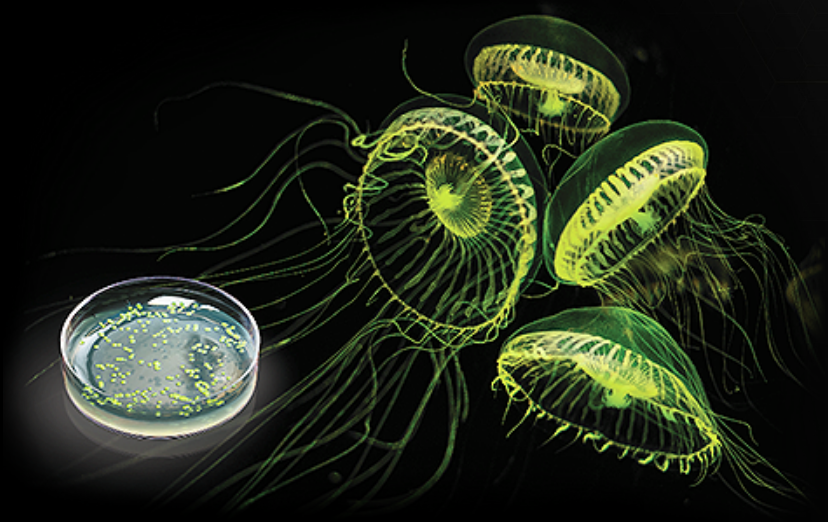
BIO-RAD Explorer

BIO-RAD

pGLO Bacterial Transformation



Student presentation for use with the pGLO Bacterial Transformation Kit

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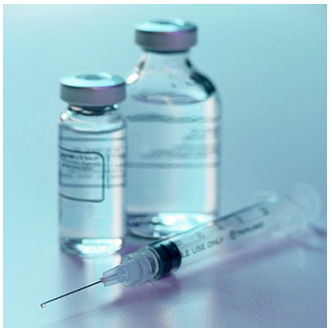
Why genetically modify organisms?

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

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

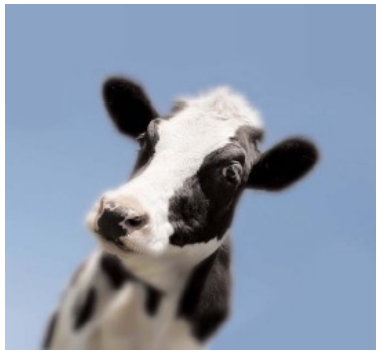
- *Modified mosquitoes to fight disease*

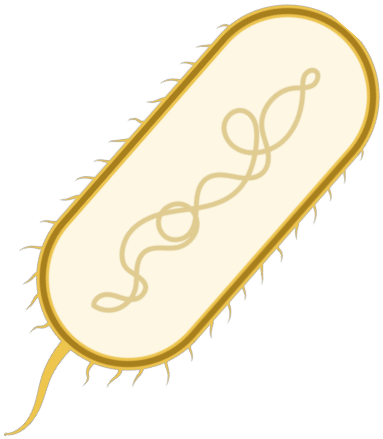


- *Drug production like insulin, hormones, vaccines, and anti-cancer drugs.*

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.



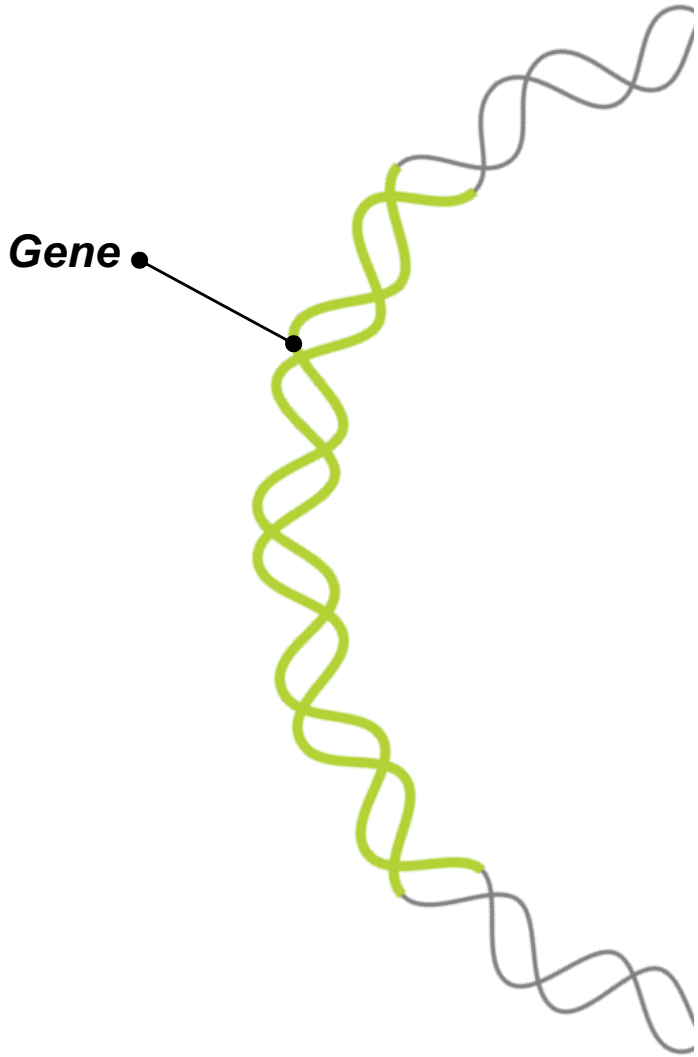


The protein products of biotech

	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

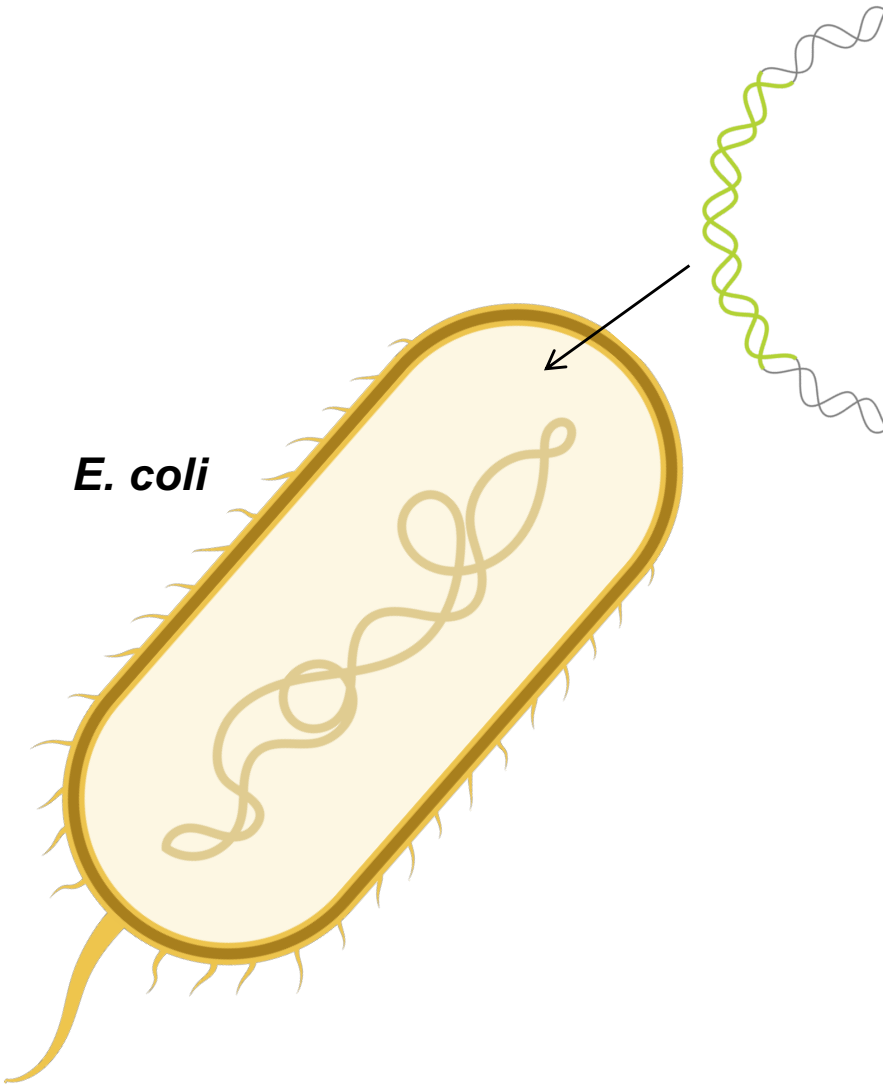
How can we make LOTS of protein?

1. Identify a gene for a protein.



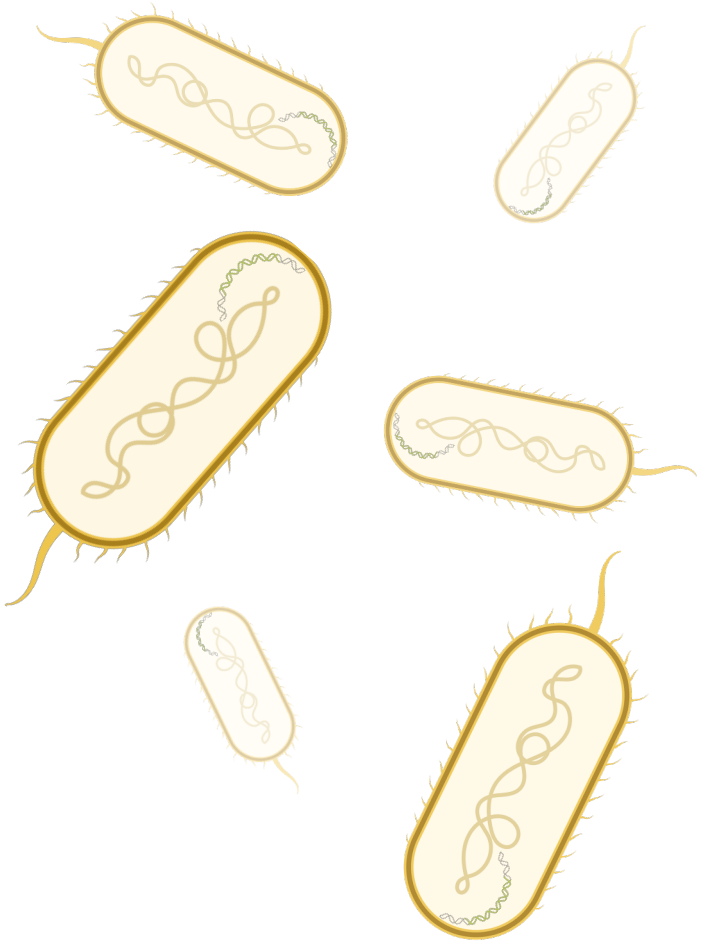
How can we make LOTS of protein?

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



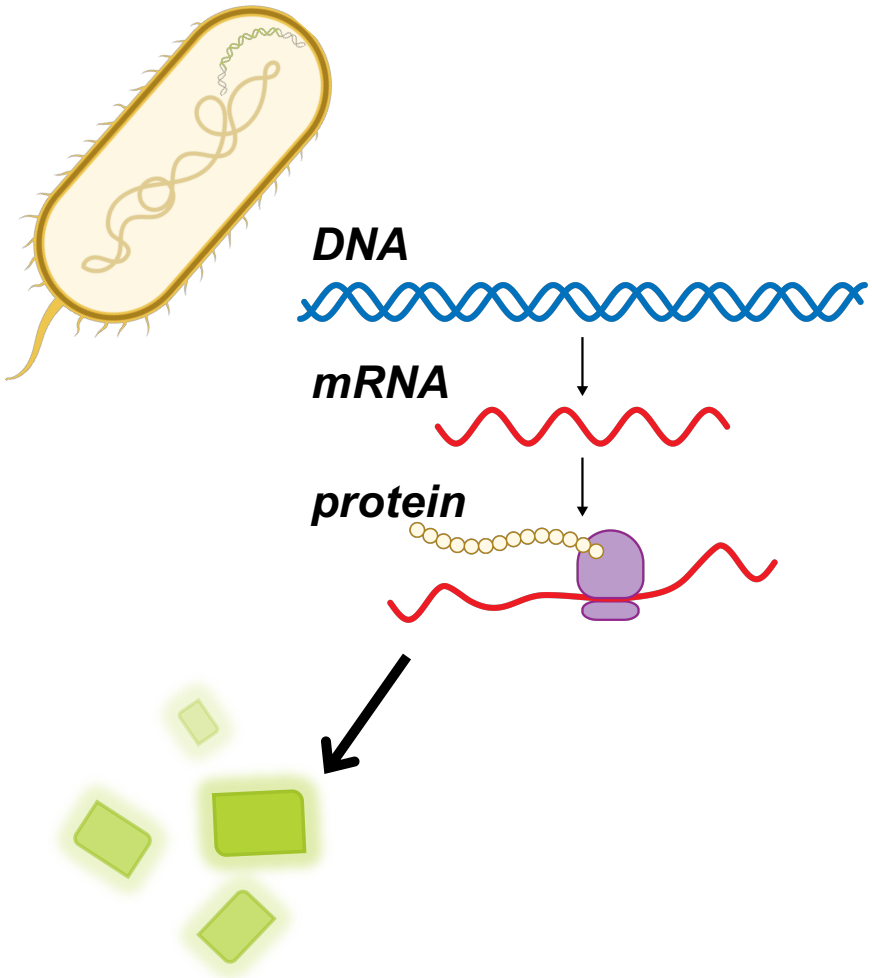
How can we make LOTS of protein?

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!



