delay(buffer3+4000);
lcd.clear();

// Set the input and output modes for each pin
// Setup the buttons
pinMode(buttonPlus,INPUT);
pinMode(buttonMinus,INPUT);
pinMode(buttonPause,INPUT);

// Setup the Arduino data pins for OUTPUT,
pinMode(HT1,OUTPUT);
pinMode(HT2,OUTPUT);
pinMode(HT3,OUTPUT);
```

```
// Turn OFF any power to the Relay channels
digitalWrite(HT1,LOW);
digitalWrite(HT2,LOW);
digitalWrite(HT3,LOW);

}

void loop() {

// Main Reaction Loop
while (programfinished == 0)
{

//Outer Setup Loop
while (setupStage == 0)
{lcd.clear();

   while (instructionsStage == 0)
   {
      lcd.setCursor(0,0); lcd.print("To continue press");
      lcd.setCursor(0,1); lcd.print("the Pause Button");

      if (digitalRead(buttonPause) == HIGH)
           {++instructionsStage;
           lcd.clear();
           delay(buffer2);}}

 // Loop determines number of cycles
  while (cyclesStage == 0) {
   lcd.setCursor(0,0); lcd.print("Please input the");
   lcd.setCursor(0,1); lcd.print("number of cycles");
   lcd.setCursor(0,3); lcd.print("Cycles:");
   lcd.setCursor(8,3); lcd.print(String(cycles)+"   ");

     buttonPresses = 0;
     while (digitalRead(buttonPlus) == HIGH)
        {++buttonPresses;
         if (buttonPresses>10)
           {cycles+=5;}
         else
           {++cycles;}
         lcd.setCursor(8,3); lcd.print(String(cycles)+"   ");
         delay(buffer1);}

     buttonPresses = 0;
     while (digitalRead(buttonMinus) == HIGH)
        {++buttonPresses;
         if (buttonPresses>10)
           {cycles-=5;}
         else
```

```
          {--cycles;}
        if (cycles <= 1)
          {cycles = 1;}
        lcd.setCursor(8,3); lcd.print(String(cycles)+"   ");
        delay(buffer1);}

    if (digitalRead(buttonPause) == HIGH)
       {++cyclesStage;
       lcd.clear();
       delay(buffer2);}}


// Loop determines number of periods per cycle
  while (cycleperiodsStage == 0)
  { lcd.setCursor(0,0); lcd.print("Please input the");
    lcd.setCursor(0,1); lcd.print("number of periods");
    lcd.setCursor(0,3); lcd.print("Periods:");
    lcd.setCursor(8,3); lcd.print(String(cycleperiods)+"   ");

    buttonPresses = 0;
    while (digitalRead(buttonPlus) == HIGH)
       {++buttonPresses;
        if (buttonPresses>10)
          {cycleperiods+=5;}
        else
          {++cycleperiods;}
        lcd.setCursor(8,3); lcd.print(String(cycleperiods)+"   ");
        delay(buffer1);}

    buttonPresses = 0;
    while (digitalRead(buttonMinus) == HIGH)
       {++buttonPresses;
        if (buttonPresses>10)
          {cycleperiods-=5;}
        else
          {--cycleperiods;}
        if (cycleperiods <= 1)
          {cycleperiods = 1;}
        lcd.setCursor(8,3); lcd.print(String(cycleperiods)+"   ");
        delay(buffer1);}

    if (digitalRead(buttonPause) == HIGH)
       {++cycleperiodsStage;
       lcd.clear();
       delay(buffer2);}}


//Loop determines time length of each period in a cycle
for (int x = 0; x < cycleperiods; x++)
{ periodlengthStage = 0;
```

```
 lcd.setCursor(0,0); lcd.print("Please input the");
 lcd.setCursor(0,1); lcd.print("time length of each");
 lcd.setCursor(0,2); lcd.print("period in minutes.");
 lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Time:"+String(periodtimelength)+"m   ");

 while (periodlengthStage == 0)
   {buttonPresses = 0;
    while (digitalRead(buttonPlus) == HIGH)
       {++buttonPresses;
        if (buttonPresses>10)
           {periodtimelength +=5;}
        else
           {++periodtimelength;}
        lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Time:"+String(periodtimelength)+"m   ");
        delay(buffer1);}

    buttonPresses = 0;
    while (digitalRead(buttonMinus) == HIGH)
       {++buttonPresses;
        if (buttonPresses>10)
           {periodtimelength-=5;}
        else
           {--periodtimelength;}
        if (periodtimelength <= 1)
           {periodtimelength = 1;}
        lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Time:"+String(periodtimelength)+"m   ");
        delay(buffer1);}

