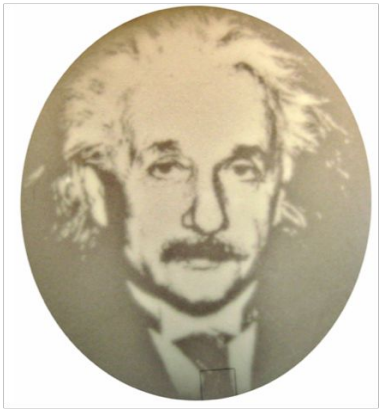
Bacterial Photography



Bacterial photography is performed using a genetically modified organism which has been engineered to see light and respond by changing color. When millions of these bacteria are spread over a surface, they will imitate any image you shine on them, capturing a bacterial photograph. This is a groundbreaking demonstration of our ability to engineer nature to perform a distributed computation in response to the environment, and a striking example of what the future holds in the exciting new field of synthetic biology. To learn more about Bacterial Photography please skip to the Scientific Background section.

The Bactograph

We have created a K12 kit which contains everything needed for a science class to create their own bacterial photographs. Teachers familiar with bacterial transformation kits will be at home executing our three day protocol. To create their bactograph film students mix the bacteria into specially formulated culture media, which they then cast into film by polymerizing the media via a temperature change. To take a picture students attach a hand drawn image to the film and incubate the bactograph. The next day students observe their own bacterial photographs and analyze their performance. To learn more about the protocol associated with this experiment please skip to the experiment section.

Can I get a Bactograph Kit?

We are currently distributing our kit free of charge to high school classrooms, universities, and museums on a limited basis. If you are interested in using our kit please send an email to contact@bactograph.org. In your email include your class size, number of bactographs requested, shipping address and when you wish to receive the kit. The kit is stable for at least 2 weeks at 4°C. If you would like to keep up to date on our progress please send an email to mailinglist@bactograph.org. To date over 800 high school students have taken Bactographs, and over 175 teachers have tested our kits, and we are excited to share this science with as many people as possible.

The Bactograph Team

The Bactograph Kit is the brainchild of two researchers, Brian Landry and Ravi Sheth, who are a part of Jeff Tabor's lab at Rice University. Jeff was part of the team which created the first bacterial photographs a decade ago, and has alway envisioned placing the experiment in the hands of young scientists. Brian is a graduate students in who is interested in creating genetically modified probiotic organisms which can address diseases such as diabetes from within the human gut. Ravi is an undergraduate who likes to pretend he is a graduate student. Together we have spent the last three years iteratively crafting a bacterial photography experiment so that it can be performed by high school students.

We hope to continue expanding the impact of this kit and are interested in any feedback you may have. Our current research and free distribution has been funded through a NSF research experience and mentoring program, but if you are interested in funding future work on this educational project please let us know. Furthermore, we would be interested in hearing anyone who has knowledge about the legal requirements for expanded distribution of these kits beyond the controlled school and university environment, so if you have specific knowledge in this area please send us an email. We can be reached at contact@bactograph.org. We are excited at the success we have had with the Bactograph Kit and want to share bacterial photography with as many people as possible.

Jeff Tabor

Brian Landry

Ravi Sheth

National Science Foundation

The Bactograph Challenge

Over the summer of 2014 the Bactograph team had a blast hosting four underrepresented undergraduates and one high school teacher in our lab for the Bactograph challenge. We challenged each of the participants to improve one portion of the kit using an iterative design process. Many aspects of the kit and teaching resources you see in this manual are the result of their hard work. They also ran two teacher workshops to demonstrate their achievements to over 50 high school teachers. This work was part of the National Science Foundation's Research and Mentoring Experience program. Crystal, Kat, and Nikitha have since continued in our lab providing help with the Bactograph kit.

From left to right: Nikitha Cherayil, Kat Sofjan, Crystal Lin, Nico Medellin

The Science behind the Bactograph

Synthetic Biology

Synthetic biology leverages engineering principles to design and construct biological systems at the DNA level for useful purposes. Biology is incredibly prevalent and important. For example, biological processes contribute to health and disease in the human body. Microbes surround our environment, populating nearly every niche on earth. The ability to both understand and engineer biology around us has important applications for nearly every major problem human society faces, such as health, food and energy.

