The UMaryland iGEM Lab-in-a-Box is a compilation of various lab hardware that allows synthetic biology research and education in settings with limited resources. It comprises of a microcentrifuge, incubator, shaker, and vortex machines in one box that is open source, compact, and customizable. While these parts will cost thousands of dollars to purchase, it can be built for under $300. The construction of the box requires no soldering, drilling, and uses all off-the-shelf and 3D printed parts to function, making construction and repair easy. With the lab-in-a-box and lab equipment found in typical high school classrooms, transformations, overnight cultures, and minipreps can be conducted, with further possible modifications to increase its functionality. We hope this guide to construction, usage, and troubleshooting our lab-in-a-box is comprehensive and easy to understand.
General Principles

Microcentrifuge 3
Shaker 4
Incubator 5

List of Parts 7

Assembly Instruction

Peltier Plate Assembly 9
Peltier Plate Mounting 13
Motor Mounting 15
Rotor Assembly 16
Electronic Parts Setup 17
Wiring Setup 17
Arduino Software 22

Basic Operations 23

Switching Out Rotors 23
Centrifuge 23
Incubated Shaker 24
Vortex 24
Incubator 24

Expanding Functionality 25

Chilled Centrifuge 25
RPM Controlled Shaker 25
Vertical Gel Box 25
Water Bath 25

Specifications 26
General Principles

This section explains the general principles behind each piece of equipment and how it is achieved in the lab-in-a-box.

Microcentrifuge

A microcentrifuge separates samples based on their mass, by spinning it at an extremely high speed. The sample is placed inside a rotor, which is spun at a high speed using a motor. This generates centrifugal force.

This centrifugal force can be used to separate components of a sample with different mass following Newton’s Second Law, which states that

\[ F = ma \]

And in rotational motion

\[ a = \frac{v^2}{r} = \omega^2 r \]

Where \( a \) is acceleration, \( v \) is velocity, and \( r \) is the radius. This means that for centrifugal force

\[ F = m \times \frac{v^2}{r} \]
Meaning that the force experienced by components of the sample depends on its mass. Because of this, when a sample is being spun, such as bacteria in media, the bacteria experience a different amount of force (because it is heavier) compared to the water or the molecules in the media. The faster the motor spins, the velocity becomes greater, meaning that there is a bigger difference in centrifugal force.

Rotational velocity can be measured in terms of RPM (revolutions per minute) or radians per second. When dealing with microcentrifuges, it is common to use RPM or RCF (relative centrifugal force) to describe the speed at which a microcentrifuge spins. While RPM measures how fast the motor spins, it does not describe the force that the sample feels, which as the equation states, is also dependant on the radius. Therefore, microcentrifuges can spin at the same RPM, but the force experienced by the sample may be different depending on how far the samples were placed from the motor.

To account for this, RCF is used to describe the force experienced by the sample. It is measured in units of g, which is the gravitational force of the Earth (9.8 m/s²). This standardizes how centrifugation is described so that a sample experiences the same amount of force regardless of what microcentrifuge is being used. To convert RPM to RCF the following formula is used:

\[
RCF = 1.12 \times r \times (\text{RPM}/1000)^2
\]

Where \(r\) is the radius of the sample from the center. In the lab-in-a box, an RPM of up to 12,000 is generated, which generates about 10,000 x g RCF.

**Shaker**

A biological shaker ensures that the media is well oxygenated and that the cells do not settle at the bottom of the culture tube. Aerobic cells such as *Escherichia coli*, which is commonly used in synthetic biology, need oxygen to replicate and grow. However, oxygen is limited by the rate of diffusion, or the rate at which the oxygen can move into the media from the air. The rate at which it moves is given by Fick’s Law, which states

\[
\text{Rate} = D_e \times A \times \Delta P / T
\]

