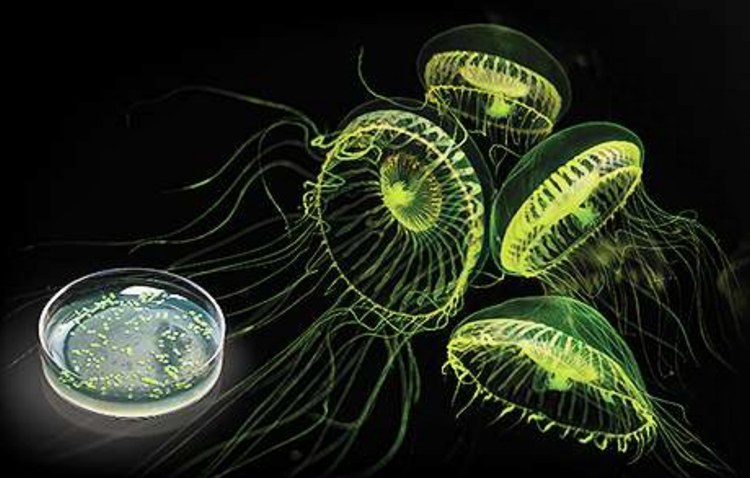
pGLO Bacterial Transformation



Student presentation for use with the pGLO Bacterial Transformation Kit for General Biology

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Getting Started

Introduction

- Bio-Rad's pGLO Bacterial Transformation Kit for General Biology is all about asking questions, designing experiments, and making claims — with glowing bacteria!
- Use and modify this slide deck with your students as needed.
- The instruction manual can be downloaded for free from the pGLO Bacterial Transformation Kit for General Biology product page.

Instructor Preparation

Required Materials

- pGLO Bacterial Transformation Kit for General Biology

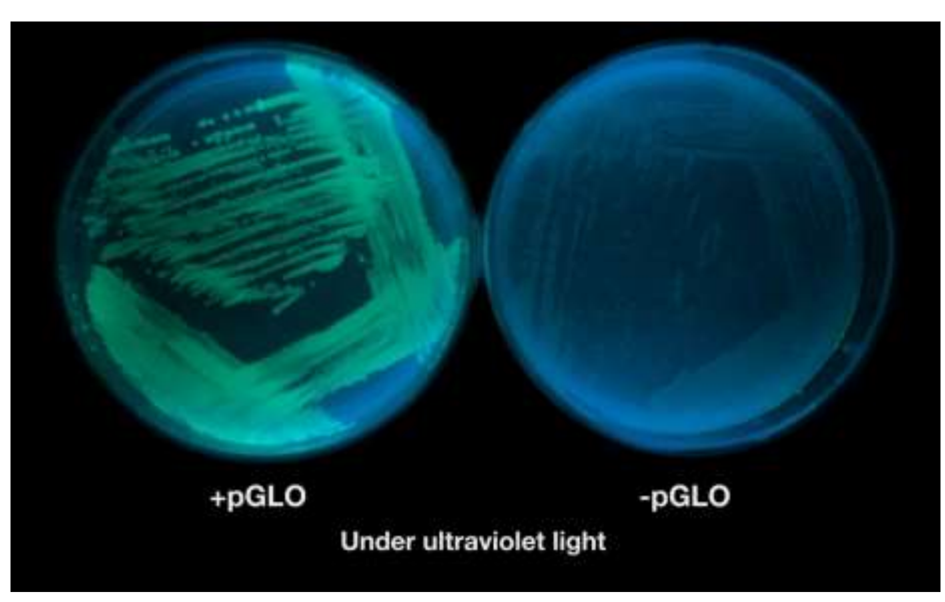
Required Materials (not included in this kit)	Quantity
Microwave oven	1
Temperature-controlled dry bath or water bath	1
Thermometer (0–60°C)	1
Erlenmeyer flask, 1 L	1
Graduated cylinder, 500 ml	1
Distilled water	500 ml
Ice bath (e.g., ice bucket or foam cup)	8
Marking pen	8
Timer to count seconds	
Laboratory tape	
Household bleach, 10% solution for cleanup	

Recommended Materials (not included in this kit)	Quantity
2–20 µl adjustable-volume micropipet and tips	1
Incubator oven, 37°C	1
Vortexer	1
Tube rack	1
Parafilm laboratory sealing film	

Activity 1

Transferring Genes between Species

Bacteria (*E. coli*)



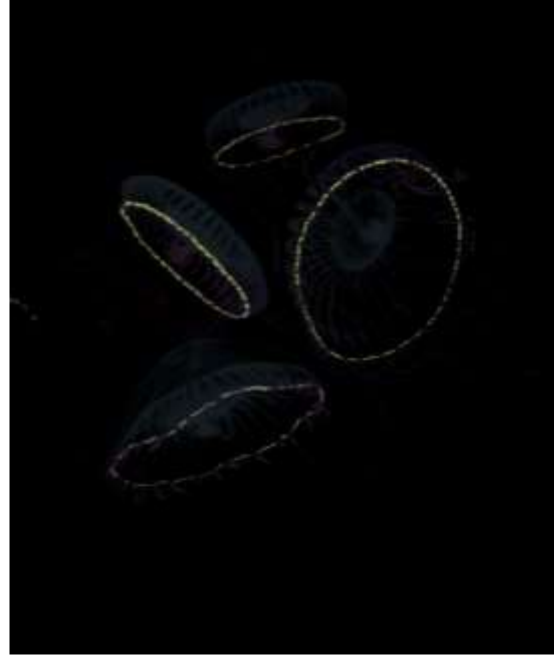
What do you notice…

- about the bacteria under visible and UV light
- about the bacteria that are +pGLO and –pGLO

Bio-rad.com/glow

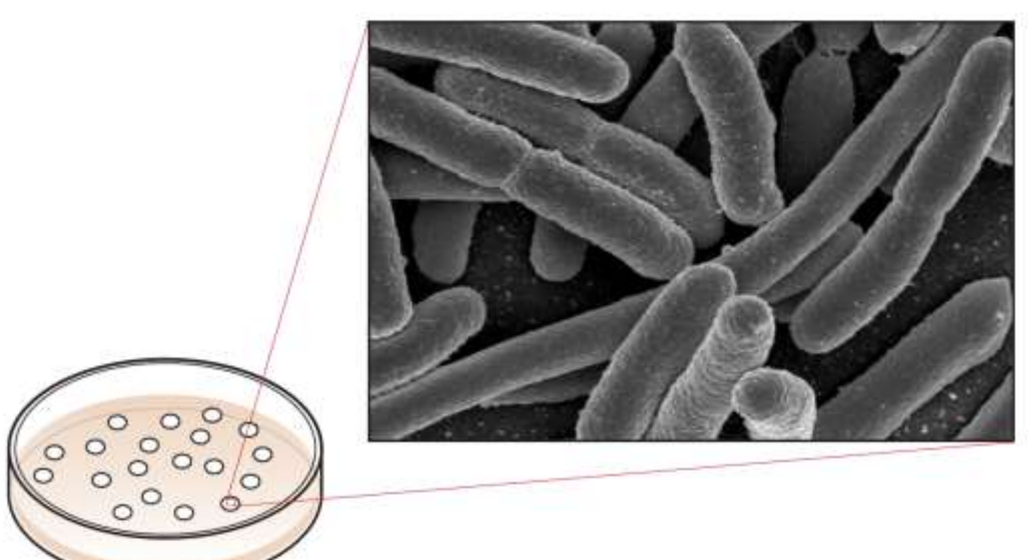
Jellyfish (*Aequorea Victoria*)

