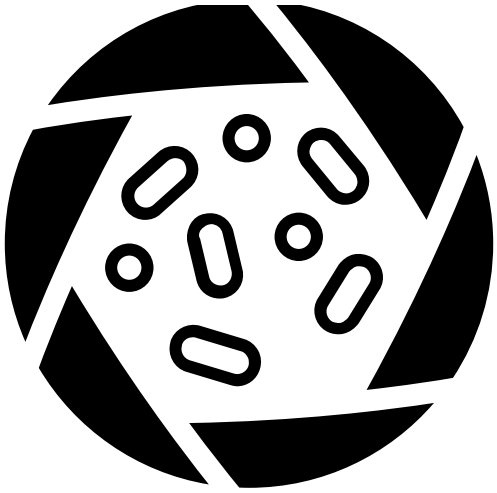

The Bactograph Kit

Taking pictures with bacteria

See page 1 for storage instructions

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Experiment Components

COMPONENT	NUMBER	STORAGE
Bactograph culture stab	1	4°C
Starter plate	1	4°C
Bactograph media tube	1	4°C
Inoculation loop	11	Room temperature
15 mL tubes	10	Room temperature
Petri dish	10	Room temperature
LED tea light	10	Room temperature
Paper cups	10	Room temperature
Black paper strips	10	Room temperature
Transparency paper	1	Room temperature
Black paper sheet	1	Room temperature
Sharpie pen	1	Room temperature

REQUIREMENTS

37°C incubator

A beaker or similar container

Boiling water

Clear tape

Scissors

When stored appropriately, this kit is stable for at least 2 weeks.

Experiment Timeline

DAY 1	Streak out bacteria and prepare media
DAY 2	Perform experiment
DAY 3	View Bactographs

Safety

The *Escherichia coli* strain used in this experiment is BW29655, which is a descendent of the *E.coli* K-12 isolate. This is a non-pathogenic strain of *E. coli*, however, it is important to follow standard microbiological safety procedures when working with the bacteria.

1. Gloves and goggles should be worn during the experiment and when viewing the Bactographs.

2. The lab bench should be wiped down with 10% bleach or a laboratory disinfectant at the end of the experiment.

3. After finishing the experiment or handling the Bactographs, students should wash their hands.

4. All materials that come in contact with the bacteria, including the Bactograph culture, starter plate, inoculation loops, media tubes, and Bactographs should be sterilized before being disposed of in the trash. Sterilization can be achieved via autoclaving or bleaching. To sterilize materials via the autoclave, run the autoclave at 121°C for 20 minutes. To sterilize materials with bleach, soak materials in 10% bleach for 10 minutes. Bag media twice before disposing it in the trash.

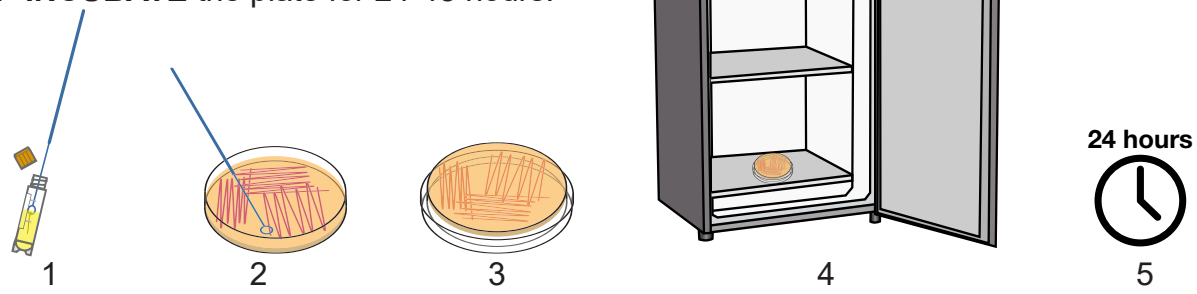
IMPORTANT

The starter plate and Bactograph media both contain the antibiotics ampicillin and spectinomycin. **Students and teachers who are allergic to ampicillin, spectinomycin, penicillin, or other related antibiotics should not partake in this experiment.**

