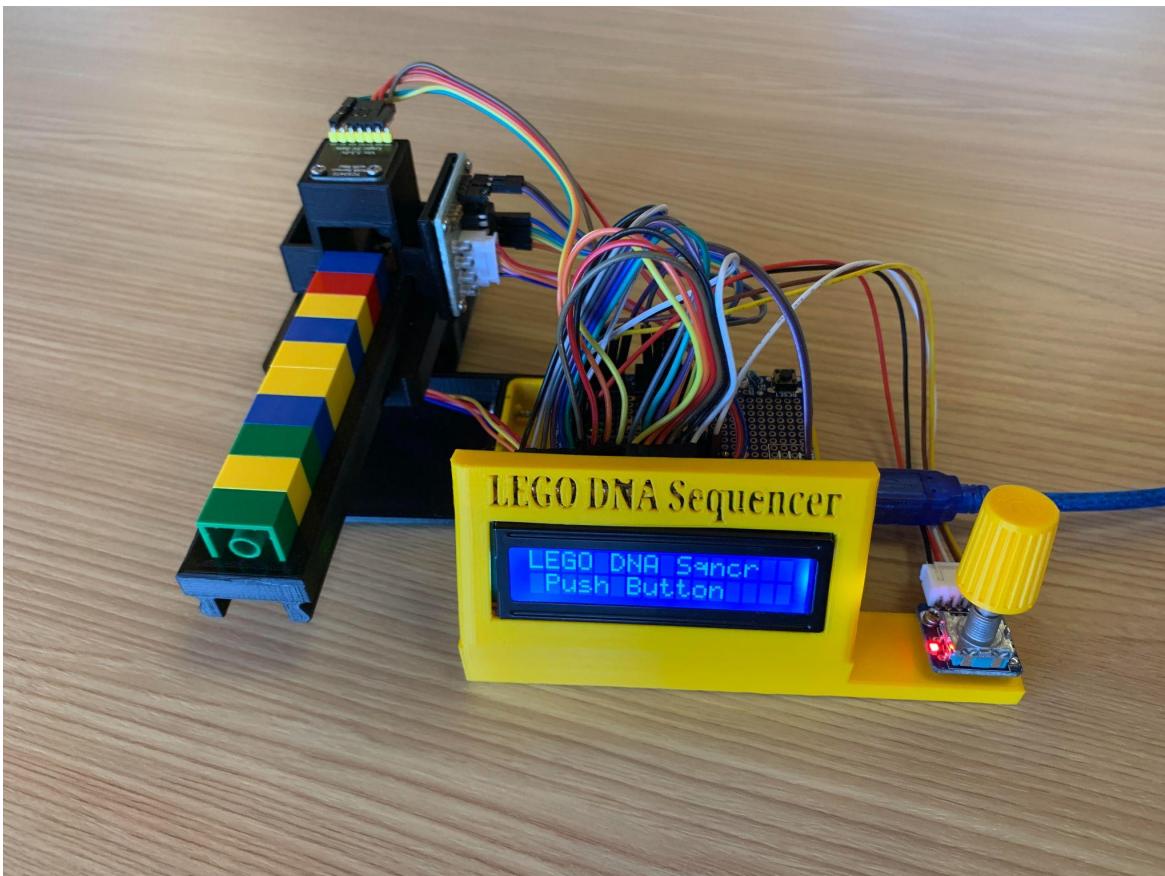


# LEGO DNA Sequencer

## User Manual

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The purpose of this LEGO DNA Sequencer User Manual is to provide instructions for the operation of this easy to use colorful tool for explaining the complex DNA sequencing operation



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# Overview

The purpose of this document is to provide a complete Users Manual that describes the operation of the LEGO DNA Sequencer. A companion document, LEGO DNA Sequencer and DIY Construction, includes additional information about the operation of the system including troubleshooting.

DNA sequencing determines the order of the four chemical building blocks - called "bases" - that make up the DNA molecule. The building blocks are composed of A (adenine), C (cytosine), G (guanine), and T (thymine). These building blocks are represented by LEGO® 2x2 bricks of **Red for G**, **Blue for T**, **Green for A**, and **Yellow for C**. Actual DNA sequencing is greatly simplified in this model by using genomes of only 10 building blocks to identify a species.

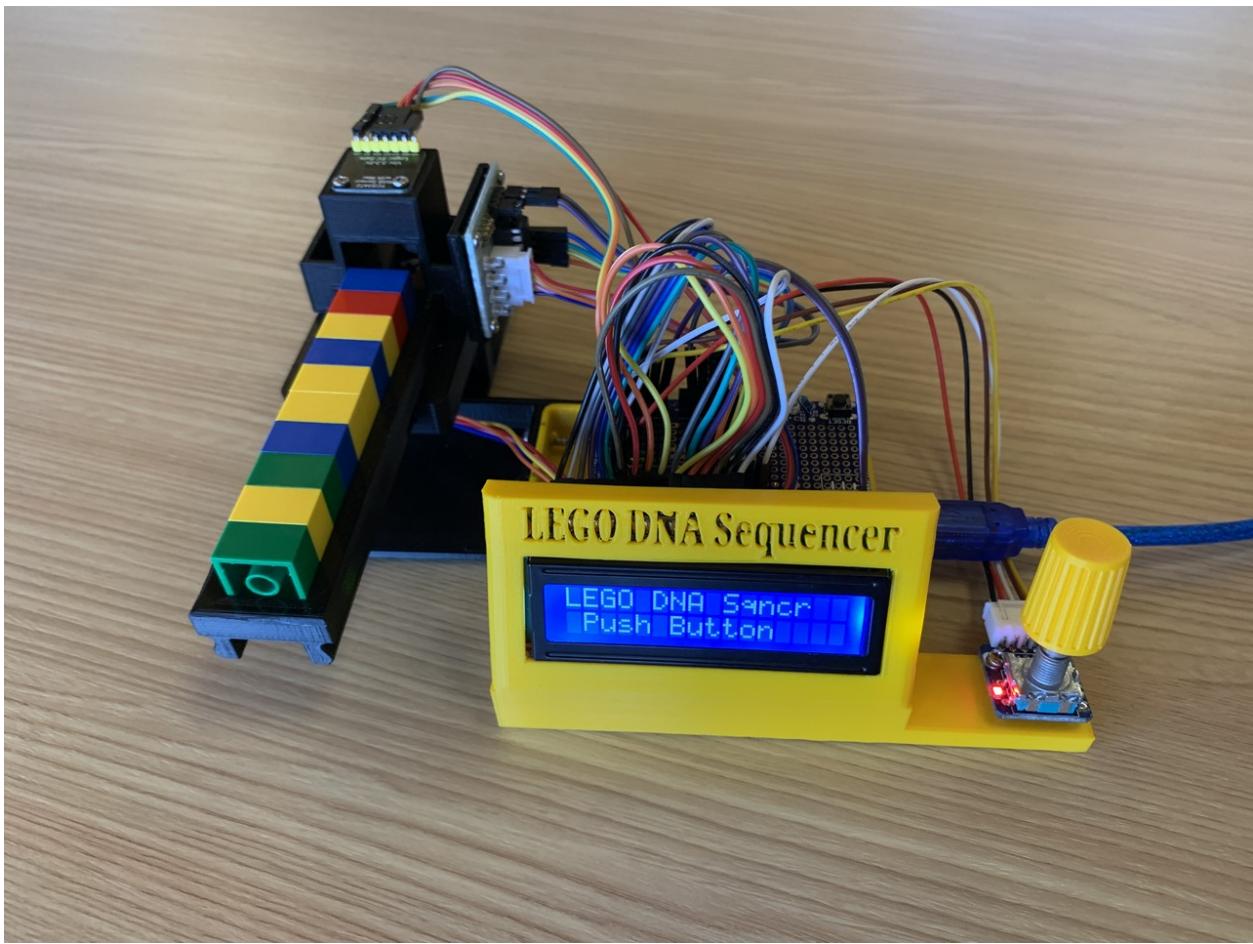
It is beyond the scope of this document to provide more details regarding actual DNA sequencing.

