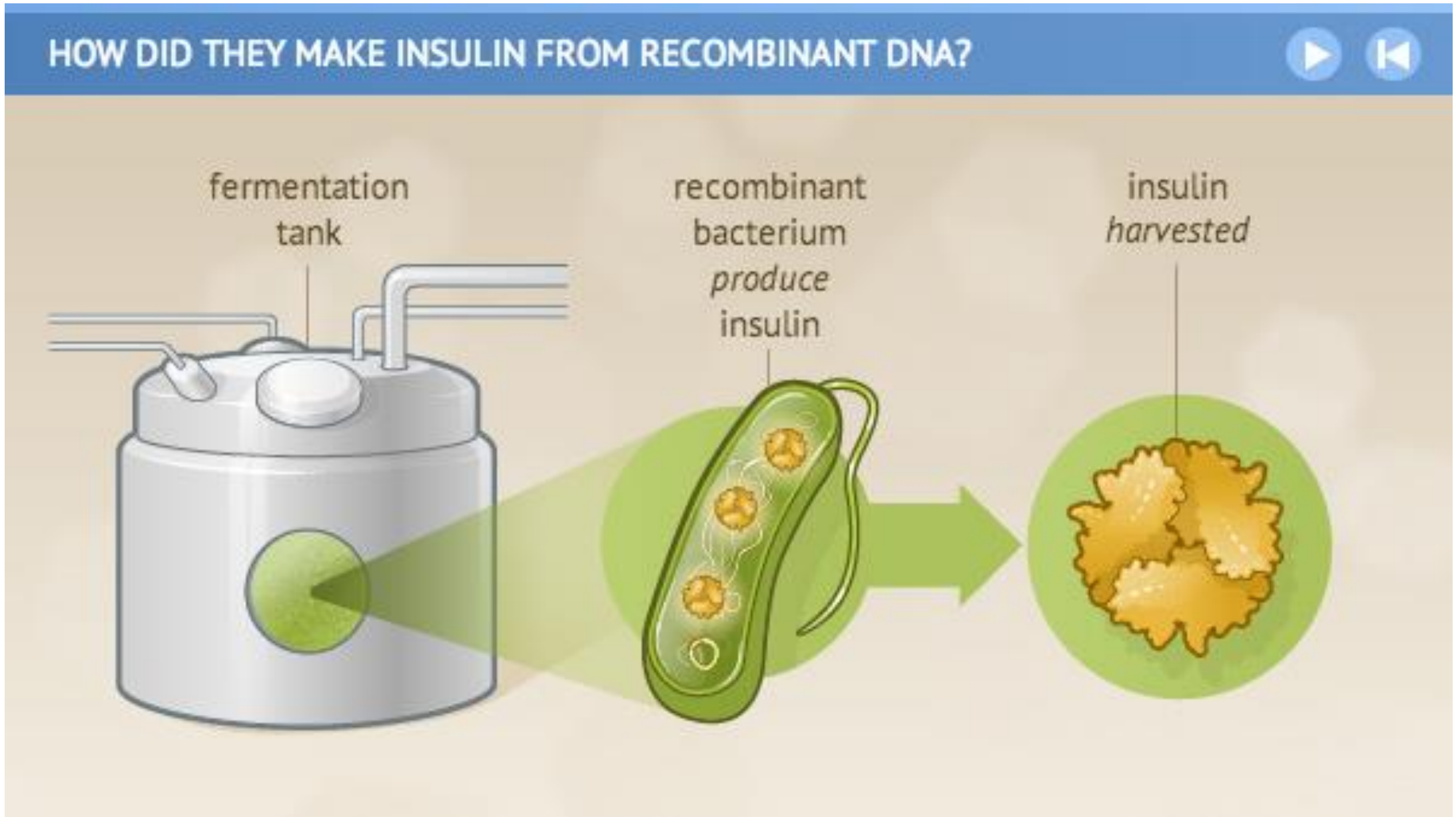
pGLO Bacterial Transformation

Genetic engineering, GFP, plasmids, and bacterial transformation

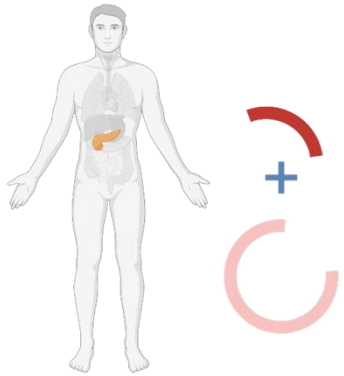

HOW DID THEY MAKE INSULIN FROM RECOMBINANT DNA?
fermentation tank
recombinant bacterium *produce* insulin
insulin *harvested*

Other Recombinant Drugs

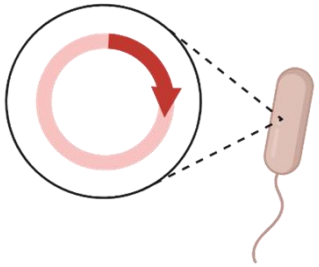
Drug (Brand)	Molecule / Mechanism	Indications	Cell Factory
Filgrastim (Neupogen)	Cytokine — stimulates growth of white blood cells	Acute lymphocytic leukemia	*E. coli*
Interferon alfa-2a	Recombinant interferon - antiviral	AIDS-related Kaposi's sarcoma, chronic hepatitis C, leukemia	*E. coli*
Hepatitis B Vaccine (Recombivax)	Subunit viral vaccine from HBsAG	Hepatitis B	*S. cerevisiae*
Trastuzumab (Herceptin)	Recombinant human monoclonal antibody	Breast cancer, stomach cancer	CHO
Etanercept (Enbrel)	Recombinant soluble fusion protein — TNF inhibitor	Rheumatoid arthritis	CHO
Adalimumab (Humira)	Recombinant human monoclonal antibody	Arthritis, plaque psoriasis, Crohn's disease, ulcerative colitis	CHO

How do we produce drugs like insulin?

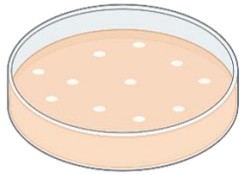
1. Clone human insulin gene into plasmid



