



Prokaryote playhouse: A low-cost, laser-cut acrylic incubator for optogenetic bacterial culture



Jin Wu^{b,c}, David Dellal^b, Steven Wasserman^a

^aDepartment of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

^bDepartment of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

^cDepartment of System Design and Management, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

Bacterial photography is a printing technique that replaces conventional photochemistry with a living film of engineered *Escherichia coli*. Many biology teaching labs have adopted monochrome bacterial photography because it offers a captivating playground for illustrating central concepts and lab techniques in biological engineering, particularly in the fields of synthetic biology and optogenetics. Recent improvements have increased the number of color channels from one to three. A key practical challenge in three-color printing is to expose a Petri dish loaded with engineered bacteria to a trichromatic image while maintaining it at 37 °C. Prokaryote Playhouse is a compact, inexpensive, open-source, benchtop incubator for light-sensitive bacterial cultures that makes bacterial photography and similar bacterial optogenetic methods more accessible to teaching labs, makerspaces, and research labs. The system includes a laser-cut, light-tight enclosure; digital thermostat; heated sample shelf; single-board computer; and miniature projector. We built a fleet of Prokaryote Playhouses that students have used to produce hundreds of bacterial photographs in a wide range of educational experiences, ranging from a four-hour introduction to synthetic biology and wet lab techniques to a six-week exploratory class for first-year students at MIT.

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Specifications table

Hardware name	Prokaryote Playhouse
Subject area	<ul style="list-style-type: none"> • Biological Sciences (e.g. Microbiology and Biochemistry)
Hardware type	<ul style="list-style-type: none"> • Biological sample handling and preparation
Open Source License	Creative Commons – Attribution-NonCommercial-ShareAlike 4.0 International
Cost of Hardware	\$386.06
Source File Repository	http://doi.org/10.17605/OSF.IO/AEUYH

E-mail address: scwass@mit.edu (S. Wasserman)

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1. Hardware in context

In 2005, a team of students participating in the Intercollegiate Genetically Engineered Machine (iGEM) competition from the University of Texas developed a photographic printing system capable of reproducing monochrome images on a thin layer of engineered *E. coli* bacteria growing in a Petri dish [1]. The team called their invention “bacterial photography.” Recognizing the student’s spectacular accomplishment, renowned synthetic biologist Prof. Drew Endy commented in *Nature News*, “This kicks ass” [2].

Bacterial photography has many characteristics that make it a compelling educational vehicle: it is freely available (the inventors published the genetic components in a registry of standard biological parts [3]); it produces a macroscale, tangible, and captivating result; it embodies central concepts of biological engineering; the necessary equipment is accessible to many teaching labs; and the reagents are not costly. Recognizing this potential, many teaching labs began offering lab modules based on bacterial photography – a task that was simplified by the development of a free bacterial photography kit for high schools, universities, and museums by researchers at Rice University [4].

In 2017, Fernandez-Rodriguez et al. published a system capable of producing trichromatic bacterial photographs [5]. Example three-color bacterial photographs produced by students using the Fernandez-Rodriguez protocol with the Prokaryote Playhouse incubator are shown in Fig. 1. The biological system design is realized with 18 genes partitioned into functional modules: sensors, logic, resource allocation, and actuators. The added elements of the three-color system make it even more compelling for educational use. In addition to producing a more eye-catching final result, the three-color design illustrates concepts that are not present in the monochrome system such as design abstraction, modularity, and crosstalk between components. The modularity of the system inspires many possibilities for new and interesting designs. For example, the actuators can be easily replaced so that the bacteria produce a print using fluorescent proteins instead of RGB pigments. Moser et al. rejiggered the system to create intricate, light-controlled patterns of cells adhered to various materials, including plastic, glass, mica and cotton [6].

The designers of the original monochrome bacterial photography system used a light-switchable gene promoter to create a strain of *E. coli* that produces an enzyme called β -galactosidase in dark conditions [4]. Exposure to red light shuts off the promoter, which in turn ceases β -galactosidase production. The function of β -galactosidase is to cleave a bond in lactose to yield glucose and galactose. (Wild-type *E. coli* express β -galactosidase only when their favorite food, glucose, is absent, but their second-favorite energy source, lactose, is present [8].) The photosensitive layer is created by mixing transformed *E. coli* into low-melting-point agar supplemented with the chemicals S-gal and ferric ammonium citrate, and then pouring a thin layer of the mixture on top of an agar plate. S-gal is a lactose analog whose structure is similar enough to lactose that β -galactosidase will cleave it. It is transparent when added, but after cleavage one of the products reacts with the iron in the ferric ammonium citrate to yield an insoluble, black precipitate [9]. Thus, bacteria exposed to red light remain clear and ones in the dark turn black. β -galactosidase production is proportional to illumination irradiance, which allows varying densities of black to be produced.

The three-color system operates on similar principles, adding two new enzymes expressed under control of green and blue light plus associated dye precursors to selectively produce red, green, and blue pigments. In outline, the printing process entails: transforming genes into *E. coli*; culturing the transformed bacteria in a selective culture plate; picking a colony and growing it in liquid culture; mixing the liquid culture into heated, low-melting-point agar supplemented with the dye precursors; and pouring a thin layer of the engineered bacteria/agar on top of an agar culture plate (Fig. 2). Detailed biological protocols can be found in the supplementary information of reference [5].

The final step is to expose the plate to the image to be printed for 18–24 h while maintaining it at 37 °C. The bacteria largely exhaust their nutrient supply during incubation, after which they cease producing color. Various apparatuses exist for exposing and incubating prepared bacterial photography plates (Fig. 3).

Reference [9] details the approach used by the developers of bacterial photography technique (Fig. 3, left panel). The apparatus is based on a commercially available incubator, such as Boekel Scientific Model 133000, with a hole in the top of its enclosure that normally accommodates a thermometer. Removing the thermometer mount provides optical access



Fig. 1. Example three-color bacterial photographs produced by students using Prokaryote Playhouse.

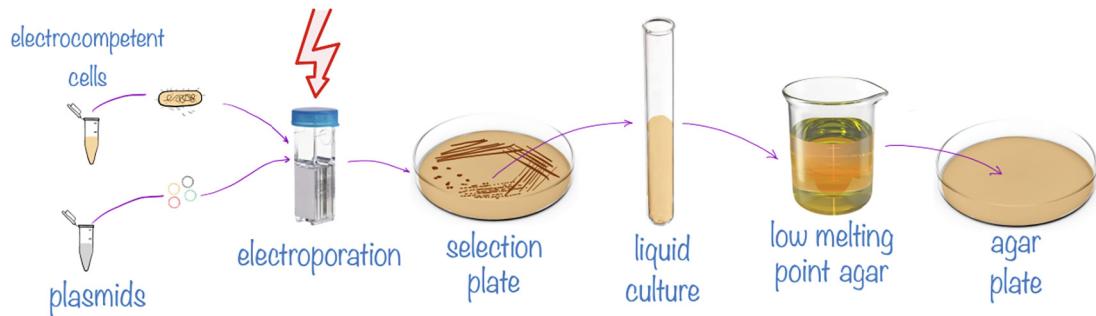


Fig. 2. Overview of bacterial printing process.

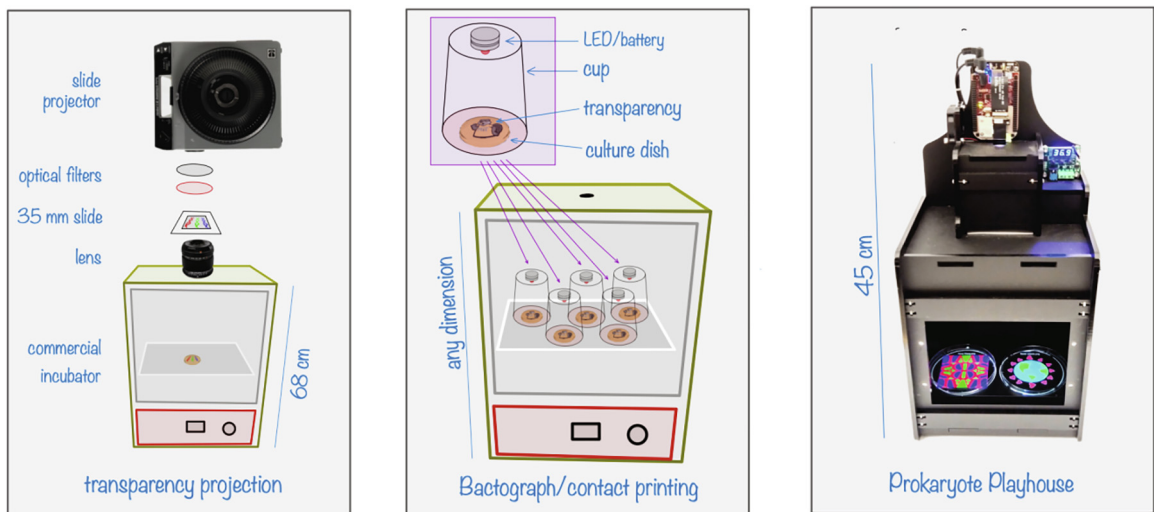


Fig. 3. Methods for exposing/incubating optogenetic bacterial cultures.

to the plate during incubation. In this method, the image to be printed is first reproduced on a 32×24 mm photographic slide. A Kodak Ektagraphic carousel slide projector mounted on a stand above the incubator illuminates the slide. The light source inside the projector is a halogen bulb. Optical filters adjust the projector's spectrum and radiant power. A lens near the opening of the incubator forms an image of the slide on the shelf of the incubator. Several characteristics of this setup make it unwieldy for teaching labs and makerspaces. The apparatus is large, difficult to move and store, requires regular maintenance (the protocol advises aspiring bacterial photographers to change the projector bulb at the beginning of each incubation), and the necessity of producing a transparency limits flexibility. Cost of this setup varies, depending on the price of the secondhand components. Used Boekel incubators sell for about \$800, and Kodak Ektagraphic III projectors range from \$150-\$900, depending on condition. In some of their biofilm experiments, Moser et al. replaced the slide projector/transparency/lens system with a short-throw LCD projector or a laser beam scanning projector (Aaxa ST200 or Cellulon PicoPro) [6]. Only the blue channel of the PicoPro was used. They reported that the output of the Aaxa ST200 projector diminished noticeably over relatively short usage intervals – likely due to the added heat load of being placed directly over the incubator opening (personal communication).

Bacterial photographs can also be produced by a method similar to photographic contact printing. In this scheme, a 1:1 scale, positive transparency is affixed to the bottom of a culture dish that is illuminated from above during incubation (Fig. 3, middle panel). The contact printing technique is appealing to teaching labs because it is cheap, simple, and scalable: a lens is not required, the standard incubator already present in most teaching labs can be MacGyvered with a suitable illuminator, and incubator shelves are generally large enough to accommodate many dishes simultaneously. For example, beginning in 2009, students in an undergraduate lab course at MIT made monochrome bacterial contact prints [7] using a setup that consisted of ultra-bright, red LEDs in optical mounts affixed to posts mounted on $2' \times 1'$ aluminum optical breadboard. The breadboard rested on a shelf inside a large, reach-in, bacterial incubator. Outside the incubator, an adjustable lab power supply in current mode drove the LEDs via wires that ran through the door opening. This apparatus offered sufficient capacity for group work, but it was expensive and heavy. The developers of the Bactograph kit devised a cleverer, cheaper, lighter, easier-to-store, and all-around more elegant solution for providing illumination inside of an incubator: a battery-powered LED rest-

ing on top of a paper cup with a small hole in the bottom. In the Bactograph system, a cup-and-LED is placed on top of each culture dish inside the incubator. Some drawbacks of contact printing are that the transparency is separated from the photosensitive layer by a thick sheet of agar, resulting in fuzzy edges and features that run together, and it is challenging to control light levels precisely enough to achieve good contrast. Three-color bacterial photography requires finer light control than monochrome printing, making it very difficult to accomplish using the contact printing technique.

Prokaryote Playhouse is a compact, inexpensive, open-source, benchtop incubator for light-sensitive bacterial cultures that makes three-color bacterial photography and similar bacterial optogenetic methods more accessible to teaching labs, makerspaces, and research labs (Fig. 3, right panel). At 21.5 cm × 18.4 cm × 45.0 cm, it is portable and easily stored. The system can be constructed by anybody with access to a laser cutter, soldering iron, basic hand tools, and the ability to drill and cut aluminum. It requires no regular maintenance. Using a software-controlled projector to illuminate the bacterial cultures eliminates the need to produce intermediate transparencies.

