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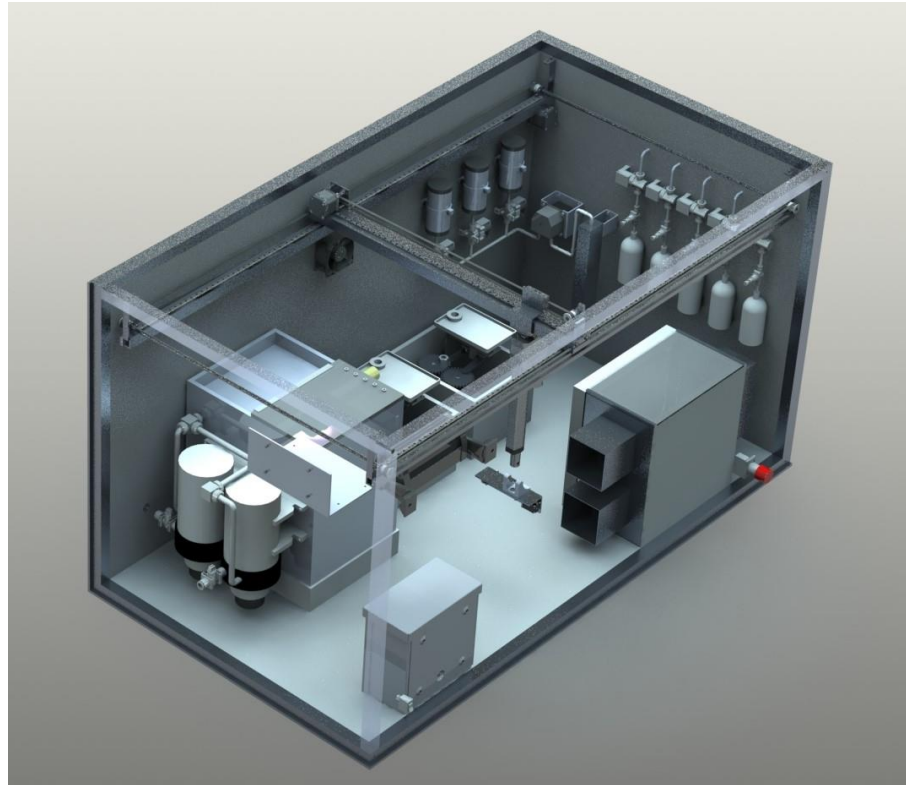
# Dr. Clean Bioreactor

*Spring 2021*

Section 22695, Group 10

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# Overall Assembly



# Hedgehog Concept

- This system will carry out an experiment from start to finish, being ready for the next experiment with little to no additional work to the user. The sanitation system incorporated in the bioreactor will be able to safely dispose of culture materials and sanitize the equipment used, making the system ready for the next experiment. The well plates and conical tubes, still filled with cell culture media, are placed into a sanitation chamber that will cull the cell cultures and dispose of solid and liquid waste, leaving sanitized well plates and tubes for future use.

# Description

- The Doctor Clean Bioreactor grows bacteria cultures with little to no additional work to the user. The sanitation system incorporated in the bioreactor will be able to safely dispose of culture materials and sanitize the equipment used, making the system ready for the next experiment. The well plates and conical tubes, still filled with cell culture media, are placed into a sanitation chamber that will cull the cell cultures and dispose of solid and liquid waste, leaving sanitized well plates and tubes for future use. The Dr. Clean Bioreactor uses a manipulator system to interact with the cell cultures and other system features, including a fluid handling system capable of providing nutrients to cultures, a shaker system capable of linear, orbital, and double orbital motions to aerate cultures, and a climate-controlled storage system. Optical density and fluorescent intensity measurements will be taken frequently to monitor cell growth within the enclosure. The assembly can maintain consistent temperature within the interior to protect electrical components as well as a reasonable temperature on the exterior to allow for movement of the overall system within a laboratory environment.
  - The Doctor Clean Bioreactor was designed for parameters set by the UF Biofoundry and to fulfill customer needs metrics outlined by industry experts. Prioritization of meeting these needs and outperforming previous iterations of bioreactor designs is critical to design evaluation and future performance in the final down-selection stages of the project.
- **Costs:**
    - OTS Parts: \$7138.17
    - Modified OTS Parts: \$182.67
    - Raw Materials: \$1001.02
    - Manufacturing Labor: \$834.78
    - Energy Consumption: \$55.44
    - Assembly Labor: \$520.00
    - Total: \$9732.08



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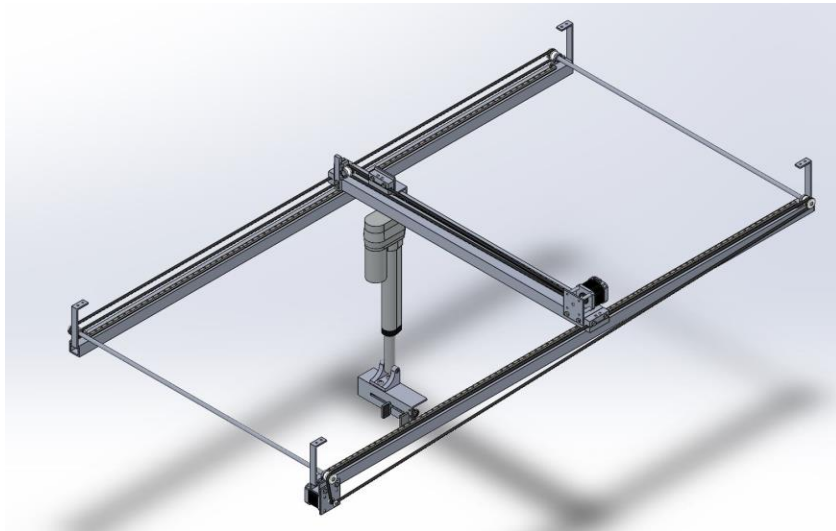
# Manipulator Subsystem

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# Addressed Customer Needs

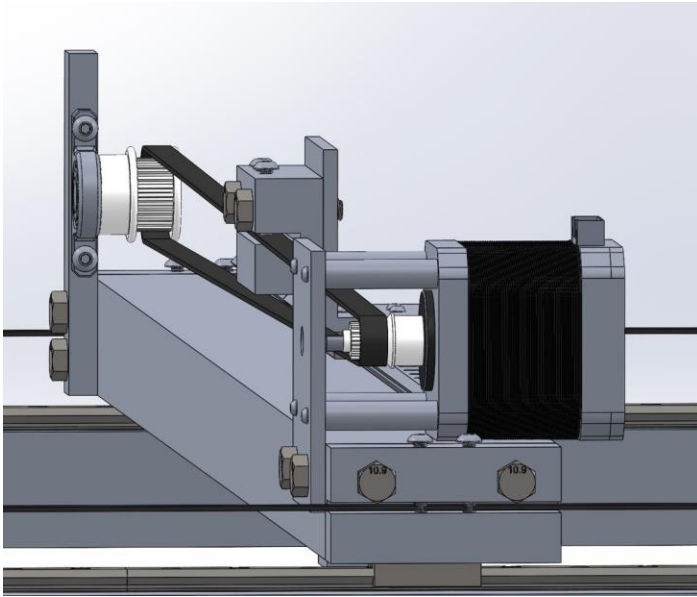
- Requirement 1: Operation lifetime of at least 10 years.
- Requirement 2: Prototype production cost does not exceed \$10,000. •
- Requirement 3: Movable by one person after disassembly.
- Requirement 5: Runs from a single standard 120 VAC wall outlet.
- Requirement 11: only nonporous materials contact cell cultures
- Requirement 12: only nonreactive materials contact lab chemicals
- Requirement 16: culture microbes are in fully enclosed vessels that are interchangeable
- Requirement 19: Is capable of incubation periods long enough to permit 1000 E. coli culture generations (~two weeks)
- Requirement 25: Accommodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384
- Requirement 26: Accommodates existing conical tubes of the following sizes: 15mL and 50 mL

# Manipulator System



- The most important needs are to make sure that the manipulator can accommodate all provided well plate and tube sizes, as well as being able to be able to interchange these units.
- The main components of this system are as follows, the Y drive, the X drive, a linear actuator system, and the gripper itself.
- Main focus of the system is to access as much of the structure as possible.