2. **Transform** bacteria with plasmid

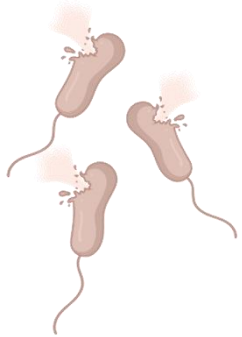


3. Select transformed bacteria

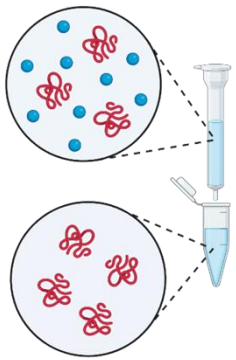


4. Culture bacteria and induce gene expression

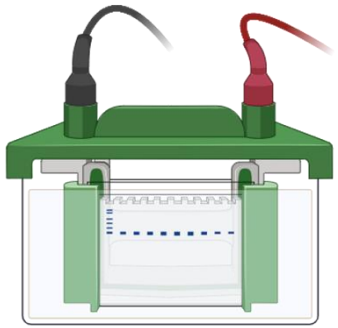
5. Lyse cells



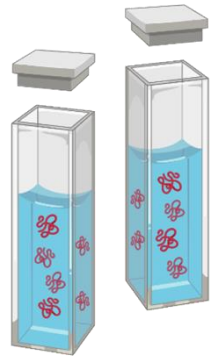