Bacterial optogenetic techniques have exploded in popularity since the early demonstrations of the technology. Biological engineers and researchers have created everything from miniature, optically-coxswained rowing crews that propel the shafts of micromotors [10] to microscopic chemical factories embedded in the guts of nematode worms that produce life-extending chemical compounds on command [11]. Although Prokaryote Playhouse was designed and tested with educational settings in mind, several features of the system make it potentially attractive in research applications. The system can be easily modified to suit specific research applications, for example by replacing the light engine or developing software to produce dynamic light patterns. The capacity, size, portability, modularity, and cost of Prokaryote Playhouse facilitate a wide range use cases in education, outreach, artistic endeavors, and research.

2. Hardware description

Fig. 4 shows an operating Prokaryote Playhouse incubator with major components and features annotated. The incubator door, which is held in place by magnets for easy removal and replacement, has been removed for the photo. The projector at the top of the photo displays a three-color light pattern on two 100 mm Petri dishes resting on the sample shelf. Optical access is provided by a clear acrylic window in the top panel of the incubator enclosure (not visible in the photo). Light baffles exclude stray light. The thermostat maintains the sample shelf at 37 ± 0.1 °C (or another programmed temperature).

The projector is a Digital Light Processing (DLP) technology evaluation module manufactured by Texas Instruments that is popular with makers and hobbyists (LightCrafter Display 2000 EVM), based on a 0.2 in., nHD resolution DLP chip and an

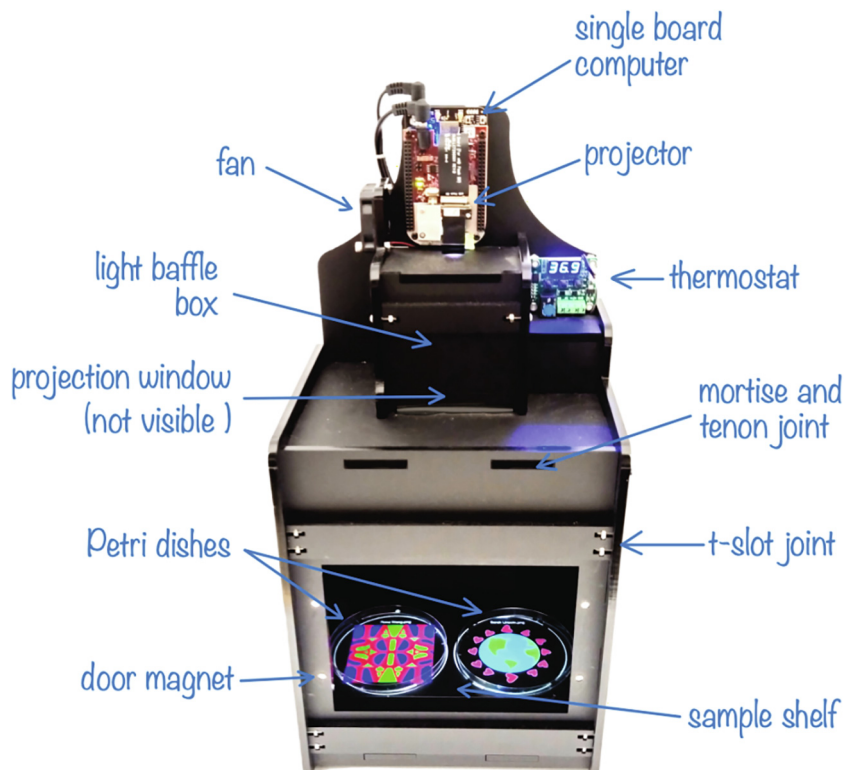


Fig. 4. Major components and features of Prokaryote Playhouse (door removed).

impressively tiny, 30 lumen, red/green/blue light engine with peak emission wavelengths of 624, 519, and 455 nm, respectively [8,9,10]. Full illumination spectra are available in references [13] and [14]. The projector operates in RGB888 format, providing 256 discrete levels for each color in each pixel. Header pins on the bottom of the module mate directly to the expansion connectors of a BeagleBone Black single-board computer. We selected the wireless version of the BeagleBone Black so that images can be downloaded and settings adjusted over Wi-Fi. The BeagleBone runs Debian Linux. Custom scripts automatically display image files downloaded to a special directory on the computer. For three-color bacterial photography, the illuminator operates at its highest output level. At this high setting, the system requires a fan to keep the illuminator from overheating.

Two cartridge heaters embedded in aluminum plates deliver approximately 14 W of heat with 12 V power applied. One of the plates serves as the sample shelf. The other plate (not visible in Fig. 4) is mounted at the back of the incubator to provide increased temperature uniformity and stability. The sample shelf is placed at the close focus of the projector. Both plates are painted black or anodized to reduce reflections. A W1209 programmable thermostat switches the heaters on and off to regulate the temperature. At under \$5.00, the W1209 is a spectacularly inexpensive thermostat with excellent features and performance, and it is very widely available. To provide the most accurate control of the sample temperature, the probe (included with the W1209) is embedded in the sample shelf and the programmable regulation range is adjusted to its minimum value of ± 0.1 °C.

With the exception of the transparent projection window, the enclosure is constructed from 6 mm, laser-cut, DP9 black acrylic sheet. Mortise and tenon joints hold the panels in alignment and provide mechanical strength. Tensioning bars hold the joints together, tightened by screws working against hex nuts captured in a T-slot (Fig. 5). This joinery system was chosen to facilitate easy and reversible assembly. Putting the T-slots in separate tension bars (instead of embedding them in the panels) provides a more air- and light-tight seal. (T-slots in the panels would allow stray light to enter and air to circulate directly between the inside and the outside of the incubator.)

Prokaryote Playhouse:

- maintains optogenetic bacterial culture plates at an adjustable incubation temperature while exposing them to spatially modulated, three-color light patterns under software control
- reduces the cost, space, and maintenance requirements of optogenetic bacterial culture
- is compact, portable, and easy to store when not in use
- supports a wide range of usage cases for educators, makers, and researchers.

3. Design files

The mechanical design was created using SOLIDWORKS CAD software. Shell scripts are written to run under Debian Linux release 8.9. All files listed in this section are published at <http://doi.org/10.17605/OSF.IO/AEUYH> under CC (BY-SA) license. A frozen version of the repository at the time of publication is available at <https://osf.io/85urz/>.

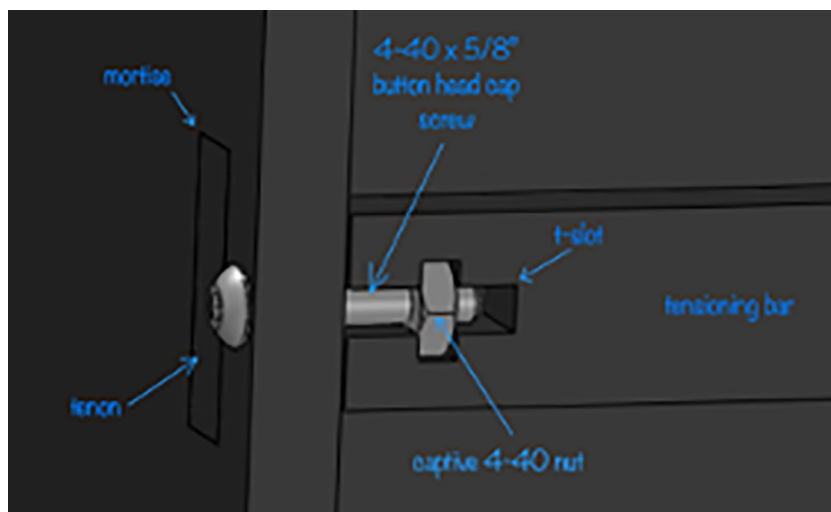


Fig. 5. Anatomy of incubator case joinery.

3.1. Top-level assembly CAD file

The top-level design was created in SOLIDWORKS and exported to eDrawing format. This file is not required to build the incubator, but very useful for visualizing how all the pieces fit together. A free eDrawings viewer is available from Dassault Systemes at <https://www.edrawingsviewer.com>. Many CAD programs can import eDrawings via plugins (Table 1).

3.2. Machined aluminum parts

Aluminum parts were created in SOLIDWORKS. Each part is provided in Drawing Interchange Format (.DXF) and a dimensioned drawing is provided in Portable Document Format (.pdf). All aluminum part files are in a subdirectory called "Aluminum" (Table 2).

3.3. Laser-cut black acrylic parts

Laser-cut acrylic parts were created in SOLIDWORKS. Each part is provided in DXF format. All files are referenced in this section reside in a subdirectory called "Black Acrylic" (Table 3).

3.4. Laser-cut clear acrylic part

A DXF file for the one laser-cut clear acrylic part is located in the "Clear Acrylic" subdirectory (Table 4).

3.5. 3D printed part

There is one 3D printed part in the design that was created in SOLIDWORKS and saved as a part file (.SLDPRT). It was exported to STL format, which works with many 3D printers. The file is located in the CAD\To 3D Print\ directory. This part is optional (Table 5).

3.6. Linux shell scripts

Linux shell script files (.sh) were written for Debian Linux 8.9 installed on the BeagleBone Black Wireless single-board computer. These scripts control projector functions and can be found in the "Scripts" directory of the repository (Table 6).

4. Bill of materials

Listed Per Incubator Cost is package cost divided by package quantity times build quantity. Package quantities of several parts are inconvenient for single incubator builds. (For example, the bill of materials quantity of item 28 is one but the package quantity is 50.) You may be able to find vendors that offer equivalent components with more convenient package quantities. Contact the authors if you are interested in sharing parts with another bacterial photographer. If there is sufficient interest, we will consider developing a parts kit.

Items marked "commodity" are standard items available from many vendors. Shop around!

4.1. Aluminum stock

The build requires 3/8" aluminum bar and small piece of 0.040" sheet stock. You need enough of the bar stock to produce two pieces with finished dimensions of 7.75" × 4" × 3/8". If you do not have easy access to a local supplier, the specific part number from McMaster-Carr referenced in the table is convenient because it only has to be crosscut to achieve the achieve the final dimensions. Only one small part is made from 0.040" stock, with final dimensions of 1.25" × 1.25" × 0.040". The exact thickness is not critical, so look around the metal shop for a suitable piece of scrap before you purchase anything (Table 7).

4.2. Acrylic stock

The bill of materials calls for two types of 6 mm acrylic sheet: black DP9 and clear. DP9 has a frosted/matte finish on one side that helps reduce stray light reflections inside the incubator. Although common, DP9 is not as readily available as stan-

Table 1
Top-level assembly CAD file.

File Name	Image	Location on Overall Assembly	Description
Prokaryote Playhouse Assembly.EASM		N/A	Top level assembly drawing of incubator with all components.

Table 2
Machined aluminum part design files.

Filename	Image	Location on Overall Assembly	Description
Sample Shelf.DXF Sample Shelf.pdf			Aluminum heated shelf to hold Petri dishes during incubation. Cartridge heater and temperature probe potted in with thermally conductive epoxy. Anodized or painted flat black to reduce reflections. Approx. 8" × 4".
Heated Vertical Plate.DXF Heated Vertical Plate.pdf			Aluminum heated vertical plate. Similar to Heated Shelf but positioned vertically on back wall. Increases temperature uniformity and thermal capacity inside box. Cartridge heater potted in. Anodized or painted flat black to reduce reflections. Approx. 8" × 4".
Power Connector Mounting Plate.DXF Power Connector Mounting Plate.pdf			Power connector mounting plate to be machined. Approx. 1 1/8" × 1 1/8" × 0.040" aluminum plate with large mounting hole for power connector and pass holes for 4 screws.

Table 3
Laser-cut 6 mm DP9 black acrylic part design files.