Under visible light



Under ultraviolet (UV) light

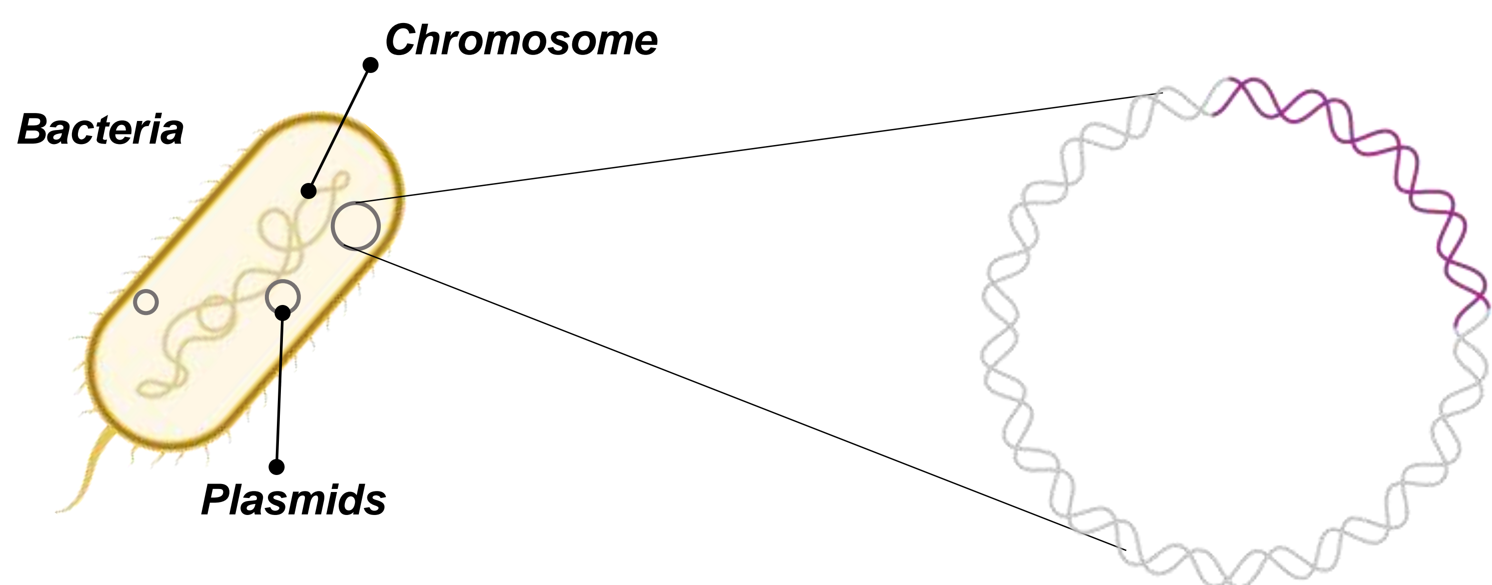
Bacteria growth



The bacterial growth you see on the plate is made of millions of individual bacteria (*E. coli*) cells.

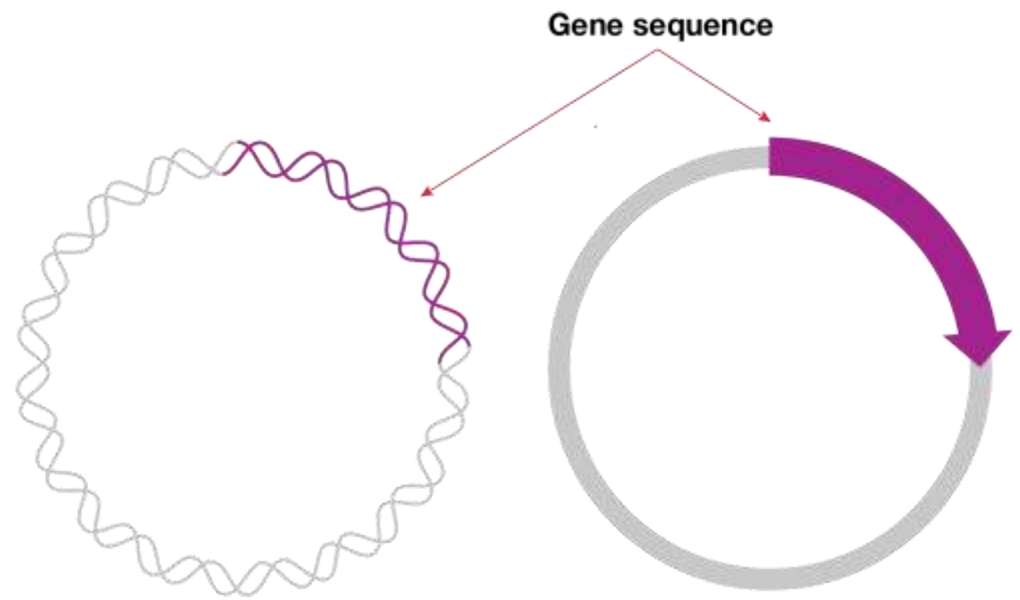
Plasmid DNA

- Bacteria often have plasmids — circular loops of DNA
- Bacteria can also take in new plasmids.



Plasmid DNA

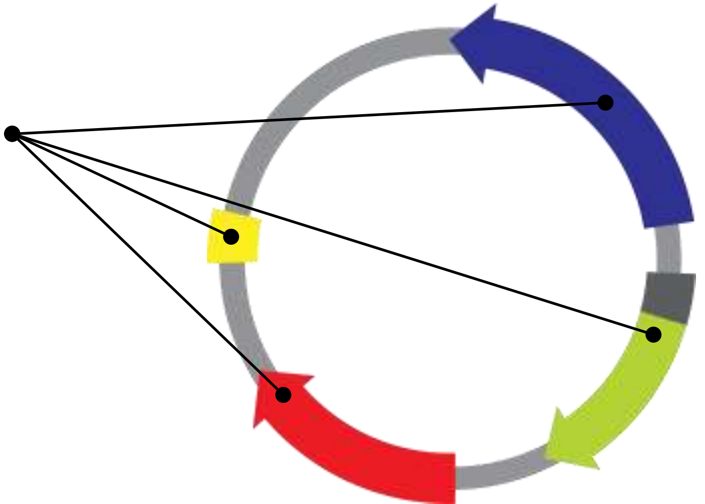
- Plasmid DNA is represented graphically in multiple ways
- Features typically have different colors and are labeled



How do you use plasmids to add genes into bacteria?

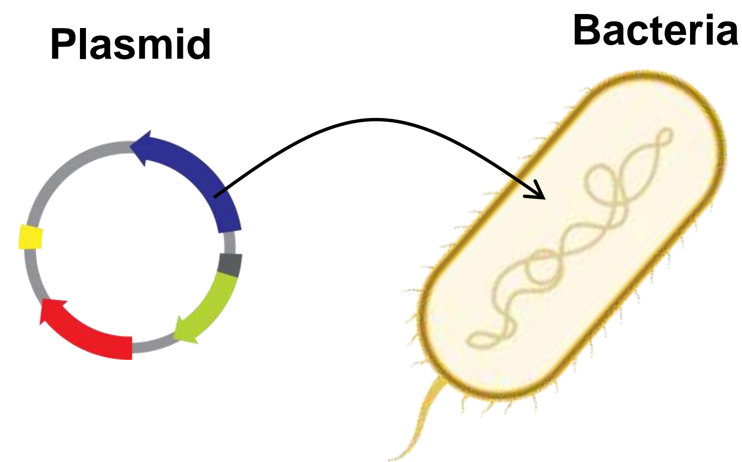
1. Scientists modify or engineer plasmids for specific purposes.

