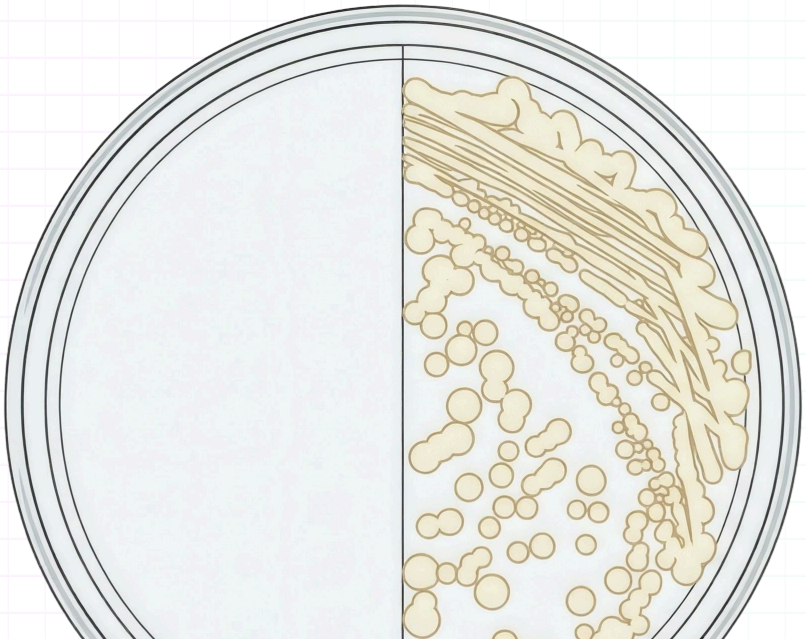




PROTOCOL

CRISPR Bacteria Gene Editing Kit

Use the same gene-editing tool behind Nobel Prize-winning research to rewrite DNA.



What You'll Do

In this experiment, you'll use the CRISPR-Cas9 system to make a precise edit to the DNA of *E. coli* bacteria. The edit changes a single DNA letter in the ribosomal gene *rpsL*, which allows the bacteria to survive on streptomycin — an antibiotic that would normally kill them.

This is a real CRISPR experiment. You'll use the same molecular tools that researchers use to edit genes in labs around the world. The bacteria's genome is over 4 million bases long, and CRISPR will find and edit exactly the one that needs to change.

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

CRISPR-Cas9 is a precision gene-editing tool with three key parts:

Cas9

The “molecular scissors”. Cas9 is a protein that binds to a specific location in the genome (guided by the gRNA) and cuts the DNA. By itself, Cas9 doesn't do any editing, it just cuts.

Guide RNA (gRNA)

A short piece of RNA that matches the DNA sequence you want to edit. The gRNA acts like a GPS — it guides Cas9 to exactly the right spot in the genome.

Template DNA

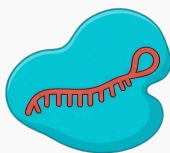
After Cas9 cuts, the cell tries to repair the break. If you flood the cell with a repair template that has a specific mutation in it, the cell will often use that template, incorporating your desired change. Our template has a single base change (A → C) that turns Lysine-43 into Threonine in the ribosomal protein rpsL. This one change prevents streptomycin from binding to the ribosome, giving the bacteria resistance.

How CRISPR Works

The *E. coli* genome has 4,000,000+ base pairs.

CRISPR edits exactly **1** of them.

That single change makes bacteria survive on streptomycin media.



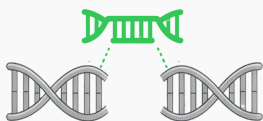
Cas9 protein + gRNA
forming complex



Complex
scanning DNA



Cas9
cutting DNA



Cell repairing
with template DNA

What's in the Kit

1. Equipment



1x

250mL glass bottle
(for melting agar)



1x

50mL tube
(for measuring water)



1x

adjustable pipette
(10–100 μ L) + pipette tips



5x

1.5mL tubes pre-filled
with LB broth



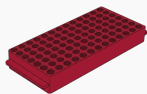
5x

inoculation
loops



5x

pairs of nitrile
gloves



1x

microcentrifuge
tube rack



14x

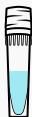
petri
plates



25x

microcentrifuge
tubes

2. Perishables [store in fridge on arrival]



1x

sterile water
tube



1x

LB agar powder
tube



1x

LB + Strep/Kan/Arabinose
agar powder tube



5x

0.1mL bacterial
transformation buffer
(CaCl_2 + PEG 8000)

3. Perishables [store in freezer on arrival]



1x

tube of Cas9 plasmid
(carries kanamycin
resistance)



1x

tube of gRNA plasmid
(carries ampicillin
resistance, targets rpsL)



1x

tube of template DNA
(contains the rpsL K43T
mutation)



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 (12-18 hours).