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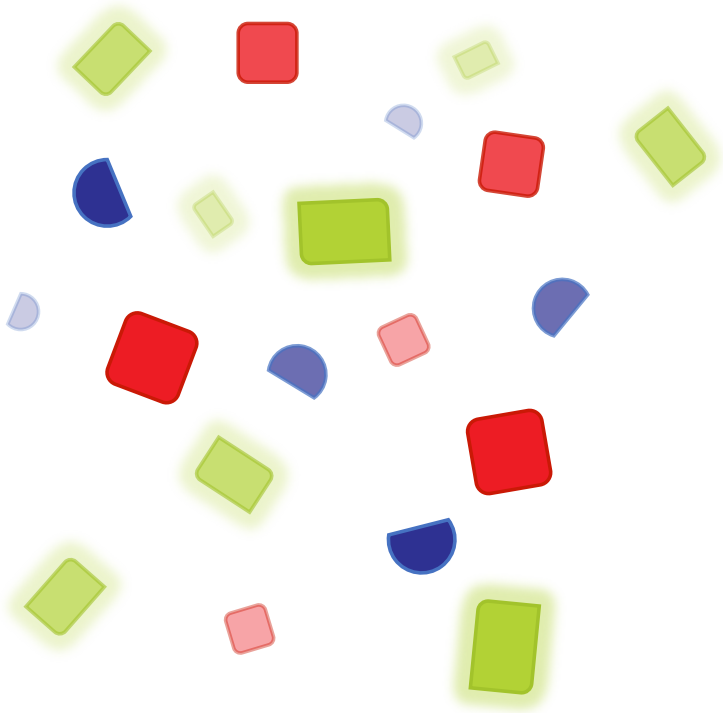
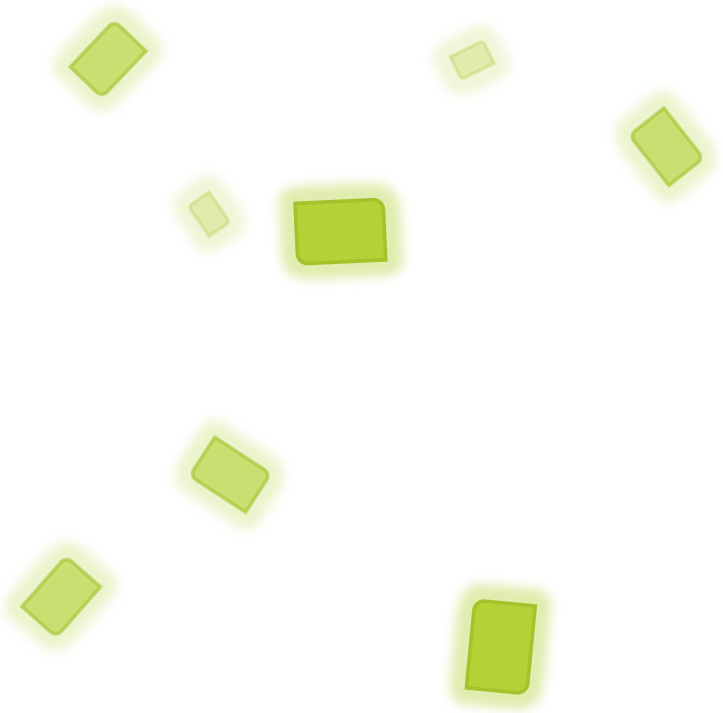


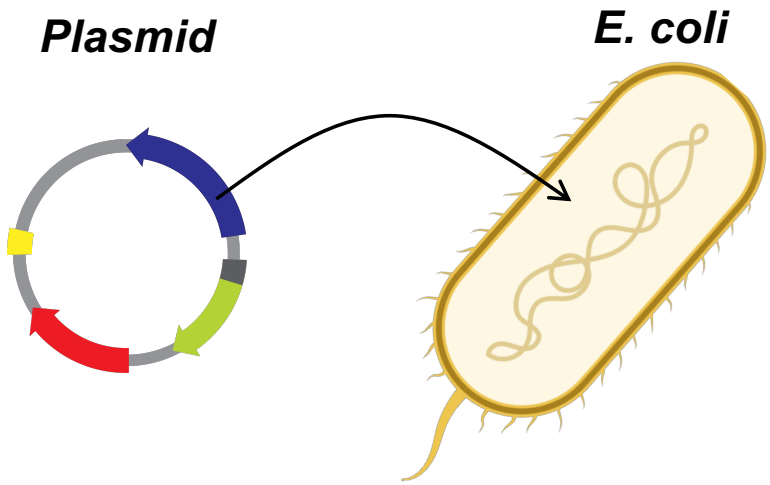
How can we make LOTS of protein?

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.



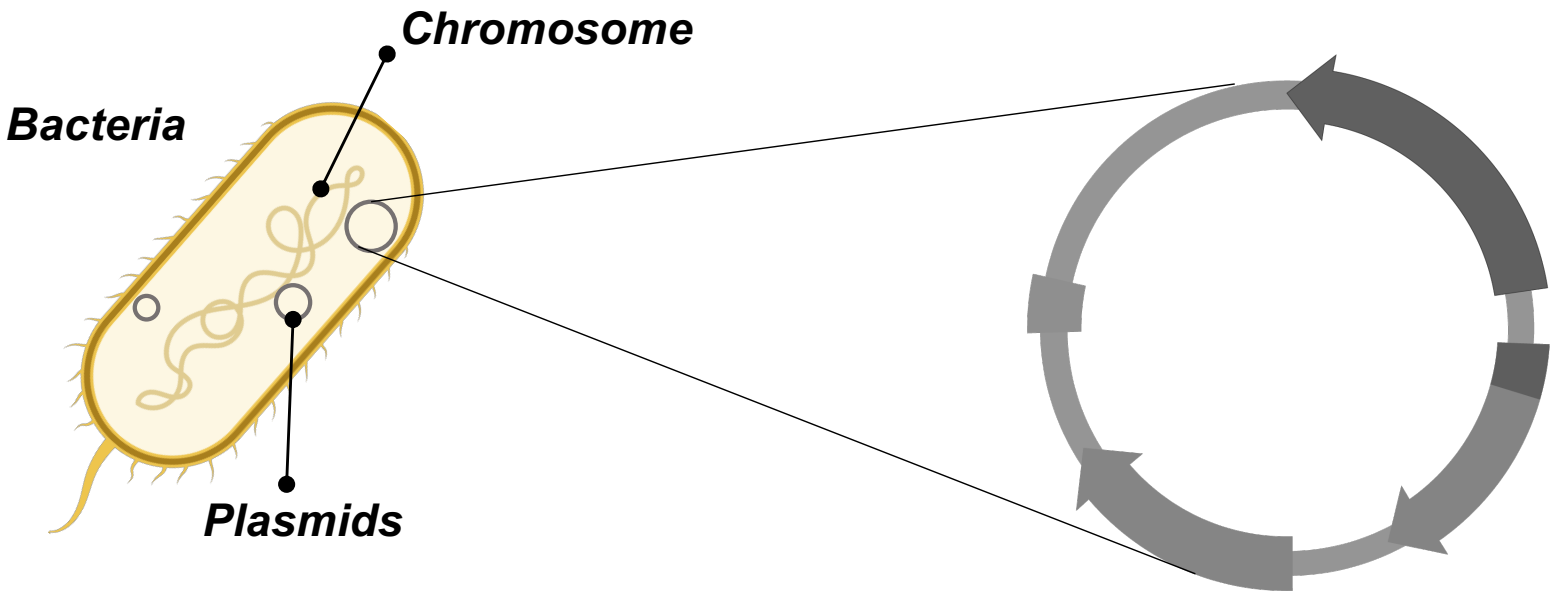
How do you get genes into bacteria?

1. Make a plasmid with your gene.
2. Do bacterial transformation. This is what you'll do in this activity.



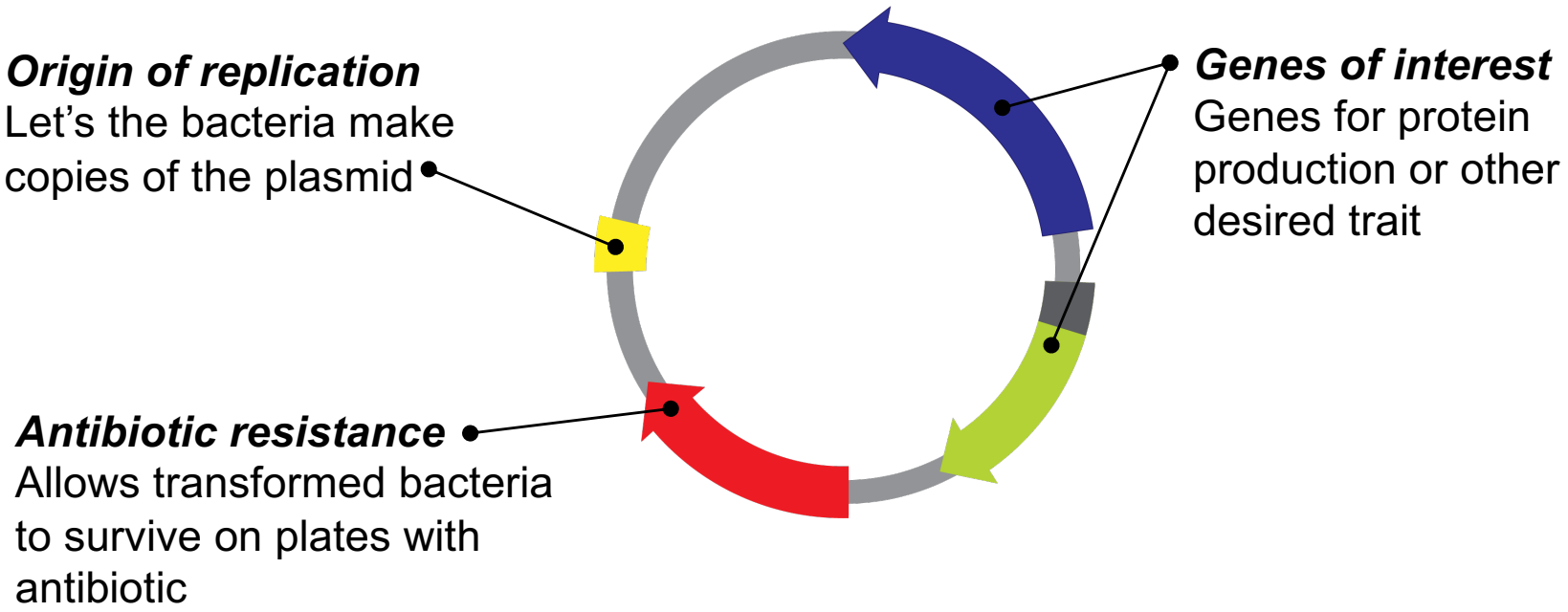
Genetic engineering using plasmids

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

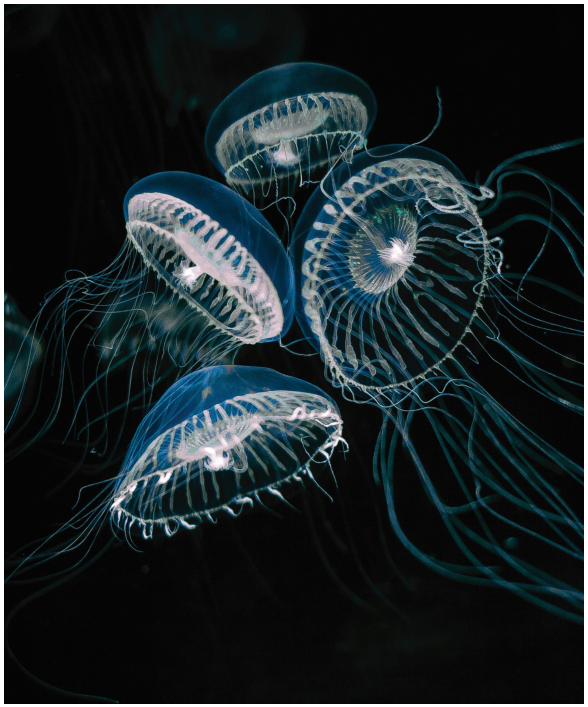


Genetic engineering using plasmids

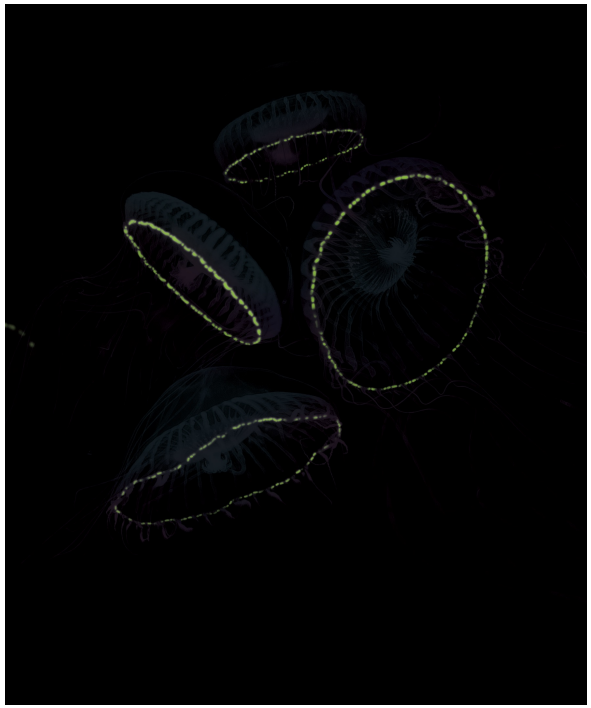
- Scientists can modify or engineer plasmids for specific purposes.