Filename	Image	Location on Overall Assembly	Description
Door.DXF			Laser-cut black acrylic panel that forms incubator door. Includes holes for magnets that hold the door in place when closed. Approx. 7.75" × 5.75".
Front Panel.DXF			Laser-cut black acrylic panel that forms the front wall of incubator box. Rectangular opening for access to Petri dishes in operation. Holes for magnets that secure door. Approx. 10.5" × 8.5".
Bottom Panel.DXF			Laser-cut black acrylic panel that forms the floor of the incubator box. The Heated Sample Shelf is affixed by standoffs. Approx. 8.5" × 6.75".
Back Panel.DXF			Laser-cut black acrylic panel that forms the back of the incubator box. Also includes mounting holes for projector, thermostat, and power connector. Approx. 17.5" × 4.5".
Top Panel.DXF			Laser-cut black acrylic panel that forms the top of incubator box. Includes opening and mounting holes for projection window. Approx. 8.5" × 6.5".
Baffle Box Front Back.DXF			Laser-cut black acrylic panel that forms the front of the baffle box, which excludes stray light from the projection window. Approx. 4" × 3.75".
Baffle Box Left.DXF			Laser-cut black acrylic panel that forms the left side of the baffle box, which excludes stray light from the projection window. Approx. 5.75" × 4.75".
Baffle Box Right.DXF			Laser-cut black acrylic panel that forms the right side of the baffle box, which excludes stray light from the projection window. Approx. 4" × 4".
Baffle Box Top.DXF			Laser-cut black acrylic panel that forms the left side of the baffle box, which excludes stray light from the projection window. Approx. 5" × 4".
Small Tensioner.DXF			Laser-cut black acrylic small tensioning rod with T-slots to accommodate cam nut and screw shaft. This applies tension to keep mortise and tenon joints of the baffle box together. Approx. 3.5" × 0.5".
Electronics Enclosure Front.DXF			Laser-cut black acrylic panel that forms front of electronics enclosure. Approx. 2.5" × 1.5".
Electronics Enclosure Top.DXF			Laser-cut black acrylic panel that forms top of electronics enclosure. Approx. 2.5" × 2.5".
Left Panel.DXF			Laser-cut black acrylic panel that forms the left wall of incubator box. Identical to right wall, except matte finish faces opposite direction. Approx. 11" × 7.25".
Right Panel.DXF			Laser-cut black acrylic panel that forms the right wall of incubator box. Identical to left wall, except matte finish faces opposite direction. Approx. 11" × 7.25". Mirror image of Left Panel.
Large Tensioner.DXF			Laser-cut black acrylic large tensioning rod with T-slots to accommodate hex nut and screw shaft. Quantity 4 of these hold the main incubator enclosure together. These apply tension to keep mortise and tenon joints of the incubator box together. Approx. 8" × 1".

Table 4
Laser-cut 6 mm clear acrylic part design files.

Filename	Image	Location on Overall Assembly	Description
Projection Window.DXF			Laser-cut clear acrylic window for projecting images inside of incubator. Approx. 3" × 2".

dard “shiny-both-sides” acrylic sheet. Shiny acrylic is an acceptable substitute if you dull interior surfaces with steel wool after cutting. The total area of black DP9 is about 4.4 square feet. The exact quantity will depend on your laser cutter work area, stock size, and layout efficiency. We purchased 8' × 10' sheets from a local vendor and had the vendor cut them down

Table 5
3D printed part design files.

Filename	Image	Location on Overall Assembly	Description
ProjectorShroud.STL ProjectorShroud.SLDPRT			3D printed plastic part to block light from entering small opening between projector lens and window in baffle box top. Optional. Approx. 0.64" × 0.5" × 0.31"

Table 6
Linux shell scripts.

Script Name	Function
TurnProjectorOn.sh	Enables projector output. Projector splash screen appears after running.
ProjectorToMaximumBrightness.sh	Configures projector light engine to produce brightest possible output.
DebianDesktopOnProjector.sh	Displays Debian desktop on projector.
DisplayFiles.sh	Scans for new image files in the directory /home/Debian/pictures and displays them full screen in a Chromium tab. After 5 tabs have been accumulated, the browser restarts. Useful for automatically displaying images. (Images can also be displayed manually.) Runs every 30 s.

Table 7
Aluminum stock.

Item #	Component Name	Manufacturer/Vendor Part Number Link	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
1	Aluminum Bar	McMaster Carr (Commodity) 8975 K426 https://www.mcmaster.com/8975K426/	2	2	ft.	\$ 26.03	\$ 26.03	24" × 4" × 3/8" 6061 Aluminum Bar
2	Aluminum Sheet	McMaster Carr (Commodity) 89015 K163 https://www.mcmaster.com/89015K163/	0.009	0.25	sq. ft.	\$ 4.21	\$ 0.15	0.040" Sheet Aluminum

Table 8
Acrylic stock.

Item #	Component Name	Source Link	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
3	Black Acrylic	Alltec Plastics (Commodity) http://altecplastics.com	4.5	32	sq. ft.	\$294.00	\$ 41.34	6 mm Black DP9 Acrylic
4	Clear Acrylic	Scrap Alternate: Alltec Plastics (Commodity) http://altecplastics.com	0.04	32	sq. ft.	\$294.00	\$ 0.38	6 mm Clear Acrylic

to the size of our laser cutter's work area. This required a special order because the vendor does not normally stock DP9, but was able to obtain it within a few days.

Only one small part, the 3" × 2" Projection Window, is made from 6 mm clear acrylic. It is likely that you will find a suitable offcut in the discard pile next to any respectable laser cutter (Table 8).

4.3. 3D printing filament or resin (optional)

The Projector Shroud is an optional part and may be omitted from the build. The source listed in Table 9 is included for cost reference. Use whatever dark colored filament or resin you have handy. If you don't already have some dark-colored filament or resin on hand, it's not worth buying any just for this. The projector works fine without the shroud. If the 3D printer is free and there is some dark filament already in it, knock yourself out (Table 9).

Table 9
3D printer filament.

Item #	Component Name	Manufacturer/Vendor Part # Link	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
5	Projector Shroud	AMZ3D (Commodity) PLA-1.75-Black-1 kg https://www.amazon.com/1-75mm-Black-PLA-Printer-Filament/dp/B01BZ5ND80	0.03	2.2	lb.	\$ 19.99	\$ 0.25	Dark-Colored PLA

4.4. Purchased electronic systems and components

Many companies sell W1209 thermostats (Item 8 below) for extraordinarily cheap prices. We purchased one batch of these that were defective. Chip markings on the defective units suggested that they were likely counterfeit. Purchase Item 8 from a trustworthy vendor (Table 10).

4.5. Fasteners and miscellaneous hardware

The 10–32 × ½" screw (Item 20) just holds the knob on the door. You can almost certainly find an appropriate spare screw without buying a whole package of 100 (Table 11).

Table 10
Purchased electronic systems and component.

Item #	Component Name	Manufacturer/Vendor Part # Link	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
6	Computer	GHI Electronics BBBWL-SC-562 BeagleBone Black Wireless https://www.digikey.com/products/en?keywords=BBBWL-SC-562	1	1	count	\$ 78.75	\$ 78.75	Preassembled electronic system
7	Projector	Texas Instruments DLPDLCR2000EVM https://www.digikey.com/products/en?keywords=DLPDLCR2000EVM	1	1	count	\$ 118.00	\$ 118.00	Preassembled electronic system
8	Thermostat Temperature Probe	Elecrow (Commodity) W1209 https://www.elecrow.com/w1209-high-precision-digital-thermostat-incubator-temperature-controller.html	1	1	count	\$ 4.60	\$ 4.60	Preassembled electronic system
9	Power Connector And Nut	CUI Devices SD-50LS https://www.digikey.com/products/en?keywords=CP-1235-ND	1	1	count	\$ 4.39	\$ 4.39	Electronic component
10	Cartridge Heater	Signstek (Commodity) SW043 https://www.amazon.com/gp/product/B00SUYVLYC	2	5	count	\$ 8.99	\$ 3.60	Electronic component
11	Fan	Sunon MF40101VX-1000U-G99 https://www.digikey.com/product-detail/en/sunon-fans/MF40101VX-1000U-G99/259-1808-ND/6564863	1	1	count	\$ 6.35	\$ 6.35	Preassembled electronic system
12	Power Supply	Mean Well USA GP50A13D-R1B https://www.digikey.com/products/en?keywords=1866-1832-ND	1	1	count	\$ 48.69	\$ 48.69	Preassembled electronic system
13	5 V Power Cable	Memory Protection Devices 172-4207 https://www.digikey.com/en/products/detail/mpd-memory-protection-devices/172-4207/2439191	2	1	count	\$ 3.47	\$ 6.94	Electronic component
14	Wire Nut	TE Connectivity 2299502-1 https://www.digikey.com/en/products/detail/te-connectivity-amp-connectors/2299502-1/6588344	4	1	Count	\$ 0.10	\$ 0.10	Electronic component

Table 11
Fasteners and miscellaneous hardware.

Item #	Component Name	Manufacturer/Vendor Part Number Link	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
Screws								
15	Black Nylon Screw	McMaster-Carr (Commodity) 94735A250 https://www.mcmaster.com/94735A250	16	200	count	\$ 7.26	\$ 0.58	Black nylon 6–32 × 5/8 pan head screw
16	4–40 × 7/16" screw	McMaster-Carr (Commodity) 92949A111 https://www.mcmaster.com/92949A111	8	100	count	\$ 3.65	\$ 0.29	18–8 Stainless steel 4–40 × 7/16" BHCS screw
17	4–40 × 5/8" screw	McMaster-Carr (Commodity) 92949A112 https://www.mcmaster.com/92949A112	28	100	count	\$ 3.90	\$ 1.09	18–8 Stainless steel 4–40 × 5/8" BHCS screw
18	8–32 × 3/4" Screw	McMaster-Carr (Commodity) 92949A197 https://www.mcmaster.com/92949A197	4	100	count	\$ 6.81	\$ 0.27	18–8 Stainless steel 8–32 × 3/4" BHCS screw
19	M2.5 × 0.45 Screw	McMaster-Carr (Commodity) 92095A459 https://www.mcmaster.com/92095A459	4	25	count	\$ 7.14	\$ 1.14	18–8 Stainless steel M2.5 × 0.45 BHCS screw
20	10–32 × 1/2" Screw	McMaster-Carr (Commodity) 91772A829 https://www.mcmaster.com/91772A829/	1	100	count	\$ 9.17	\$ 0.09	18–8 Stainless steel 10–32 × 1/2" Screw BHCS screw
Nuts								
21	4–40 nut	McMaster-Carr (Commodity) 91841A005 https://www.mcmaster.com/91841A005	36	100	count	\$ 3.02	\$ 1.09	18–8 Stainless steel 4–40 hex nut
22	8–32 Nut	McMaster-Carr (Commodity) 90730A010 https://www.mcmaster.com/90730A010	4	100	count	\$ 6.80	\$ 0.27	18–8 Stainless steel 8–32 hex nut
Standoffs								
23	Projector Standoff	McMaster-Carr (Commodity) 94868A167 https://www.mcmaster.com/94868A167	4	1	count	\$ 1.30	\$ 5.20	18–8 stainless steel hex standoff, 4.5 mm × 12 mm long, M2.5 × 0.45 mm thread
24	Computer Standoff	McMaster-Carr (Commodity) 93655A090 https://www.mcmaster.com/93655A090	4	1	count	\$ 3.35	\$ 13.40	18–8 Stainless steel hex standoff, 4.5 mm × 5 mm Long, M2.5 × 0.45 mm thread
25	Thermostat Standoff	McMaster-Carr (Commodity) 92745A321 https://www.mcmaster.com/92745A321	4	1	count	\$ 0.38	\$ 1.52	Nylon hex standoff, 1/4" × 3/8" Long, 4–40 to 4–40 thread
26	Heated Shelf Standoff	McMaster-Carr (Commodity) 92319A666 https://www.mcmaster.com/92319A666	4	2	count	\$ 2.34	\$ 4.68	Nylon hex standoff, 1/4" × 1" Long, 6–32 female thread
27	Heated Back Plate Standoff	McMaster-Carr (Commodity) 92319A658 https://www.mcmaster.com/92319A658	4	2	count	\$ 2.11	\$ 4.22	Nylon hex standoff, 1/4" × 3/4" Long, 6–32 female thread
Miscellaneous Hardware								
28	Grommet	McMaster-Carr (Commodity) 9600 K54 https://www.mcmaster.com/9600 K54	1	50	count	\$ 11.57	\$ 0.23	Rubber
29	Knob	McMaster-Carr (Commodity) 6046 K42 https://www.mcmaster.com/	1	1	count	\$ 1.37	\$ 1.37	Phenolic knob with metal 10–32 thread

Table 11 (continued)

Item #	Component Name	Manufacturer/Vendor Link	Part Number	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
30	Magnets	6046 K42 Apex Magnets (Commodity) M5x5mmCYL		8	30	count	\$ 8.99	\$ 2.40	Rare earth magnet

4.6. Adhesives and paint

We used 3M TC-2810 thermal epoxy (Item 31) to mount the cartridge heaters and temperature probe. This is a high-quality and well-characterized product, but at 50 ml the package quantity is much too large for a single incubator build. Furthermore, the product is best dispensed with an expensive gun (~\$100, not included in BOM) and mixing nozzle (~\$4, not included). Many suitable alternatives exist that are available in friendlier quantities and packaging. One example is MG Chemicals 8329TCM-6ML, which comes in a 6 ml, self-dispensing package for about \$15 (<https://www.mgchemicals.com/products/adhesives/thermally-conductive-adhesives/thermal-adhesive-high-tc/>). Choose an epoxy with high thermal conductivity and good bonding properties to help ensure temperature uniformity of the sample shelf. The five-minute epoxy holds the magnets in their holes. Most any brand will do (Table 12).

