Experiment Components

KIT CONTENTS

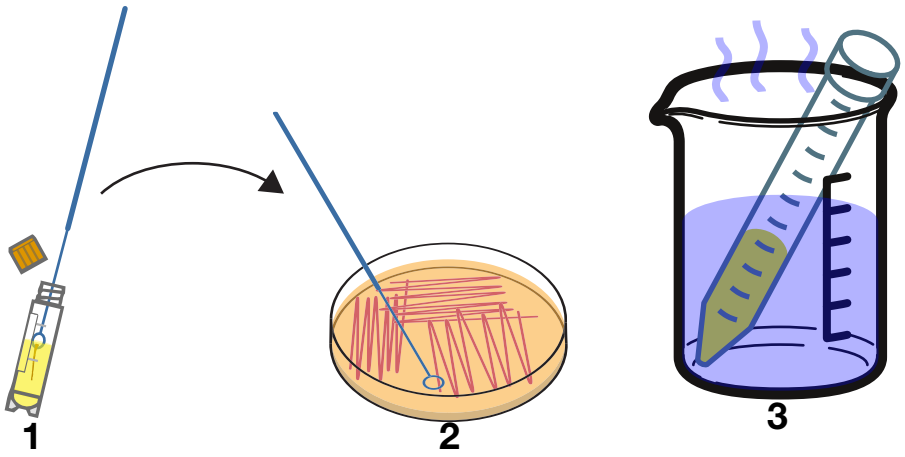
- 1 bactograph culture*
- 1 starter plate*
- 10 tubes of bactograph media*
- 10 petri dishes
- 1 Transparency sheet
- 11 innoculating loops
- 30 LED lights

* These components are stable for 2 weeks when store at 4°C

Required Materials

- A 37°C incubator
- Transparent Tape
- Permenant marker
- Boiling water

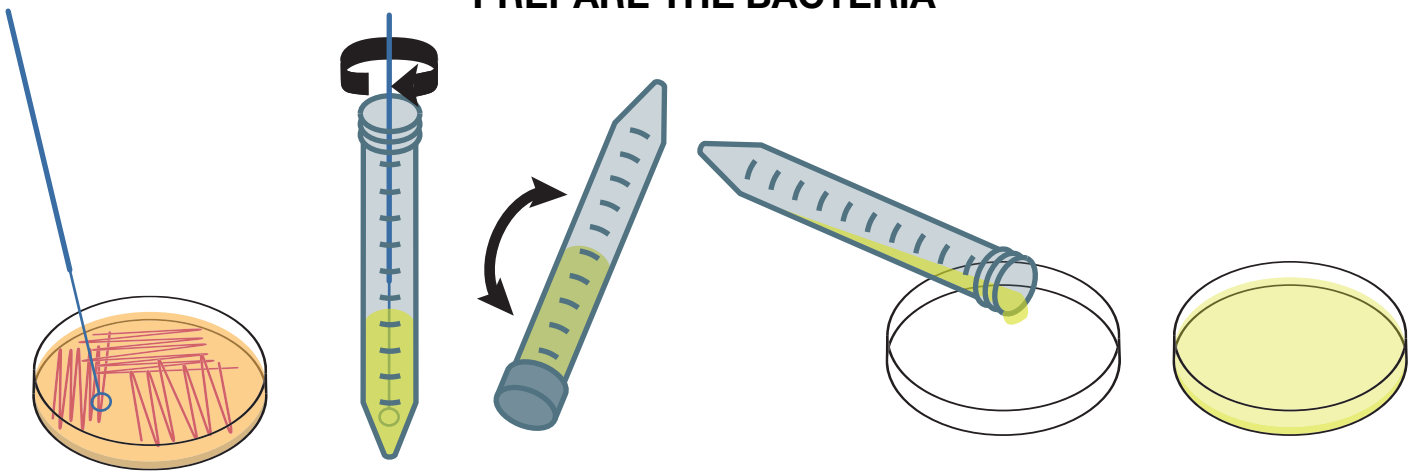
Day Before Teacher Preparation



1. **INOCULATE** the loop with bacteria by inserting it into the agar stab.
2. **STREAK** the bacteria on the starter plate and **INCUBATE** the bacteria at 37°C for 24-36 hours
3. **MELT** the agar tubes by submerging them in a boiling water bath for 15 minutes. **STORE** melted agar at 37°C.

Student Protocol

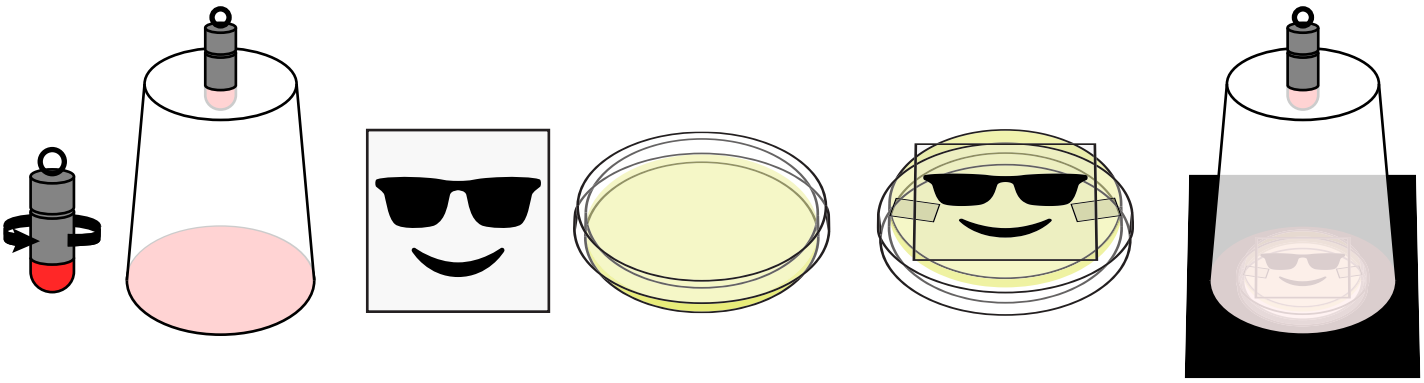
PREPARE THE BACTERIA



1 2 3 4 5

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.

TAKE THE BACTOGRAPH



6 7 8 9 10 11

6. **TURN ON** the LED by unscrewing it slightly, removing the 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 a Bactograph of on the sheet of transparency.
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 on top of the petri dish.
12. **INCUBATE** the bacteria for 12-24 hours.

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 effect 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!