Green fluorescent protein



Under visible light

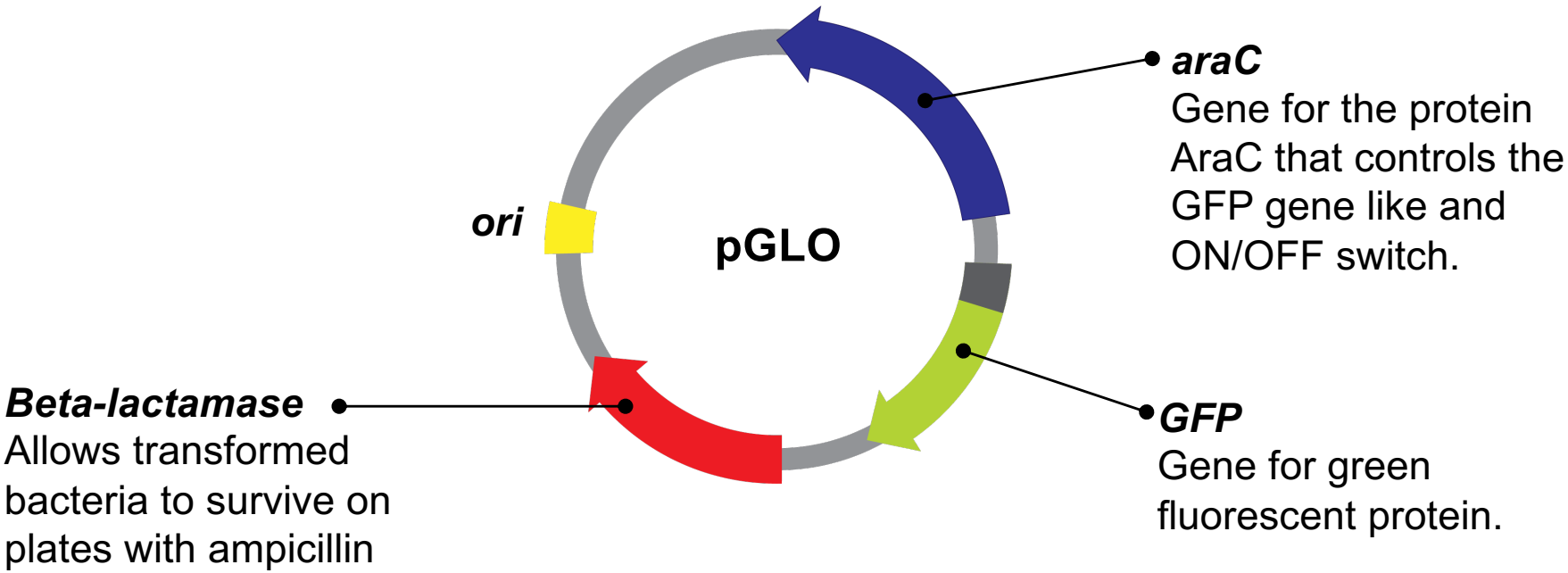


Under ultraviolet (UV) light

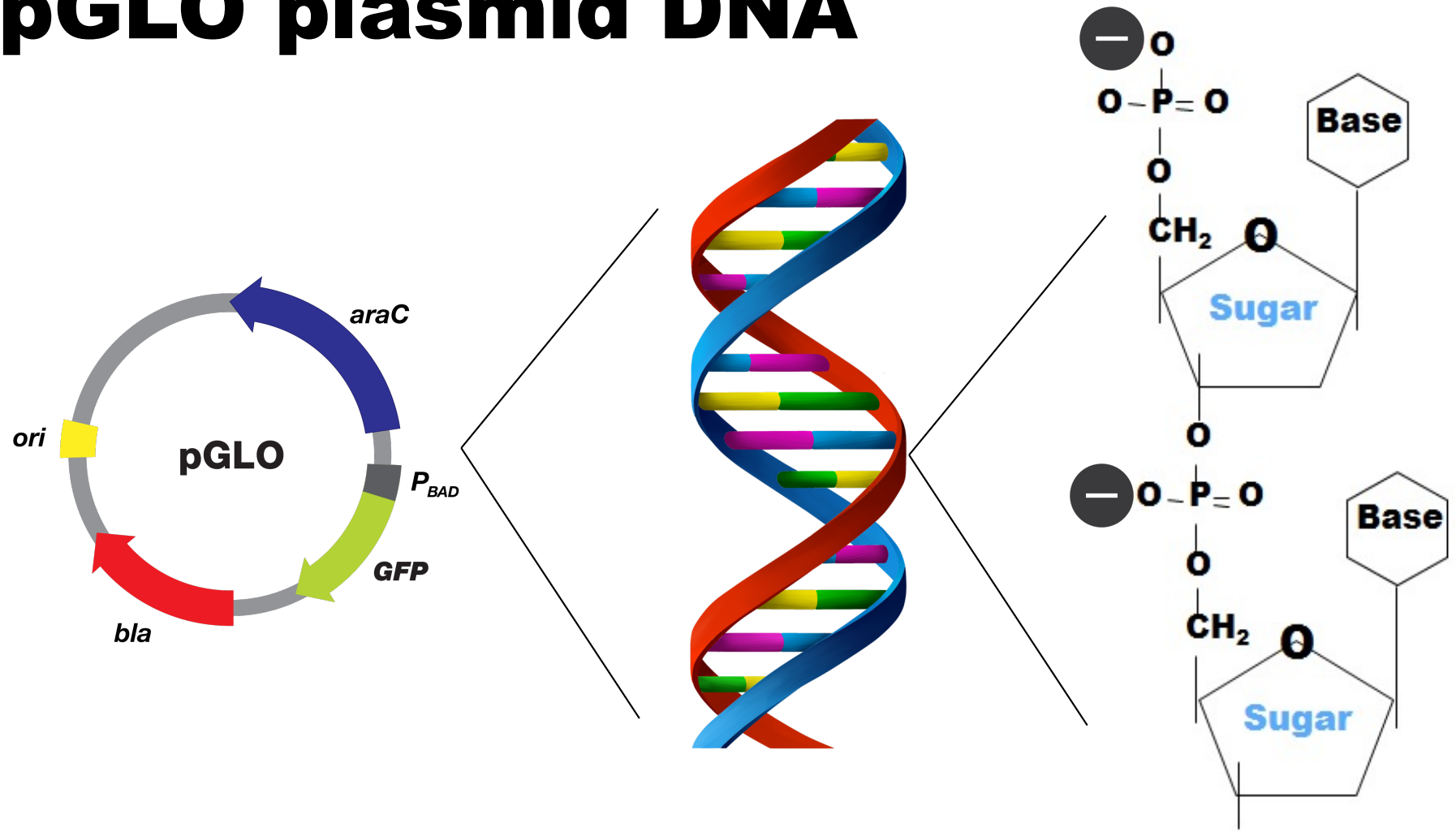
The jellyfish *Aequorea Victoria* has a gene for green fluorescent protein which glows green under UV light.

pGLO plasmid

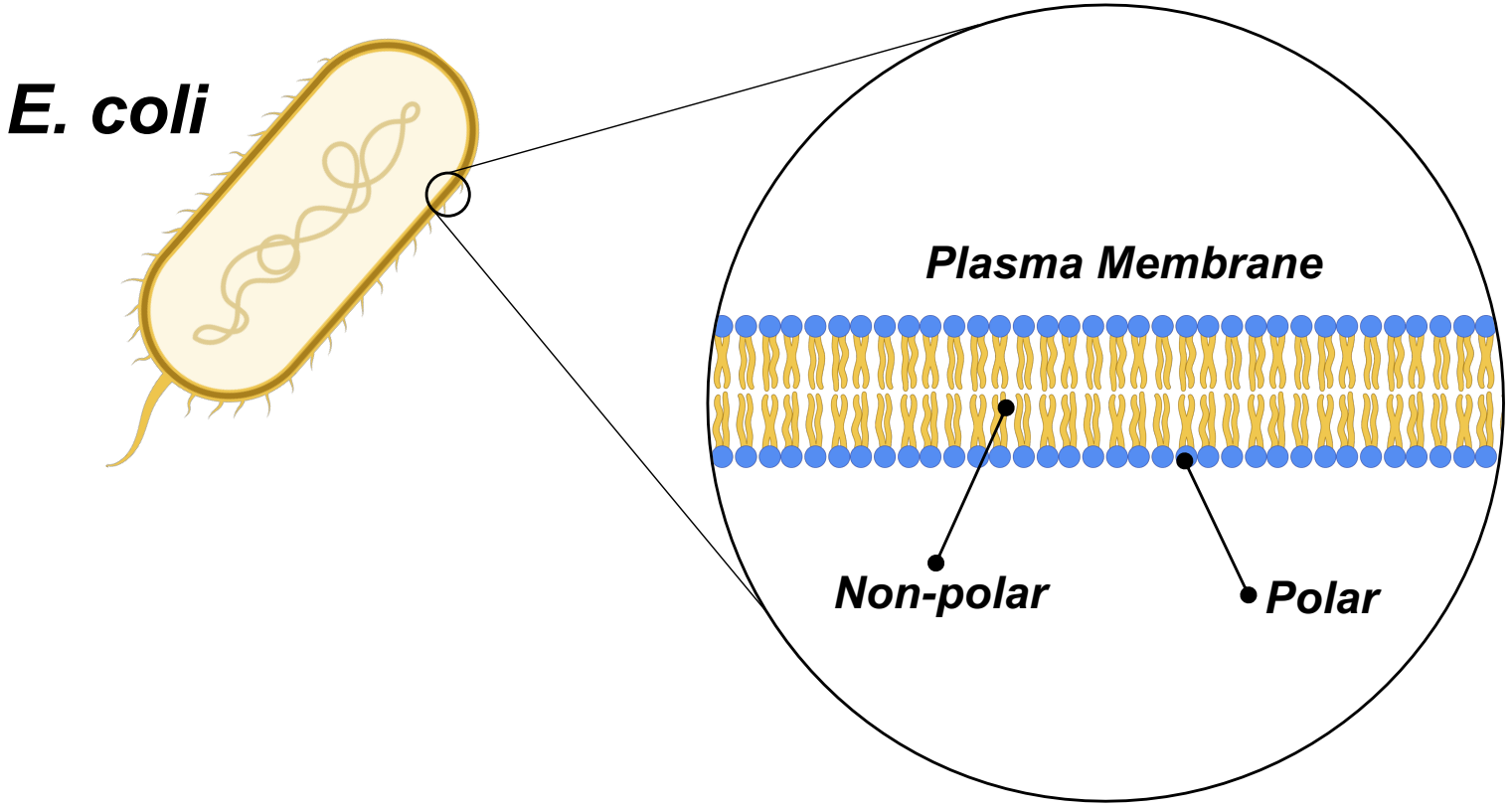
- The pGLO plasmid is engineered to have the *GFP* gene from *Aequorea victoria*.



