

## Construction of Automated Home-Cage System

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### 1. Build an enclosure

- Lay out six **9" construction rails** (XE25L09) and two **20" rails** (XE25L20). Place three of the 9" rails in a "U" shape, with the bottom rail laying on bottom of the two side rails. Join the rails using two **setscrews** (SS25S075) and **drop-in T-nuts** (XE25T1).
- Repeat above step to make another "U" shape.
- Connect two 'U' shape together with 20" construction rails connecting each of top corners. For one side, join the rails with two setscrews and drop-in T-nuts. For the other side, Fasten with two **right angle brackets** (AB90H), **drop-in T nuts**, and 1/4-20 screws.
- Cut one 9" x 22" **thick acrylic board** (8505K758) and fix it at the bottom of the enclosure using ¼-20 screws and drop-in T-nuts.
- Cut two 9" x 10", two 10" x 22" and one 9" x 22" **thin acrylic boards** (8505K745). Attach one 9" x 10" and one 10" x 22" board on the back and right side of the enclosure, respectively, with ¼-20 screws and drop-in T-nuts.
- Cut ten 9" and four 22" strips of **magnetic tape**. Pair together and place adhesive of one of the two paired strips on left side and front rails.
- Remove adhesive on other strip of magnetic tape pairs. Carefully align 9" x 10" and 10" x 22" acrylic sheets with their respective sides. Press firmly to secure adhesive to edges of acrylic sheet.
- Cut a rectangular hole (7" x 7") on the 9" x 22" board and cover it with **metal mesh**. Place the board on the top to cover the enclosure.

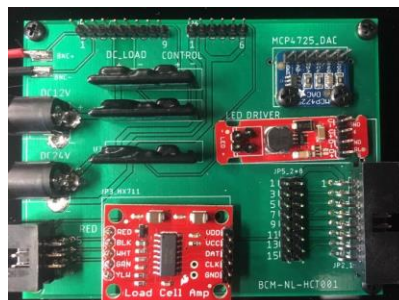


## 2. Fix components on the enclosure side boards

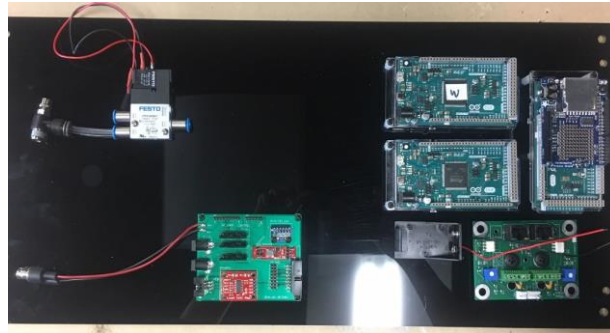
- Get an **analog pressure regulator** (FESTO, 557773). Screw one **L-shape push-in-fit** (5225K564) in the inlet hole, and another **L-shape push-in-fit** (5779K425) in the outlet hole. Screw a **silencer** (9836K21) at the exhaust hole (hole no. 3)
- Get a **manual pressure regulator** (LR-M5-D-7-MICRO) and fit with two **push-in fitting** (QSM-M5-6).
- Fix these two regulators on the back board of the enclosure.



- Get a custom **breakout PCB board**. Soldering three **solid state relays** (CC1139-ND), one **HX711 amplifier** (SparkFun, SEN-13879), one **MCP4725 DAC** (Adafruit, 935), one **LED driver** (SparkFun, SS25S075), one **6-pin 2x3 box header connector** (JP1), one **16-pin 2x8 box header connector** (JP2), one 9-pin (JP4), one 6-pin (JP6) and one 16-pin 2x8 straight header connectors (JP5), on the PCB board.

