Creating DNA from Scratch for DNA-Based Data Storage

Team 12

Client: Iowa State University

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Nathan Armstrong: Electrical Engineering → LCD/LED Hardware Development

Lucas Heimer: Electrical Engineering, Minor in Biomedical Engineering → Microfluidic System Development

Kyle Riggs: Software Engineering → GUI Design

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Revised: 12/2/2022 - Version 4

Executive Summary

Development Standards & Practices Used:

- Coding syntax for C# and C++
- Hardware development
- 3D modeling software (Solidworks and Fusion 360)
- Circuit element testing (power)

Engineering Standards Applied:

- IEEE 802.11 - Wireless Networking “WiFi”
- IEEE 260.1 - Standard Letter Symbols for Units of Measurement
- IEEE 830 - Software Requirements Specifications
- IEEE 1588 - Precision Time Protocol
- IEEE 802.6 - Standards for information exchange between systems
- IEEE 1074 - Software Development Life Cycle
- IEEE 1471 - Software Architecture / System Architecture

Summary of Requirements:

- Design an affordable and autonomous system for synthesizing DNA
- Accurately encode digital information into the DNA strand
- Design a user friendly interface to control the DNA microarray cycling.
- Use UV light to denature DNA and serve as catalyst for nucleotide to nucleotide bonding
- Use LCD screen to selectively control the location of UV light exposure on the microarray
- Design a microfluidic system to flow the nucleotides through the system sequentially

Applicable Courses from Iowa State University Curriculum:

- E E 201: Electric Circuits
- E E 230: Electronic Circuits and Systems
- ARTIS 308: Computer Modeling, Rendering and Virtual Photography
- E E/BME 341: BioMEMS and Nanotechnology
- BIO 212: Principles of Biology II
- CPRE 281: Digital Logic
- Com S 227: Object-Oriented Programming
- Com S 228: Introduction to Data Structures
- SE 319: Introduction to Design Structure

New Skills/Knowledge acquired that was not taught in courses:

- Understanding of how an LCD screen works
- New software used (Fluigent's OxyGEN software)
- Understanding of the current DNA synthesis technologies
- Components and design of microfluidic systems
- Spin coating and photolithography methods
- Windows Presentation Foundation User Interface design and Data Binding
- C# code design and interfacing backend/frontend logic within one code base

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Definitions

DNA synthesis: process of bonding individual nucleotides to create a desired DNA sequence

Flow cell: device used to serve as active region for bonding or chemical reactions in microfluidics

Microfluidics: system designed to flow small volumes of fluid through a given space

1 Team

1.1 Team Members

Connor Larson, Kyle Riggs, Brandon Stark, Lucas Heimer, & Nathan Armstrong

1.2 Required Skill Sets for Your Project

The required skills for this project include: programming, DNA sequencing, and hardware testing. We are required to program our own UI so we are able to precisely synthesize DNA autonomously. We also needed to gain a background knowledge of biology to aid in understanding the necessary processes for synthesizing DNA with the provided technology. Finally, we are required to apply a variety of practices in hardware testing so we are able to verify the sufficient amount of UV exposure is resulting in the expected bonding of nucleotides.

1.3 Skill Sets covered by the Team

- Knowledge of coding: Connor Larson & Kyle Riggs
- DNA sequencing and biology principles: Kyle Riggs & Lucas Heimer
- Hardware development and testing: Brandon Stark, Nathan Armstrong, & Lucas Heimer

1.4 Project Management Style Adopted by the team

Each member is assigned to a sub-team working on a specific component of the project. Week by week we are assigned a set of goals to accomplish. These goals are determined at the end of every meeting we have with our advisor, Professor Meng Lu. Project resources and progress are shared via slack and google drive.

1.5 Project Management Roles

- Connor Larson keeps track of the team organization as well as supporting the firmware side of the project in regards to controlling the microfluidic system and LED light source
- Kyle Riggs leads the GUI development for sending and receiving user input as well as client interaction for software issues.
- Brandon Stark leads client interaction in regards to the electrical engineering aspect of the project while working with the LCD and LED.
- Lucas Heimer leads the component design of the electrical engineering part of the project and develops the microfluidic system

- Nathan Armstrong is focused on the testing aspect of the project and develops unit tests to verify the functionality of the LCD and LED screen integration with the additional components in the overall system.

2 Introduction

2.1 Problem Statement

To implement an automated and affordable system to complete DNA synthesis for the purpose of digital data storage to keep up with the increasing demand for long-term data storage solutions.

2.2 Requirements & Constraints

Requirements:

- User interface to simultaneously control the LCD, UV light LED module, and microfluidic system
- User interface to set the dimensions of the bonding area to fit within the flow cell channel
- Design a microfluidic system to flow nucleotides through the flow cell channel on the LCD screen where it will undergo UV light exposure
- Integrate the microfluidic system and user interface to autonomously flow the necessary sequence of nucleotides and buffer solution to create the desired DNA strand
- Ensure the design is capable of synthesizing full strands of DNA in a timely manner

Constraints:

- Ensure the DNA is synthesized accurately to store and maintain the integrity of the digital information
- Build a bug free and operator-friendly user interface to control the system
- Dynamic microarray sizes in conjunction with preset DNA sequences
- Use affordable parts to make this design financially appealing
- The LCD screen is connected to the user interface via an HDMI connection
- Softwa
    - Integrating dynamic matrix code with printing code for specific gene

sequences.

        - For sequence printing, the matrix must be a certain size or there is an incorrect DNA strand printed. We used a 4x4 matrix for our use case.
- Hardware (LED/LCD)
    - Using a heatsink to cool down the LCD when needed.
        - When running the system, the LED is so powerful that it temporarily damages the LCD screen due to heat creating a black dot in the middle of it.

2.3 Engineering Standards

- IEEE 802.11 - Wireless Networking "WiFi": This standard applies to our project because we use wireless networking to communicate between our computer application, the software controller (raspberry pi), and the 3D printer itself.

- IEEE 260.1 - Standard Letter Symbols for Units of Measurement: This standard applies because we are working with a microfluidic system to make a DNA microarray. This means we will be working with very miniscule amounts of liquid pushed through holes that may just be a few millimeters wide.

- IEEE 830 - Software Requirements Specifications: We apply this standard because our project has certain parameters that we must abide by, so we have certain software requirements we must follow to develop our software to meet our specifications.

- IEEE 1588 - Precision Time Protocol: This standard is applied through our use of a 3D printer. The fluid must be applied to create the microarray at specific intervals to avoid mistakes and actually create an accurate end product.

