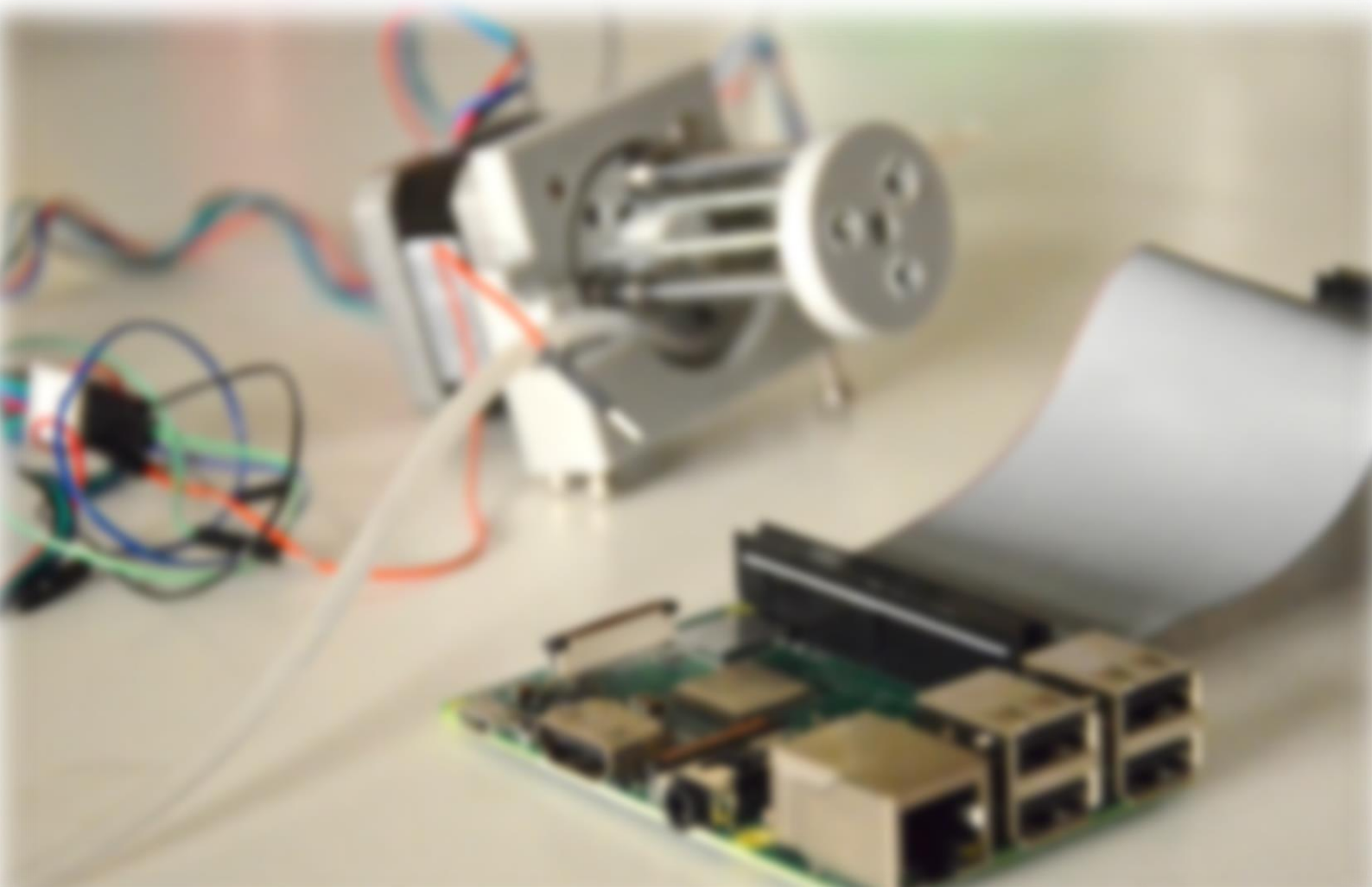
Morbidostat

Manual for DIY Assembly

iGEM

Athens 2019



Contents

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Materials

1x Raspberry Pi (at least a model 3)
1x Raspberry Pi 40 pin Ribbon (Optional
1x Raspberry Pi Cobbler (Optional)
1x SD card (Preferably 16GB and up)
1x Medium Size Breadboard
3x NEMA 17 Stepper Motors
3x Stepper Motor Driver A4988
3x 47 uF 50V electrolytic capacitors
1x ADS1115 ADC Converter
1x TIP120 Transistor
1x Diode
1x 300 Ohm resistor
1x DC fan for Raspberry Pi (We used a 30x30x10mm 12V fan)
1x 12V 2A DC Power supply
1x 2.5A 5V USB Power supply for the Raspberry Pi
1x Bright Red LED (624 nm)
1x LED holder
1x Light Dependent Resistor (LDR)
2x 4x2 mm diameter Neodymium magnets
1x 15 mL Falcon tube Cap
1x Magnetic Stir unit, 4 cm in length
Loads of jumper wires
Autoclave safe tubing, about 2 meters (0.8 mm inner diameter, 1.6 mm wall thickness)
3x Pyrex vial for Media of whatever volume
1x 50 mL Pyrex vial for the culture itself