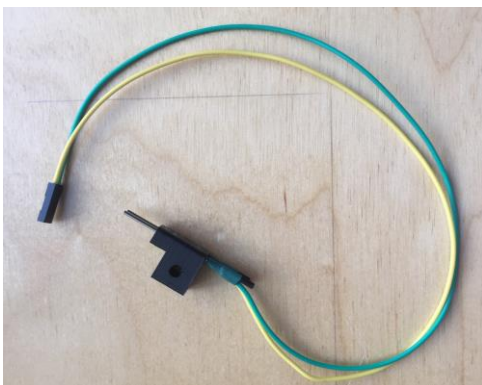


- Fix three **Arduino boards** (Mouse, A000062), one custom **lickometer PCB**, one **9-V battery case**, one **3/2-way solenoid valve** (FESTO, 196847) and custom breakout PCB on the enclosure right board.
- Assemble a **SD card shield** (<https://www.adafruit.com/product/1141>), and insert into Arduino\_Master board.

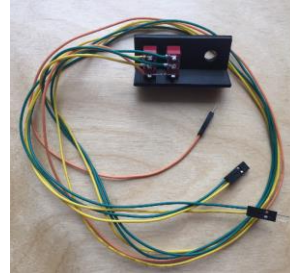


### 3. Lickport Station

- Cut two 18", two 9", and two 2" pieces of 1/32" **plastic tubing** (Tygon E-3603).
- Remove plungers from two **30 mL syringes**. Tape syringes together at top with regular tape. Attach **female luers** (45508-02) to syringes. Attach 18" tubing to luers.
- Attach 9" tubing to top port of two Lee company **solenoid valves** (LHDA1233215H), and 18" tubing from 30 mL syringes to middle ports. Tie each of 2" pieces in knot and slide one end over the bottom ports.
- Cut two 2" pieces of **18G stainless steel tubing** with Dremel tool. Smooth at least one end for licking.
- Slide steel tubing into top two holes of **custom lickport holder**. These holes may need to be reamed out with a #51 drill bit. Place tubes securely with ~ 1/2" sticking out of front end. Glue if necessary.
- Get two 10" wires (different color) and solder one end to steel tubing. Slide piece of **heat shrink** over tubing and soldered wire. Heat with heat gun to shrink material and create tight fit. Crimp the other end to a Female pin connector.
- Slide free ends of 9" plastic tubing over steel tubing on the heat shrink side.

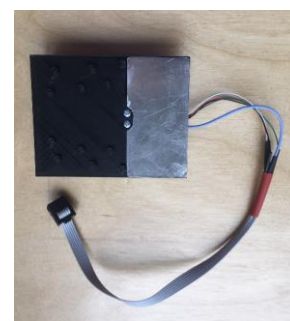
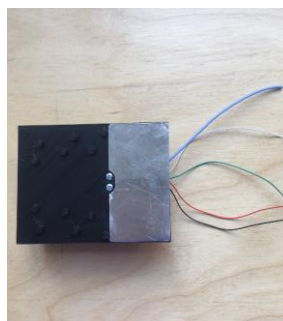
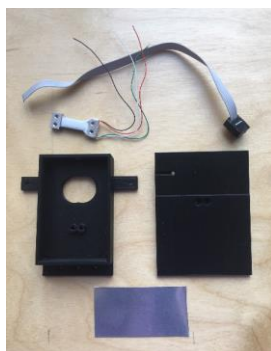


- Get 3D-printed **switch holder** and two **mini toggle switches**, one for left solenoid and one for right solenoid. Fix the switches to the holder.
- Get five of 24" cables. Solder these 5 cables to the switches' pin1, pin2 and pin3 accordingly as shown in the picture.



