University of Central Florida

College of Engineering and Computer Science

Department of Electrical and Computer Engineering

Initial Project Document

Portable PCR Diagnostics

A convenient portable diagnostics system for quantitative viral detection with wireless capabilities

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Introduction

Summary

In the field of medical diagnostics, polymerase chain reaction (PCR) is a technique used to amplify a specific section of DNA. This process that generates millions to trillions of copies of the target DNA or gene, usually from a specific virus being tested for, such as HIV. Since developed in the 1980s it has changed the face of biology, for its applications are vast and has not yet reached its full potential. Common work done using PCR include DNA and gene detecting, paternity testing, forensics, genotyping, cloning, and mutation detection. PCR is important to molecular biology because the quantity of DNA in a given sample typically isn't enough to run tests, as the detection equipment is not sensitive enough.

The process of PCR relies on the thermal cycling of a sample between approximately 60 and 95 degrees Celsius. When the sample is at 95 degrees, the DNA in the sample is split into its two strands. The sample is then cooled to 60 degrees where the polymerase in the sample begins to reconstruct the opposite half of the DNA strands, the entire thermal cycle results in a doubling of the quantity of DNA in the sample. When repeating this thermal cycle 40 times, the amount of DNA in the sample effectively increases 2^{40} times. This amplification is the means by which PCR has become the industry standard in viral detection because it allows for the easy detection of the virus concentration. For example, if there is one segment of HIV virus DNA in the sample at the beginning of the process, there will be more than one trillion copies of that DNA at the end. This massive quantity of DNA at the end of the process is much more easily detected than a single segment and the primary reason PCR has become the industry standard.

The detection method usually relies on some form of optical detection. These methods rely on some form of primer which mates to a specific sequence of DNA. When bonded to DNA, this primer fluoresces if excited by a particular wavelength of light and emits light on another wavelength. In physical implementations of a fluorescent detection system, the sample is excited by an LED or laser usually passed through an optical bandpass filter. This is so the excitation occurs on the exact wavelength required by the specific primer. The system then observes the sample using a photodiode or similar detector behind another optical bandpass filter centered on the specific wavelength emitted by the primer. Since the primer will only bond to a specific sequence of DNA, a PCR system equipped with a fluorescent detection system can be extremely sensitive at measuring very specific target DNA. Since the primers can be designed to bond to any sequence of DNA, a primer can be made that will bond to a sequence only found in a virus, such as HIV. Using this feature, a PCR device can be made to search for and detect any virus present in a sample, thus allowing for accurate and reliable diagnosis of a patient.

Motivation

PCR being the key player that it is for molecular biology makes producing a portable PCR machine very appealing. Currently the PCR machines today are large in size and very expensive. This group desires to produce a device that is the contrary; small, cheap, and portable. The portability feature by itself, allows for a great impact on the science community and, indirectly, to the world. Being portable, more research can be done around the world. Scientists

and forensics don't have the privileges of taking their work or their lab where they need to, since most of their equipment is large and too expensive to risk damaging. For this reason, much of the testing process is delayed, which could have dramatic consequences for individuals with diseases. With a portable device, fast test results would greatly increase and more data from around the world would be acquired, allowing for doctors to better plan strategies for treating disease outbreaks.

Outbreaks are a main focus to molecular biologists and a world concern when they occur. There have been countless times in the United States that an outbreak has caught the attention and stimulated fear of the citizens. Examples such as the Zika virus, Ebola virus, and H1N1 virus are well known. When the United States heard reported cases of the Zika virus in Miami, studies ensued. A portable PCR machine would have been perfect for this situation. Scientists in the area, and many others sent to do studies on the situation, would have the ability to test patients who are suspected of having the virus in a quick manner. Currently the processes is slow. Scientists take samples from the patients then send them to the lab for evaluation. Since a scare is in a specific location, there are a finite number of PCR machines also in that area. Samples must wait to be tested or sent elsewhere, which also hinders results. For any outbreak minimum delay is crucial. Although our device is capable of running a PCR in a normal operation, our design is specific to viral detection. With quantitative viral detection, our PCR machine will give results to the presence of the virus immediately after running the PCR. Most PCR machines today do not have viral detection and require additional equipment and additional steps to yield those results. Scientists can avoid these hiccups with a portable quantitative PCR device that we are proposing. The device will provide quick results, which allows for more data to be collected over a shorter period of time, reducing risks of the virus spreading.