Plasmid Features
Sequences that allow replication, genes for protein production or other desired traits



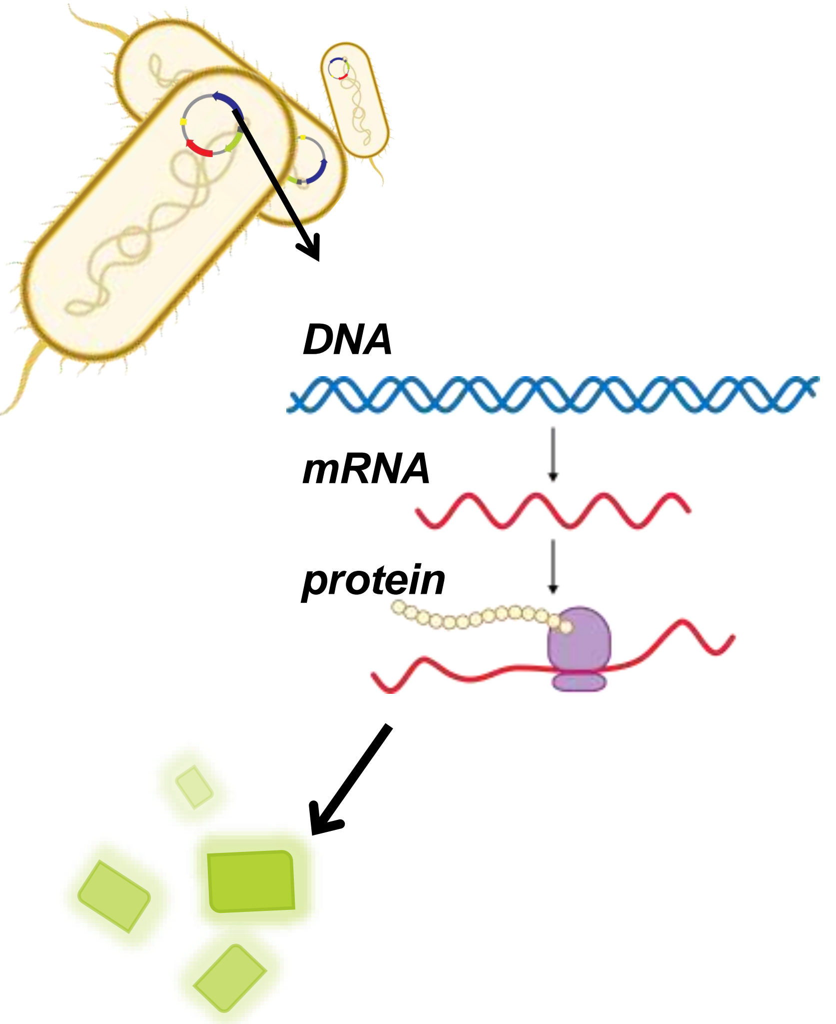
How do you use plasmids to add genes into bacteria?

1. Transform bacteria with the plasmid. This is what you'll do in this activity.



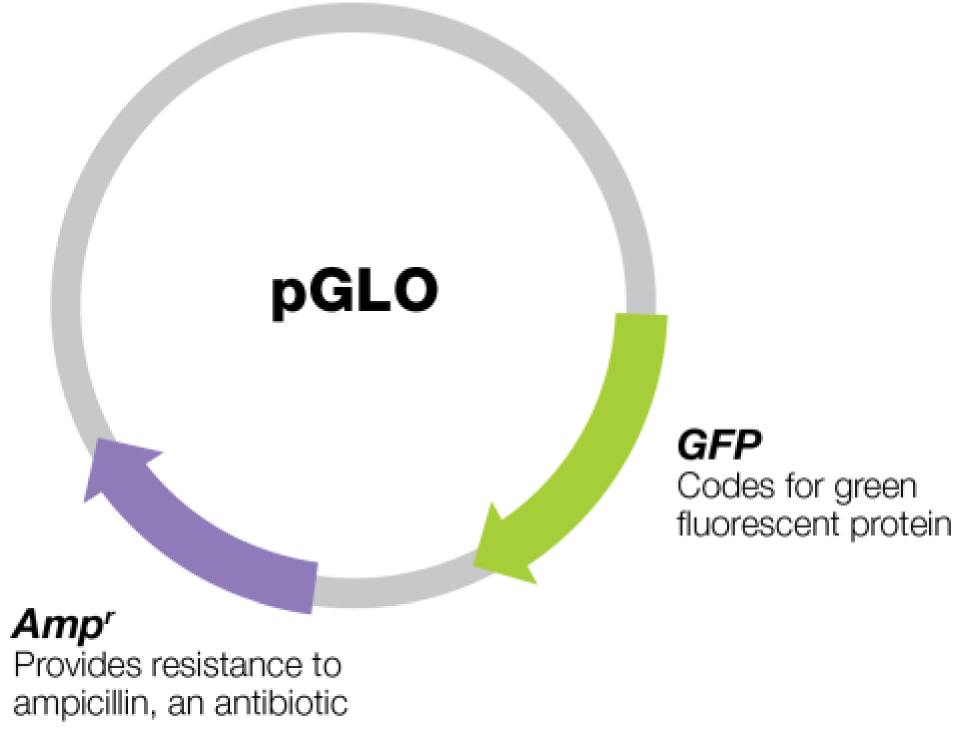
What happens after transformation?

1. Grow lots of the bacteria.
2. The bacteria transcribe and translate the gene — mini protein factories!



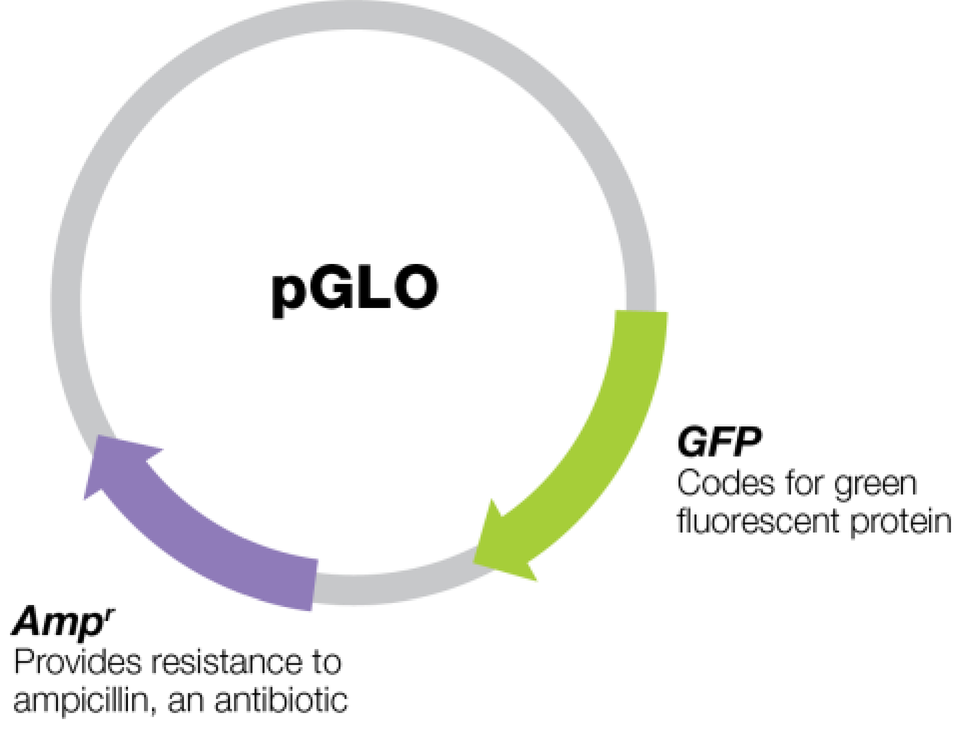
pGLO Plasmid

- The pGLO plasmid is engineered to have the *GFP* gene from *Aequorea victoria*.



pGLO Plasmid

- What traits might you expect a bacterial cell to have if it has been transformed with this plasmid?