- IEEE 802.6 - Standards for information exchange between systems: This standard is applied to our project because we will use a Raspberry PI to transfer data to a LCD and a nucleotide fluid dispenser.

- IEEE 1074 - Software Development Life Cycle: We apply this standard to our project because we will need to create a UI and a program to control the LCD and nucleotide fluid dispensation system. This code we create for this task will need to go through the Software Development Life Cycle.

- IEEE 1471 - Software Architecture / System Architecture : We apply this standard through the development of our application that the user will be interacting with as well as the system that communicates between the 3D printer and software controller. Good architecture will help immensely in the runtime of our application and how easy it will be to implement.

2.4 Intended Users and Uses

Users

- Geneticists
- Data Analysts
- Pathologists
- Biologists

Uses

- Synthetic DNA genes can be used for characterization of a genome
- Synthesized DNA is used as a form of digital data storage
- Synthetic DNA can be used for disease research
- The system can be used to simulate the naturally occurring process

2.5 Current Market Solutions

The current market for DNA synthesis systems for the purposes of digital data is relatively small as there are not many companies specialized for this product. However, several companies have proposed new systems for general DNA synthesis in the areas of genome sequencing and other medical applications. The use of UV light exposure is also a relatively new procedure for a DNA synthesis process known as phosphoramidite synthesis. This new method of synthesis replaces the older methods by using a more stable version of DNA molecules known as nucleosides. This has allowed the process to become more efficient and accurate for such a complex system that is prone to errors. This process is generally expensive, but one of the goals for our system is to use inexpensive components to work around this major constraint in an effort to make this process more accessible to a broader range of consumers desiring a solution for digital data storage.

3 Project Plan

3.1 Project Management/Tracking Procedures

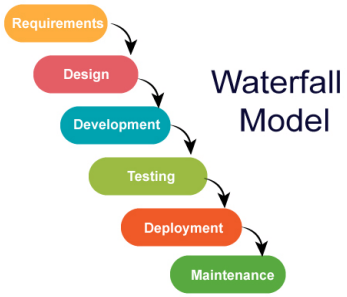
a. *Project Management*

The project management style we adopted for our design is waterfall. Due to the technical nature of our project we require a well thought out process and series of steps which allows for the best progression of our project. In order to fulfill the necessary requirements for DNA synthesis, we need to first have a method of controlling where and how the DNA oligomers bond on the microarray. Then we need to create a process which allows for the flow of DNA molecules into the system to complete the DNA synthesis. Since these steps are complex and dependent upon previous steps, we needed to create deadlines to be completed in sequential order.

b. *Tracking Procedures*

To keep track of our progress we utilized a few mediums. First, we communicate as a group with our advisor through Slack. As well, we use Google Drive to take weekly meeting notes. This helps us keep track of our weekly discussions in regards to tasks to be worked on for the given week. It also helps us store knowledge we gather from our advisor. We have made use of Git throughout the progression of the development of programming our user interface.

Figure 1: Summary of Waterfall Management Model



3.2 Task Decomposition

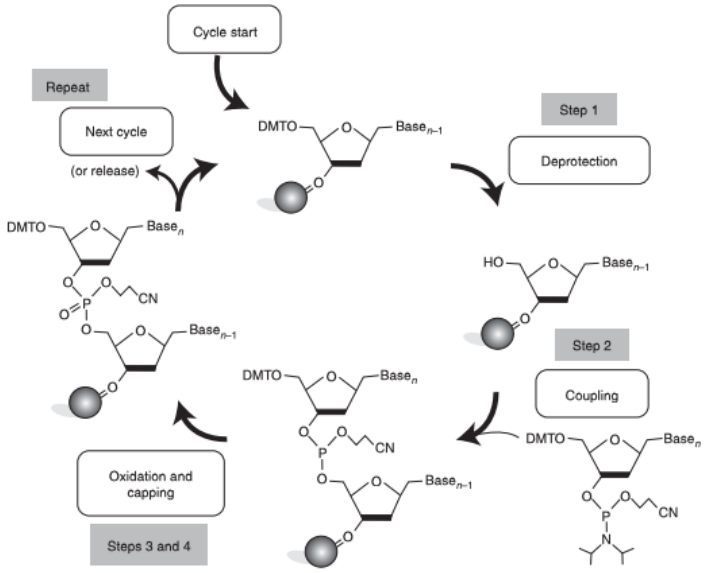
Tasks:

- Controlling the LCD/LED
- Developing the microfluidic system
- Coding a GUI
- Integration of all components into single process

Subtasks:

- Research biology principles of DNA synthesis
- Development of components to withstand necessary pressure requirements of microfluidic system
- Programming DNA sequence to be calculated into microarrays to be displayed on the LCD
- Design program to control microfluidic system
- Develop code to control LED through an Arduino and also be compatible with the rest of the software
- Communicate with LCD screen via created code

Figure 2: Summary of DNA Synthesis Process



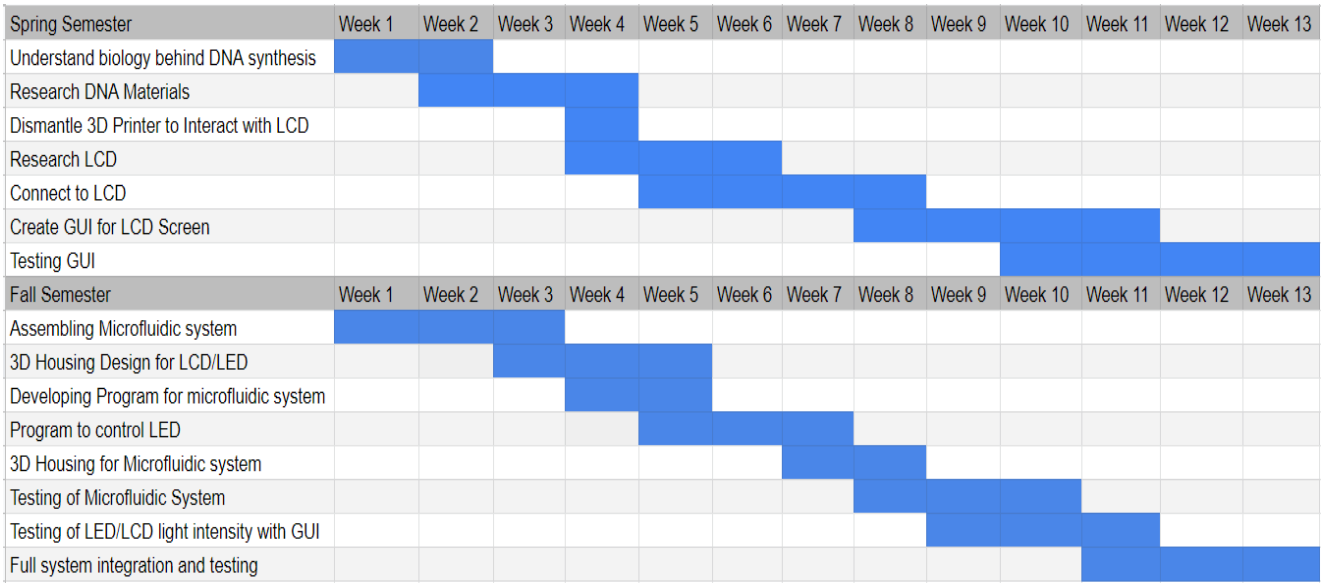
3.3 Project Milestones, Metrics, and Evaluation Criteria

