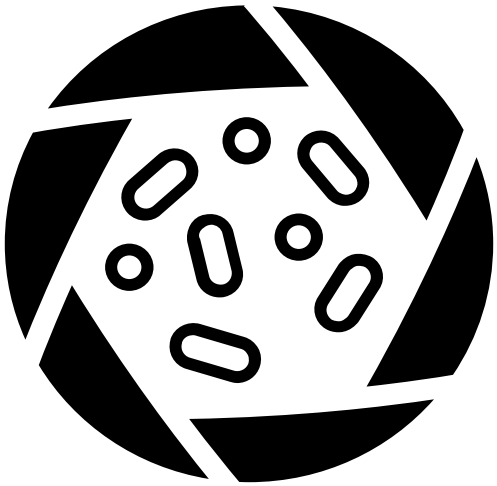


The Bactograph Kit

Taking pictures with bacteria

See page 1 for storage instructions

Experiment Components

COMPONENT	NUMBER	STORAGE
Bactograph culture	1	4°C
Starter plate	1	4°C
Bactograph media tube	1	4°C
Innoculation loop	11	Room temperature
15mL tubes	10	Room temperature
Petri dish	10	Room temperature
Battery LED	10	Room temperature
Paper cups	10	Room temperature
Permanent marker	2	Room temperature
Black paper	1	Room temperature
Transparency paper	1	Room temperature

REQUIREMENTS

A 37°C incubator

A beaker or similar container

Boiling water

Clear tape

Scissors

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

Experiment Timeline

DAY 1 Streak out bacteria, prepare media

DAY 2 Perform experiment

DAY 3 View bactographs

Safety

The *Escherichia coli* strain used in this experiment is BW29655, which is a decesdent of the *E.coli* K-12 isolate. This is a non-pathogenic strain of E. coli, nevertheless it is important to follow standard microbiological saftey 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 handeling the Bactographs students should wash their hands.

4. All materials that come in contact with the bacteria, including the bactograph culture, starter plate, innoculating loops, media tubes, and bactographs should be sterilized before being disposed of in the trash. Sterilization can be achieved via autoclaving or bleaching. To sterilized 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. Double bag media prior to disposing it in the trash.

IMPORTANT

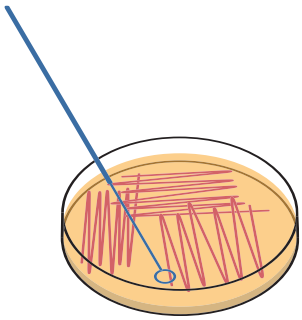
The starter plate and bactograph media contain the antibiotics ampicillin and spectinomycin. Students and teachers who are allergic to the antibiotics ampicillin, penicillin or related other antibiotics should not partake in this experiment.

Teacher Protocol

PREPARE THE STARTER PLATE

1.

2.



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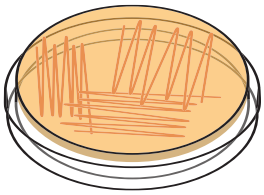

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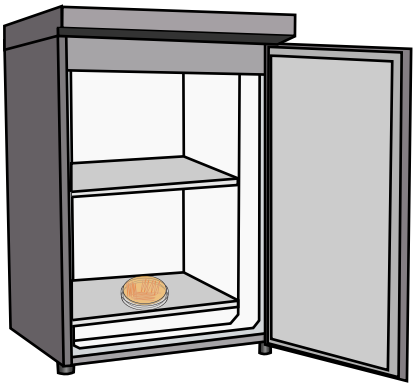


5.

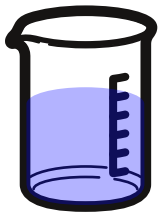
24 hours

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 gouging of the media.
3. **COVER** and **INVERT** the plate.
4. **PLACE** the plate in a 37°C incubator.
5. **INCUBATE** the plate for 24-48 hours.

Teacher Protocol

PREPARE THE MEDIA

6.