Living Machines

A core principle of synthetic biology is applying engineering principles to rapidly engineer new organisms with unnatural functions. A NASA engineer could quickly program a space robot by connecting sensors (cameras, temperature sensors, radars) to a computational unit which could process these inputs and then relay appropriate commands to actuators (wheels, robotic arm). Similarly, synthetic biologists seek to engineer individual cells to sense environmental signals, perform computations on these inputs, and respond with appropriate actions.

A key technology that synthetic biologists utilize is “genetic engineering” tools. These technologies allow scientists to quickly rearrange and assemble DNA sequences — the basic unit of biological information — in new ways. By transferring and creating new DNA sequences that encode novel functions, synthetic biologists can engineer organisms to accomplish completely novel functions.

Engineering E. coli to See Light

Researchers at the University of Texas (including Jeff Tabor) in 2005 (the early years of the synthetic biology field) were able to engineer *Escherichia coli* - a laboratory strain of bacteria that normally lives in the dark human gut - to “see light”. First, they transferred DNA sequences encoding sensor proteins from photosynthetic bacteria that live in ponds to the model *E. coli.* Then, they linked an actuator that produces a dark pigment to these sensors, creating a “bacterial photograph” circuit. By embedding these bacteria in a petri dish, and shining light on them, they showed that these synthetic bacteria could sense and respond to the light, creating a bacterial photograph.

Jeff Tabor continued work on these light sensors, creating complex circuits with them and characterizing completely new light sensors, and now directs a research lab at Rice University. In 2013, researchers in his lab adapted the bacterial photography protocol from 2005 for the K12 classroom, allowing for the students to complete the highly visual experiment in their own classroom, at low cost.

The Bacterial Photography Circuit

The bacterial photograph kit consists of an engineered light sensor connected to a pigment production module. The light sensor, consisting of two genes named “Cph8 and OmpR” was engineered as a fusion of a cyanobacteria light sensor two a native E. coli signaling molecule. This signaling pathway is an example of a common prokaryotic signaling pathway called a “Two Component System”. Bacteria use these networks to sense and respond to different environment conditions; a sensor “Histidine Kinase” can sense an input (for example a metabolite, mechanical stress on the cell wall, or even light), and then phosphorylates a

corresponding “Response Regulator” which can then affect some type of response in the cell, including transcription of a specific gene.
In this specific circuit, the Cph8/OmpR two component system is turned on in the dark, and turned off in red light; we have connected the output to a gene that produces indigo (the same pigment that is used to dye your jeans!). Thus, when red light reaches the cells they turn off production of indigo; conversely when the cells are in the dark they turn on indigo. This allows you to reproduce your stencil in bacteria!
Learn more about the lab the Bactograph was developed in at: taborlab.rice.edu

Protocol

Kit contents

- Bactograph solid media
- Bactograph liquid media
- Agar stab of bacteria
- 9 60mm petri dishes
- 1 transparency sheet
- 1 sharpie
- 2 disposable pipettes
- 1 15-LED light strip
- 2 watch batteries

Note: when multiple kits are requested, only a single LED light strip and Agar stab of bacteria will be sent

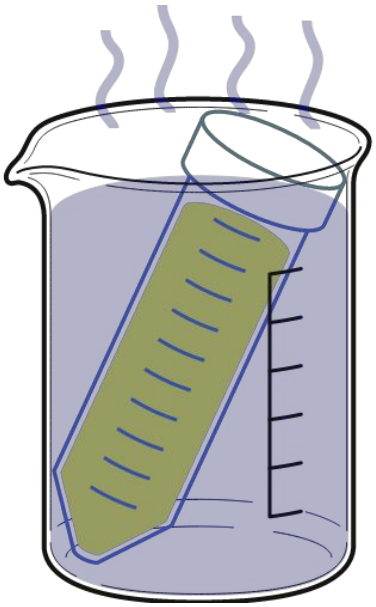
Required Materials

- A 37°C incubator
- A beaker or similar container
- Boiling water
- Transparent tape
- Scissors