# Users Manual

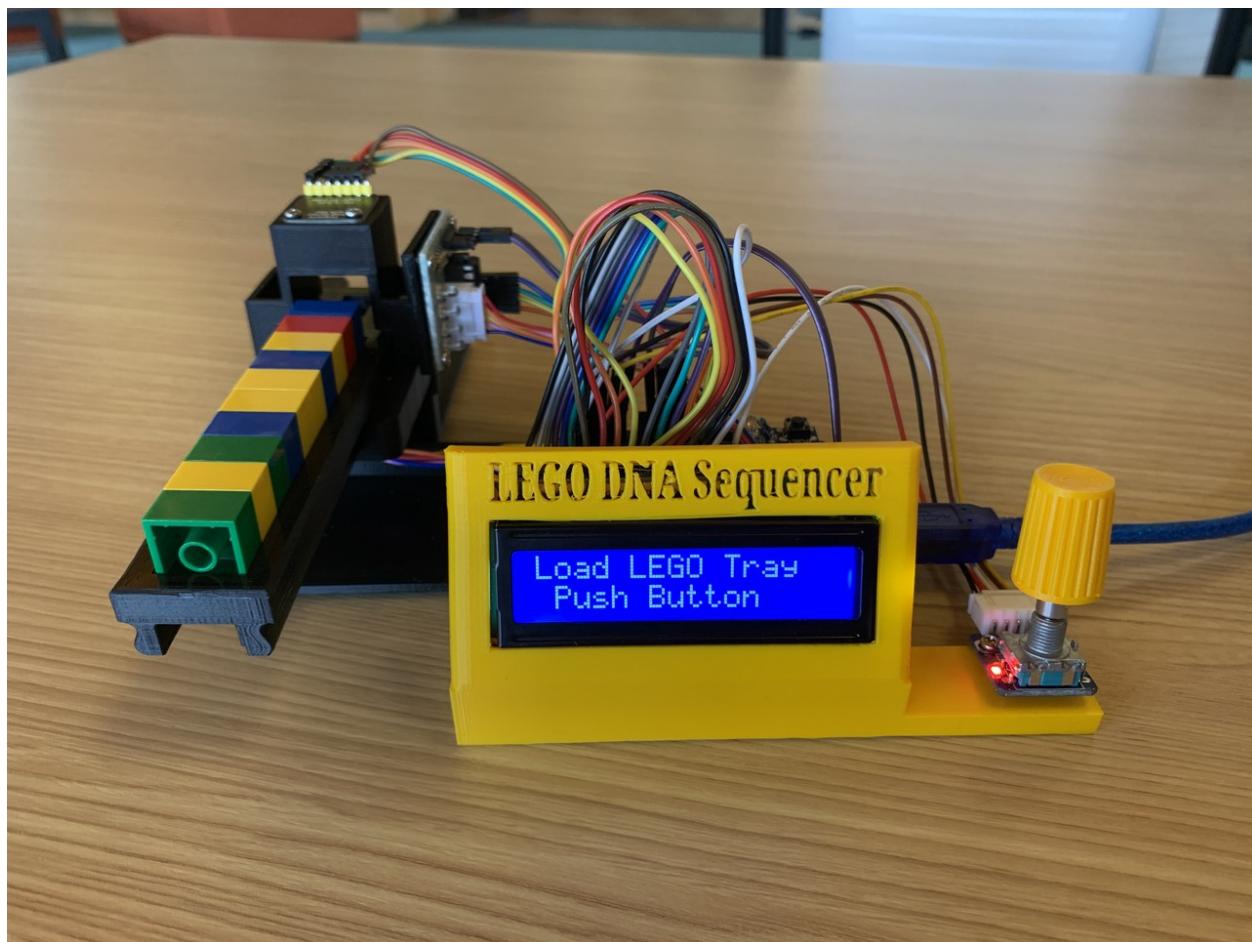
The LEGO DNA Sequencer is operated with a simple user interface. The UI includes an LCD display, a buzzer, and a rotary encoder / button which is much like a car radio knob.

## Starting the System

Power on the system by connecting the USB cable from the Arduino into a USB power adapter. There is no ON/OFF switch. When the system is plugged in it will beep three times and display the following screen.



Push the button and you will be ready to perform DNA sequencing.



To operate the sequencer you will need to assemble 10 of the LEGO 2x2 bricks into a genome for sequencing. The system has 6 built in genomes that it will recognize as shown in the following illustration.

