DEVELOPMENT OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) BASED BIOSENSOR SET FOR DETECTION OF GENETICALLY MODIFIED ORGANISMS (GMO) AND *Escherichia coli* (*E. Coli*)

By

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The worldwide usage of products with genetically modified organisms (GMOs) is constrained by different legal frameworks. GMO's usage limits are determined by the related regulations in each countries. "Regulation on Genetically Modified Organisms and Products" named law – effective since 2010 – draws the aforementioned conditions in Turkey. As a consequence, a variety of products are monitored by undergoing a GMO detection and classification processes. Researchers have been proposing different works regarding the specifically important problem of GMO-based products' detection. Various methods such as Polymerase Chain Reaction, Ligase Chain Reaction, Rotating Circle Amplification are commonly used for DNA amplification, a crucial preprocessing step of the detection process. In this work, the loop-based isothermal amplification (LAMP) method which provides fast results and requires fewer temperature cycles is used. For the LAMP method to be properly implemented, the sample should be kept at 60-65^{o}C between 35-120 minutes. Conventional laboratory devices used for this purpose can be characterized - on average – to be heavy (1-12 kg), expensive (1300-15000 €), unmovable and only operable by trained experts. In this thesis, we present the design, implementation and performance analysis of two prototype GMO-based product and bacteria detection devices that are portable (108-240 g) , fast (<30-40 minutes), affordable (<25-30 €) and pocket-size (6x6x3-9.7x6.8x5 cm) provided with battery system that allows its functionality outside laboratory environment. The temperature level controller, heater design, feedback circuit and the exterior of the device are prepared via different mechanical and electronic design software. A Proportional control based feedback control scheme is used to adjust the temperature with a high accuracy of (± 0.2^{o}C).

In this work, we extended the devices uses to *E.coli* bacteria detection as well. The detection was done using the LAMP method and is presented as the second device with its customized design providing high output (105 microchannels). Roundup Ready Soybean (RRS), gts40-3-2) with 0%, 0.1%, 1% and 10% GMO content were used for GMO analysis part and *E. Coli* ATCC 10536 bacteria for bacterial analysis. Both GMO and Bacteria detection and analysis results were done by the proposed devices showed similar performance in terms of accuracy and sensitivity when compared to the laboratory or commercial correspondents in the market while successfully outperforming them in the other already mentioned aspects.

GENETİĞİ DEĞİŞTİRİLMİŞ ORGANİZMALARIN (GDO) ve *Escherichia coli* BAKTERİSİNİN (*E. Coli*) TESPİTİ İÇİN İLMİĞE DAYALI İZOTERMAL ÇOĞALTMA (LAMP) TABANLI BİYOSENSÖR SETİ GELİŞTİRİLMESİ

DOĞUKAN KAYGUSUZ

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Genetiği değiştirilmiş organizmalara (GDO) sahip ürünlerin Dünya çapında kullanımı yasal çerçeveler altında olmaktadır. GDO'nun kullanım sınırları, ülkelerin yönetmelikleri ile belirlenmiştir. Türkiye'de 2010 yılında yürürlüğe giren "Genetik Yapısı Değiştirilmiş Organizmalar ve Ürünlerine Dair Yönetmelik" ile bu sınırlar çizilmiştir. Bu yönetmelik sonucunda GDO'lu ürünlerin kullanım kontrolü tespit ve sınıflandırma sürecinden geçmektedir. Araştırma camiası bağlamında önemli sayılan GDO ürün tespitine yönelik çeşitli çalışmalar yapılmaktadır. GDO tespiti için Polimeraz Zincir Reaksiyonu, Ligaz Zincir Reaksiyonu, Dönen Daire Çoğaltması yöntemleri gibi çeşitli DNA amplifikasyon metotları vardır. Bu çalışmada hızlı sonuç veren ve daha az sıcaklık çevrimi gerektirmesiyle ilmiğe dayalı izotermal çoğaltma (LAMP) metodu kullanılmıştır. LAMP metodunun gerçekleşmesi için, numunenin 60-65^{o}C derecede 35-120 dakika arasında tutulması gerekmektedir. Ayrıca, bu metot için kullanılan geleneksel laboratuvar cihazları; ağır (1-12 kg), pahalı (1300-15000 €), taşınamaz ve teknik bir bilgiye sahip kullanıcı tarafından çalıştırılabilecek özelliklere sahiptir. Bu çalışma, GDO ve Bakteri tespitinde LAMP metodunun laboratuvar ortamı haricinde kullanılabilmesine olanak sağlayacak taşınabilir (108-240 g) bataryalı sistemi ile her yerde kullanılabilir, hızlı (< 30-40 dakika), maliyeti düşük (< 25-30 €) ve cep boyutlarına (6x6x3-9.7x6.8x5 cm) sahip iki adet prototip cihazın tasarımı, imalatı ve performans analizini sunmaktadır. Bu cihazlar içinde sıcaklığı hassas bir şekilde tutan denetleyici, ısıtıcı tasarımı, geri bildirim devresi ve cihazın dış kısmı mekanik ve elektronik tasarım programları ile hazırlanmıştır. Hazırlanan elektronik devre ve ısıtıcı PCB cihazında, cihazın dış kısmı 3B yazıcı ile üretilmiştir. Elektronik devre, sıcaklığın hassas bir şekilde (±0.2^{o}C) kalmasını sağlayan geri beslemeli olarak yazılan oransal kontrol kodu ile çalışmaktadır.

Bu çalışma, *E.coli* bakterisinin tespitine yönelik çalışmaları da içermektedir. Tespit yöntemi LAMP metodu kullanılarak yapılmış ve yüksek çıktı (105 mikro kanal) alınmasını sağlayan ikinci bir cihaz tasarımı olarak sunulmuştur. GDO analiz kısmı için %0, %0.1, %1 ve %10 GDO içeriğine sahip Roundup Ready Soya (Round Ready Soybean (RRS), gts40-3-2) ve bakteri analizi için de *E. Coli* ATCC 10536 bakteri çeşidi kullanılmıştır. Hem GDO analizi, hem de Bakteri analizi hem cihazlarla hem de ticari cihazlarla yapılarak karşılaştırılmış ve benzer hassasiyet ve doğruluk değerleri elde edilmiştir.

ACKNOWLEDGEMENTS

I will never forget what I experienced during the thesis period…

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1. INTRODUCTION

Preventive medicine promotes the well-being of the population by avoiding potential risk factors for diseases. Harm inflicted by the consumption of Genetically Modified Organisms (GMOs) and infections caused by Escherichia coli (*E. coli*) are among these potential health risks. Since their first introduction in 1972, GMOs have been a subject of controversy regarding their adverse health effects. Although being a part of our natural bacterial flora, certain types of *E. coli* are responsible for serious health issues such as hospital acquired infections.

In order to prevent these negative conditions, the quick, affordable and in situ detection of both GMO and *E. coli* bacteria is necessary. Detection studies performed in traditional methods are generally in the laboratory environment. While the equipment used is expensive, these devices are suitable for use by people with technical knowledge. It is the greatest motivation to prevent these situations and to develop and deliver the sets of devices that can be carried to the desired location without the need for technical knowledge.

The aim of this thesis is to provide fast, easy and simple detection of GMO products and *E. coli* bacteria and to provide a device that can be used in home, garden and field as an alternative to traditional devices used in the laboratory. The devices can be easily printed from the 3D printer and these devices can be used by the non-professional.

1.1 Health Risks associated with GMOs and E. Coli

In this section we provide background information on genetically modified organisms (GMOs) and the Escherichia coli (*E. coli*) family of bacteria.

1.1.1 Genetically Modified Organisms (GMOs) and Potential Hazards

With the end of World War II, the world population started to increase rapidly, and in order to meet the nutritional needs of this growing population, a development called the Green Revolution was experienced in the 1950s. This revolution was based on the use of pesticides, chemical fertilizers, and excess water in order to obtain the highest level of crops in a narrow area [1].

Indeed, with the green revolution, agricultural production has increased prominently, but by the 1970s its impact on both the environment and human health began to be heatedly discussed in the world public and in the academic community. Incorrectly used pesticides and chemical fertilizers have harmed human health and some pesticides have been banned [2]. The green revolution, which was shown as a savior when it emerged, left behind serious side effects such as health problems and environmental pollution. Soils have been polluted by fertilization and pesticide use, and water resources have begun to decrease rapidly.

After these developments, new methods were sought to feed the growing world population. In 1972, Paul Berg formed the first genetically modified DNA molecule, and a year later Stanley Cohen, Annie Chang, and Herbert Boyer produced the first genetically modified organism. In 1983, the first genetically modified plant sample was produced by four different study teams [3]. In the following years, corn cultivation with Bacillus thuringiensis (Bt) was carried out and in 1998 GMOs labeling rules were determined. The first purpose in GMOs production; it was the use of this technology in the healthcare sector as medicine and vaccine, but as the health-related sectors were under strict control, the profits from this technology were diminishing [4]. Thereupon, a new health-related but less supervised field was found; the food industry. As a result, Genetically Modified Organisms (GMOs or transgenic products) were put into practice and offered to humanity as a solution to hunger in the world. Moreover, this time, unlike the health sector, the sheath was ready because there was hunger in the world.

It was unclear what the health effects of GMO products, which were cultivated in a very short time, without sufficient research and studies, and which decorated our tables, would have a health impact. It has been shown that it may cause allergic reactions, accumulate in the food chain, cause toxic effects and produce antibiotic resistance.

1.1.2 *Escherichia coli* (*E.coli*) and Potential Hazards

The *Enterobacteriaceae* family is the largest and most heterogeneous group of medically gram-negative bacilli. Fifty genera and hundreds of species and subspecies have been identified. In the family of *Enterobacteriaceae*, there are many species of bacteria that are medically important. *Enterobacteriacea*e are very common microorganisms found throughout the world, in soil, water, plants and normal intestinal flora of humans and many

animals. Bacterial species of the *Enterobacteriaceae* family can cause many diseases in humans and animals. These bacteria are responsible for the majority of bacteremia in humans [5].

These bacteria also cause diseases such as septicemia, meningitis, surgical wound infections, pneumonia and urinary tract infections in humans. These bacteria have many organs and tissue involvement. Examples of important genera in this family are *Escherichia, Klebsiella, Salmonella, Shigella, Citrobacter, Enterobacter, Proteus, Yersinia, Morganella, Serratia, Providencia* [6].

Escherichia is a member of the *Enterobacteriaceae* family. *Escherichia coli* (*E. coli*) was first discovered in 1885 by the German Bacteriologist Theodor Escherich *E. coli* has been widely used for biological laboratory experiments and research since then. Enteric *E. coli* can be divided into five categories depending on their virulence characteristics. These are enterotoxigenic *E. coli* (ETEC), *enteropathogenic E. coli* (EPEC), *enteroinvasive E. coli* (EIEC), *enterohemorrhagic E. coli* (EHEC), *enteroadherent aggregate E. coli* (EAggEC) and *verotoxigenic E. coli* (VTEC). *E. coli* can cause intestinal and extraintestinal infections. Bloody diarrhea occurs in the intestine. Outside the intestine; *E. coli* can cause urinary tract infections, neonatal meningitis, pneumonia, septic arthritis, skin and soft tissue infections [7].

Urinary tract infections are the most common bacterial infections in the community and in the hospital environment. The susceptibility to bacteria and antibiotics responsible for urinary tract infections changes over time. *E. coli* is known to be the most common causative agent in these infections. Urinary tract infections are often treated with antibiotics and are therefore an important potential source for the selection and emergence of resistant bacteria [8].

1.2 Contribution of the Thesis

In this thesis, two portable and low-cost devices for the detection of GMOs and *E. coli* were designed, manufactured and tested. The first device, which was named as DaimonDNA, was developed for GMO analysis. DaimonDNA is lightweight (108-240 g), portable, low-cost (<25-30 €) and pocket size (6x6x3-9.7x6.8x5 cm). Four PCR tubes with biological sample can be placed in DaimonDNA and heated. The color change is observed during the Loop Mediated

Isothermal Amplification (LAMP) reaction with the help of the window on the front of the DaimonDNA device.