Table 1: Description of Project Milestones

Milestone	**Measure Progress**
Connect to the LCD	Proper, stable connection and ability to display/mirror our display to the desired LCD.
Develop Microfluidic System	Test solutions are able to flow through the entire system at a constant pressure without blockages or other hindering factors
Coding a microarray	DNA is accurately bonded to microarray based on input from GUI
Coding a GUI	Proper coding connection to display and ability to project image to desired LCD.
Communicate with LED	When the GUI application is run, the state of the LED is controlled based on the timing set in the created code
Control of the microfluidic system	The correct sequence of DNA nucleotides and buffer solution are passed through the flow cell based on created code
Create Full System Housing	Use of Fusion 360 to 3D model and print a housing structure for the full system
Software displays DNA sequence split into appropriate microarrays	Software calculates and divides set DNA sequences and correctly displays them with appropriate timing to the LCD screen.

3.4 Project Timeline/Schedule

Figure 3: Project Schedule



3.5 Risks And Risk Management/Mitigation

Table 2: Risks and Mitigations

Task:	**Risk:**	**Risk Factor (1-10): 1 = Low, 10 = High**	**Resolution or Explanation:**
Displaying to the LCD	LCD does not display what is intended	5	Replace LCD, or use a different way to connect to the LCD
Developing microfluidic system	Trying to understand the biology behind this task and implement it	7	Further understand task and get access to required
Coding GUI	Code does not function as intended	5	Debugging and implementing individual use case tests.
Communicating with LCD screen	GUI and LCD screen are not compatible and do not	8	Change how we communicate with the LCD or use a

	communicate		different process behind the GUI
LED Backlight for LCD	LCD gets too hot from LED source and damages the screen if exposed to for a long time	9	Heatsink needed to cool down LCD or limited exposure time from LED

3.6 Personnel Effort Requirements

Table 3: Allocated Project Hours

Task	Hours
Biology/DNA Research	20
Connect/Research LCD/LED	30
Creating Microarray	25
Coding GUI	30
Communication between LCD/LED and Created Code	20
Developing Microfluidic System	80
Fusion 360 Modeled Housing	30
TOTAL	235

3.7 Other Resource Requirements

- Fabrication of flow cell channel
- DNA oligomers to be used in microfluidic system
- 3D printer for development of system housings
- Use of available lab equipment to prepare photoresist samples
- Use of ETG for manufacturing of various components

4 Design

4.1 Design Context

4.1.1 Broader Context

Table 4: Ethical Design Considerations

Area	**Description**	**How our Project Relates**
Public health, safety, and welfare	How does your project affect the general well-being of various stakeholder groups? These groups may be direct users or may be indirectly affected (e.g., solution is implemented in their communities)	This method of DNA synthesis uses UV light to break down and bond the DNA. If not regulated the UV light could be too intense and prolonged exposure could lead to potential harm of users such as geneticists or other scientists
Global, cultural, and social	How well does your project reflect the values, practices, and aims of the cultural groups it affects? Groups may include but are not limited to specific communities, nations, professions, workplaces, and ethnic cultures.	Development of DNA synthesis technology assists with researchers who are attempting to keep up with the increasing data storage demand year to year. DNA can be a viable solution for containing copious amount of data in a single structure
Environmental	What environmental impact might your project have? This can include indirect effects, such as deforestation or unsustainable practices related to materials manufacture or procurement.	Data centers are currently used to hold the digital information for companies such as Amazon or Facebook. DNA storage capabilities could reduce the required space and therefore the required energy to store data, from the size of a data center to the size of a small room.
Economic	What economic impact might your project have? This can include the financial viability of your product within your team or company, cost to consumers, or broader economic effects on communities, markets, nations, and other groups.	This product reduces the cost of current DNA synthesis by using simpler components. This would allow bioinformatics personnel the opportunity to budget more resources towards storing data in DNA. This process could also eventually be used by geneticists for patients who require some form of gene therapy.

4.1.2 User Needs

- Geneticists need a cheaper way to synthesize DNA that will end up saving them money and time in the end.
- Data analysts need a long term alternative for digital information to keep up with the increasing demand of a digital world
- Pathologists need a method of studying disease causing genes without needing to extract these molecules from a human sample

4.1.3 Prior Work/Solutions

There are no DNA 3D printers on the marketplace currently. Although, there are a few companies that can perform DNA synthesis. The first of these companies are San Diego-based Molecular Assemblies, Ansa Biotechnologies, and Paris-based DNAScript. Currently a single character in a genetic sequence costs roughly $1 to print, or $1 per base pair. In perspective, if you wanted to print the entire sequence of a human, it would cost around $2 billion.

4.1.4 Technical Complexity

1. The following lists outlines the major components used in each of the main subsystems of the design
    a. LED Light Module
    b. LCD Screen
    c. Microfluidic System
        i. Flow cell
        ii. Fluigent Flow EZ Pressure Controller
        iii. Fluigent M-Switch Valve
        iv. Air filter
        v. Safety Valve
        vi. DNA oligomer samples
        vii. Air compressor
    d. Software/GUI
        i. WPF
        ii. C#
        iii. XAML
        iv. C++
        v. Arduino Nano
        vi. Fluigent’s OXYgen software library
2. The DNA synthesis process requires the use of several scientific principles which can be realized using the above components.
    a. UV light polarization via control of liquid crystal display
    b. DNA oligomer denaturing via UV light

    c. DNA phosphoramidite synthesis
3. The problem scope contains multiple challenging requirements that match or exceed the current solutions or industry standards.
    a. More affordable alternative to the current industry processes
    b. Accuracy of DNA sequencing to reflect the current industry standard
        i. Minimal substitutions, omissions, or additions of DNA base pairs during sequencing
        ii. Proper dispersion of UV light exposure to all regions of the flow cell
    c. User friendly interface to allow for ease of use
        i. Variability in DNA strand array size
        ii. Easy input of desired sequence