# LEGO DNA Sequencer

**Sea Urchin**

CGATGACTAG



**White Shark**

TACGCTAGCT



**Sea Star**

GATCCGATGC



**Anchovy**

ACTGATCGAT



**Giant Kelp**

TAGCTAGCTA



**Pelican**

CAGTACCGATC



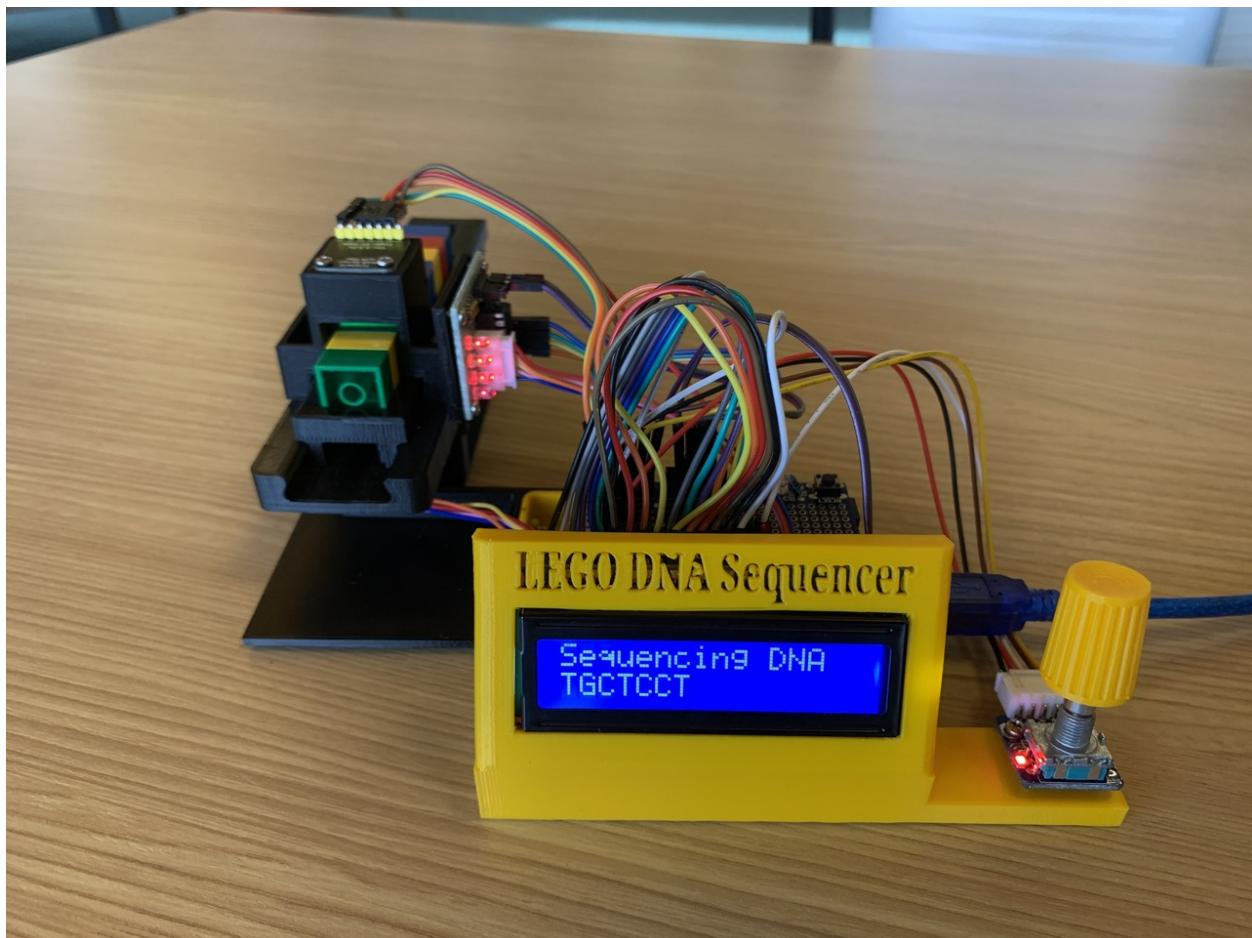
## Loading the LEGO Tray

When you have assembled a genome of 10 LEGO bricks, place them in the slot in the tray with the first brick in the sequence at the forward end of the tray. Note that the first brick will be inside the color sensor detection unit. The beginning of the second brick should align with the face of the color sensor.