Where \(D_e\) is the diffusion coefficient, \(A\) is the area, \(\Delta P\) is the difference in pressure, and \(T\) is the temperature. The value of \(D_e\) is around \(2.10 \times 10^{-5}\) for air, making the rate of diffusion very slow. This means that the cells that are at the bottom of the culture tube grow slower compared to the cells that live near the surface. The cells at the bottom can then either undergo slow anaerobic (without oxygen) respiration to grow, or die.
Cells are also subject to gravitational force, even when suspended in media. Gravity pulls the cells towards the bottom of the tube, where they aggregate. Some species of bacteria can form pellicles, which suspends them at the surface of the media, or form biofilms which makes them stick to the side of the culture tube, but species or strains of bacteria used in labs commonly lack this ability. Therefore, settling of the cells combined with the lack of oxygen at the bottom of the tube means that cells grow very slowly when not being shaken.

The lab-in-a-box uses an unbalanced rotation in order to shake the culture. First, the rotation of the motor is not balanced because where the motor connects to the rotor is off center. This causes shaking motions due to the rotation force being unevenly distributed. In addition, the culture tubes are laid at a slight angle and the combination of these two mechanisms causes the culture tubes to be shaken and well oxygenated.

**Incubator**

Electrical work is converted to a differential in heat through the use of a Peltier plate. The inner workings of a Peltier plate are beyond the scope of this guide, but it is most important to know that there is a hot side and a cold side to a Peltier plate, and that these sides can be changed based on the direction of the current. When voltage is put through the plate, it transfers heat from one side to the other. This heat is quantified using Q, which is the amount of thermal energy that is moved from one side to the other. The temperature difference created in the box is determined by the equation:

\[ Q = mc\Delta T \]

Where \( m \) is the mass, \( c \) is the heat capacity, and \( T \) is the temperature. The value for \( Q \) is given in the specification sheet of the Peltier plate, found online.
From Thermonamic Module for the Peltier used in the lab-in-a-box.

Based on the voltage and amperage used, the DT (change in temperature assuming perfect dissipation of heat) and Q could be found. However, it's important to keep in mind that specifications give only an idea of the amount of cooling provided, and that under real conditions, the efficiencies are much lower.

In the lab-in-a-box, the Peltier plate is controlled by an H-bridge, which can control the power delivered to the Peltier plate and the direction of the current. The direction of current can switch whether or not the inside of the box becomes hotter or colder, depending on the application. The power delivery is controlled by a PWM (pulse width modulation) which sends pulses of electricity to the Peltier plate. The faster the pulses are sent, the more power goes to the Peltier, and the hotter or colder the Peltier gets, depending on the direction of the current.

There is a feedback loop for temperature, which is sensed by a thermsister. The thermsister changes the value of its resistance depending on the temperature. The colder the box, the
higher the resistance becomes. This change in resistance can be sent to the Arduino to determine whether the box needs to be cooled or warmed.

**List of Parts**

The following are the list of materials needed to build the lab-in-a-box in its most basic configuration. The prices were found mostly on eBay as of October 12th, 2017.

The 3D printed rotor files may be downloaded from the 2017 UMaryland iGEM Website:

http://2017.igem.org/Team:UMaryland/Hardware

And may be printed using a commercial 3D printing service. We recommend printing with ABS rather than PLA due to its higher strength.