The second device was designed to examine *E.coli* bacteria. Called DaimonDNA-E, the device is also lightweight (190-300 g), portable, and low-cost. DaimonDNA-E is capable of examining 105 samples and the device also is powered by battery.

In both devices, a microcontroller based temperature controller and associated heater elements were used to maintain process temperatures for LAMP.

1.3 Outline of the Thesis

Chapter 2 addresses background and theory. First, a background of GMO and *E.coli* bacteria is given. DNA analysis and the methods used for the detection of GMOs and *E.coli* bacteria are mentioned as well. Then, literature studies about heating, electronics, mechanical design, and coded process have been also introduced.

Chapter 3 describes mechanical design of the devices for GMO and *E. coli* bacteria analysis. Chapter 4 describes the preparation of electronic circuits for temperature control for GMO and *E.coli* bacteria analysis. At first, the processes from the first prototype to the final circuit design are explained. Then, normal and flexible PCB prints of these circuits are discussed. In the last section, the determination of control parameters is described.

Chapter 5 presents the results. The results of GMO and bacteria analysis on the device were given and the results obtained by performing the same experiments in the traditional devices were compared and the sensitivity and selectivity of the device were calculated. The results obtained in real-time with color change during the analysis were compared using gel electrophoresis.

The conclusion is given in Chapter 6.

1.4 Publications and Patents

- Kaygusuz, D., Vural, S., Aytekin, A. Ö., Lucas, S. J., & Elitas, M. (2019). DaimonDNA: A portable, low-cost loop-mediated isothermal amplification platform for naked-eye detection of genetically modified organisms in resource-limited settings. Biosensors and Bioelectronics, 141, 111409.

- A device for use in nucleic acid testing. TURKPATENT. Sabanci University, Sabanci Sabanci University Nanotechnology Research and Application Center, Meltem Elitaş, Doğukan Kaygusuz, Sümeyra Vural, Ali Özhan Aytekin, Stuart J. Lucas. (in progress)

2. BACKGROUND AND THEORY

2.1 Genetically Modified Organisms (GMOs)

The term GMO is used to describe organisms that have been altered in nature other than through natural crossing or natural recombination. GMO is a biological system that can multiply itself and transfer its genetic material. Gene transformation between unrelated species and the process of obtaining function from them is defined as genetic transformation.

2.2 GMOs Threshold in the World

To determine the amount of GMO in a variety, quantification is required. This is determined by correlating the plant-specific gene in the genome of the plant with the gene that has been altered. In EU approved products, a threshold value of 0.9% is applied [9]. Products with GMOs above this value have to be labeled as GMOs. In the US, there is no labeling rule. The GMO threshold for labeling in Japan is 5%, in Korea is 3% and in Russia is 0.9% [9]. The basis of these restrictions is the harmful effects that GMO products can have on human and animal health as well as on biodiversity in the short and long term. Turkey has Biosecurity Law and regulations on the use of GMO products. A threshold limit of 0.9% in the approved GMO products are applied in Turkey [10] [11]. (Table 1)

Country	Mandatory Labelling	Labeling Threshold
United States	No	N/A
France	Yes	0.9 – 1%
Germany	Yes	0.9 – 1%
Russia	Yes	0.9 – 1%
Saudi Arabia	Yes	0.9 – 1%
Turkey	Yes	0.9 – 1%
United Kingdom	Yes	0.9 – 1%
China	Yes	1% or < 1%

Table 1. Labelling Law and Threshold in some Countries

2.3 How are GMOs detected?

Transgenic plants are characterized by the addition of new genes to their genomes. A new protein is expressed for this transferred gene. The basis of GMOs diagnostic logic is the use of this difference between the unmodified variety and the transgenic plant. This can be done by identifying the transferred new DNA fragment or the expressed new protein. Also it can be done by using chemical analysis methods to detect the products of enzymatic reactions.

2.4 *E.coli* Bacteria

E. coli is approximately 2-6 µm long and 1.0-1.5 µm wide. *E.coli* flat, rounded ends. *E.coli* may have small, short shapes or long, branching shapes in their cultures. They move slowly. There are also still strains. Some strains are encapsulated. They are well stained with bacteriological dyes and are gram (-) bacteria [12].

The optimum breeding temperature is 37 ° C. It can also grow between 7 and 46 degrees. It can survive in the pH range of 4.4 to 9. *E. coli* has the ability to ferment many sugars, glucose and lactose. Thus, it differs from other *Enterobacteriacae* members [13].

E.coli has O, H and K antigens. O antigens are present in the polysaccharide portion of the lipopolysaccharide layer. It is heat resistant and is used to identify serological types of most gram negative bacilli. The H antigen is found in the flagella structure responsible for movement in many *Enterobacteriaceae* members, as in *E. coli*. K antigens are usually associated with the capsule and less frequently with the fimbriae [12]

2.5 Amplification of DNA

DNA contains all the genetic information that fully describes the structure and function of an organism. Three different processes are responsible for the transmission of genetic information [14]:

- Amplification
- Transcription
- Translation

During amplification, a double-stranded nucleic acid molecule is amplified one-to-one to give identical copies. This process ensures the continuity of genetic information by keeping it unchanged. During transcription, the DNA fragment corresponding to a gene is read and expressed by a single-stranded RNA sequence. This RNA molecule moves from the nucleus to the cytoplasm. During translation, the RNA sequence is translated into the protein-forming amino acid chain in the cytoplasm [14].

DNA amplification is the process on which PCR amplification is based. During amplification, the double helix structure of the DNA molecule is unwound and each strand becomes ancestor for the synthesis of a new complementary strand. Each offspring molecule consists of an old DNA strand and a replica of the parent molecule [14].

2.6 DNA Analysis Methods

2.6.1 Loop-mediated Isothermal Amplification Method (LAMP)

LAMP is the technique of amplification of isothermal nucleic acids in which the amplification and detection of target genes is completed in a single step at a constant temperature. LAMP is characterized by the use of four different primers that recognize six different regions on the target [15].

An internal primer pair containing significant and insignificant sequences of target DNA followed by an outer primer pair initiates the LAMP reaction progressing at a constant temperature [16]. The addition of loop primers increases the specificity and time efficiency of LAMP assays [17]. LAMP products show a ladder-like pattern on agarose gel. It can be monitored either using turbidimetry or by measuring fluorescence with real-time LAMP.

Primers used in the LAMP method [18]:

- FIP: Forward Inner Primer
- BIP: Backward Inner Primer
- F3:FOP: Forward Outer Primer
- B3:BOP: Backward Outer Primer

2.6.2 Polymerase Chain Reaction (PCR)

PCR is an in vitro technique in which a particular DNA segment extending between two known segments of a DNA chain is enzymatically amplified. Initially, only a small part of a particular gene can be obtained, while millions of copies can be amplified from a single gene copy within a few hours using PCR [19].

DNA is a molecule with a right-sided helical structure that occurs when two parallel chains of phosphoric acid and deoxyribose units are crosslinked by some of the purine and pyrimidine, which store the genetic information encoded in the sequence of the nucleotides it carries. In eukaryotic cells, most of the DNA is found in the nucleus and is called chromosomal DNA. It is separated from the rest of the cell (cytoplasm) by a bilayer membrane. DNA contains all the genetic information that fully describes the structure and function of an organism.

Three different processes are responsible for the transmission of genetic information. These are Amplification, Transcription and Translation. During amplification, a double-stranded nucleic acid molecule is amplified one-to-one to give identical copies. This process ensures the continuity of genetic information by keeping it unchanged. During transcription, the DNA fragment corresponding to a gene is read and expressed by a single-stranded RNA sequence. This RNA molecule moves from the nucleus to the cytoplasm. During translation, the RNA sequence is translated into the protein-forming amino acid chain in the cytoplasm [14]

2.6.3 Stages of LAMP Method [18]

1. After denaturing the target DNA region, FIP initiates synthesis from the 3’ to the 5’ of the F2 region.
2. The external forward primer (F3) initiates synthesis from the 3‘ to the 5’ of the F2c region of DNA. It separates and extends the strand to which the inner forward primer (FIP) is bonded. The separated strand forms a ring at the 5’.
3. Single DNA with a ring at the 5’ serves as a template for the internal reverse primer (BIP). B2 at the 5’ of the BIP initiates synthesis from this DNA to the 3 5 to 5’. Eventually, the ring at the 5’ opens.
4. The outer reverse primer (B3) initiates synthesis from the 3’ to the 5’ of the B2c region of DNA. It separates and stretches the strand to which the BIP is attached. The separated strand forms a ring at the 5’.

5. The dumbbell-shaped DNA is transformed into a root loop structure. This structure serves as the initiator for the second stage of the LAMP reaction.
6. The FIP root loop is adapted to the DNA structure to initiate the LAMP cycle. The synthesis of the stand is started here. The F1 yarn is displaced and a new ring structure is formed at the 3'.
7. Inserting the nucleotides into the 3' of B1 produces a new DNA.
8. In the subsequent reaction, the BIP acts as a template for the displacement reaction. Thus, a LAMP target sequence grows 13-fold per half turn. The final products obtained are DNAs of various root lengths and cauliflower-like structures with multiple cycles.

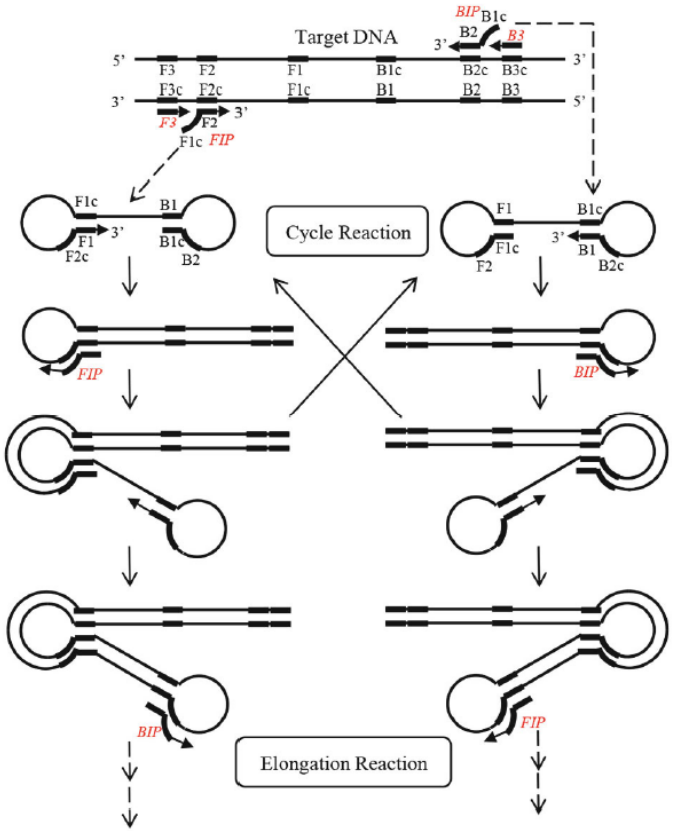

Figure 1. Stages of LAMP method [18]

2.7 Soybean

Soybean is a member of the Fabaceae family. It is a diploidized tetraploid with 2n = 40 chromosomes [20] Cholesterol-free structure, high-quality protein content and leguminous digestible product with the feature of a variety of uses is a vegetable food. Soya grains can

be germinated and eaten sprouts as vegetables or processed so that soy oil, soy flour, soy meat, soy milk, soy sauce, tofu, soy coffee and soy cream can be obtained.

Soybeans are mainly used in the animal feed sector in Turkey. The reason for the rapid increase in soy consumption in the world is not only because it is a beneficial nutrient for human health and it is used as feed [21].