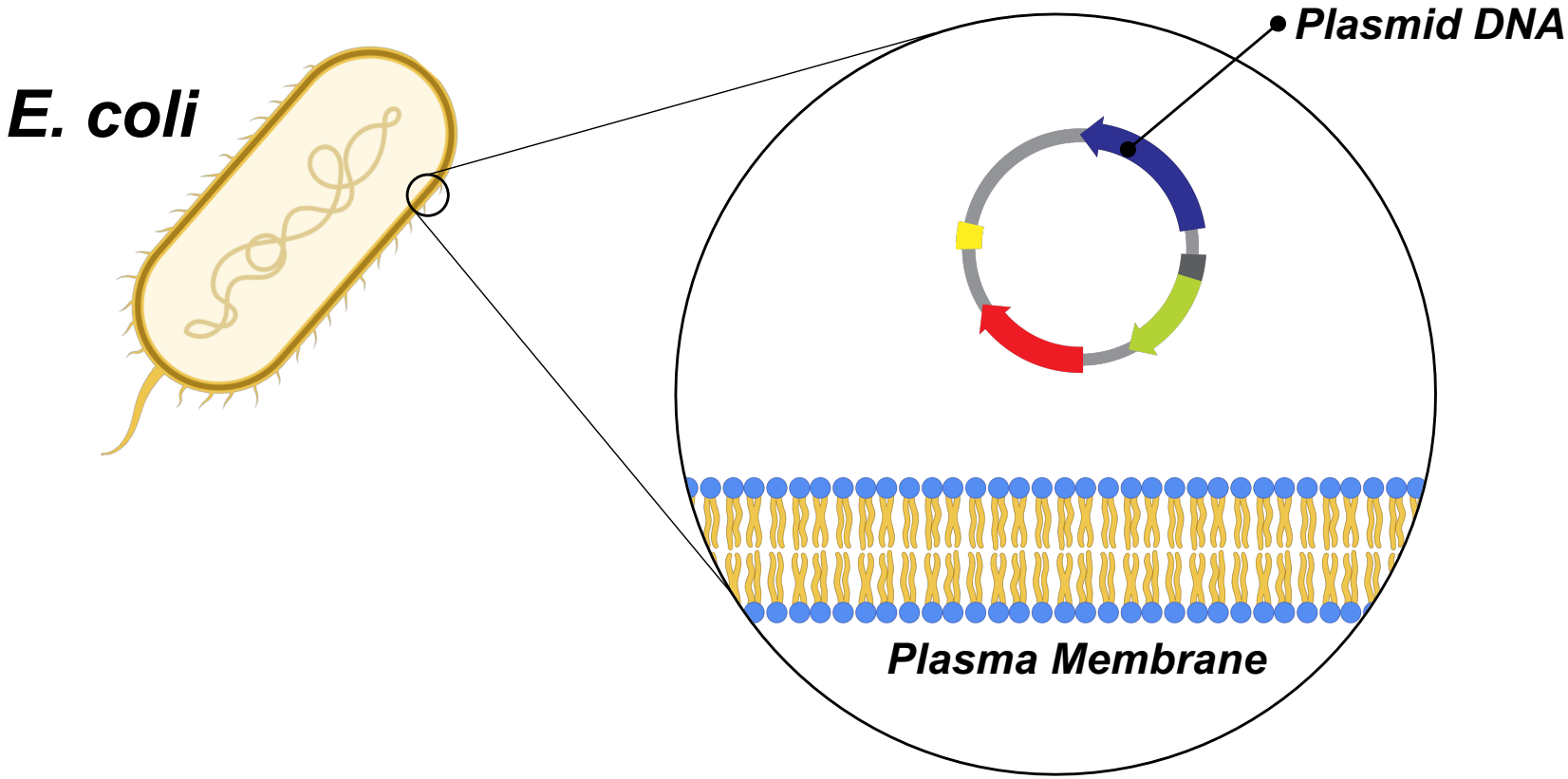
pGLO plasmid DNA



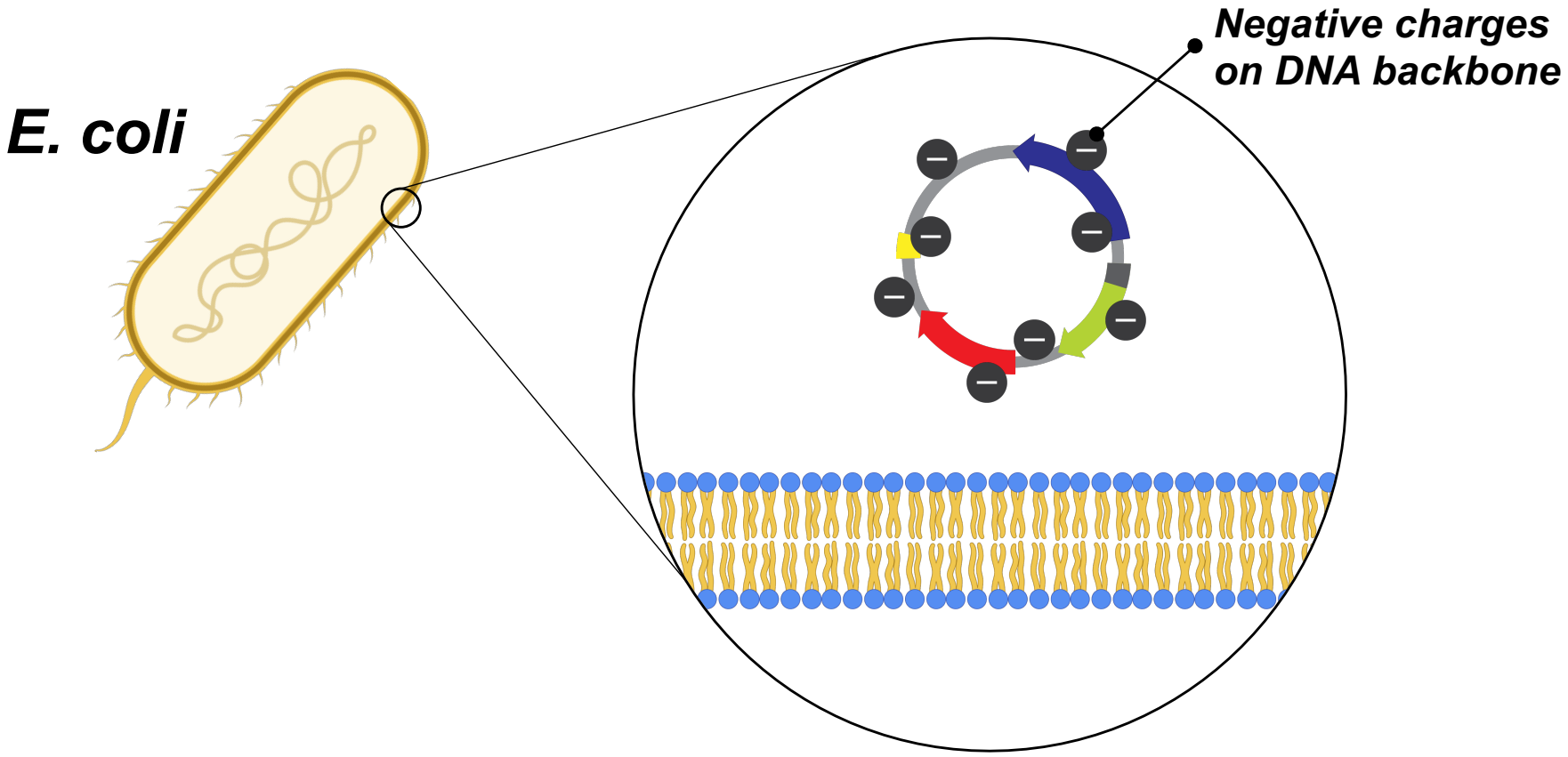
Bacterial Membrane



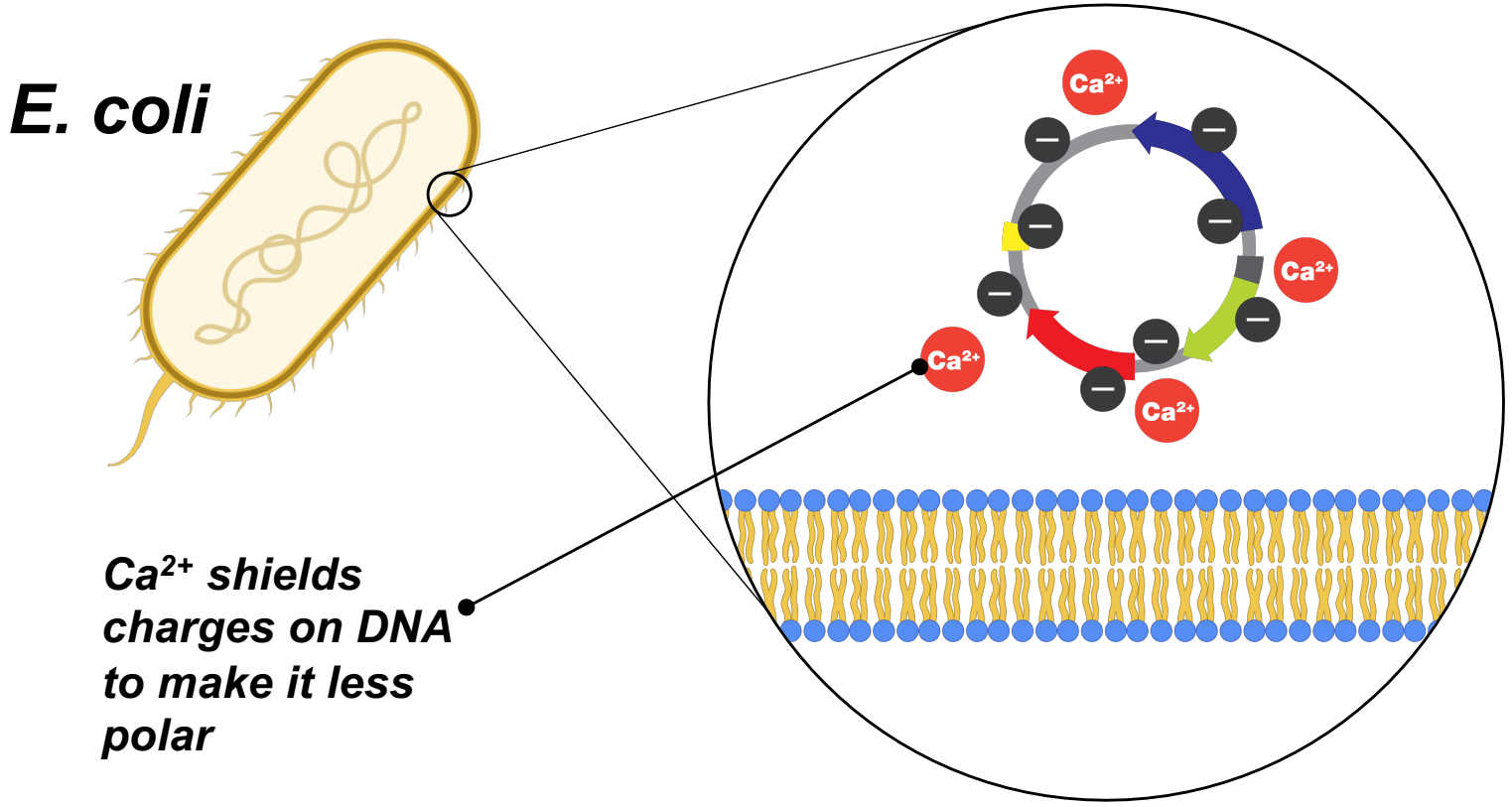
Add plasmid



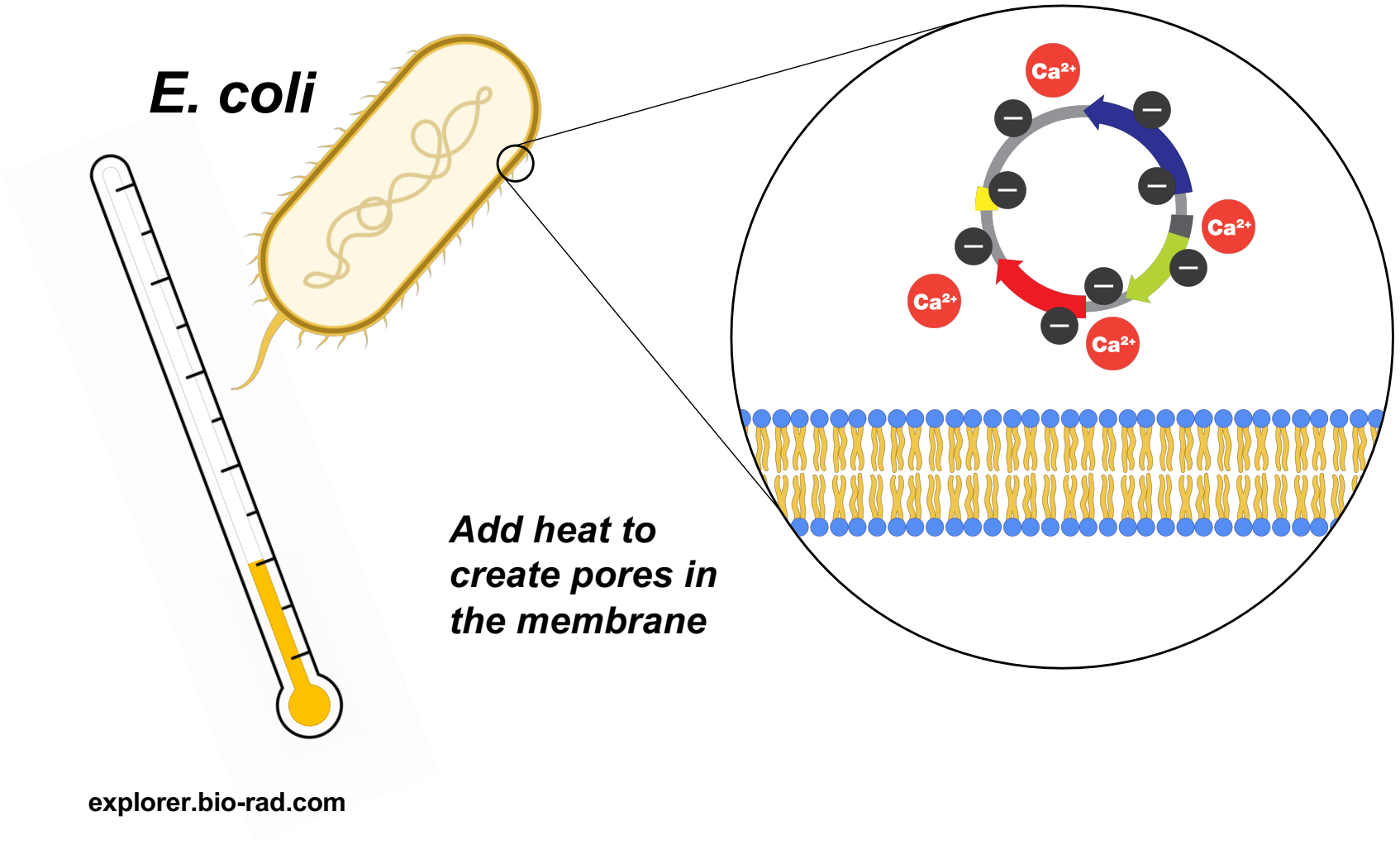
Add plasmid



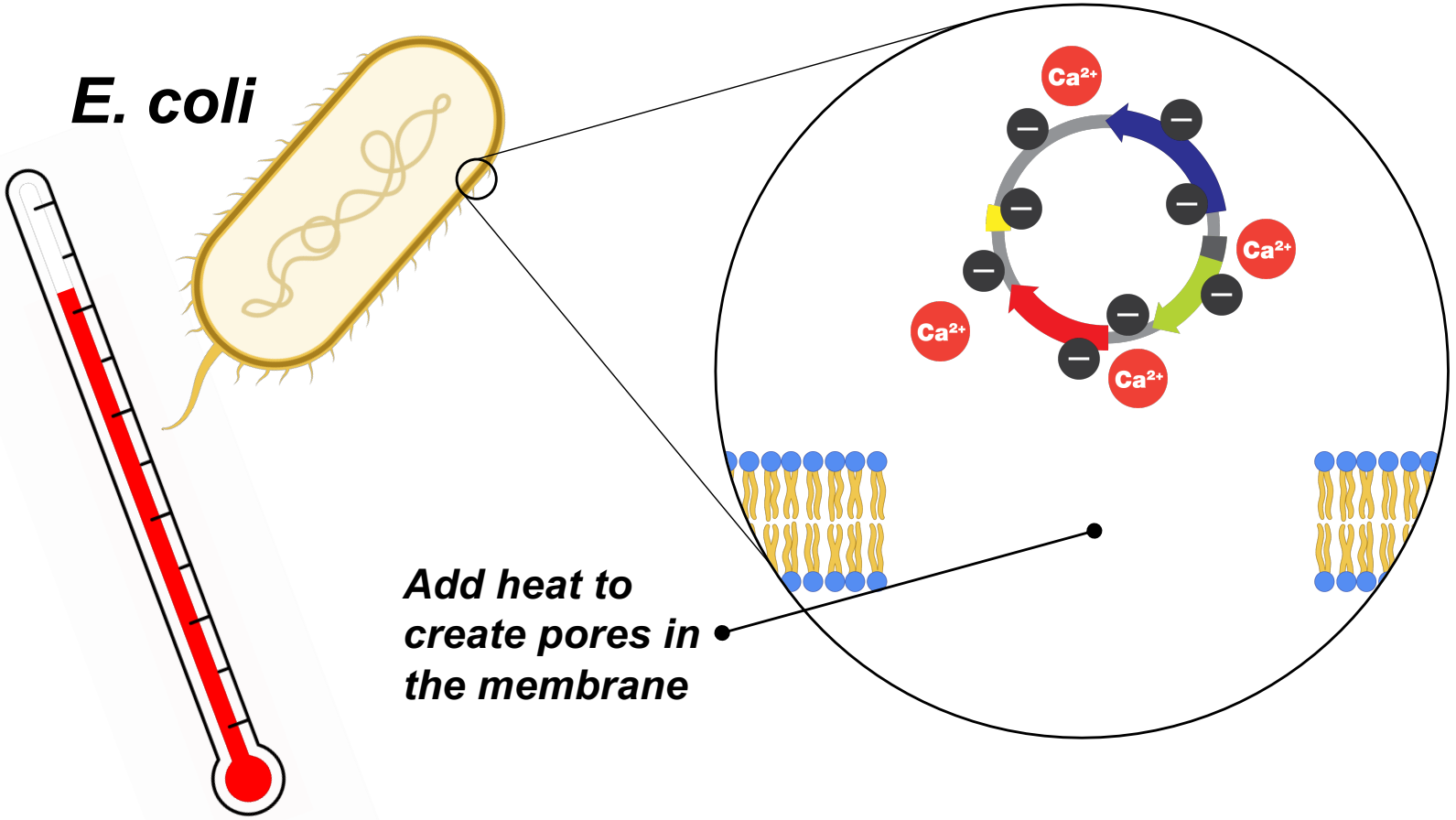
Add transformation solution ($CaCl_2$)



Heat Shock


E. coli
Ca2+
Ca2+
Ca2+
Ca2+
Add heat to create pores in the membrane

Heat Shock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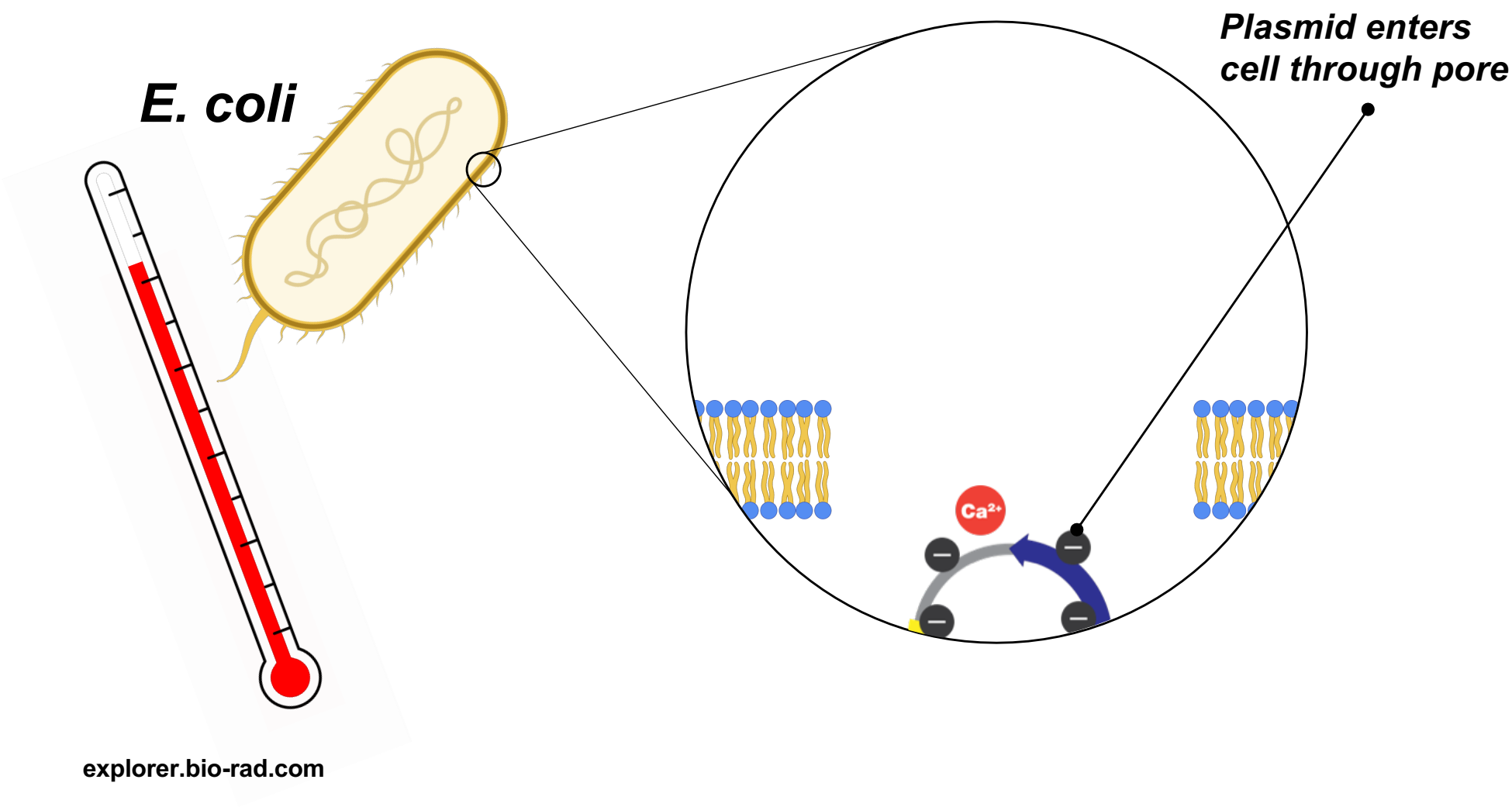