6. Purify protein with chromatography



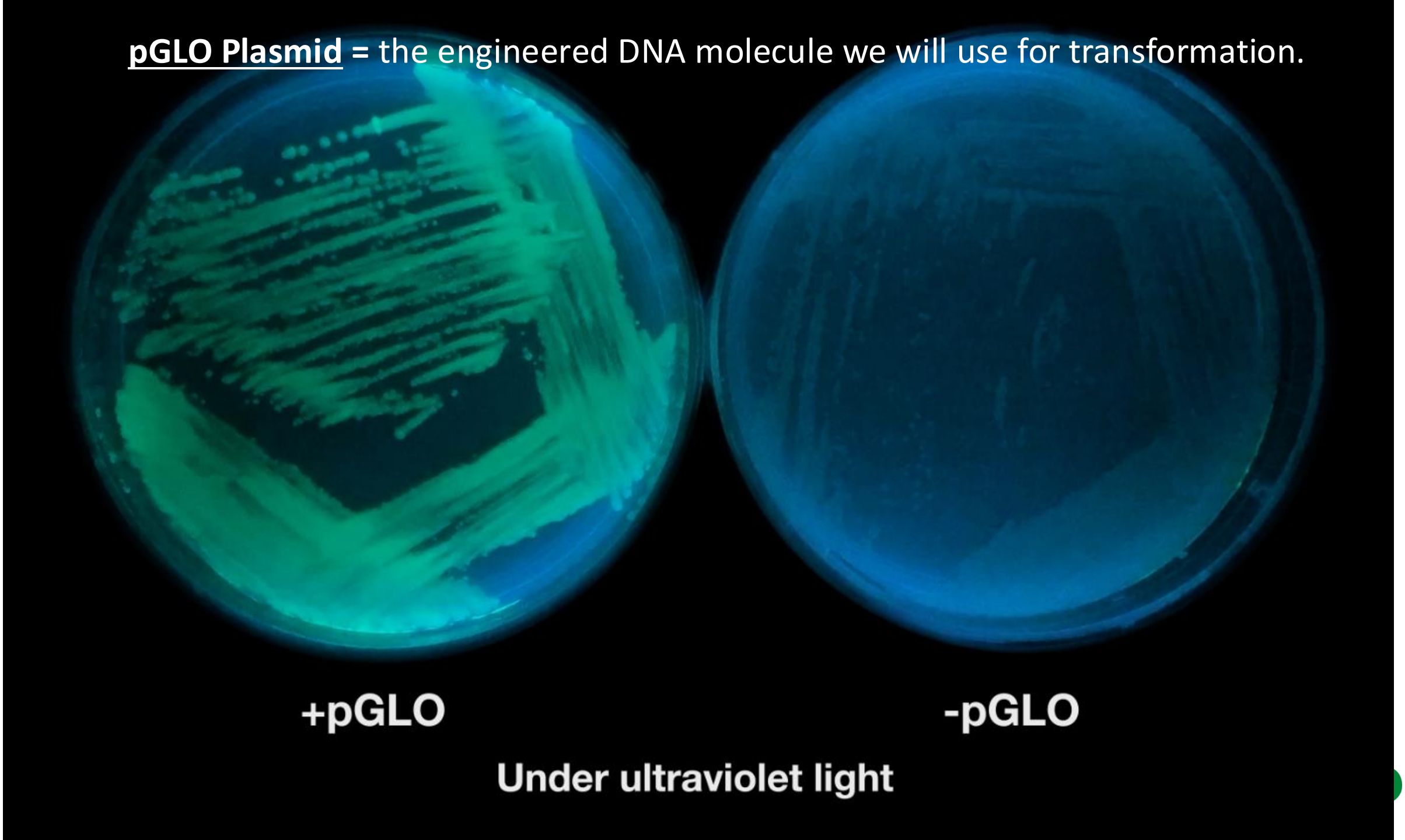
7. Analyze purification with gels



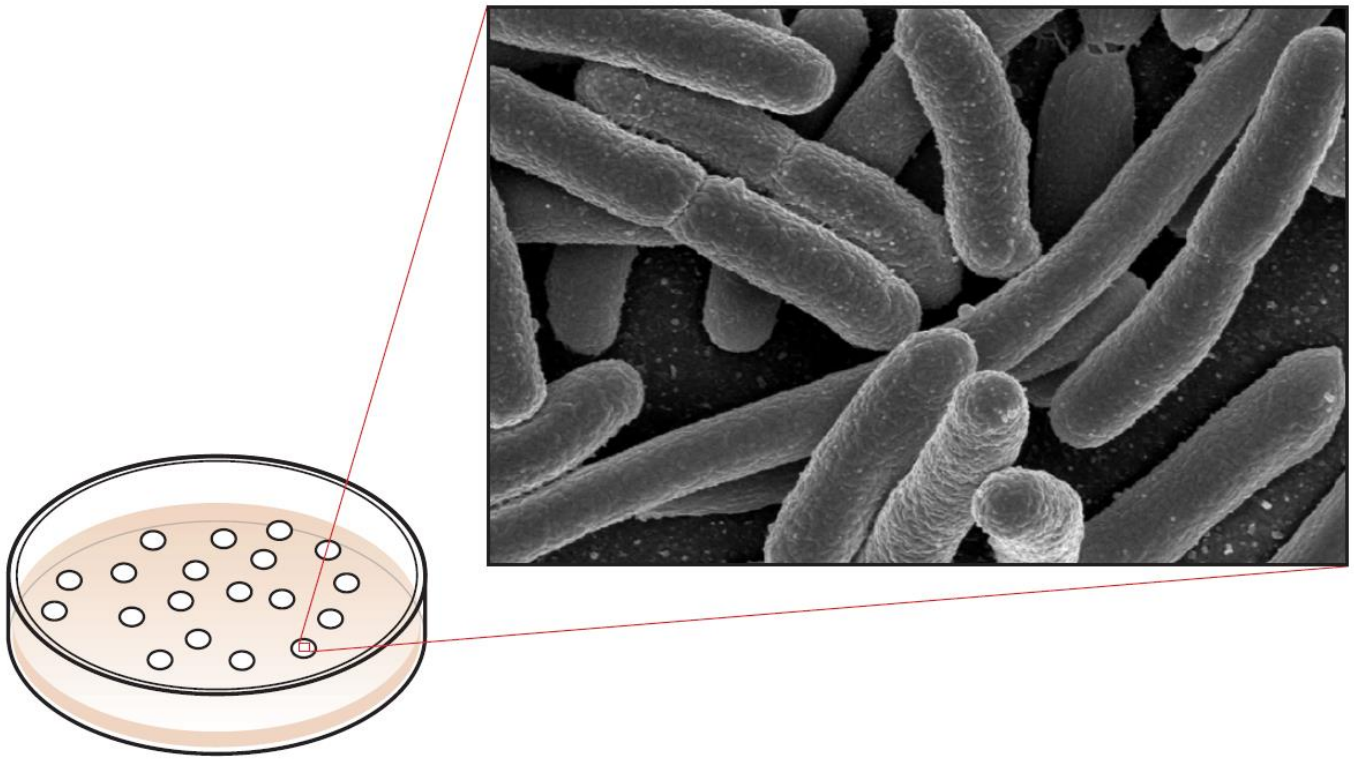
8. Assay protein activity



pGLO Plasmid = the engineered DNA molecule we will use for transformation.