#### 4. Weighting stage

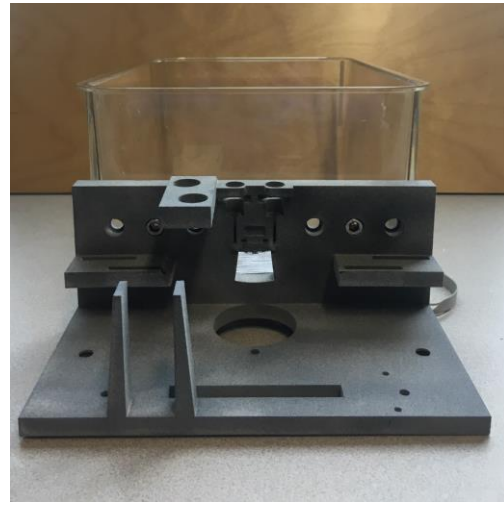
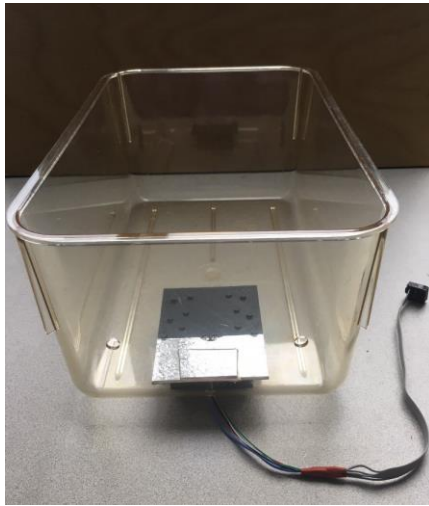
- Get 3D-printed parts **WeightingStage\_bottom** and **WeightingStage\_top**;
- Fix the **micro load cell** (Phidgets, 100g) on the bottom part using two screws (M2.5, 12 mm) and two nuts. Make sure the arrow on the load cell is downward.
- Screw another four screws (M2.5, 8 mm) at the back of bottom part. You can adjust the height of the stage by these screws if necessary.
- Cut a piece of **aluminum sheet** (2.5" by 1.25" by 0.016"). Strip two ends of a wire. Using **aluminum tape** to tape one end of the striped wire on the aluminum sheet. Route the other end of the wire out of the hole of WeightingStage\_top part and then out of the hole of WeightingStage\_bottom part. Glue the aluminum sheet on top of WeightingStage\_top part and let the side with the wire attached facing down.
- Secure the top part on the load cell using another two screws and nuts.
- Get a 40cm long of flat Ribbon cable with both ends of 6-pin (2x3) female connector and cut to two 20cm long cables. Solder the 4 wires from load cell and one wire from the aluminum sheet to the open end of the ribbon cable (see Table1 for the connector pinout).



**Table 1. 6Pin (2x3) female connector Pinout**

| Connector Pin# | Weighting Stage Wire |
|----------------|----------------------|
| Pin 4          | AL Sheet             |
| Pin 2          | RED                  |
| Pin 5          | BLACK                |
| Pin 3          | WHITE                |
| Pin 1          | GREEN                |

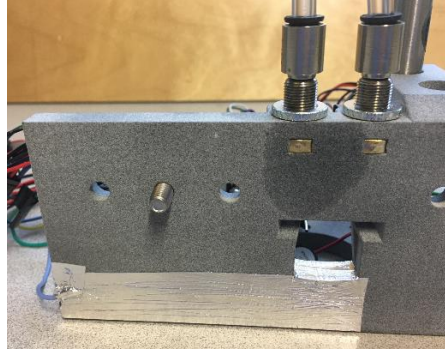
- Align the custom 3D-printed **headport** and **weighting stage** assembled above against one side of a **home-cage**. According to the positions of the headport, drill two holes (7 mm) and one opening (~30 mm x 30 mm). The headport should be attached to the home-cage such that the lower edge of the home-cage opening is trimmed with the weighting stage and the lower edge of the headport opening is a bit higher than the weighting stage.
- Drill a small opening (~15mm x 10mm) on the lower side of the home-cage to allow the weighting stage ribbon cable connector (2x3 female connector) go through.
- Align the weighting stage on the floor of the home-cage, centered around the opening. Mark the mounting locations and drill 4 of threaded holes (4-40) on the floor. Fix the weighting stage to the threaded holes using size 4-40 screws.



## 5. Headport

- **Apply aluminum tape on the headport:**
  - Apply **aluminum tape** on the floor of the opening in the headport and route it to the side of the headport by another long piece of aluminum tape.
  - Get a **wire**, strip two ends and attach one end to the aluminum tape and fix them to side of the headport. The other end will be soldered to the Headport ribbon cable.