Teacher Protocol

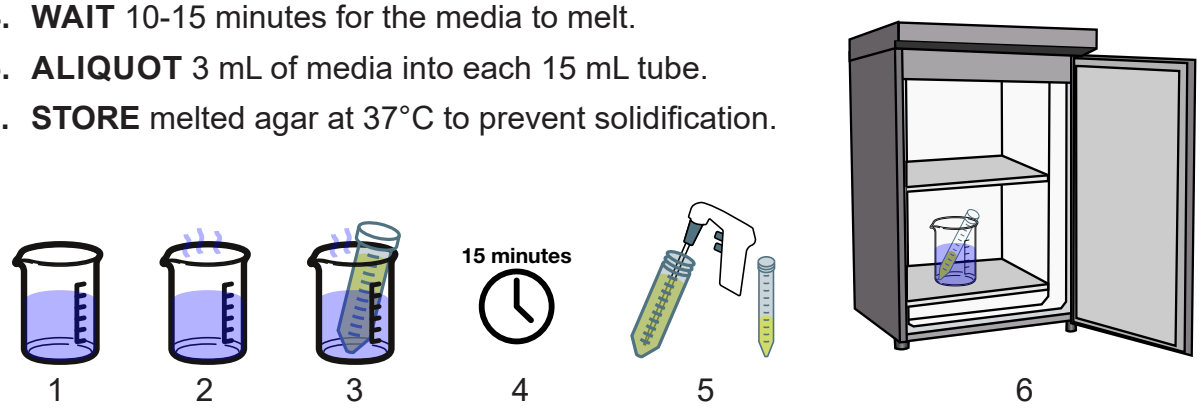
PREPARE THE STARTER PLATE

1. **INOCULATE** the loop with bacteria by inserting it into the agar stab.
2. **STREAK** the loop across the starter plate to spread the bacteria. Use a light touch to prevent puncturing the media. *Steps 1 and 2 should be repeated 2 to 3 times to ensure enough bacteria grow for the experiment.
3. **COVER** and **INVERT** the plate.
4. **PLACE** the plate in a 37°C incubator.
5. **INCUBATE** the plate for 24-48 hours.



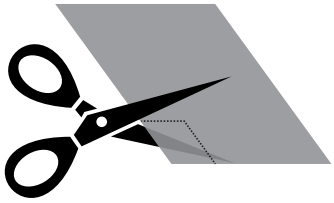
PREPARE THE MEDIA

1. **FILL** a beaker with 5 inches of water.
2. **BOIL** the water using a microwave or hot plate.
3. **SUBMERGE** the Bactograph media tube in the beaker.
4. **WAIT** 10-15 minutes for the media to melt.
5. **ALIQUOT** 3 mL of media into each 15 mL tube.
6. **STORE** melted agar at 37°C to prevent solidification.



PREPARE THE TRANSPARENCY

1. **CUT** the provided sheet of transparency paper into 2.25 inch squares.



FAQs

Can I perform this experiment at room temperature?
No, we have found that Bactographs do not develop at room temperature.

How long can I store the Bactograph kit?
The Bactograph kit is stable for at least two weeks when stored as specified in this manual.

What is the antibiotic resistance of the bacteria?
The Bactograph strain carries two plasmids, which convey resistance to 100 µg/mL spectinomycin and 50 µg/mL ampicillin.

What type of media is used?
The media contains LB as a nutrient source, 2.4 g/L tryptophan as a substrate for indole production, 100 µg/mL spectinomycin and 50 µg/mL ampicillin for plasmid maintenance, and 0.9% low-melt agarose, which makes it stable as both a gel and a liquid at 37°C.

Can normal agar be used?
We have had success using 0.75% normal agar, which solidifies at approximately 45°C. The main problem with using normal agar is that it is much easier to accidentally heat kill the Bactograph bacteria.

Can I store the bacteria and use my own reagents
Yes, these bacteria can be stored like any other E. coli strain, and the media can be made from the formula above.

Other Resources

The bactograph website: A pdf of this manual can be downloaded at www.bactograph.org

The creation of bacterial photography
Levskaya A., et al. Nature, 438. 441. doi:10.1038 http://www.nature.com/nature/journal/v438/n7067/full/nature04405.html (2005).
Blog post: http://www.nature.com/news/2005/051121/full/news051121-8.html

3-color bacterial photography
Fernandez-Rodriguez, J., Moser, F., Song, M. & Voigt, C. A. Nature Chem. Biol. http://dx.doi.org/10.1038/nchembio.2390 (2017).
Blog post: https://www.nature.com/news/light-sensitive-e-coli-paint-a-colourful-picture-1.22026

Biobuilder: A complimentary synthetic biology curriculm with a lesson plan focused on computational modelling of bacterial photography
Book: Hart, N. K. P., Rachel Bernstein, Karen Ingram, Kathryn M. (2015). BioBuilder.
Website: http://biobuilder.org/picture-this/