4.2 Design Exploration

4.2.1 Design Decisions

1. Connection between the LCD and the computer will be made directly via a HDMI cable
2. The UV LED light trigger will be controlled by the main computer via an Arduino Nano
3. The variables users will have control over within the software will consists of array size, DNA strand, and cell spacing

4.2.2 Ideation

Several considerations were taken into account when determining the best course of action for the connection and control of the LCD screen and LED. The ideas were generated through brainstorming sessions during team meetings and new ideas continued to be generated as we gained more background information from our advisor. The following options were considered for this decision in the design:

1. Connection to the LED via USB port
2. Connection to the LED via Arduino Nano
3. Connection between the LCD and computer via an HDMI to MIPI adapter
4. Various alternative LCD screens with built in HDMI connections

4.2.3 Decision-Making and Trade-Off

For each of the options we went through and researched the current products available on the market to determine its viability. We also took into account financial considerations since one of the major goals of our project is to create an affordable design. The different variations of LCD screens were more expensive than our current screen so we wanted to find a method to make it work without the need for a new part. Research was done on the wifi connection and USB connections to the 3D printer and it became evident that extensive reconfiguration of the microcontroller would be required to control the LCD and allow for an image file to be passed through the printer to the LCD. This option was mainly in consideration because it did not require us to dismantle the printer. The

Raspberry Pi and MIPI adapter options were appealing because they would be simple to implement. The main issue was that they required introducing an additional part to our system which could be bypassed by using an LCD with a direct HDMI connection. This option allows for the LCD screen to essentially act as a second monitor to our computer and we can project any image we want to the screen for the purposes of DNA synthesis. We are no longer using the 3D printer parts, but instead using a UV LED light and a LCD screen without a diffuser.

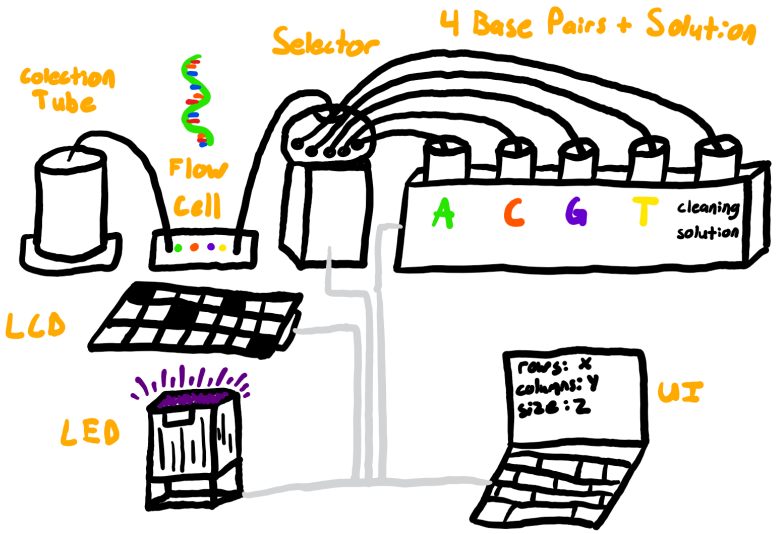
4.3 Proposed Design

Development of a DNA synthesis process that will satisfy a new medium of digital storage to keep up with increasing demand at an affordable price. Our design will consist of three major sub-components: LCD screen and UV light control, a user interface, and a microfluidic system.

4.3.1 Design Visual and Description

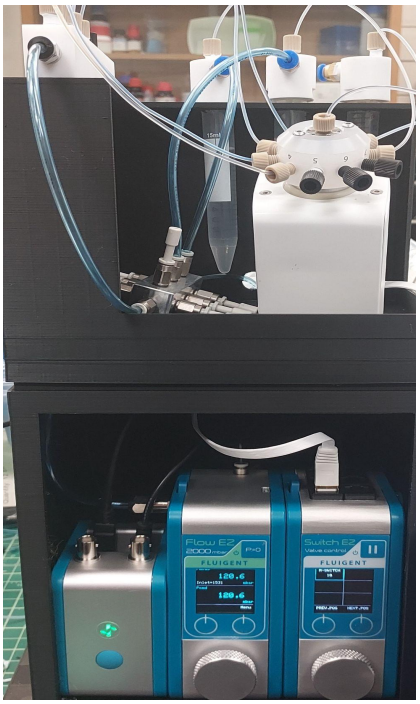
The diagram below depicts the interaction between the whole system. The array parameters and digital information are uploaded into the designed software. The digital information will be divided up into two-bit segments, each of which will be correlated to any given DNA base pair based on its value. The software will then break the string of data down into relatively equal segments to fill each of the positions on the array. The segments will be parsed layer by layer in each of the array locations and an image will be generated to be passed to the LCD screen. The images will configure the LCD screen in a way which selectively lets the UV light through and into the flow cell where it will initiate the bonding between the DNA base pair and the growing DNA segment.

Figure 4: Integration of Entire System



The microfluidic system is further detailed in the image below. There will be a total of four nucleotide base pairs and one buffer solution that will be used in each cycle to create a single layer. The rotating M-Switch in the middle of the image switches at each step and opens up the channel between the reagents and the flow cells. The entire system is operated using a vacuum and compressed air to push the reagents through the system.

Figure 5: Image of Microfluidic System



Example of the User Interface. Each cell in the matrix will eventually be separated (surrounded by black to match the background) and also be able to switch colors to completely blend in with the background. Also, as mentioned above, we currently receive user input to allow the entire matrix to be customizable to the DNA standards that they want. Namely different DNA sequences.

Figure 6: Example of User Interface Dimension Selection

4.3.2 Functionality

The DNA synthesis is initiated by a user dictating the DNA sequence. The digital information which is to be encoded by the DNA is translated by assigning a two-bit value to each of the four base pairs. Each time a different base pair is introduced to the flow cell a new black and white image will be displayed on the LCD screen. The white locations allow UV light to pass through and thus allow bonding to occur between the current base pair and the DNA strand being synthesized in that position of the array. This process is repeated for each base pair at any given level until the desired strand length is reached. Once completed, the individual strands can be bonded together to create one coherent strand containing all of the digital information.

