INCU-GATOR

Herbert Wertheim College of Engineering
Department of Mechanical & Aerospace Engineering
UNIVERSITY of FLORIDA

Group 6: Edward Deumens, Jessica Johnsen, Mikayla Lamb, Carlie Taylor, Daniel Tremblay, Zac Tupper

GATOR Engineering UNIVERSITY OF FLORIDA

Abstract:

The Incu-Gator is an autonomous microbioreactor developed for the UF Biofoundry with the objective of providing lab equipment at an accessible cost to researchers while emphasizing the importance of modular subsystems. The decreased price comes primarily from the use of integrated high-quality off-the-shelf components and capitalization of sub-system synergy, rather than compromising design quality. The Incu-Gator can hold a combination of six well plates or conical tube fixtures, which are agitated through the stepper motor-driven orbital vibration module. However, the module can be swapped out if the user desires other shaking patterns. The system's fluid delivery system is comprised of two of the assembly's subsystems: a 3-axis servo belt motor system and an attached mechanically actuated plunger. To prevent cross-contamination, there is a tip ejector to allow for the use of disposable pipette tips. The servo belt system allows for precise movement of the pipette and attached laser used for optical measurement. A sliding tray with an array of photodiodes is located under the samples, which allows for easy positioning when measuring optical density, and filters can be placed on the module to measure fluorescent intensity. The atmospheric composition is controlled using gas flow through solenoid valves and temperature is regulated through a combined resistive heating element and compressed-refrigerant system. This closed-loop environmental control system is housed in a separate compartment to increase efficiency. All the components are controlled using an integrated raspberry pi system and bio-safety standards were taken into consideration throughout the entire design.

Product Summary:

When a user chooses the Incu-Gator for their experiment, they are opting for a fully-customized experience. The user can alter the internal controlled environment of the bioreactor for their application. Before cultures are placed in the main compartment, the desired environment is set by adjusting temperature and atmospheric composition. The user chooses appropriate vessels for their experiment and places them in the main base plate, which can be removed from the bioreactor to make experiment set-up easier. With the reinstallation of the base plate and the achievement of a stable environment, the user can fill fluid modules and begin running the system. The 3-axis movement system guides an automated micropipette to move fluid as desired by the user. Orbital culture shaking may be initiated if desired. The user may choose to perform OD or FI measurements at any point and utilize a sliding system to move optical modules as needed. Fluid levels as well as temperature and atmospheric conditions are monitored for the duration of the experiment. For an extensive experiment, fluid may need to be refilled periodically by the user.

Cost Overview:

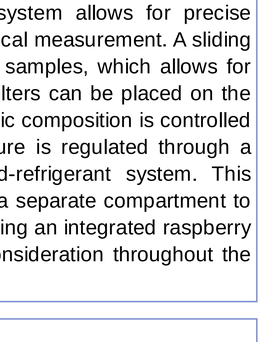
- OTS Parts: $3,349.46
- Raw Materials: $1,510.12
- Manufacturing and MFG Labor: $2,020.85
- Energy Consumption: $215.76*
- Assembly Labor: $34.78
- **Total Cost: $7,130.96**

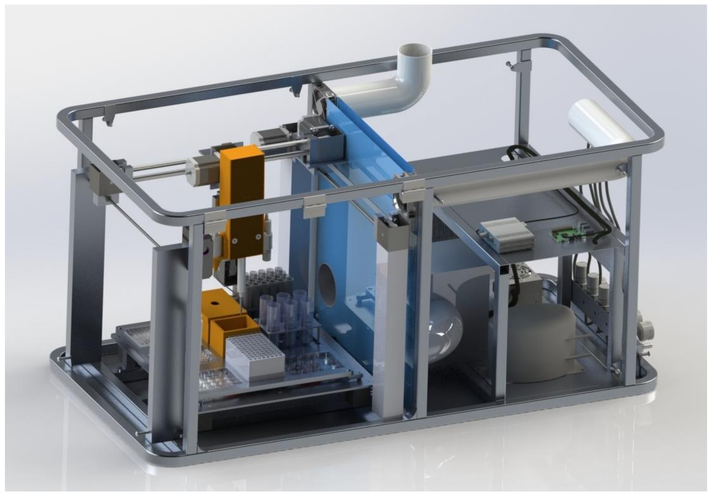
*Energy cost per year

Liquid Handling Subsystem:

Liquid dispensing is controlled by a mechanically actuated plunger. The micropipette system uses standard disposable pipette tips to draw and dispense liquid. Risk of contamination between stock and samples is minimized by a tip ejection system. There is potential to swap the single pipette with an 8-channel to make filling well plates faster.