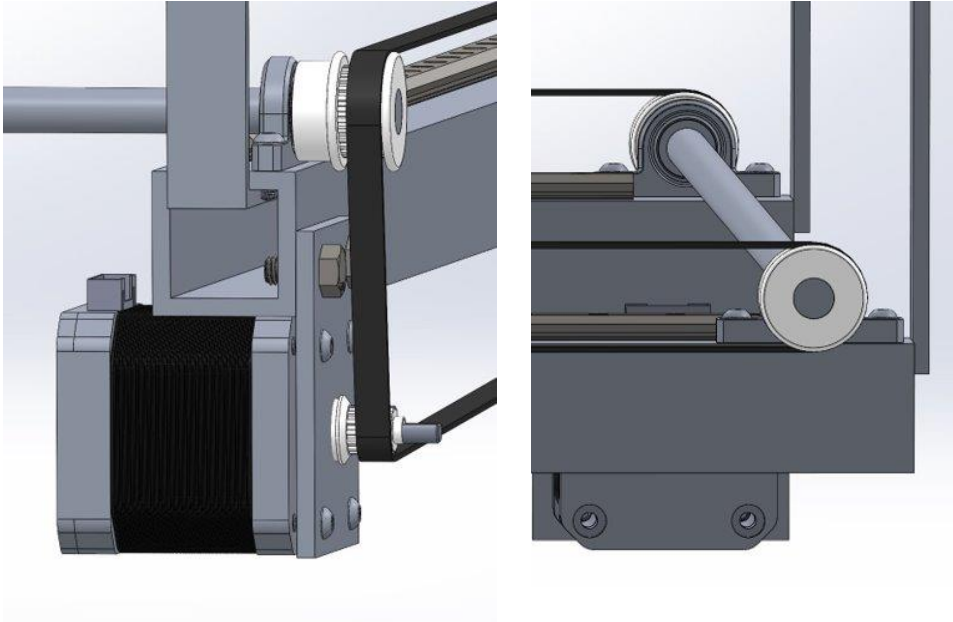
# X-Drive



- The X drive is composed of a belt drive pulley system, powered by a Nema-17 motor, and complete with a tensioning system
- The X drive is attached to the belt with a clamp system, which is equipped with set screws to press down onto the belt.
- A low profile ball bearing is attached on the opposite side of the drive to allow for smooth rotation of the driven pulley.
- Although the power to move the system is provided by the belt system, the weight of the system is supported by a gliding rail.

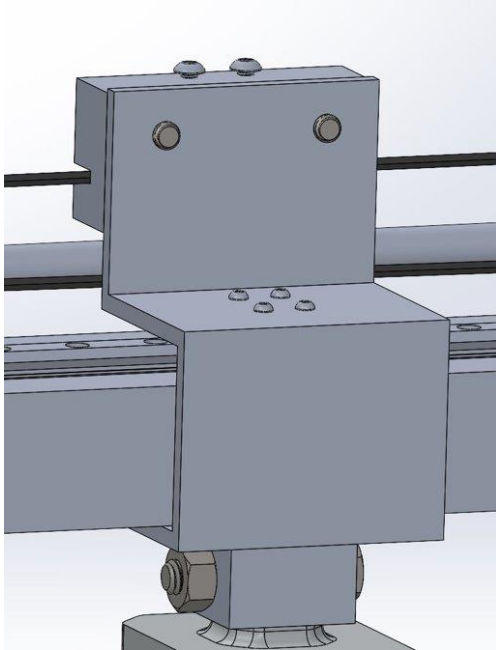


# Y-Drive



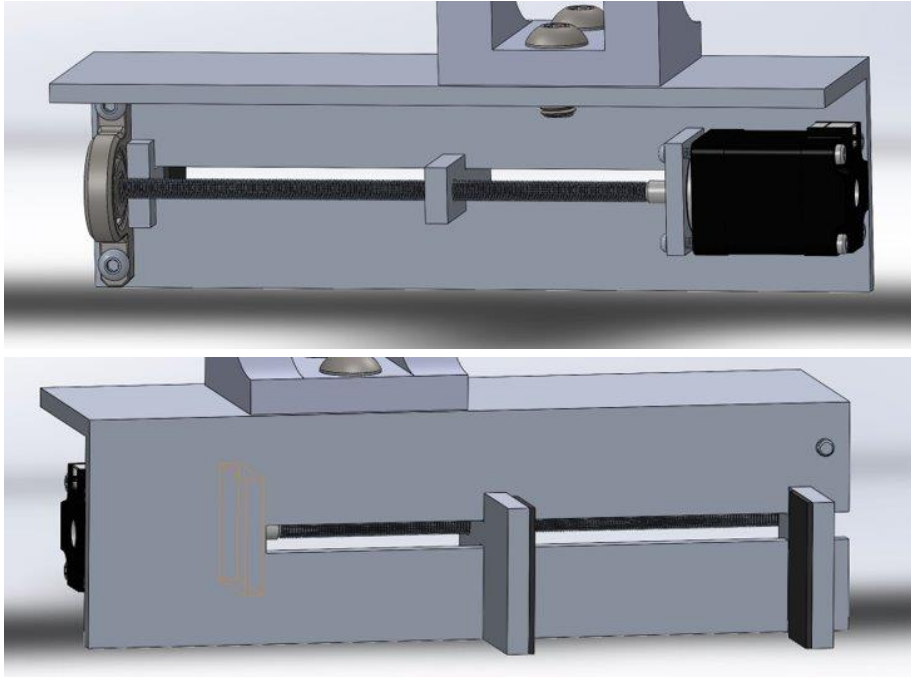
- The Y drive is composed of a belt drive pulley system, which is also powered by a Nema-17 motor.
- The Y drive is attached to the belt with the same clamp system as the X drive.
- Aluminum shafts connect the driving side of the subsystem to the driven side, equalizing power distribution on each side.

# Linear Actuator



- The 6" linear actuator allows for the gripper to move in the Z axis, and it is mounted to the X drive using a custom built bracket.
- The bracket is mounted onto the guide rail of the X drive.
- The linear actuator is mounted onto the bracket using a screw.

# Gripper

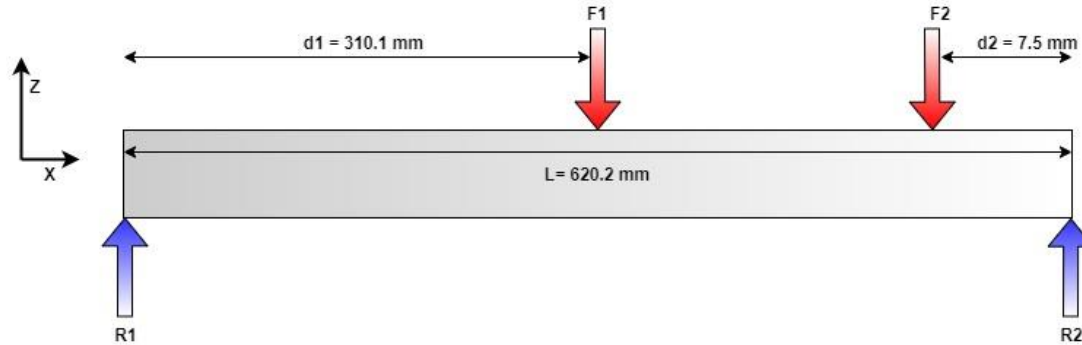


- The gripper is inspired by the mechanism in adjustable wrenches.
- While one gripper jaw is fixed, the other is moved by using a Nema-8 motor, a lead screw, and a bearing mounted opposite of the motor.
- The jaws are fitted with rubber pads which increase the friction on the gripper, raising the reliability of the mechanism.

# Motor Torque Calculations

- Assuming that the max torque  $T_{total}$  needed from the motors is to accelerate the Y drive, the following calculations were made.
- $T_{total} = T_{const.} + T_{acc.}$  where  $T_{const.}$  is the torque needed to sustain a velocity, and  $T_{acc.}$  is the torque needed to accelerate the system.
- $T_{const.} = \frac{F(N) \times r(mm)}{1000 \frac{mm}{m} \times \gamma}$  where  $F = mass \times g \times \mu$ ,  $\mu$  being the friction coefficient of the rails and  $g$  is the force due to gravity.  $r$  is the radius of the driving pulley, and  $\gamma$  is the belt system efficiency (assumed to be 95%).
- $T_{const.} = J_{total} \times \alpha$  where  $J_{total}$  is the total inertia of the mass and  $\alpha$  is the max angular speed.  $J_{total} = mass \times (r(m))^2$  and  $\alpha = \frac{2\pi \times N}{60 \times t}$  where  $N$  is the desired speed and  $t$  is the time required.
- After plugging in values for these equations, the required torque to move and sustain motion in the system is found to be 0.001928 Newton meters, which is well under the provided torque of the Nema-17 motor (0.313813 Newton meters).