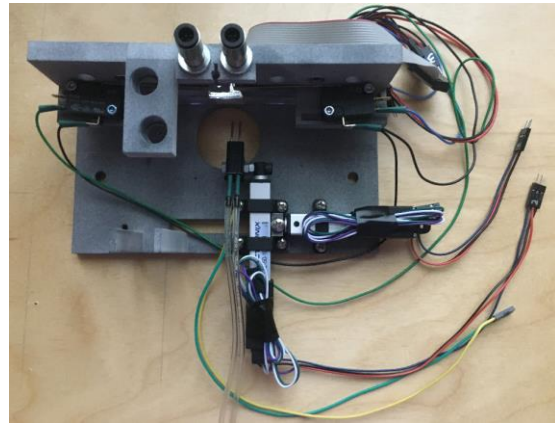
- **Set up switches:**

- Get two **snap action switches (D429-R1ML-G2)**.
- Bend the first 3 mm part of the lever outward to form a 90-degree angle.
- Fix the two switches on each side of the headport's switch holder with **25 mm screws**. Adjust the position to make sure the switches can be triggered only when the headbar reaches the end of the headport track.
- Connect pin1 of the two switches by soldering a jump wire.
- Solder jumper wires to pin4 of each switch and pin1 of one switch; these wires should be with the other end free so that they can be soldered to the Headport ribbon cable.



- **Set up motors:**

- Get one 50 mm and one 30 mm stroke **motor (Actuonix, L12)**.
- Get a 3D-printed **motor holder** and thread the holes with 6-32 and 8-32 tap. Fix the 50 mm motor on the tip of the 30 mm one in an orthogonal way using the 3D-printed holder with 6-32 x 1/4" screws and 8-32 x 1/2" screw.
- Fix the lickport holder on the tip of 50 mm motor (using one 25 mm M4 screw and three nuts).
- Thread the two holes located at the lower right side of the headport floor using 6-32 tap. Fix the motors on the headport floor using a bracket set which is came with the motor and 6-32 x 3/8" screws. Be sure to put a thin washer under the bracket to adjust the gap between the motor and the headport floor.
- From each motor, take red, black and blue wires and put them together into a 1x3 pin connector housing to get ready to connect to the Headport ribbon cable.



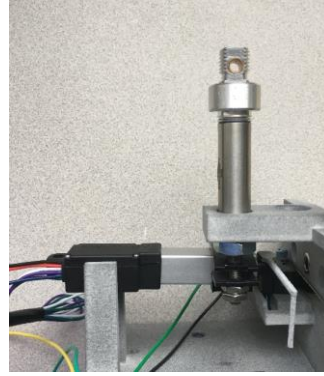
- **Set up pistons:**

- Get two 1/4" OD Air Cylinder Pistons (McMaster, 6604K11) (with hex nuts). Grind the hex nuts outer shape to match the headport by Dremel as shown in the picture.
- Insert two shaped hex nuts in the head port, screw the pistons in the piston holes, and fix them with two Panel Nuts (McMaster, 5601396540).
- Screw two Push-in-Fitting connectors (McMaster, 5779K246) in the air inlet holes of the pistons.



- **Set up pole mechanism:**

- Get one Air Cylinder Pistons (McMaster, 6498K999). Insert the piston into the hole of pole holder of headport and screw one hex nut (10-32) on the rod of the piston until flush and.
- Get one 3D printed pole and one 30 mm stroke motor (Actuonix, L12-30). Insert the pole into the hole at the tip of motor. Take red, black and blue wires from the motor and put them together into a 1x3 pin connector housing to get ready to connect to the Headport ribbon cable.
- Fix the motor at the end of the piston with 3D printed bracket. Screw two hex nuts (3/8"-24) at the end until flush.
- Screw one Push-in-Fitting connectors (McMaster, 5779K246) in the air inlet holes of the pistons.