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Miscellaneous

M3 2.5 Allen key
Glue
Screwdrivers of various sizes
Dremel
Vice
Electric Screwdriver or Drill
9x Straight Pins 4 mm in diameter x N in length
18x Flanged Needle bearings 4x8x3 mm (depending on the tubing)
9x Screws of length N
12x M3 Screws of length 25 mm
12x M3 Screws of length 8 mm
Access to 3D printer
Computer
HDMI Cable
Keyboard Mouse
Wi-Fi connection
Multimeter
Accurate scale (4 decimals preferred)


LOGO


HDMI

Assembly process

Hardware

Most holes are not made exactly to match the screws or the motor axes that are supposed to go through them. Feel free to drill them to the necessary diameter. We suggest doing the drilling before putting everything together, to avoid unnecessary accidents, but it can be done during the building process as well

Troubleshooting

Pumps: If you notice that the pumps are not turning but the stepper motor axis is, you can try two things: Drill the edges where the pump gets stuck and serrate them, but you will need to recalibrate the pumps and/or apply some glue on the axis and then quickly ass the pump on it, so that you can increase friction.

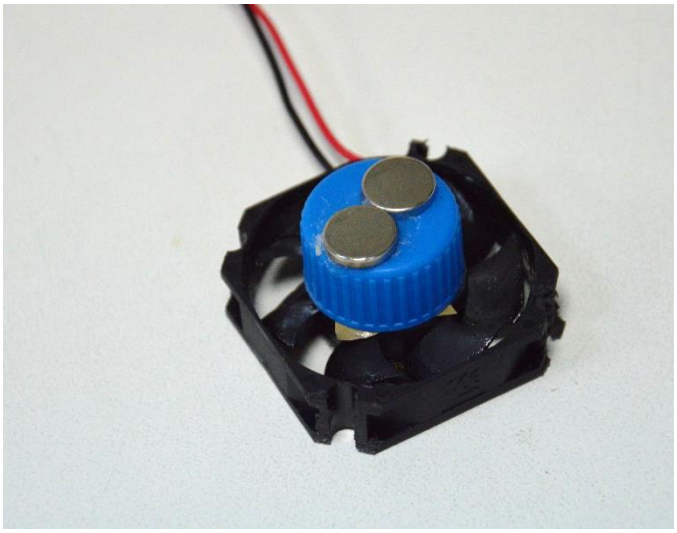
Peristaltic Pumps

Needs to be repeated threefold

1. Print the peristaltic pump parts
2. Mount peristaltic pump back onto the motor axis.
3. Screw in place the peristaltic pump frame with the 8mm screws. Make sure it is on the level plane with the pump's back.
4. Add the straight pins in the peristaltic pump' s indentations. You should add 3 pins per pump.
5. Fit 2 Flanged Needle bearings onto each pin, making sure that the flanges are on the outer sites.
6. Glue the bottom Needle bearing to the pump's back and the top needle bearing to the bottom one.
7. Add the peristaltic pump' s front and tighten with the screws that correspond to the pins' length.
8. Add the pump's cap and screw it with the frame.
9. Fit the tubing onto the pump.
10. Fasten the screws that tighten the tubing, keeping it in place. Be warned, if you tighten them too much, the pump will not function. Also, if you change the tension, you will need to recalibrate your pump.
11. Plug male to male jumpers to each of the motor's 4 female pins.
12. Use a multimeter to figure out which 2 pairs of pins are coiled together and write that down. We suggest using 4 colors so that you certainly don't forget the order, as this can fry your stepper motor.

Fan

1. Glue the Falcon cap onto the fan.
2. Glue the two magnets onto the cap. This is done to lessen the magnetic burden that the fan faces when the magnets are too close to it, as it can grind the fan to a halt.



LED and LDR system

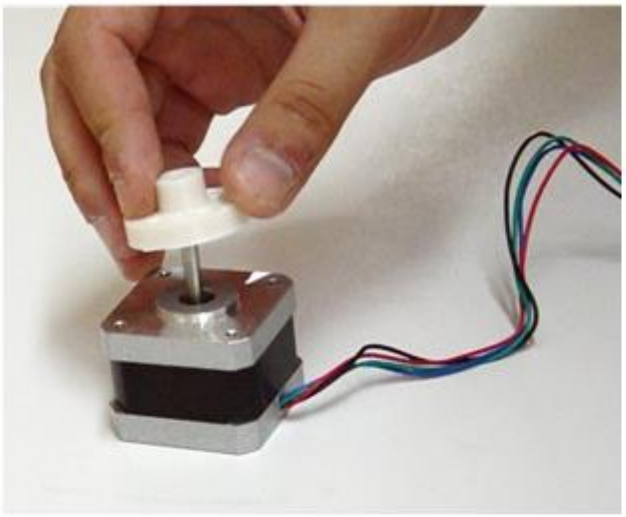
1. Add female to male jumpers to both the LED and the LDR to extend their range.
2. Mount the LED into the LED holder.

