



PROTOCOL

Bioluminescent Bacteria Culturing Kit

Grow GMO bacteria that glow in the dark.



What You'll Do

You're going to revive freeze-dried bioluminescent bacteria (*E. coli* DH10B carrying the pJE202 bioluminescence plasmid), grow them on nutrient media, and watch living bacteria produce real light using only biochemistry — no electricity, no dye.

Bioluminescent bacteria naturally glow at room temperature. Your bacteria carry the *lux* gene cluster — the same biochemical pathway that makes deep-sea squid and some fish glow.

Safety Notes



The *E. coli* in this kit is non-pathogenic and non-hazardous. It cannot make you sick.



Wear gloves when handling bacteria.



Wash hands after the experiment.



To dispose of bacteria: pour 5% bleach on any plates, wait 10 minutes, then trash.



Keep your work area clean. Wipe surfaces with rubbing alcohol before you start.

What You'll Learn

By the end of this experiment, you'll understand:



How bioluminescence works at the molecular level
(luciferase + FMNH₂ + O₂ → photon).



Basic sterile technique and bacterial culture.



How genetic engineering moves traits between organisms.



Freeze-dried bacteria



Inoculate LB Amp broth



Glowing culture in dark room



Shake

How Bioluminescence Works

Your E. coli have been engineered with the lux operon – five genes (luxA, luxB, luxC, luxD, luxE) from a bioluminescent marine bacterium, inserted into a plasmid.

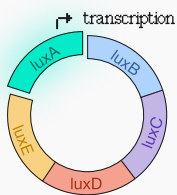
The bacteria carry this plasmid like extra instructions: grow, glow, repeat.

The Chemistry

The luciferase enzyme (encoded by luxA + luxB) catalyses a reaction between reduced flavin mononucleotide (FMNH_2), a long-chain fatty aldehyde, and oxygen. The result is light at 476nm – a cold, blue-green glow produced continuously while the bacteria are alive.

Why LB Ampicillin?

The lux plasmid also carries an ampicillin-resistance gene. LB Amp media selects for only the bacteria that kept the plasmid – any that lost it die off. This keeps your culture pure and glowing without additional purification steps.



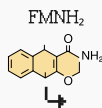
Lux operon on plasmid



Luciferase enzyme



Blue-green light



fatty aldehyde

 O_2 

Reaction inputs

What's in the Kit

1. Equipment



1x

250mL glass bottle
(for melting agar)



1x

50mL tube
(for measuring water)



5x

disposable
pipettes



5x

inoculation
loops



5x

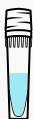
pairs of nitrile
gloves



7x

petri
plates

2. Perishables 🌡️ (store in fridge on arrival)



1x

sterile water
tube



1x

LB Amp agar powder
tube



1x

LB Amp broth
powder tube

3. Perishables ❄️ (store in freezer on arrival)



1x

tube of E. coli DH10B
with pJE202 plasmid,
freeze dried

Timeline at a Glance



DAY 1, AFTERNOON (~1 HOUR)

Make agar plates. Streak bacteria onto a plate.
Start liquid broth culture from freeze-dried bacteria.



DAY 1, EVENING

Let bacteria grow overnight (12-18 hours).



DAY 2, MORNING (~2 HOURS)

Check broth in dark —should be cloudy and glowing. Check plates for growth.



DAY 2-4

Propagate: add drops to fresh broth. Restreak plates as needed.

60-Second Video Guides

Before starting your experiment, check out these fast 60-second video protocols.

They walk you through every step of making agar plates, from melting and pouring to cooling and storage, so you can avoid common mistakes and set up your lab work for success.



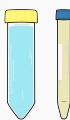
60 Second Video Protocols
“How to Make Agar Plates”.

STEP: 1 TIME: ~1h

Make Agar Plates

LB is a nutrient medium used to grow bacteria.

You'll use LB + ampicillin in two forms: solid agar plates for growing colonies and liquid broth for growing cultures.



1 Pour the contents of the LB Agar tube into the 250mL glass bottle.

☐

2 Use the 50mL measuring tube to add 150mL of tap water to the bottle. Shake vigorously.

☐

3 Loosely place the lid on top – do **NOT** screw it down tight. Place the bottle in the microwave.

☐

4 Microwave in 20–30 second bursts, swirling between each burst. Stop if you see boiling.

☐

5 Continue until the liquid looks completely clear and yellow with no visible grains or cloudiness. Total time: about 2–3 minutes of microwaving.

☐

6 Remove carefully (bottle is hot). Let cool 20–30 minutes until you can hold it comfortably.

☐

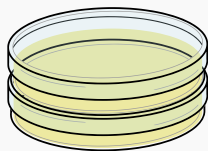
7 While still warm, pour into 7 petri plates: lift each lid, pour just enough to cover the bottom half, replace the lid. Don't move them until solid.

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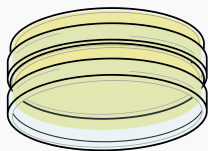
8 Let cool for at least 1 hour. Label the plates clearly “LB Amp Agar”.

☐

9 Store plates upside down in the fridge (4°C). They'll last 1–2 months.



Petri plate columns with labels:
“LB Amp agar”



Correct upside-down storage in fridge

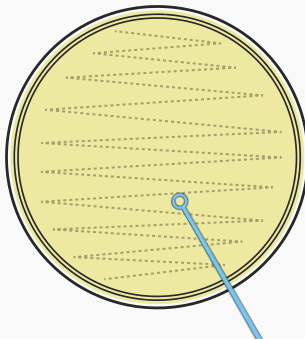
STEP: 2 TIME: ~10min

Prepare the Bacteria

Your *E. coli* arrives freeze-dried. You'll rehydrate it and streak it onto the LB Amp agar plate to grow a fresh colony overnight.