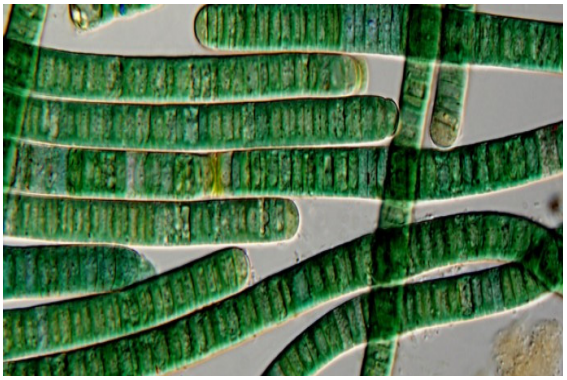
TEDx talk on bacterial photography: https://youtu.be/Q_g_dvWUPXU

Introduction to bacterial photography

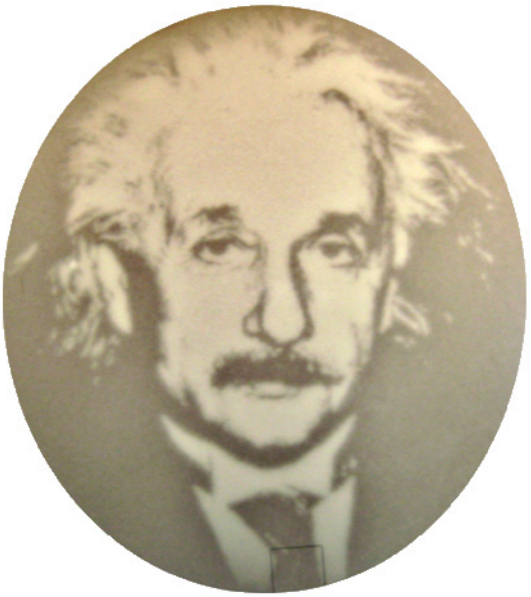
Bacteria have developed a variety of methods to see light. This enables bacteria to determine what time of day it is, if they are on the surface of a pond, or if they are experiencing harmful UV radiation. A specific protein was identified in 1998 in the pond scum, *Synechocystis* PCC6803, that was found to bind to the light absorbing molecule phycocyanobilin (PCB), which allowed the bacteria to detect red light.

A few years later a group of synthetic biologists - engineers that create new bacteria using genetic transformation - wanted to create bacteria which could take photographs. To accomplish this they needed to make bacteria that changed color when exposed to light, much like film photography. To make the common laboratory bacteria *E. coli* see light, they took the portion of the previously discovered *Synechocystis* PCC6803 protein which senses light, and combined it with a *E. coli* signaling protein. After transforming the bacteria with DNA encoding for this combined protein as well as the enzymes which produce PCB, the bacteria were able to sense light. The synthetic biologists then transformed a second piece of DNA, which caused the bacteria to change colors after sensing light. This create a completely new bacteria that had been engineered to take pictures.

To take a photograph, billions of these bacteria were embedded within agar in a petri dish. An image was then shown on the plate, and bacteria which sensed they were in the presence of light changed color, and bacteria in the dark did not. In this way the bacteria replicated the image that had been shown on the plate, creating the first bacterial photographs.



Synechocystis PCC6803



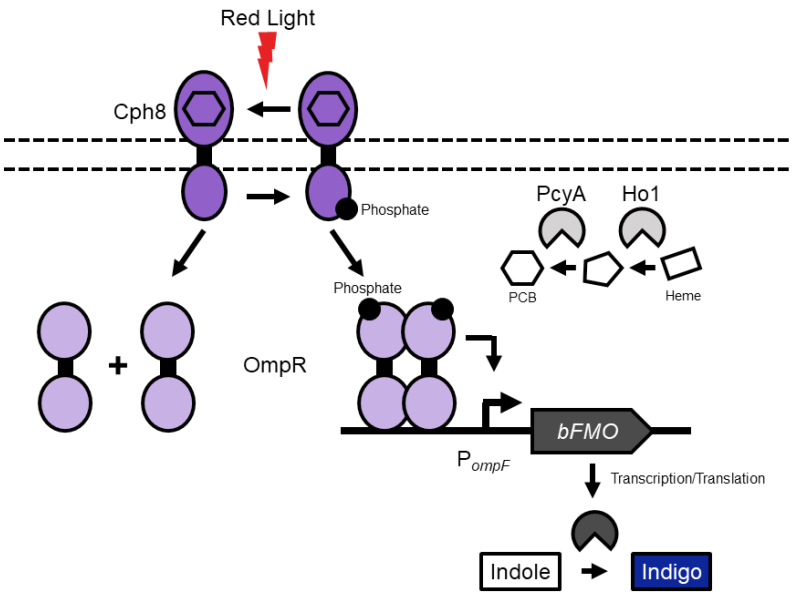
A bacterial photograph of Einstein

Introduction to the Bactograph

To take Bactographs, we have created a next generation bacterial photography strain. This strain was made by transforming two plasmids into *E. coli*. These plasmids contain the DNA for the enzymes, signaling proteins, and promoters needed to produce light-sensing bacteria.