    if (digitalRead(buttonPause) == HIGH)
       {++periodlengthStage;
       lcd.clear();
       delay(buffer2);}}

 myPeriodLengths[x] = periodtimelength;
 periodtimelength = 0;}

// Loop determines temperature of each period in a cycle
for (int x = 0; x < cycleperiods; x++)
{periodtemperatureStage = 0;

 lcd.setCursor(0,0); lcd.print("Please input the");
 lcd.setCursor(0,1); lcd.print("temperature of each");
 lcd.setCursor(0,2); lcd.print("period in deg C.");
 lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Temp:"+String(periodtemperature)+"C   ");

 while (periodtemperatureStage == 0)
   {buttonPresses = 0;
    while (digitalRead(buttonPlus) == HIGH)
       {++buttonPresses;
```

```
        if (buttonPresses>10)
          {periodtemperature +=5;}
        else
          {++periodtemperature;}
        lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Temp:"+String(periodtemperature)+"C   ");
        delay(buffer1);}

    buttonPresses = 0;
    while (digitalRead(buttonMinus) == HIGH)
       {++buttonPresses;
        if (buttonPresses>10)
          {periodtemperature-=5;}
        else
          {--periodtemperature;}
        lcd.setCursor(0,3); lcd.print("P:"+String(x+1)+" Temp:"+String(periodtemperature)+"C   ");
        delay(buffer1);}

    if (digitalRead(buttonPause) == HIGH)
       {++periodtemperatureStage;
       lcd.clear();
       delay(buffer2);}}

 myPeriodTemperatures[x] = periodtemperature;
 periodtemperature = 0;}

++setupStage; } //exits loop

if (done == 0)
{lcd.setCursor(0,0); lcd.print("RUNNING");
lcd.setCursor(3,1); lcd.print("Cdtns:");
++done;}

//Checks if time period is finished, resets time parameters, transitions to next time period in cycle
if (timeelapsed > timesetpoint)
{++currentperiod;
 timeelapsed = 0;
 time1 = 0;
 referencetime = 0;
 totalpausedtime = 0;}

//Checks if cycle is finished, resets time period, counts the number of cycles initiated, ends program once
cycles complete
if (currentperiod == cycleperiods)
  {if (cyclecount < cycles)
      {currentperiod = 0;
      ++cyclecount;}
   else
      {++programfinished;
      done = 0;
      lcd.clear();}} //exits the main loop, reaction conditions finished
```

```
sp = myPeriodTemperatures[currentperiod];

timesetpoint = myPeriodLengths[currentperiod];

// Define inputs and variables for temperature calculation
int analogValue = analogRead(analogPin); //A0
int correctedAnalogValue = map(analogValue,0,1023.0,1023.0,0);
// Calculate and round the temperature using the Thermistor function
T = Thermistor(correctedAnalogValue);

// Refreshes measured temperature on screen every 100 loops
++ refreshTemp;
if (refreshTemp == 1) // Prints temperature
 {refreshedT = T;
 lcd.setCursor(0,2); lcd.print("Temp:"+String(refreshedT,1)+"C Time:"+String(timeelapsed)+"m   ");
 lcd.setCursor(0,3); lcd.print("Period:"+String(currentperiod+1)+" Cycle:"+String(cyclecount)+"   ");
 }
else if (refreshTemp == 100) // Resets loop counter
 {refreshTemp = 0;}

if (T < (sp - 10))  // Temperature offset > 10 C, use all heaters
   {digitalWrite(HT1,HIGH);
    digitalWrite(HT2,HIGH);
    digitalWrite(HT3,HIGH);
    lcd.setCursor(17,0); lcd.print("1");
    lcd.setCursor(18,0); lcd.print("1");
    lcd.setCursor(19,0); lcd.print("1");
    }
else if (T < (sp - 5))   // Temperature offset > 5 C, use three heaters
   {digitalWrite(HT1,HIGH);
    digitalWrite(HT2,HIGH);
    digitalWrite(HT3,LOW);
    lcd.setCursor(17,0); lcd.print("0");
    lcd.setCursor(18,0); lcd.print("1");
    lcd.setCursor(19,0); lcd.print("1");
    }
else if (T < (sp))   // Temperature offset > 1 C, use single heater
   {digitalWrite(HT1,HIGH);
    digitalWrite(HT2,LOW);
    digitalWrite(HT3,LOW);
    lcd.setCursor(17,0); lcd.print("0");
    lcd.setCursor(18,0); lcd.print("0");
    lcd.setCursor(19,0); lcd.print("1");
    }
else if (T>(sp+3))
   {digitalWrite(HT1,LOW);
    digitalWrite(HT2,LOW);
    digitalWrite(HT3,LOW);
    lcd.setCursor(17,0); lcd.print("0");
```