4.7. Total cost

Total parts cost per incubator is \$386.06.

5. Build instructions

The incubator build requires familiarity with cutting and drilling aluminum, laser cutting acrylic, soldering, basic hand tools, and optionally 3D printing. Seek out a local expert if you need to develop new skills for the build. Follow your institution's safety protocols during all machining and assembly steps.

The following tools and resources are required to complete the build:

- MicroSD card
- USB mouse and keyboard
- USB cable (type B micro to whatever kind of USB your computer has)
- Laser cutter
- Bandsaw or other type of saw that can cut 3/8" aluminum
- Drill or drill press
- Drill bits:

Table 12
Adhesives and paint.

Item #	Component Name	Source	Build Qty	Package Qty	Unit	Package Cost	Per Incubator Cost	Material type
31	Thermal Epoxy	3 M (Commodity) TC-2810 50ML https://www.digikey.com/en/products/detail/mg-chemicals/8329TCM-6ML/6035511	4	50	ml	\$ 83.93	\$ 6.71	Thermally conductive adhesive
32	Five Minute Epoxy	Loctite (Commodity) 1,365,868 https://www.amazon.com/Loctite-1365868-6-Minute-Instant-Syringes/dp/B0044F9KFI/	4	50	ml	\$ 3.59	\$ 0.29	Thermally conductive adhesive
33	Flat Black Spray Paint	Rustoleum (Commodity) 245,198 https://www.rustoleum.com/product-catalog/consumer-brands/universal/universal-flat-spray-paint	4	12	oz	\$ 3.99	\$ 1.33	Paint

- o #30 (0.1285")
- o #25 (0.1495")
- o 7 mm (0.1575")
- o #1 (0.2280")
- o 23/32" or 18.5 mm (0.7284")
- Steel wool (if using standard acrylic)
- Center punch
- Clamps or fixtures to hold stock during drilling
- Backer board
- Metal finishing tools (some combination of file, sandpaper, and deburring tool)
- Suitable location to apply spray paint (if not anodizing)
- Epoxy mixing/application sticks (popsicle stick, toothpick, ...)
- Disposable mixing surface (piece of scrap cardboard)
- Wire cutter/stripper
- Soldering iron
- Solder
- Needle nose pliers
- Electronic multimeter
- 1/16" hex driver
- 3/32" hex driver
- ~1/4" flat head screwdriver
- #1 Phillips screwdriver

5.1. Prepare the projector and single-board computer

In this step you will connect the projector to the single board computer, as shown in Fig. 6. You will also install the operating system and custom scripts on the computer.

Parts needed:

- BeagleBone Black Wireless single-board computer
- Texas Instruments LightCrafter Display 2000 EVM DLP evaluation module
- Computer Standoffs (Item 24, quantity 4)
- Projector Standoffs (Item 23, quantity 4)

Tools needed:

- microSD card
- USB mouse and keyboard

5.1.1. Install standoffs and mate the projector to the computer

Insert the externally threaded end of a Computer Standoff into a mounting hole on the BeagleBone Black Wireless and secure it with a Projector Standoff, as shown in Fig. 6. Install three more sets of standoffs in the remaining mounting holes. Mate the Projector to the Computer by aligning the header pins on the Projector with the matching sockets on the Computer and squeezing the two together, as per the instructions on the manufacturer's website [12].

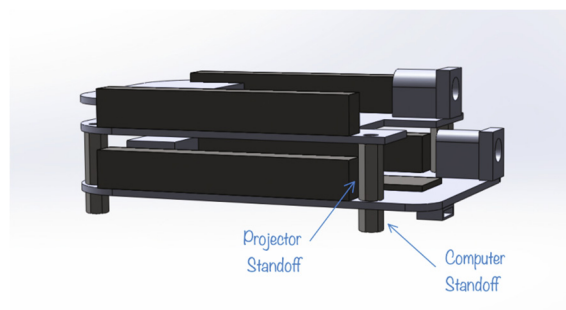


Fig. 6. Projector mated to BeagleBone Black Wireless.

5.1.2. Install operating system

The BeagleBone Black and the Projector must be connected to power for this step. The computer can be powered either through its USB connector or the external power connector, but the projector requires an external +5 V power source with a 2.1 mm × 5.1 mm round plug that has the positive voltage on the center terminal and ground on the outside. This style of power adapter is fairly common – check the junk drawer in your lab or borrow one from another piece of equipment if possible. You'll only need it for an hour or so. If you can't find one, follow the instructions in step 5.7 and then wire up a temporary power supply using the Power Connector, Power Supply, and 5 V Power Cable (Items 9, 12, and 13). Wire +5 V to the center conductor and ground to the outer conductor, as shown in Fig. 27.

Connect the mouse and keyboard to the BeagleBone Black. Install Debian Linux as per the manufacturer's instructions. There are several helpful resources on the internet. References [15,16], and the FAQ maintained by the manufacturer [17] are particularly worthwhile.

5.1.3. Install script files

Download Prokaryote Playhouse script files from "Scripts" folder of the design repository (<http://doi.org/10.17605/OSF.IO/AEUYH>). Follow the instructions in the file "HowToInstall.html" to install script files. After installation, images copied to the `/home/debian/pictures` directory of the BeagleBone Black Wireless will be projected automatically within 30 s.

Set the prepared projector and computer aside in a safe place.

5.2. Fabricate laser-cut acrylic parts

In this step you will fabricate the acrylic parts that form the incubator enclosure.

Materials needed:

- 6 mm black DP9 acrylic (Item 3, approx. 4.5 sq feet)
- 6 mm clear acrylic (Item 4, approx. 3" × 2")

Tools needed:

- laser cutter

5.2.1. Cut a test piece

Acrylic sheet thickness varies from manufacturer to manufacturer and batch to batch. Key laser cutter performance parameters such as accuracy and kerf width also differ from machine to machine (and even sometimes from location to location in a cutter's work area). As such, it is wise to cut a test mortise and tenon or two before slicing up a lot of plastic. If you notice more than slight resistance as the test tenon slides into its mortise, the fit is too tight. Resist the temptation to force a tenon into a mortise that is too small. If you force it in, the tenon is very likely to break off in such a way that the remnant is virtually impossible to remove. This situation usually results in a good deal of swearing. A little extra play is preferable to an undersized mortise. The tensioning bars will hold the joints together firmly. Nonetheless, a sloppy fit is also undesirable. Depending on the laser cutter and software you use, various options exist to adjust the fit. One useful tool is the kerf correction extension for Inkscape (https://github.com/braingram/inkscape_kerf_correction). Small adjustments to tight-fitting mortises can be made with a flat needle file. (We used 3 different laser cutters and 2 batches of acrylic stock to cut parts for 10 incubators. Only one mortise required a small adjustment with a needle file.)

5.2.2. Laser cut 6 mm DP9 black acrylic parts

Table 13 catalogs the parts to be laser cut from 6 mm black DP9 acrylic sheet stock. The process for submitting DXF files to the laser cutter depends on the make, model, and software version of the cutter. Many laser cutters are configured to accept DXF files directly. We used an open-source program called Inkscape (<https://inkscape.org>) to convert the DXF files to SVG format for use on a Dremel LC40 laser cutter that accepts only PDF and SVG files.

Lay the parts out efficiently within your laser cutter's work area and cut the quantities indicated. Approximate sizes are shown in the table. Drawing scales vary. The Left Panel and Right Panel are mirror images so that the frosted side of both panels faces the inside of the incubator.

5.2.3. Laser cut 6 mm clear part

Laser cut the Projection Window from 6 mm clear acrylic sheet stock.

5.3. Fabricate and paint or anodize aluminum parts

In this step you will make the Heated Sample Shelf, Heated Vertical Plate, and Power Connector Mounting Plate from aluminum stock. Dimensioned drawings in PDF format and DXF files of the parts are available in the Aluminum directory of the repository.

Materials needed:

Table 13
Laser-cut black acrylic parts.

Door Approx. 7.75" × 5.75" Quantity 1	Front Panel Approx. 10.5" × 8.5" Quantity 1	Bottom Panel Approx. 8.5" × 6.75" Quantity 1
Back Panel Approx. 17.5" × 4.5" Quantity 1	Top Panel Approx. 8.5" × 6.5" Quantity 1	Baffle Box Front Back Approx. 4" × 3.75" Quantity 2
Baffle Box Left Approx. 5.75" × 4.75" Quantity 1	Baffle Box Right Approx. 4" × 4" Quantity 1	Baffle Box Top Approx. 5" × 4" Quantity 1
Small Tension Bar Approx. 3.5" × 0.5" Quantity 1	Electronics Enclosure Front Approx. 2.5" × 2.5" Quantity 1	Electronics Enclosure Top Approx. 2.5" × 2.5" Quantity 1
Left Panel Approx. 11" × 7.25" Quantity 1	Right Panel Approx. 11" × 7.25" Quantity 1	Large Tension bar Approx. 11" × 7.25" Quantity 4

- 3/8" aluminum stock (Item 1)
- ~0.040" aluminum stock (Item 2)
- Black spray paint (Item 33, only if not anodizing)

Tools needed:

- Bandsaw or other type of saw that can cut 3/8" aluminum
- Drill
- Drill bits:
 - #30 (0.1285")
 - #25 (0.1495")
 - 8 mm (0.1575")
 - #1 (0.2280")
 - 23/32" or 18.5 mm (0.7284")
- Center punch
- Clamps to hold stock during drilling
- Backer board
- File, sandpaper, or deburring tool

5.3.1. Cut and drill the parts

It is possible to cut the aluminum parts on a water jet cutter, but an old-school bandsaw and drill press is probably simpler. Cut the Heated Sample Shelf and Heated Vertical Plate to size. You may find it more convenient to cut the Power Connector Mounting Plate to size after drilling the holes. Lay out the hole locations on the stock using a center punch. Drill holes. Ensure that workpieces are securely clamped during drilling. Use a backing board to prevent tearout and distortion. Clean up all surfaces with file, sandpaper, or deburring tool.

5.3.2. Paint or anodize the heated sample shelf and heated vertical plate

Spray paint or anodize the Heated Sample Shelf and Heated Vertical Plate. Anodization looks snazzier, but the process is more expensive and takes a few days to complete at an outside shop. The cost per piece to anodize a small batch may be very high. To ensure good thermal conductivity, do not paint the insides of the heater and temperature probe holes. Remove any paint that gets inside the holes with a fine, round needle file or sandpaper.

5.4. Fabricate 3D printed part (optional)

This step is optional. In this step you will produce a small 3D printed part that goes between the lens of the projector and the opening of the Baffle Box (Fig. 7). Light tightness of the incubator is satisfactory without the Light Shroud, so you can skip this step if you want.

Materials needed:

- Dark-colored 3D printing filament or resin, depending on printing process (Item 5)

Tools needed:

- 3D printer

If you would like to include the optional light shroud, 3D print the file `ProjectorShroud.STL` using a dark, nonreflective material.

5.5. Preassembly

In this step, you will epoxy the cartridge heaters, temperature probe, and magnets in place.

Parts needed:

- Heated Shelf from step 5.3
- Heated Vertical Plate from step 5.3
- Thermal Epoxy (Item 31)

Tools needed:

- Epoxy mixing sticks
- Disposable mixing surface

5.5.1. Secure cartridge heaters and temperature probe with thermal epoxy

Mix a small quantity of thermal epoxy per manufacturer's instructions. Cement the Cartridge Heaters and Temperature Probe in the holes indicated by [Fig. 8](#). Install the heaters and temperature probes so their top surfaces are flush with or slightly proud of the plate. Wires from the heater and probe should exit in the same direction, from the bottom side of the Heated Bottom.

5.6. Epoxy magnets in incubator door and incubator front

In this step, you will bond eight rare-earth magnets into their mounting holes and attach the doorknob to the door with a screw, as shown in [Fig. 9](#). The magnets hold the Door in place when the incubator is in operation.

Parts and materials needed:

- Front Panel from step 5.2.2
- Door from step 5.2.2
- Magnets (Item 38, quantity 8)
- Five-Minute Epoxy (Item 32)
- Knob (Item 29)
- 10–32 × ½" screw (Item 20)

Tools needed:

- Epoxy mixing stick
- Disposable mixing surface

Test fit the magnets and adjust the mounting holes with a file or sandpaper if necessary. Mix a small quantity of Five-Minute Epoxy on a disposable mixing surface per manufacturer's instructions. Bond a magnet in each hole. The ends of the magnets should be slightly shy of the acrylic surfaces. Remove excess epoxy to ensure that the Door lies flat against the Front Panel. Make sure that the magnet poles all face the same direction so that the door will function properly. (If you are building more than one incubator, it's a good idea to orient the poles the same way on all of them so the doors

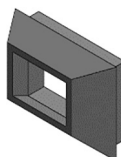


Fig. 7. Light shroud.