A wire stripper, Phillips and flathead screwdrivers, and scissors will be required for assembly.
<table>
<thead>
<tr>
<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Styrofoam Box (minimum 14”x14”x7”)</td>
<td>14.94</td>
</tr>
<tr>
<td>Golden Power A2212-10 1400KV Brushless Motor</td>
<td>11.50</td>
</tr>
<tr>
<td>Velotech Magic Multirotor 30A Electronic Speed Controller with BEC</td>
<td>13.00</td>
</tr>
<tr>
<td>12V 33A 400W Power Supply</td>
<td>20.99</td>
</tr>
<tr>
<td>3D Printed Rotors</td>
<td>47.70</td>
</tr>
<tr>
<td>Thermoelectric Peltier Refrigeration Cooling System Kit</td>
<td>16.99</td>
</tr>
<tr>
<td>Elegoo Arduino Mega</td>
<td>13.89</td>
</tr>
<tr>
<td>400 Point Solderless Breadboard</td>
<td>6.98</td>
</tr>
<tr>
<td>Irf3205 H-Bridge DC Dual Motor Driver PWM Module 3-36V 10A, Peak 30A</td>
<td>33.32</td>
</tr>
<tr>
<td>TEC1-12712 Thermoelectric Cooler</td>
<td>14.95</td>
</tr>
<tr>
<td>Thermal Paste</td>
<td>2.95</td>
</tr>
<tr>
<td>NTC Thermistor Temperature Sensor 10K 1% 3950 Waterproof Probe 1m N178</td>
<td>3.50</td>
</tr>
<tr>
<td>18 AWG Wires and Jumper Cables for Arduino (male to male, male to female, and female to female)</td>
<td>12.42</td>
</tr>
<tr>
<td>Arduino LCD Shield</td>
<td>6.99</td>
</tr>
<tr>
<td>18 AWG Wire Nuts</td>
<td>2.99</td>
</tr>
<tr>
<td>Gorilla Glue + Electrical Tape</td>
<td>13.00</td>
</tr>
<tr>
<td>10K Ohm Resistor</td>
<td>2.50</td>
</tr>
<tr>
<td>#10 Nylon Lock Hex Nut</td>
<td>3.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>241.61</strong></td>
</tr>
</tbody>
</table>
Assembly Instruction

SAFETY WARNING - Please wear protective eye equipment when working with any parts during assembly and use. Do not plug in the power supply until everything has been connected and verified to be correct. If any malfunction is noticed, promptly disconnect the power supply.

Basic guide to working with electricity - voltage flows from the positive pole to the negative pole, as denoted by V- and V+ on the power supply. It may also be denoted as Ground for negative and Power for positive. Red cables denote V+ and black cables denote V-. Do not let the black and red cables touch!

Peltier Plate Assembly

1. Put the large heatsink piece with the flat side up on a tabletop
2. Peel off the plastic covering the sticky side of the foam mount and attach it to the large heatsink.
3. Use electrical tape to cover the areas outside of the foam mount
4. Cover the area enclosed by the foam mount with thermal paste, spread evenly
5. Cover the side of the large heatsink with 1 width of the electrical tape

6. Place the Peltier plate with the side with letters printed on it face up and the red wire facing to the right
   7. Cover the Peltier plate with thermal paste, spread evenly

8. Place the small heatsink on top of the Peltier plate.
9. Flip the entire heatsink assembly upside down.
10. Insert the short but wide screw into the plastic casings, then insert into the screw holes on the large heatsink

11. Line up the screw holes on the large and small heatsinks and tighten
12. Attach the fan and grill to the large heatsink

13. Attach the small fan to the small heatsink, making sure that the power cords are all facing the same side of the assembly. The small screws insert directly into the heatsink rather than inside screw holes.
Peltier Plate Mounting

1. Mark a 100 mm x 100 mm hole on one side of the box towards the bottom edge

2. Using either a shorted 9V battery or a sharp knife, cut out the styrofoam. The shorted 9V battery method can be found at: https://hackaday.com/2016/07/27/build-a-foam-cutter-right-now/

3. Clean out the edges of the styrofoam to ensure a flat edge.

4. Pull the small fan power cord from the inside to the outside of the box
5. Attach the heatsink to the outside of the box, ensuring a tight fit to not allow air to circulate
6. Use electrical tape (preferred) or Gorilla glue to seal any gaps between the heatsink assembly and the box
7. Use jumper cables to connect the fans to the power supply, using multiple male to female connectors to extend the cable from the power supply to the fan. Connect the red side of the fan power cable to the V+ line and the black side of the fan power cable to the V- side. It does not matter which V+ or V- line the cable is attached to.
8. Open a 30 mm x 30 mm hole on a different side of the box near the heatsink assembly