Plasmid DNA

O
O – P = O
O
CH_2
O
Sugar
Base
O
O – P = O
O
CH_2
O
Sugar
Base

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Bacterial Membrane

E. coli

Plasma Membrane

Non-polar

Polar

Add plasmid

E. coli

Plasmid DNA

Plasma Membrane

Add plasmid

E. coli

Negative charges on DNA backbone

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Add transformation solution ($CaCl_2$)

E. coli

Ca^{2+} shields charges on DNA to make it less polar

Ca²⁺

Heat Shock

E. coli

Add heat to create pores in the membrane

Ca^{2+} Ca^{2+} Ca^{2+} Ca^{2+}

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Heat Shock

E. coli

Ca²⁺

Add heat to create pores in the membrane

Heat Shock

E. coli

Plasmid enters cell through pore

Ca^{2+}

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Recovery on ice, 2 min

E. coli

Pores close

Ca2+

Ca2+

Ca2+

Ca2+

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Add LB broth, allow gene expression, 10 min

E. coli

Add LB broth, allow gene expression, 10 min

E. coli

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Selective media – ampicillin

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Selective media – ampicillin

Beta-lactamase, ampicillin resistance

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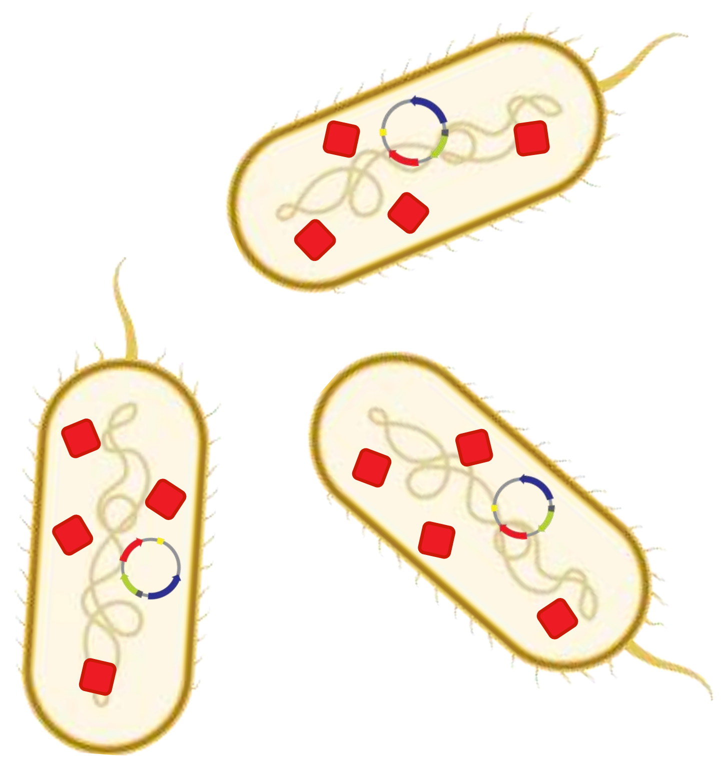
Selective media – ampicillin

Ampicillin on the plate

Bacteria without the plasmid cannot grow in the presence of ampicillin

Selective media – ampicillin

- Transformed bacteria (with the plasmid) will make beta-lactamase ■ , which breaks down ampicillin. This enables them to grow on ampicillin plates
- Bacteria without the plasmid (NOT transformed) cannot grow on plates with ampicillin.



LB Broth

- LB (Lysogeny broth or Luria Bertani) broth is like chicken noodle soup for bacteria. It has all the nutrients bacteria need to grow:
  - Carbohydrates
  - Amino acids
  - Nucleotides
  - Salts
  - Vitamins

Transformation summary

1.	$CaCl_2$ transformation solution	Shields negative charge on DNA.
2.	Pre-heat shock incubation on ice	Slows fluid plasma membrane for greater shock.
3.	Heat shock	Increases permeability of cell membranes.
4.	Post-heat shock incubation on ice	Restores cell membrane.
5.	Incubation at room temperature with LB broth	Allows beta-lactamase expression so bacteria can grow on plates with ampicillin.
6.	Spread on plates	Allows bacterial growth.

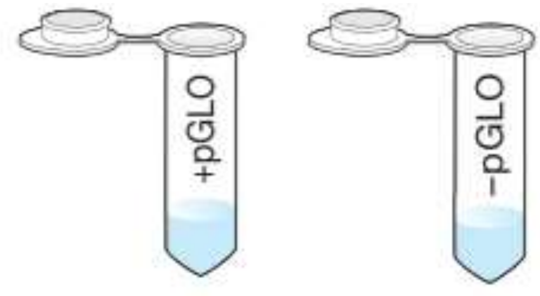
Label tubes

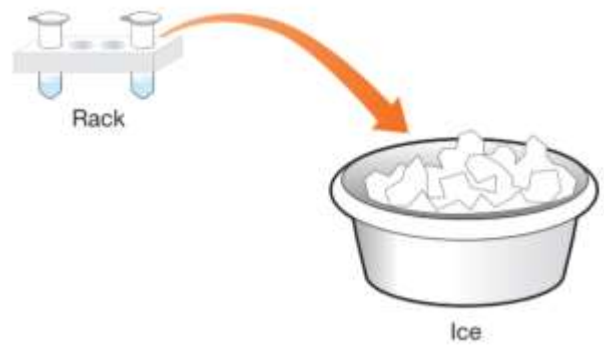
You have tubes with 250 µl transformation solution.

1. Label one **+pGLO** and the other **–pGLO**.

   Add your initials.

   Place into foam rack and on ice.