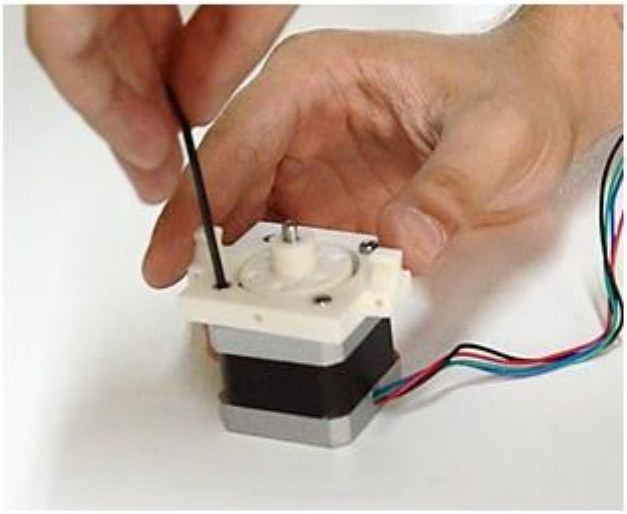
ADS1115

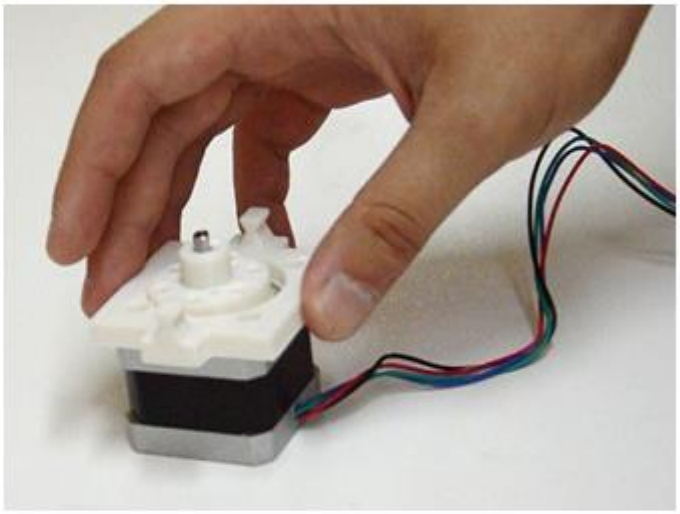
If you bought the pins and the ADS1115 separately, you will need to solder them together to establish electrical contact.