9. Mount the temperature sensor above the hole using tape or paper clips
Motor Mounting

1. Screw the mounting bracket included with the motor into the bottom of the motor
2. Mark 110 mm from two sides of the box, this will the center position of the motor
3. Place the center of the motor over this mark, with the motor power cords facing towards the 10 mm x 10 mm hole, and screw into the styrofoam
4. Remove the screws and add Gorilla glue into the holes made by the screws
5. Use a wet paper towel to wet the screws, then insert into the holes
6. Unscrew the propeller mount from the motor
7. Add Gorilla glue to the shaft of the motor without the threaded adaptor piece
8. Insert the threaded adapter onto the shaft
9. Leave the motor and shaft to dry overnight
10. Attach the ESC to the motor as pictured

**Rotor Assembly**

1. Wet the nylon lock hex nut with a wet paper towel
2. Add Gorilla glue to the inside edge of the hex nut mount and the bottom edge
3. Place the hex nut on top of the hex nut mount hole

4. Ensure that the rotor is placed on a flat surface and that the hex nut is parallel to the bottom of the rotor piece
5. Repeat with all three rotor pieces
6. Leave to dry overnight
Electronic Parts Setup

1. Place electrical tape on the top surface of the power supply as seen in the picture, leaving half of the fan vent open for cooling. This will prevent the metal surface from short-circuiting the electronics.

2. Attach the Arduino Mega to the power supply using electrical tape
3. Mount the LCD shield on top, making sure to line up the pins properly. Match analog pins on the LCD to the Arduino Mega to ensure a correct assembly
4. Attach the H-bridge to the right side of the power supply surface using electrical tape
5. Attach the breadboard to the left side of the power supply surface using electrical tape

Wiring Setup

The Fritzing diagram indicates the wiring setup for the lab-in-a-box. Written explanation follows the visual representation. Follow the explanations while looking at the diagram. A breadboard is used to easily create circuits and distribute power throughout the system. On the sides with the red and blue lines are connected vertically, with red indicating positive voltage and blue indicating negative voltage or ground. In the middle section, all of the horizontal rows are connected, meaning that any two components that connects to the same row are connected to each other.
Temperature Sensor / Thermsister
1. Connect the thermsister to two different rows spaced two rows apart
2. Connect the top row to the blue (ground) line on the ends
3. Connect the other row to analog input 10 (A10) on the Arduino
4. Connect the row with the thermsister and analog input to another row using a 10K Ohm resistor
5. Connect the other end of the resistor to the +V (red) line on the ends

Motor / ESC
1. Using the speaker cables (thick red / black wires) connect the thicker red end of the ESC to the +V screw on the power supply and the thicker black end of the ESC to the -V screw on the power supply. This is done by stripping the wires on the speaker cable, wrapping them around the power cord on the ESC, and screwing them together using a wire nut (orange cone)

2. Using a jumper cable, connect the thin red wire from the ESC to the top of the red line on the breadboard, the thin black wire to the blue line, and the thin yellow line to one of the empty center rows towards the top
3. Connect the top row to one of the empty bottom rows, then connect this row to digital pin 30.
**Peltier / H-Bridge**

1. Using a speaker cable (thick red / black wires) with stripped ends, connect the H-bridge to the power supply, the POWER screw should attach to the +V on the power supply and the GROUND screw should attach to the -V on the power supply.
2. Using another speaker cable (thick red / black wires), connect the Peltier plate to the H-bridge. Connect the wires on the peltier to the speaker cable using a wire nut, then connect the other end of the speaker cable to the H-bridge. Attach the red cable to the MOTOR1 screw closest to the POWER screw, and the black cable to the MOTOR1 screw closest to the GROUND screw.