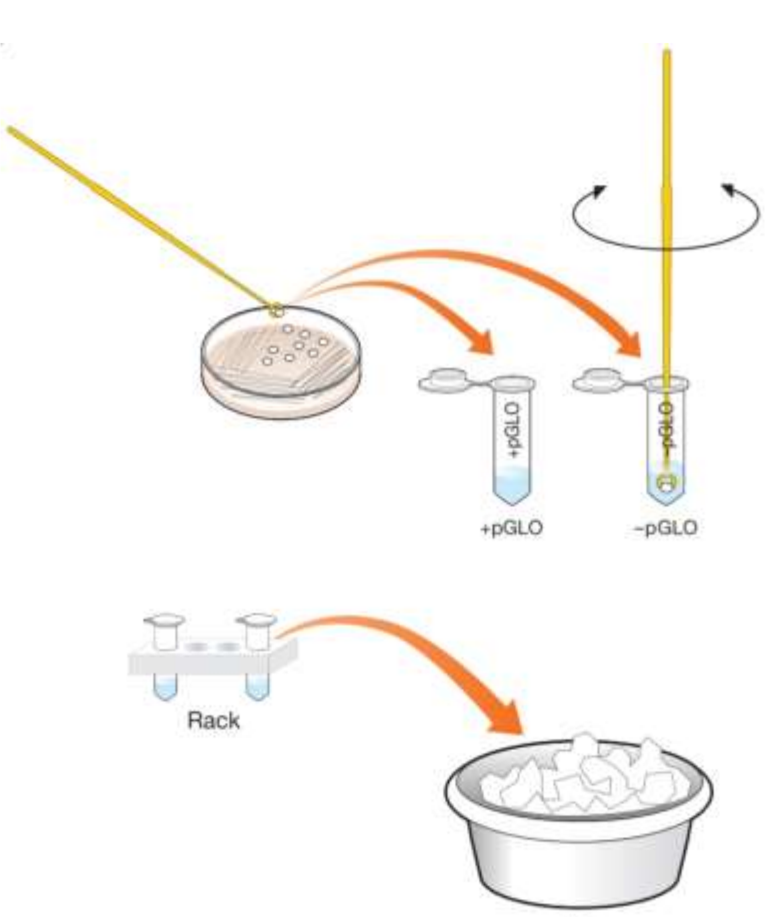




Pick colonies

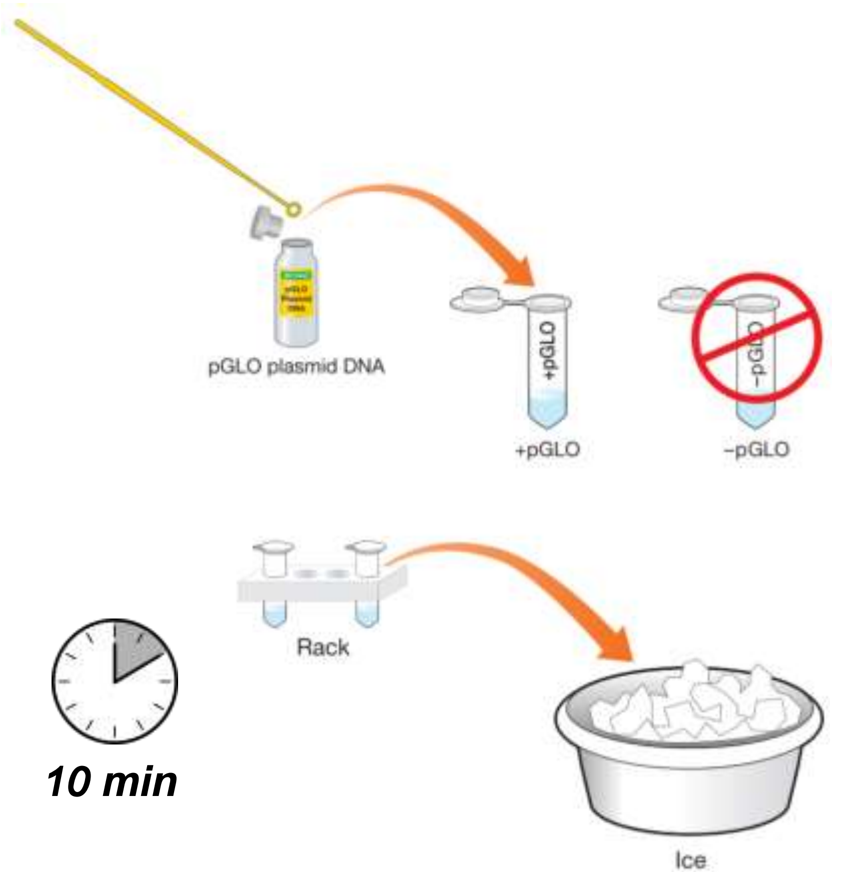
2. ***Using a sterile loop*** pick 2–4 large *E. coli* colonies.
3. Add to the **+pGLO** tube. Spin the loop to disperse the bacteria. No clumps!
4. Using a ***new*** loop, at 1–2 colonies to **–pGLO** tube.

   Place tubes into foam rack and on ice.



Add plasmid DNA

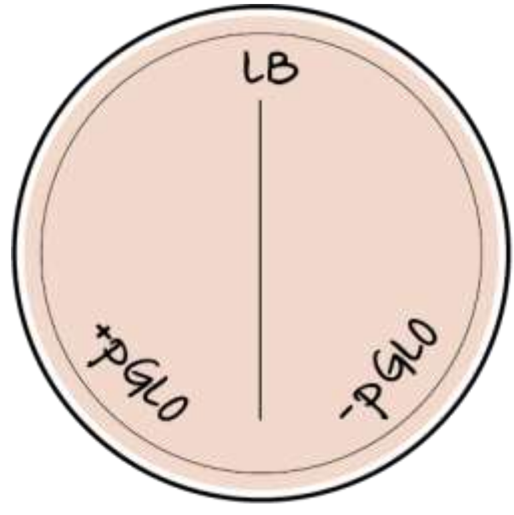
5. Add 10 µl (1 loop full) pGLO plasmid to **+pGLO** tube. ***DO NOT ADD TO –pGLO tube.***
6. Place tubes into foam rack and on ice for 10 min.

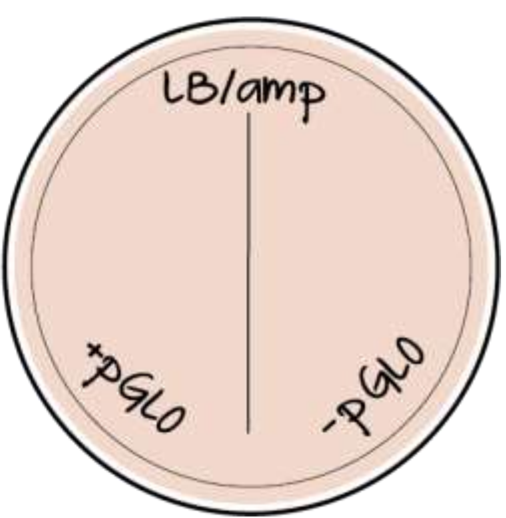


Label plates

7. While your tubes are on ice, label the ***bottom*** of your plates. As shown below.

   Add your group ID or initials.

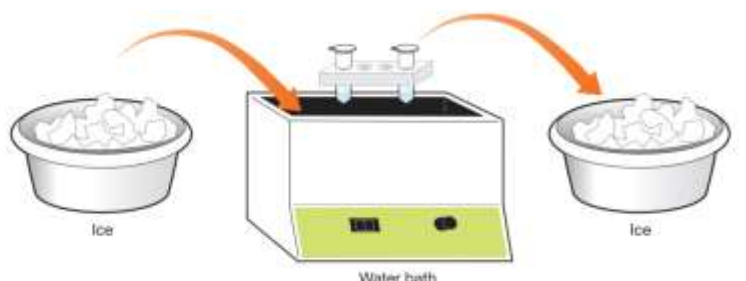


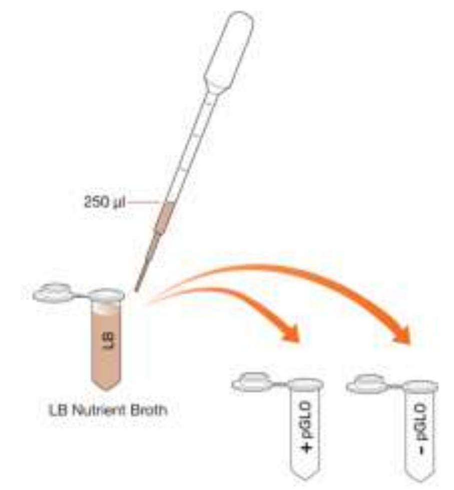


Heat shock

Get your timers ready!