1. The enzymes, PcyA and Ho1, convert the naturally occuring Heme molecule into PCB, a light absorbing molecule.
2. The signaling protein, Cph8, binds PCB, which allows it to sense red light.
3. In the presence of red light, the signalling protein is unable to autophosphorylate and indigo is not produced.
4. In the dark, autophosphorylated Cph8 binds OmpR and chemically modifies it with a phosphate molecule.
5. This enables OmpR to bind to DNA and induces transcription at the P_{ompF} promoter.
6. From this promoter, the bFMO gene is transcribed and translated into an enzyme.
7. This enzyme converts indole into indigo, the blue pigment found in jeans.

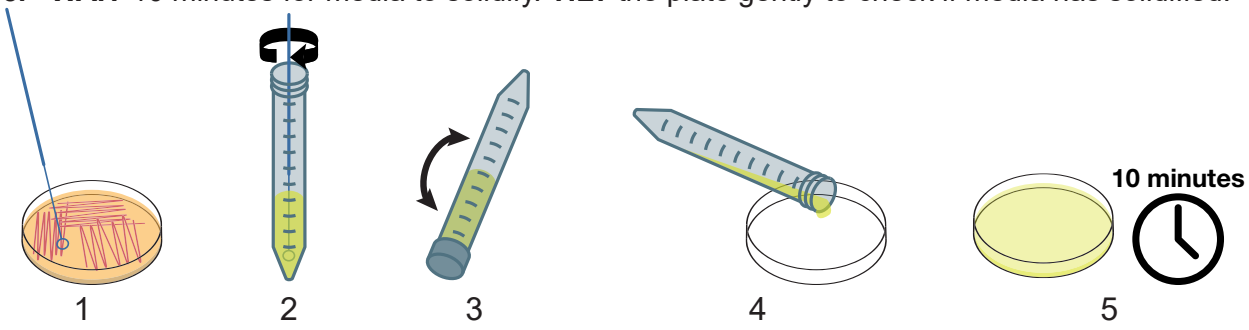
Each of the proteins, enzymes, and promoters listed above were selected with a specific well-defined purpose in mind. When these components are placed into bacteria, the bacteria are able to sense the presence or absence of light and respond by regulating the production of indigo. This regulation allows us to create bacterial photographs. This is a striking demonstration of the ability of synthetic biologists to use genetic transformation to make bacteria with new capabilities.



Student Protocol

PREPARE THE BACTOGRAPH PLATE

1. **SCRAPE** 2-4 colonies of bacteria off of the class plate with an inoculating loop.
2. **SHAKE** the inoculating loop in media for 10 seconds.
3. **CAP** the media tube. **INVERT** the media tube back and forth for 20 seconds to mix bacteria.
4. **REMOVE** the cover to the petri dish and **POUR** the media into it.
5. **WAIT** 10 minutes for media to solidify. **TILT** the plate gently to check if media has solidified.



SET UP THE BACTOGRAPH

1. **TURN ON** the LED tea light by unscrewing it, removing the white plastic divider, and then screwing it back together tightly.
2. **INSERT** the LED tea light into the hole of the paper cup.
3. **DRAW** the image you want to take a Bactograph of on the sheet of transparency using the Sharpie provided. Dark and thick lines will result in a better Bactograph.
4. **CAP** the petri dish with the larger dish once it has solidified.
5. **INVERT** the petri dish and **TAPE** your transparency image to the bottom. **TAPE** the black paper strip so that it fits around the Bactograph plate.
6. **PLACE** the petri dish and taped black paper strip on the black paper sheet in the incubator. **PLACE** the LED tea light and cup upside down over the black paper strip and petri dish.
7. **INCUBATE** the bacteria for 12-24 hours (longer incubation produces better Bactographs).
8. **REMOVE** the transparency and **VIEW** the Bactograph by placing the petri dish on a white surface. Bactographs can be parafilmed and stored for several months at 4°C.