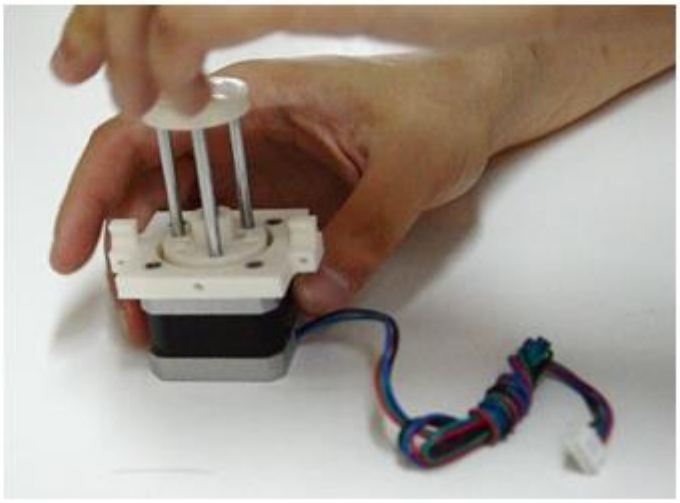
Bottle

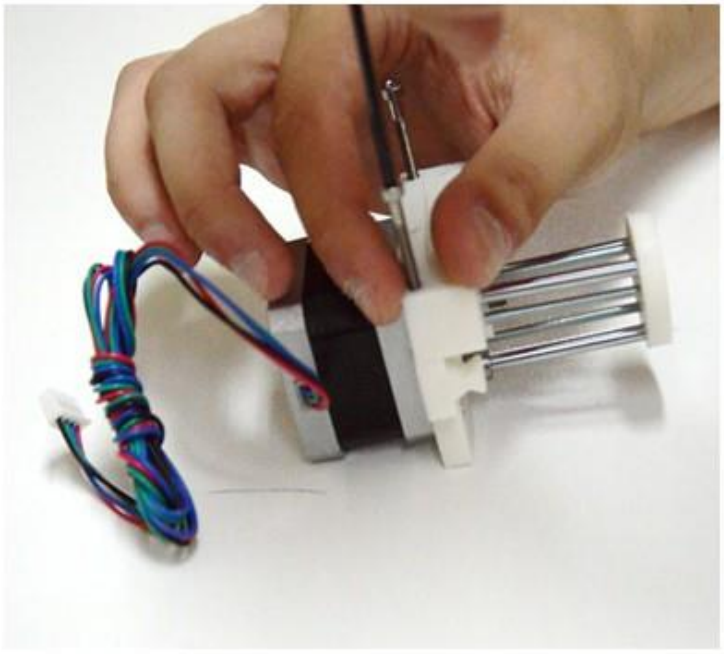
1. Drill 3 holes on top of the bottle, fit tubing inside and seal the rest using silicone.
2. Pass tubing through the holes on top of the bottle. Fill all gaps with silicon.
3. Close the bottle, not too tightly.
4. Wrap it in Parafilm so that oxygen can flow through.