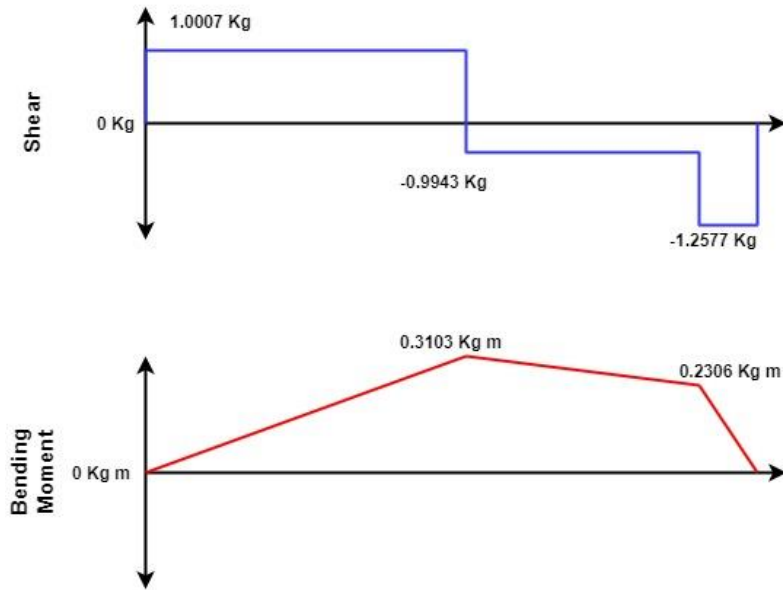
# Bending Moment Calculations



- To make sure that the X-Drive could handle the weight of the linear actuator and manipulator, a bending moment problem was set up as shown above.
- The force  $F_1$  is equal to 1.99495 Kg and represents the weight of the linear actuator and manipulator, while the force  $F_2$  is equal to 0.2634 Kg and it represents the weight of the Nema17 motor as well as its motor mount.
- By setting the moments in the X axis equal to zero:  $\sum M_x = 0 = -(F_1 \times d_1) - (F_2 \times d_2) + (R_2 \times L)$  and solving for  $R_2$ , we find it is equal to 1.2577 Kg
- Furthermore, by setting the sum of forces in the Z axis equal to zero, we find that  $R_1$  is equal to 1.0007 Kg:

$$\sum F_z = 0 = R_1 - F_1 - F_2 - R_2$$

# Bending Moment Calculations Cont'd



- Using the calculated values for  $R_1$  and  $R_2$ , the shear and bending moment diagrams are sketched as shown.
- Assuming the stress due to shear is negligible, the max bending stress is then calculated.
- $\sigma_{b,max} = \frac{M \times y}{I}$  where  $I = \frac{BH^3 - bh^3}{12}$  for a rectangular tube
- $\sigma_{b,max} = 180331.4 \frac{\text{kg f}}{\text{mm}^2} = 1.7685 \text{ MPa}$
- This is well under the yielding stress of 1060 aluminum alloy, which is  $S_y = 75.8 \text{ MPa}$  so therefore the beam will not fail.

# Screw Stress Calculations

- Calculations were made to ensure that the screws could support the weight of mounting the manipulator to the ceiling of the structure.
- The factor of safety was calculated with the following equation,  $n = \frac{S_p A_t}{CP + F_i}$ , where  $S_p$  is the proof load,  $A_t$  is the tensile stress area of the screw,  $C$  is the stiffness constant,  $P$  is the load per screw, and  $F_i$  is the preload.
- The stiffness constant factors in the screw stiffness and the member stiffness,  $C = \frac{k_b}{k_b + k_m}$
- The bolt stiffness is derived as such,  $k_b = \frac{A_t E}{l_t}$
- The member stiffness is found by solving this equation,  $k_m = \frac{P}{\delta} = \frac{\pi E d \tan(\alpha)}{\ln \left[ \frac{(2t \tan(\alpha) + D - d)(D + d)}{(2t \tan(\alpha) + D + d)(D - d)} \right]}$
- The preload is determined by  $F_i = 0.75 S_p A_t$
- After all the variables were substituted by known values, the factor of safety came out to be  $n=1.2411$ , which means that the structure would be securely fastened with these screws.



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# Shaker Subsystem

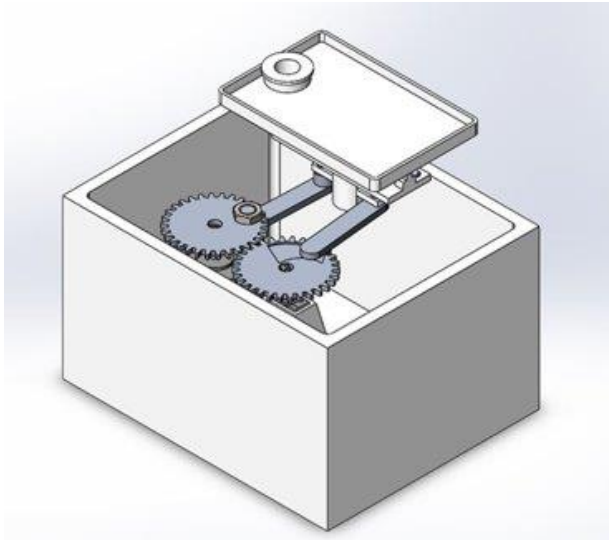
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# Addressed Customer Needs

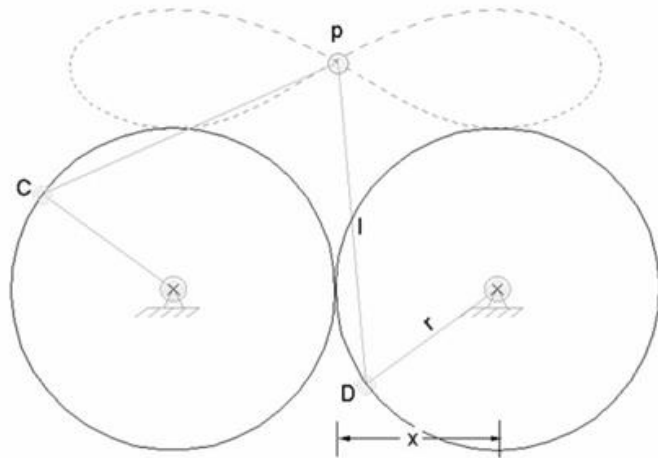
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- Requirement 19: Is capable of incubation periods long enough to permit 1000 E. coli culture generations (~two weeks)
- Requirement 25: Accommodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384
- Requirement 26: Accommodates existing conical tubes of the following sizes: 15mL and 50 mL
- Requirement 28: Shakes well plates and tubes in linear, orbital, and double orbital patterns
- Requirement 29: Has shaking patterns that are independent for each well plate or tube
- Requirement 40: No cross contamination between individual wells/tubes during liquid handling

# Shaker System



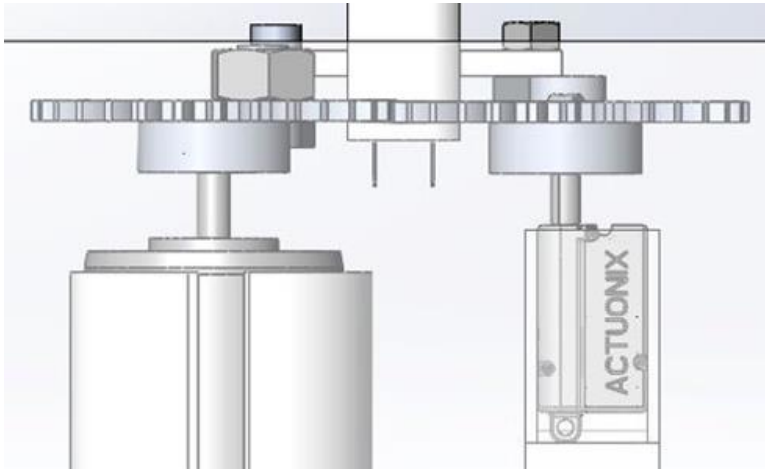
- Shaker enclosure protects gear and motor systems

# Double Orbital Motion



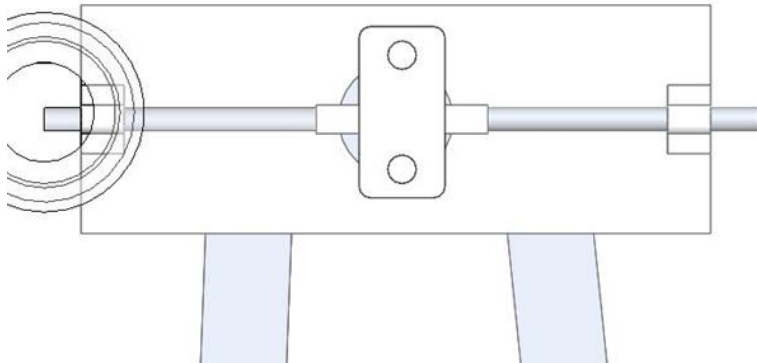
- The double orbital motion is created using two identical gears with rods attached at their ends, and one gear being driven by a motor
- Connector point  $P$  is the motor for the linear motion, which is centered on the shaker platform

# Single Orbital Motion



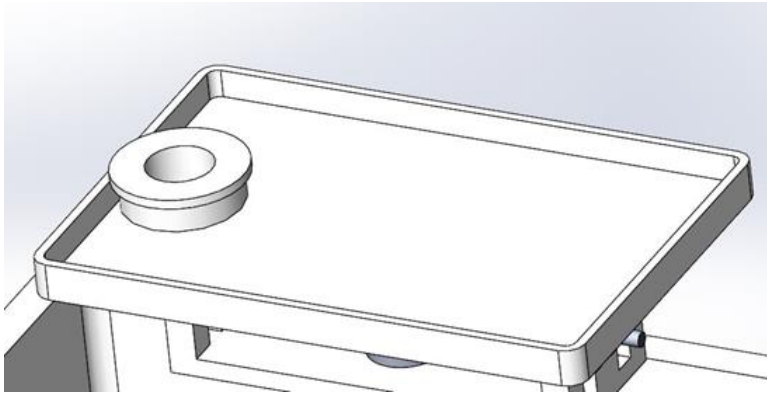
- Linear actuator used as gear shifter disengages second gear, causing single orbital motion from single gear and motor

# Linear Motion



- A scotch yoke mechanism creates linear motion

# Shaker Tray



- Tray can accommodate well plates of all sizes
- Both tube sizes are accounted for with an insertable plug seen to the left of the tray

# Shaker Calculations

- Calculations were done to determine motor requirements, strength requirements of the rods connecting the gears to the scotch yoke mechanism, and to model what a spring damper system's requirements would be to be able to model different values for the spring stiffness and damping coefficient.