3. Connect the ground jumper cable pin (located on the other side of the screws) to the blue line on the breadboard, and the +5 V jumper cable pin to the red line on the breadboard.
4. Connect the DIR1 pin to digital pin 31 on the Arduino, and the PWM1 pin to digital pin 1 (D1) on the LCD shield.

Connect the Arduino LCD Shield Vin to the red line on the breadboard and the GND pin on the Arduino LCD Shield.
Run all cables from inside of the box to the outside through the 30 mm x 30 mm hole. Fix the cables onto the box using paper clips, then cover the hole with electrical tape.

Confirm that everything is connected correctly before proceeding.

Strip the female end of the AC power cable, then Attach the green/lime cable to ground, blue cable to N, and brown cable to L. Connect the AC power cable to a power strip (make sure the power strip is set to the OFF position) and connect the power strip into an outlet.

Double check that everything is connected correctly. The input/ouput of the AC power supply should look as follows.
Turn on the power strip and look for any possible short circuits (smoke or parts getting extremely hot) and verify that the fans are running. The ESC should beep three times to signal that it has been connected correctly. If these are not heard, then promptly shut down the power strip and verify that the wires have been connected properly.

If everything has been verified, connect the USB cable from the Arduino to the computer.

**Arduino Software**

Download the Arduino IDE software from

https://www.arduino.cc/en/Main/Software

And install it, making sure to install all of the necessary drivers in the process.

Download the Lab-in-a-box script from

http://2017.igem.org/Team:UMaryland/Hardware

Open the script using the Arduino IDE software

Under Tools → Board → Select the “Arduino/Genuino Mega or Mega 2560”
Under Tools → Processor → Select “ATmega2560 (Mega 2560)”
Under Tools → Port → Select the port COM port that is attached to the Arduino Mega 2560

If you do not see anything listed under Port, make sure the USB cable is connected to the Arduino and that the correct drivers have been installed.

Press Upload to add the script to the Arduino. After uploading, the USB cable no longer has to be attached to the Arduino and can be unplugged.
Basic Operations

SAFETY WARNINGS
- Please wear protective eye equipment when operating the lab-in-a-box
- Do not operate the machine while the lid is open, unless using the vortex feature
- Whenever touching or operating on electronic parts, make sure the power is unplugged and the green LED on the power supply is off

Switching Out Rotors

1. Ensure that the centrifugation, vortex, or shaker function is enabled
2. Disconnect the jumper cable from Arudino digit pin 31 to prevent accidental activation of the motor
3. Hold the side of the motor with one hand, while screwing in the rotor with the other
4. Continue to spin the rotor to check for a solid connection between the hex nut and the 3D printed plastic, and to tighten the nut
5. Reconnect the Arduino digital pin 31 to enable control of the motor
6. Press the RST button to reset the Arduino

Centrifuge

1. Place samples inside the centrifuge, ensuring that they are balanced, meaning that there is an even distribution of samples inside the rotor.
2. Close the lid on the box, then place a heavy object on top to prevent movement
3. Select the centrifuge function using the up and down push buttons on the LCD, then press the select button
4. Use the up and down buttons to increase / decrease the speed (ranging from 2000 to 12000 RPM), then press the select button
5. Set the centrifugation duration (up to 5 minutes) then press the select button

**Incubated Shaker**

1. Place the culture tubes inside the shaker, then place the lid on the box
2. On the LCD, select the shaker function
3. Use the up and down arrows to select the desired temperature (8-50 C)
4. Input the number of samples, which will determine the frequency and the speed of the shaker
5. The LCD will display the current temperature of the shaker
6. After the desired amount of time, press the RST button to stop the shaker

**Vortex**

1. Select the vortex function on the LCD
2. Place the sample to be vortexed slightly inside the rotor so that the sides are barely touching. Do not press against the bottom of the rotor.
3. Hold the sample in place until the sample has been sufficiently vortexed
4. Press the RST button to stop the vortex

**Incubator**

1. Select the incubator function on the LCD
2. Place the agar places or other samples inside of the box
3. Use the up and down arrows to select the desired temperature, then press select
4. The LCD will display the current temperature inside the box, it may take up to 30 minutes for the incubator to reach the desired temperature
5. Press the RST button to stop the incubator
Expanding Functionality

These are ideas for functions that we at UMaryland iGEM have proposed but did not have the opportunity to build or test. It presents an opportunity to modify the box depending on your particular needs.