4.3.3 Areas of Concern and Development

One of the major areas of concern is the accuracy of the DNA synthesis process. Bonding errors, such as omissions, additions, or substitutions, are potential issues to arise if the surface of the flow cell is not properly cleansed between each base pair being introduced or insufficient light allowing for the activation of the bonding reaction. This issue was addressed by determining the best compounds to use as intermediate fluids to cleanse the surface of each DNA base pair. Extensive testing was also completed to determine the accuracy of the process and where errors are occurring. Another major area of concern is having dynamic array sizes in conjunction with set sequences. Dynamic matrices were implemented, however, getting this code functionality to work with specific sequences is a large issue.

4.4 Technology Considerations

Table 5: Evaluation of Technology Utilized

Technology	Strengths	Weaknesses	Trade-offs
LCD Screen	Directly connects via HDMI port Significant difference in UV intensity between white and black regions Easy control of produced image	Lifespan of RGB screen Overheating due to proximity of UV light	Shorter lifespan compared to monochromatic, but improved UV intensity difference The overheating was initially an issue, but implementation of a cooling system made the necessary correction
LED	Very easy to control and manipulate	Produces a lot of heat	UV light intensity is strong enough for intended project use
Software	C# capable of encompassing all requirements for project Fluigent's OxyGEN built in commands for microfluidics OxyGEN easily integrated with C#	Requires application to run and be connected through a computer	Program being run through the computer makes the design simpler and cheaper but makes setup more complex.
Microfluidics	All components are compatible Simple assembly Autonomous capabilities Components are easily replicable	Expensive components Self manufactured components with sealing concerns	Saved money by manufacturing some of our own components, but found issues with sealing to ensure no pressure leaks

Design Alternative Suggestions:

The potential for alternative solutions from those outlined above could relieve some of the concerns and weaknesses described. A different model of adapter could be designed to be compatible with a monochromatic LCD screen which would alleviate the lifespan concerns associated with an RGB LCD screen. In regards to the microfluidic components, manufacturing our own versions of the normally expensive components has saved us money, but resulted in insufficient sealing when placed under pressure. In order to resolve this issue we have revised our design from using an epoxy sealant to designing the components with threaded ferrule fittings.

4.5 Design Analysis

The current state of the design is a fully integrated system which utilizes the three major subsystems that have been outlined throughout this report. There was individual verification of functionality of each component prior to the addition of each subsequent system in an effort to effectively troubleshoot any issues that arose. The system is fully capable of loading in a given DNA sequence, dividing it, and evenly distributing it into the programmed defined matrix size. Once the user initiates the synthesis the design will systematically flow individual solutions across the flow channel and the LCD will display a pattern to selectively allow UV light to pass through the positions of the matrix which require bonding of the given solution at that time. After a sufficient amount of UV exposure a buffer solution will be passed through to clean out the channel before the next nucleotide solution is transported into the channel. This sequence will continue until the entire DNA sequence has been completed.

4.6 Design Plan

The current design has been broken down into three major sub-components. Those being the LCD screen and UV light control, the user interface, and the microfluidic system. Each member of the group has been designated a role in designing at least one of these components. In the end, the LCD screen, UV light control, and microfluidic system will fit all on one housing space controlled by the UI via USB and HDMI.

LCD Screen/UV Light Control:

The different iterations of LCD screen control methods we have tried have been outlined in the above sections. The final product to be used was a LCD screen with no diffusion layer, no backlighting, and a built-in HDMI port adapter. Testing was completed to verify this screen could create a large enough differential in UV intensity between white and black spots on the defined matrix. This was an important factor in determining the time of exposure for the DNA base pairs when they pass through the system. The UV light is the catalyst for the bonding reaction so understanding the intensity of light available is critical to ensuring the DNA strands are denatured to the point where bonding can occur. A housing was designed to effectively focus the UV light towards the flow cell without any unnecessary dissipation. Due to the proximity of the LCD screen to the UV light, a heat dissipation solution was required to prevent overheating and long-term damage to it. Mineral oil compressed between the LCD screen and an additional glass piece served as an effective solution to allow the LCD screen to withstand the heat exposure during the required time of denaturing while the DNA was in the flow cell channel. The UV light is controlled by an Arduino Nano sending a trigger voltage whenever the DNA cycle is started and completed.

User Interface:

For the software application that users will visually interact with we decided to go with a WPF framework application that uses C# for the backend functionality and XAML for the GUI. The GUI ideally allows users to define matrix size, matrix position spacing, and the DNA sequence to be loaded into the system. While the program is running, there is beginning implementation to simultaneously control the microfluidic system, UV light source, and display corresponding images on the LCD screen. The Fluigent library for its OXYgen software was used to call functions for the individual control of the different components of the microfluidic system. The state of the UV light source is controlled by a digital output port on an Arduino Nano. The Arduino has a simple code script uploaded to it that will trigger the output to change states based on a serial communication from the computer program. All of these functions are running on the same clock which allows the program to initiate the following sequence of events. Once the user defined sequence is set and the process is started, the first matrix pattern will be displayed on the LCD. The correct position on the valve switch will be selected, then the pressure will be set. After a delay to allow the solution to reach the flow cell channel, the pressure will be reset to zero and the UV light will be turned on. Another delay allows the necessary time for UV exposure to initiate nucleotide bonding. The valve position is changed to the buffer solution and the pressure is set once again to flush the channel. The same process then repeats continuously and sequentially for each base pair until the entire DNA sequence is completed.

Microfluidic System:

The microfluidic system is completely composed of parts from Fluigent. An air compressor, safety valve, and air filter prepare the pressurized air flow to be input into the microfluidic system. A Flow EZ Pressure controller communicates with the software to pressurize the containers for the different solutions used. The M-Switch valve controls which solution is being transported to the flow cell channel and eventually into the waste container if it is not exposed to UV light. Due to the number of components involved in this system, a housing was created to condense them all into a single structure. This was an important aspect to the design in order to make it easier to transport to different locations. Another important aspect of the microfluidic system was the development of test tube fittings that could withstand the pressurized system. There were some initial issues with leakages that caused the level of input pressure to be limited and in turn the system to be slow. By designing caps to the test tubes housing the various solutions we were able to create a durable seal that could withstand much higher pressures.