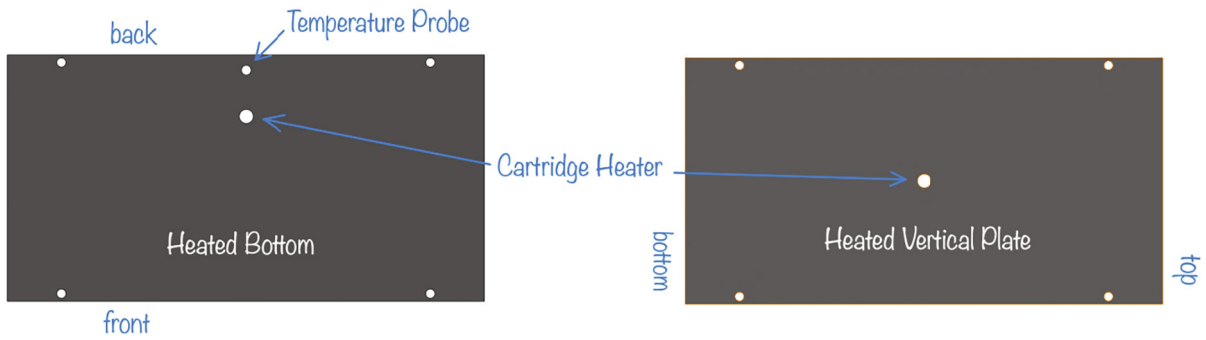


Fig. 8. Cartridge heater and temperature probe locations.

are interchangeable.) Do not allow the parts to come together or place them near ferrous metal until the epoxy sets to avoid dislodging the magnets.

After the epoxy sets, attach the knob with a 10–32 × 1/2" screw.

5.7. Solder wires to power connector contacts

In this step, you will prepare the power connector by soldering short lengths of wire to each of the pins. Parts needed for this step:

- Power Connector (Item 9)
- Cutoffs from Cartridge Heater leads (Item 10) or other suitable wire

Tools needed for this step:

- Wire cutter/stripper
- Soldering iron
- Solder

Cut five approximately 2" lengths of wire from the excess length of the Cartridge Heater leads. (Or use any suitable wire you have at hand. The maximum current is 5 A and the maximum voltage is 24 V.) Strip one end of each wire very short, about 1/8–1/4", for soldering to the lugs of the Power Connector. Strip 1/2" to 3/4" from the other end. The heat resistant insu-

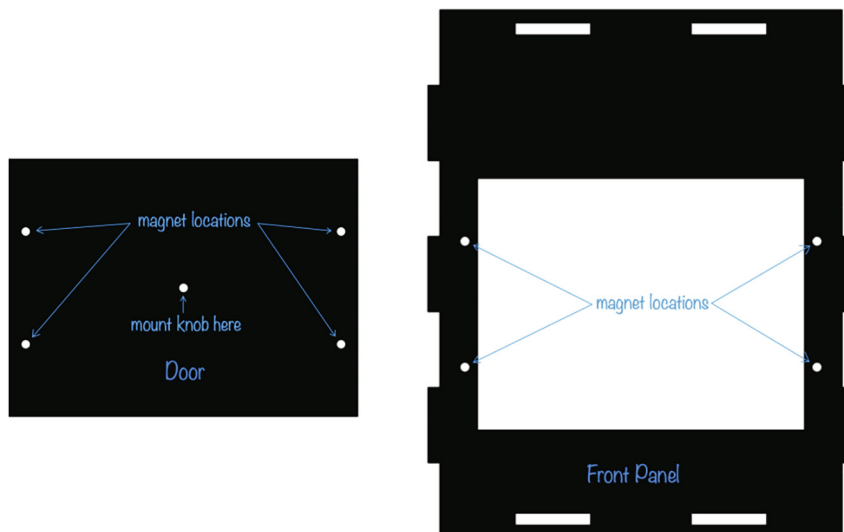


Fig. 9. Magnet locations.

lation on the Cartridge Heater wires is a little fiddlier to work with than regular wire. Connections to the long ends will be made with a wire nut. Solder the short end of a wire to each pin of the power connector.

5.8. Assemble the incubator bottom

In this step, you will assemble the Incubator Bottom, as shown in [Fig. 10](#).

Parts needed:

- Bottom Panel from step 5.2.2
- Heated Shelf from step 5.5.1 (with Cartridge Heater and Temperature Probe installed)
- Black Nylon 6–32 × 5/8" Screws (Item 15, quantity 8)
- Heated Shelf Standoffs (Item 26, quantity 4)

Tools needed:

- ~1/4" flat head screwdriver
- 1/16" hex driver

Mount the Heated Shelf on the Back Panel using four Heated Shelf Standoffs and eight Black Nylon 6–32 × 5/8 screws, as shown in [Fig. 10](#), with the wires from the Cartridge Heater and Temperature Probe exiting toward the back. Be sure to use the correct standoffs – the Heated Shelf Standoffs are similar to the Heated Vertical Plate Standoffs (Items 23 and 24). Use the shorter ones for this step.

5.9. Assemble incubator back

In this step, you will assemble the components of the Incubator Back as shown in [Fig. 11](#). (Wires soldered to the Power Connector are omitted for clarity.)

Parts needed for this step:

- Back Panel from step 5.2.2
- Heated Vertical Plate from step 5.5.1 (with Cartridge Heater installed)
- Power Connector Mounting Plate from step 5.3
- Black Nylon 6–32 × 5/8" Screws (Item 15, quantity 8)
- Heated Back Plate Standoffs (Item 27, quantity 4)
- 4–40 × 7/16" Screws (Item 16, quantity 4)
- 4–40 Nuts (Item 21, quantity 4)
- Power Connector and nut from step 5.7 (with wires soldered on)

Tools needed for this step:

- ~1/4" flat head screwdriver

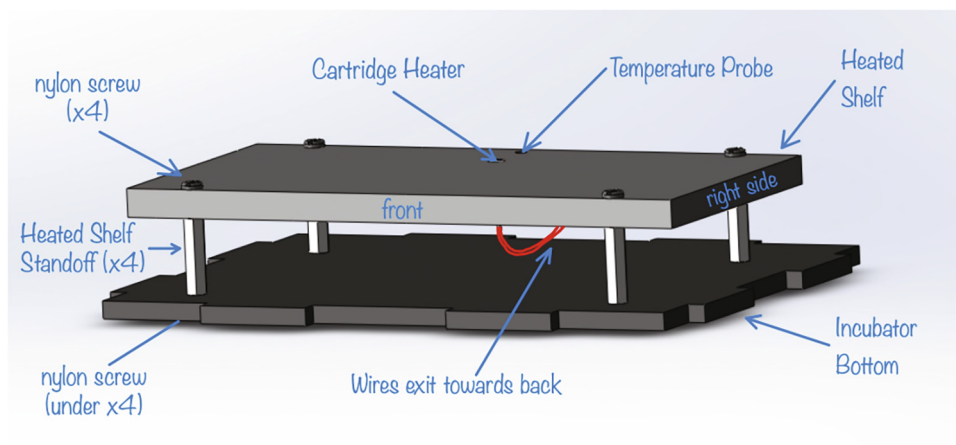


Fig. 10. Incubator Bottom.

- 1/16" hex driver
- Needle nose pliers or other tool suitable for tightening Power Connector Nut

5.9.1. Attach the power connector to the back panel

Attach the Power Connector Mounting Plate to the Back Panel using four 4-40 \times 7/8" screws and four 4-40 nuts, as shown in Fig. 12. Use the nut provided with the power connector to secure the Power Connector in its mounting plate.

5.9.2. Mount heated back plate on back panel

Mount the Heated Back Plate on the Back Panel using 4 Heated Back Plate Standoffs and 8 Black Nylon 6-32 \times 5/8 Screws. Route the wires from the Cartridge Heater through the top set of standoffs, as shown in Fig. 11.

5.10. Assemble the incubator top

In this step, you will assemble the top of the incubator enclosure and the light baffle box, as shown in Fig. 13.

Parts needed for this step:

- Baffle Box Front Back from step 5.2.2 (quantity 2)
- Top Panel from step 5.2.2
- Projection Window from step 5.2.3
- Baffle Box Left from step 5.2.2
- Baffle Box Right from step 5.2.2
- Baffle Box Top from step 5.2.2
- Small Tensioner with T Slots from step 5.2.2
- 4-40 nut, quantity 8 (Item 27)
- 4-40 \times 5/8" screw, quantity 8 (Item 28)

Tools needed for this step:

- 1/16" hex driver
- 3/32" hex driver

5.10.1. Install projection window on incubator top

Secure the Projection Window to the Top Panel using four 4-40 \times 5/8" Screws and four 4-40 Nuts, as shown in Fig. 14. Confirm the orientation of the Top Panel by verifying that the hole is in the right, rear of the part and there is a matte finish is on the bottom.

5.10.2. Assemble the baffle box

Assemble the Baffle Box as shown in Fig. 15, with the projection window facing towards the back side of the box.

5.10.3. Insert the baffle box into the top panel

Slide the tenons on the bottom of the Baffle Box into the matching mortises in the Top Panel so that the Baffle Box encloses the Projection Window, as shown in Fig. 16.

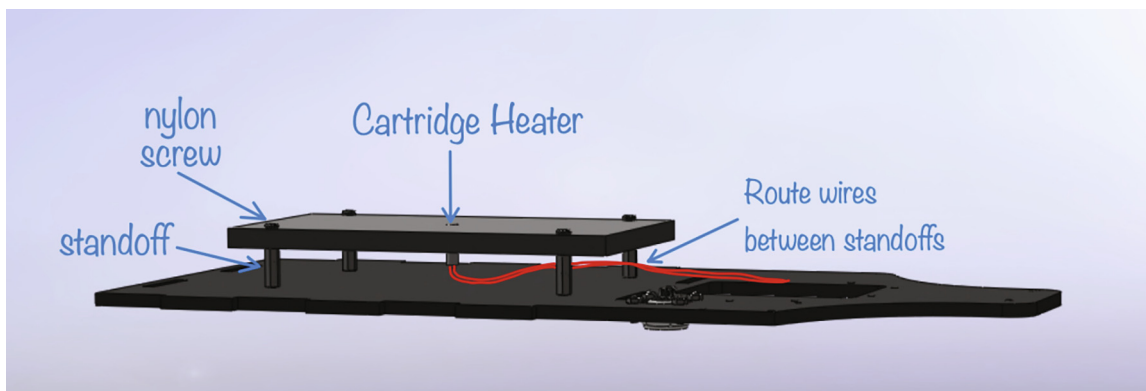


Fig. 11. Assembled Incubator Back with Heated Vertical Plate and Power Connector Mounting Plate mounted on Back Panel.

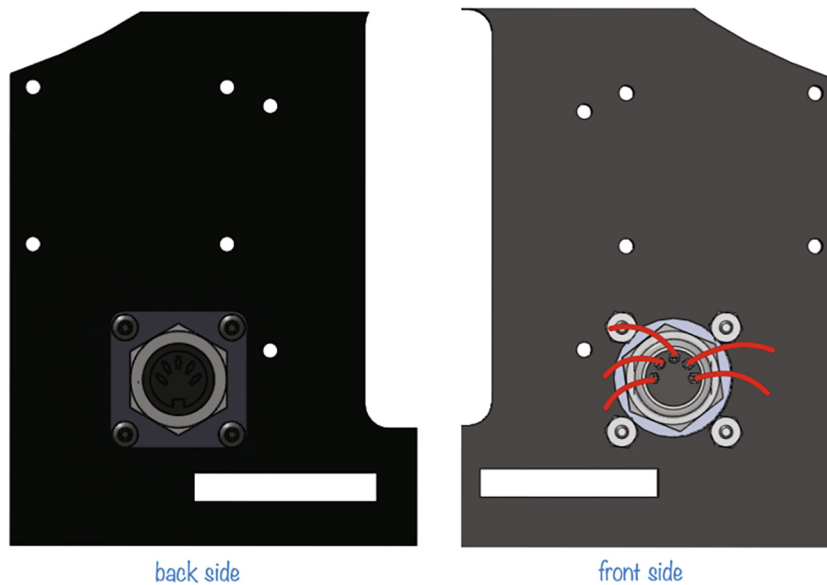


Fig. 12. Power connector mounted in Back Panel.