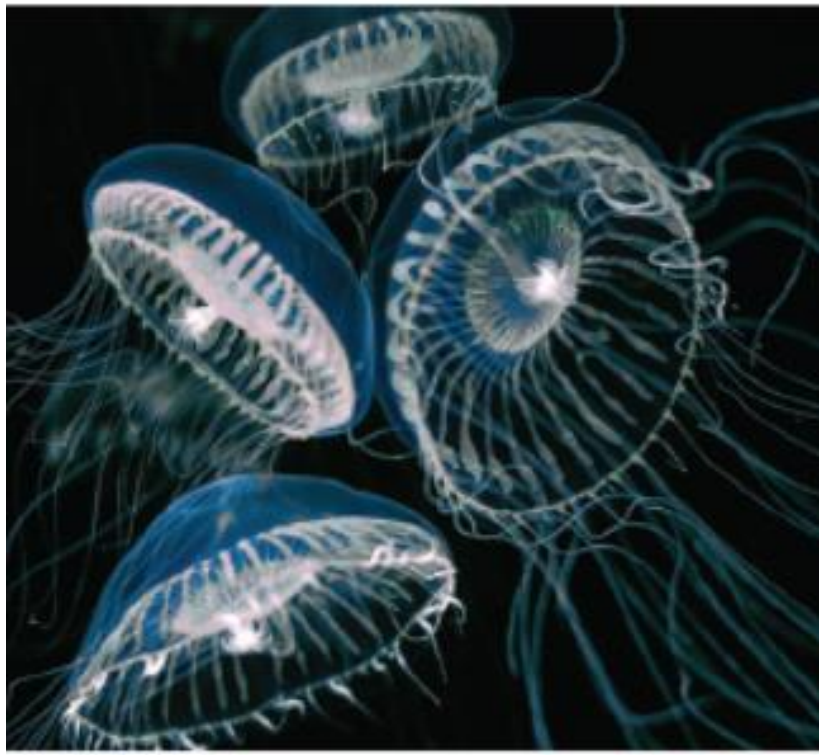


E. coli colonies

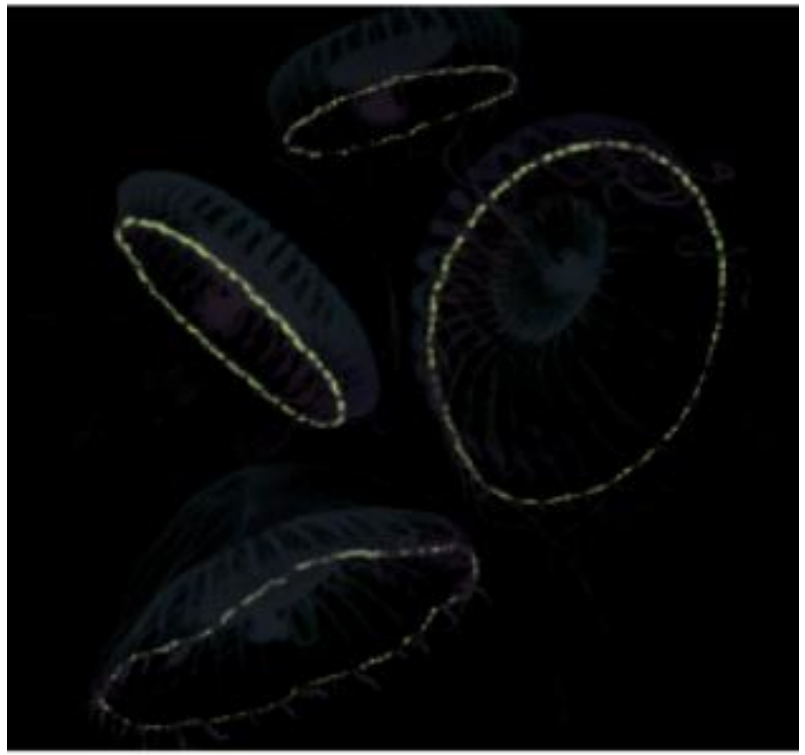


The bacterial growth you see on the plate is made of millions of individual bacterial (*E. coli*) cells.

Aequorea Victoria - jellyfish that makes green fluorescent protein (there is a gene that encodes for it).