- Motor Requirement Calculations

- Drive motor:

- $T = 0.1312 \text{ Nm}$      $T = FR = mgR$

- $P = 4.123 \text{ W}$      $P = T\omega$

- Scotch Yoke motor:

- $T = 0.0116 \text{ Nm}$

- $P = 0.121 \text{ W}$

- Bending in support rods

- Stress w/ 1.2 FoS = 5734.4 Pa     $\sigma = Mc/I$

$$I = bt^3/12$$

- Vibrational analysis

- $F_{max} = 0.445 \text{ N}$

$$m\ddot{x} + c\dot{x} + kx = F_{max}$$

$$x = r\sin(\omega t)$$

$$v = \frac{dx}{dt} = -x\omega\cos(\omega t)$$

$$a = \frac{dv}{dt} = -x\omega^2\sin(\omega t)$$

$$F_{max} = ma_{max} = -m\omega^2 = m\alpha$$

- Natural frequencies

- Structure: 3251.6 Hz

- Shaker system: 263.8 Hz

- Shaker Rods: 584.1 Hz



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# Liquid Handling Subsystem

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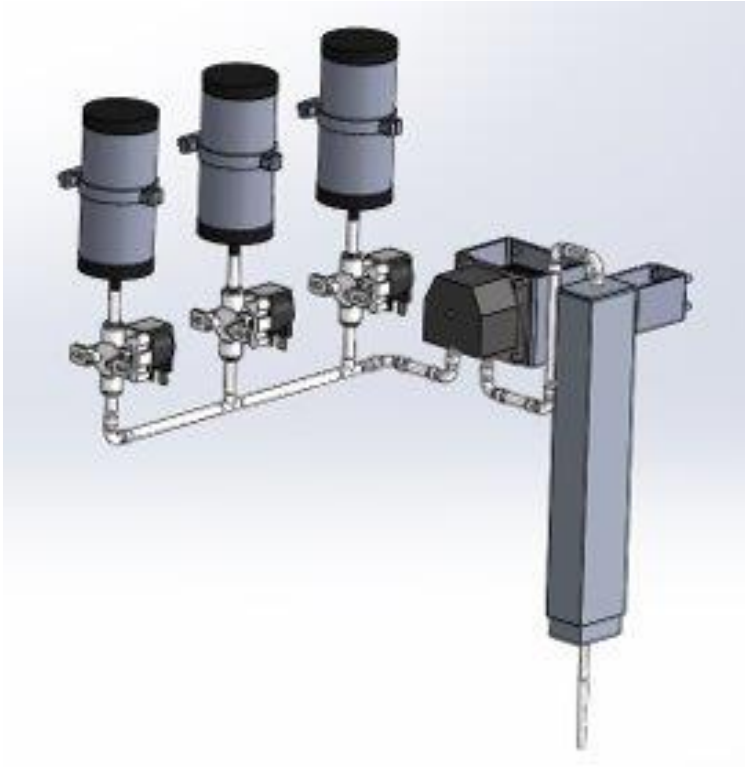


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- Requirement 11: only nonporous materials contact cell cultures
- Requirement 12: only nonreactive materials contact lab chemicals
- Requirement 16: culture microbes are in fully enclosed vessels that are interchangeable
- Requirement 18: Is capable of safely injecting, measuring, and regulating the composition of the following gases in each compartment holding a well plate or tube: N<sub>2</sub>, O<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>
- Requirement 19: Is capable of incubation periods long enough to permit 1000 E. coli culture generations (~two weeks)
- Requirement 25: Accommodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384
- Requirement 26: Accommodates existing conical tubes of the following sizes: 15mL and 50 mL
- Requirement 34: automated liquid handling with fluid addition/subtraction from each well or tube
- Requirement 35: Dispenses fluid without creating aerosols
- Requirement 36: Achieves dispense rates from 225 uL/s to 300 uL/s
- Requirement 37: Deposits a minimum/maximum aliquot fluid volume from 5–20,000 µL
- Requirement 38: Achieves dispensing volume accuracy of  $\pm 0.1$  µL
- Requirement 39: Achieves dispensing volume precision  $\pm 0.01$  µL
- Requirement 40: No cross contamination between individual wells/tubes during liquid handling

# Fluid Handling System



- The fluid handling subsystem addresses the needs for satisfactory moving cultures suspended in liquid between compartments and providing nutrients in media to cultures.
  - No aerosol creation
  - No cross contamination
- This fluid handling system will be static and well plates and tubes will be transported by the manipulator.
- Parts of this subsystem:
  - Deposits
  - Solenoid valves
  - Pump
  - Fluid dispenser valve
  - Piping

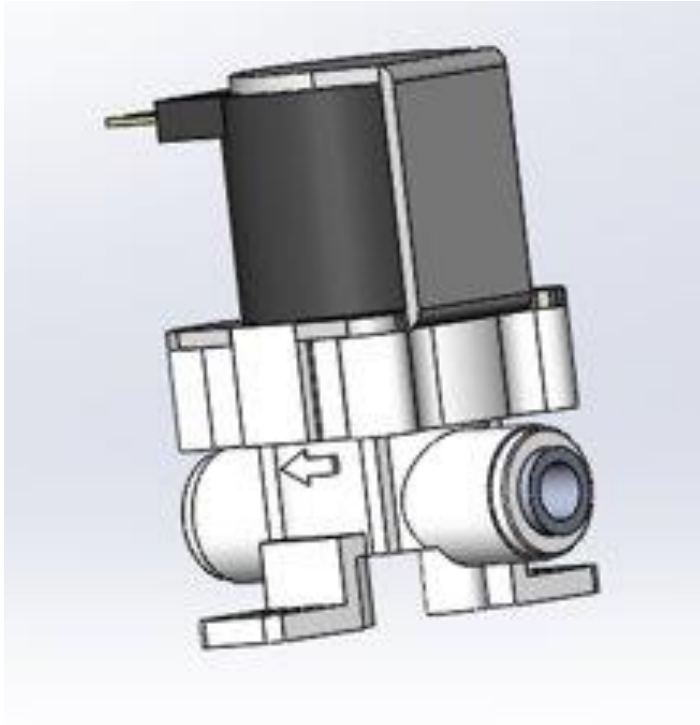
# Three Reservoirs

- The micro bioreactor will include 3 separated sealed reservoirs for each of the following liquids:
  - Nutrients: any liquid used for the cell culturing.
  - Bleach: liquid chosen for killing E-coli, avoiding cross contamination between experiments.
  - Sterilized water: this liquid will be used to neutralize and remove any waste in the tubes.

- Dimensions:  $D=5\text{ cm}$ ,  $h=10\text{ cm}$ , Volume: 196,35 ml/each.
- Materials:
  - Top and bottom parts: rigid PVC [37,9-54.8 MPa]
  - (reduction of weight and cost).
  - Main cylinder: PMMA [68,8 –83,4MPa] (see-through)

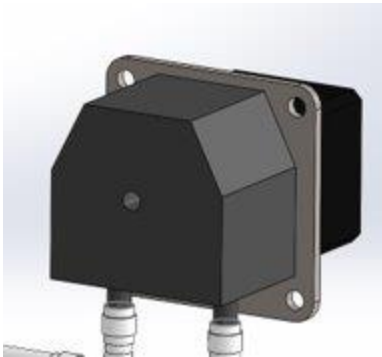
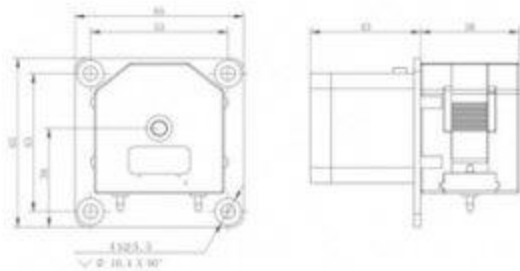