Global research is also a focus of ours for designing our project. There are many countries to this day that are very poor or underfunded and their citizens are suffering from diseases. We would like for either those countries themselves to be able to afford a PCR machine so they can conduct their own research and take action according to their data, or for scientists from around the world to be able to travel to these areas with a device that is cheap and portable. Therefore we are designing our project with expenses in mind, we want the device to be far cheaper than current PCR machines on the market, which vary from a thousand dollars to several thousand dollars. With our goal of limiting its cost, we envision the device being able to be produced in many areas in the world.

As one can see, having a portable PCR machine is beneficial in many ways. Therefore our design focuses on being convenient for travel. Our design will run on rechargeable batteries, and can run many tests before needing to recharge. In addition, our device will have the ability to wirelessly connect to a smartphone and have an app available to interact with the device. This will make the device easier to use by providing an interface most people are already familiar with and allows the device to be smaller, which improves its portability. One of the best features our design has to offer is its quantitative viral detection. As mentioned, the majority of PCR machines do not provide quantitative viral detection, and given the situation where third world countries are in need of testing or a viral outbreak occurs, our design would provide the instant feedback desired without the need for additional devices and extra procedures because third world countries can't afford them and, unfortunately, outbreaks do not wait.

Partnership

Through the course of our project we will be working with a UCF faculty member. Dr. Brian N. Kim will be sponsoring our efforts. Dr. Kim's lab specializes in the field of bioelectronics which includes diagnostic devices similar to the one being proposed by the group. Additionally, Dr. Kim's lab is located at the Burnett School of Biomedical Sciences Lake Nona Campus, which will allow the group to perform medical experiments in the lab in order to confirm the functionality of the device. Without access to a biosafety lab, these experiments would not be able to be performed. Dr. Kim is appointed by both the College of Engineering and Computer Science and the College of Medicine.

Specifications:

Heating:	Must be able to heat to 95C in under 30sec
Cooling:	Must be able to cool to 60C in under 30sec
Location:	Using GPS must be able to determine location with +/-100m accuracy
Data logging:	Must be able to log all data locally using a user friendly memory device (sd, flash, etc..)
Wireless Communication:	Must be able to transmit all data via a wireless technology to a mobile device (WiFi, Bluetooth, etc..)
Optical Filtering:	Detecting 518 nm wavelength
Optical Sensing Accuracy:	+95% accurate detection range
Charge Time:	< 1 hour for full charge
Temperature Accuracy:	± 2C
Solar Charging Capacity: (optional)**	5+ Watts
Voltage Compatibility:	3-14V Capable for compatibility with mobile charging and automobile systems
Portable Mode Battery Life:	> 2 Hours of continuous use
Dimensions:	Volume < 1000 cubic centimeters
Cost:	< 500$
Resistance to Outdoor Environments:	At least an IP67 Rating
Weight:	< 7.5 lb

Manufacturability:	Must be easy to scale up for mass production
Cost of Biological Agents:	Must be able to use low cost biological agents
App Integration:	Must be able to communicate all data completely to the app and display it as the PCR machine would
Impact Resistance:	Must survive 10 ft drop fall

Additional Electrical Specifications:

- The solar panel must be portable if our group decides to include that portion into our project.
- Total Time for a complete detection test should be less than 1 hour.
- Sleep current should not exceed 500 microAmps

House of Quality:

To further help develop our design, we have constructed a matrix consisting of marketing requirements and engineering requirements. The matrix allows us to understand the relationships between both engineering requirements and marketing requirements, while also relating the engineering requirements individually with one another. With these comparisons, the matrix represents how our design correlates to customer needs.

Key

+ = **Positive polarity, Increasing the Requirement**

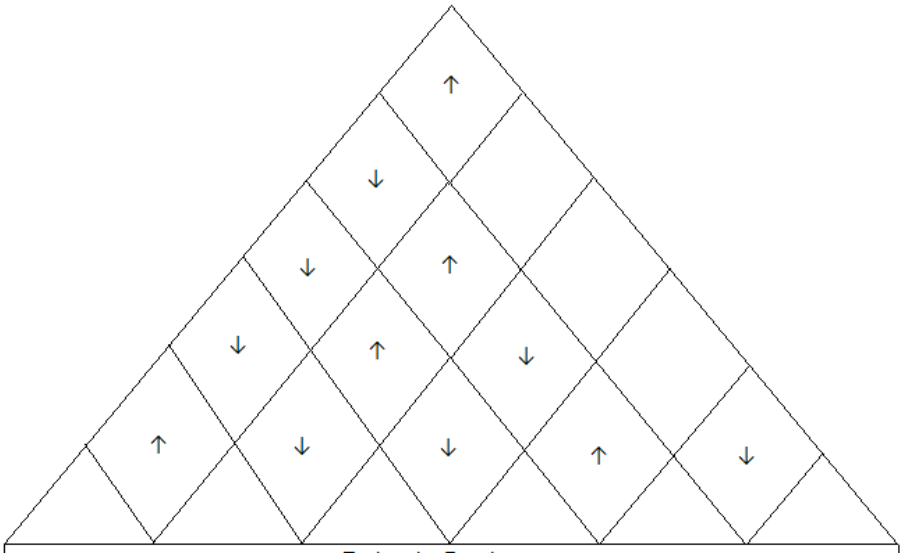
- = **Negative Polarity, Decreasing the Requirement**