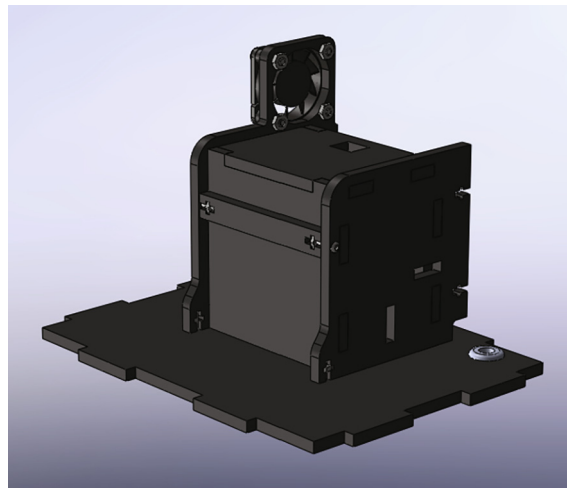


Fig. 13. Assembled Incubator Top.

5.10.4. Install the Small Tensioner with T-Slot

Place the Small Tensioner with T-Slot in-between the left and right walls of the Baffle Box so that the slot is aligned with the matching holes in the sides. Insert a 4–40 nut into one of the slots and thread a 4–40 × 5/8" screw through the matching hole. Tighten just enough to hold in place. Repeat for the other slot (Fig. 17.).

5.10.5. Fasten the baffle box to the top panel

Install 4–40 nuts in the two T-slots at the bottom of the Baffle Box and thread two 4–40 × 5/8" screws through the matching holes in the Top Panel to, as shown in Fig. 18.

5.10.6. Mount the fan

Use four 8–32 × 3/4" screws and four 8–32 nuts to mount the Fan as shown in Fig. 19. The wires should exit toward the bottom of the Baffle Box.

5.10.7. Insert the Rubber Grommet

Insert the Rubber Grommet into the matching hole in the Top Panel, as shown in Fig. 20.

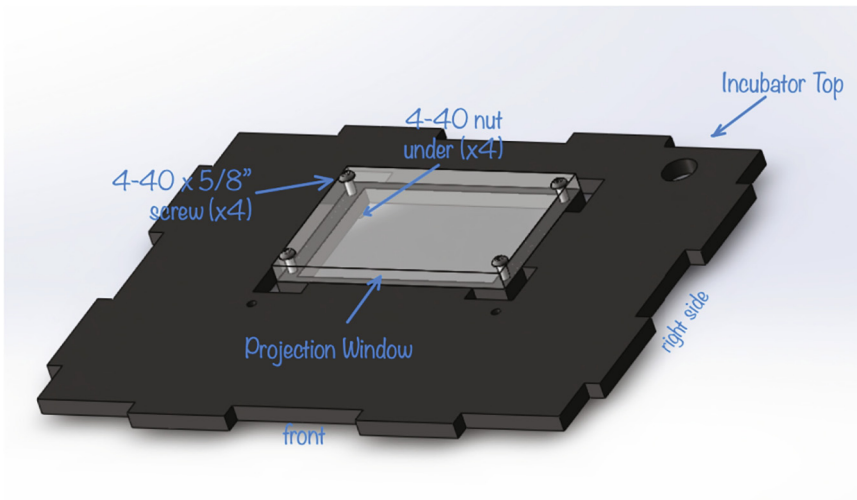


Fig. 14. Projection Window mounted on Top Panel.



Fig. 15. Baffle Box assembly.

5.11. Assemble the incubator frame

Parts needed for this step:

- Assembled Incubator Back from step 5.9.
- Assembled Incubator Bottom from step 5.8
- Assembled Incubator Top from step 5.10
- Assembled Incubator Front from step 5.6

Tools needed for this step:

- 1/16" hex driver
- 3/32" hex driver

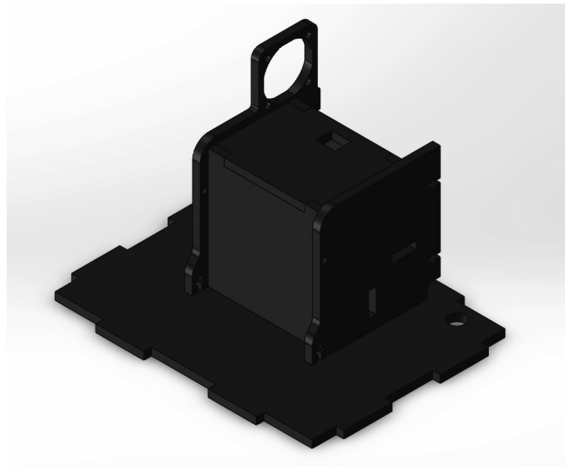


Fig. 16. Baffle Box inserted into Top Panel.

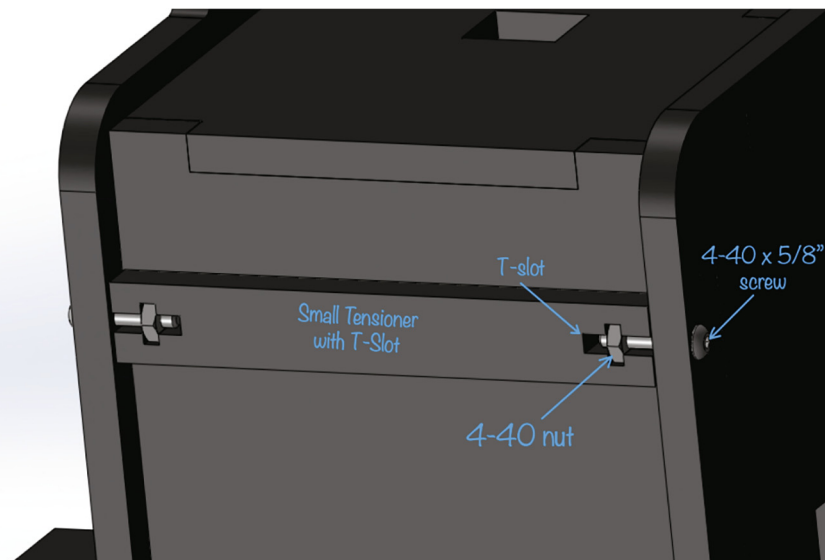


Fig. 17. Small Tensioner with T-Slot Detail.

5.11.1. Route wires

Lay the Incubator Bottom and Back Panel next to each other on a table. Route the wires as shown in Fig. 21. (Wires soldered to the Power Connector are not shown for clarity.)

5.11.2. Assemble the Incubator Bottom to the Back Panel

Insert the back tenons on the Incubator Bottom into the matching mortises in the Back Panel, as shown in Fig. 22. Be careful not to allow excessive torque on the tenons while the Incubator Bottom is unsupported.

5.11.3. Assemble the Top Panel to the Back Panel

Assemble the Top Panel to the Back Panel, as shown in Fig. 23. Route the wires from both Cartridge Heaters and the Temperature Probe through the hole in the Top Panel.

5.11.4. Assemble Incubator Front and Incubator Left Side

Add the Incubator Front and Incubator Left Side, as shown in Fig. 24. Do not add the Incubator Right Side at this time (Fig. 25).

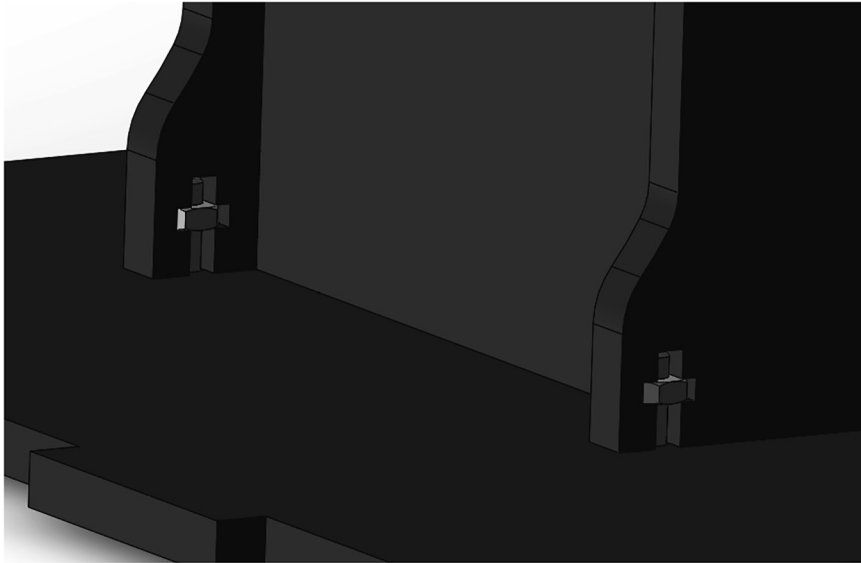


Fig. 18. Fastening the Baffle Box to the Top Panel.

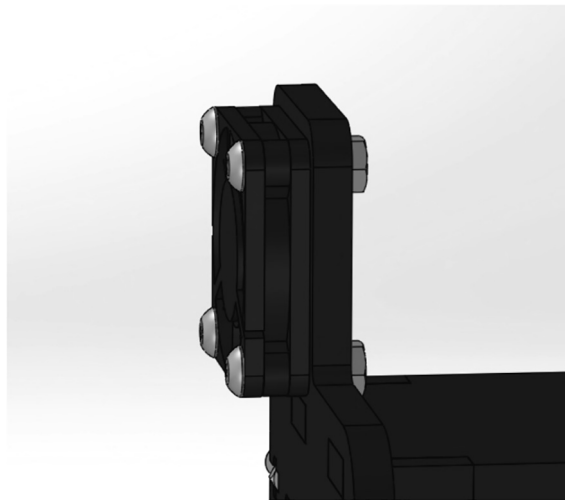


Fig. 19. Mounting the Fan.

5.12. Electronics installation and wiring

In this step, you will wire power to the computer, projector, thermostat, and fan. You will also wire the cartridge heaters and temperature probe.

Parts needed:

- Assembled Computer and Projector (from step 5.1)
- M2.5 × 0.45 Screws (Item 19, quantity 4)
- Thermostat (Item 8)
- Thermostat Standoffs (Item 25)
- 4–40 × 7/16" Screws (Item 16, quantity 4)
- 9 V Power Cables (Item 13, quantity 2)
- Wire nuts (Item 14)
- Electronics Enclosure Top (from step 5.2.2)
- Electronics Enclosure Front (from step 5.2.2)

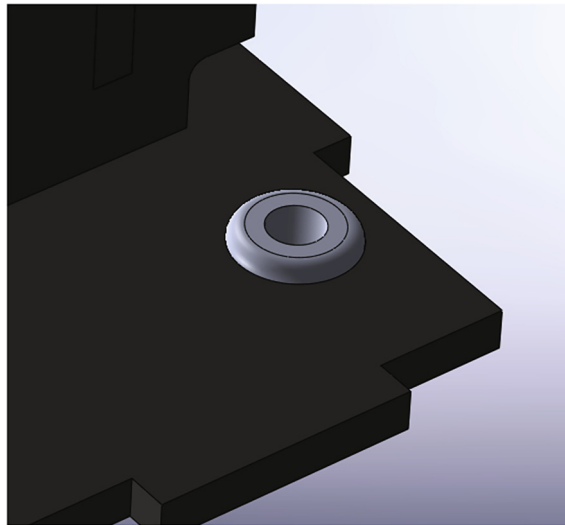


Fig. 20. Rubber Grommet.

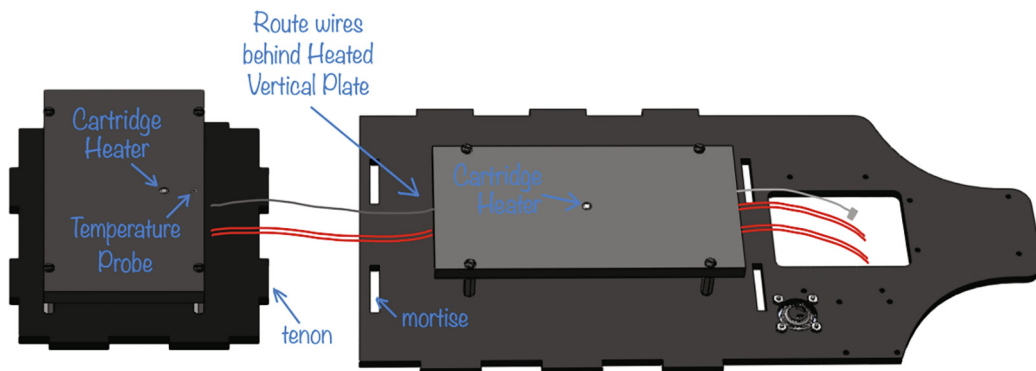


Fig. 21. Cartridge Heater and Temperature Probe wire routing.

Tools needed:

- Wire cutter/stripper
- 1/16" hex driver
- #1 Phillips screwdriver
- Electronic multimeter

5.12.1. Mount thermostat and projector

Secure the Computer and Projector in place from the back using four M2.5 × 0.45 Screws (Item 19).