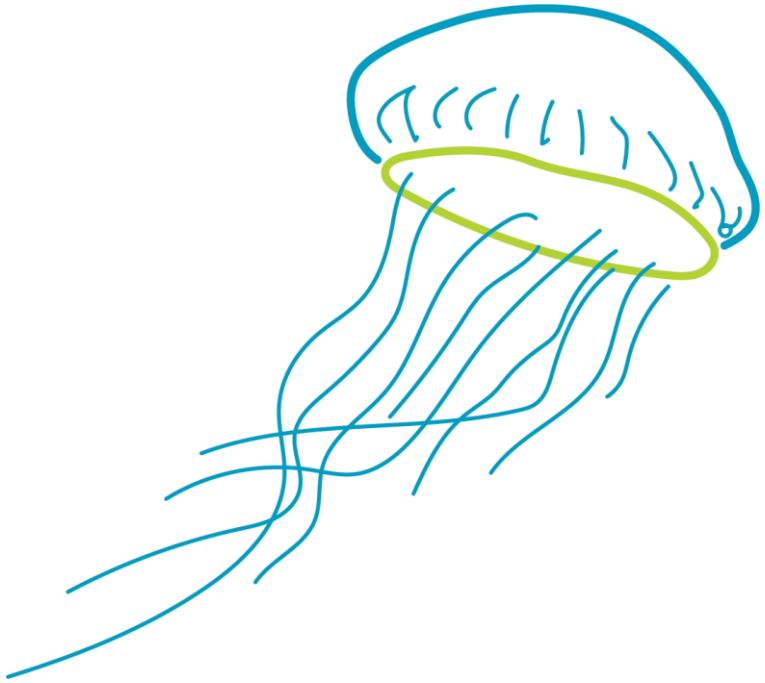


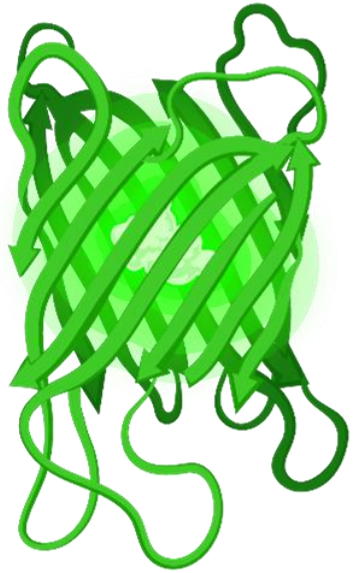
Visible light



UV light

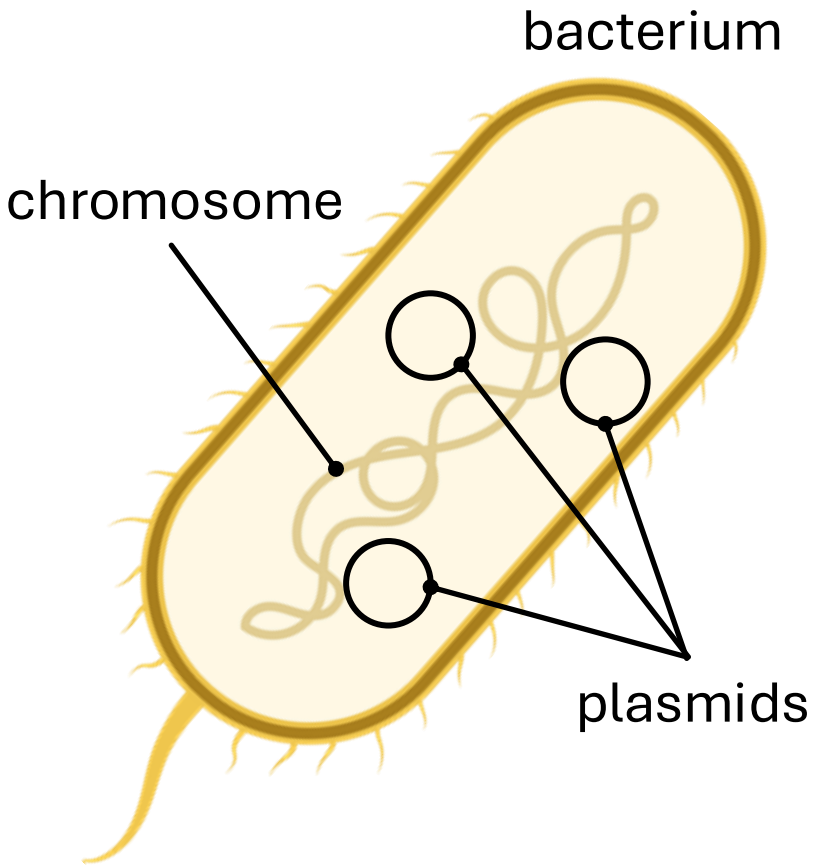
Green Fluorescent Protein (GFP) is what you are seeing in pGlo bacteria