# Solenoid Valves



- A solenoid valve will be placed in each of the tubes connecting the reservoirs to the pump.
- Solenoid valves are electromechanically operated control elements and their tasks are to shut off, release or dose fluid.
- Main characteristics:
  - Material: Pom-C plastic
  - Dimensions: 2.83"x2.05"x1.42"
  - Push-to-connect
  - Weight: 198,45 g/each

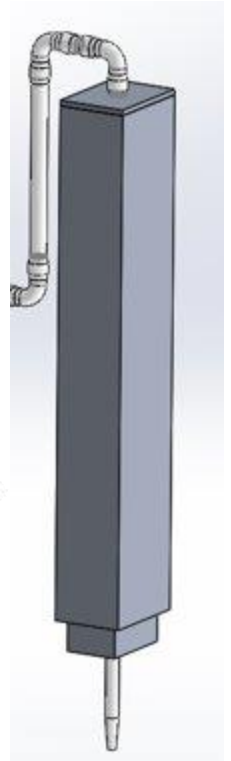
# Pumping



- The pump will force the liquid to move along the tubes at the desirable flow rate ( $225 \mu\text{L/s}$ - $300 \mu\text{L/s}$ ).
- For the analyses the maximum flow rate has been taken into account, which corresponds to  $18 \text{ mL/min}$ . Reynolds number was also used to make sure there is no risk of aerosol creation ( $Re < 500$  to avoid atomizing).
- Pump selected: Peristaltic pump OEM b01
- Main characteristics:
  - Flow rate:  $0.0024$ - $190 \text{ mL/min}$
  - 42 Stepper motor-NEMA 17 stepper motor
  - Weight:  $250 \text{ g}$

# Fluid dispenser (K8MDC dispensing valve)

- An automated dispensing valve with a variable nozzle diameter will ensure the fluid aliquots are deposited with the highest accuracy.
- The K8MDC will be enclosed in its 3D-printed case made from rigid PVC [37.9-54.8 MPa].
- Features:
  - Inner nozzle diameter: 0.5-1.8mm
  - Weight: 150g
  - Accuracy: >98% dispensing tolerance.
  - Interchangeable tips of different volumes



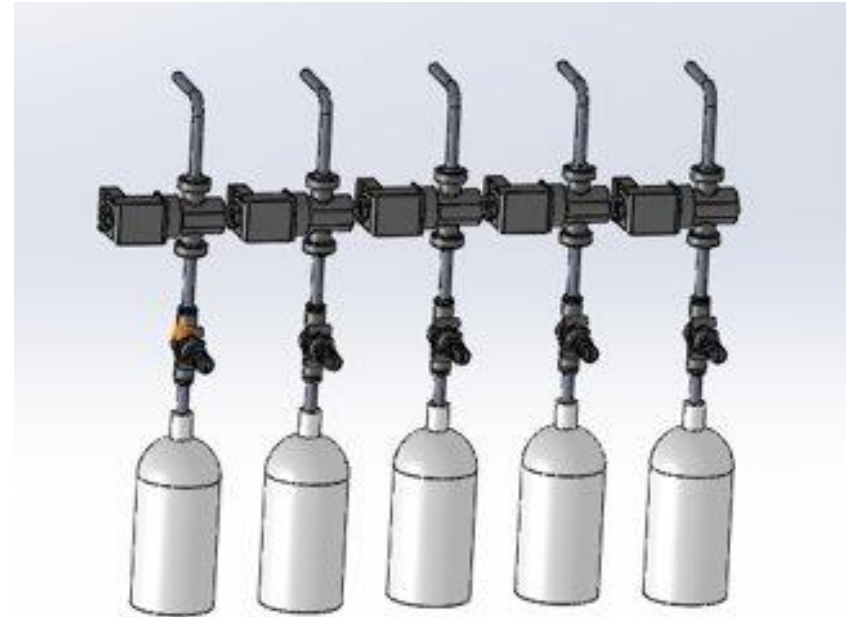
# Conical Tube Opener



- This mechanism will be attached to the fluid handling system and together with the manipulator will unscrew and screw back again the conical tubes' caps.

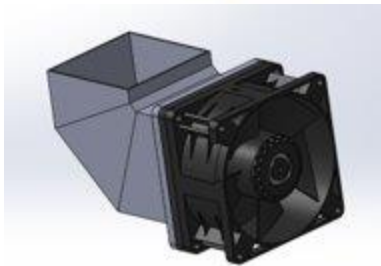
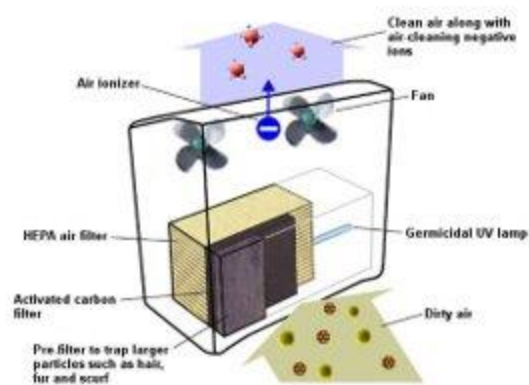
# Gas Injection

- The micro bioreactor is capable of injecting, measuring, and regulating the composition of N<sub>2</sub>, O<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub>.
- Gases are kept in well insulated customized air tanks that will be placed outside of the main structure.
- The system designed for gas injection will have pressure regulators and valves that will enable the user to adjust the desired gas composition and the flow rate from the controller.





# Gas Evacuation System



- The system needs to be able to evacuate those fluids out of the main enclosure in order to avoid contamination in future experiments. For that purpose, a vacuum-like system has been suggested.
- This mechanism will have a motor that will turn on a ventilator that will reduce the pressure making vacuum. Once the gases are inside the exit tubing, the fluids will go through a filter to purify them. For proper waste treatment, this gas evacuation system will have tubes connected to the open air.



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# Sanitation Subsystem

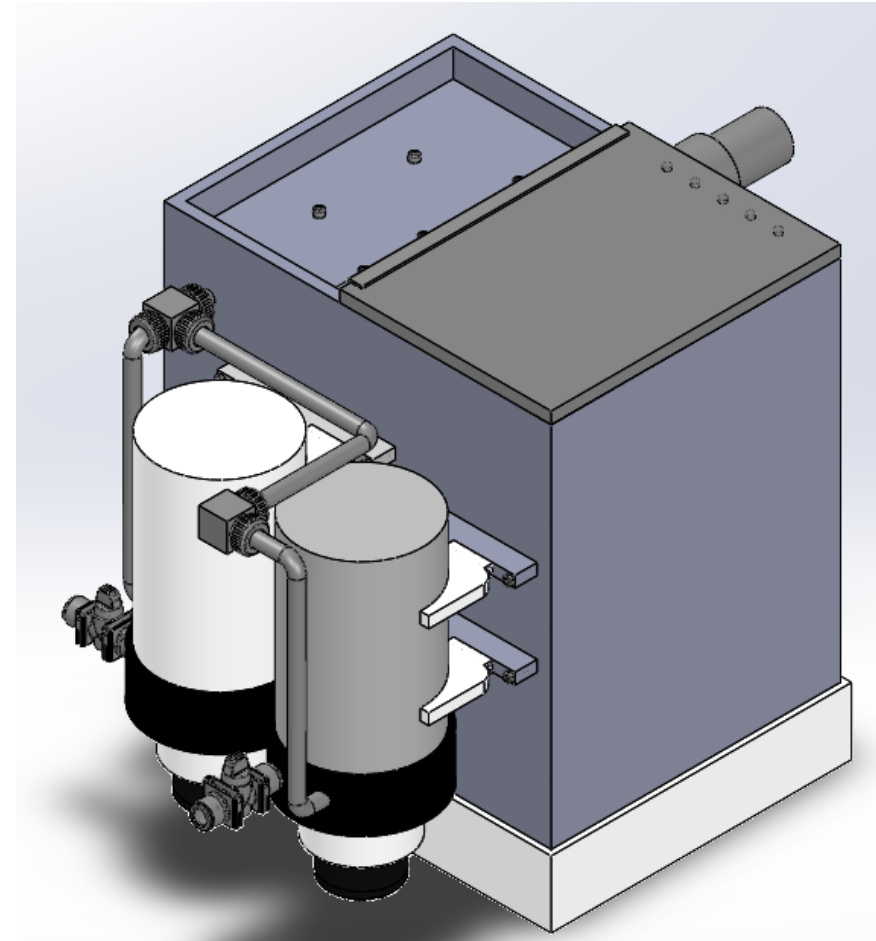
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- Requirement 15: capable of sequestering and neutralizing its own liquid and solid waste
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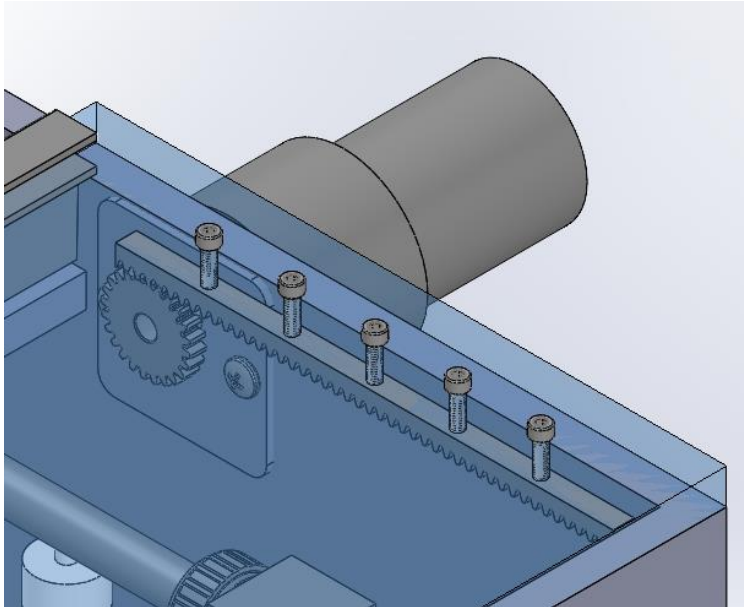
# Sterilizing plates and bacteria