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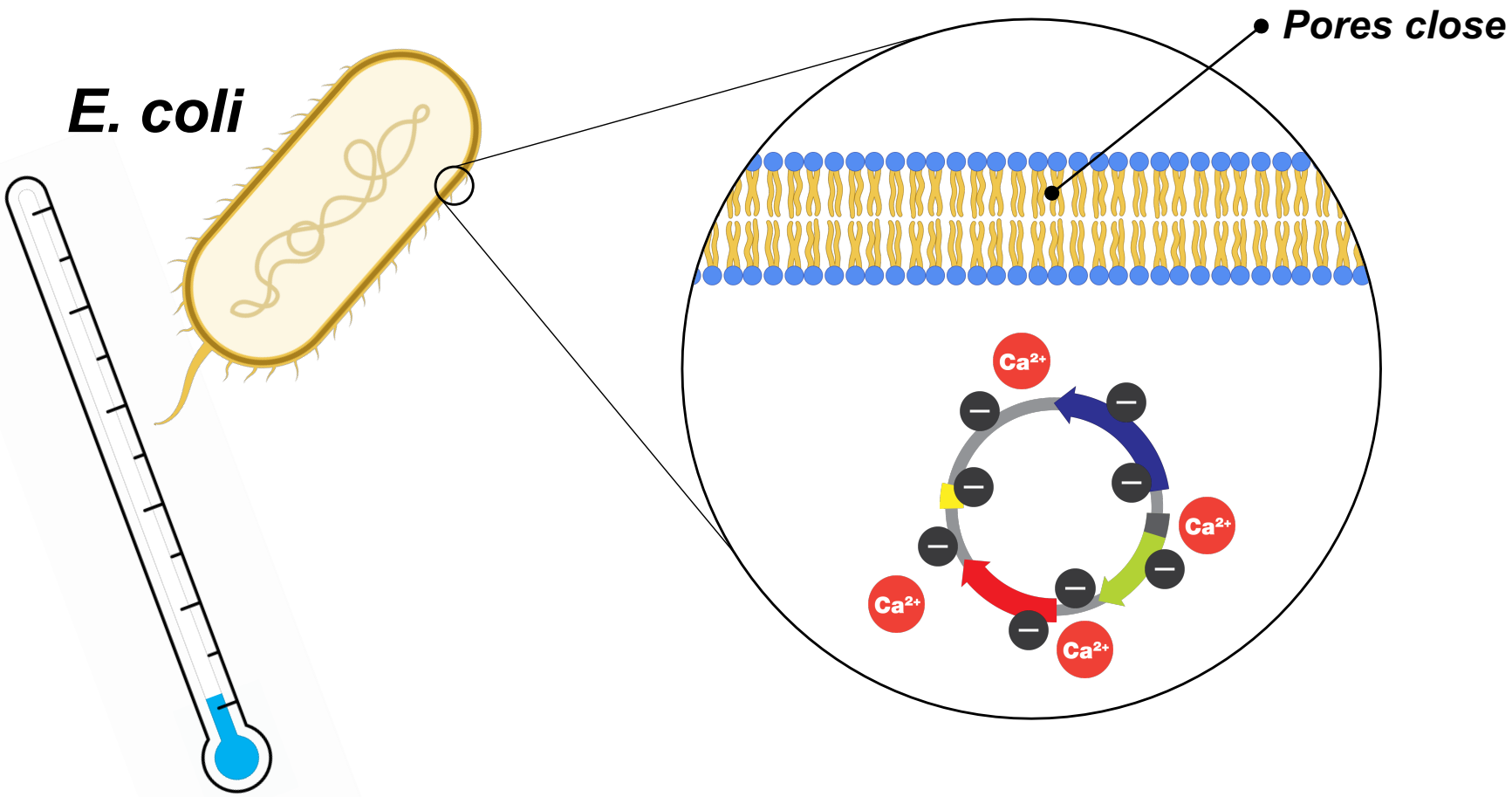
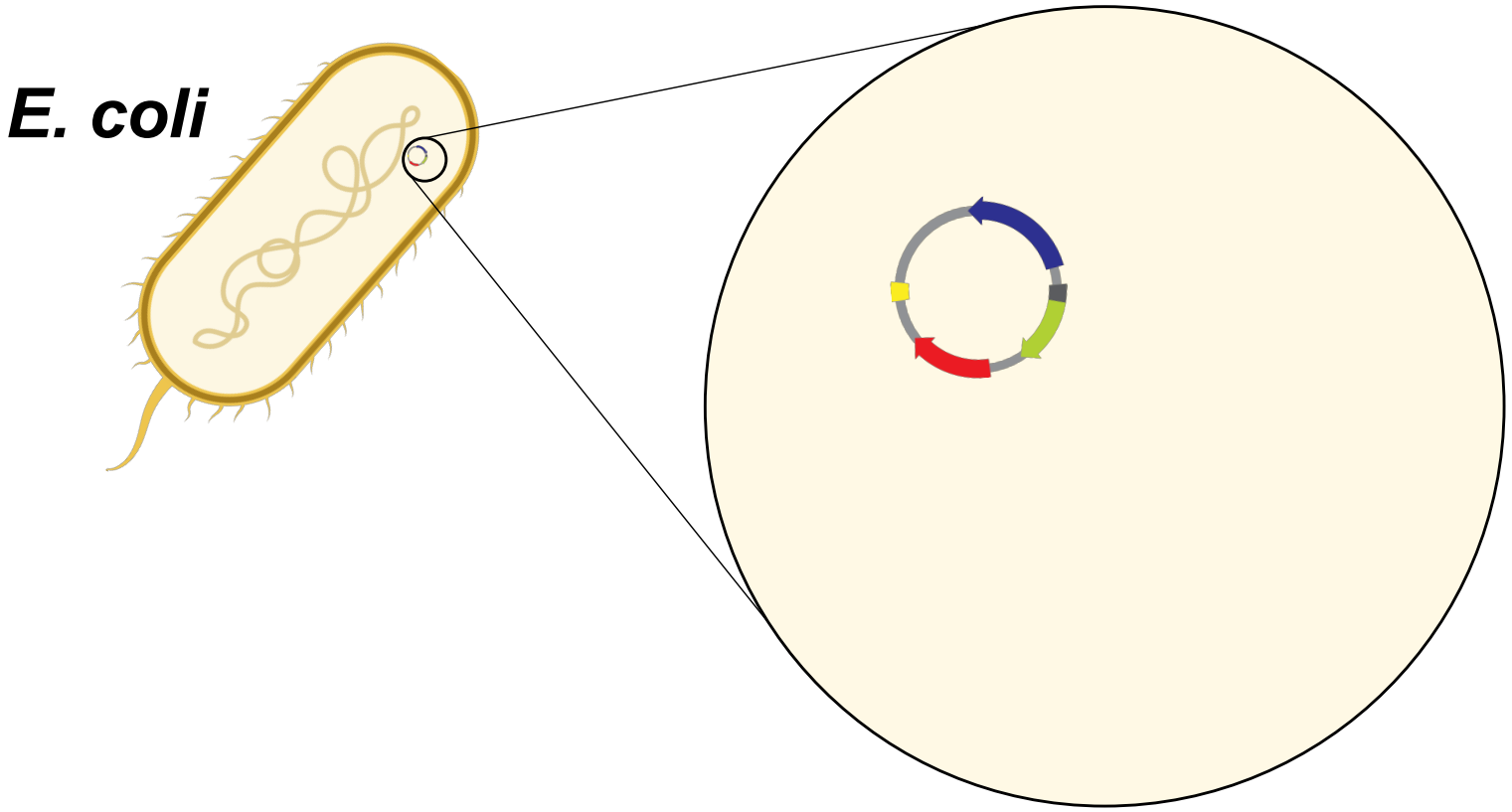
Recovery on ice, 2 min



Add LB broth, allow gene expression, 10 min



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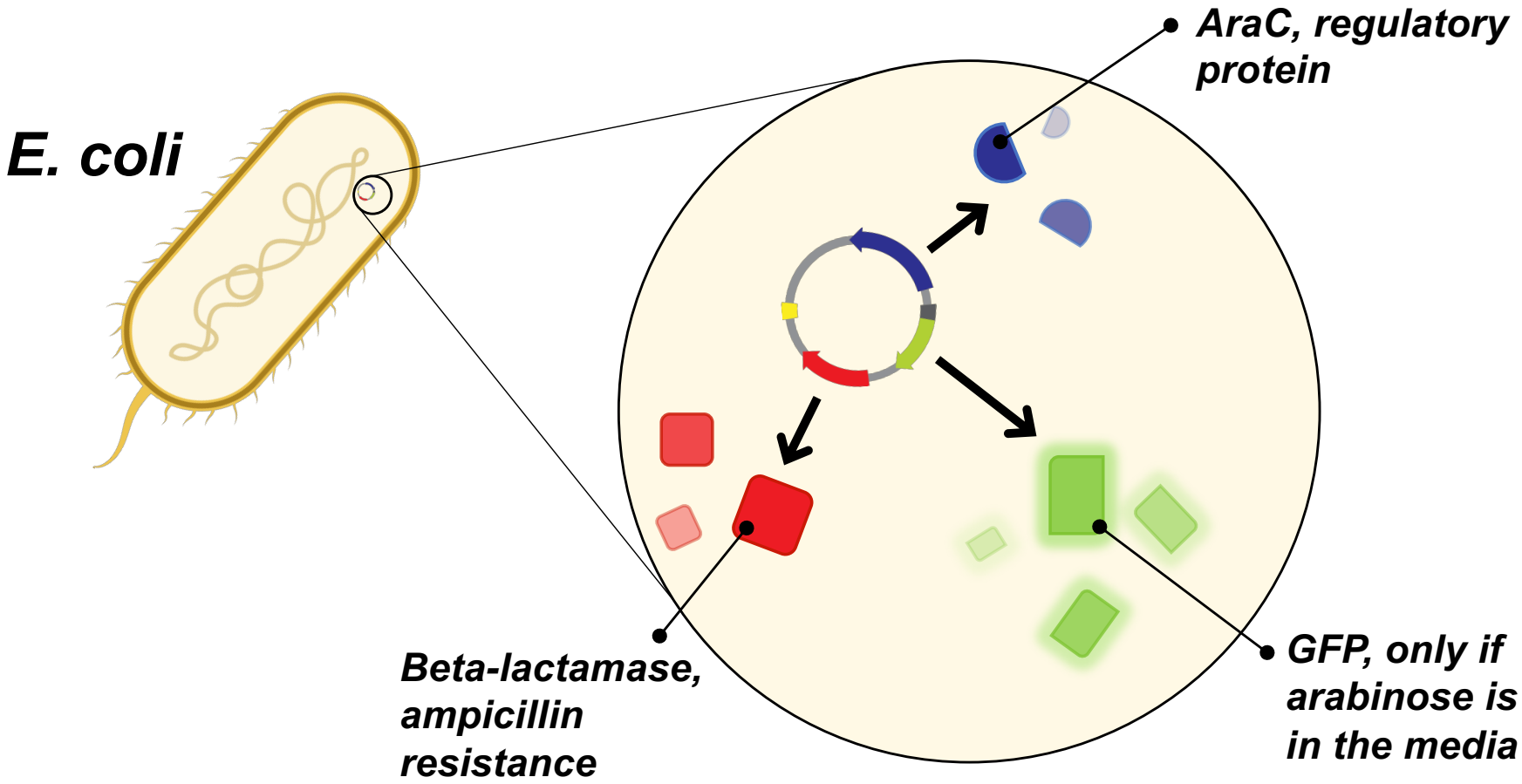
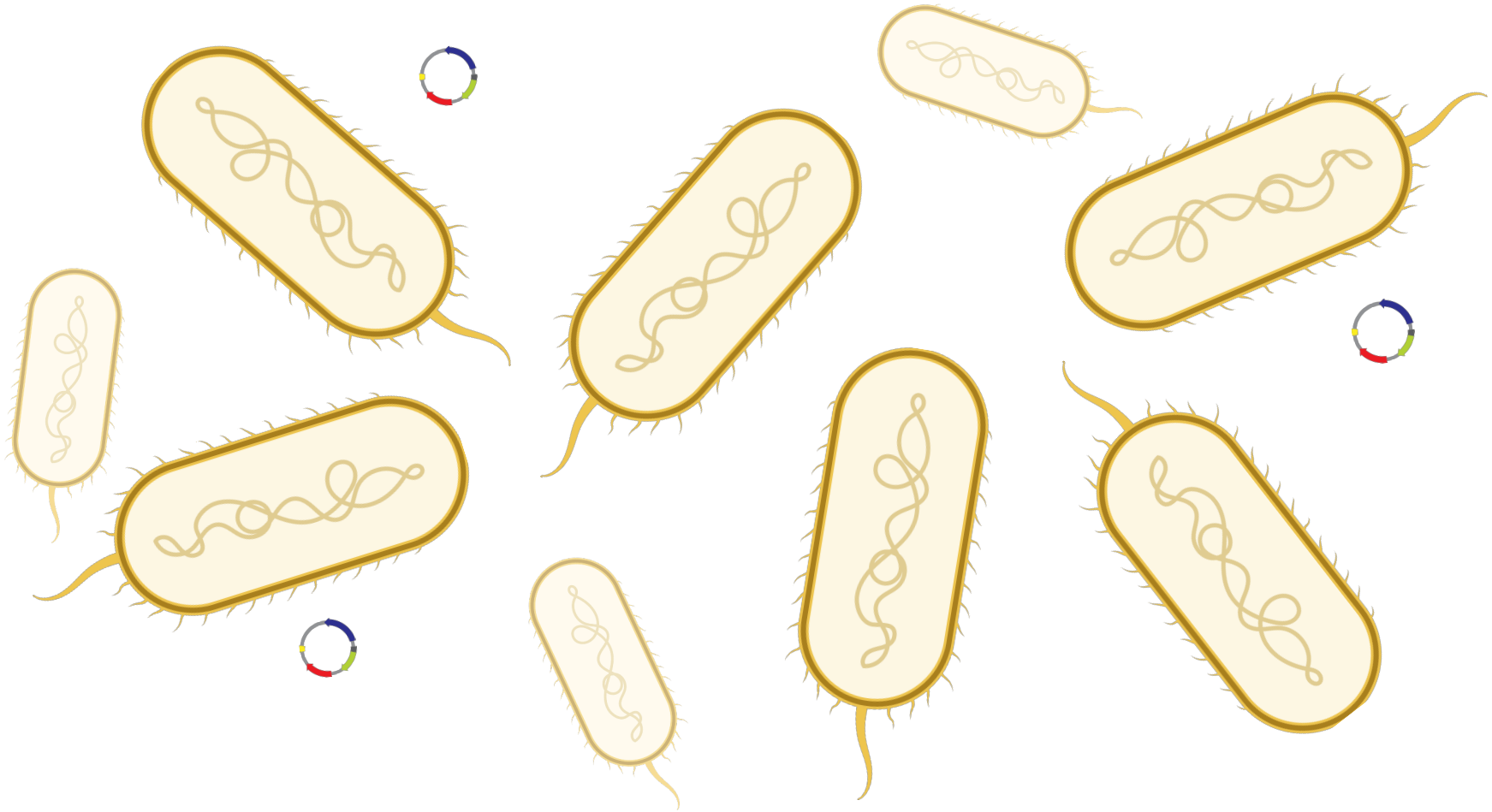