8. Heat shock tubes at 42°C for exactly 50 sec.
9. ***Immediately*** return tubes to ice for 2 min.
10. Transfer your rack and tubes to the benchtop (no more ice)
11. Add 250 µl LB broth to both tubes.
12. Incubate at room temperature for 10 min.





Meanwhile…

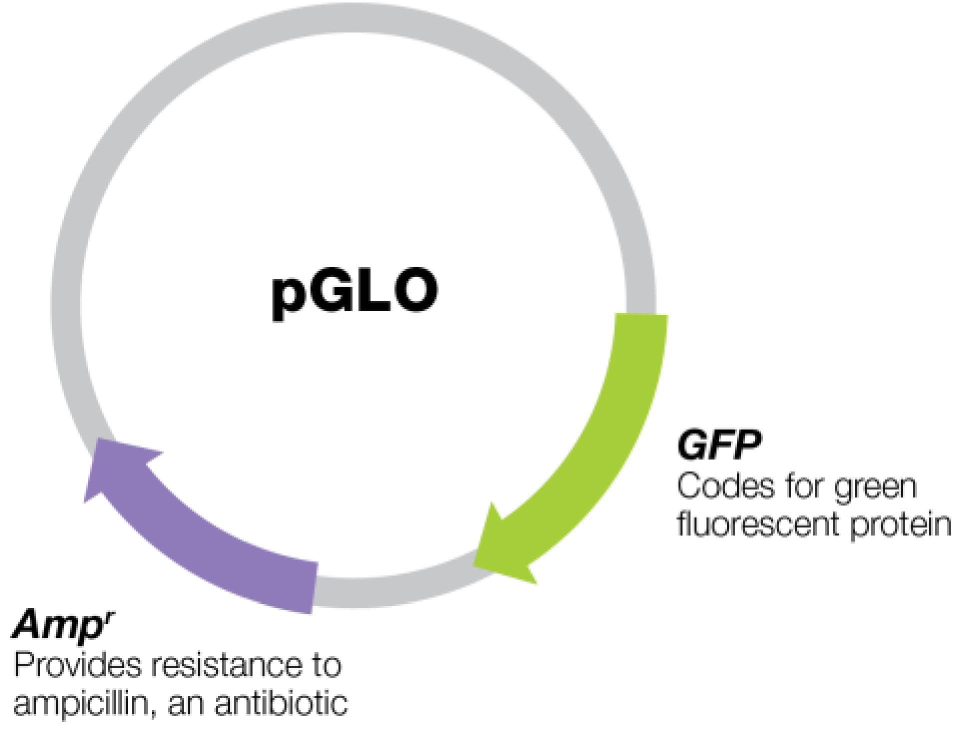
E. coli

Plasmid genes are expressed.

E. coli

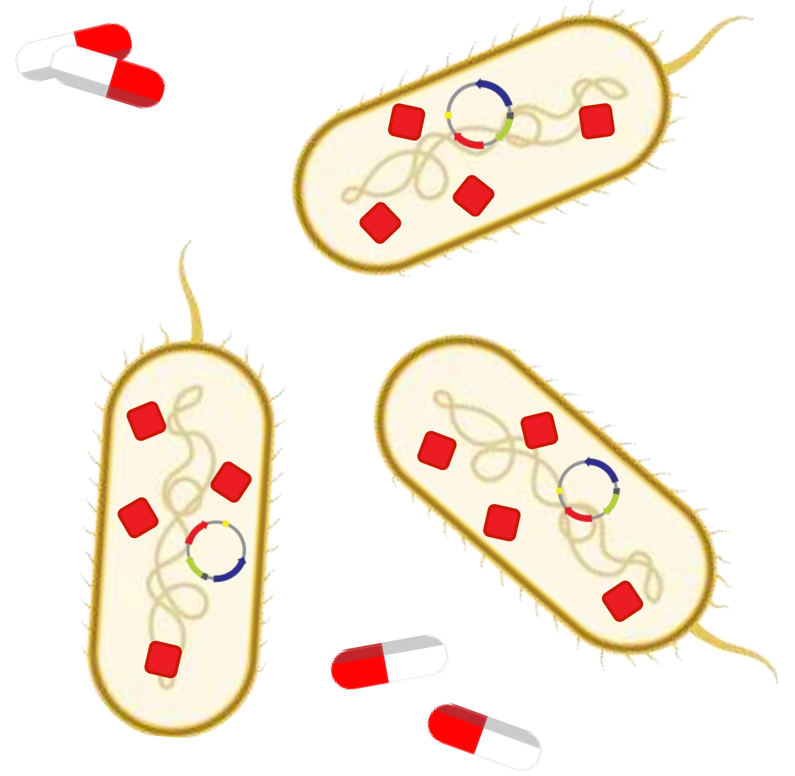
pGLO Plasmid

- What proteins might you expect a bacterial cell to have if it has been transformed with this plasmid?



Beta-lactamase makes *E. coli* resistant to ampicillin

- Transformed bacteria (with the plasmid) will make beta-lactamase ■ , which breaks down ampicillin ⊂■ . This enables them to grow on ampicillin plates
- Bacteria without the plasmid (NOT transformed) cannot grow on plates with ampicillin.

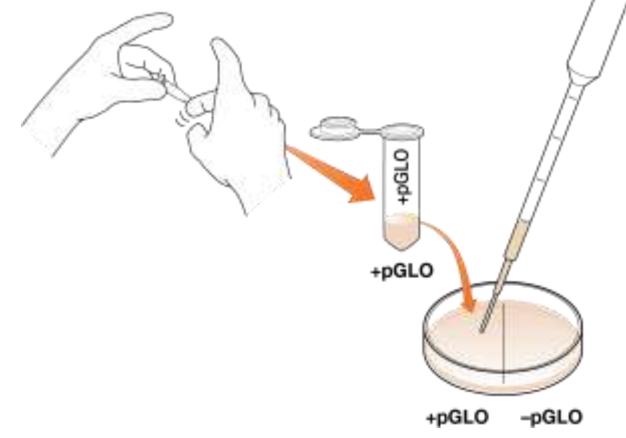


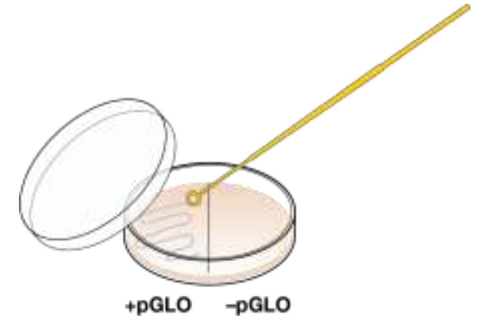
Plating Bacteria

13. Flick tubes to mix.

    Using a new sterile pipet, add one drop of bacteria from **+pGLO** to the appropriate half of each plate.