Although soybean originated in the Far East, its largest producer today is the United States. In parallel with the widespread use of the soybean which has an important place in world consumption. Other countries that produce soybean are Brazil, Argentina, People's Republic of China and India. In Turkey, the Black Sea region was started in October 1930 and also since the last 20 years is grown in irrigated areas in the Mediterranean region [22] [23].

2.7.1 Transgenic Soybean

Each genetic modification process is unique. Even if the target plant and transgene are the same, the integration of the transgene with the genome is independent of each other in each modified cell. For this reason, each product produced as a result of genetic modification is called a transgenic race [24]

In total 25 plant species, 65 maize, 39 cotton, 15 canola and 14 soybeans are sown or imported in many countries with a total of 196 SE varieties [25]. Although there are only 14 varieties of GM soy on the market at the moment, soy is the first among GM plants with a plantation area of 75.4 million hectares [25]. Transgenic soy-containing foods are widely available in the world market. Roundup Ready® is resistant to glyphosate herbicide from GD soybeans, was the first transgenic plant variety approved in 1996 to be used in food production, but it still remains the most sold and cultivated seed among all GM varieties [26]. Biotechnological methods have been applied to give herbicide tolerance in all transgenic soy breeds approved by European Union GTS 40-3-2, A2704-12, and MON 89788.

2.7.2 Characteristics of GTS 40-3-2 Soybean

Known as “Roundup Ready®” soybean, the GTS 40-3-2 transgenic soybean line was developed by Monsanto to allow the use of glyphosate as an alternative system for the control of harmful plants in soybean production [27]. The development of the GTS 40-3-2 strain was generated by particle bombardment of the gene encoding the glyphosate

resistant EPSPS enzyme isolated from *agrobacterium tumefaciens* strain CP4 to commercial soybean variety A5403 [28].

Plasmid PV-GMGT04 contains three gene cassettes designed for insertion. Two of these include the CP4 EPSPS encoding sequence and one of the uidA encoding sequences [33]. However, as a result of the analysis, only a single integration region containing the glyphosate tolerance gene was found in GTS 40-3-2 [29]. The transferred EPSPS gene is controlled by 35S and NOS terminator from A. tumefaciens, which is a strong constitutive promoter from CaMV. The DNA sequence encoding plant-derived chloroplast transit peptide was cloned into the 5' end of the glyphosate tolerance gene [29].

The signal peptide coupled to the EPSPS gene allows the newly synthesized enzyme to pass into the chloroplasts, where the cyclate pathway is present and where the glyphosate acts. Once the transition has taken place, the transit peptide is separated from the enzyme by a specific protease and rapidly degraded.

2.8 MON89788

Plasmid PV-GMGOX20 carries the CP4 epsps gene expression cassette that tolerates glyphosate herbicide in plants within the right and left boundary sequences of the T-DNA region of *agrobacterium tumefaciens* [30]. The 5-enolpyruvylshikimate-3-phosphate synthase gene, which is codon optimized for high expression in plants from the CP4 strain of *agrobacterium tumefaciens*, is linked to the Tsw1 gene promoter linked to this promoter by the Tsf1 gene promoter encoding the EF-1 alpha elongation factor from *arabidopsis thaliana* [36]. The transcriptional termination and polyadenylation region of the RbcS2 gene are encoding the subunit of RuBisCo of pea origin [31]. In addition this cassette and 5' unreadable DNA base sequences of the Tsf1 gene to enhance expression of the protein. A3244 elite soybean seeds germinated embryos developed by germination of the tissue, the above-described PVGMGOX20 plasmid carrying *agrobacterium tumefaciens* inoculated by gene transfer [32].

2.9 Electromechanical Devices for DNA Detection

A portion of the all the more striking advancements in DNA discovery have happened in the field of infection identification, particularly concerning test structure. Specialists who analyzed detecting of infections explore different catch tests that are labeled with redox

species. In one methodology, polythiolated DNA test labeled with ferrocene was assessed [33].

At the point when the catch test was not hybridized, the ferrocene atom on the adaptable ssDNA is increasingly versatile and moves to the cathode surface. At the point when the objective DNA hybridizes with the test, a firm twofold helix is shaped, which fundamentally diminishes the DPV signal due to generally stable ferrocene. Utilization of peptide nucleic acids (PNA) as catch tests has likewise been researched, as it accommodates unbiased DNA analogs, which brings down electrostatic shock, and structure triplex with dsDNA [34]. We look at three guides to delineate the strategies that are incredibly touchy, directed without enhancement, or utilize novel tests.

In the investigation revealed by Aguilar and Fritsch, an electrochemical methodology focused on 121-mer mRNA of the hsp70 heat stun protein in Cryptosporidium parvum [35]. An example of 2.6×10^{6} oocysts/ml was heat stunned for 10 min to prompt interpretation of hsp70. A catch DNA test was immobilized onto an aminated Au/SiO2 wafer. At that point, 500 µl of a 50-µg/ml arrangement of warmth stunned oocysts was brooded with the functionalized wafer for 1 h for hybridization to be finished. In this way, hatching with a columnist test comprising of 42-base ssDNA conjugated to soluble phosphatase was utilized. The adjusted wafer was flushed and moved to an answer containing the substrate for the chemical, p-aminophenyl phosphate (PAPP). Over a 12-h period, soluble phosphatase created the electroactive species, p-aminophenyl, which was estimated by cyclic voltammetry. The creators built up that there was extremely restricted cross-reactivity with a few normal pathogens, for example, Cr. lamblia, Listeria monocytogenes, Campylobacter lari, *E. coli* O157:H7, and Salmonella typhi. An alignment bend was set up utilizing manufactured hsp RNA as an objective. The pinnacle flows shifted directly in the 5-to 50-µg/ml fixation run, and the cutoff of identification was determined as 2 µg/ml (146 nm), which is unassuming contrasted with crafted by Liao et al. who got a location breaking point of 1 mol [36].

Chen et al. utilized PCR related to QCM for identifying *E. coli* O157:H7 with a consolidated point of confinement of location of 1.2×10^{4} cfu/ml. Every single surface adjustment and estimations were completed with the sensor introduced in a stream cell. A ssDNA test correlative to the eaeA quality objective was immobilized onto the gold-covered QCM surface for location. Far lower utmost of discovery of 1.2×10^{2} cfu/ml was acknowledged by intensifying the QCM signal with a second gold nanoparticle-marked ssDNA correlative to

the gathered objective eaeA quality caught on the sensor. The point of confinement of identification relates to the focus in the first cell suspensions before DNA extraction and the PCR intensification step. The creators showed that their technique was explicit to the objective by leading detecting tries different things with DNA strands got from other bacterial strains. The nearness of incidental strands caused insignificant change in sensor reaction. The Au nanoparticle intensification diminished the probability of bogus negatives. The agents detailed that the recurrence reaction was split when meat tests vaccinated with *E. coli* O157:H7 were utilized, however didn't evaluate as far as possible for the perplexing lattice [37].

To clarify the oxidation component of purine puts together, electrodeposition of gold nanoparticles on single-walled carbon nanotubes (CNTs) terminal was done under streamlined conditions to get the best surface-upgraded Raman spectroelectrochemistry reaction. This investigation was seen as accommodating to recognize the oxidation intermediates and various directions during various advances. The adenine base was found to have direction that didn't change during the entire procedure. Besides, the atom kept up a parallel design after the initial step of oxidation and displayed just a somewhat tilted direction. Then again, the direction of guanine base totally changed during oxidation. At first, it was opposite to the gold nanoparticles. After the initial step, the atom indicated to some degree tilted direction that was totally changed to parallel direction regarding the anode surface after the second step of oxidation [38].

The CNT disclosure has a significant job in the improvement of electrochemical DNA biosensor for the examination of DNA. CNTs encourage immobilization of DNA and are additionally useful as ground-breaking speaker by enhancing the transduction sign of hybridization. The DNA chip having showed CNT needs modest quantity of test and offers a significant job in CNT-based biosensor advancement for DNA-based diagnostics [39].

The peptide nucleic corrosive (PNA)- based information has opened new entryways for DNA biosensors. In PNA, sugar phosphate spine is subbed with pseudopeptide. The arrangement stage PNA has particular basic hybridization and acknowledgment viewpoints to plan profoundly specific DNA biosensors [40].

In late 1990s, the consideration was centered on the assembling of fast, cheap and high-delicate detection gadgets for the biological systems. DNA identification by utilizing stripping voltammetry brought about low-cost electrochemical method [41].

3. MECHANICAL DESIGN OF DEVICES FOR GMO AND BACTERIA ANALYSIS

In order to detect GMO and *E.coli*, we need to keep biological samples in the appropriate temperature range under LAMP conditions. For this purpose, a device for simultaneously analyzing a substantial amount of samples was designed, the details of which are shown in Figure 2. The biological samples are placed in several chambers on the well-plate. The chambers have a diameter of 3.5 mm and a depth of 1 mm, hence a volume of 9.62 mm^3. These chambers should be kept at 65^oC to meet the requirements of the LAMP protocol. In order to provide an even temperature distribution, the well-plate is placed over an aluminum block which is heated by means of a PCB heater in contact with the lower surface.

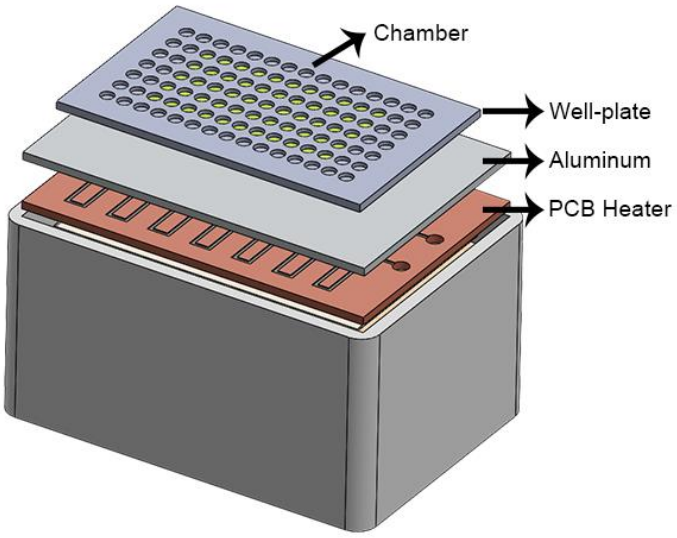

Figure 2. Heating parts of device

Two prototype devices of varying size and sample capacity were designed, which were used for GMO and *E. coli* analysis. Mechanical designs of these devices were performed in SOLIDWORKS 2016, while prototypes were manufactured by an Ultimaker 3 model 3D printer (Ultimaker B.V., Utrecht, Netherlands) using Acrylonitrile Butadiene Styrene (ABS). PCBs for the electronic circuits and heater units were designed using version 5.0.1 of the open source EDA tool KiCad and were manufactured using a Trotec Speedy300 Flex PCB printing machine (Trotec Laser GmbH, Marchtrenk, Austria).

3.1 Prototype for GMO Analysis

In general, GMO analyses are performed in the laboratory and control centers. The prototype, which is called DaimonDNA was designed to perform GMO analysis anywhere without the need for a laboratory. DaimonDNA has 4 sample storage chambers. One of these chambers was used for negative control and the other three for triplicate. Samples are placed in PCR tubes. During the LAMP process, the color change was observed from the front window.