- 10 Add 100 μ L (~4 drops) of sterile water to the freeze-dried *E. coli* tube using the transfer pipette. ☐
- 11 Shake for 1 minute until the bacteria are fully dissolved. ☐
- 12 Pipette all 100 μ L of the bacteria suspension onto the LB Amp agar plate. ☐
- 13 Use an inoculation loop to gently streak the bacteria across the plate in a zigzag pattern. ☐
- 14 Place the lid back on and let the plate grow at room temperature overnight (12–18 hours). **DO NOT REFRIGERATE.** Colonies are ready to use when they appear as visible white or cream-colored dots spread across the plate. ☐



Zigzag pattern across plate

Warning:



If your room is cold (below 18°C), bacteria may take longer – up to 24 hours – to grow.



Do not grow bacteria above 28°C. It damages the bacteria and the experiment will fail.

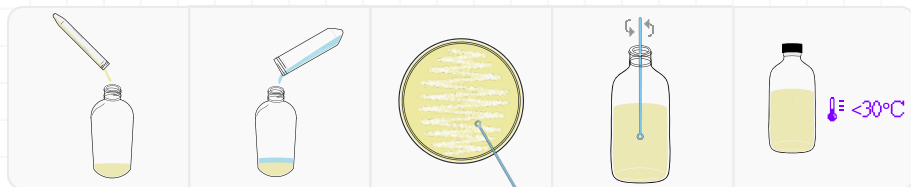


If agar solidifies in the bottle before you finish, briefly re-microwave to liquefy it again.

STEP: 3 TIME: ~10m

Start a Liquid Culture

Liquid culture shows bioluminescence most clearly.



- 15 Pour the contents of one LB Amp media tube into the glass bottle. Add about 200mL of tap water. Shake gently to dissolve. ☐
- 16 Use an inoculation loop to scrape a small amount of bacteria from the plate with freshly grown bacteria. ☐
- 17 Swirl the loop in the broth to transfer the bacteria. **DON'T** shake the bottle while inoculating. ☐
- 18 Rest the cap loosely on top, bacteria need oxygen to glow. Leave at room temperature below 30°C. ☐

Warning:



Do **NOT** shake or seal the bottle while incubating. Bacteria need oxygen and stillness to produce maximum glow.

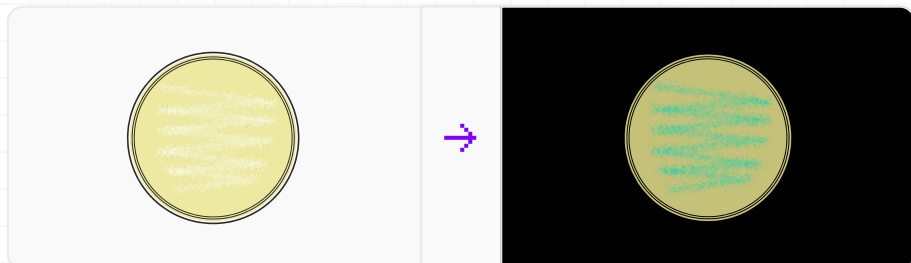
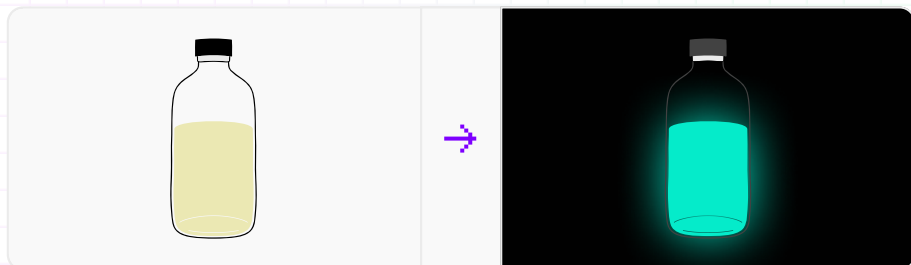
STEP: 4 TIME: ~6-10h after incubation

Observe the Glow

Check your liquid culture 6–10 hours after inoculating.

Glow intensity depends on bacterial density, gently swirl the bottle before checking.

- 19 Take your bottle to a completely dark room. Wait 2–3 minutes for your eyes to adapt. Secure the cap briefly, gently swirl the bottle, then loosen the cap again. Oxygen contact boosts glow briefly. ☐
- 20 Look for a faint blue-green glow throughout the liquid. It may be subtle at first, give your eyes time. ☐
- 21 For photos: use Night Mode or long-exposure (5–15 sec), ISO 3200+. Place the bottle on a dark surface. ☐
- 22 Check your plate the same way. ☐



Success:



Cloudy broth glowing blue-green in total darkness, even a dim glow at hour 6 is working.



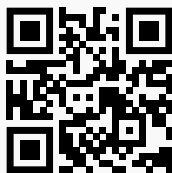
Glowing dots on the plate are individual bioluminescent colonies.

Record Your Results Here



This Is Just the Beginning

Explore more experiments at the-odin.com



Need Help?

Contact us at odin@the-odin.com