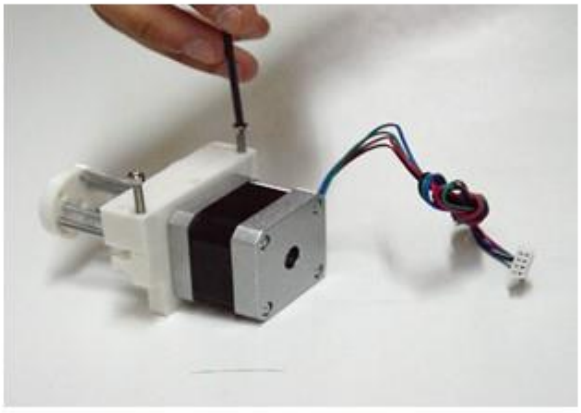












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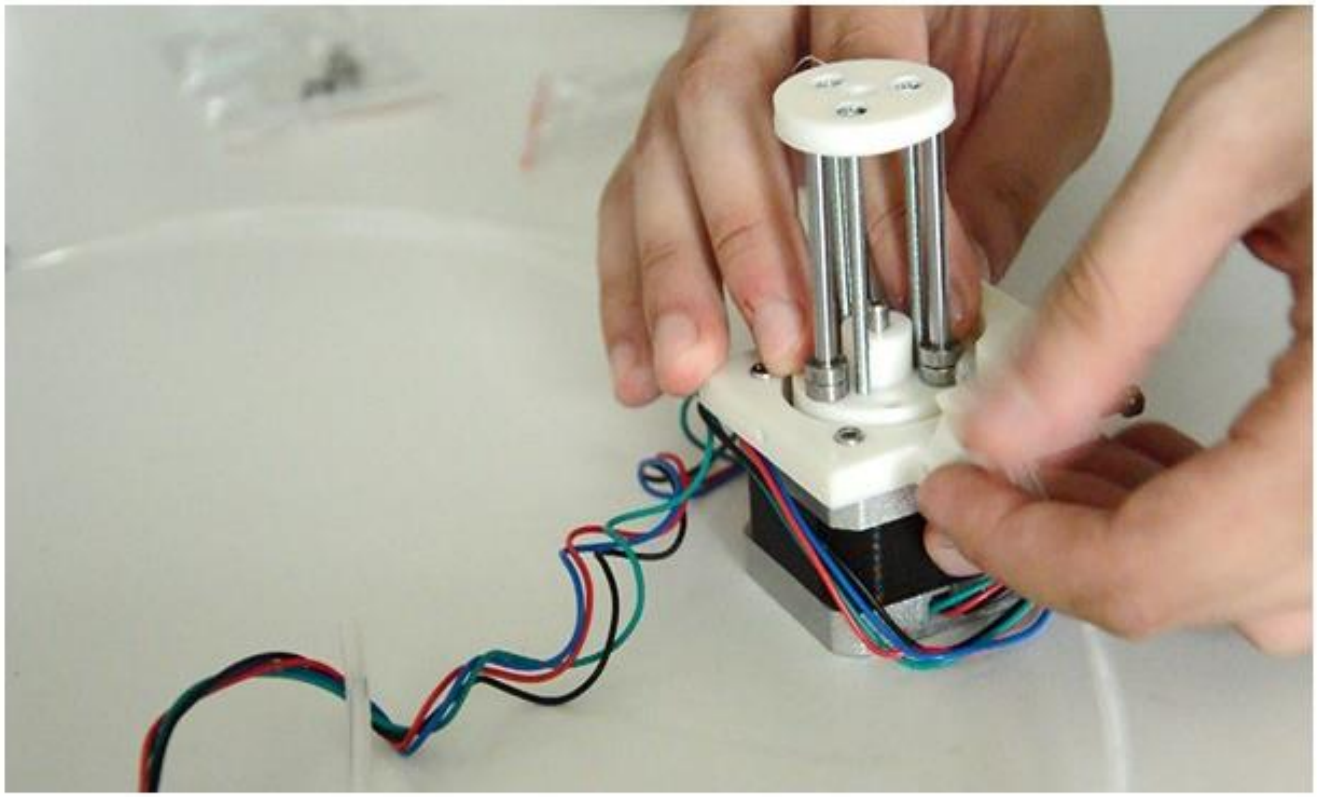