- This enclosure is for dispensing bacteria and cleaning the well plates or tubes. A manipulator places the vessel in the system, a face mounted motor attached to a rack and pinion would move the top retractable panel to seal the structure. A pump and reservoir combo is used to move bleach from the reservoir into the fluid dispensing system in the structure and sterilize the area. The bleach and bacteria would drain into a waste reservoir and distilled water from another reservoir is pumped in to remove any bleach residue.
- There is one fixed panel above the fluid system where the PVC of the fluid spray system is mounted using small sheet metal brackets.

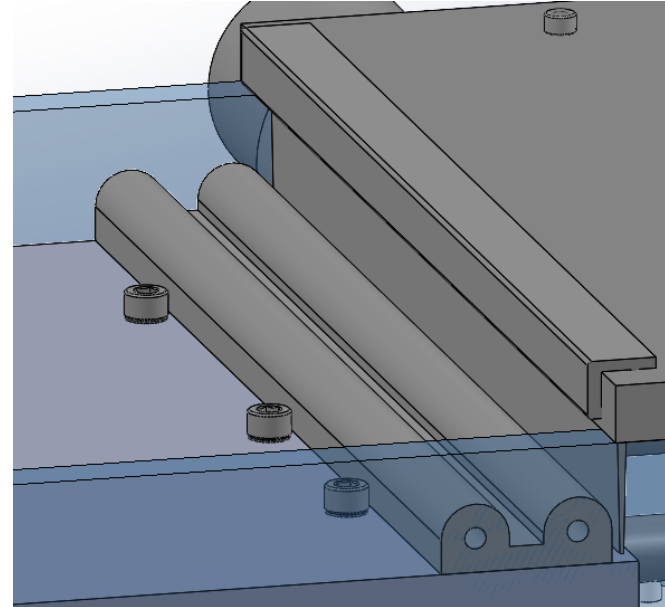


# Retractable Panel

- The rack is 15cm long and mounted using screws to the retractable panel. There is a 24 tooth spur gear connected to a 7 RPM mini gear motor.

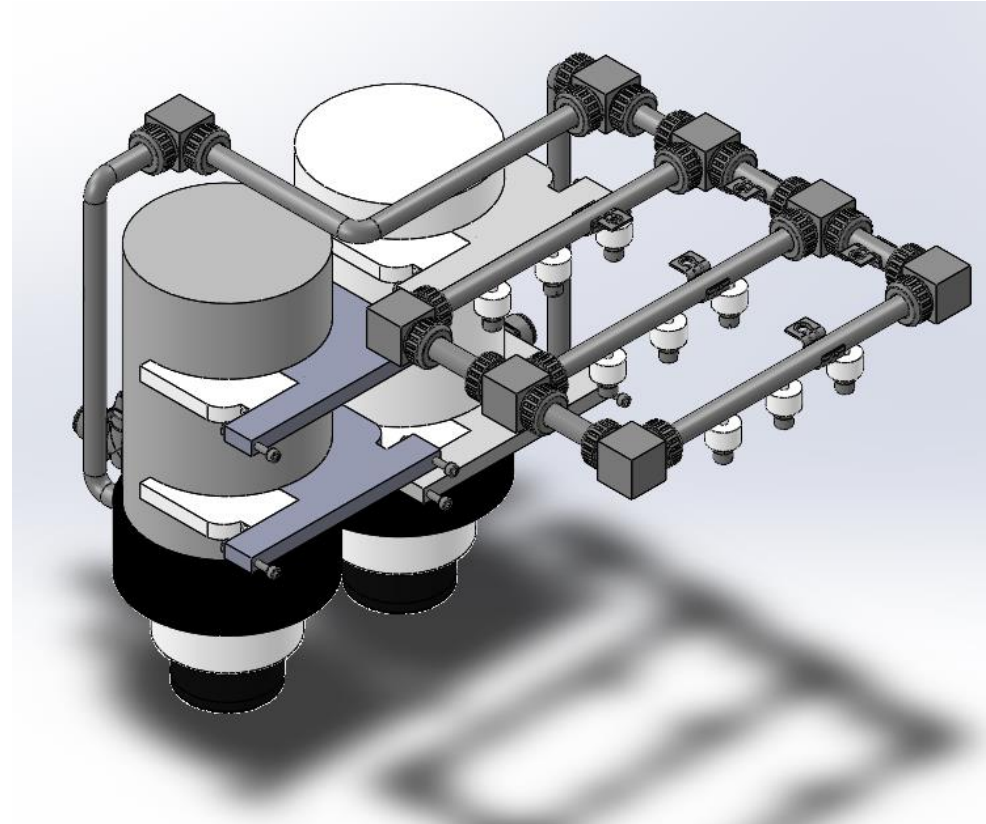


- A seal is created using a push on seal with a wiper and a surface mounted rubber edge seal.



# Fluid Spray System

- This system uses hard plastic 10mm inner diameter tubing and compression-based connectors to disperse the fluid in the structure. This is mounted to the right side of the structure. Each tube would have small holes where a nozzle adapter and nozzle head is threaded for fluid dispensing at a 65 degree angle. The pipes connecting the nozzles to the reservoir are mounted to the fixed panel of the subsystem structure using pipe clamps.
- The are where the tubes meet the housing structure and each other will be sealed using O-rings so that no fluid leaks.





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# Environmental Management Subsystem

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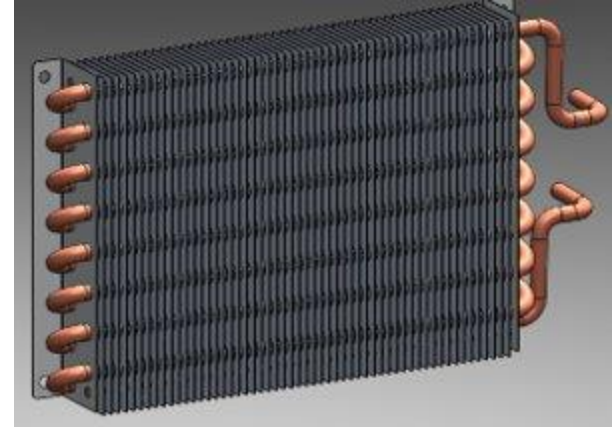
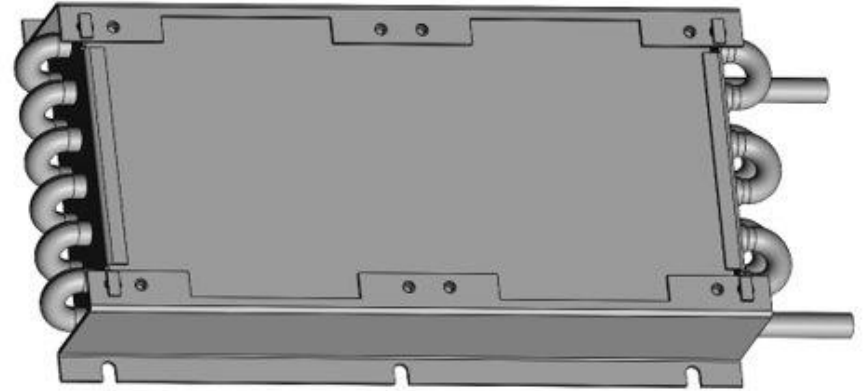
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- Requirement 5: Runs from a single standard 120V AC wall outlet.
- Requirement 11: Only nonporous materials contact cell cultures.
- Requirement 12: Only nonreactive materials contact lab chemicals.
- Requirement 14: Has an exterior surface that is not too hot to comfortably touch.
- Requirement 17: Maintains environmental conditions independently for each well plate.
- Requirement 20: Well plate, test tubes, and testing environment should maintain a constant temperature between 4° C and 70° C.
- Requirement 21: Maintains internal set point temperature  $\pm 2.5^{\circ}$  C.
- Requirement 22: Uniformly heats and cools wells and tubes within the desired temperature range.
- Requirement 24: Environment has the capability of reaching the set point temperature targeted within a 15 minute time frame