Selective media – ampicillin



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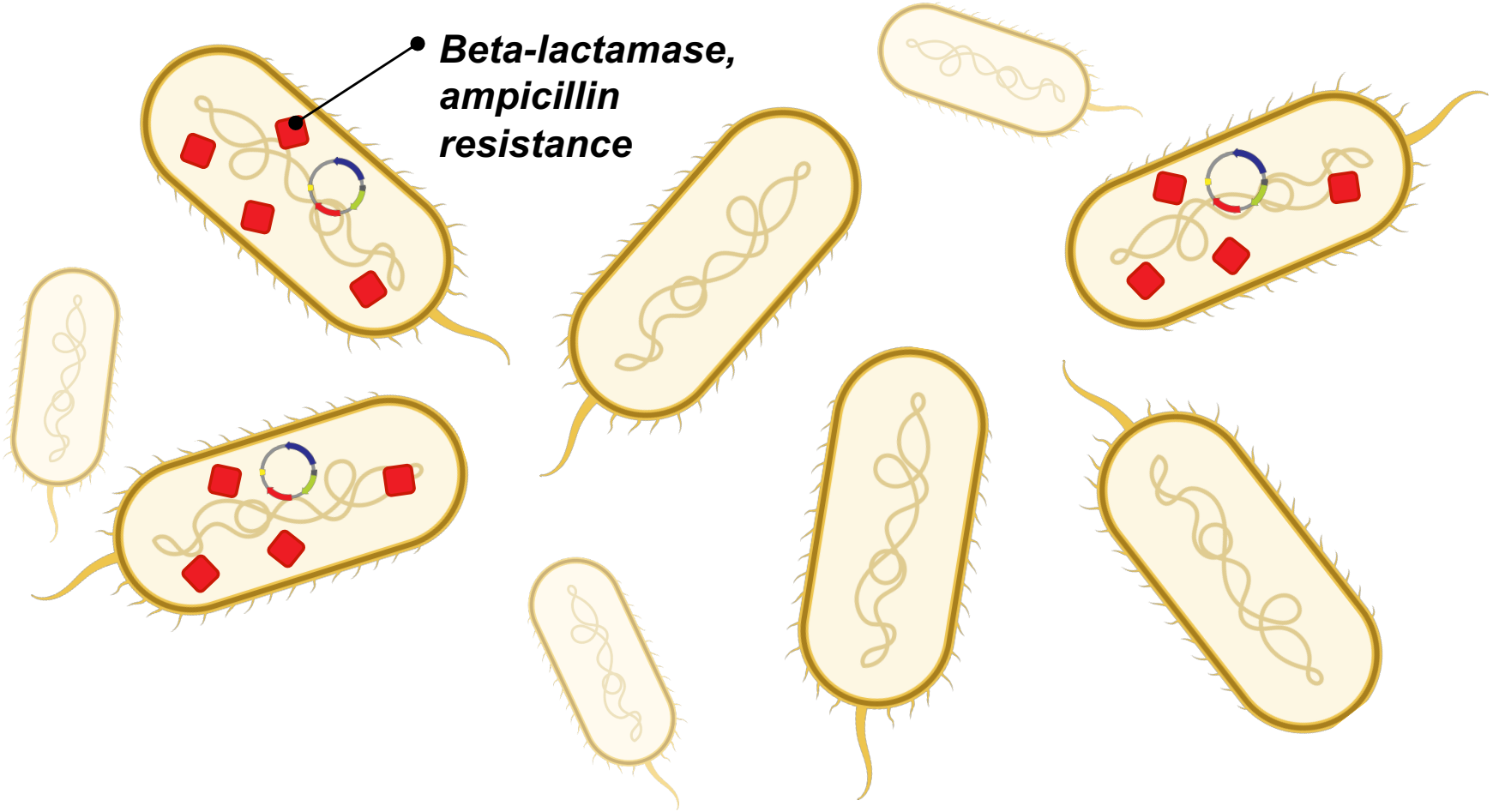

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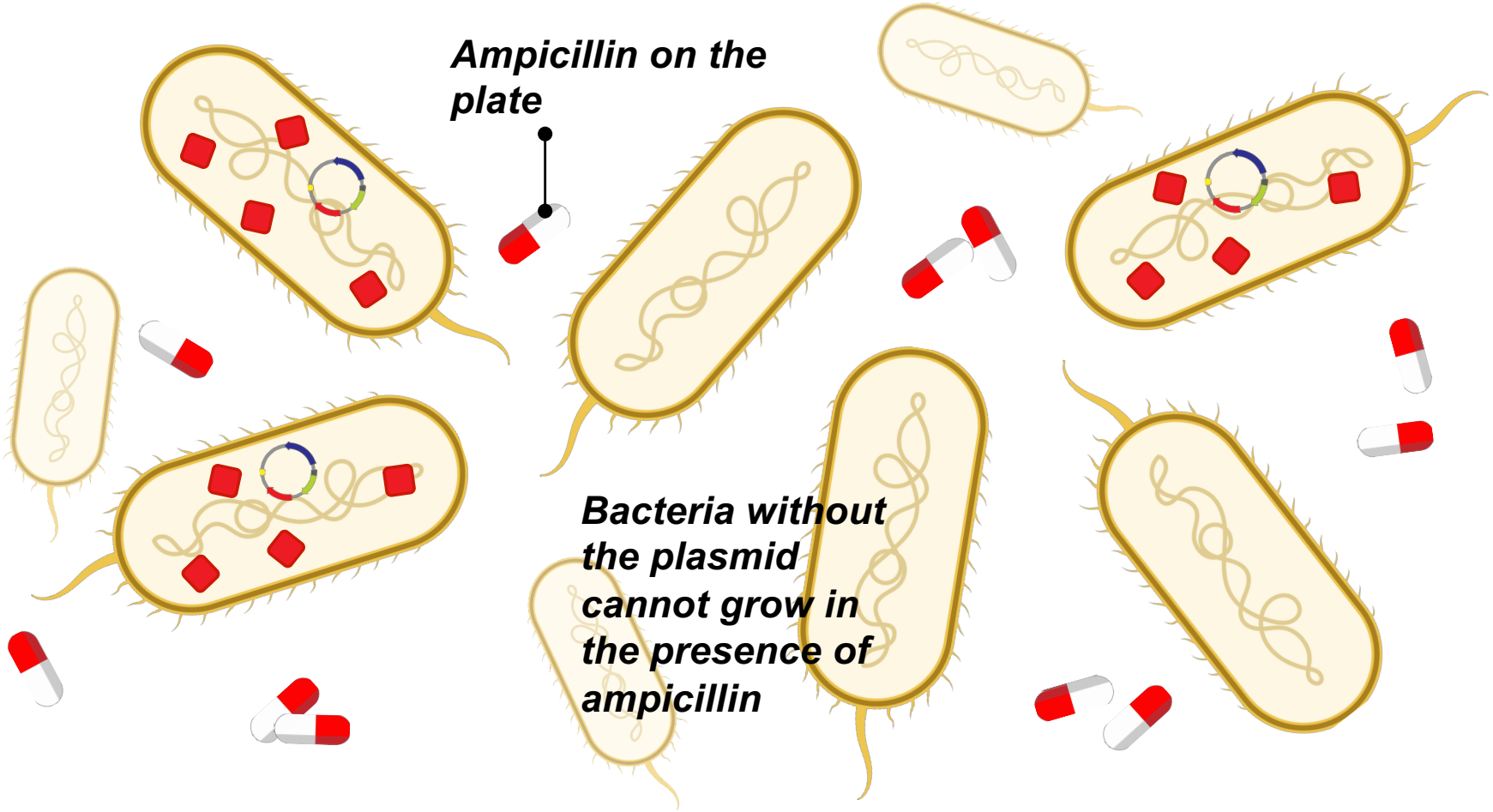
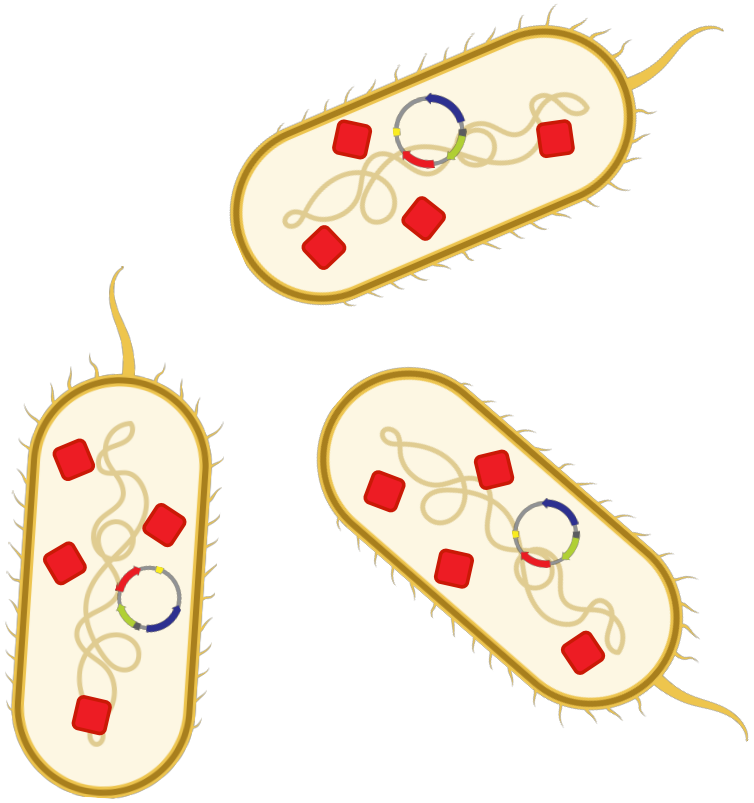


Selective media – ampicillin

- 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

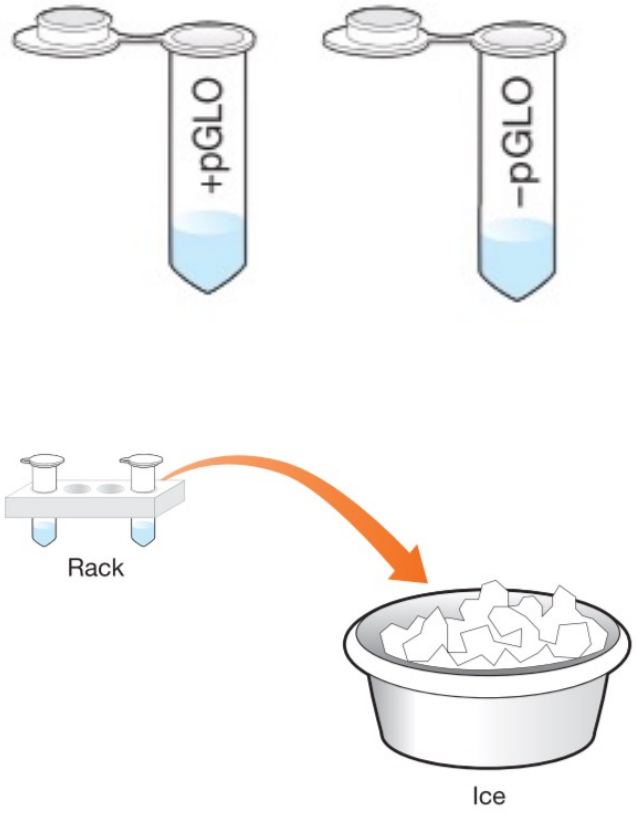
Transformation summary

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 LB/amp plates	Selects for transformed bacteria and allows formation of colonies.

Label tubes

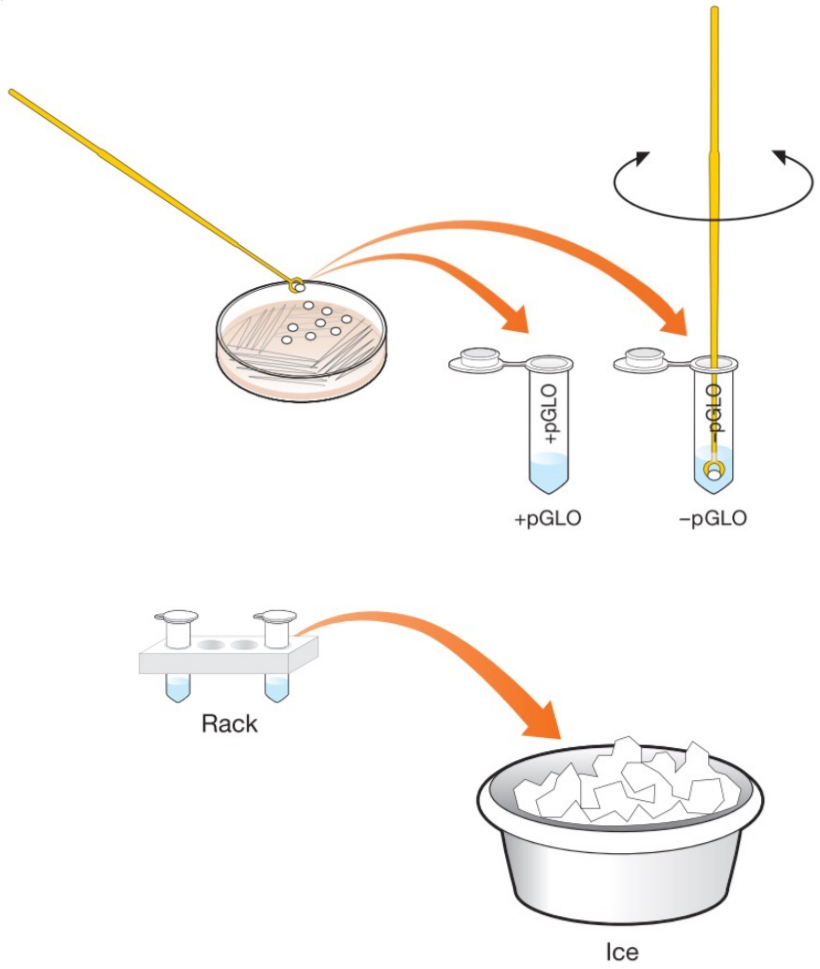
You have tubes with 250 µl transformation solution.

1. Label one **+pGLO** and the other **–pGLO**.
2. Add your initials.
3. Place into foam rack and on ice.



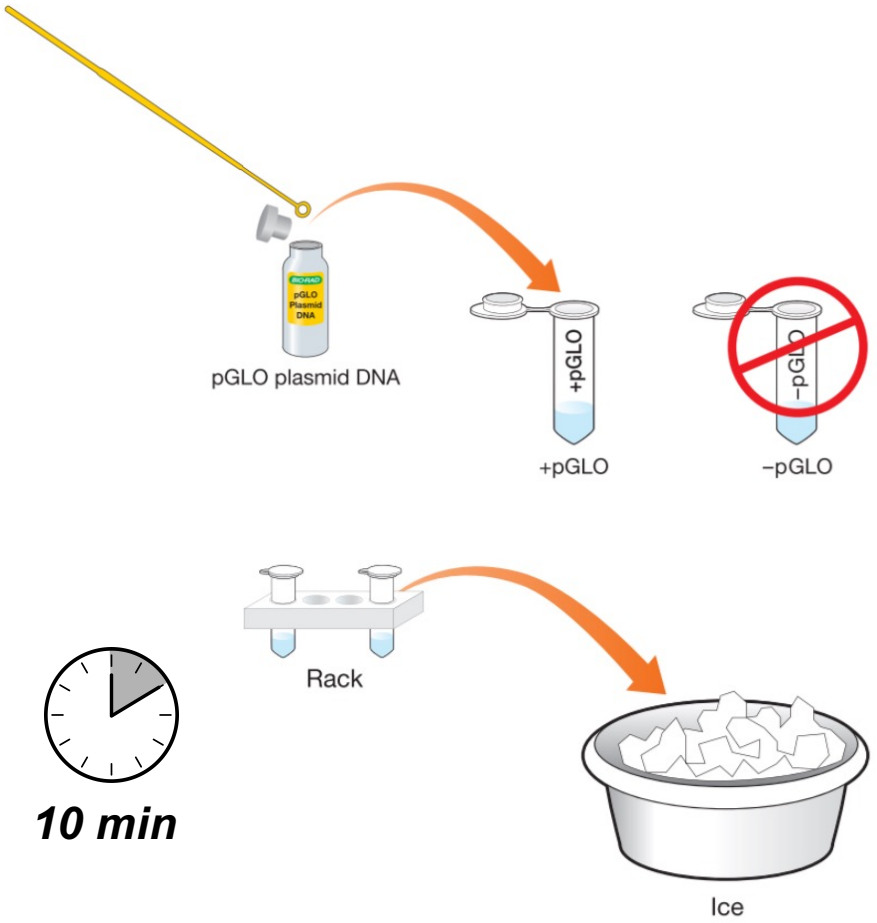
Pick colonies

4. ***Using a sterile loop*** pick 1–2 large *E. coli* colonies.
5. Add to the **+pGLO** tube. Spin the loop to disperse the bacteria. No clumps!
6. Using a ***new*** loop, at 1–2 colonies to **–pGLO** tube.
7. Place tubes into foam rack and on ice.