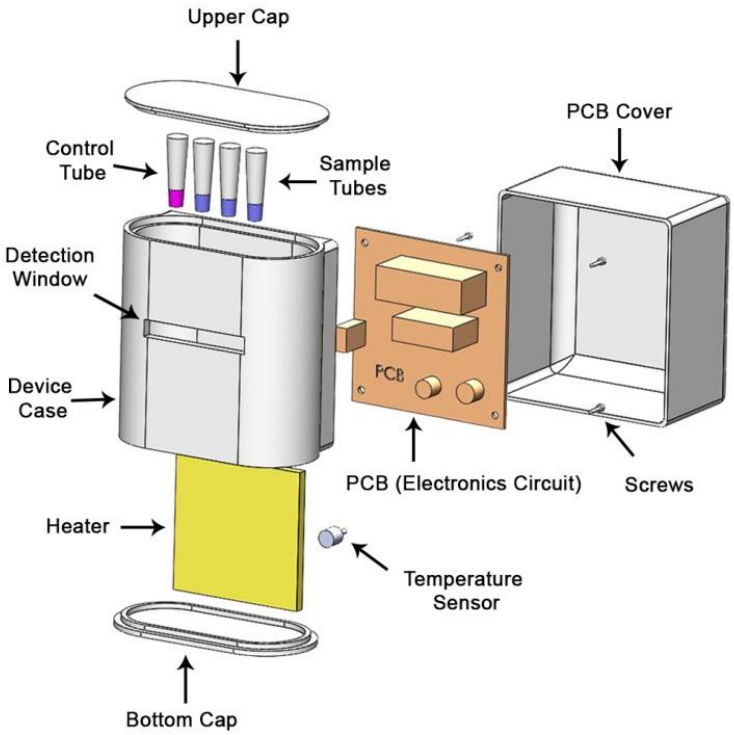

Figure 3. Prototype for GMO analysis [50]

Figure 4Figure 5 show the dimensions of DiamonDNA. The assembled device has a size of 60×56×65 (h×w×d, all in mm). There are 4 perforated structures on the top of the device to accommodate the PCR tubes. These holes were plotted according to standard PCR tube sizes. The diameter of the holes is 7 mm. The distance between the centers is 8.5 mm. A plexiglass covered rectangular window of size 5 mm × 32.5 mm enables the viewing of the samples during the process.

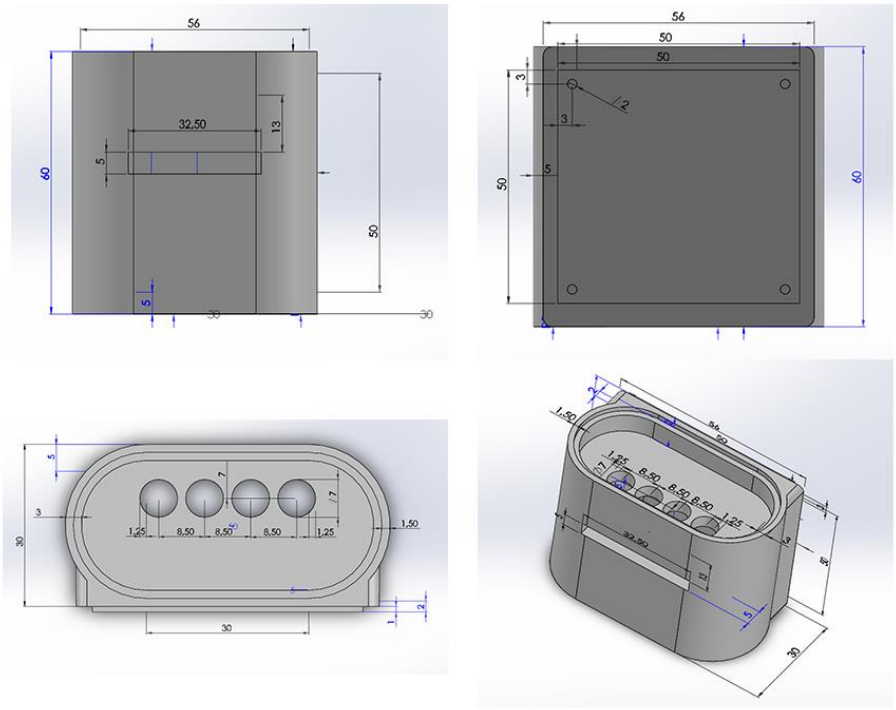

Figure 4. Dimensions of DaimonDNA's base part

Figure 5 shows the covers used to close the top and bottom of the device and a PCB cover portion. A 2 mm fillet is made with sharp corners to increase strength and reduce the brittleness of the device.

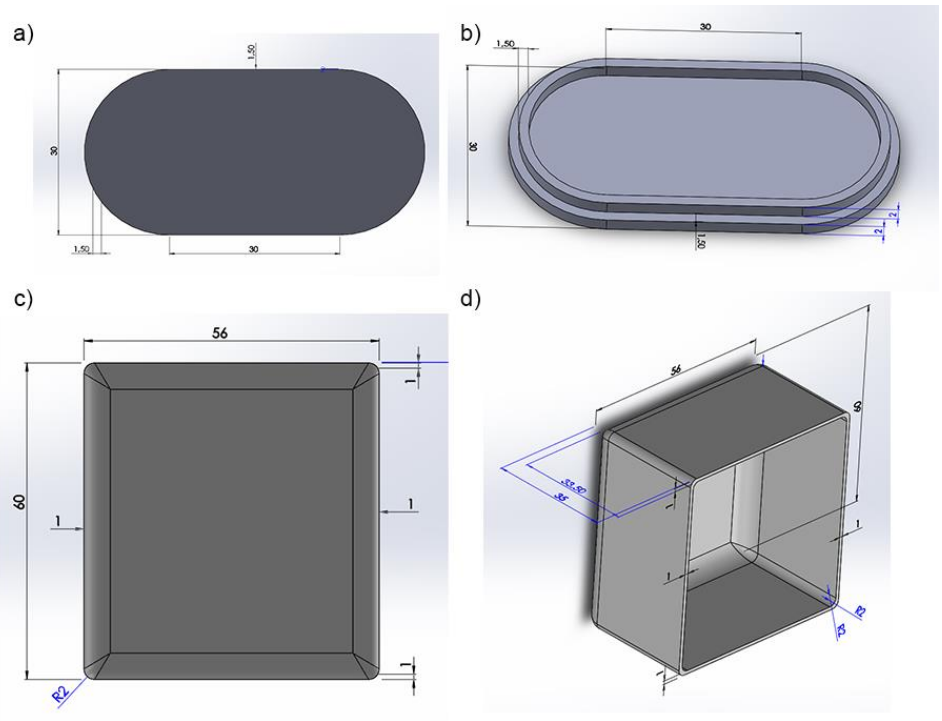

Figure 5. Dimensions of DaimonDNA's other parts

3.2 Prototype for *E.coli* Analysis

Figure 6 shows DaimonDNA-E, the device made for *E. coli* analysis. DaimonDNA-E is designed to analyze 105 samples. Color change during the procedure was observed through the PDMS top cover of the device.

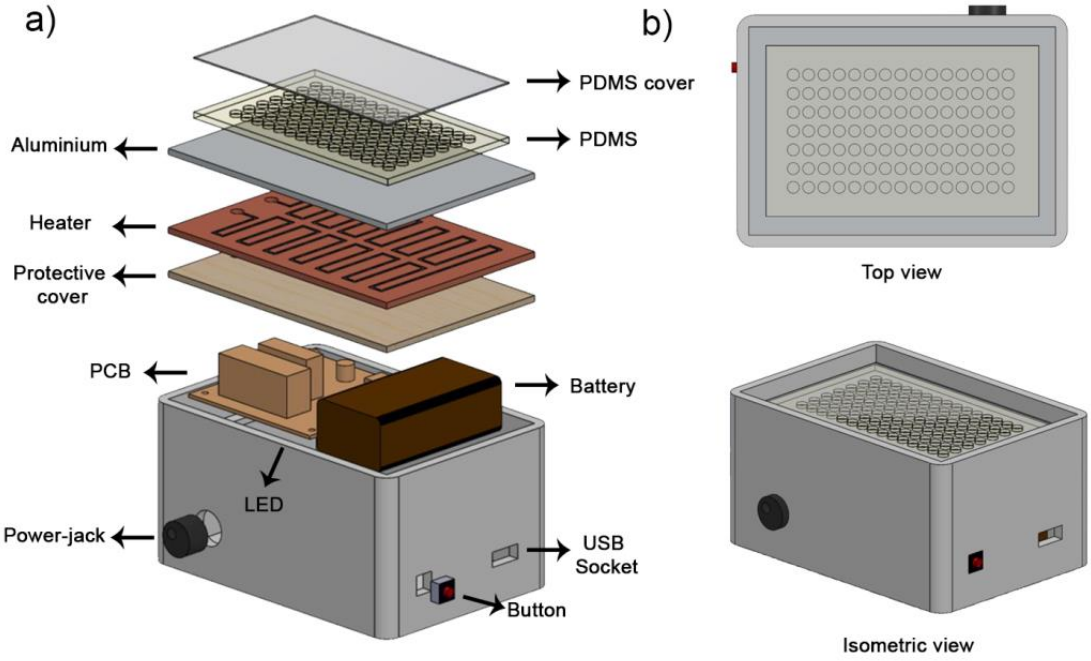

Figure 6. DaimonDNA-E Prototype for *E. coli* analysis, a) components of the colony-LAMP platform, b) different views of the platform

Figure 7Figure 8 show the dimensions of the DaimonDNA-E. The PCB heater is made longer and wider than the dimensions of the 105-well plate made of PDMS. Thus, the temperature will reach the same distribution for all the samples put into the chamber made of PDMS. The chamber made of PDMS consists of 105 holes. These holes are 1 mm deep and have a diameter of 3.5 mm.