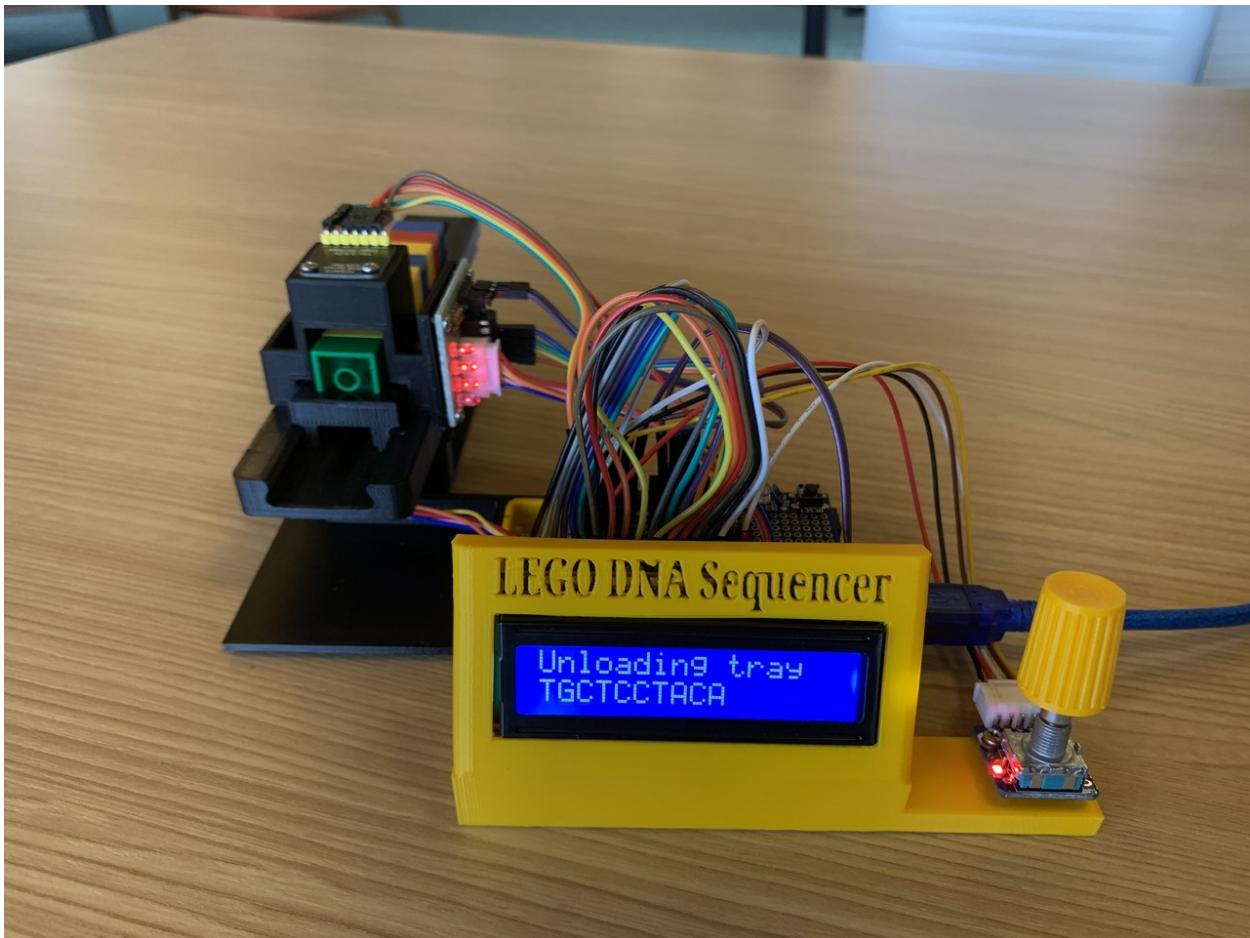
## Beginning the Sequencing

Push the button to begin sequencing. You will hear a buzzer click with each brick that is sequenced and the appropriate A, C, G, or T will be displayed.