- **Set up Piezo Buzzer**

- Get a **Piezo Buzzer** (490-CPE-160).
- Get one cable with pre-crimped terminals and cut it to two cables. Solder the free end of the cable to the Buzzer and have the other end ready to connect to the headport ribbon cable.
- Fix the buzzer on the Headport floor.



- **Built the headport ribbon cable**

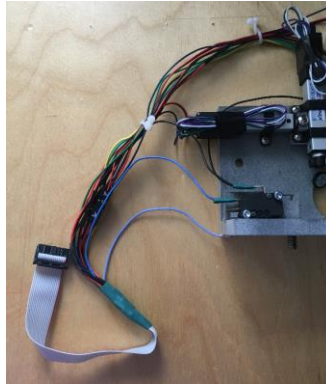
- Get a 40cm long of **flat Ribbon cable** with both ends of 16Pin (2x8) female connector and cut it to two 20cm long cables to make the headport ribbon cable.
- Connect all the electric components located at the headport to the headport ribbon cable (see Table2 for the connector pinout).

**Table2. 16Pin (2x8) female connector Pinout:**

| Connector Pin# | Headport Electric Components Wires                                    |
|----------------|---|
| Pin 1,3,5,7,16 | NC  |
| Pin 9          | Piezo Buzzer + (Red)  |
| Pin 11         | Switch L (pin4)   |
| Pin 13         | Switch R (pin 4)  |
| Pin 15         | Motor LR/FB/Pole GND (Black), Switch Com(pin1), Piezo Buzzer- (Black) |
| Pin 2          | Motor LR/FB/Pole Power (+12v, Red)                                    |
| Pin 4          | Motor LR Input (Blue)   |



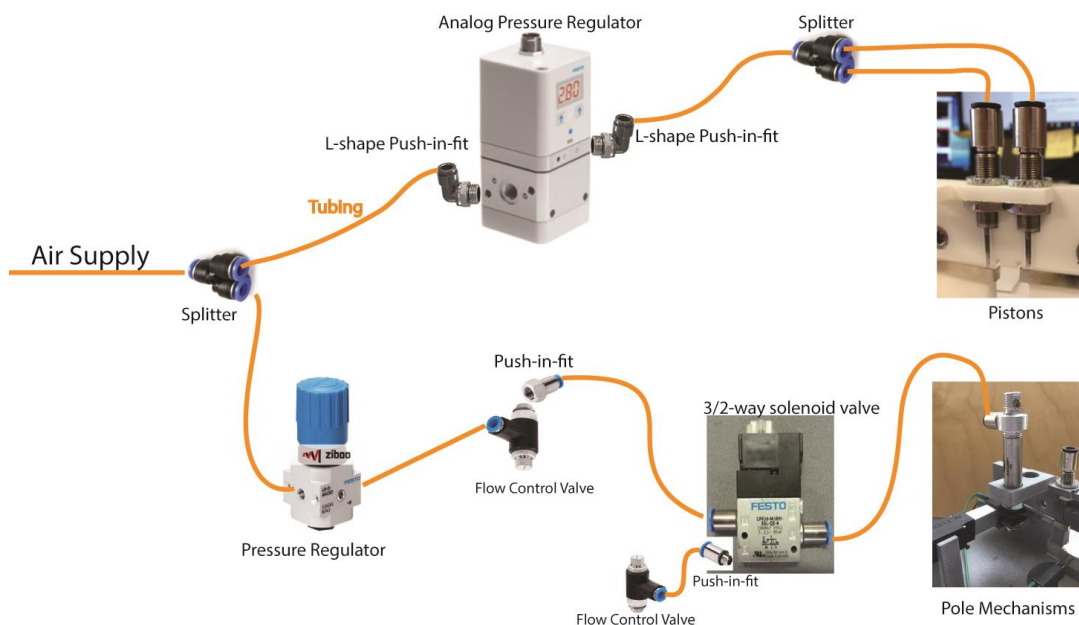
|        |                         |
|--------|-------------------------|
| Pin 6  | Motor FB Input (Blue)   |
| Pin 8  | Motor Pole Input (Blue) |
| Pin 10 | Lickport Right          |
| Pin 12 | Lickport Left           |
| Pin 14 | Headport AL Tape        |