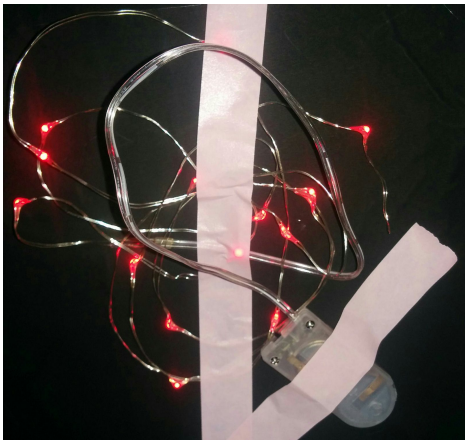
Preparation Day

Melt Media

Take the media tube and submerge it in a container of boiling water until it completely liquefies (approximately 10 minutes). Store the melted media withe the container in a 37 degree C incubator. This can be done any time at least 2 hours prior to the experimental day protocol, since the media simply has to cool to 37 degrees before use.



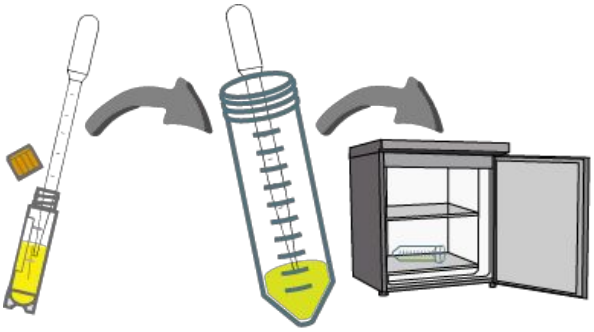
Set Up Lights



1. Place the batteries provided in the LED light strip.
2. Tape the LED light strip to the top of your 37C incubator such that it fairly evenly illuminates the entire incubator. Make sure the lights work and can be toggled on/off.
3. If your incubator has a transparent door block cover it with something which blocks light from entering.
4. If you have the option of choosing a shelf height chose a height of ~9 inches between the plates and lights, however a wide range of heights should still work.

Culture Bacteria

Using a sterile object (pipette tips work) scrap bacteria from the hole in the agar stab and then shake off the tip in the culture media. Place the culture media on its side in a 37 degree incubator and allow it to grow for 48 hours.



Experimental Day

Prepare supplies

The class needs the following:

- Bacterial culture
- Melted photography media
- Disposable Pipette

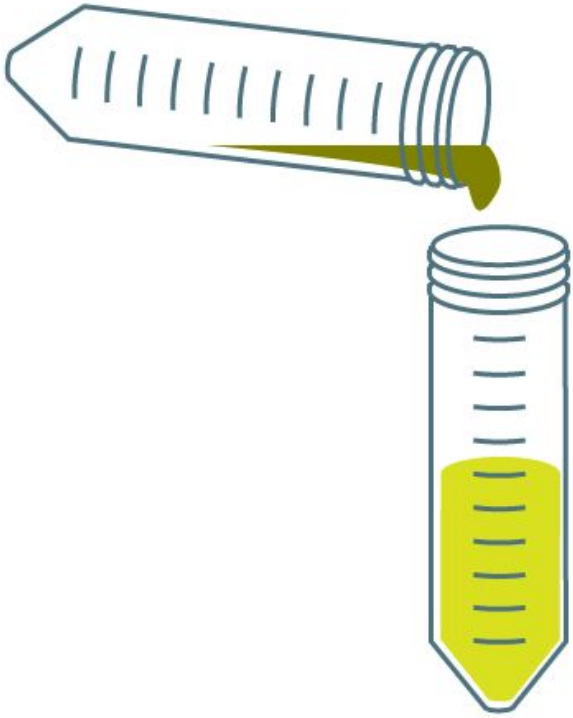
Each group needs the following:

- Petri dish
- Petri dish sized section of transparency
- Tape
- Permanent marker

Add bacteria to photography media

Remove the photography media and water container from the incubator. Keeping the photography in the water container will keep it warm and prevent it from solidifying for approximately 30 minutes. Take the bacterial culture and pour it into the photography tube.