4.7 Design Plan Evolution

The design plan evolved a bit over the course of the second iteration of development, but not too drastically. For the user interface, design components were changed to make it easier for the user to understand and operate. Also, although dynamic matrix sizes were implemented for the sake of having the user control how big they want their arrays, this led to issues when printing preset sequences as well as fitting into the flow cell. Widely different sizes will affect bonding within the limited space of the flow cell. Furthermore, specific DNA sequences would begin to be printed inaccurately if matrix size changed. So, we left the option to change matrix sizes as something to be worked on in the future, but for sake of testing/printing the DNA sequence was calculated and divided into a four by four microarray. As for the LCD/LED system, we went from using the 3D printer as our UV light source to modifying our own LED to produce the UV light required. This LED with the UV light attached can be easily modified to what is desired by the user. We also tested multiple LCD screens. Our current screen is much larger than the original one that came with the 3D printer. It does not have a diffuser which is necessary in order to bond the DNA base pairs. Without the diffuser, we obtain the required minimal power measured through the LCD screen.

5 Testing

5.1 Unit Testing

- UV Light Intensity - This was tested using an available power meter in order to determine the difference between white matrix spots and dark matrix spots. It is critical to the design that we were able to have a significant difference to attempt to prevent any unnecessary bonding during UV light exposure on the DNA nucleotides, as well as the light density being strong enough to denature the DNA.

- LCD Screen Resolution - Verification of the resolution of the LCD screen with the user interface was important to ensure the matrix cell sizes were displaying proportionally as intended. Any distortions could have caused a potential merging of cells on the screen or misplacement of matrix cells displaying below the flow cell channel.

- Systems Software - All of the different software components within the system had to work in tandem. To make sure this works, analysis of each system was completed. Errors occurred throughout the development process for controlling the user interface, UV light control, and microfluidic system. Debugging was completed periodically to resolve these issues and ensure the internal functions were producing the expected outputs.

5.2 Interface Testing

We have a user interface that formulates microarrays to a specific DNA sequence. The interface will request and accept user input regarding matrix size, cell spacing, and DNA sequences to be created. The testing of the user interface was completed by updating different aspects of the design and through WPF being able to see changes in real time. Orientation and dimensions of the microarray were the major features observed on the LCD screen.

5.3 Integration Testing

The integration of the subcomponents of the system occurred one at a time to ensure functionality and provide a simple way of performing regression testing after each added piece. Integration of the user interface with control of the LCD screen was critical for the design because the user interface controls the LCD screen to selectively designate where the UV light passes through to catalyze the bonding reaction between DNA base pairs. A variety of input matrix combinations were programmed to be sent to the LCD screen and dynamically changed after a set time period.The matrix patterns on the user interface were compared to the final display on the LCD to check for any orientation discrepancies and size distortions.

Integration of the microfluidic system with user interface was completed by implementing the pre-defined functions created by Fluigent to control the pressure and valve positions. It was important to note that the response time for the pressure controller to reach the specified value from the user interface was only a matter of seconds so as to not greatly affect the timing of other parts of the full system.

Integration of the UV light source and the user interface was completed by developing a program that could trigger a port on an Arduino Nano through serial communication. The trigger would be able to turn the LED on and off as desired.

Once all three of the main hardware components were functioning properly with the control of the user interface we were able to integrate all of the code into a single file for the final system testing.

5.4 System Testing

System testing can be seen as complete end to end testing for a specific system. The two main systems that require testing are the user interface and the microfluidic system.

User interface:

The user interface required testing on multiple aspects. We created unit tests that inject user inputs to test and see if we get the desired output. When creating these tests and inputs we ensured to encompass corner cases. We also needed to perform some automation tests to assure the image was properly rendered.

Microfluidic system:

The major aspects to be tested for the microfluidic system individually were the pressure limits and the flow rate of the different solutions. The pressure limitation was mostly affected by the flow cell because the sealant used to connect it to the tubing was the most susceptible to breaking down under pressure. Gradual increases in pressure were tested until the sealant produced a leakage. Once a suitable pressure was determined, we were able to proceed with measuring the flow rate of the different solutions to be used. The pressure was fixed and the time to reach the flow cell channel from the reservoir was measured in several trials.

LCD/LED Assembly:

The testing of the LCD's capability to selectively allow UV exposure in designated regions of the matrix was visually represented through photolithography. Since it is nearly impossible to get real time feedback during the synthesis of DNA, we determined using photolithography to observe UV exposure through the matrix would accurately represent how the system will function when it begins the DNA synthesis process. We prepared some slides coated with photoresist and displayed several different patterns on the matrix. By varying the time of exposure we were able to observe the effects of varying times of exposure to get an estimated time requirement for the DNA nucleotides to receive the necessary dosage for bonding.

5.5 Regression Testing

Any new additions we had, such as a new LCD screen, we immediately tested with our system to make sure everything functions. We also only attempted to integrate one additional component at a time for ease of troubleshooting. This testing was required since each step builds off one another, so if for example the screen did not work then the rest of the project would not produce the correct output. Testing the UV light with the power meter over the LCD screen also gave us crucial data regarding the cellular bonding of DNA.

In regards to the user interface code, we continuously built branches with multiple commits. This ensured we did not lose any critical progress as well as ensured we were able to revert back to a working version whenever required.

5.6 Acceptance Testing

- Demonstrate physically that the user interface works as intended in our weekly meetings with the client.
- Successfully and accurately design a system to synthesize DNA with correct coding
- Timing of the system allows for sufficient UV exposure to promote nucleotide bonding

5.7 Results

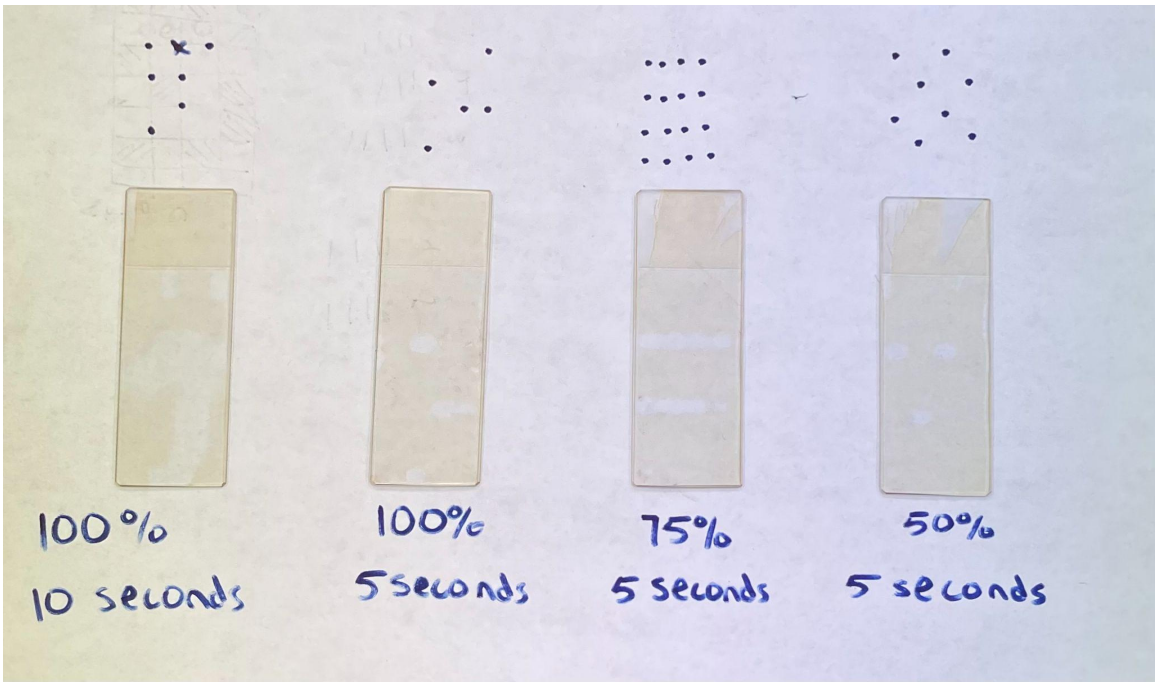
- Our testing followed in accordance to the acceptance testing in that our results, when compiled together show proof of the design being capable of synthesizing DNA.
- For the user interface, the results of our testing checked the output that was presented to us was the one that we were expecting based on the input of the user interface. Since the nature of our project allows us to know what the output should look like before entering any input, we can simply see how the program reacts to what we enter into it.
- The testing of the microfluidic system pressure and flow rate are as follows:

Table 6: Microfluidic testing results

Parameter	Tested Value
Burst Pressure	150 mbar
Flow Rate (At 120 mbar)	33.3 µl/s
Time from reservoir to flow cell channel	20 s

- The results of the photolithography tests with the LCD and UV light source are as follows:

Figure 7: Photolithography test with glass slides, different Voltages and exposure times, and the matrix design shown above in black dots



6 Closing Material

6.1 Discussion

The final design of our project is a fully functional system with the ability to simultaneously control the individual subsystems through a single program script. The program allows the user to input a matrix array size, cell spacing, and a desired DNA sequence to be synthesized. Once the user initiates the start of the program it will sequentially flow the four base pairs and intermediate buffer solution. The LED and LCD will be the factors that control the synthesis of DNA in specific locations within the flow cell channel. Preliminary testing has shown the overall system functionality prior to tests with actual DNA samples.

Figure 8: Microfluidic System

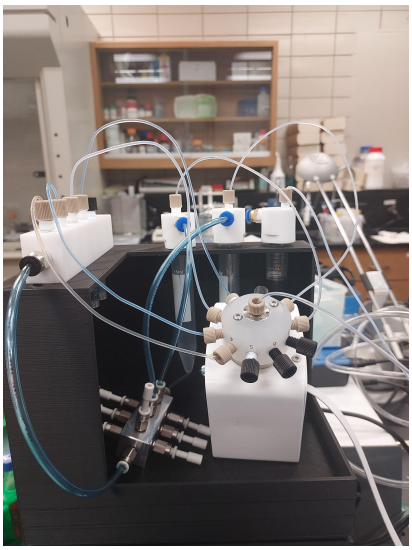
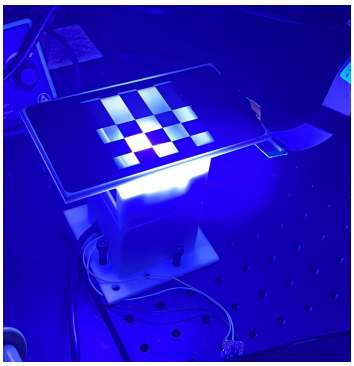


Figure 9: Displayed Image on LCD Screen



6.2 Conclusion

In conclusion, the goal for our project was to create a system which can autonomously synthesize DNA for the purpose of digital data storage. The increasing conversion of information to a digital medium is causing an increased demand for storage space. DNA serves as a stable, durable, and compact medium for digital data storage. Our system has been developed through the use of an LCD screen, UV light module, user interface, and microfluidic system. The choice of LCD screen allowed us to use an HDMI cable to transmit images from our user interface directly to the screen. Based on the white and black regions of the screen, UV light is able to selectively pass through the screen and is the catalyst for nucleotide bonding. The microfluidic system has been designed using parts from Fluigent and a simple flow cell created with the assistance of ETG.

Several iterations to the design were required in order to reach this stage of the project. Multiple different types of LCD screens and methods of connections were tested to determine the best method for displaying the matrix array while still maintaining an acceptable amount of UV exposure to pass through the screen. The microfluidic system required the development of new components which could withstand the required pressure to allow the system to be fast and efficient in synthesizing long strands of DNA. Throughout the entire design process, the software faced several delays due to major bugs and errors which required extensive troubleshooting to correct. Integrating multiple different libraries and versions of code needed meticulous review to ensure the integrity of the system was maintained and capable of being automated. Despite the delays in each of the subsystems, the design was able to be completely integrated and automated.

This system is able to take an input of digital information and translate it into a DNA sequence by correlating two bit values to a specific base pair. The DNA sequence is then divided into segments which can be used to produce images representative of the structure of each segment at any given layer. The user interface controls the microfluidic system by activating the pressure controller and M-switch to flow each of the base pairs sequentially through the flow cell. DNA synthesis is regulated by exposure of UV light produced by the LED. The process continuously runs until it reaches the end of the strand. At this point, a new process is required to detach the individual segments and then fuse them back together. The completed strand can then be stored for future reference whenever the digital information is needed.

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6.3 References

[1] Bleugh, “Anycubic Photon Mono Teardown 3,” Bleugh.Biz Blog Thingy, 16-Jan-2021. [Online]. Available: https://bleughbleugh.wordpress.com/2020/10/18/anycubic-photon-mono-teardown-3/amp/. [Accessed: 24-Apr-2022].

[2] “Shaping the future,” Fusion 360 Tutorials. [Online]. Available: https://warwick.ac.uk/fac/sci/wmg/about/outreach/resources/fusion_tutorials/. [Accessed: 24-Apr-2022].