```
    lcd.setCursor(18,0); lcd.print("0");
    lcd.setCursor(19,0); lcd.print("0");
    }
else // If temperature is greater than the setpoint within 3 degrees
   {digitalWrite(HT1,LOW);
    digitalWrite(HT2,LOW);
    digitalWrite(HT3,LOW);
    lcd.setCursor(17,0); lcd.print("0");
    lcd.setCursor(18,0); lcd.print("0");
    lcd.setCursor(19,0); lcd.print("0");

   if (referencetime == 0)
    {referencetime = millis(); // A starting point for time sample has been kept at desired temperature
    }

   time1 = millis() - referencetime - netpausedtime - totalpausedtime; // time1 refers to time elapsed of
sample at desired temperature
   timeelapsed = time1 / 60000; // Converts time elapsed into minutes

   }

if ((digitalRead(buttonPause) == HIGH) && (digitalRead(buttonPlus) == LOW))
{if (startpause == 0)
   {startpause = millis(); // Start Time of pause period
   runningpause = millis(); // Initiates Running Time of pause period
   lcd.setCursor(0,0); lcd.print("PAUSED ");
   delay(buffer2);}
 else
   {startpause = 0; // Resets Start Time of a pause period
   runningpause = 0; // Resets Running Time of a pause period
   totalpausedtime += netpausedtime; // Holds total pausetime for all pause periods in a time period
   netpausedtime = 0; //Resets Paused Time of a pause period
   lcd.setCursor(0,0); lcd.print("RUNNING");
   delay(buffer2);}
   }

if (startpause != 0)
{runningpause = millis();
 netpausedtime = runningpause - startpause;
}

// Manual option to exit the Main Reaction Loop
if ((digitalRead(buttonPause) == HIGH) && (digitalRead(buttonPlus) == HIGH) &&
(digitalRead(buttonMinus) == HIGH))
{++programfinished;
done = 0;
lcd.clear();
delay(buffer2);}}
if (done == 0){
lcd.setCursor(3,0); lcd.print("STANDBY MODE");
```

```
lcd.setCursor(0,1); lcd.print("Press Pause to set");
lcd.setCursor(0,2); lcd.print("new parameters.");
++done;}


//As safety, turns off heaters
 digitalWrite(HT1,LOW);
 digitalWrite(HT2,LOW);
 digitalWrite(HT3,LOW);
 lcd.setCursor(17,0); lcd.print("0");
 lcd.setCursor(18,0); lcd.print("0");
 lcd.setCursor(19,0); lcd.print("0");

//Perhaps to show temperature cooling down after the reaction conditions have been satisfied and
completed
// Define inputs and variables for temperature calculation
int analogValue = analogRead(analogPin); //A0
int correctedAnalogValue = map(analogValue,0,1023.0,1023.0,0);
// Calculate and round the temperature using the Thermistor function
T = Thermistor(correctedAnalogValue);

// Refreshes measured temperature on screen every 200 loops
++ refreshTemp;
if (refreshTemp == 1) // Prints temperature
  {refreshedT = T;
  lcd.setCursor(0,3); lcd.print("Cooling Temp:"+String(refreshedT,1)+"C   ");  }
else if (refreshTemp == 200) // Resets loop counter
  {refreshTemp = 0;}

// To setup another set of reaction conditions, all original variable values reassigned
if (digitalRead(buttonPause) == HIGH)
{programfinished = 0; //Allows for rentry into main loop
setupStage = 0;
cyclesStage = 0;
cycleperiodsStage = 0;
periodlengthStage = 0;
periodtemperatureStage = 0;
instructionsStage = 0;
timesetpoint = 10000;
cyclecount = 1;
refreshTemp = 0;

cycles = 1; // not necessary, useful to leave out so previously selected number of cycles still available
cycleperiods = 1; // not necessary, useful to leave out so previously selected number of periods per cycle
still available

startpause = 0; // not necessary, safe to keep
runningpause = 0; // not necessary, safe to keep
netpausedtime = 0; // not necessary, safe to keep
totalpausedtime = 0; // not necessary, safe to keep
```

```
timeelapsed = 0; // not necessary, safe to keep
time1 = 0; // not necessary, safe to keep
referencetime = 0; // not necessary, safe to keep

periodtimelength = 0; // not necessary, safer to keep
periodtemperature = 0; // not necessary, safer to keep
currentperiod = 0; // not necessary, safer to keep

done = 0;

delay(buffer2);}}
```