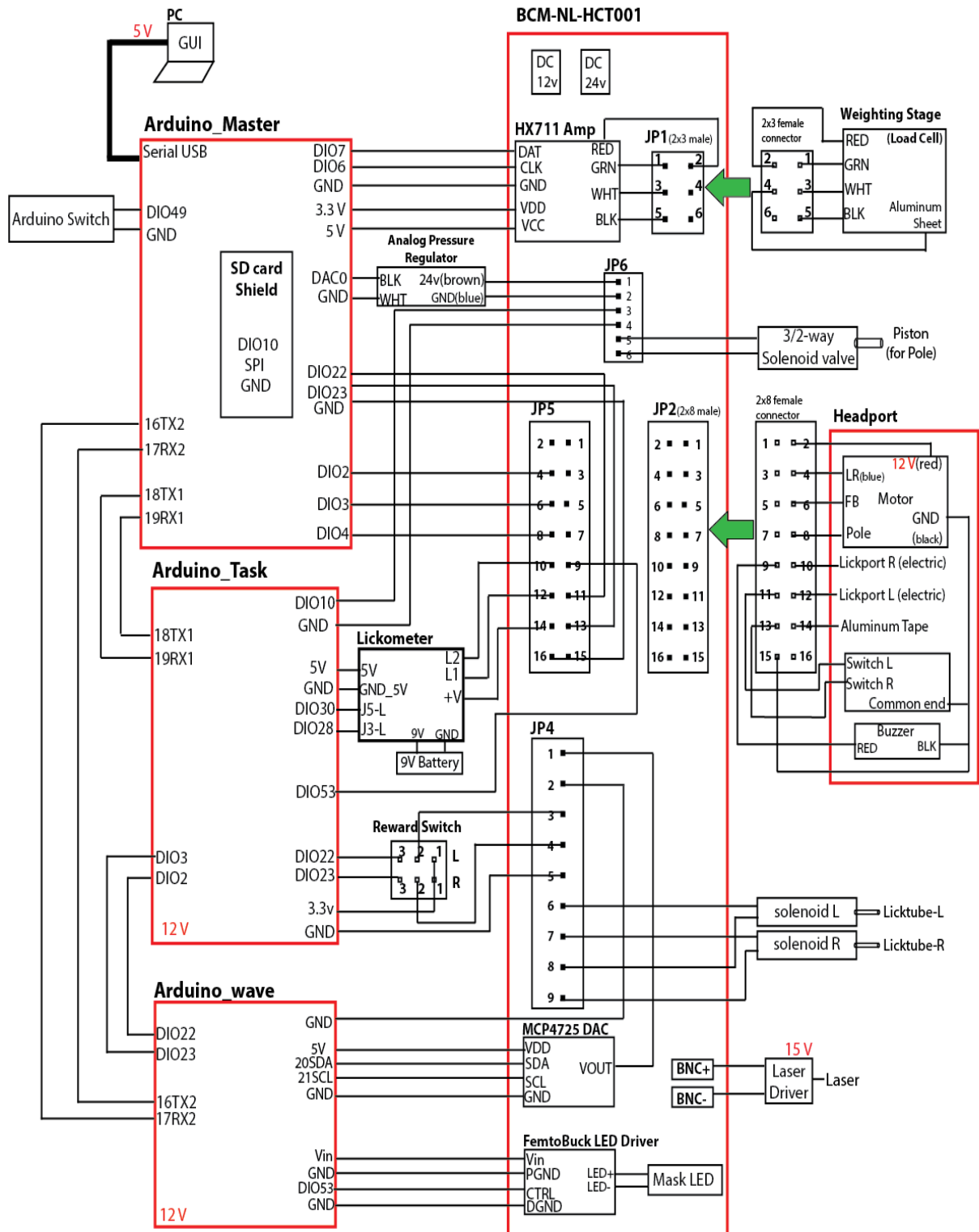
## 6. Pneumatics

- **Set up plumbing:**

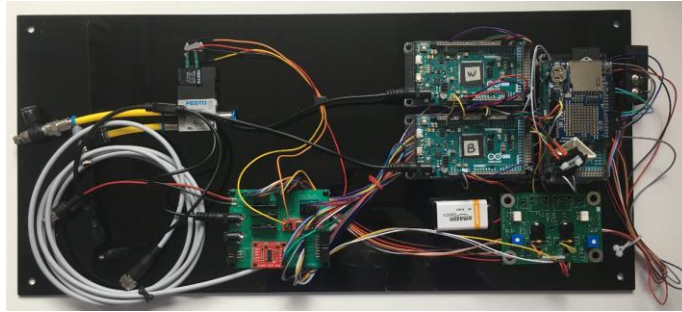
- Attach a **push-in-fit** (QSM-M7-6-I) in the exhaust hole of the 3/2-way solenoid valve (hole no. 3). Connect to a **flow control valve** (GRLA-1/8-QS-6-RS-D) using **6-mm tubing** (Freelin-Wade, 1E-158-08)
- Get another flow control valve, attach a **female push-in-fit** (QSF-1/8-6-B) on the threaded port.
- Connect all the pneumatic parts using 6-mm tubing according to chart below.
- Attach **power/control cable** (SIM-M12-5GD-2,5-PU) to the analog pressure regulator and connect to the Arduino board (see below).



## 7. Connect wires and assemble



- Connect the electric circuit according to the above diagram.



- Mount the Lee solenoids, reward switches and Arduino switch on the enclosure frame.