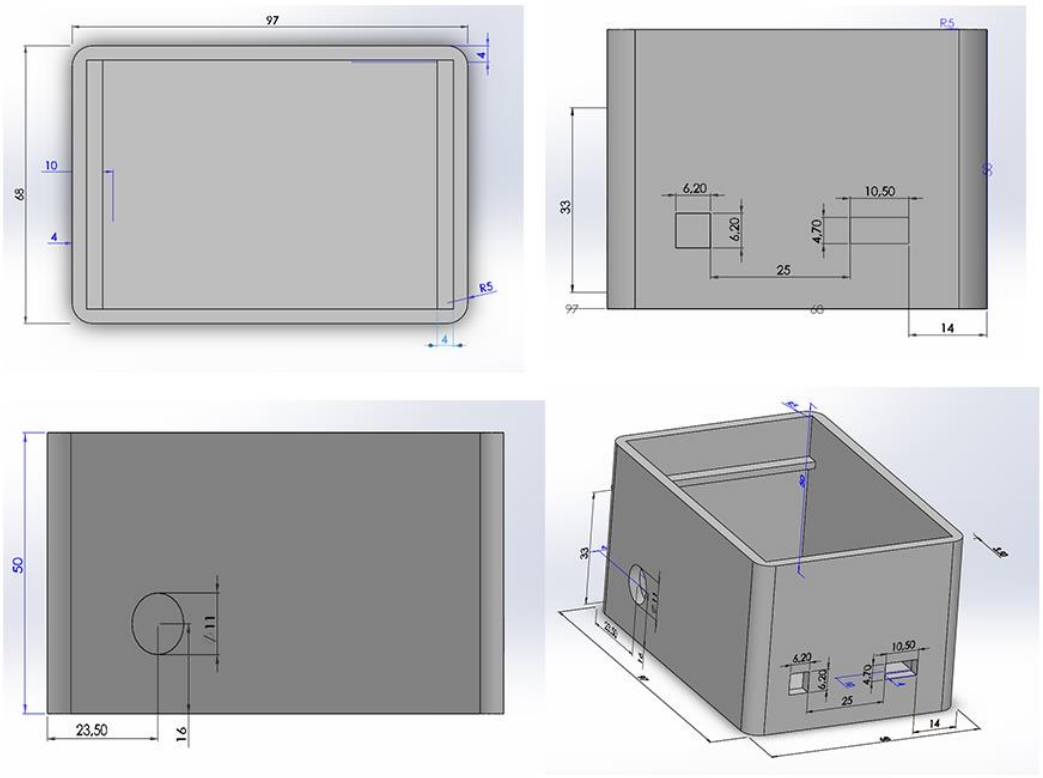

Figure 7. Dimensions of DaimonDNA-E's main part

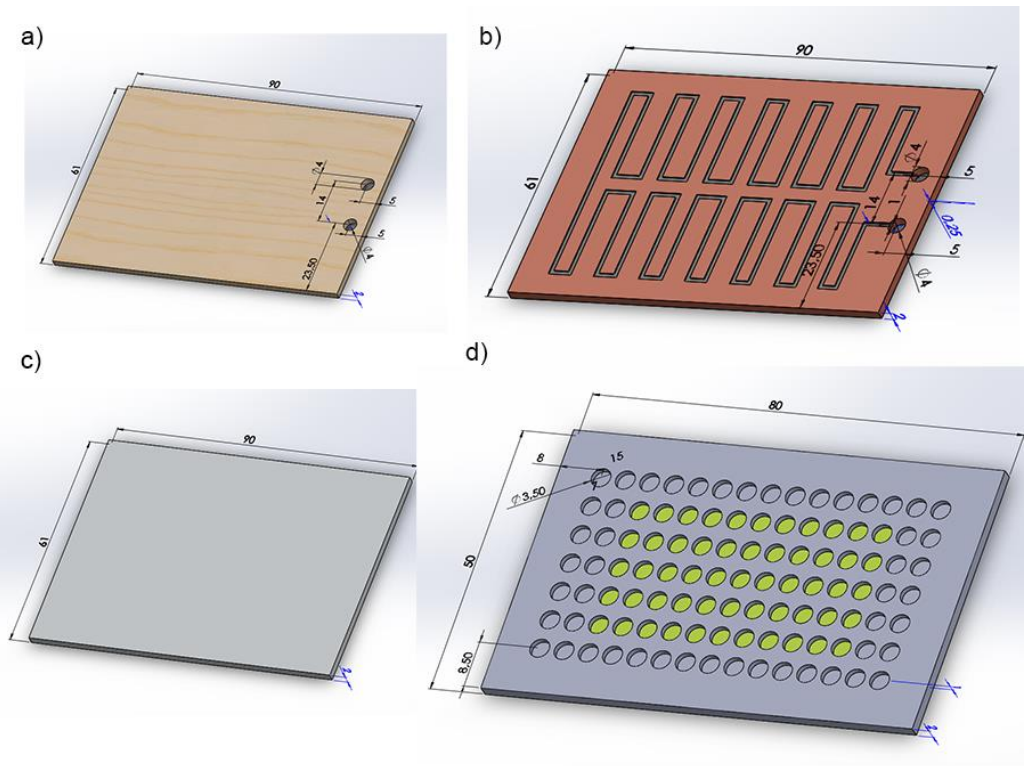

Figure 8. Dimensions of DaimonDNA-E's other parts. a) protective cover, b) PCB heater, c) aluminum plate, d) 105-well plate

DaimonDNA-E is intended to have more sample storage vessels, compared to DaimonDNA. The 105 sample wells of the device were made of polydimethylsiloxane (PDMS) by molding. Figure 9 shows the PDMS mold design, the manufactured mold using 3D printer, and the molded PDMS sample chamber. The PDMS plate has dimensions of 95 × 60 × 17 mm. The mold was designed with SOLIDWORKS, and 3D printed using. The channel height was 10 mm to accommodate 25 µL of LAMP mixture. For the construction of the device, PDMS and curing agent were mixed in 10:1 ratio and poured into the mold in a 60 mm width and 13 mm depth container, degassed in a desecrator, and cured at 75 °C for 60 min in an oven. The PDMS pieces were cut and gently peeled off from the mold on the container.

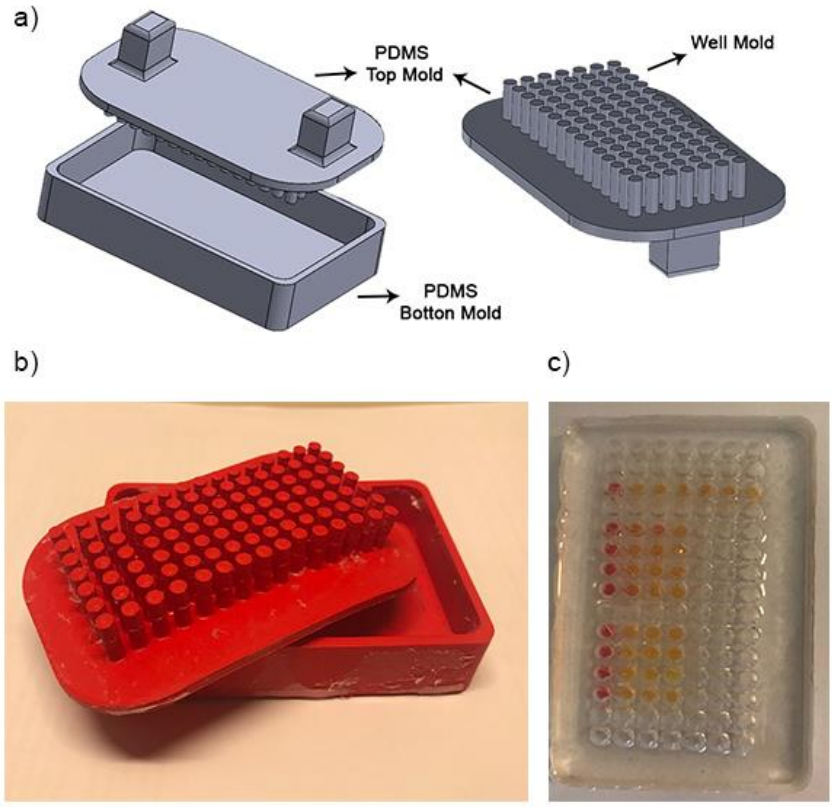

Figure 9. a) PDMS mold prototype, b) PDMS mold 3D print, c) PDMS reservoir where samples are placed.

4. ELECTRONIC DESIGN

The temperature of the heater element needs to be closely monitored for the success of the LAMP process. The most convenient and affordable method for temperature control is the use of a microcontroller in conjunction with a thermocouple for temperature sensing, and a driver for controlling the power applied to the heater element.

4.1 Heater and Controller

Heating dynamics of the DaimonDNA-E was first analyzed by the use of ceramic resistors. When 40 watts of power is applied to the platform, it reaches a temperature of 65^{o}C for approximately 20 seconds. The plot in Figure 10 was recorded using the Arduino Serial Plotter software. With a peak power of 40 watts and supply voltage of 12 Volts, the resistance of the heater element is calculated as 3.6 Ω, while the peak current is 3.3 A.

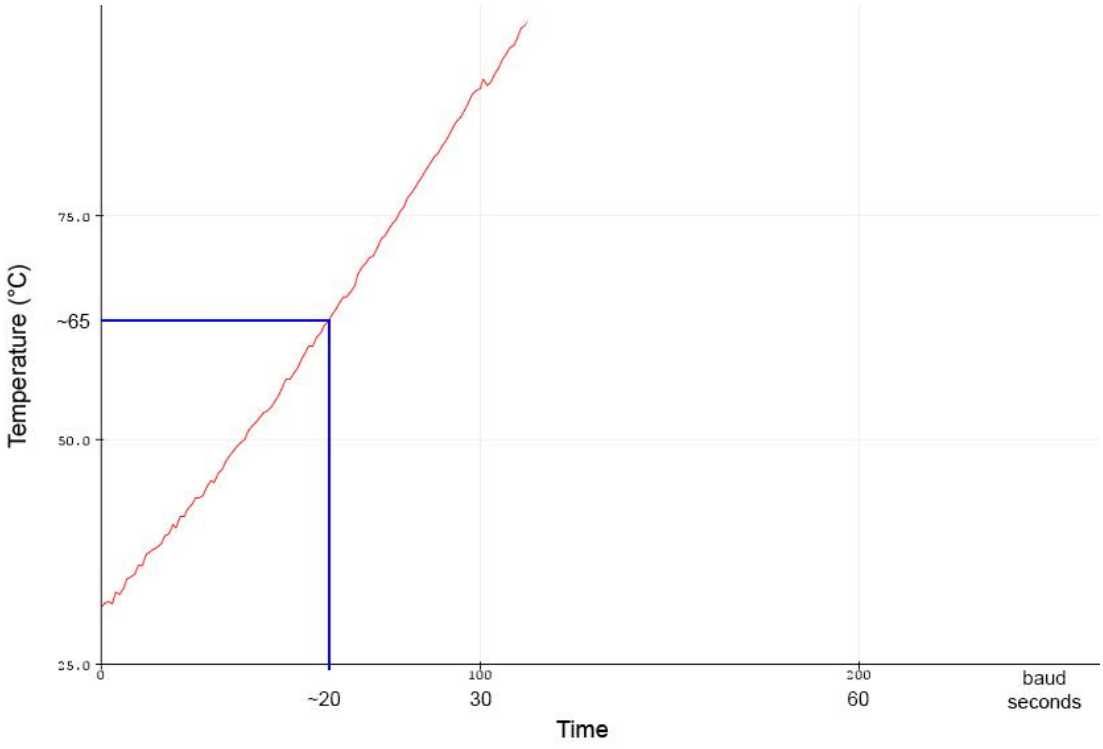

Figure 10. Heating graph with 40W

The temperature controller used in the DaimonDNA-E is based on an Arduino Nano board with an Atmel ATmega328 microcontroller. Power regulation was performed using pulse-width modulation (PWM) where a IRF510 n-channel power MOSFET was used as the switching element, which is capable of continuously driving 5.6 A of current. The relatively large gate capacitance of the power MOS required the use of a driver BJT, for which an S8050 npn was chosen. The plate temperature is measured using a K-type thermocouple, which is interfaced to the microcontroller through a MAX6675 Cold-Junction-Compensated K-Thermocouple-to-Digital Converter, which provides a sensitivity of 0.25°C in a temperature range from -20 to +80°C. The complete schematics is shown in Figure 11.

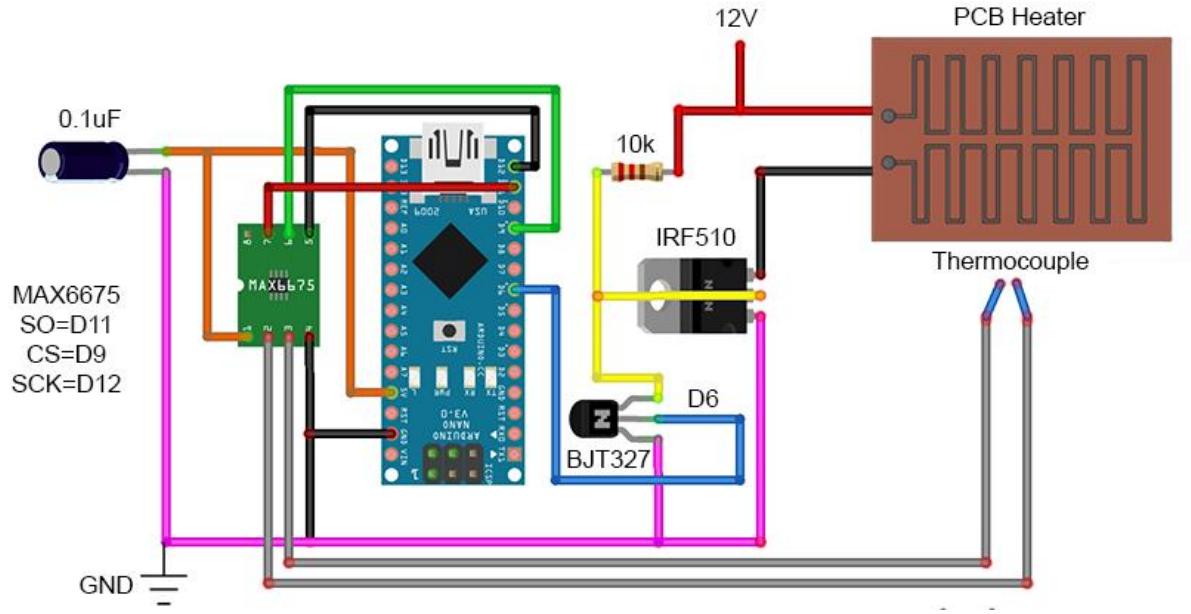

Figure 11. Schematic of heater and temperature controller

Trace widths for the PCB heater were found using online calculation site [42]. The traces of the heater were drawn with the KiCad program and produced with a PCB printing machine. Figure 12 shows the designed and manufactured heater. All components of the controller are then assembled on a PCB, which was again designed using KiCad. PCB dimensions for DaimonDNA and DaimonDNA-E are 5cm×5cm and 7cm×3.5cm, respectively. The PCBs are shown in Figure 13.