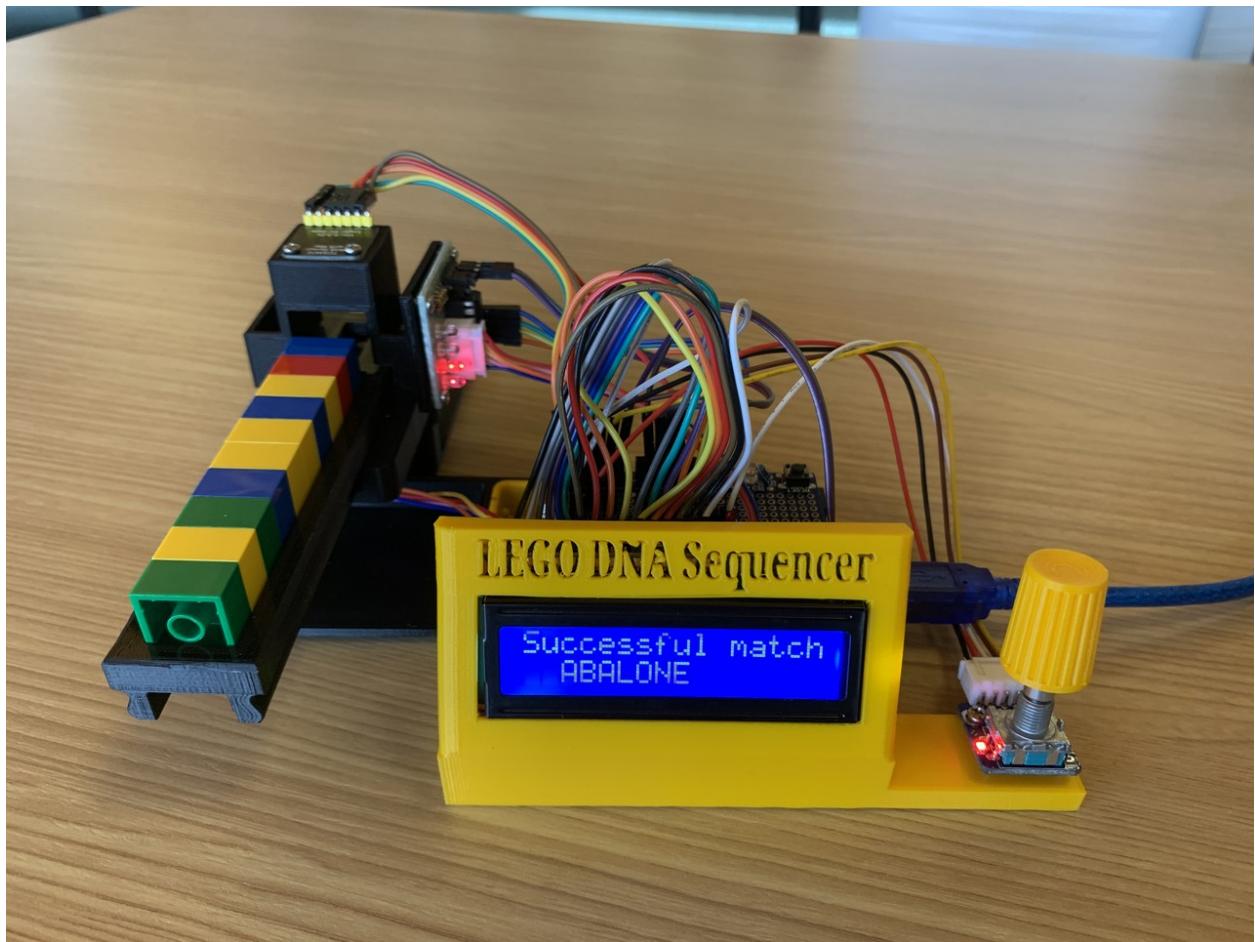


## Unloading the LEGO Tray

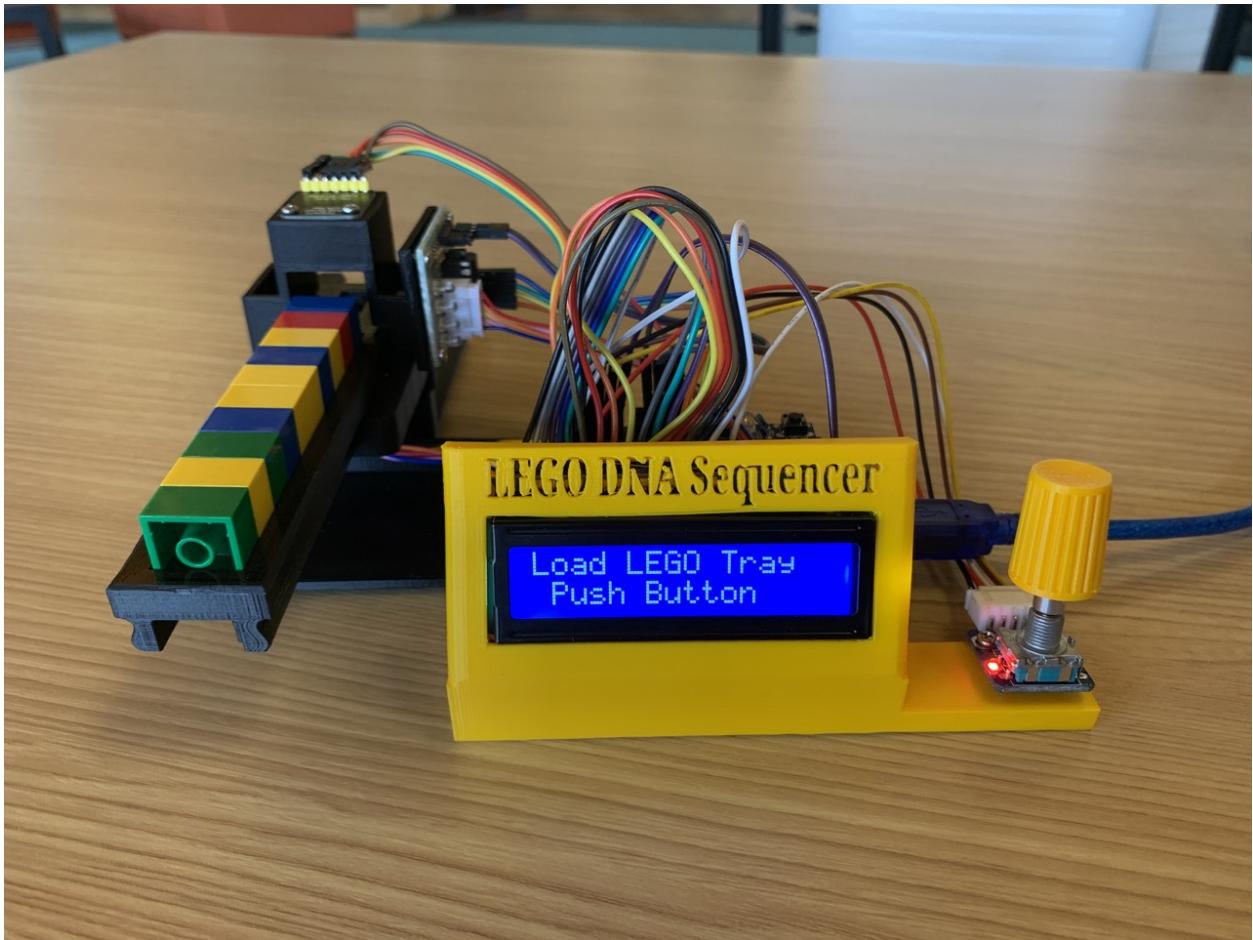
When all 10 of the bricks have been sequenced the LEGO tray will be unloaded, returning the tray to the initial position.



At that point you should see a “Successful match” and the name of the species that has been sequenced.

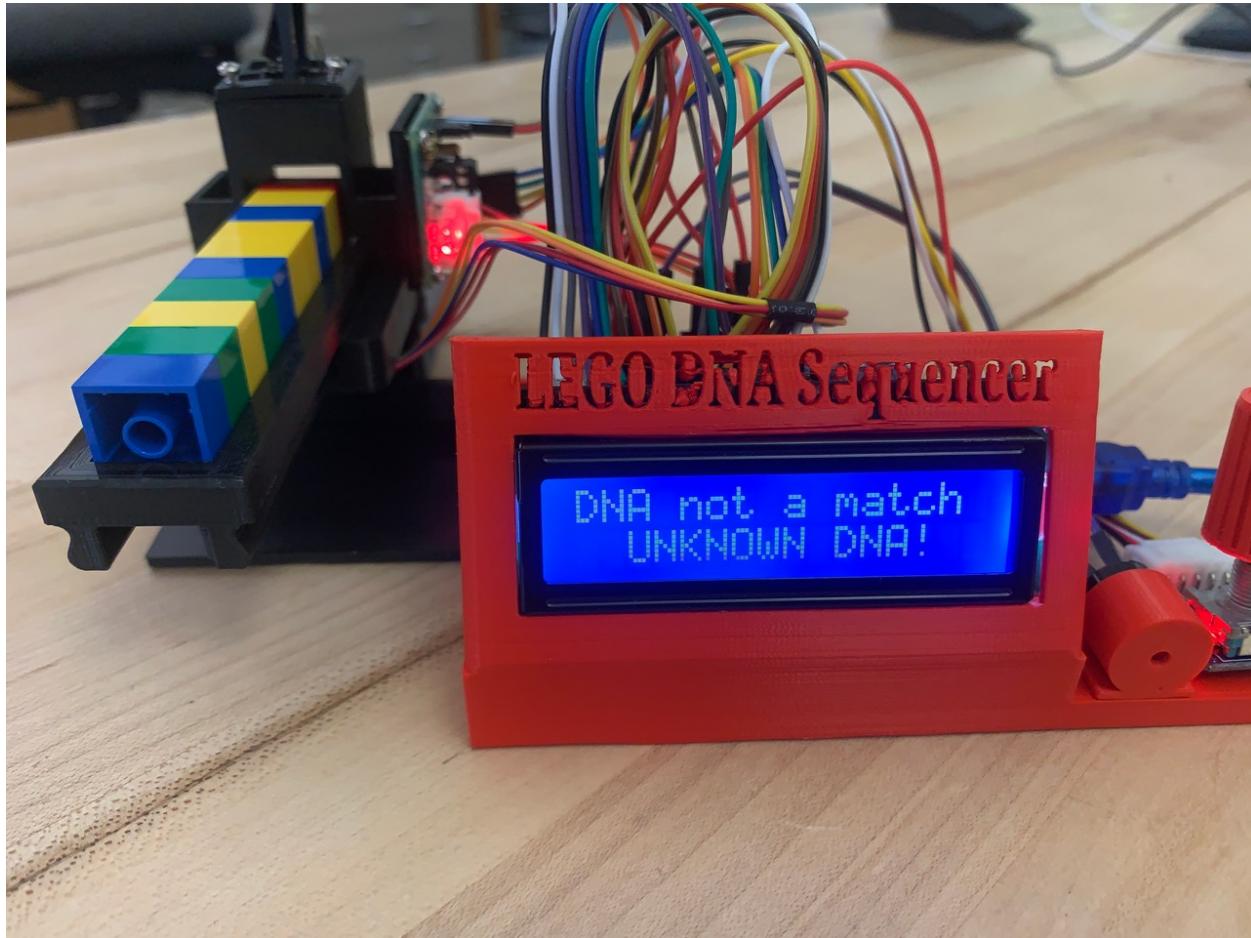


Pushing the button will ready the system to perform another sequencing operation.