DAY 2, MORNING (~2 HOURS)

Prepare competent cells, add CRISPR plasmids + template, heat shock, plate.



DAY 2-4

Wait for colonies to grow, check plates for colonies that survived streptomycin.

Before You Start: Pipette Practice

Before running the experiment, practice drawing up 10 μ L of water accurately. The liquid should reach the first line on the pipette tip with no air bubbles.



See our video guide for a full walkthrough.

Warning:

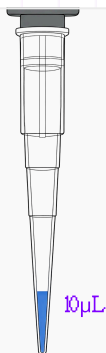


Pipette accuracy is critical for CRISPR to work.
The volumes in this kit are small.

Plasmid Preparation and Handling

Your plasmid tube contains freeze-dried DNA and it may look empty. The DNA is dried on the tube walls and will dissolve when you add water.

Thaw your plasmid tubes and flick them with your wrist to collect liquid at the bottom. Practice drawing up 10 μ L of water accurately before you begin.



Correct 10 μ L volume in pipette tip — liquid reaching the first demarcation mark, no air bubbles.

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.



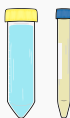
60 Second Video Protocols
“How to Make Agar Plates”.

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 + Kan/Strep/Arabinose (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.

☐

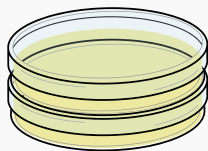
6 Remove carefully (bottle is hot). Let cool 20–30 minutes until you can hold it comfortably.

☐

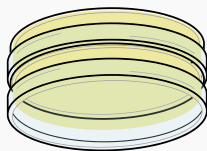
7 While still warm, pour into 7 petri plates: lift each lid, pour just enough to cover the bottom half, replace the lid. Don't move them until solid.

☐

- 8 Let cool at least 1 hour. Repeat the whole process for the LB + Kan/Strep/Arabinose agar tube, making 7 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/Strep/Arabinose”

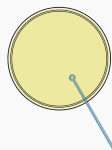


Correct upside-down storage in fridge

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 100 μ L of sterile water to the freeze-dried *E. coli* tube using the adjustable transfer pipette. Remember to always change pipette tips in between uses to keep from accidentally mixing leftovers in the tip. ☐
- 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**). ☐

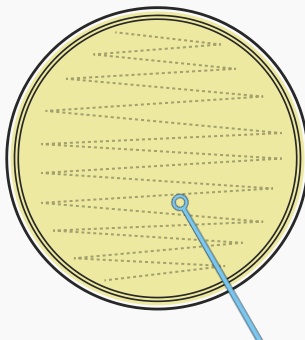
13

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 – up to 24 hours – to grow.



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



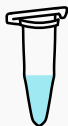
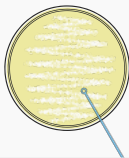
If agar solidifies in the bottle before you finish, briefly re-microwave to liquefy it again.

STEP: 3 TIME: 2h

Transformation: Adding the CRISPR

This is the main event. You'll combine the bacteria with both CRISPR plasmids (Cas9 + gRNA) and the template DNA. The transformation buffer makes the cells temporarily leaky so the DNA can enter.

3.1 Prepare 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. The liquid should look cloudy. Break up clumps by pipetting gently up and down.



3.2 Add the CRISPR Components

17

Take the Cas9 plasmid tube. Add 10 μ L of sterile water and shake for 1 minute to rehydrate the freeze-dried components. Repeat same process for gRNA plasmid and template DNA. Change tips between each.



18

Add 10 μ L of the rehydrated Cas9 plasmid to your bacteria tube. Repeat same process for gRNA plasmid and template DNA. Change tips between each. **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.



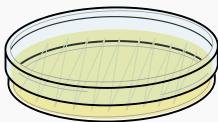
23

Incubate at 37°C for 1–2 hours, or at room temperature for at least 4 hours. This recovery is important, it lets Cas9 do its editing work inside the cells.

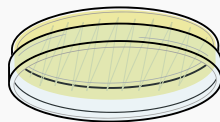


3.6 Plate the Bacteria

- 24 Take an LB + Kan/Strep/Arabinose plate out of the fridge and let it warm to room temperature. ☐
- 25 Pipette a few drops (~10 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

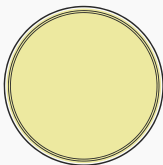


Flip upside-down for incubation

STEP: 4 TIME: 1-3d

Check Your Results

After incubation, look for colonies on the selection plate.
Only bacteria whose *rpsL* gene was successfully edited will survive on streptomycin.



Success:



White or yellow colonies visible on the selection plate after incubation.



If no colonies appear:

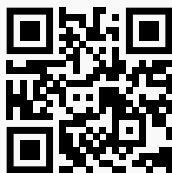
The editing efficiency may have been low. Try again with fresh bacteria.

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