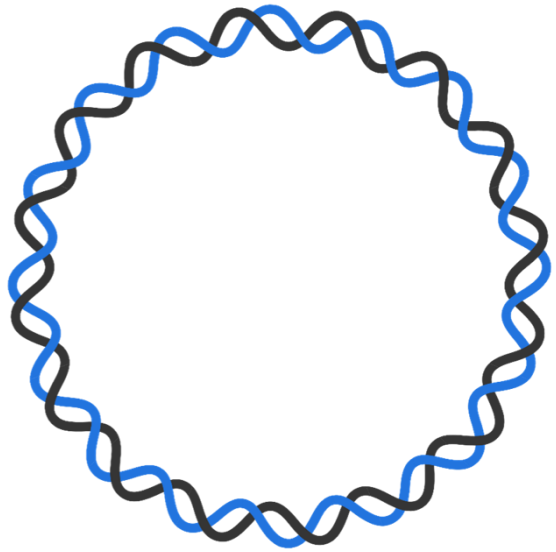




GFP allows us to visualize protein expression with UV light

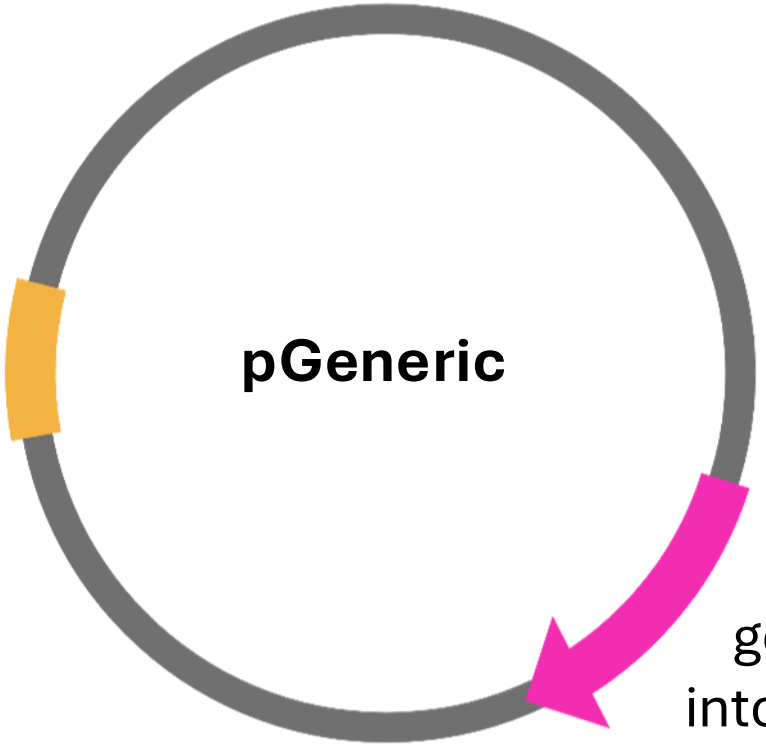
Genetic engineering using plasmids