7 Appendices

7.1 Appendix I - Operation manual

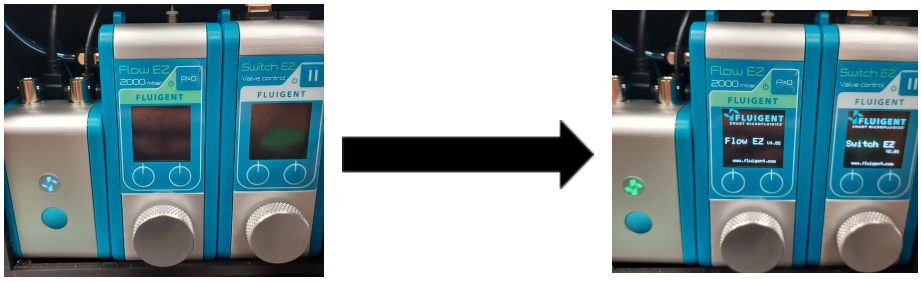
Setup:
The following steps are required to prepare the system prior to initiation of the process.

1. Fill the supply reservoirs with a sufficient volume of DNA nucleotide solutions and buffer solutions.
    a. If necessary, empty the waste collection reservoir
2. Place each of the reservoirs in the respective locations in the microfluidic system based on Figure 10 below. Note: all of the test tubes and microfluidic tubing fittings need to be firmly tightened into their respective threaded slots to prevent any leakages.
    a. If leakages occur, perform individual testing of each of the connections by plugging all other connection ports.

Figure 10: Outline of Reservoir Locations

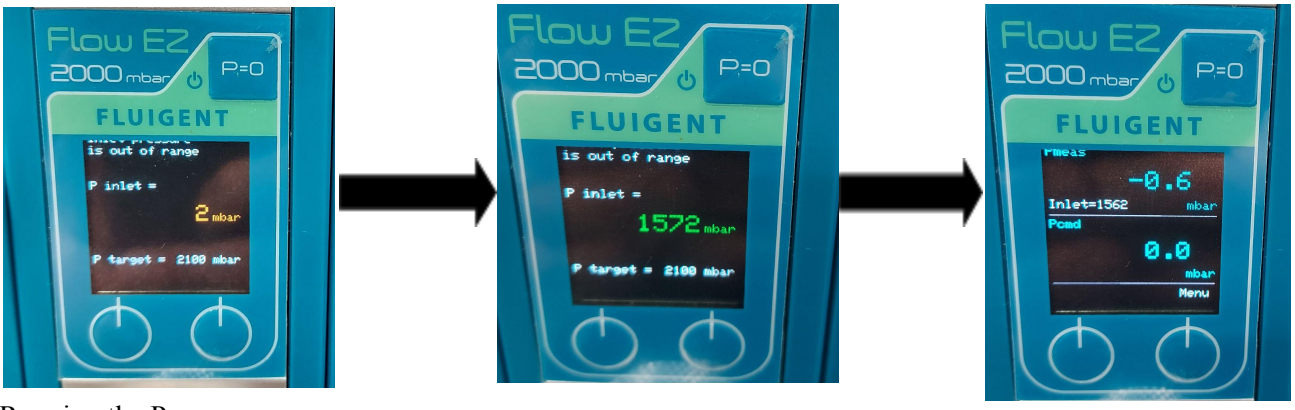
3. Connect the M-Switch to the Switch EZ controller
4. Connect Fluigent software controller to your computer via USB
5. Connect the Arduino Nano to your computer via USB
    a. The current software requires the use of the COM3 port to initiate serial communication with the Arduino. If COM3 is unavailable, the source code can be reconfigured to select a different port
6. Power on the Fluigent software controller.
    a. Once plugged in, the light should initially flash blue. Press it once and it will turn all three components in the lineup on as shown in Figure 11.

Figure 11: Fluigent System Startup Sequence



7. Power on the air compressor
8. Set the pressure to 2.00 Bar on the air compressor
    a. As the pressure begins to increase in the system, monitor the Flow EZ pressure controller. Verify that it reaches the minimum threshold input pressure and the screen transitions as shown in Figure 12.

Figure 12: Flow EZ Pressure Controller Target Pressure Transition



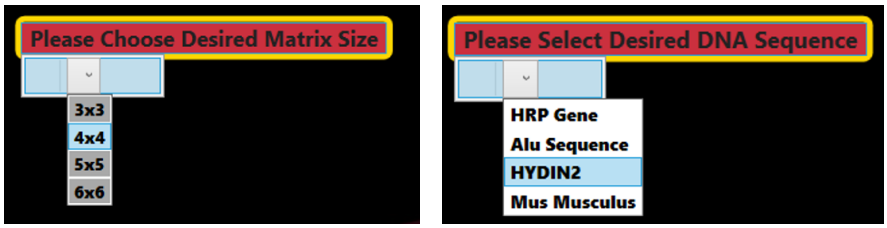
Running the Program:

1. Upon startup of the program, the user interface will present the image in Figure 13.

Figure 13: User Interface Home Screen

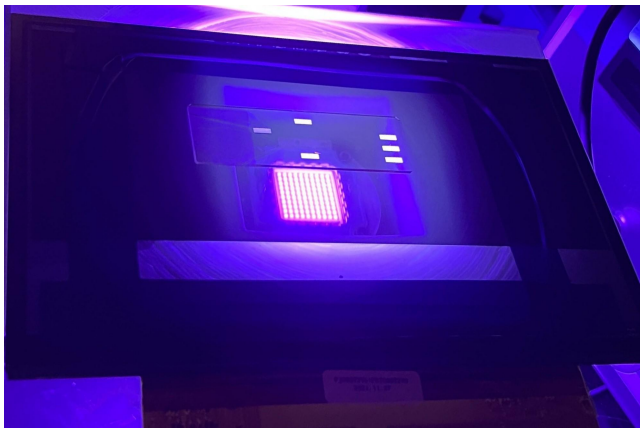
2. Select the desired inputs for matrix size and DNA sequence as shown in Figure 14

Figure 14: User Interface Custom Selection Options



3. After the necessary fields are completed, hit the start button
4. The system should then place an image on the LCD screen and begin pumping the first nucleotide solution to the flow cell channel. The process will continue to autonomously iterate through the entire loaded DNA sequence until complete. Figure 15 shows the matrix displayed on the LCD while the UV light is on.

Figure 15: Matrix Displayed on LCD



Shut Down:

1. After the process is complete, ensure the pressure on the Flow EZ controller is set back to 0 mbar and the UV LED light is off.
2. Switch off the air compressor
3. Press the button on the Fluigent software controller to shut down the lineup

7.2 Appendix II - Alternative/Other Initial Versions of Design

LCD: We have gone through roughly two versions of the LCD screen.

1) 6x3.5 inch LCD with a diffusion layer
2) 8.5x6 inch LCD without a diffusion layer

LED: Initially we used a UV light that was built into a 3D printer. The base “Stratus LED” has been the same, the only thing we modified was replacing the default LED with a UV light.