## UNKNOWN DNA

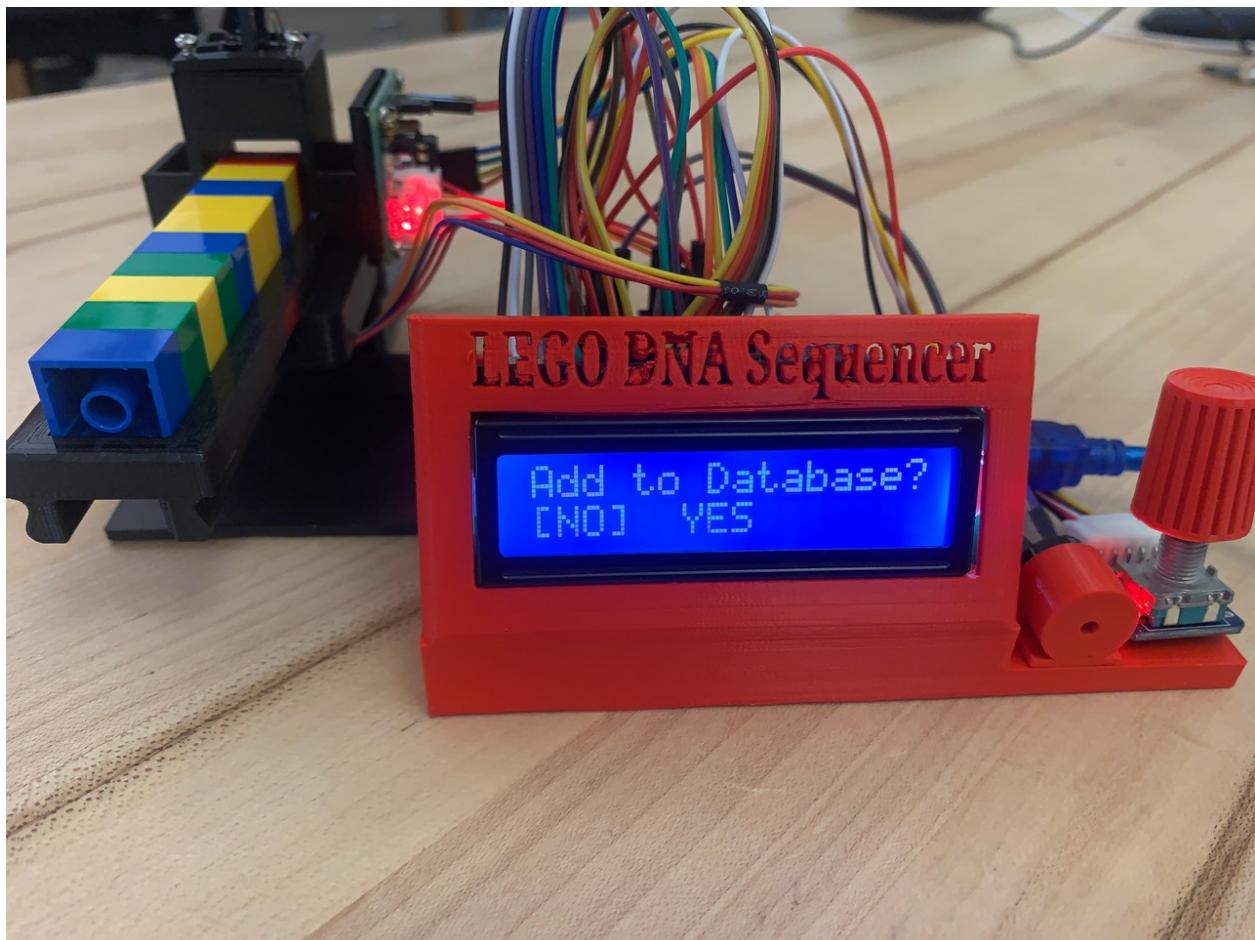
In the event that the bricks you have assembled do not match those of a known genome the system will display the following message.



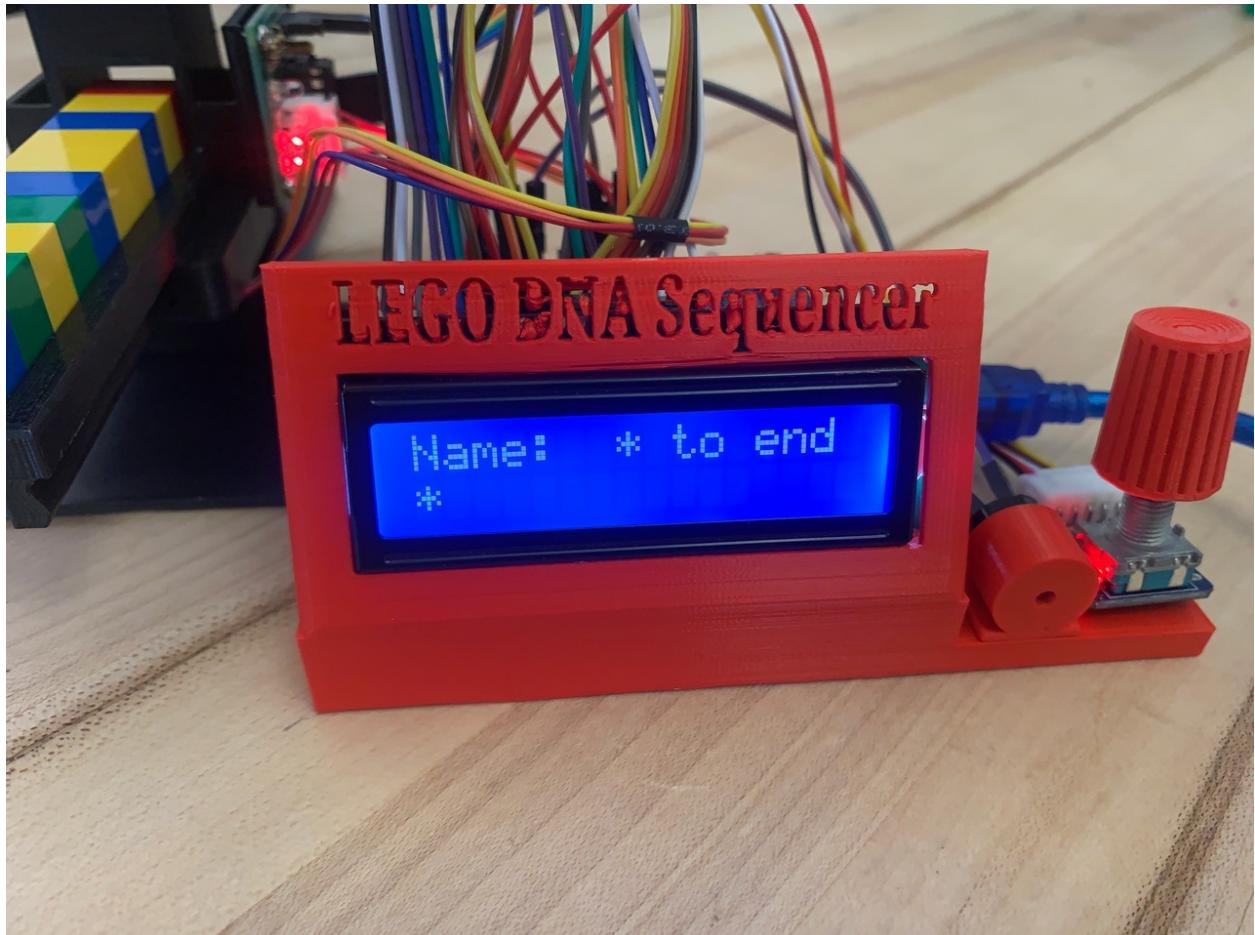
## Updating the Database with a new DNA

Push the button and you will be asked if you want to add the sequence to the database of known species genomes. Rotating the knob will move the selection back and forth between the choices of NO and YES. If you select NO, which is the default selection, the system will return to the state where it is ready to start another sequence operation.