Media above 37 degrees C has a chance of killing cells when they are added, storing it for a day at 37C insures this does not happen.

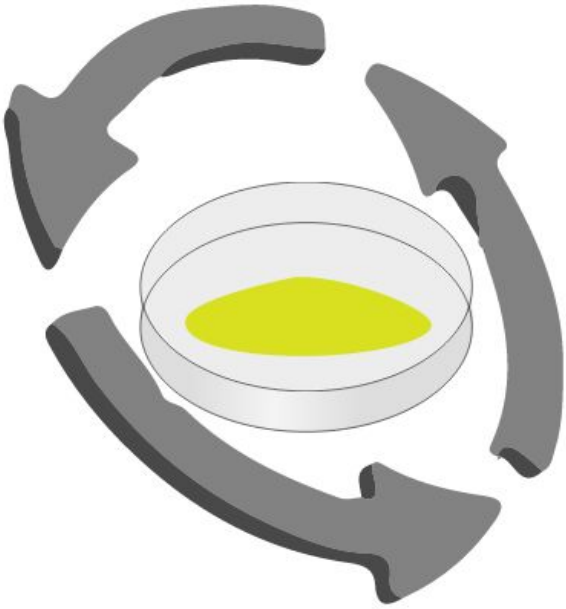


Mix in bacteria

Gently invert the photography media 10 times to mix the cells thoroughly. Try not to form too many bubbles

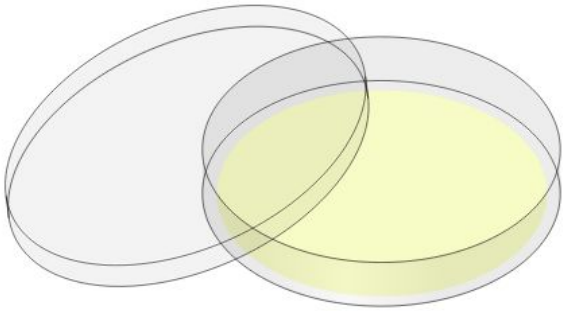
Cast plate

Add approximately 2mL of photography media to each petri dish, this can be done with a pipette or by careful pouring. The provided disposable pipette will pipette 1mL at a type. Immediately after pipetting rotate the plate so that media is spread evenly over the entire bottom of the plate, waiting too long will cause the media to solidify.



Solidify media

Allow the plate to sit uncovered for approximately 5 minutes to allow the media to solidify. Place a lid on the plate and gently tilt it 45 degrees to check that the media has solidified.

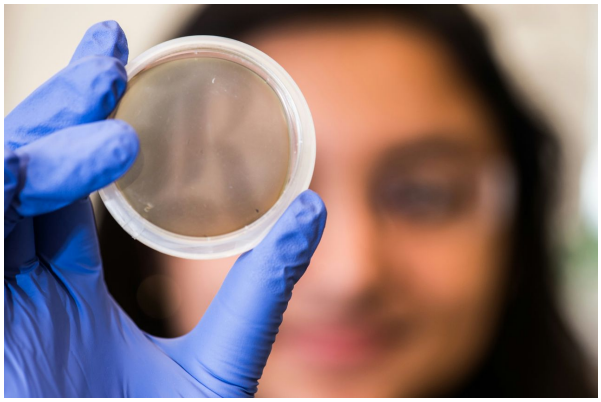


Attach image and incubate

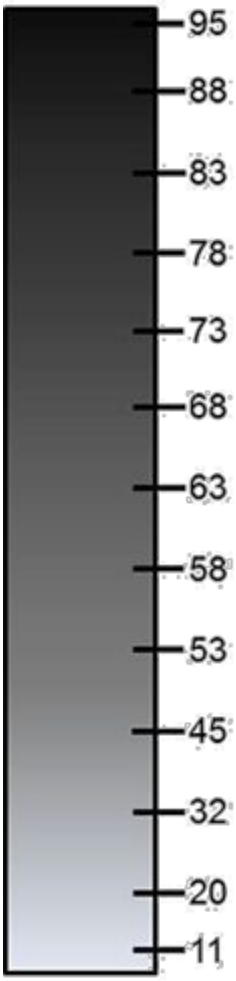
Draw the picture you want to capture in the Bactograph on a piece of transparency and cut it out. Cap the the petri dish and flip it upside down. Attach the cut out piece of transparency to the petri dish using clear tape. Place the dish in the illuminated incubator.