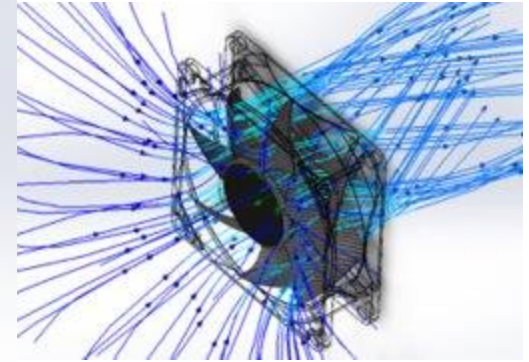
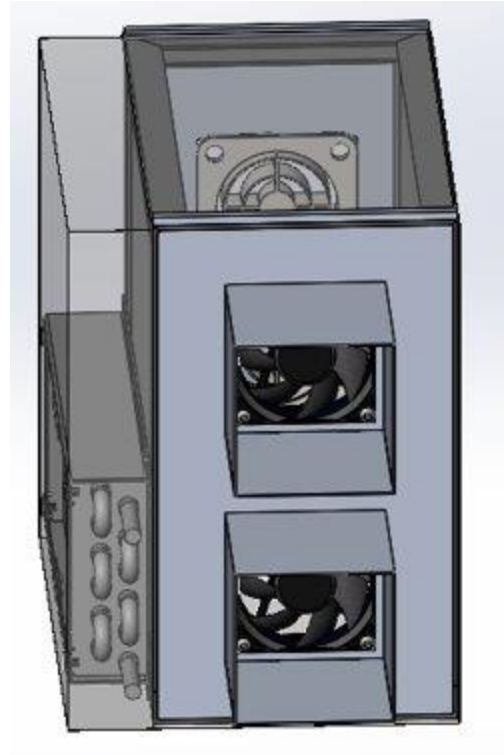
# Condenser

- R134-a Freon Fridge Unit
  - Mini heat exchanger for vapor compression cycle
  - 8mm copper tubing within encased .125mm aluminum housing which will include a layer of insulation
  - Estimated 2200 BTU/hr thermal capacity in cooling assuming ideal gases
  - Customer needs were met for environmental management with the concept, #2,5,11,12,14,17,20-24



# Air Intake

- Corsair Fan w/ Rotor, Set Screw
  - 80mm classic CPU fans
  - Basic OTS deliberately curved to simulate flow within enclosure as in a singular direction
  - Two vented exits for hot air exhaust to the rear of the assembly
  - Movable roof with linear actuators to access interior of subsystem





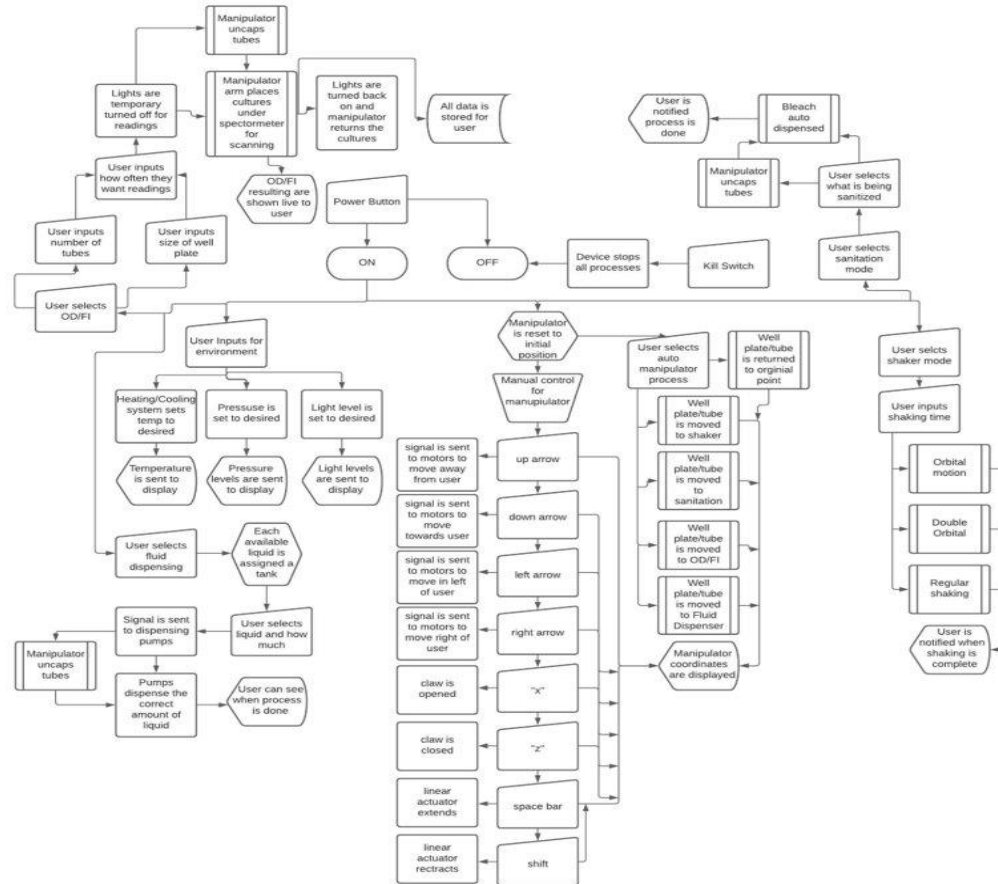
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# Controls Subsystem

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# Addressed Customer Needs

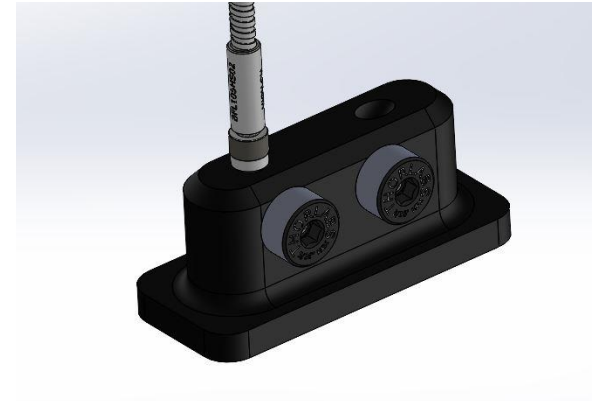
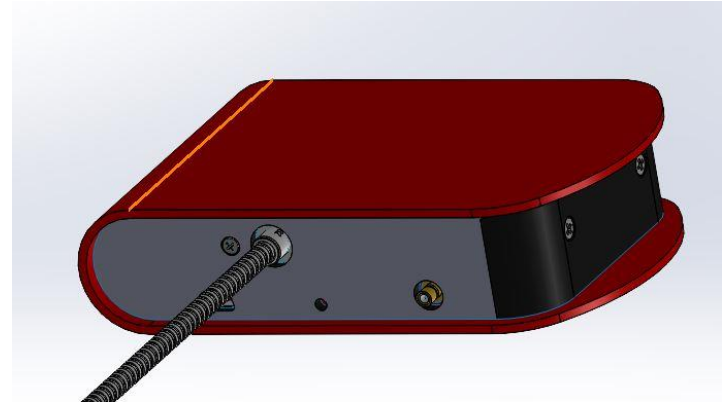
- Requirement 1: Operation lifetime of at least 10 years.
- Requirement 2: Prototype production cost does not exceed \$10,000.
- Requirement 3: Movable by one person after disassembly.
- Requirement 5: Runs from a single standard 120 VAC wall outlet.
- Requirement 7: Includes an easily actuated emergency shut-off that safely stops all functions.
- Requirement 8: Has an intuitive user interface.
- Requirement 10: Has an easily seen visual indicator showing: process mode, elapsed process time and remaining process time, error.
- Requirement 19: Is capable of incubation periods long enough to permit 1,000 E. coli culture generations (two weeks).
- Requirement 27: Includes a photobioreactor mode to illuminate photosynthesis-capable cell cultures (e.g., cyanobacteria) with white light.
- Requirement 30: Measures optical density (OD) in all individual well and conical tubes.
- Requirement 31: Measures fluorescent intensity (FI) in all individual wells and conical tubes.
- Requirement 32: For OD/FI measurement, sustains adequate light intensity to make measurements at wavelengths not lethal to cells.
- Requirement 33: Processes a 384 well plate through OD/FI measurements in less than 6.5 minutes.