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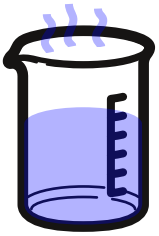

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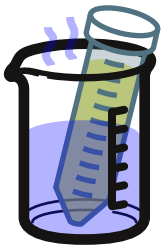
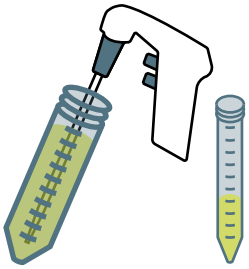


9.

15 minutes

10.



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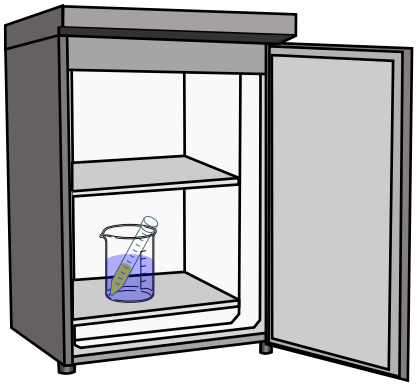
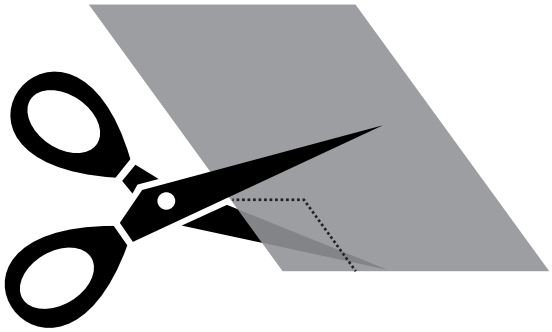


6. **FILL** a beaker with 5 inches of water.
7. **BOIL** the water using a microwave or hot plate.
8. **SUBMERGE** the bactograph media tubes in the beaker.
9. **WAIT** 10-15 minutes for the media to melt.
10. **ALIQUOTE** media into ten 15mL tubes
11. **STORE** melted agar at 37°C to prevent solidification.

PREPARE THE TRANSPARENCY

11.



12. **CUT** the provided sheet of transpency paper into 2.25 inch squares.

FAQ

Can I do this 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

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

What is the media used?
The media contains LB as a nutrient source, 1g/L tryptophan as a substrate for indole production, 100µg/mL spectinomycin and 50µg/mL ampicillin for plasmid maintenance, and 0.9% SeaPlaque low melt agarose which makes it stable as both a gel and liquid at 37°C.

Can normal agar be used?
We have had some success using 0.75% normal agar, this agar solifies slowly around 45°C. Therefore its possible to cool the agar to 45°C (so bactograph bacteria are not heat killed), add the bacteria, mix, and pour a bactograph plate quickly before the media solidifies. However, this is much more difficult than using 0.9% SeaPlaque agar.

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 from here www.bactograph.org

The creation of bacterial photography
Levskaya A., et al. Nature, 438. 441 - 442 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: This 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/

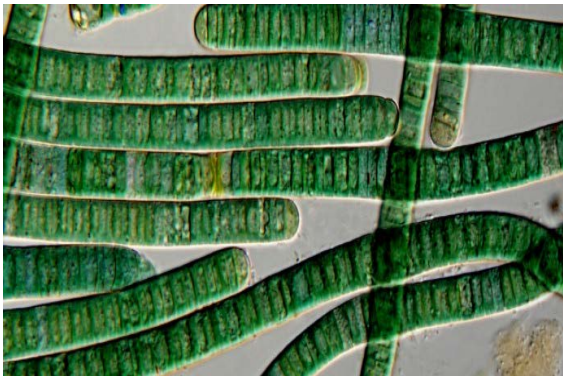
TEDx talk on bacterial photography
https://youtu.be/Q_g_dvWUPXU