↑ = **Positive correlation**

↑↑ = **Strong positive correlation**

↓ = **Negative correlation**

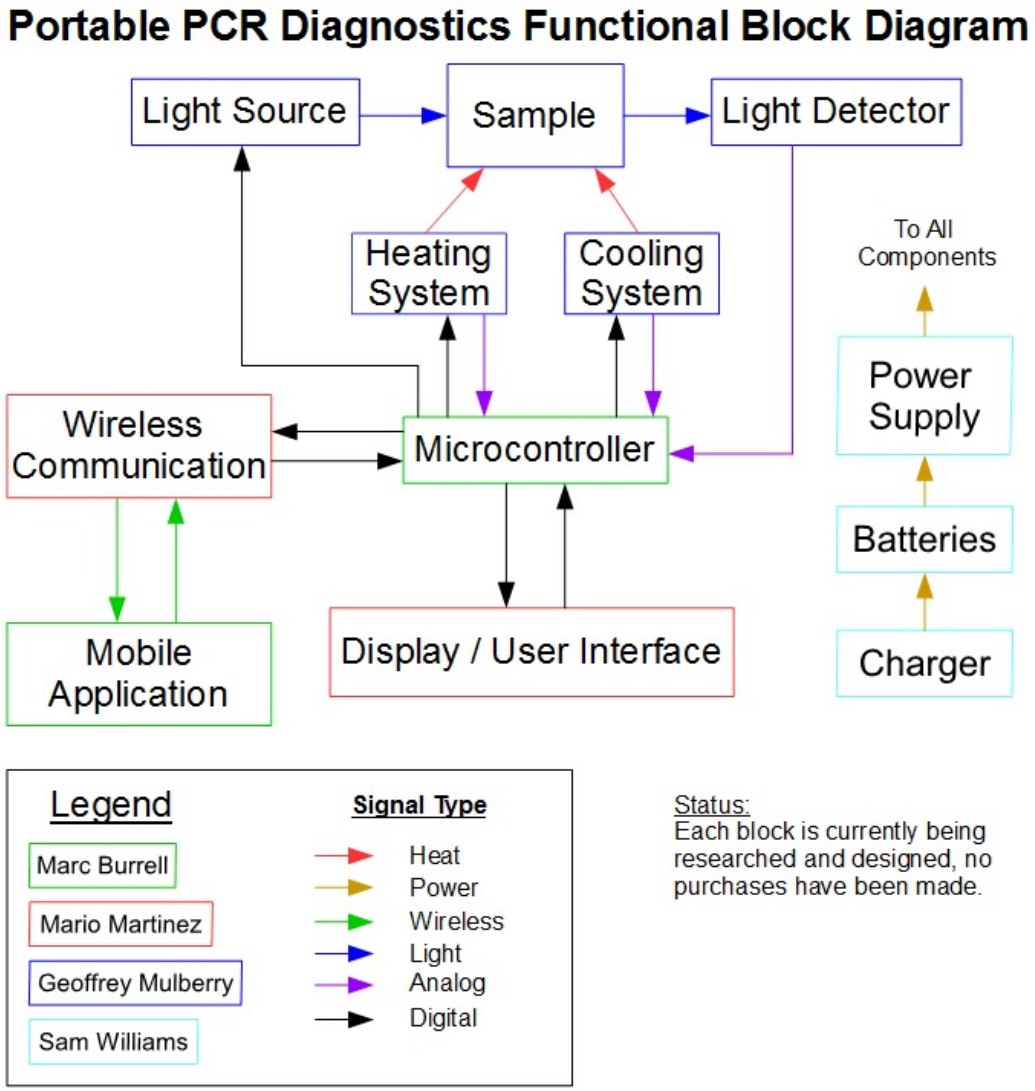
↓↓ = **Strong negative correlation**



			Engineering Requirements					
			Cost	Battery Life	Cycle Time	Weight	Dimentions	Thermal Accuracy
			-	+	-	-	-	+
Marketing Reqirements	Cheaper than Commercial Devices	-	↑↑	↓↓	↑	↑↑	↑	↑↑
	Number of Tests	+	↓↓	↑↑	↑↑		↓↓	
	Durability	+	↓↓			↑		↑
	Size	-	↓	↓↓		↑↑	↑↑	↑
	Wireless Compatability	+	↓↓	↑↑		↑	↓	↓↓
	Accuracy	+	↓		↑↑	↓		↑↑
	Portability	+	↓↓	↑		↑↑	↑↑	↓
	Safety	+	↓					↑↑
	Targets for Engieering Requirements		< 500$	2 Tests	< 1.25 min	< 2.5 lbs	<100cm^3	+/- 2°C