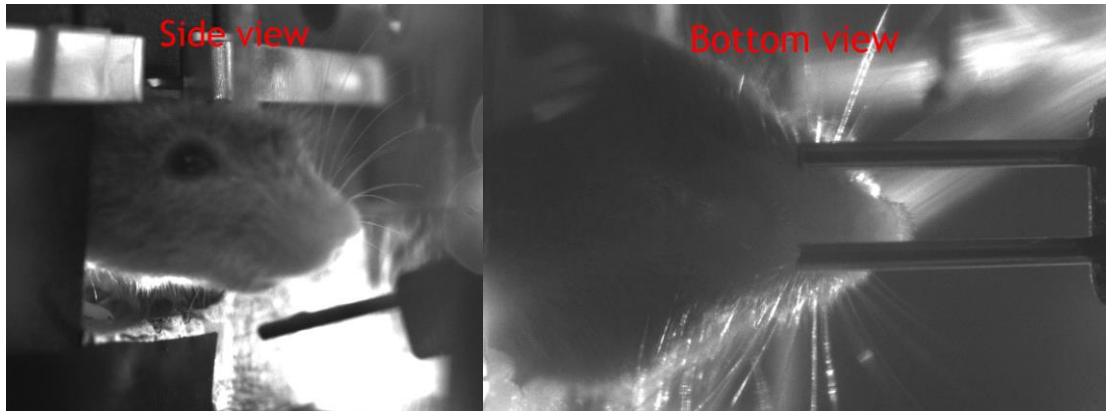
## 8. Testing and Operating

- Supply the plumbing system with **4-bar air pressure**; adjust the pressure regulator for the pole to **2 bars**. Make sure there is no gas leaking.
- Power up the **12V and 24V adapters**, manually connect male wire in pin 10 of Bpod to 3.3 V to see if the **piston for pole** can drop smoothly. When finished, unplug the **12V and 24V adapters**.
- Insert a battery to SD card shield. Copy all the files in the folder “final config files” to a SD card and insert it to SD card shield.
- Upload “**correctTime.ino**” to Arduino\_Master board to correct the on-board Date/Time.
- Upload “**Weight\_Calibration.ino**” to Arduino\_Master board and open real\_term.exe to receive serial info. Put an object with known weight and make sure the readings roughly match the weight.
- Upload “**waveSurfer.in**”, “**Bpod\_Firmware\_Home-cage.ino**” and “**Controller\_3motors.ino**” to Arduino\_Wave, Arduino\_Task and Arduino\_Master board, respectively.
- Power up the **12V and 24V adapter**. Open **home-cage\_gui** program in MATLAB (or **realterm.exe**) to receive serial information.
- Turn on the **Arduino Switch** connected with the Arduino\_Master, the **left/right motor** should move to the initial position 70. Adjust the location of the **left/right motor** to make sure the lickport is roughly at the center of the head port. Also adjust the location of the **forward/backward motor** to make sure the tip of the lickport is around 14 mm from the wall of the headport (when the motor position is around 100, it should just touch the wall).
- Fill the **water reservoir** with water and toggle the reward switch to “Flush” side (pin1) to turn on the solenoid valve and fill the whole tube with water.
- Trigger the two switches in the headport. Make sure the following items working properly:

- **Pistons for head-fixation:** coming down and retracting smoothly, fixation for 60sec.
- **Piston for pole:** dropping and retracting smoothly, otherwise, adjust the flow control valve.
- **Motor for pole:** moving forward and backward smoothly.
- **Masking LED:** flashing during trial for ~5 sec.
- **Sound buzzer:** making a go cue sound.
- **Weighting stage:** during head-fixation, push the weight stage, the pistons for head-fixation should be released.
- Check if the **lickometer board** can detect licking by measuring the voltage between the leftmost and rightmost pins in J3 and J5. The resting voltage is around 0.3 V and the voltage goes to 3.8 V when you connect the lickport with the aluminum tape by your hand.