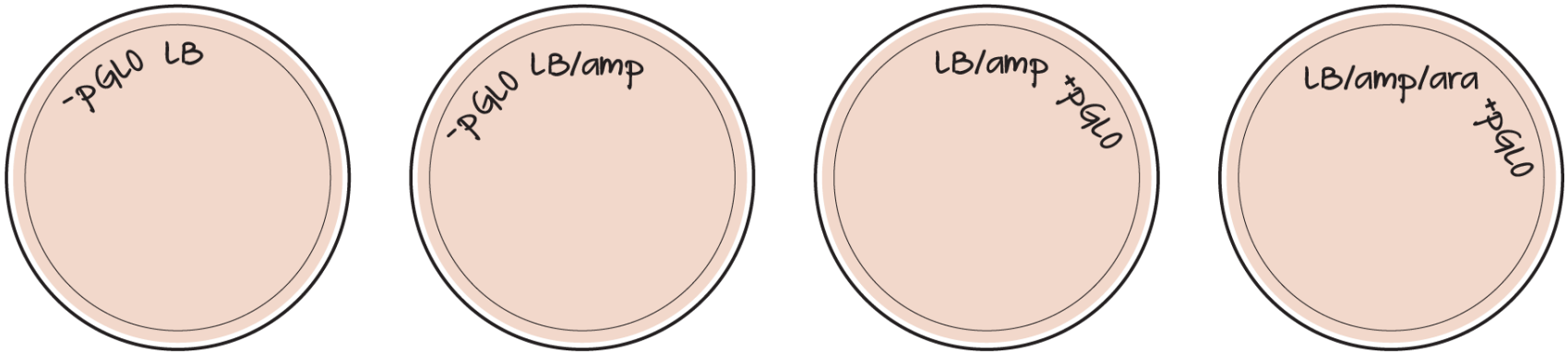
Add plasmid DNA

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



Label plates

10. While your tubes are on ice, label the ***bottom*** of your plates.
11. Add your group ID or initials.



LB
–pGLO

LB/amp
–pGLO

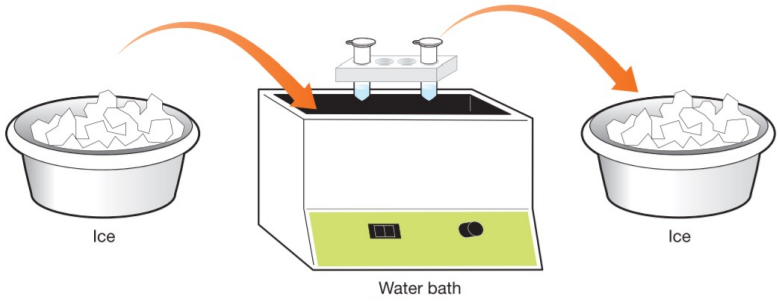
LB/amp
+pGLO

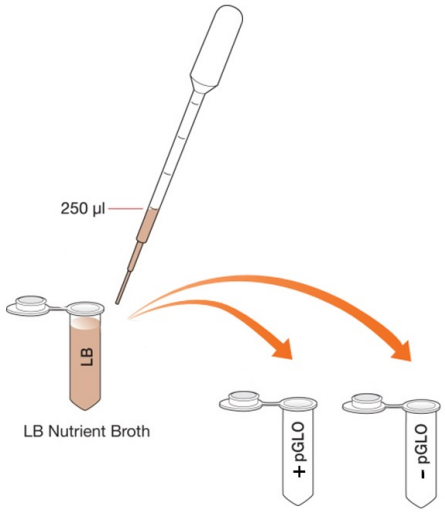
LB/amp/ara
+pGLO

Heat shock

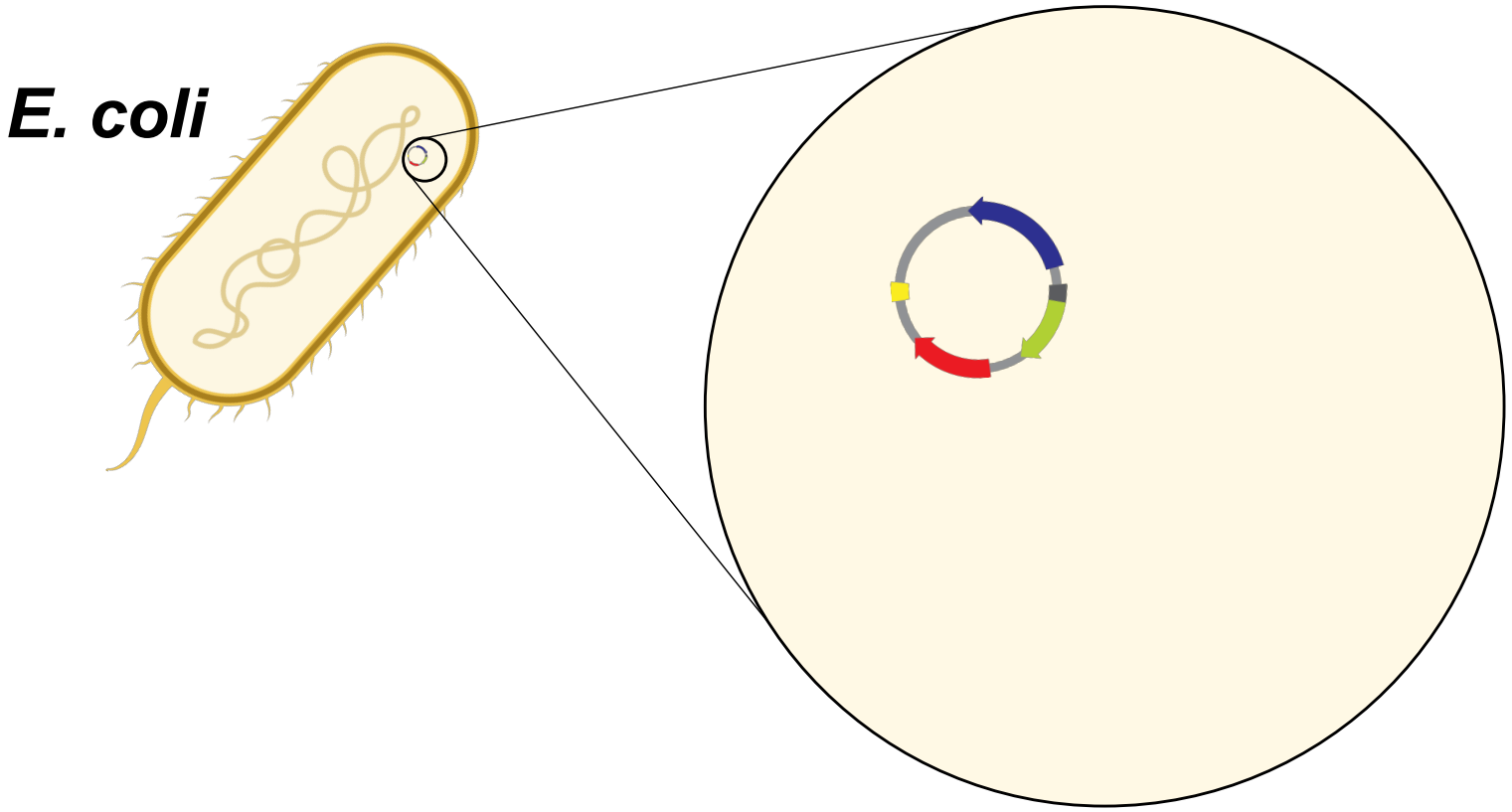
Get your timers ready!

12. Heat shock tubes at 42°C for exactly 50 sec.
13. ***Immediately*** return tubes to ice for 2 min.
14. Add 250 µl LB broth to both tubes.
15. Leave at room temperature for 10 min.

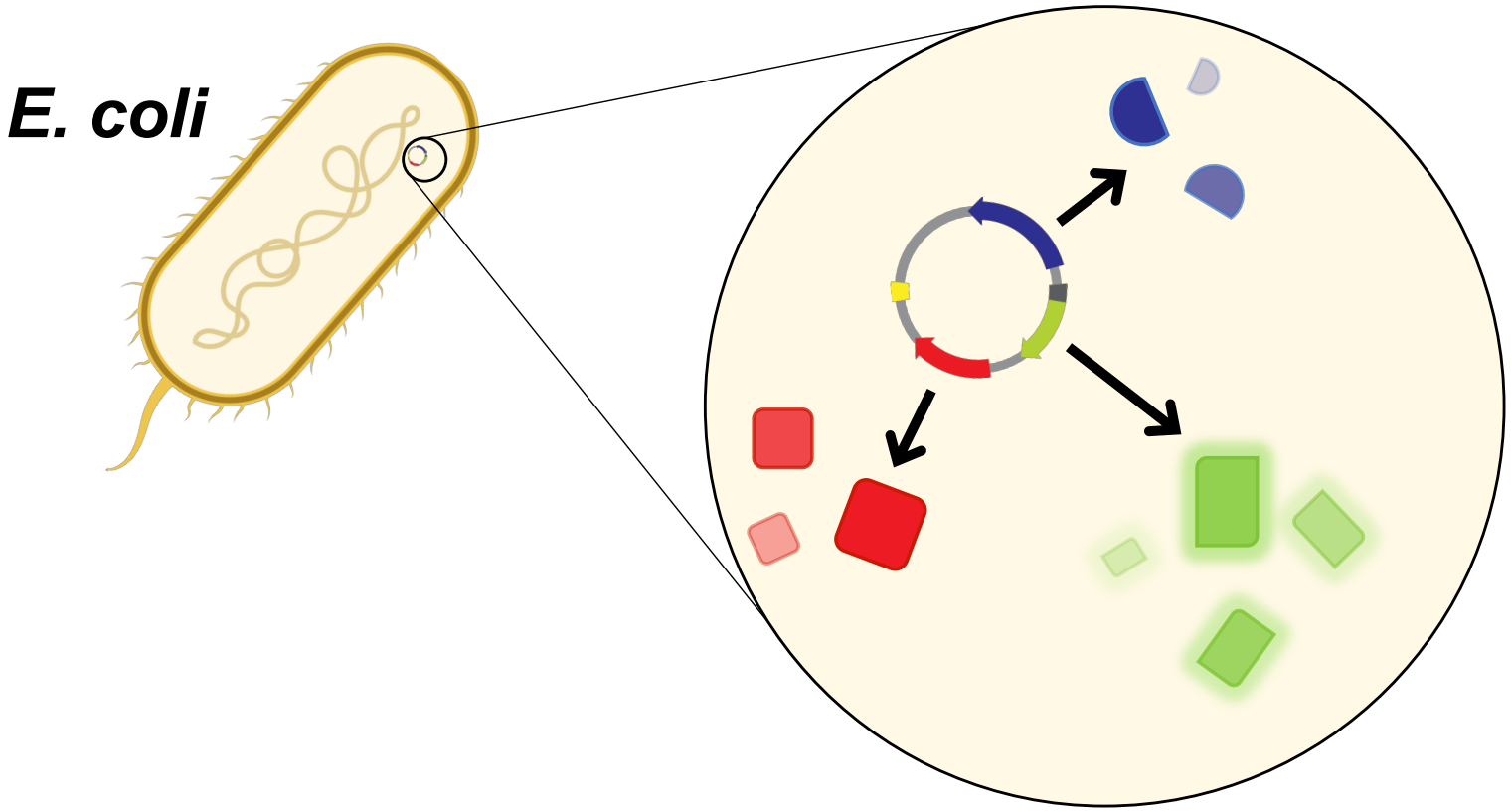




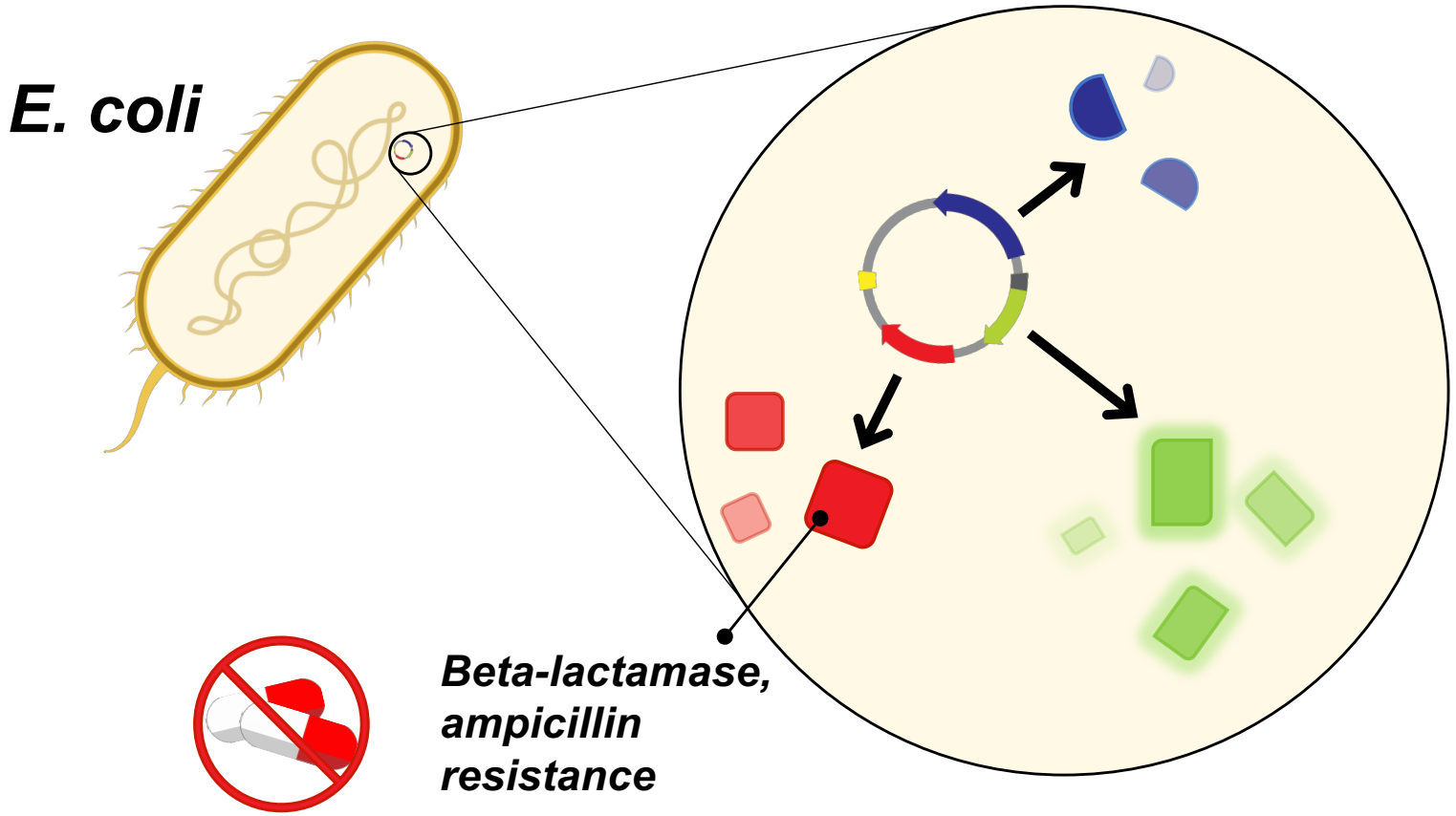
Meanwhile...



