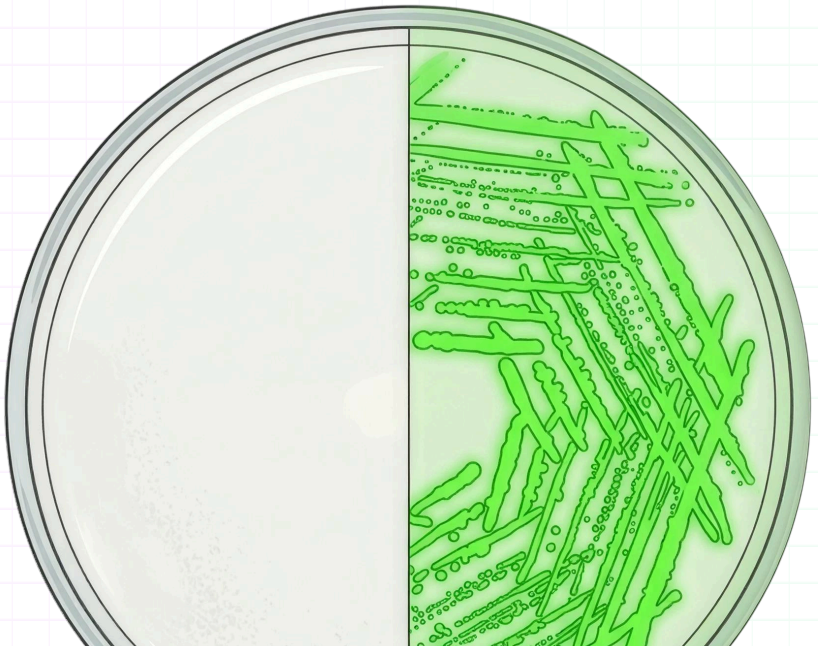




PROTOCOL

Glow-in-the-Dark Bacteria Engineering Kit

Make Bacteria Glow with Jellyfish DNA.



What You'll Do

In this experiment, you'll insert a gene from a jellyfish into harmless *E. coli* bacteria.

That gene makes a protein called Green Fluorescent Protein (GFP) – the same protein that makes jellyfish glow.

When you shine blue light on the bacteria through the orange filter included in this kit, any bacteria that successfully took up the gene will glow bright green.

This is called bacterial transformation, and it's one of the most foundational techniques in genetic engineering.

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.

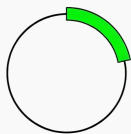
How It Works

Cells use DNA as an instruction manual for making proteins. Every 3 letters of DNA code for one amino acid, and chains of amino acids make proteins.

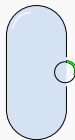
GFP is a protein that naturally fluoresces, it absorbs blue light and emits green light.

The GFP gene is carried on a tiny circular piece of DNA called a plasmid. We'll mix the bacteria with chemicals that temporarily make their cell walls leaky, then add the plasmid.

Some bacteria will absorb the plasmid and start producing GFP. Only the transformed bacteria survive on our antibiotic plates, which glow green in blue light.



Plasmid with
GFP gene



Bacteria



Bacteria
glowing green

What's in the Kit

1. Equipment



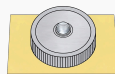
1x

250mL glass bottle
(for melting agar)



1x

50mL tube
(for measuring water)



1x

orange UV filter sheet +
blue LED light



5x

1.5mL tubes pre-filled
with LB broth



5x

inoculation
loops



5x

pairs of nitrile
gloves



9x

disposable
transfer pipettes



7x

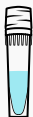
petri
plates



25x

microcentrifuge
tubes

2. Perishables 🌡️ [store in fridge on arrival]



1x

sterile water
tube



1x

LB agar powder
tube



1x

LB + Kanamycin agar
powder tube



5x

0.1 mL bacterial
transformation buffer
(CaCl_2 + PEG 8000)

3. Perishables ❄️ [store in freezer on arrival]



5x

tubes of GFP plasmid
(jellyfish gene),
20 ng/ μL



2x

tubes of non-pathogenic
E. coli DH5a,
freeze dried

Timeline at a Glance



DAY 1, AFTERNOON (~1 HOUR)

Make agar plates. Streak bacteria onto a plate.



DAY 1, EVENING

Let bacteria grow overnight (12-24 hours).



DAY 2 (~2 HOURS)

Prepare competent cells, add plasmid, heat shock, plate.



DAY 2-4

Wait for colonies to grow, then check for fluorescence.

Before You Start: 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.



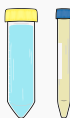
60 Second Video Protocols
“How to Make Agar Plates”.

STEP: 1 TIME: ~1h

Make Agar Plates

Agar plates are solid nutrient surfaces for bacteria to grow on.

You'll make two types: plain LB agar (for growing bacteria) and LB + Kanamycin agar (antibiotic plates – only transformed bacteria survive here).



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



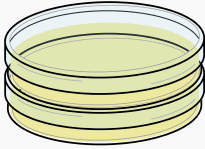
8

Let cool at least 1 hour. Repeat the whole process for the LB + Kanamycin agar tube, making 5 more plates. Label them clearly.