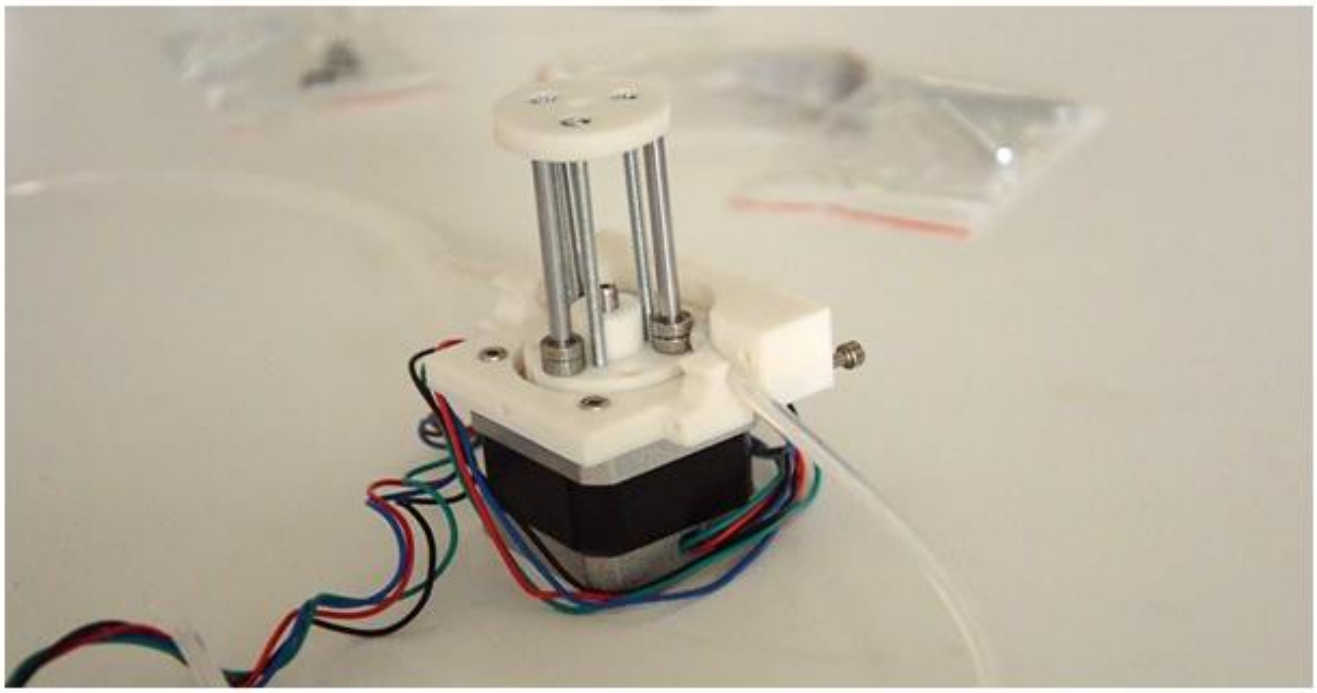
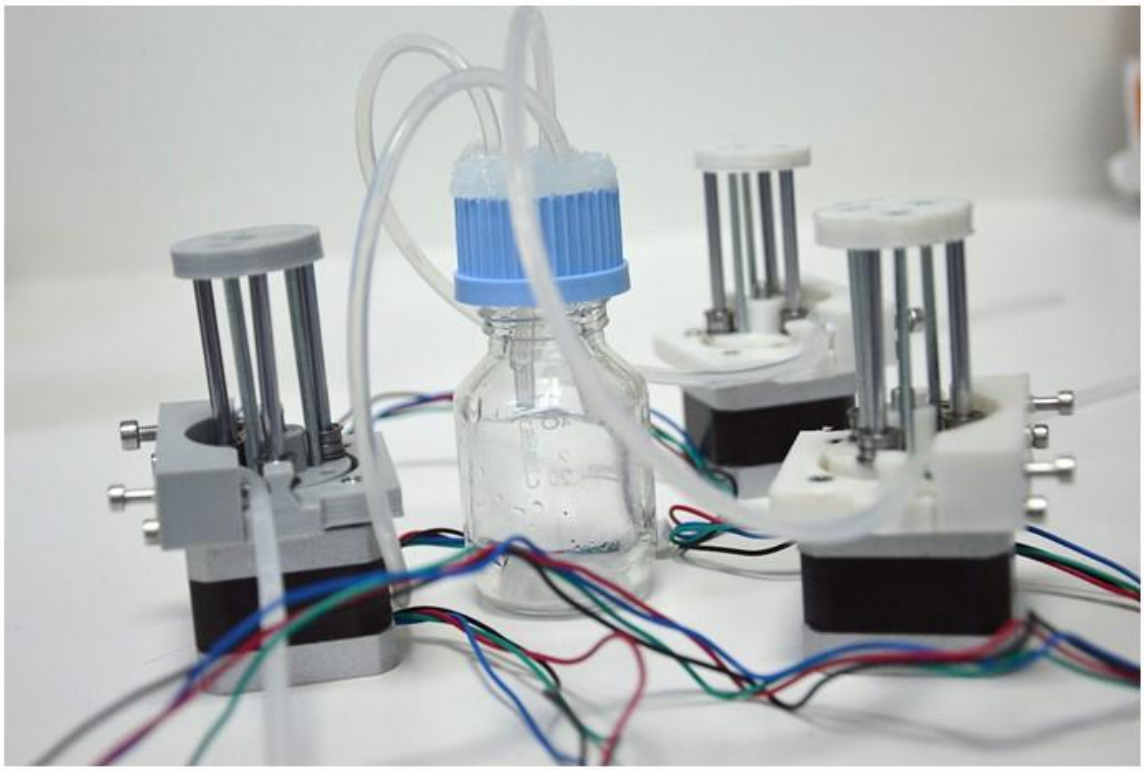
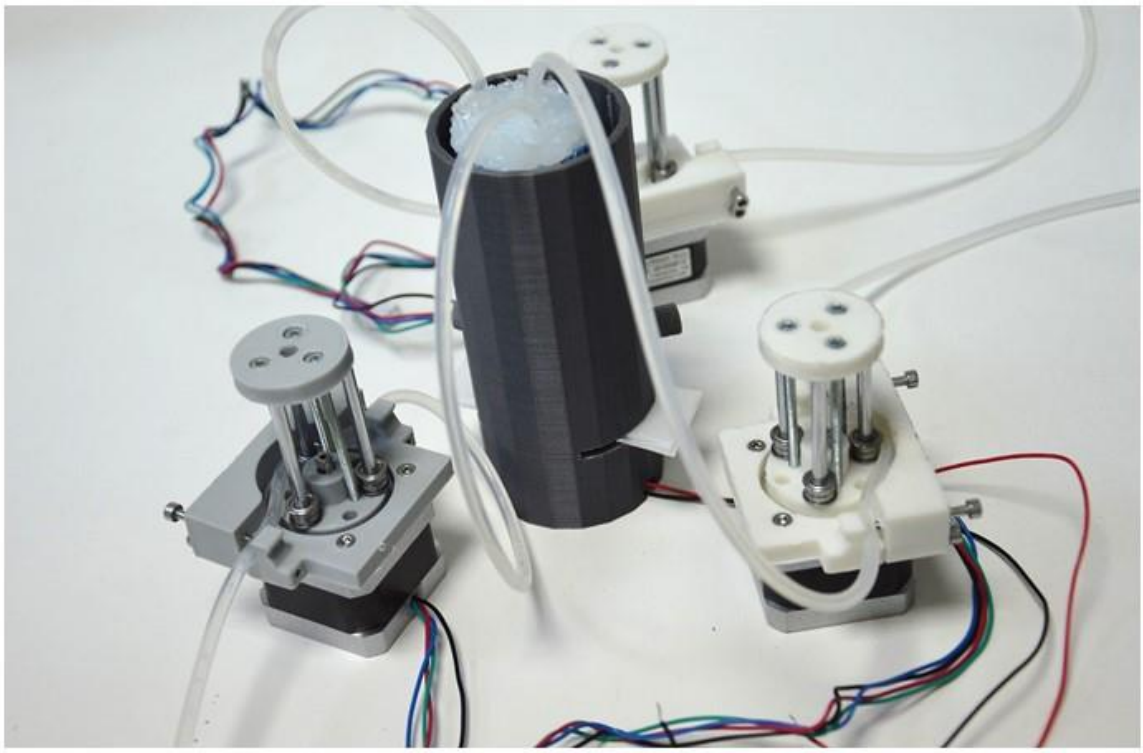