- Check if the left and right lickports can be detected separately and correctly by connecting either the left or right lickports with the aluminum tape by your hand right after you heard the go cue sound in each trial.
- Toggle the **Arduino Switch** connected to the Controller. You should see a drop of water coming out of both lickports when switch is toggled from OFF to ON.
- To turn off the system, you should turn off **Arduino Switch**, unplug USB cable from PC, unplug 12V adapter and 24V adapter from power outlet, turn off air supply sequentially.
- To turn on the system, you should turn on the air supply, plug 12V adapter and 24V adapter to power outlet, plug USB cable to PC, turn on the **Arduino Switch** sequentially.
- **Note:**
  - if you want to reset all three Arduino boards, reset Arduino\_Task and Arduino\_Wave first and then Arduino\_Master.
  - If you want to remove SD card from Arduino\_Master, make sure the switch is turned OFF; turn it ON after you re-insert the card.

## 9. Launch the auto-training cage

- Move the whole enclosure box onto the shelf.
- Supply the plumbing system with 4-bar air pressure.
- Copy '**PARAF.TXT**' and '**PARAS.TXT**' files under folder '**initial config files**' to a blank SD card and insert the SD Card into the SD card shield. Be sure the **Arduino switch** is toggled to the OFF position.
- Adjust the height of the lickport to make sure it is higher than the bottom edge of headport opening.
- Power up the 12V and 24V adapter.
- Connect Arduino\_Master to USB hub via USB cable. The lickport will be moved into the headport opening.
- Turn the switch to ON, the pole will drop down and trial 1 started. Manually touching the lickport will trigger another trial.
- Open MATLAB on PC and start **home-cage\_gui** program. Set the mouse name, start date and the com port in the corresponding cage block. Click open button to start receiving data from Controller.
- Put bedding, food, hiding tube into the home-cage.
- Put the mouse into the cage and Close lid and the enclosure.
- During Auto-training, check and adjust the lickport position periodically:



- When the lickport retracts out of the headport, adjust its height to the level of the bottom edge of headport opening (a little higher) and make sure it is still at the center of the head port opening in left-right direction.
- When lickport reaches the final position, there is a one-time adjustment of the position to make sure the mouse can access both of the lickports and they are roughly in the center (see bottom view above). To adjust the forward/backward position, unscrew the motor and move it forward/backward so that the mouse can just reach the lickport. To move the motor in left/right direction, change the parameters in Matlab GUI (lower right corner).