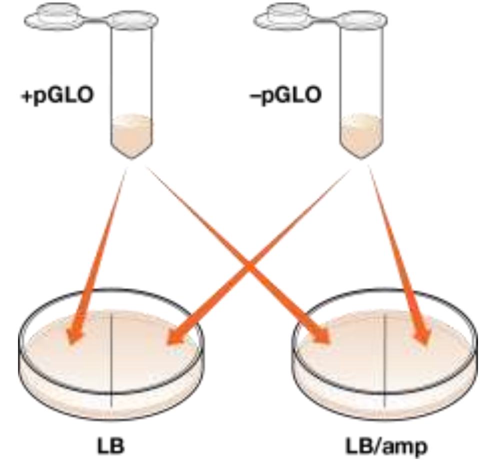
14. Use a loop to spread bacteria evenly.



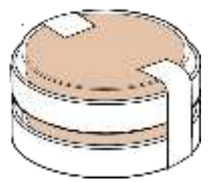


Plating Bacteria

15. Using a new sterile pipet, add one drop of bacteria from **–pGLO** to the appropriate plate.
16. Use a new loop to spread bacteria evenly.



17. Incubate overnight at 37°C or for 2 days at room temperature.



Plates

Components	LB −pGLO	LB +pGLO	LB/amp −pGLO	LB/amp +pGLO
Bacteria	✓	✓	✓	✓
DNA				
Ampicillin				
Grow?				
Glow?				

Appendix

Additional graphics and supplementary slides

Why genetically modify organisms?

- *Modified animal models for research*
- *Cancer, obesity, heart disease, etc.*

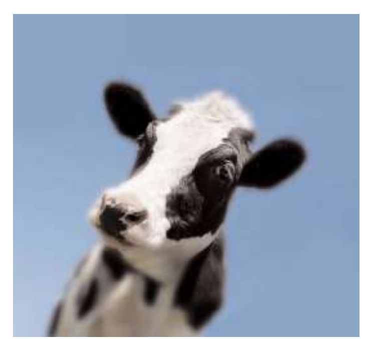
- *Modified mosquitoes to fight disease*

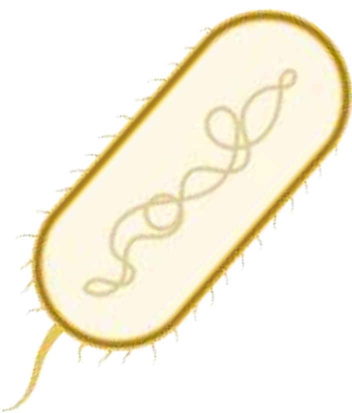
- *Disease/drought/pest resistance.*
- *Increased nutrition*

- *Drug production like insulin, hormones, vaccines, and anti-cancer drugs.*

Brief history of insulin

- 1922 – Canadian researchers isolate insulin, cure diabetics using bovine insulin, and win the Nobel Prize in 1923. Previously, diabetes had been a virtual death sentence – there was no treatment.
- 1978 – scientists at Genentech produce human insulin using genetically engineered E. coli (recombinant DNA, or rDNA).
- 1982 – Humulin approved by the FDA.



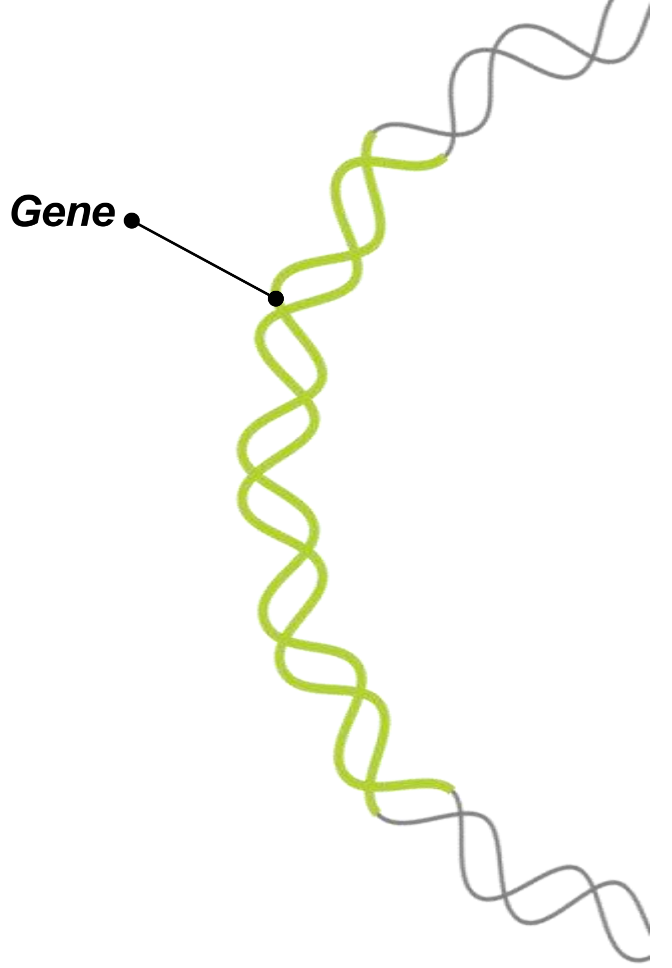


The protein products of biotech

	Used to treat	Made in	Price per gram
Gold	N/A	N/A	$40
Insulin	Diabetes	*E. coli*	$60
Human Growth Hormone	Growth disorders	*E. coli*	$227,000
Granulocyte Colony Stimulating Factor	Cancers	*E. coli*	$1,357,000

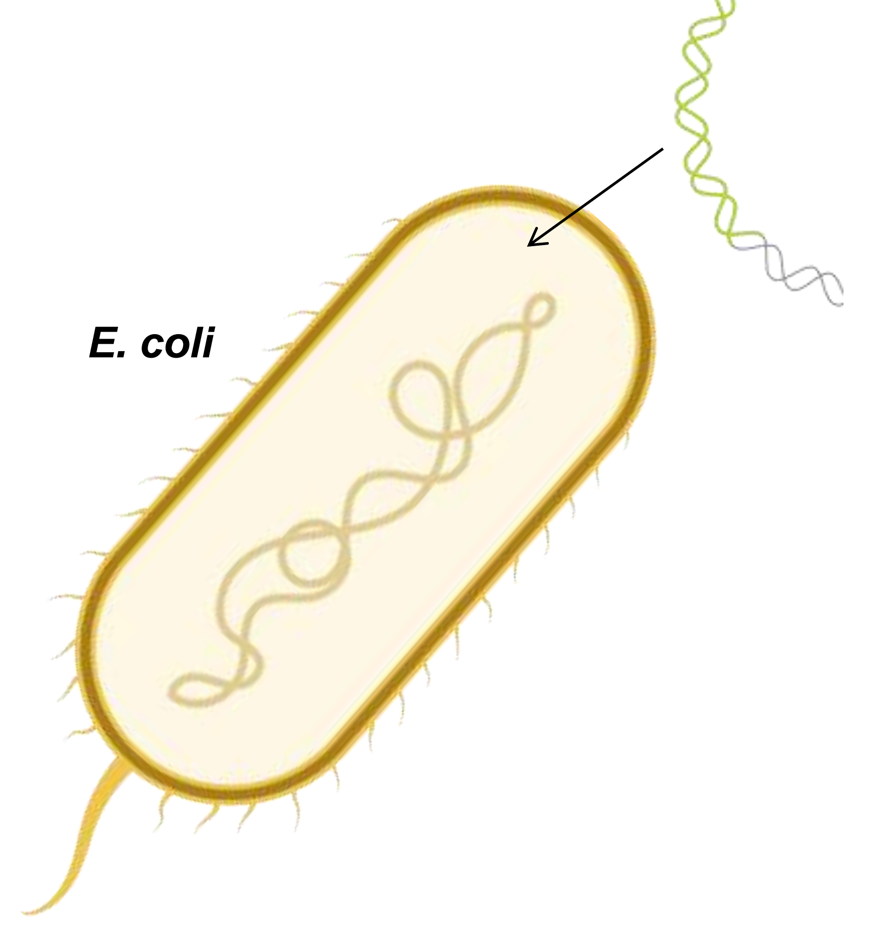
How can we make LOTS of protein?