# Spectrometer

## Thors Labs Compact Spectrometer CCS175

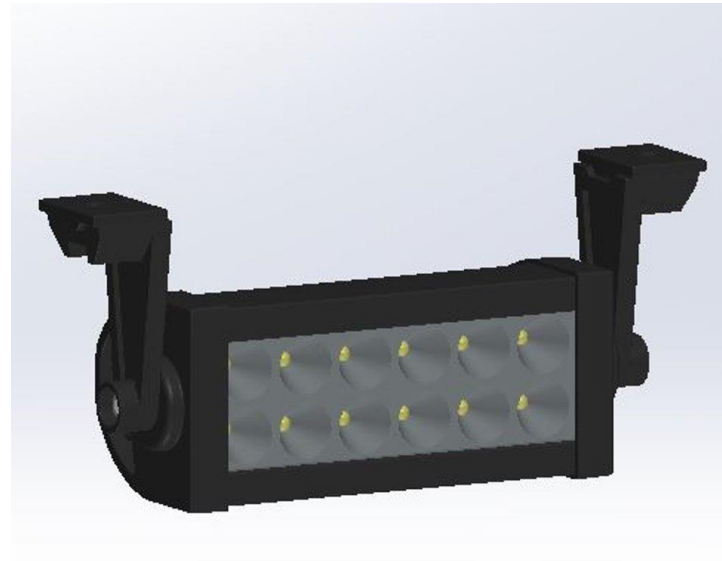
- Internal mount is secured in place using epoxy and fiber cable is secured in the mount
- Housed outside of main unit so temperature and other internal factors will not interfere with operations
- High-Speed USB Connection Allows up to 200 Scans per Second
- Quick and easy interchangeable systems for different wavelength measurements if necessary



# Photobioreactor

## Nilight - 10001S-A36W LED Light Bar

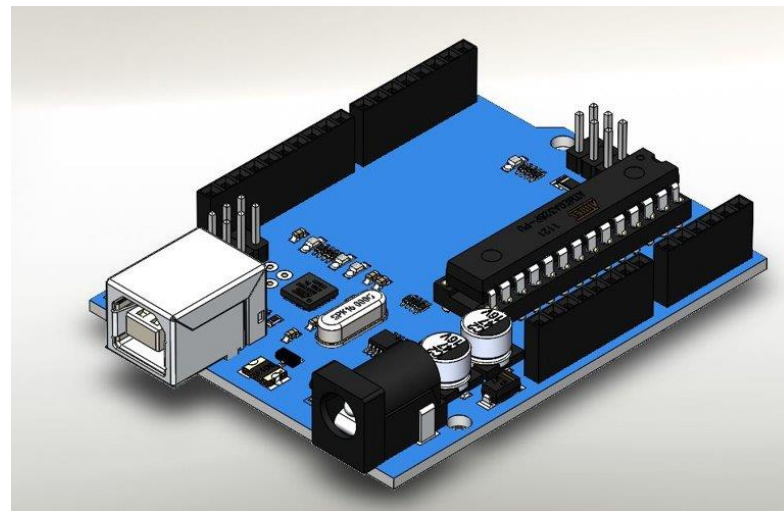
- High-quality heat conduction silicone gel together with redesigned aluminum alloy housing, providing superior cooling effect
- LED lighting will be interchanged for red, green, and blue which will be ideal for bacterial growth
- Easy to mount and position for best directional use of the lighting



# Microcontroller

## Arduino Uno R3

- Arduino is a cheap option to provide information the operations within the Bioreactor
- Connected laptops or computers will handle the user inputs and data output provided from the bioreactor instruments
- Visual display of a HD screen is optimal for multiple viewers and clear understanding numbers and graphs
- 3 Arduinos will be used to handle processes of the Bioreactor







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# General Structure

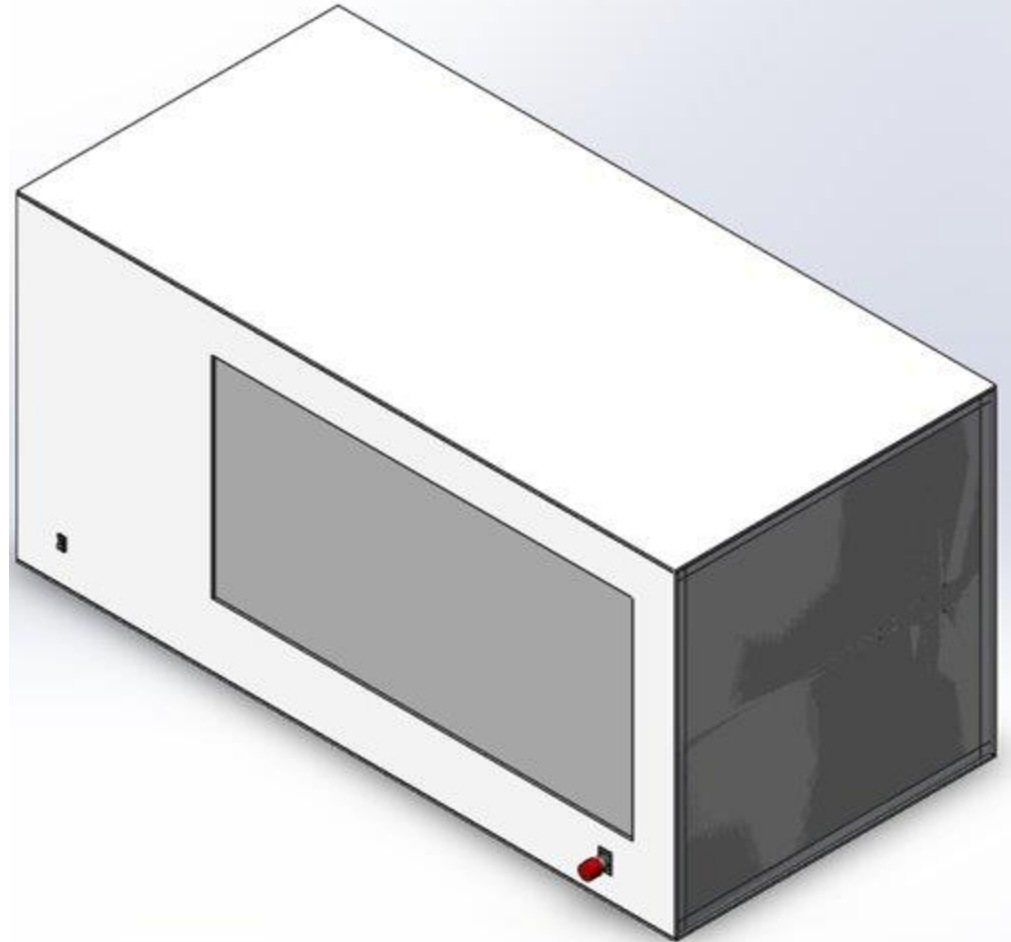
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# Addressed Customer Needs

- Requirement 1: Operation lifetime of at least 10 years.
- Requirement 2: Prototype production cost does not exceed \$10,000.
- Requirement 3: Movable by one person after disassembly.
- Requirement 4: Fits on a research benchtop
- Requirement 6: Has an easily accessible interior for cleaning
- Requirement 11: Only nonporous materials contact cell cultures
- Requirement 12: Only nonreactive materials contact lab chemicals
- Requirement 13: Appropriate for operation in a BSL-2 space
- Requirement 14: Has exterior surface that is not too hot to comfortably touch
- Requirement 19: Is capable of incubation periods long enough to permit 1,000 E. coli culture generations (two weeks).
- Requirement 21: Maintains internal set point temperature (+/- 2.5 C variation)
- Requirement 25: Accommodates existing culture well plates of the following sizes: 6, 24, 48, 96, deep 96, 384
- Requirement 26: Accommodates existing conical tubes of the following sizes: 15mL and 50 mL

# CAD Model

- Holds all other subsystems and provides insulation – 3D printer style
- 5 main components: bar frame, outer panels, inner panels, insulation and window
- 3 minor components: on/off switch, emergency shutoff switch and window film



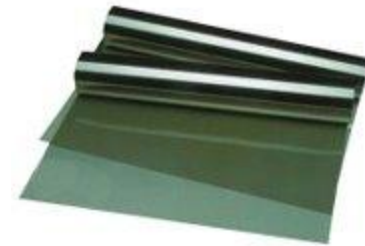
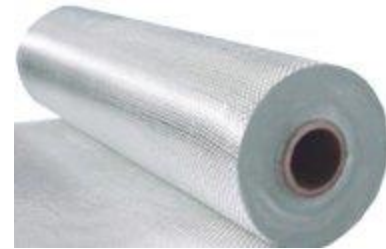
# Frame and Panels

- Aluminum 2024 rectangular bar frame supports entire structure
- ABS Plastic panels on all sides for general housing
- Stainless Steel sheet metal for upper and lower inner panels to support other subsystems
- Al 2024 Yield Strength: 324 MPa
- Total Compressive Stress: 257.8 kPa (<0.1%)



# Insulation and Windows

- Front transparent glass window for visibility
- Polarizer (polymer chemical film) over glass window for light intensity
- Entire structure has S-fiberglass cloth insulation
- Thermal conductivity average of  $k=1.3$  W/m-K for S-fiberglass
- Water (0.6) vs. Steel (30.0)



# Conclusion

- This bioreactor was designed to make the scientists jobs easier
- Programmable presets could remove the need of having to supervise the experiment
- Sanitation system aids in the clean up process, removing another step from the experimental process



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