A: housing & solenoid actuator
B: lead screw and motor
C: plunger
D: tip ejection mechanism
E: disposable tip



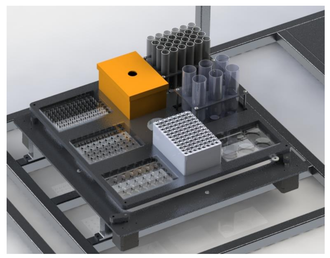


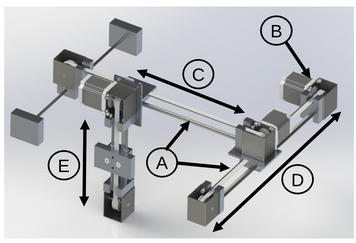
Removable Panels Key Feature:

The simple panel removal process maximizes access to the Incu-Gator components for ease of use as well as maintenance and repair purposes. Screws are used for easy removal. A seal system and insulation within the panels help to maintain the internal environment.

Well Plate Modularity Key Feature:

The Incu-Gator accommodates various well plate and conical tube sizes using an easy swap-in/swap-out approach. The main base plate can hold any combination of 8 of the following: conical tube racks, well plates, pipette tip racks, and fluid compartments. Additionally, the entire base plate may be removed to easily swap these modules.





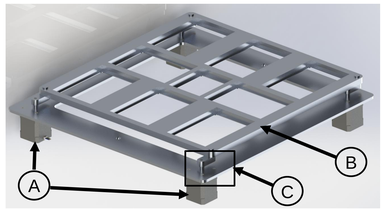
Liquid Movement Subsystem:

There are 3 axes of motion all controlled on a servo driven belt system for precise control. The subsystem features a simple mounting system for the liquid handling subsystem.

A: belts
B: motor
C: x-axis
D: y-axis
E: z-axis

Culture Vibration Subsystem:

The Incu-Gator is equipped with an orbital shaking pattern. Four stepper motors control the orbital pattern and there is potential for the shaking module to be swapped out and replaced to accommodate other shaking patterns. The subsystem includes a removeable base plate which aids in the setup process.

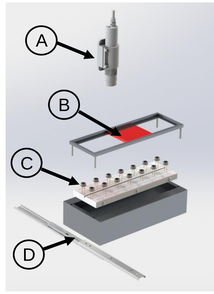


A: stepper motors
B: base plate
C: orbit arm

OD/FI Measurement Subsystem:

This system automates measurement of optical density and fluorescent intensity. The laser is mounted to the liquid movement system making it able to reach any well or tray. For optical density measurement, the laser shines through the sample directly onto the OPD, which is compared to a baseline value of just the well tray. Fluorescent Intensity is measured by adding the optical filter, so the only light absorbed by the OPD is from re-mission by the sample in the fluorescent spectrum.

A: laser
B: optical filter
C: OPD sensors and breadboard
D: tray slides



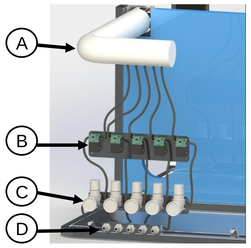
Optical Modularity Key Feature:

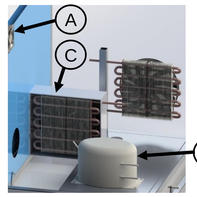
Modules are on slides to allow for easy positioning under individual well plates. Additionally, the filter can be positioned to measure fluorescent intensity in the desired locations. This allows for a combination of sensors capable of measuring OD or FI at any or all locations.

Atmosphere Control Subsystem:

The Incu-Gator's atmospheric conditions can be manipulated and monitored to meet the needs of the user. The controls system can inject and regulate Nitrogen, Oxygen, Carbon Dioxide, Methane, and Hydrogen supplied by external tanks using solenoid valves.

A: PVC delivery
B: solenoid valves
C: regulators
D: hose fittings





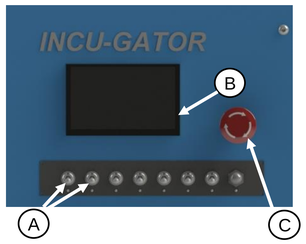
A: resistive-heater
B: compressor
C: refrigeration system

Temperature Control Subsystem:

The culture environment's temperature will be controlled via a resistive heater and compressor-refrigerant system. The resistive heater is a 150 W cartridge heater in aluminum casting with a built-in fan to facilitate heat transfer to the environment, allowing for it to be heated to at least 70 ºC. The compressor-refrigerant system is a commercially available coolant compressor rated for 112 W of cooling at 0 ºC, so it will meet the requirement of cooling to 4 ºC during use.

User Interface & Safety Key Features:

This system has 8 mechanical programmable toggle switches and an LCD screen as well as USB connection for detailed customization. The twist release E-Stop allows for protection of the user and the equipment. Due to the use of a Raspberry Pi and large data storage the user can customize closed loop experiments based on feedback from this system's numerous sensors and rely on large data tables for conditional response.