5.12.2. Connect electronics

Connect a 5 V Power Cable to the Projector and Computer. Route the wires along with the Fan power leads behind the Baffle Box and through the opening shown in Fig. 26 so they run just below the Power Connector. Trim wires to length, leaving enough slack to make a connection with the leads soldered to the Power Connector.

Make the power connections shown in Fig. 27 by twisting wires together and securing with a wire nut. Double check the + 5 V and ground connections to the Projector and Computer power cables. (An error in wiring can destroy both components.) Connect one lead of each Cartridge Heater to + 12 V. (The leads are interchangeable.) Connect the remaining Cartridge Heater leads to one of the relay terminals on the thermostat. Use a short length of excess wire from one of the offcuts to wire the other thermostat relay terminal to ground.

Run the Temperature Probe wire behind the Thermostat and plug the connector into its matching socket.

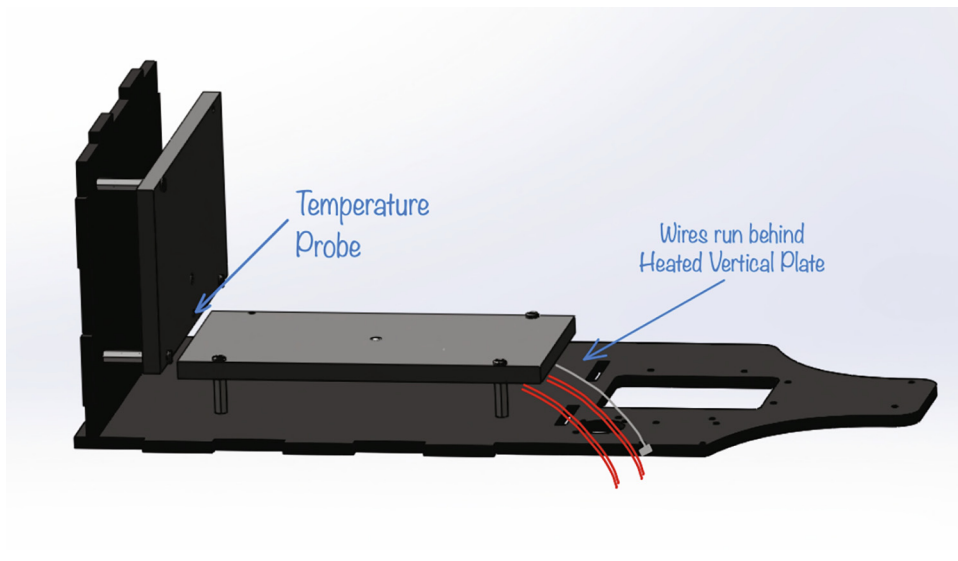


Fig. 22. Incubator Bottom assembled to Back Panel.

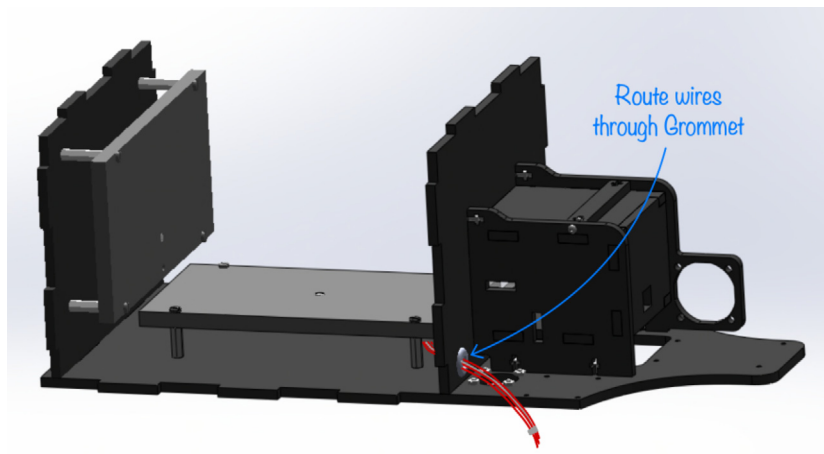


Fig. 23. Top Panel assembled to Back Panel.

5.12.3. Test the electronics

Plug in the Power Supply and connect it to the Power Connector. Verify that the computer, projector, thermostat, and fan power on. Configure the thermostat:

1. Press and hold the “SET” button for at least 5 s until the display reads “P0.”
2. Press “SET” again to configure heating/cooling mode.
3. If the display reads “C” press the “+” button. The display should now read “H” for heating mode.
4. Press the set button again to save the setting.
5. Press the “+” button. “P1” should now be displayed.
6. Use the “-” button to change the hysteresis setting to 0.1 (the minimum value).
7. Press set again.
8. After about 5 s, the temperature should be displayed.
9. Now press “SET” until the display flashes.
10. Use the “+” and “-” buttons to set the temperature to 37 °C or another desired temperature.

A red LED indicates that the relay is closed and the heaters are on. The thermostat will maintain its setting, even if it is unplugged.

Make sure the aluminum plates heat up. Disconnect the power.

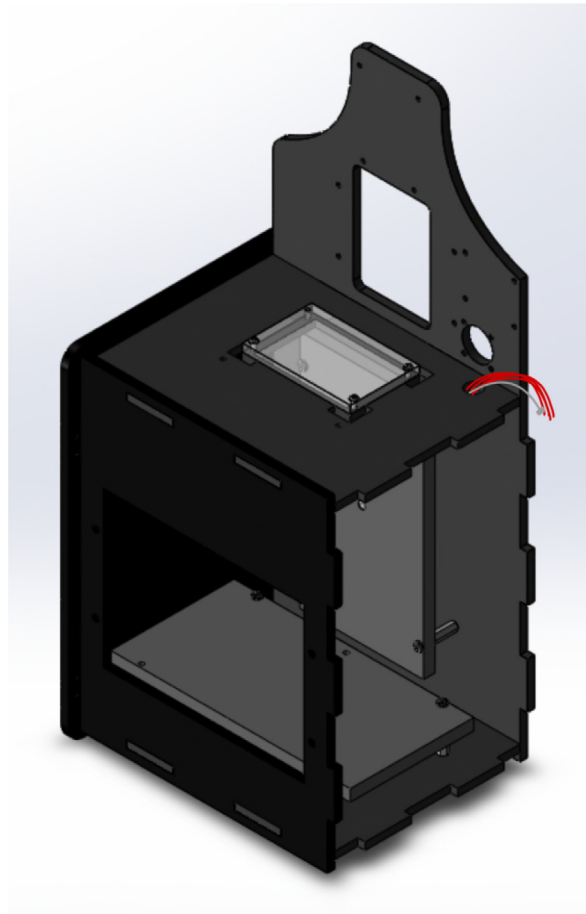


Fig. 24. Partially assembled incubator enclosure.

5.13. Complete assembly

Parts needed:

- Electronics Enclosure Top (from step 5.2.2)
- Electronics Enclosure Front (from step 5.2.2)
- Right Panel (from step 5.2.2)
- Large Tension Bar with T-Slots (from step 5.2.2, quantity 4)
- 4–40 × 5/8" Screws (Item 17, quantity 16)
- 4–40 Nuts (Item 21, quantity 16)

Tools needed:

- 1/16" hex driver

5.13.1. Install Electronics Enclosure

Install the Electronics Enclosure as shown in [Fig. 28](#). Tuck the wire nuts inside the enclosure.

5.13.2. Complete assembly

Install the Right Panel. Secure the incubator case with four Large Tension Bars, sixteen 4–40 × 7/8" screws, and sixteen 4–40 Nuts, as shown in figure [Fig. 29](#) Final assembly.

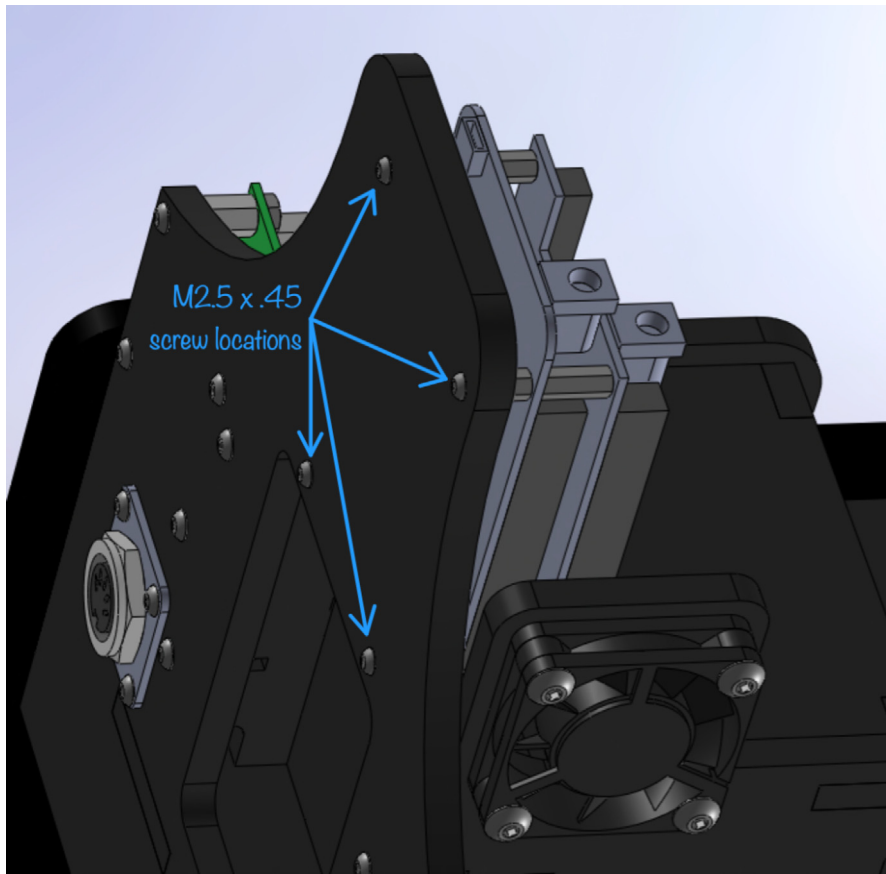


Fig. 25. Projector screw locations.

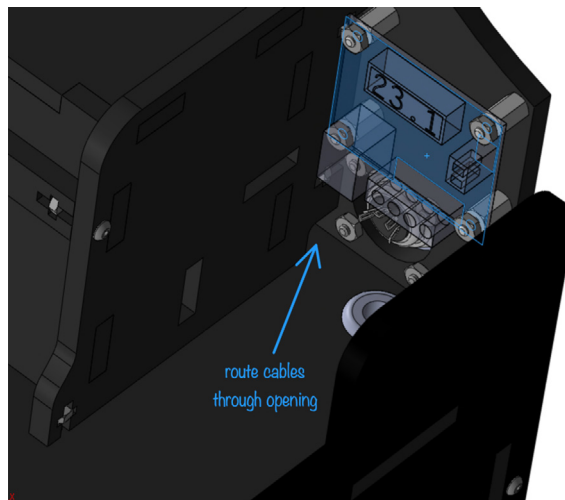


Fig. 26. Projector, computer, and fan power wire routing.

5.14. Focus projector

Put a sheet of paper on the Sample Shelf. Apply power to the system and focus the Projector (Item No. 21). Use the locking screw that comes with the projector to secure the focusing adjustment.