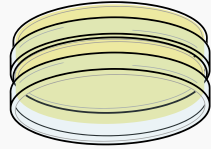


9

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



Petri plate columns with labels:
“LB Agar” and “LB + Kan Agar”

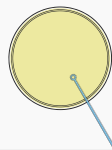


Correct upside-down storage in fridge

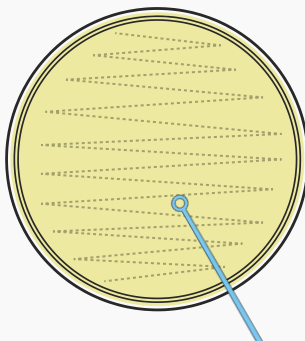
STEP: 2 TIME: ~10min

Prepare the Bacteria

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



- 10 Add 4 drops (~100 μ L) of sterile water to the freeze-dried bacteria tube using the disposable transfer pipette. ☐
- 11 Shake for 1 minute until the bacteria are fully dissolved. ☐
- 12 Pipette all 100 μ L of the bacteria suspension onto a plain LB agar plate (**NOT THE ANTIBIOTIC 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 to grow – up to 24 hours.



Do not grow bacteria above 37°C. It damages the bacteria and the experiment will fail.



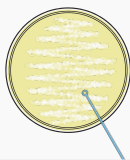
If agar solidifies in the bottle before you finish, briefly re-microwave to liquefy it again.

STEP: 3 TIME: 2h

Transformation: Adding the GFP Gene

This is the main event. You'll mix bacteria with the transformation buffer (which temporarily makes cell walls porous), add the plasmid DNA, then heat shock the mixture to drive the plasmid inside.

3.1 Prepare Competent Cells



15

Take an inoculation loop and gently scrape a small amount of bacteria from your overnight plate. You want the loop to be visibly coated. A thin film is enough.



16

Transfer the bacteria into a microcentrifuge tube containing 100 μ L of transformation buffer. Swirl the loop to release the bacteria. Mix gently until any clumps dissolve.



3.2 Add the Plasmid DNA

17

Take the GFP plasmid tube. Add 10 μ L of sterile water and shake for 1 minute to rehydrate the freeze-dried DNA.



18

Add 10 μ L of the rehydrated GFP plasmid to your bacteria tube. **MIX GENTLY.**



3.3 Cold Incubation

19

Place the tube in the fridge (4°C) for 30 minutes.
DO NOT FREEZE IT.



3.4 Heat Shock

20

Heat shock the tube in warm water at 42°C (108°F) **FOR EXACTLY 30 SECONDS**. You can test temperature with your wrist. It should feel hot but not painful.



21

Immediately move the tube to room temperature.



🌡️ = 42°C/108°F

3.5 Recovery

22

Add 900µL of LB broth (from one of the pre-filled LB tubes) to your transformation tube.



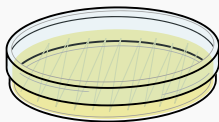
23

Incubate at 37°C for 1–2 hours, or at room temperature for at least 4 hours. This lets the bacteria recover and start expressing GFP.

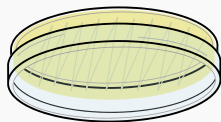


3.6 Plate the Bacteria

- 24 Take an LB + Kanamycin plate out of the fridge and let it warm to room temperature. ☐
- 25 Pipette a few drops (~100 μ L) of your transformation mixture onto the antibiotic plate. ☐
- 26 Use an inoculation loop or spreader to gently distribute the bacteria across the plate. ☐
- 27 Let the plate sit closed for 10 minutes so the liquid absorbs. ☐
- 28 Flip the plate upside down, put the lid on, and incubate at 37°C for 16–24 hours, or at room temperature for 24–48 hours. ☐



Petri plate with bacteria spread evenly



Flip upside-down for incubation

STEP: 4 TIME: 1-3d

Check Your Results

After incubation, look for white or cream-colored dots on the plate. These are bacterial colonies. Only transformed bacteria survive on the antibiotic plate.

29 To check for fluorescence, hold the orange filter up to your eyes.



30 Shine the blue LED light onto the plate.



31 Transformed colonies will glow bright green through the filter.
Non-transformed colonies won't survive on the antibiotic plate.



Success:



Bright green glowing dots visible through the orange filter.



If no colonies appear:

The agar plate may have been too hot when bacteria were plated. Try again.



If colonies appear but don't glow:

The plasmid may not have been taken up. Retry with fresh bacteria.

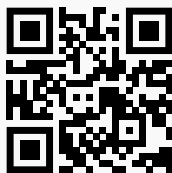
Record Your Results Here

A large, light gray rectangular area with rounded corners, containing horizontal lines for recording results. The area is designed to look like a notepad or a form for data entry. It has a subtle drop shadow and is set against a background of a light green grid.



This Is Just the Beginning

Explore more experiments at the-odin.com



Need Help?

Contact us at odin@the-odin.com