1. Identify a gene for a protein.



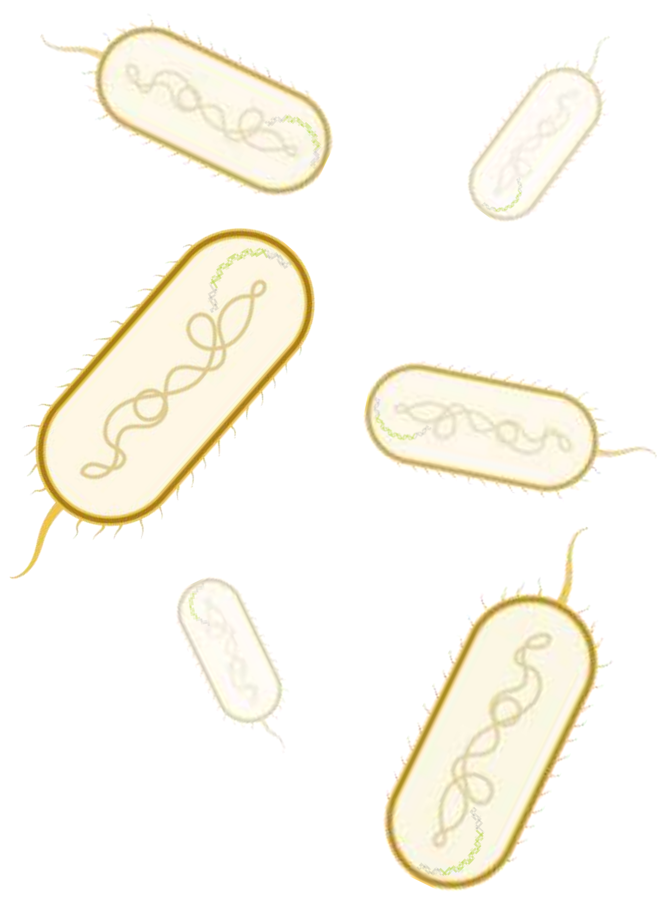
How can we make LOTS of protein?

1. Identify a gene for a protein.
2. Put the gene into bacteria.



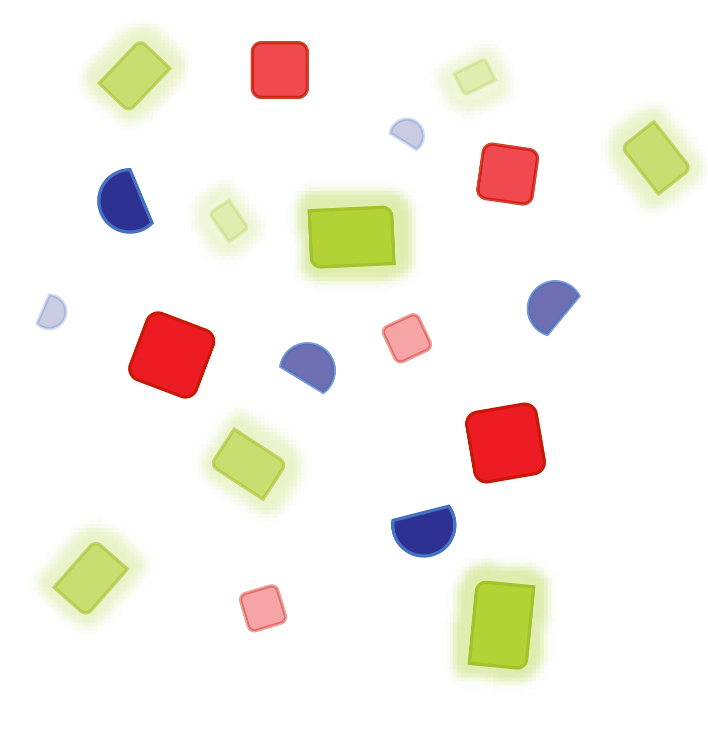
How can we make LOTS of protein?

1. Identify a gene for a protein.
2. Put the gene into bacteria.
3. Grow lots of the bacteria.



How can we make LOTS of protein?

1. Identify a gene for a protein.
2. Put the gene into bacteria.
3. Grow lots of the bacteria.
4. The bacteria transcribe and translate the gene — mini protein factories!



How can we make LOTS of protein?

1. Identify a gene for a protein.
2. Put the gene into bacteria.
3. Grow lots of the bacteria.
4. The bacteria transcribe and translate the gene — mini protein factories!
5. Purify the protein.

Beta-lactamase

E. coli

Beta-lactamase, ampicillin resistance

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AraC

E. coli

AraC, regulatory protein

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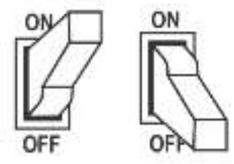
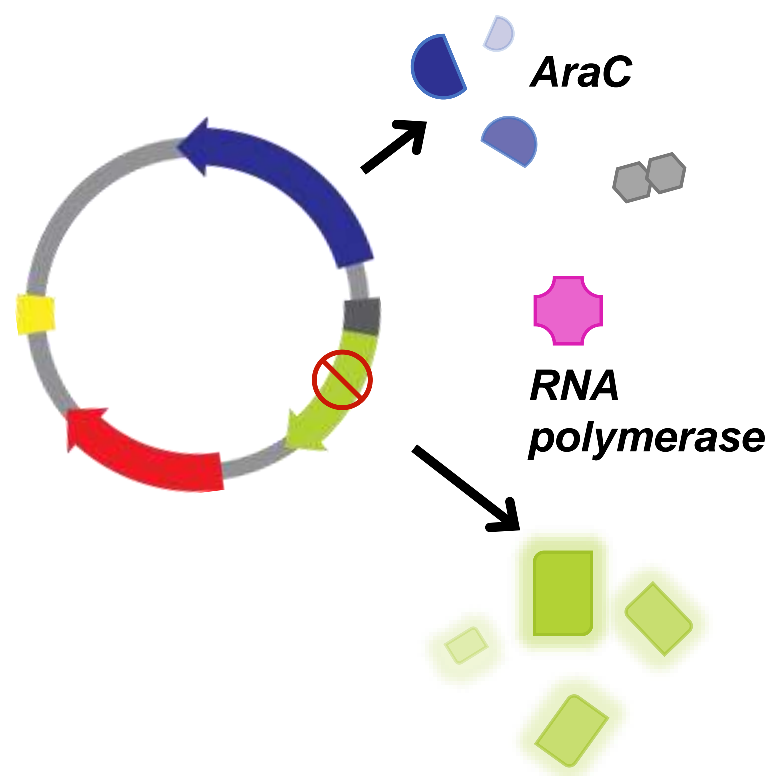
AraC Controls Expression of GFP

- Arabinose (a sugar) works like a switch.



- ***Without*** arabinose, the switch is OFF. AraC blocks RNA polymerase, and the GFP gene is not transcribed.
- ***With*** arabinose, the switch is ON. AraC changes shape and RNA transcribes the *GFP* gene.



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Green Fluorescent Protein (GFP)

E. coli

GFP, only if arabinose is in the media

Extra graphics