Note that due to Arduino memory limitations you will only be able to add 3 sequences. The added sequences can be removed by using the operation "ZERO NEW DNA".



If you selected YES to add the new sequence to the database, you will be prompted to enter a Name for the new genome. Rotating the knob will show you the characters that you can choose for the next letter of the name: A-Z, 0-9, and ‘‘.



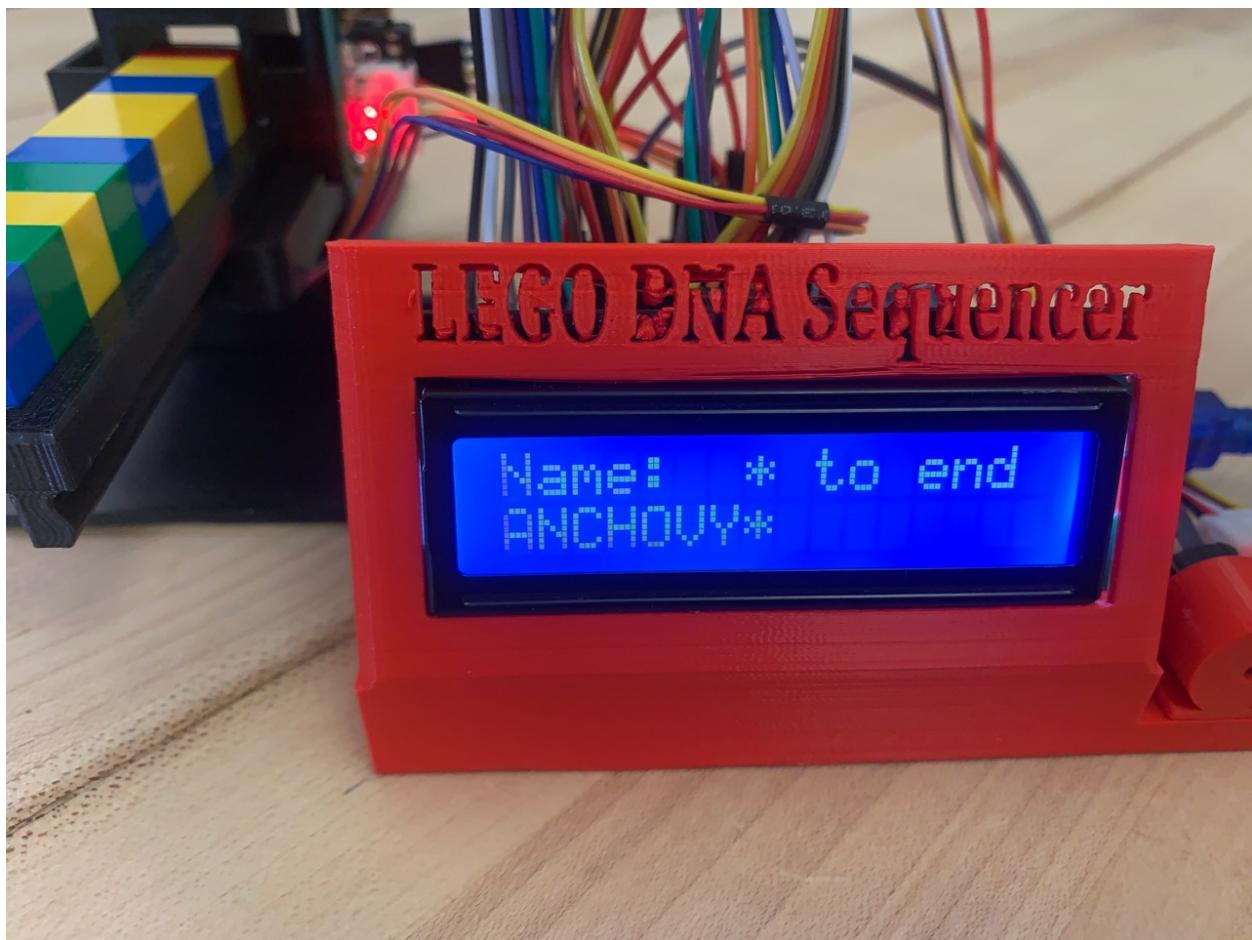
To select a character for the name, press the button and the display will advance for you to enter the next character.



If you wish to delete a previous character, select the '<' character and push the button.



When you have completed entering the name, select the '\*' character.



You will then be prompted NO or YES to accept the database entry.



You should then be able sequence the same tray of LEGO bricks and see that your new genome is successfully matched.



## Troubleshooting

In the event that the sequencer is not correctly identifying a known genome sequence it will be necessary to make some adjustments to either the positioning of the LEGO tray and/or adjustments to the color channel threshold levels.

### Adjustment of the LEGO Tray Position

The “home” initial position of the LEGO tray can be manually adjusted with the maintenance User Interface. **NOTE: DO NOT MANUALLY FORCE THE TRAY!**

The maintenance User Interface is entered by rotating the knob when the system is in the state where it is ready to begin sequencing with the display showing, “Load LEGO Tray / Push Button”. Each click of the rotating knob will advance to another option. Select “Cancel” to exit from maintenance mode.