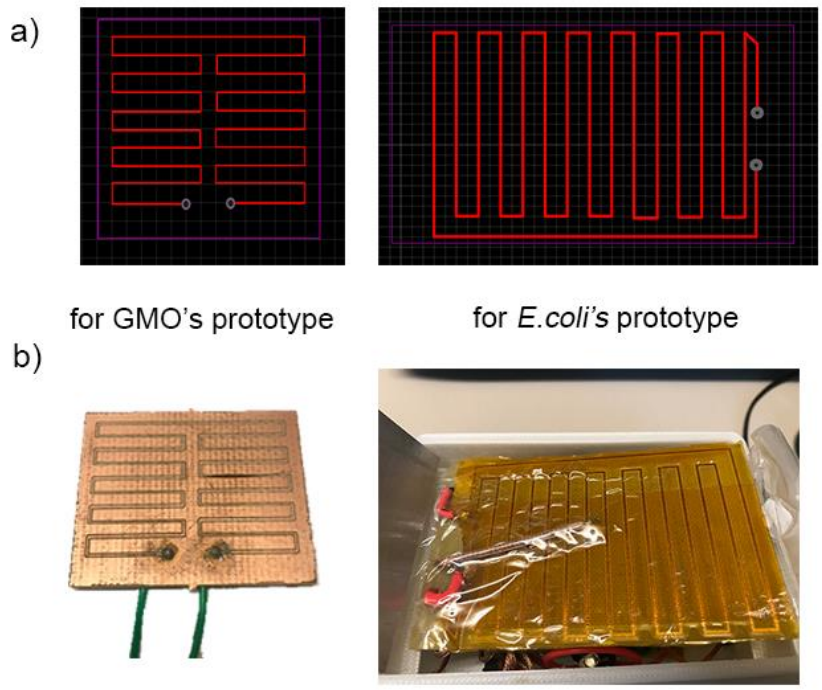

Figure 12. a) Heaters are drawn with KiCad program b) The physical PCB heaters

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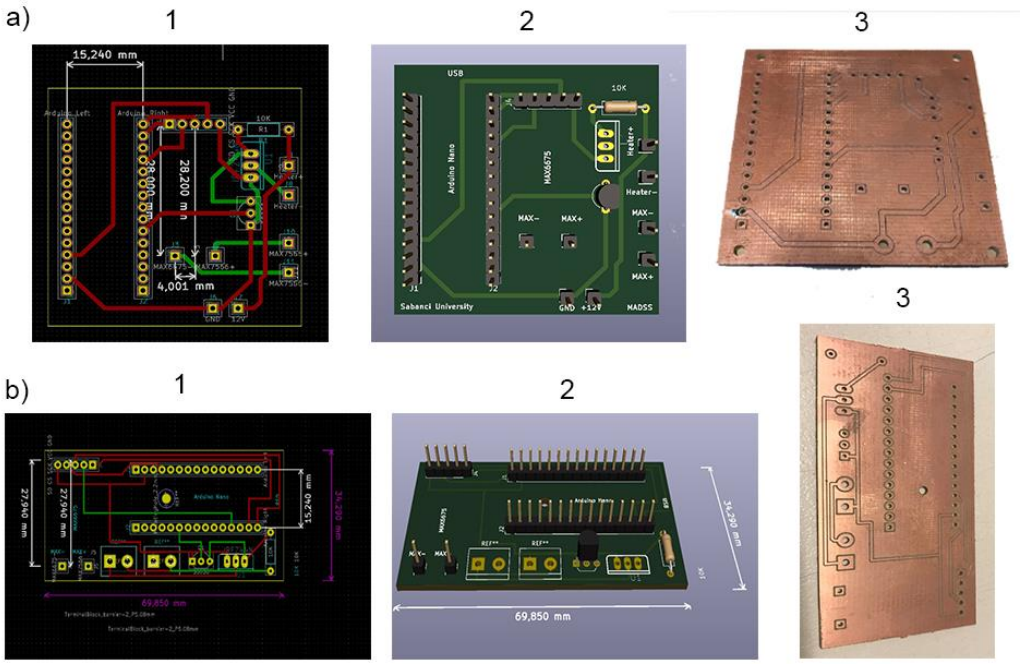

Figure 13. a) PCB printing for GMO analysis b) PCB printing for *E. coli* analysis

4.2 Controller Firmware and Control Parameters

The firmware of the Arduino based temperature controller was adopted form [54]. The open-loop heating experiment result in Figure 10 revealed that there is insignificant time delay in the heater, hence the system is expected to be inherently stable even with simple proportional control. Keeping K_i=0 and K_d=0 in all cases, the controller was evaluated for various gain settings.

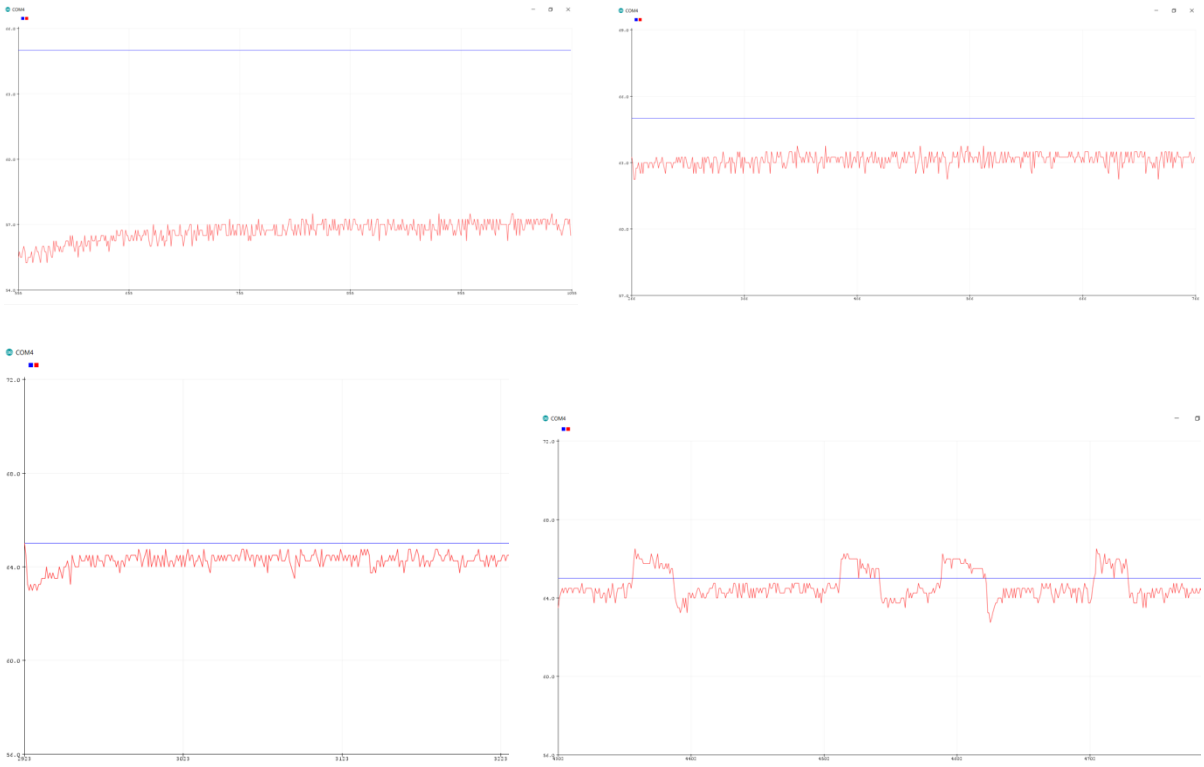

Figure 14. Temperature vs time. $K_p = 1$, $K_p = 5$, $K_p = 12$ and $K_p = 14$.

Results in Figure 14 reveal that for a gain setting of 14 the temperature response starts to fluctuate due to numerical problems in the microcontroller. Hence, the gain is set to 12. This resulted in a response as shown in Figure 15.

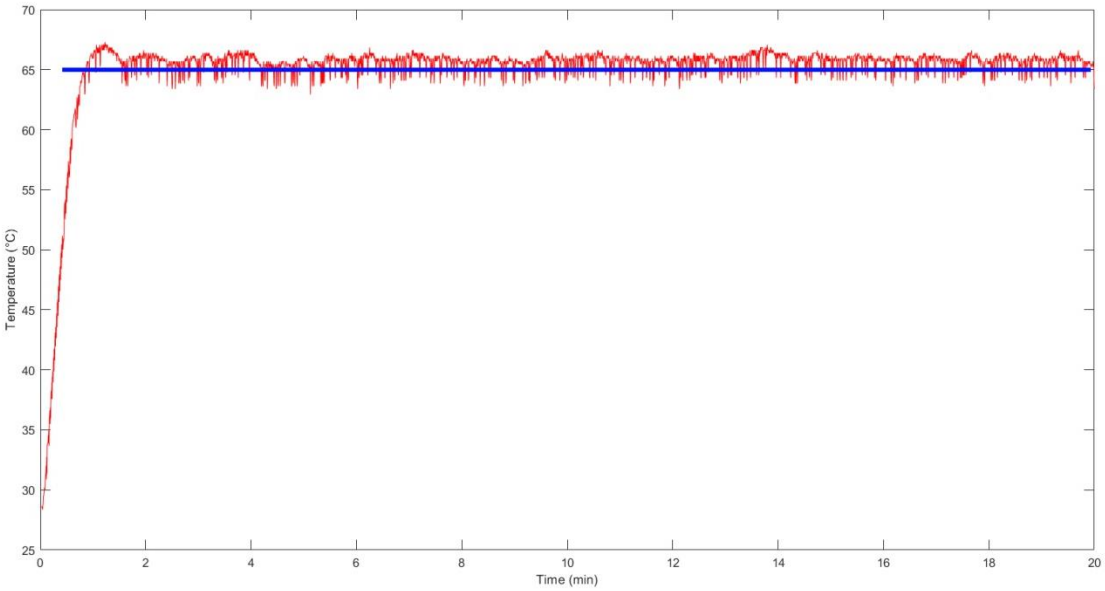

Figure 15. Temperature curve of the system

Since biological materials are expensive, the first experiments were carried out using water. Two heating graphs were obtained with and without water. Rise time is determined by the operation performed here. The average time of the desired temperature, which is 64-65 ^{o}C, was calculated. When the PID is controlled, it slows down to 65 degrees asymptotically. Therefore reaction will start within 2 min. The temperature graph with the water in the tubes is shown in Figure 33.

When the tubes are completely filled with water, the time to reach the desired temperature is approximately 120 seconds. The same test was tested without any liquid in the tubes. In this case, it reached the desired temperature in about 70 seconds.