Introduction to bacterial photography

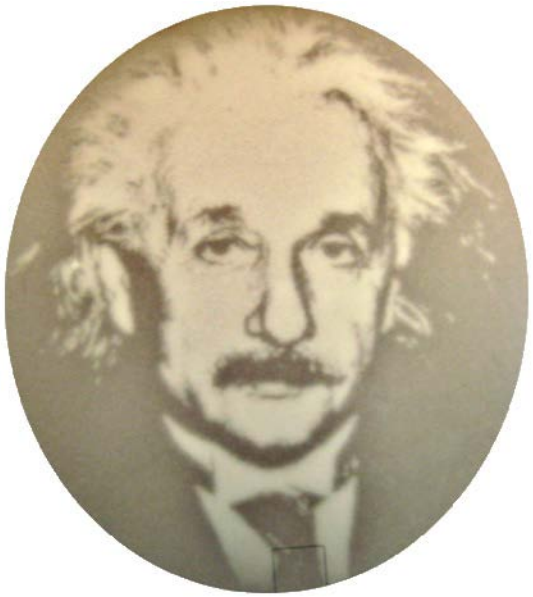
Bacteria have developed a variety of methods to see light. This enables bacteria to determine what time of day it is, if they are on the surface of a pond, or if they are experiencing harmful UV radiation. A specific protein was identified in 1998 in the pond scum Synechocystis PCC6803 that was found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

A few years later a group of synthetic biologists, who are 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, imitating old photography film. 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 signalling 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. A 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.



Synechocysits PCC6803



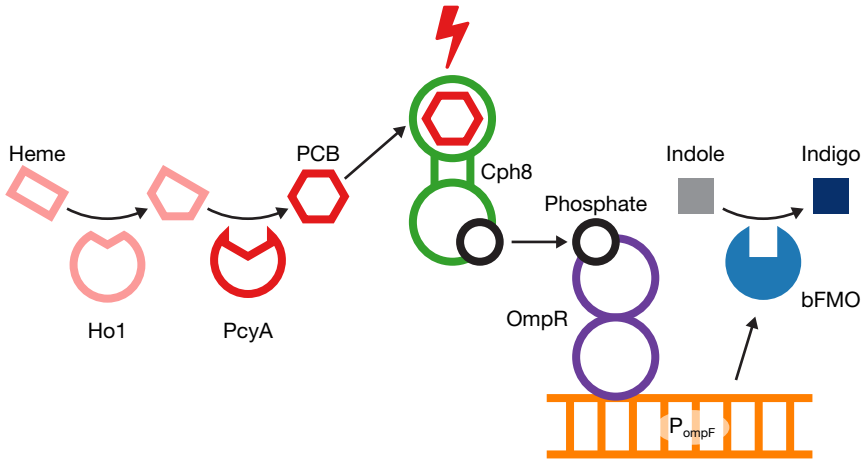
A bacterial photograph of einstein

Introduction to the Bactograph

To take bactographs, we created a next generation bacterial photography strain. It was made by transforming two seperate plasmids which contain the following enzymes, signalling proteins, and promoter encoded on their DNA.

1. The enzymes PcyA and Ho1 convert the naturally occuring molecule heme into PCB, a light absorbing molecule.
2. The signalling protein Cph8 binds PCB, and when PCB absorbs light, Cph8 becomes active by changing its shape. Activated Cph8 binds OmpR and chemically modifies it by adding a phosphate molecule to it.
3. The signalling protein OmpR is activated when Cph8 chemically modifies it, activated OmpR binds DNA and activates transcription from the P_{ompF} promoter.
4. The PompF promoter is activated by OmpR and transcribes the bFMO gene.
5. The enzyme bFMO converts the natually occuring molecule indol to indigo, the blue pigment found in jeans.