Plasmid genes are expressed

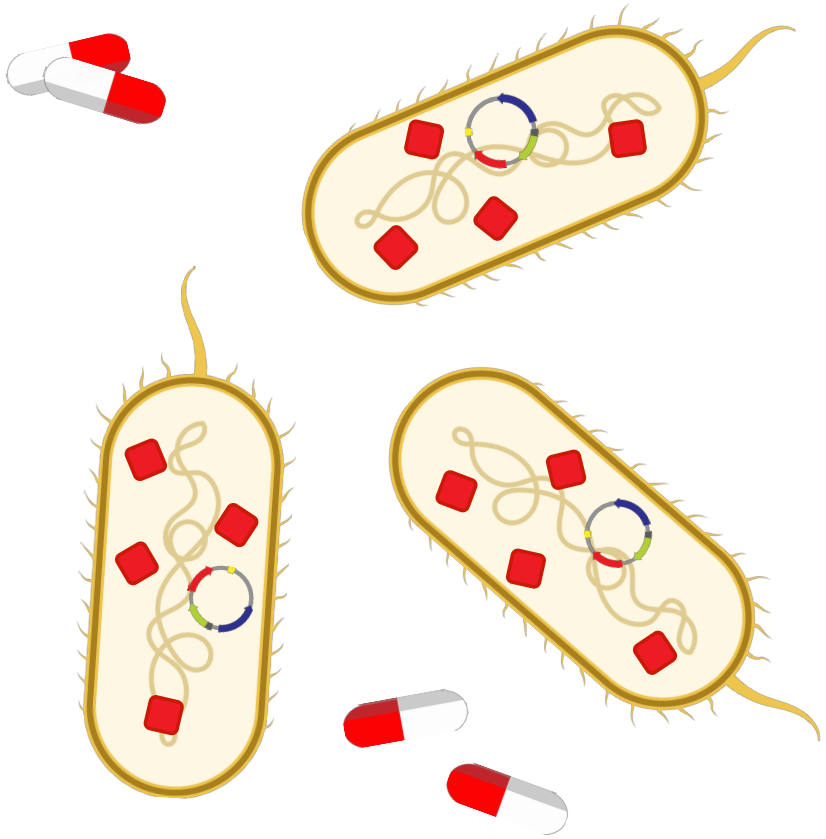


Beta-lactamase

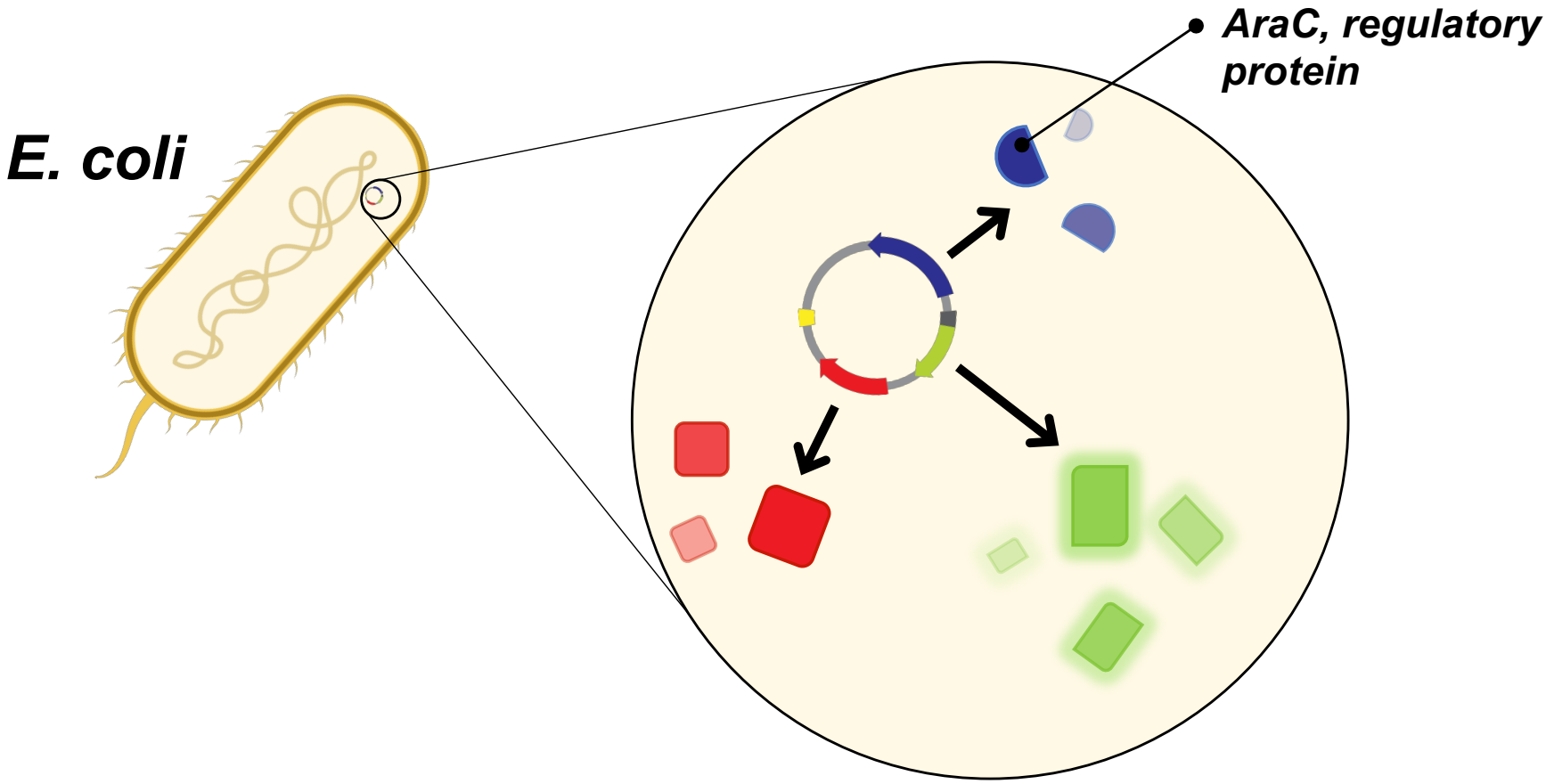


Beta-lactamase makes *E. coli* resistant to ampicillin

- 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.

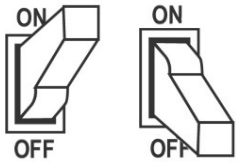


AraC



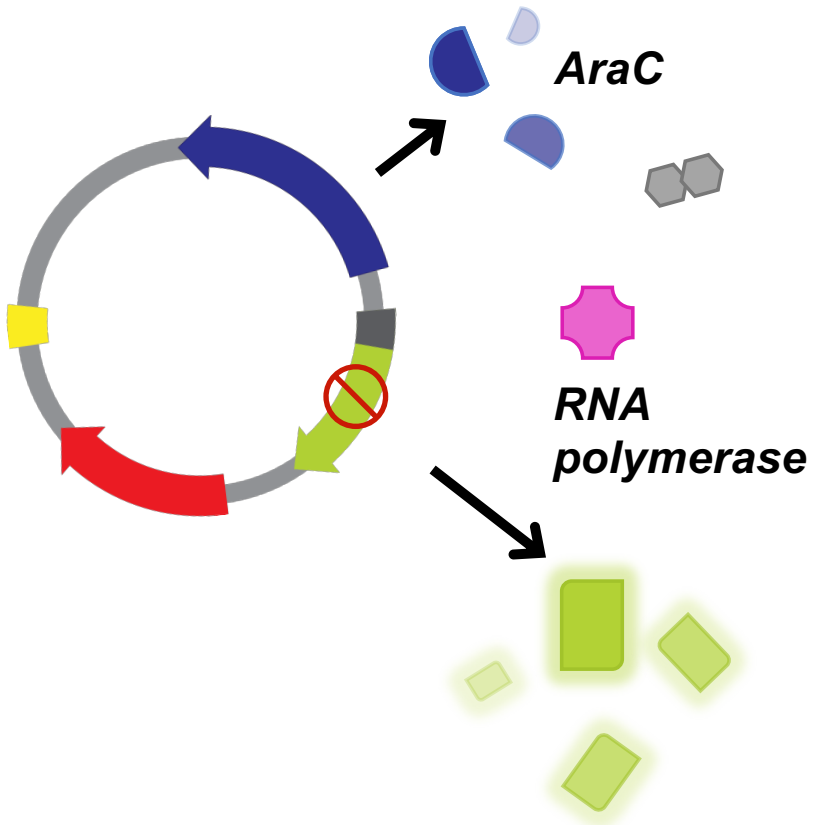
AraC Controls Expression of GFP

- Arabinose (a sugar) works like a switch.

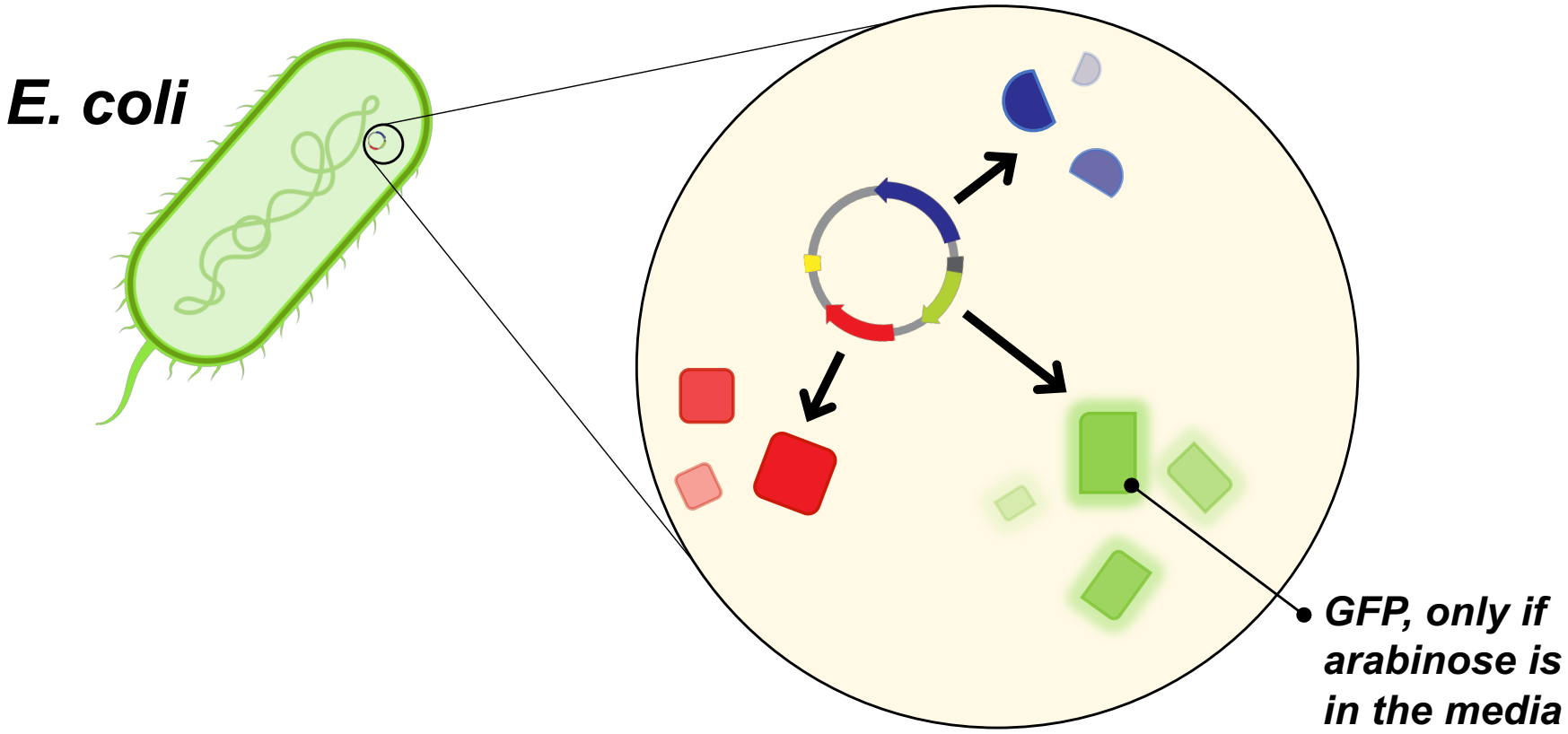


- ***Without*** arabinose, the switch is OFF. AraC blocks RNA polymerase, and the GFP gene is not transcribed.

- ***With*** arabinose, the switch is ON. AraC changes shape and RNA transcribes the *GFP* gene.

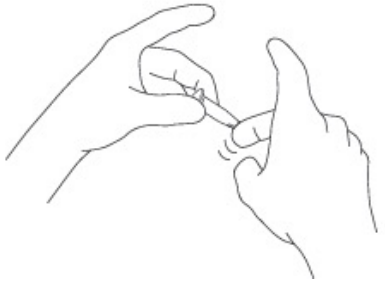


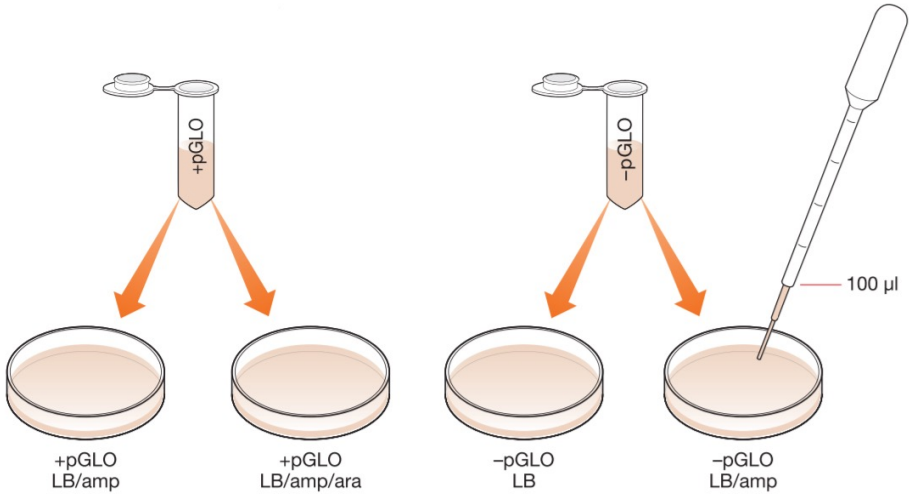
Green Fluorescent Protein (GFP)



Plating Bacteria

16. Flick tubes to mix.
17. Using a new sterile pipet, add 100 µl bacteria to appropriate plates (**+pGLO** or **–pGLO**).
18. Use a loop to spread bacteria evenly.
    Use a new loop for each plate.
19. Incubate overnight at 37°C or for 2 days at room temperature.





Components	Plates: –pGLO LB	Plates: –pGLO LB/amp	Plates: +pGLO LB/amp	Plates: +pGLO LB/amp/ara
Bacteria	✓	✓	✓	✓
DNA				
Ampicillin				
Arabinose				
Grow?				
Glow?				

Transformation Efficiency

- How successful was your transformation?
  You can calculate the transformation efficiency and compare with other groups.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in µg)}}$$

Example

$$\frac{\text{87 colonies growing on plate}}{\text{0.16 µg of DNA spread}} = 543 \text{ transformants/µg}$$

(or 5.4×10^2 transformants/µg)