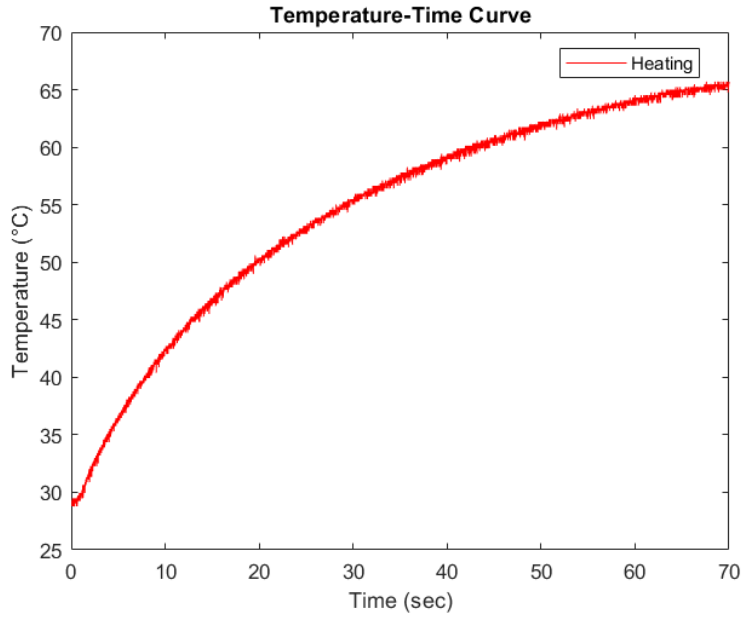

Figure 16. Heating without any liquid in the tubes

After heating the tubes containing water, the heater was switched off and the device was allowed to cool. According to the data obtained from the temperature sensor, a cooling graph was obtained. The graph is shown in Figure 35. Accordingly, it falls below 40 ^{o}C in approximately 250 seconds by itself.

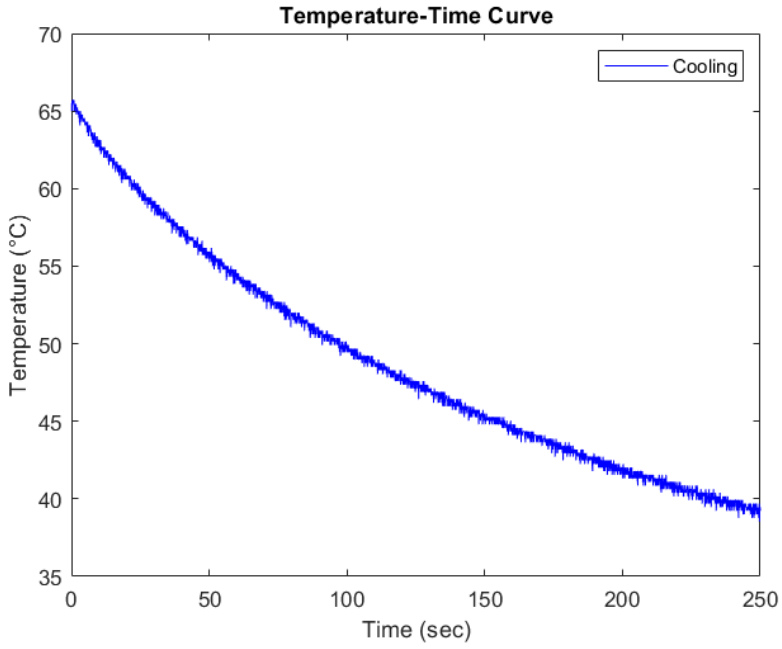

Figure 17. Graph of cooling of tubes with water

5. EXPERIMENTAL RESULTS

5.1 Reaction Time and Reaction Temperature

Separate analyses were performed for GMOs and *E.coli*. The reaction time for GMO analysis is about 30 minutes [43]. For *E.coli* bacterial analysis, it takes about 40 minutes. In our experiments, the relationship between time and color change was examined. For GMO, we can clearly see the color change in 30 minutes at 65 ^{o}C. For *E.coli* we detect color change in about 40 minutes at 65 ^{o}C.

Our reaction temperature was 65 ^{o}C for both analyzes. This temperature value was chosen because it is ideal for 65 ^{o}C by performing cycles in various temperature ranges. In the previous experiments were examined to Temperature and Time values. Then many experiments were performed to find temperature and time values. According to the results of these experiments temperature (65-66 ^{o}C) and time (30-40 mins) values were found.

Result of the experiment to find the appropriate temperature range. According to the results of this experiment, the appropriate temperature range was found to be 64-65 ^{o}C [43].

5.2 Detection of GM Soybean Genes

This section shows the results of color change and gel electrophoresis for GMO analysis. The LAMP responses were proceeded as three duplicates utilizing the thermal cycler and the DaimonDNA biosensor. The LAMP responses were done utilizing the lectin primer set and 100 ng of MON89788 template DNA per reaction. Figure 36a and b, by naked eye the color of the negative LAMP response shifted from indigo to violet, while the positive responses consistently became sky blue. The light blue color demonstrates positive; the violet shading shows negative outcomes in the thermal cycler utilizing HNB. Figure 36c, by agarose gel electrophoresis of the LAMP amplified products, we affirmed the ladder pattern of LAMP in positive reaction. Figure 36d, the intensity measurements of DNA bands from Figure 37c showing mean values of three duplicates with their standard deviations [43].

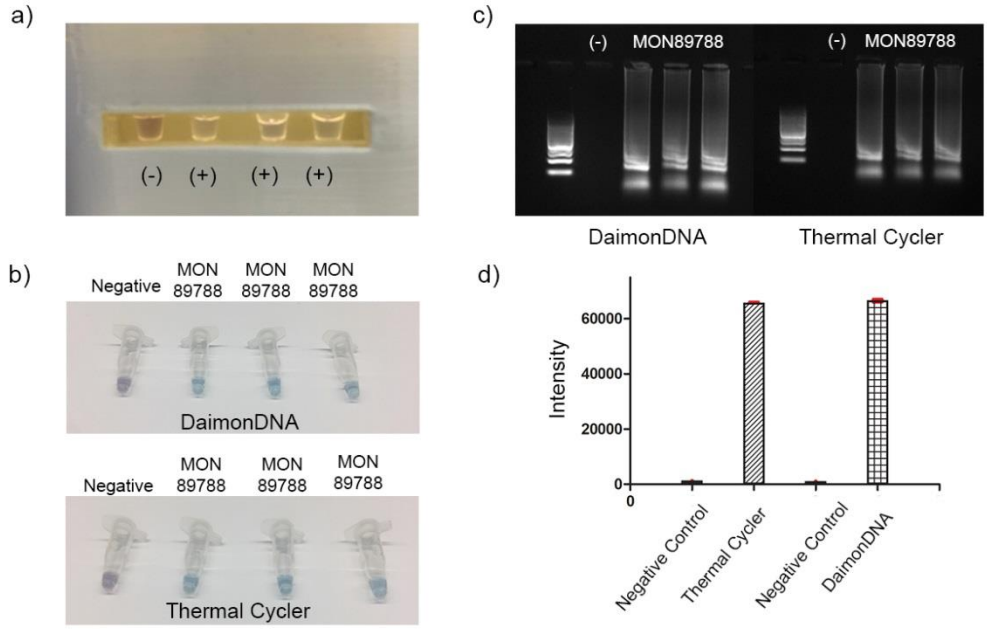

Figure 18. LAMP reactions [43].

5.5 Sensitivity and Selectivity of Device

To assess sensitivity, we utilized the P35S primer set and analyzed the genomic DNA at three different serial dilutions: 0.1%, 1%, and 10%. They represent low, medium, and high RRS concentrations, respectively. The concentration of genomic DNA was calculated as copies number as follows.

$$Copy\ number\ of\ RRS\ DNA = \frac{weight\ of\ gDNA\ used\ in\ the\ reaction}{genome\ size\ of\ soybean}\ x\ \frac{\%\ GMO}{100} \qquad (6.5)$$

Figure 37a displays copy number samples of RRS by LAMP amplification, both in the DaimonDNA and the heatblock are 76.92, 769.2 and 7692. Figure 37b demonstrates the agarose gel electrophoresis of the LAMP amplified products [43].

Figure 37c shows the selectivity of the LAMP reactions as colorimetric readouts in the PCR tubes. Figure 37d confirms the selectivity of the LAMP reactions using P35S and lectin sets by agarose gel electrophoresis; specifically the LAMP reaction for P35S with MON89788 DNA did not provide color change, and bands in the gel electrophoresis [43].

Although this method allowed detection of the color change by naked eye as long as the LAMP reaction occurs, it was not eligible to distinguish the gradient of the color change

according to serial dilutions of DNA concentrations in the PCR tubes. In order to test the specificity of the DiamonDNA biosensor, we performed the LAMP reactions with (i) Lectin primer set as a species specific control, which should give positive results for both CRMs, (ii) P35S as a GMO-specific primer set, which should give positive results for RRS but not MON89788, as the latter variety does not contain this element [43].

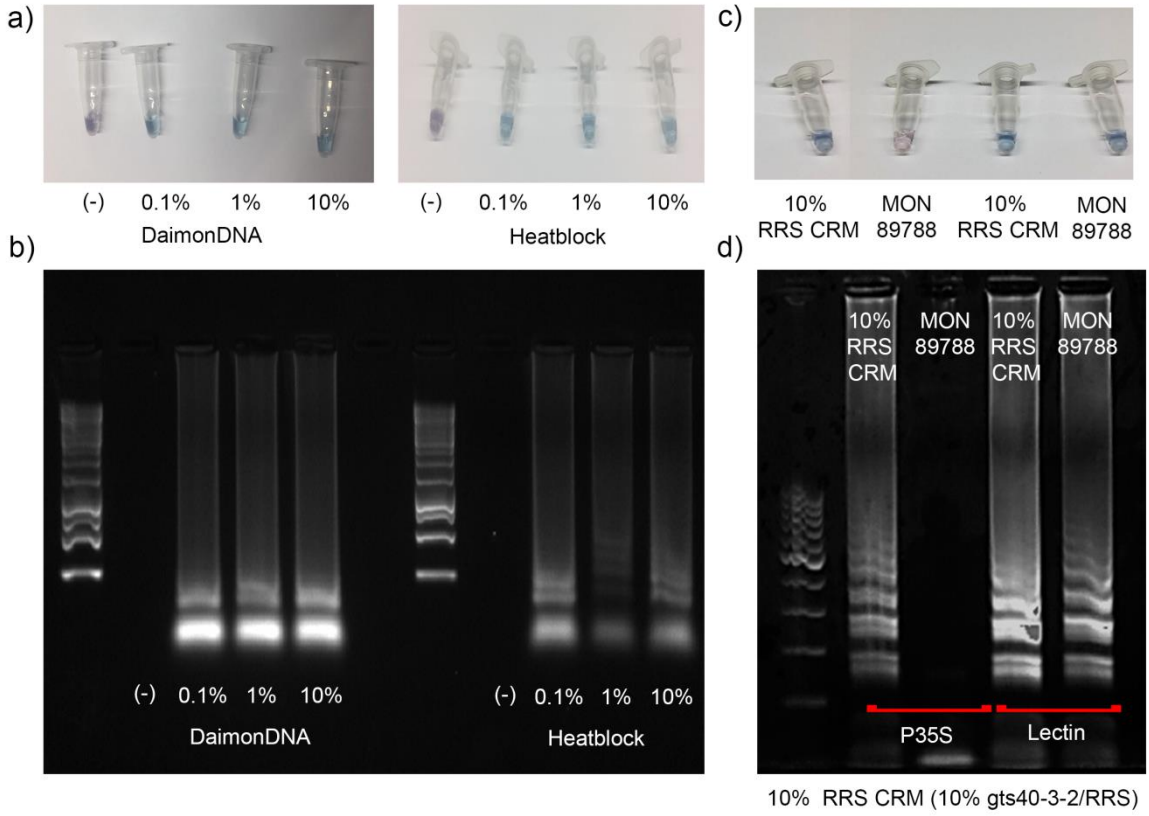

Figure 19. Characterization of DaimonDNA biosensor [43].

5.6 DNA preparation and LAMP reactions in high-throughput colony LAMP platform

In order to reduce contamination caused by external factors, rapid and accurate detection methods are highly required. This study aimed to establish simple and rapid testing methods based on direct bacterial LAMP assay with the colony for the detection of *E. coli*. Figure 38 describes the organization of reactions on the platform with the PDMS 105-well plate and the reactions that took place in each well. With inner and outer primers recognizing six distinct regions, and with the reaction under isothermal without thermal cycler, LAMP showed its advantages as rapid, specific, sensitive, cost-effective and easy-operating, with which LAMP was an alternative for detection of clinical pathogens. The LAMP assay was less

affected by various components of clinical samples as well. Our previous research demonstrated that LAMP was a helpful method for rapid DNA detection platforms [43].