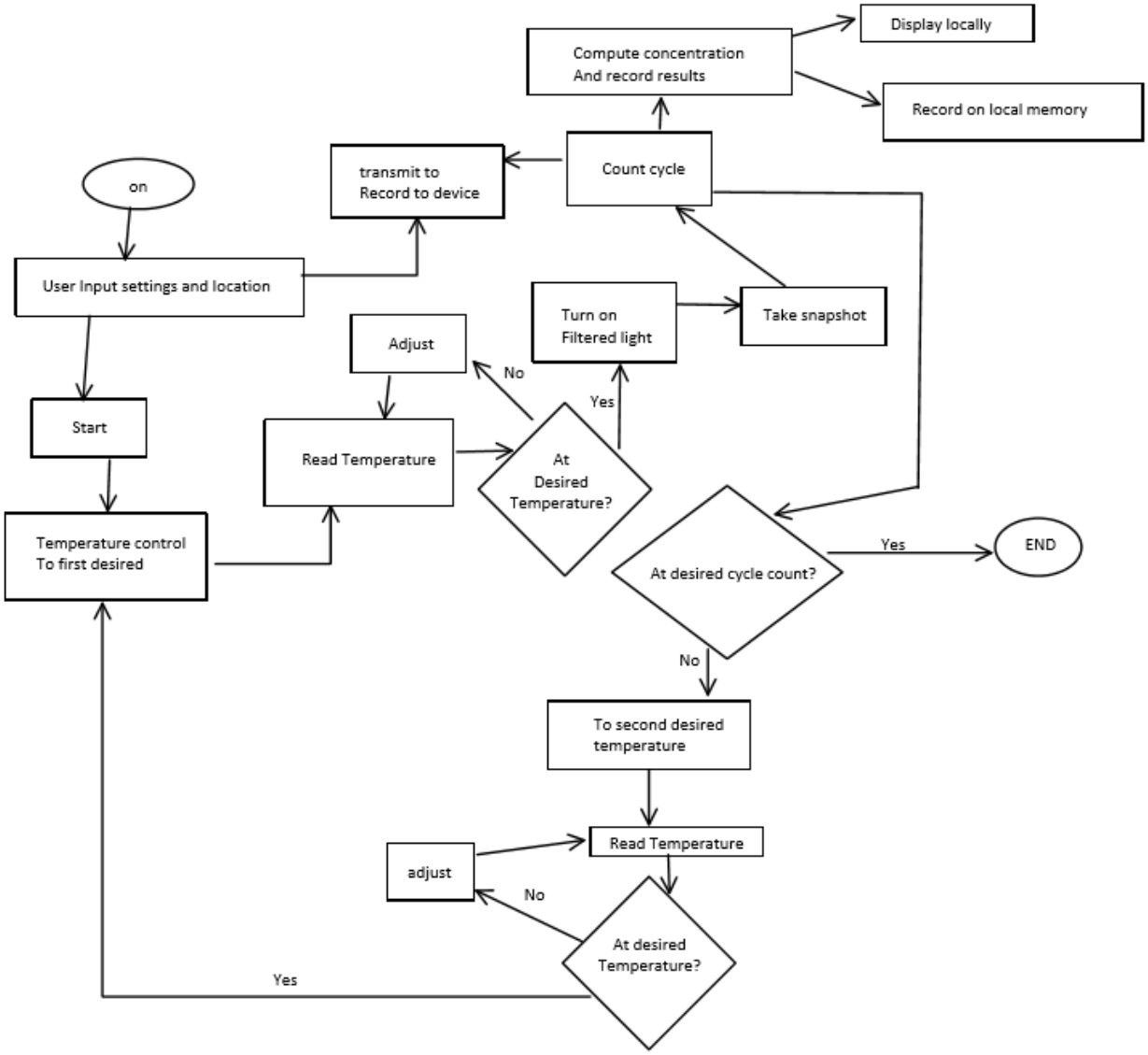
Block Diagrams

Physical Diagram



The proposed system consists of the above elements. The main component of the device is the sample, which contains (or does not contain) the virus being detected. Two systems, the heating and cooling systems are controlled by the microcontroller and are responsible for the transfer of heat into and out of the sample to facilitate PCR. The light source and light detector, work together to determine the concentration of target DNA in the sample. This data is recorded by the microcontroller and sent to the mobile application for additional processing via the wireless communication system. The system also consists of a display and user interface so that the device is still functional without the use of a mobile phone.

Software Diagram



This flowchart shows the proposed program flow used to control the PCR machine.

Estimated Budget:

Product Name:	Price:	Quantity:	Total:	Link:	Reasoning:
GPS Receiver - GP-735	$39.95	1	$39.95	https://www.sparkfun.com/products/13670	Local GPS location
18650 Li-ion Battery	$8.99	4	$35.96	http://www.myvaporstore.com/LG-HG2-18650-LiMn-3000mAh-Battery-35-Amp-p/ef6503035.htm	High Current Draw and High Capacity
10k NTC Thermistor	$1.49	5	$7.45	http://www.mouser.com/ProductDetail/US-Sensor/103JG1F/?qs=sGAEpiMZZMuBd0%252bwiCVS22TnLIlDljZTH0kjwnBuZBkTWoICC%252bNbSg%3d%3d	Temp Sensing
Arduino Mega 2560	$45.95	1	$45.95	https://www.sparkfun.com/products/11061	Main MCU
SD Card Module	$4.95	1	$4.95	https://www.sparkfun.com/products/13743	Local Data Logging
RedBearLab BLE Nano	$17.95	1	$17.95	https://www.sparkfun.com/products/13729	Bluetooth communication to phone
Servo's	$10.00	2	$20.00	https://hobbyking.com/en_us/servos/hobbyking.html	Possibly use for movement functions