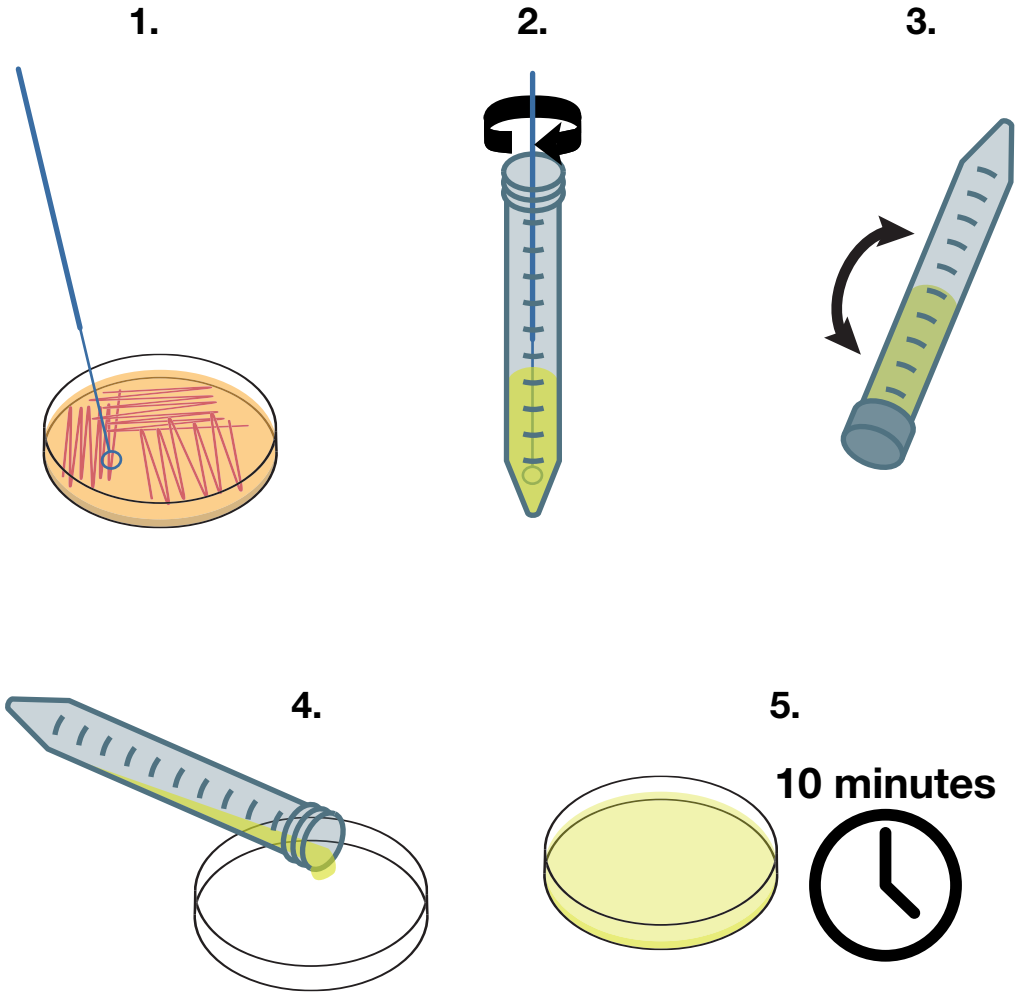
Each of the proteins, enzymes and promoter listed above was selected with a specific well-defined purpose, and when combined allowed the bacteria to see light and then decide to change color in response, creating bacterial photographs. This is a striking demonstration of the ability of synthetic biologists to use genetic transformation to create new bacteria with new capabilities. In addition to using these proteins to capture photographs, synthetic biologists are using them to mimic the patterns of gene expression seen in development in order to better understand how we grow from a single cell to a complex differentiated oragnism. They are also used in biotechnology to control the expression of the enzymes used to produce chemicals in large bioreactors.