Raspberry Pi

Preparing the SD card with the OS

1. Download Raspbian from the official website (https://www.raspberrypi.org/downloads/raspbian/)
2. Download the Real VNC Viewer off the official website (https://www.realvnc.com/en/connect/download/viewer/)
3. Connect the SD card to your computer
4. Use balenaEtcher or any other similar program to flash Raspbian onto the Raspberry Pi (https://www.raspberrypi.org/documentation/installation/installing-images/)

Setting up VNC and enabling useful protocols

1. Unplug your SD card and plug it into the Pi
2. Connect the Pi to a monitor using the HDMI cable
3. Connect a keyboard to the Pi
4. Connect a mouse to the Pi
5. Plug the Pi into the power supply, booting it up
6. Go through the setup (Make sure to connect to your Wi - Fi)
7. VNC (https://learn.adafruit.com/adafruit-raspberry-pi-lesson-7-remote-control-with-vnc/installing-vnc):

Run the following code to set up your VNC password and make it run at startup:

```
sudo apt-get -y update && sudo apt-get -y upgrade
 sudo apt-get -y install tightvncserver
Setup your password following the onscreen instructions
Now run the following commands to make it open on startup
cd /home/pi/.config
 mkdir autostart
cd autostart
echo """ [Desktop Entry]
Type=Application
Name=TightVNC
Exec=vncserver :1
StartupNotify=false""" > tightvnc.desktop
sudo raspi-config
Navigate to interfacing options and enable I2C, as well as SPI
sudo shutdown now
```

Test that it all works

1. Unplug the HDMI and USB cables from Pi (even the power supply)
2. Plug the power supply back in
3. From your computer, enter ifconfig and find the Pi's new IP address, suppose it's 192.168.1.6
4. Open VNCViewer and connect to 192.168.1.6:1 (the last numbre is the port the VNC server is running on)

Electronics

Breadboard initial setup:

1. Connect 40 pin ribbon to Raspberry Pi
2. Connect Ribbon to Raspberry Pi Cobbler T
3. Connect the Cobbler to the top of the Breadboard
4. Below that, connect the 3x A4988 Stepper motors
5. Below that, connect the ADS1115 ADC Converter on the right side of your breadboard
6. On that level, connect the TIP120 transistor on the left side of your breadboard

Connecting the pumps

(This step should be repeated 3 times)

1. Using the provided schematic of the A4988, connect the capacitors positive pin to the VMOT and the negative pin to the GND.
2. After connecting the two sides of the negative and positive rails to the side, ground each of the stepper motor drivers (We use black for grounds).
3. To provide them with power, connect the VMOT for each of the stepper motor drivers to the positive rail (We use red for power).
4. Connect the logic ground to the Ground rail.
5. Connect the Pi 3V3 pin to the right side positive rail.
6. Connect the top and bottom part of the rails together.
7. Connect each VDD to a Raspberry Pi 3V3 pin (we also used Red for this).
8. Connect each STEP to a different GPIO pin (we used Green for this).
9. Connect each SLEEP & each RESET pin to a 3V3 supply on the Pi (we used Orange for this).

10. Connect jumpers to the power supply on the left rail and connect the Power Supply, as well as boot up the Raspberry Pi.
11. Connect Pi ground to the ground rail
12. Using a Multimeter, place the negative pin on a grounded pin and the positive one on the screw on the top of the A4988 (pic unavailable due to having too few hands).
13. Screw (to increase) or unscrew (to decrease) Vref for the stepper motor driver. The Vref you should set is given by the following formula. MaxCurrent = Vref * 2.5. Make sure that your Vref does not provide too much or too little current.
14. Since you figured out which pins on each pump are coiled, connect the one coil to A1 and B1 and the other one to A2 and B2. Repeat for all stepper motor drivers. We used colors that matched the motor's wires to the best of our ability.
15. At this point, you should calibrate the pumps by using the calibrate_pumps.py script in the repository.

Connecting the fan

1. Connect the fan's positive end to the positive rail.
2. Connect the fan's negative end to the TIP120's collector.
3. Connect the TIP120's base to a GPIO.
4. Connect the emitter of the TIP120 to the common ground.
5. Edit fan_constant.py with your settings. You might need to steady the fan, since it might move the hole base holder, when spinning at high speeds.

Connecting the Light Dependent Resistor (LDR)

1. Connect the LDR's one end to the ADC's ground.
2. Connect the LDR's other end to the A0, A1 or A2 port.

Connecting the ADC

1. Connect the VDD to a constant 3V3 pin on the Pi.
2. Connect the SDA and SCL to the appropriate Pi pins.
3. Connect the Ground to the common ground.