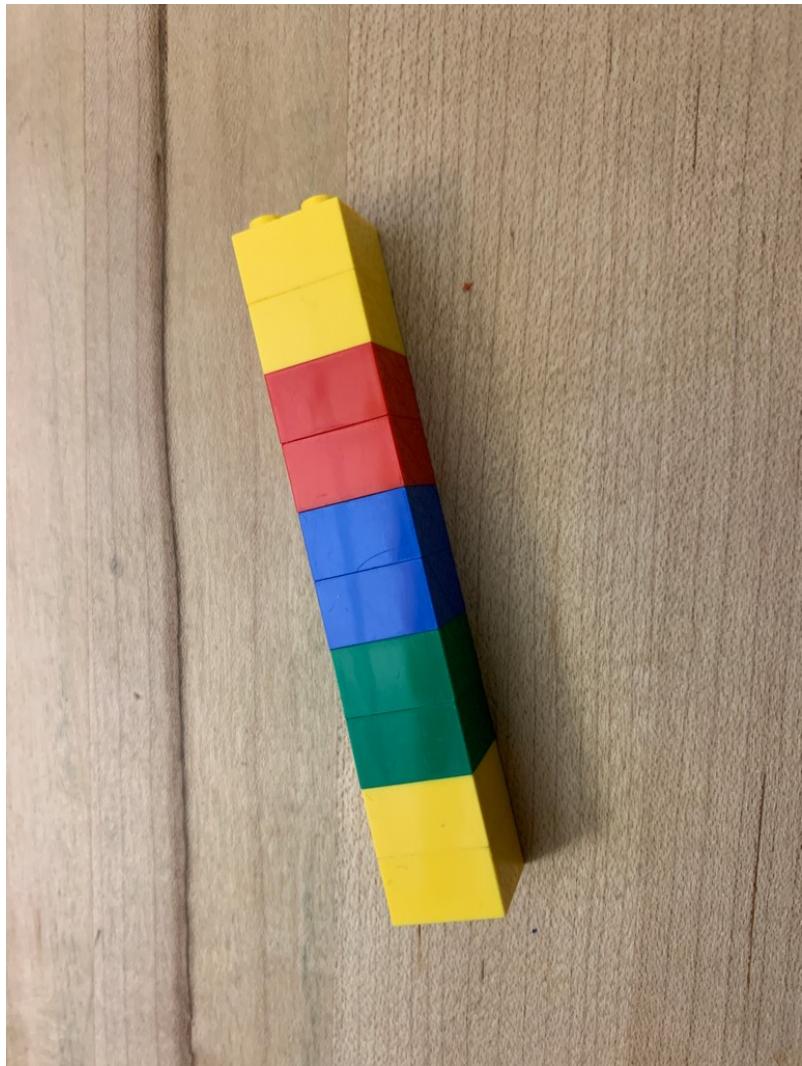
A suggested method of adjusting the LEGO Tray Position is to first perform the UNLOAD TRAY operation. The tray should then be easily pulled out and reinserted. The LEGO tray should be inserted until it reaches the gear. This will position the tray about 1” from the face of the color sensor. Gently advance the tray forward when you push the “LOAD TRAY”. The tray should automatically move forward to the position where it should be ready to start sequencing.

When you push the button to select “POSITION TRAY”, rotating the knob clockwise will advance the tray forward and rotating the knob counterclockwise will move the tray backward. Push the button to finish when you have correctly positioned the tray so that the face of the 2nd brick aligns with the face of the color sensor. At that point you should re-sequence and verify the results of your operation. If this fails it is likely that you will need to make adjustments to the color channel threshold levels. (see the next section)



## Adjustments to the Color Channel Threshold Levels

The first step in adjusting the color channel threshold levels is to assemble and sequence the YYRRRBGGYY test bricks.



Sequencing this known order of bricks will enable the system to adjust the channel threshold levels used to identify the Red, Green, Blue, and Yellow colors detected by the color sensor. After sequencing, and failing to recognize the Test genome, you will need to go to the advanced additional operation for AUTO CHANNEL. It will then display the current channel threshold values for CLEAR, RED, and BLUE. After clicking the button It will show the new recommended values and allow you to update the system with those values.

## Additional Operations

The Additional Operations are accessed by rotating the knob when the system is ready to begin sequencing with the display showing, “Load LEGO Tray / Push Button”.

### CANCEL

Exit from additional operations and return to sequencing LEGO operations.

### UNLOAD TRAY

The LEGO tray will be backed out, removing it from the system

### LOAD TRAY

The LEGO tray should be inserted until it reaches the gear. This will position the tray about 1” from the face of the color sensor. Gently advance the tray forward when you push the “LOAD TRAY”. The tray should automatically move forward to the position where it should be ready to start sequencing.

### POSITION TRAY

Rotating the knob clockwise will advance the tray forward and counterclockwise in reverse direction. Pushing the button will exit positioning the tray.

### ZERO NEW DNA

This operation will remove any genomes that were added to the database. Note that there is limited storage for additional genomes.

### BUZZER OFF

This will turn OFF the buzzer which is used to announce operations and identify progress during sequencing.

## **BUZZER ON**

This will turn OFF the buzzer which is used to announce operations and identify progress during sequencing.

## **ADVANCED**

This selection provides access to system operations.

### **Advanced Operations**

The Advanced Operations include selections that adjust the color channel threshold levels, auto positioning the LEGO tray, displaying the LCD output on an external monitor, and rebooting the system.

## **CANCEL**

Exit from advanced operations and return to sequencing LEGO operations.

## **CLEAR CHANNEL**

This selection displays the current CLEAR channel threshold level and allows you to edit the value.

## **RED CHANNEL**

This selection displays the current RED channel threshold level and allows you to edit the value.

## **BLUE CHANNEL**

This selection displays the current BLUE channel threshold level and allows you to edit the value.

## AUTO CHANNEL

This selection requires that you have scanned the LEGO test bricks YYRRBBGGYY. It will then display the current channel threshold values for CLEAR, RED, and BLUE. After clicking the button It will show the new recommended values and allow you to update the system with those values.

## AUTO POSITION

The auto positioning is also accomplished using the LEGO test bricks YYRRBBGGYY. You should begin with the POSITION TRAY operation to get the tray near to the desired start position. This operation advances the tray to the two RED bricks and then backs up to the estimated start position.

## REMOTE LCD ON

This selection enables the system serial output to show the contents of the LCD. It requires that the RemoteLCD.py program is operating on the laptop computer used to display the large font output. See the next chapter on [Remote LCD](#).

## REMOTE LCD OFF

This selection disables the system serial output to show the contents of the LCD.

## REBOOT

This will immediately Reboot the system and return to the startup screen.

## Credits

This project has been clearly inspired by the LEGO DNA Sequencer created by Sam Nicholls and Tom Blanchard at [Monster DNA Lab](#). It is a very well conceived open source project.

Peter Shum was a Post Doc at the Hopkins Marine Station of Stanford University and implemented the [MonsterLab Sequencer](#) for a Hopkins Open House Day in 2018.

Dr. Amanda Whitmire is the Head Librarian & Bibliographer of the Miller Library at the Hopkins Marine Station and has provided the facilities of her Fabrication Lab for this project.