The LAMP reactions were performed as three replicates using the colony-LAMP platform. By eye, the color of the negative LAMP reaction remained pink, while the positive reactions changed from pink to yellow as indicated in the NEB Colorimetric Assay catalog.

	a	b	c	d	e	f	g
1-	Negative	*E. coli* Genomic DNA LAMP with yaiO	*E. coli* Genomic DNA LAMP with yaiO	*E. coli* Genomic DNA LAMP with yaiO	*E. coli* Colony LAMP with yaiO	*E. coli* Colony LAMP with yaiO	*E. coli* Colony LAMP with yaiO
2-	Negative	*E. coli* Colony LAMP in different sizes with malB (Rmin)	*E. coli* Colony LAMP in different sizes with malB (Rmean)	*E. coli* Colony LAMP in different sizes with malB (Rmax)			
3-	Negative	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (10%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (50%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (100%)			
4-	Negative	*E. coli* Colony LAMP in different sizes with yaiO (Rmin)	*E. coli* Colony LAMP in different sizes with yaiO (Rmean)	*E. coli* Colony LAMP in different sizes with yaiO (Rmax)			
5-	Negative	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (10%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (50%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (100%)			
6-	Negative	*E. coli* Colony LAMP in different sizes with malB (Rmin)	*E. coli* Colony LAMP in different sizes with malB (Rmean)	*E. coli* Colony LAMP in different sizes with malB (Rmax)			
7-	Negative	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (10%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (50%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with malB (100%)			
8-	Negative	*E. coli* Colony LAMP in different sizes with yaiO (Rmin)	*E. coli* Colony LAMP in different sizes with yaiO (Rmean)	*E. coli* Colony LAMP in different sizes with yaiO (Rmax)			
9-	Negative	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (10%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (50%)	*E. coli* Genomic DNA LAMP in different DNA Concentrations with yaiO (100%)			
10-	Negative	*P. aeruginosa* Colony LAMP with yaiO	*P. aeruginosa* Colony LAMP with yaiO	*P. aeruginosa* Colony LAMP with yaiO			

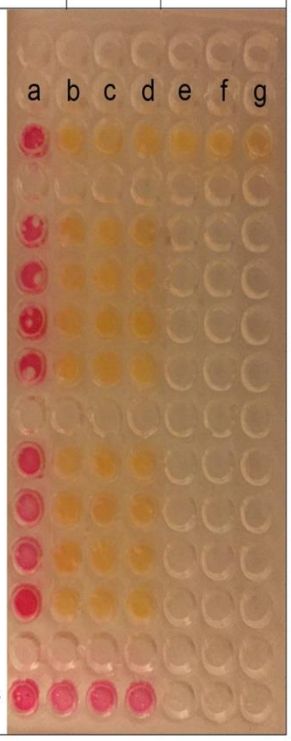

Figure 20. LAMP Reactions in the high throughput colony-LAMP platform.

In the first row of the PDMS 105-well plate, the genomic DNA isolated from *E. coli* was assayed for triplicate with yaiO primer in the first three separate wells (1b, 1c, 1d). The second three well of the first row was assayed with *E. coli* colony-LAMP. For each well one specified colony was chosen and directly added into the well with LAMP mixture.

In the second row, the *E. coli* colony-LAMP reaction was performed via malB primers with colonies of different sizes from minimum to maximum. In order to show the smallest size that can be studied with colony-LAMP, colonies with a radius of almost 1, 2 and 3 were selected R_{min}, R_{mean}, R_{max}.

In the third row, we used *E. coli* genomic DNA via malB at different concentrations to show that more than one type of reaction can be carried out simultaneously on the platform and to show the precise operating range of the platform in terms of DNA concentration.

In the fourth and fifth rows, the same experiments followed in the second and third rows were tested with yaiO primers this time to prove the platform's high-throughput capability.

In the last row, in order to demonstrate the specificity of the platform, *P.aureginosa* samples were tested with yaiO primers and showed that there was no cross-contamination.

In order to demonstrate the high-throughput of the platform, we also made both colony LAMP and normal LAMP with four different *E.coli* samples. These *E.coli* species are as follows; BL21, DH5alpha, Top10 and K12. As can be seen in the supplementary document, this platform has the power to test multiple samples with the same accuracy at the same time.

5.7 Specificity of High-Throughput Colony Lamp Platform

In order to test the specificity of the high throughput colony-LAMP platform, we performed the LAMP reactions *P.aeruginosa* with *yaiO* primer set as a species-specific control, which should give negative results, as the latter variety does not contain this element. Figure 39a displays the agarose gel electrophoresis results of colony-LAMP with *P.aeruginosa* in high throughput colony-LAMP platform; Figure 39b demonstrates the agarose gel electrophoresis of *E.coli* colony-LAMP amplified products. As shown in Figure 39c, the reaction color at well number 1e, 1f and 1g marked on the platform show color change from pink to yellow and it was seen to be positive at the end of incubation. In contrast, *yaiO* primer set, which provides *E. coli* amplification, did not work with *P.aeruginosa* because of the species-specific property of our high throughput colony-LAMP platform. The well number 10b, 10c and 10d on the platform contain LAMP mixture and *P.aeruginosa*. Color changement did not occur and the reactions remained pink.

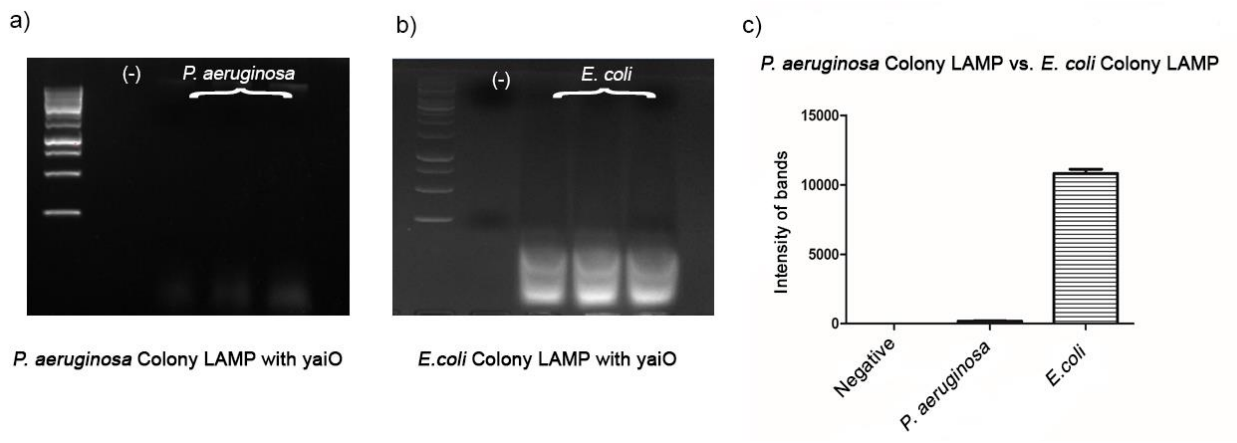

Figure 21. The specificity of Colony-LAMP reactions

5.8 Sensitivity of High-Throughput Colony Lamp Platform

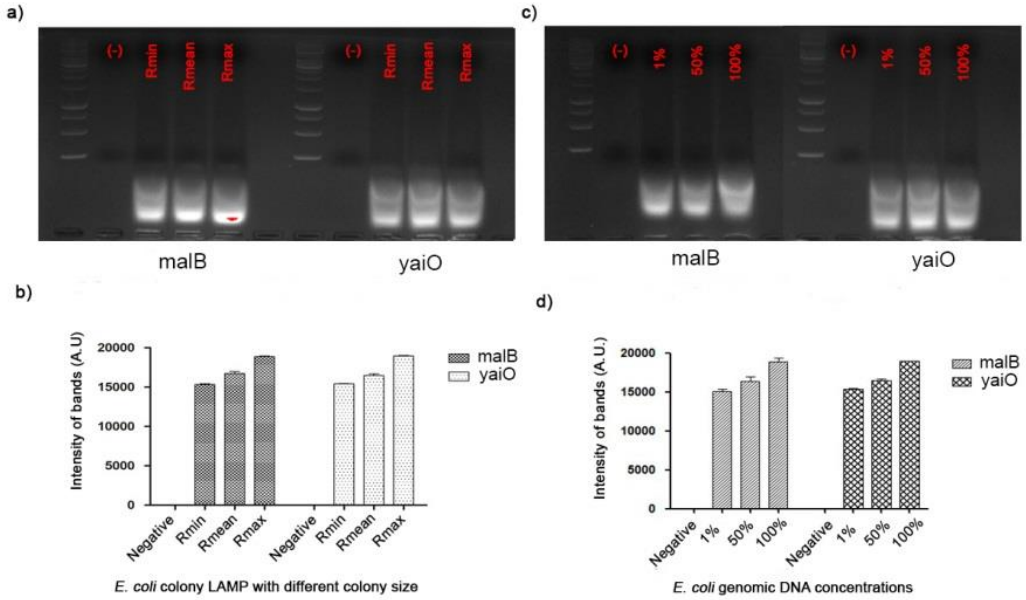

Figure 22. The sensitivity of the high throughput colony-LAMP platform

Figure 40a shows gel electrophoresis results of the colony-LAMP assay with Rmin, Rmean, and R_{max} and and Figure 40b area calculations of gel band intensities. Figure 40c shows gel electrophoresis results of *E. coli* genomic DNA LAMP and 1-kbp DNA ladder is used for the gel electrophoresis and Figure 40d shows the graph of area calculations of band intensities.

Because of the troublesomeness conditions of the DNA preparation steps, still, it requires a lot of time and labor as well. In the present study, an improved and innovative technique for direct LAMP amplification with colony-LAMP as layouts were set up and optimized. High temperature could cause the break of bacterial cells, after which various DNA was unleashed, which was adequate of sample for colony-LAMP reaction. The genomic DNA

released from spilled cells are less proficient compared with conventional DNA extraction, DNA amount was enough to yield positive LAMP amplification. When compared with the usual LAMP technique, the colony-LAMP showed extra advantages on rapid and easily applicable particularly when the colony was directly applied in the LAMP mixture. The complete detection time including amplification and results perception was around 45 min. This method eliminates long DNA isolation steps and saves both labor and time. In addition, since the platform has 105 wells, 105 different reactions can be tested at the same time in only 45 minutes.

6. CONCLUSION

In this thesis, two platforms for the detection of Genetically Modified Organisms (GMOs) and Escherichia coli (*E. coli*) bacteria are designed, manufactured and tested. Named as DaimonDNA and DaimonDNA-E, these biosensor platforms were fabricated using 3D printers and incorporate low-cost and simple electronic components. While the initial prototype, DaimonDNA, was designed to process four samples, DaimonDNA-E was extended to simultaneously handle 105 samples, proving a high throughput colony-LAMP platform. These devices are capable of providing great quality DNA amplification utilizing a LAMP assay and simultaneous HNB colorimetric amplicon identification.

DaimonDNA and DaimonDNA-E can be simply operated at 65 °C for 30 min and 40 min respectively. The devices can perform LAMP operation without the need for qualified labor and laboratory facilities. They provide low-cost, speed, and naked-eye readout and necessary sensitivity and specificity.

In the tests with GMO reference materials, it was able specifically detect fewer than 77 copies of the target P35S element. This was equivalent to a gts40-3-2 concentration of 0.1% (w/w) in a non- GMO background, which is the limit required for detection of unauthorized GMOs by EU regulations. These platforms can be used as a high throughput screening tool especially in low-income countries as a pre-clinical diagnostic tool.

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