Chilled Centrifuge

Currently, the Peltier plate is limited by the rate of cooling of the hot side, limiting the temperature of the cold side to around 8 °C. However, the Peltier plate has the ability to become even colder for special applications with better cooling. Self contained water loops, such as the Corsair Hydro Series H60 (~$60), presents a better cooling solution, but at an increased cost. The size of the hole on the styrofoam box can be increased to accommodate the radiator.

RPM Controlled Shaker

The method of RPM control is relying on the shaker returning to rest before shaking again. This prevents buildup of momentum and out of control spinning of the shaker that results in leaking of culture. However, to get a more consistent shaking rather than returning to rest, a feedback loop with the current RPM being input could be made. An infrared reflectance sensor such as the TCRT5000 Infrared Reflectance Track Sensor could be used to detect when one revolution has occurred. The body of the motor is normally shiny and reflects light, but a thin piece of electrical tape that absorbs infrared light can be sensed.

Vertical Gel Box

Gel electrophoresis is a crucial technique in synthetic biology because it allows for the verification of the genetic parts. However, gel boxes and power supplies can cost hundreds of dollars. A vertical gel box would compactly fit into a box and could be 3D printed. A gel box produces high voltages but small current, allowing the DNA to move through the gel rather than passing electricity through it. A 48 V power supply could be used with a MOSFET to control the current that flows through the electrode.

Water Bath

A water bath keeps a stable temperature for sensitive procedures such as transformations. The temperature probe used is waterproof and can be submerged with no harm. A metal or conducting container could be attached to the heatsink inside the box with the temperature probe submerged. This way, the water bath could be heated to the correct temperature and maintained.
Specifications

Motor - A2212/10T

No. of Cells: 2 - 3 Li-Poly
Kv: 1400 RPM/V
Max Efficiency: 78%
Max Efficiency Current: 6 - 12A (>75%)
No Load Current: 0.7A @10V
Resistance: 0.065 ohms
Max Current: 16A for 60S
Max Watts: 180W
Weight: 51.5 g / 1.82 oz
Size: 27.8 mm x 31 mm
Shaft Diameter: 3.2 mm
Poles: 14

ESC - VELOTECH MAGIC MULTIROTOR SPEED CONTROLLER 30A WITH 2A BEC

Continuous Current: 30A
Voltage Range: 2-4S LiPo
BEC Output: 2A, 5V
Refresh Rate: 600Hz
Motor Wires: 14 gauge soft silicone wires
Battery Wires: 14 gauge soft silicone wires
Signal Cable: 30cm long, Futaba & JR compatible
Weight: 27g (approximate, including wires)
Dimensions: 48x26x10mm (approximate, including capacitor)

H-Bridge - Dual Motor Driver H-bridge DC MOSFET IRF3205 3-36V 15A Peak 30A

Rated voltage: DC 3V-36V
Rated Current: 15A
Peak current: 30A
Product size: approx. 100*70*21mm/3.93*2.75*0.82"
Net weight: approx. 53g
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_h$ (°C)</td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td>$DT_{\text{max}}$ (°C)</td>
<td>70</td>
<td>79</td>
</tr>
<tr>
<td>$U_{\text{max}}$ (V)</td>
<td>16.0</td>
<td>17.2</td>
</tr>
<tr>
<td>$I_{\text{max}}$ (A)</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>$Q_{\text{cmax}}$ (W)</td>
<td>115.2</td>
<td>125.8</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.1</td>
<td>1.21</td>
</tr>
</tbody>
</table>