Display Module	$45.00	1	$45.00	https://www.google.com/webhp?sourceid=chrome-instant&ion=1&espv=2&ie=UTF-8#q=sparkfun+arduino+color+display	To display information to the user
PushButtons	$0.24	10	$2.38	http://www.mouser.com/ProductDetail/Schurter/13019308/?qs=sGAEpiMZZMvxtGF7dlGNpine0yr6cgj2V5u7KJyYHcU%3d	User Interface
JST GPS Connector Cable	$1.50	1	$1.50	https://www.sparkfun.com/products/10361	GPS Connector Cable
MOSFET's	$2.00	8	$16.00	http://www.mouser.com/Semiconductors/Discrete-	H-Bridge Pelteir Driver

				Semiconductors/Transistors/MOSFET/_/N-ax1sf?P=1z0y3zr	
Heatsink Compound	$7.93	1	$7.93	https://www.amazon.com/Arctic-Silver-AS5-3-5G-Thermal-Paste/dp/B0087X728K/ref=pd_bxgy_147_2?_encoding=UTF8&psc=1&refRID=J3VXCDVZ0GSQPFTD3A7F	High Quality Heatsink Compound
Cooler Master Hyper T2	$16.99	2	$33.98	https://www.amazon.com/dp/B00K7809O2/ref=twister_B01IEXUS9W?th=1	Dissipation of heat
LED's	$2.00	5	$10.00	http://www.mouser.com/Optoelectronics/LED-Lighting/_/N-74g9t	For Photodiode Illumination
Photodiodes	$2.00	2	$4.00	http://www.mouser.com/Optoelectronics/Optical-Detectors-and-Sensors/Photodiodes/_/N-6jjuh/	For Bioluminescene detection
Switch Mode IC's	$4.00	2	$8.00	http://www.ti.com/lsds/ti/power-management/non-isolated-dc-dc-switching-regulator-overview.page	Efficent Voltage Regulation
Optical Filters	$5.00	3	$15.00	https://www.newport.com/c/optical-filters	For Wavelength Filtering
			$316.00		

The above table lists several items already selected by the group's initial research and gives a reasonable reflection of the proposed cost of the device.

Timeline and Milestones:

Initial project milestones for both semesters.

January 2017:
Research

February 2017:
3D modelling and component selection

March 2017:
Initial testing and PCB design

April 2017:
Initial PCB population and testing

May 2017:
PCB revisions

June 2017:
Final assembly and testing

July 2017:
Fine tuning and optimization

August 2017:
Final presentation