View bacterial photograph

After incubating for at least 24 hours remove the petri dish from the incubator. Detach the transparency and admire your bacterial photograph!



Analyze Results: Cell Signalling Activity



The bacteria you used to capture your bactograph contain proteins which enable them to sense light, and then change color in response. As with any engineered system, it is important to ask, how well did our bacteria perform? A key metric synthetic biologist's use to characterize how well a cell is capable of sensing signals in its environment (such as light), is the **dynamic range** of the cell to its signal. Dynamic Range (DR) is defined as the ratio of the high to low levels of output of an engineered system, and is calculated by the following formula:

$$DR = (High\ State)/(Low\ State)$$

To determine the DR of your bacteria quantify how dark the darkest area of your Bactograph is and how light the lightest area of your Bactograph is using the scale the right. Calculate the dynamic range by dividing the value of the darkest area by the value of the lightest area. How does the DR of your bactograph compare to the DR of the first picture ever taken?

Bactograph Dark: _____ Photograph Dark: _____

Bactograph Light: _____ Photograph Light: _____

Photograph DR: _____ Photograph DR: _____

"View from the Window at Le Gras." The earliest photograph taken with a camera from 1826. It took several days to capture this image. (Source: Harry Ransom Center at the University of Texas at Austin)

Calculating Dynamic Range

Dynamic range data is frequently collected using a fluorescent reporter such as green fluorescent protein (GFP). A machine called a flow cytometer is used to measure the amount of GFP expressed by the cell in arbitrary units. This means that the measurements only have value relative to each other. Then, a scientist can compare the amount of GFP present in cell which has been induced in relation to a cell from a non-induced or repressed condition and have a measure of gene expression referred to as “fold induction”.

For example, imagine a system in which GFP expression is induced by green light and repressed by red light. If cells subjected to green light had an average fluorescence of 60 and cells subjected to red light had an average fluorescence of 15, the system would have “4-fold induction”.

In our experiment, the output expressed is indigo. If we can quantify the amount of pigment present, we can calculate the system’s dynamic range. The following image contains a gradient of colors you may observe in your Bactograph. The pigmentation index on the right side was calculated by comparing sections of the gradient to a black sample. The index is an expression of percent darkness, such that a higher pigmentation index is representative of a higher level of gene expression. Slide your plate up and down the left side of the page to identify the sections of the gradient that are closest to the darkest and lightest colors on your plate. Using this data, calculate the dynamic range

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Troubleshooting and FAQs

What are the reusable components of the kit?

Liquid bacterial culture media: 8mL of LB with 50ug/mL ampicillin, 100ug/mL spectinomycin
Solid bacterial culture media: 23.5mL of LB with 20mg/mL SeaPrep agarose, 50ug/mL ampicillin, 100ug/mL spectinomycin
Tryptophan solution: 3.5mL of 10g/L Tryptophan
9 60mm tissue culture dishes

What do I need to supply to use the kit?

A 37°C incubator
A beaker or similar container
Boiling water
Transparent Tape

How should I grow my bacterial starter culture?

The bacteria can be cultured simply by placing the inoculated culture tube in a 37°C incubator for 48 hours. However, if you have a shaking incubator the culture can be grown for 24 hours at 37°C and this will result in much more colorful photographs.

How should I store the kit once I receive it?

The solid Bactograph media, liquid Bactograph media and agar stab of bacteria should be stored in a refrigerator and should last for at least a month. The rest of the kit can be kept at room temperature. Alternative you can just stick the whole shipping tube in the refrigerator upon receiving it.