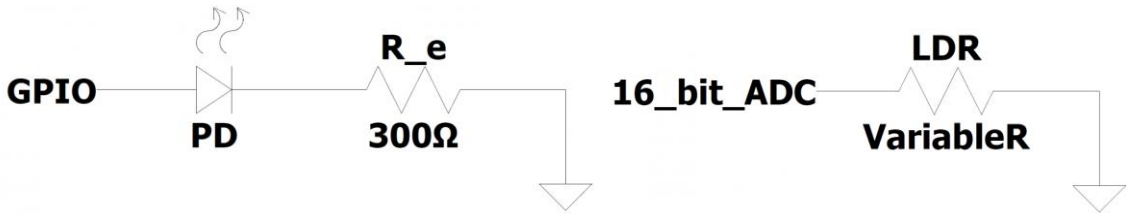
Connecting the LED

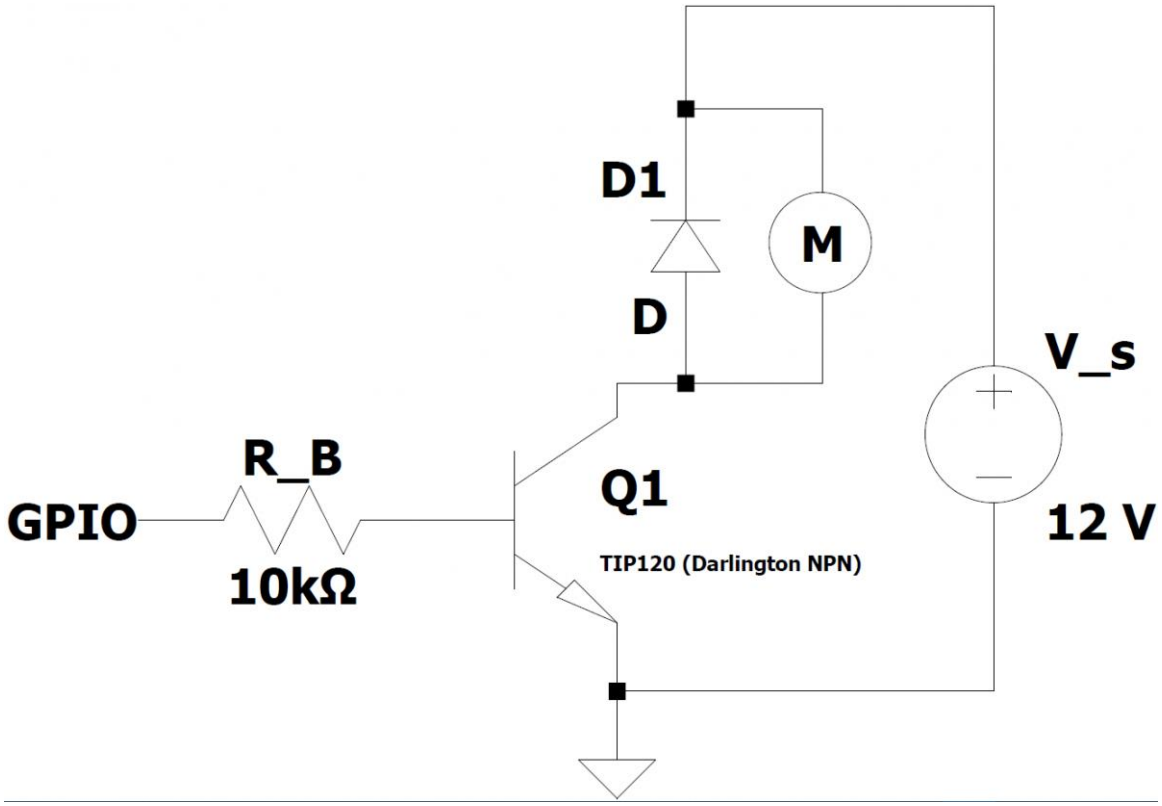
1. Connect the Positive side to a Pi GPIO.
2. Connect the Negative side to a 300 Ohm resistor, which is in turn connected to the common ground.

Other handiwork

1. Drill 3 holes on the bottle cap and fit the tubing through, do not apply too much pressure as the tubing will be rendered unusable, as the forces exerted overpower the ones from the pump.
2. Cover the bottle with ductape and apply silicone to seal gaps.
3. Use parafilm to facilitate oxygen exchange between the culture.
4. Use a small, but hard material that is of approximate dimensions at least 40x2 mm. We just tied two glass slides with ductape.
5. Fit the fan, then the glass slides in the tube holder. Fit the LED and LDR (you might need some padding, we used Blue Tac). Lastly, innoculate 20 mL of your preferred medium with 100 uL of your favourite microorganism, pour it in the vial, and connect the pumps to a medium, an antibiotic and a waste pump.

Lastly, we made use of the code provided in the paper of the Evolutionary Bioreactor (EVE). Clone that repository and follow their instructions to start the container. You should probably edit the file eve-pi/scripts/live/sample-conf.ini.




D1
M
D
V_s
R_B
GPIO
Q1
12 V
10kΩ
TIP120 (Darlington NPN)