The enzymes, signalling proteins and promoters used in the bactograph

Student Protocol

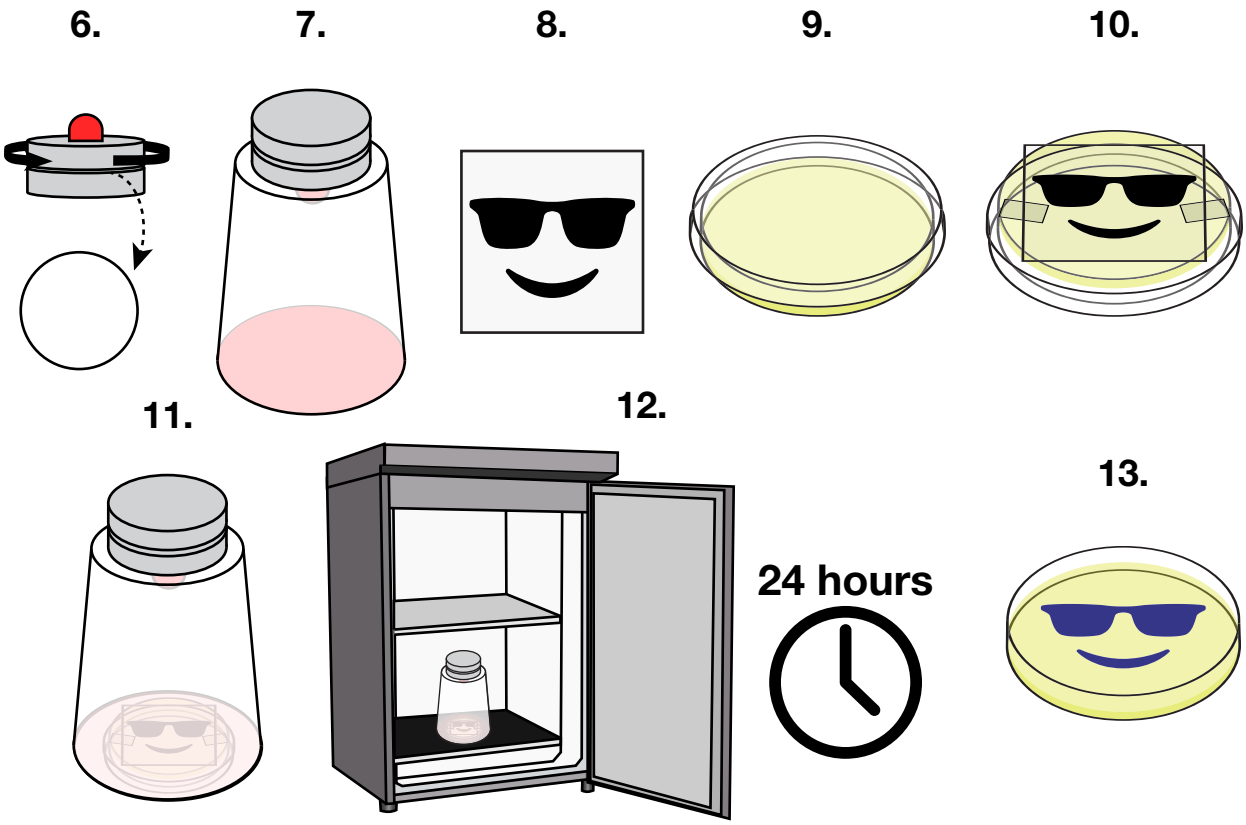
PREPARE THE BACTOGRAPH PLATE



1. **SCRAPE** bacteria off the class plate with an innoculating loop until the loop is filled with bacteria.
2. **SHAKE** off inocculating loop in media for 10 seconds.
3. **CAP** the media tube. **INVERT** the media tube quickly for 20 seconds to mix bacteria.
4. **POUR** media into the bottom of the petri dish.
5. **WAIT** 10 minutes for media to solidify. **TILT** plate gently to check if media has solidified.

Student Protocol

TAKE THE BACTOGRAPH



6. **TURN ON** the LED by unscrewing it, removing the white plastic divider, and then screwing it back together tightly.
7. **INSERT** the LED into the hole of the paper cup to illuminate the inside of the cup.
8. **DRAW** the image you want to take a Bactograph of on the sheet of transparency. Dark, thick lines will result in a better bactograph.
9. **CAP** the petri dish once it has solidified in step 5.
10. **INVERT** the petri dish and **TAPE** the transparency to the bottom.
11. **PLACE** the petri dish upside down on the sheet of black paper in the incubator, **PLACE** the cup ipside down over the petri dish.
12. **INCUBATE** the bacteria for 12-24 hours.
13. **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.