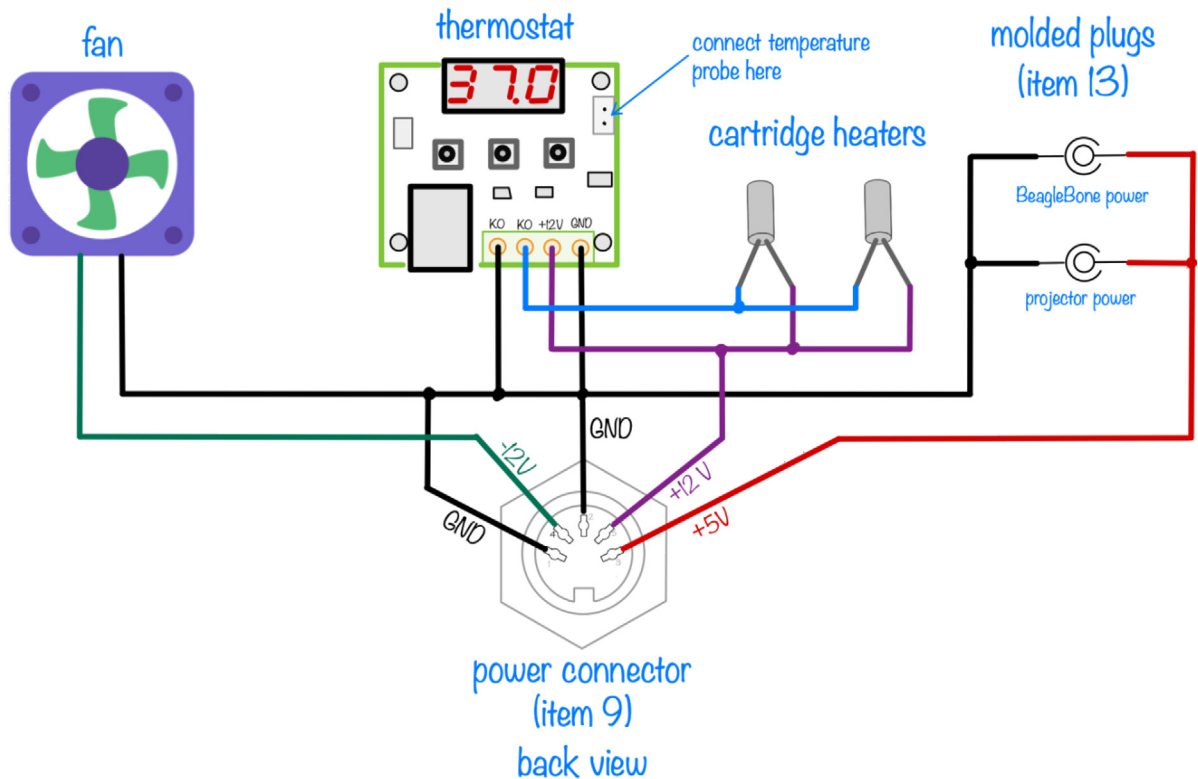


Fig. 27. Wiring diagram.

6. Operating instructions

To produce a bacterial photograph:

1. Follow the protocol in the supplementary information of reference [5] or a similar protocol to prepare a photosensitive bacterial culture plate.
2. Close the incubator door.
3. Apply power to the incubator.
4. Ensure the temperature setting is correct.
5. Allow the temperature to reach the setpoint.
6. Connect to the BeagleBone's wireless network.
7. Launch the SCP client of your choice.
8. Connect to 192.168.7.2 with the username `debian` and password `tempPWD`
9. Copy the image of your choice to `/home/debian/pictures` on the BeagleBone. For best results, choose an image with saturated reds, greens, and blues.
10. Verify that the image is displayed inside the incubator (may take up to 30 s).
11. Open the door and place a prepared plate on the sample shelf. Incubate as per the biological protocol you are using. Resist the temptation to peek.
12. Remove the plate at the end of the incubation period.
13. Enjoy your print.

7. Validation and characterization

7.1. Thermal performance

According to the manufacturer's specification, the reference incubator has a temperature stability of ± 1.5 °C [18]. At an incubation temperature of 37.0 °C and regulation tolerance set to 0.1 °C, the sample shelf temperature was not observed to deviate by more than ± 0.1 °C over > 10 h of operation, as measured by the embedded Temperature Probe. The specified measurement accuracy of the temperature probe is ± 0.1 °C [19]. The measurement was conducted with the incubator door

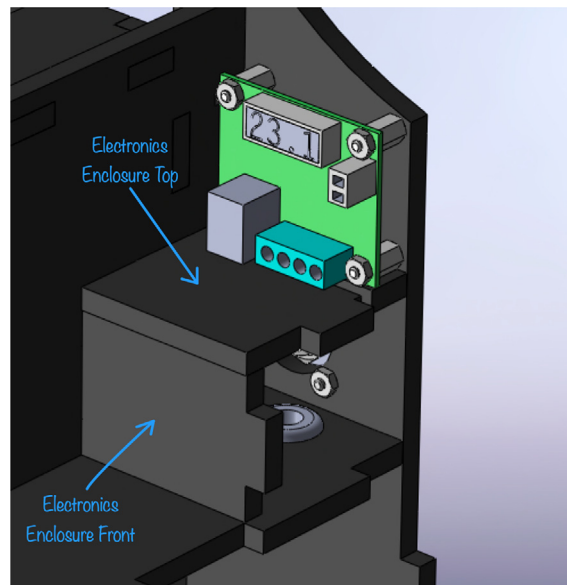


Fig. 28. Electronics Enclosure.

closed, in a large lab with an ambient temperature of 22 °C and a ventilation system that provides 6 complete air exchanges per hour. Prokaryote Playhouse appears to exceed the temperature stability of the reference incubator.

Steady-state thermal loss under the same settings and conditions was 7.0 W. This was determined by measuring the average power consumption of the heaters over 10 min with the incubator operating at steady state. The thermal time constant of the system was measured to be 1580 s by disconnecting power from the heaters and allowing the incubator to cool naturally from 37.0 °C to 27.5 °C. Using a lumped element model of the system with one thermal capacitor and one thermal resistor, the implied resistance from the heaters to outside air is 2.1 °K/W and the thermal capacitance is 752 J/°C.

Thermal imaging revealed a small, negative temperature gradient from the middle of the sample shelf to the edges of ~0.5 °C. This exceeds the temperature uniformity specified for the reference incubator of ± 1 °C [6,10]. In critical applications, the gradient could be reduced by replacing the cartridge heater with rectangular, flexible, polyimide heaters that cover a larger percentage of the sample shelf – a solution that we tried in a prototype system. We elected to stay with the cartridge heater because the gradient is less than the reference incubator specification, it did not cause noticeable problems in printed images, and the cartridge heaters are much less expensive and more available.

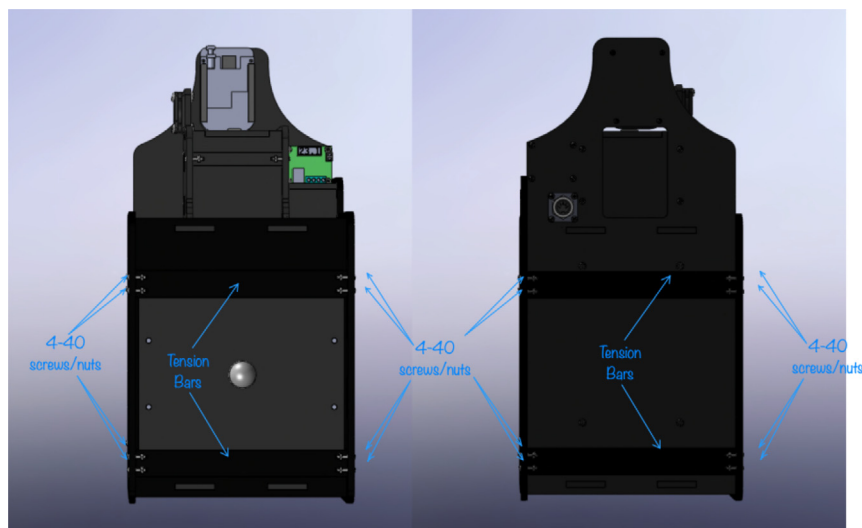


Fig. 29. Final assembly.

7.2. Optical characteristics

At the highest setting, irradiance was 2.0 W/m^2 for red, 2.7 W/m^2 for green, and 0.93 W/m^2 for blue. Measurements were made by running the shell script `ProjectorToMaximumBrightness.sh` (in the “scripts” directory of the repository) and then displaying full-screen, single-color, fully-saturated test images for each primary color (i.e. red = 255, blue = 0, green = 0; red = 0, green = 255, blue = 0, ...). The photodiode sensor of an optical power meter (Thorlabs PM100) was placed in the middle of the sample shelf, with the sensitivity set to the peak emission wavelength of the corresponding LED. Uniformity was assessed by moving the detector from the middle of the sample shelf to the extremes, which showed that the irradiance is greater at the back, middle of the shelf (roughly in line with the projector lens) by approximately 12% and smaller at the front by a similar amount, which is comparable to the manufacturer’s specification of 75% brightness uniformity [12]. On some prints, we noted a small difference in saturation over the area of a plate. In a critical application, the illumination nonuniformity could be corrected by digital image processing prior to display, with approximately 25% loss of peak irradiance. We did not have the opportunity to measure the illumination uniformity of the reference incubator for comparison.

7.3. Functional testing

The most important step in the design validation was to run some plates. We printed test patterns using the protocol from reference [5] without modification. Full- and half-width colorbar test patterns were particularly useful for establishing exposure and background levels (Fig. 30). Unfortunately, the two plates were photographed under very different lighting conditions. Perhaps a nice, laser-cut light box would be a good project in the near future. Contrast of the upper left image has been increased with digital postprocessing.) The color gradient prints demonstrate that the dynamic range of the projector, optical properties of the acrylic projection window, and light tightness of the enclosure are adequate for bacterial photography in an educational setting.

Colorbar images were created at $400 \times 400 \times 3$ resolution, rescaled, and then copied into the appropriate location on a $1280 \times 724 \times 3$ black background. The pattern consists of horizontal bars with red, green, and blue gradients separated by thin lines of cyan, magenta, and yellow gradients. From left to right, the value of each color component decreases smoothly from 255 (the maximum possible value) to 0. The red, green, and blue bars each have one nonzero component. The cyan, magenta, and yellow bars have two equal, nonzero components. (For example, the yellow bar has nonzero red and green components with blue equal to zero.) In the half-width colorbar image, the values approach zero twice as fast from left to right so that the right half of the image is completely black. Implementers with more demanding applications who are concerned about the optical properties of the clear acrylic Projection Window can substitute a rectangle of glass or fused silica bonded to the Incubator Top. It also may be possible to run the system with the Projection Window omitted, but we did not test that configuration.

A curious feature of the prints in Fig. 30 (and all prints using this biological system) is that red, green, and blue tones reproduce accurately, but cyan, magenta, and yellow do not. This happens because the pigments produced by the three-color bacterial photography correspond to the additive primary colors (red, green, and blue); however, bacterial photographs are inherently subtractive in nature. The hues in bacterial photographs arise from wavelengths that are *subtracted* from

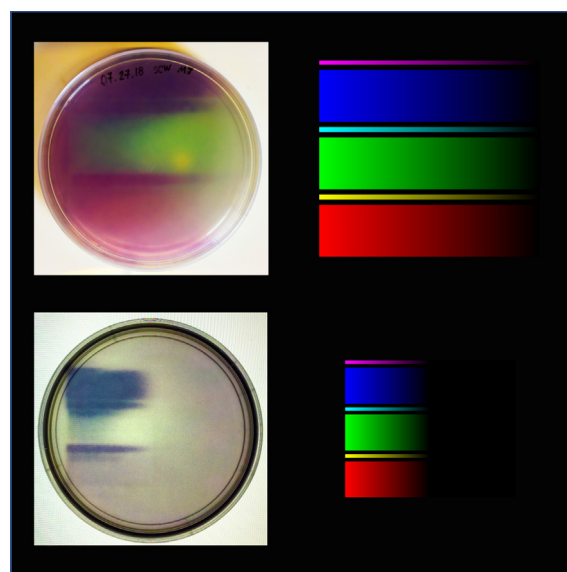


Fig. 30. RGB gradient pattern.



Fig. 31. Prokaryote Playhouse incubators ready to be loaded with student-prepared plates.

white light passing through. From a color theory point of view, bacterial photographs have more in common with subtractive systems like color laser printers (that use CMY primaries) than additive systems like computer monitors (that use RGB primaries). More accurate color mixing could be achieved by reengineering the chemical/biological system to produce pigments closer in hue to the subtractive primaries. We hope to launch an effort to optimize the fidelity of bacterial photographs in the near future.

Another feature of the colorbar patterns is that the blue bar is more saturated than the other colors. Color balance can be adjusted by changing the values of LED current in the shell script `ProjectorToMaximumBrightness.sh` or by digital image pre-processing. Because of the color mixing issue, selection of the optimal levels is both subjective and image dependent.

7.4. Battle testing

After successful prototype testing, we went on to build a fleet of five incubators, which can be seen in [Fig. 31](#). These have been battle tested by students working in groups of up to 10 at a time, who have used them to produce hundreds of bacterial photographic prints. To foster good results, we ask students to select images that consist primarily of saturated, primary colors – where one color channel is near 255 and the others are near zero. It has been our observation that some students heed this advice better than others. On the bright side, a poorly chosen image offers an ideal pretext for a deep discussion of color theory. A software script resamples the student images and composites them on a black background inside a grey circle to help position plates accurately.

8. Future work

We are considering several projects moving forward:

- Investigating new projectors and light sources.
- Optimizing the biological system design to improve color fidelity and range.
- Reducing cost and parts count.
- Developing a kit to make Prokaryote Playhouse more accessible to teachers, makers, and researchers.
- Evaluating the feasibility of modifying the DLPDLCR2000EVM light engine or using different projector models.
- Improving functionality of script files.
- Developing new form factors for bacterial photographs.

Human and animal rights

No human or animal subjects were used in this research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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