A: toggle switches
B: LCD screen
C: E-stop

Acknowledgements:

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- 5 All components and subsystems are designed with a factor of safety of 1.15 applied to their yield strengths and lifetimes
- 6 Maximum emergency shut-off time for the whole system is 1 s.
- 11 Maximum production cost is $10,000.
- 13 Must have at least 1 control feature that can be programmed for experiments.
- 15 Material chemical compatibility will be used to verify that components are non-porous and non-reactive
- 18 Must be able to continuously operate for up to 2 weeks.
- 19 At least 1 output variable that controls the start of next cycle.
- 25 Must dispense and remove liquids with a precision of 2%.
- 33 A flow rate of 300 µL/s and minimum of 225 µL/s is achieved.
- 34 Fluid can be dispensed with an accuracy of ±1 µL (or 2%) and with a precision of ≤2% at 50-200 µL. It must also dispense 50-20,000 µL in 1 µL increments.
- 35 Disposable tips will neutralize the system in compliance material compatibility standards.

Liquid Handling

- Pipette Mechanism
- Disposable Tips
- Pipette Material
- Actuation Control

- 4 Maximum total assembly time is 8 hours with 5 tools needed.
- 10 Minimal operational lifetime of 10 years.
- 11 Maximum production cost is $10,000.
- 13 Must have at least 1 control feature that can be programmed for experiments.
- 16 BSL-2 standards and design requirements are met.
- 19 At least 1 output variable that controls the start of next cycle.
- 26 Must capture 100% of the waste gas produced by cultures.
- 27 Must be able to inject/reject 5 different gases with a controller.

Atmosphere Control

- Solenoid Valves
- Gas Tubes
- Atmosphere Sensors
- User Interface
- Attachment to Fume Hood

- 11 Maximum production cost is $10,000.
- 14 Must take less than 1 hr to train operator on how to use device.
- 28 Measurements must be able to be recorded for each well plate/tube within 1 hour.
- 29 Must detect more 50% of the source intensity to measure FI.
- 30 Must maintain a light intensity of 1 kW/cm^2.

OD/FI Measurement

- Attachment to Liquid Movement system
- Laser
- OPD Sensor Tray
- Removable Filter

- 5 All components and subsystems are designed with a factor of safety of 1.15 applied to their yield strengths and lifetimes
- 10 Minimal operational lifetime of 10 years.
- 11 Maximum production cost is $10,000.
- 13 Must have at least 1 control feature that can be programmed for experiments.
- 19 At least 1 output variable that controls the start of next cycle.
- 38 A 384 well plate is able to be processed in 6.5 minutes or less.

Liquid Movement

- Servo Motors
- Position Tracking
- 3-axis Belt System
- Culture Media Station

- 1 Depth of assembly must not exceed 400 mm and no length may exceed 864mm.
- 2 Must run off power provided by standard wall socket: 120 V and 15-amp breaker
- 3 Individual components must not exceed 51 lbs and height must be 1125±100 mm.
- 4 Maximum total assembly time is 8 hours with 5 tools needed.
- 5 All components and subsystems are designed with a factor of safety of 1.15 applied to their yield strengths and lifetimes
- 6 Maximum emergency shut-off time for the whole system is 1 s.
- 7 An alert is automatically sent to operator's phone during the 1 second shutoff time.
- 8 Must automatically shut off if at least 1 set-up protocol
- 9 Must have a visual indicator that can be seen around the machine in a field of view of at least 0˚.
- 10 Minimal operational lifetime of 10 years.
- 11 Maximum production cost is $10,000.
- 12 Zero external support features.
- 13 Must have at least 1 control feature that can be programmed for experiments.
- 14 Must take less than 1 hour to train operator on how to use device.
- 16 BSL-2 standards and design requirements are met.
- 17 Exterior temperature may not exceed 50 ˚C.
- 19 At least 1 output variable that controls the start of next cycle.
- 21 Compartments must be able to be changed in less than 3 mins and have 1 feature for media exchange.
- 22 Must be able to hold 6 different sizes well plates.
- 23 Must be able to hold 2 different sizes of conical tubes.
- 31 Must achieve an acceleration range of 0.29-5.49 m/s^2.
- 32 Minimum of 4 chambers for well plates/tubes.
- 36 The footprint does not exceed 0.61 m height × 1.22 m length × 0.61 m depth.
- 37 The design is modular, allowing for modifications to functions to be made as technology advances.

Vibration & Structure

- Size of Frame
- Frame Material
- Raspberry Pi
- Fastener Selection
- Shut-off Button
- Removable Panels
- Access Door
- Stepper Motors
- LCD User Interface
- Insulation on Panels
- Modular Plate Holders

- 2 Must run off power provided by standard wall socket: 120 V and 15-amp breaker
- 11 Maximum production cost is $10,000.
- 13 Must have at least 1 control feature that can be programmed for experiments.
- 16 BSL-2 standards and design requirements are met.
- 19 At least 1 output variable that controls the start of next cycle.
- 20 Must be able to operate at temperatures from 4˚ C to 70˚C.
- 24 System temperature does not vary more than 1˚C.

Temperature Control

- Compressor
- Refrigerant System
- Resistive Heater
- Temperature Sensors
- User Interface
- PVC Delivery

Key

- # Customer Need
- Quantified Metric
- Sub-system
- Key Feature