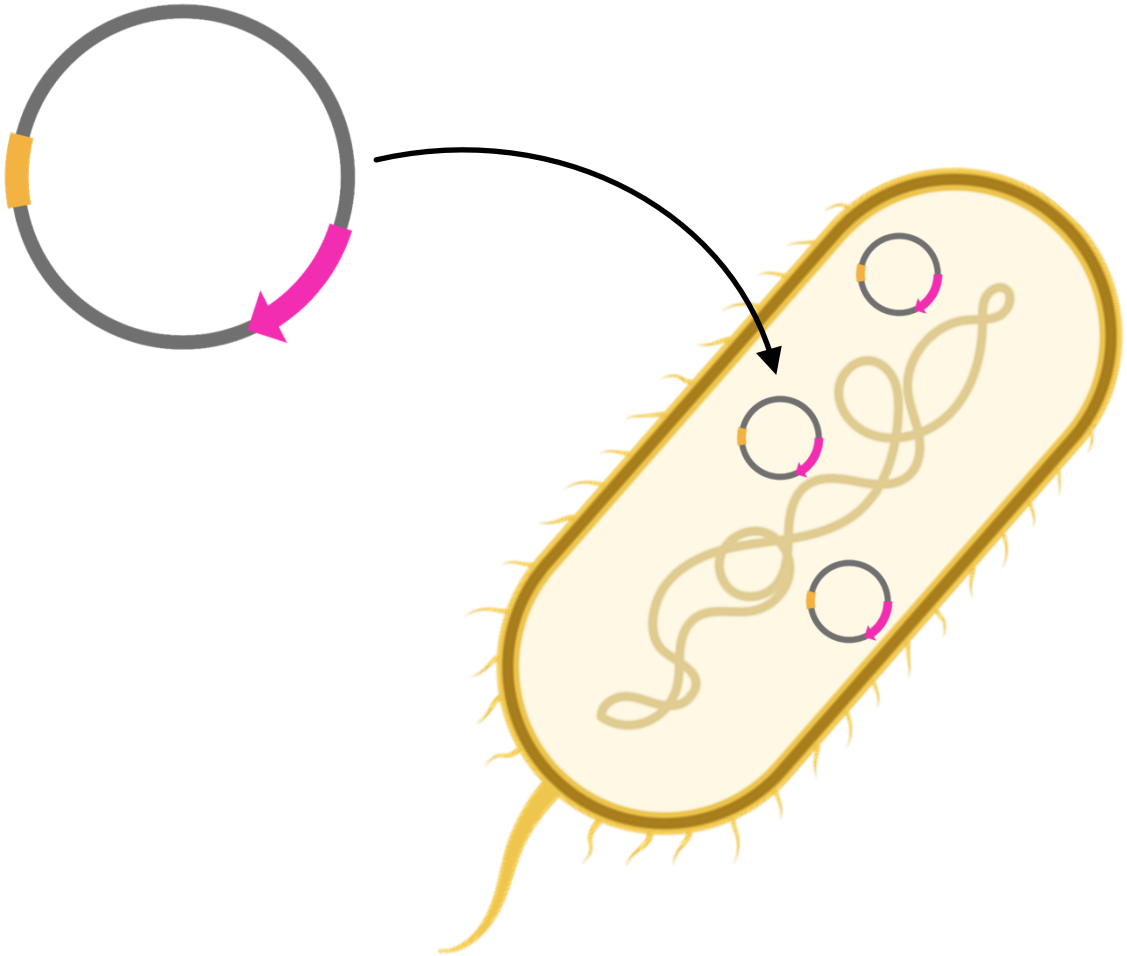
Generic plasmid

ori
origin of replication
allows bacteria to make
copies of plasmid



gene
gene of interest cloned
into plasmid

Bacterial transformation

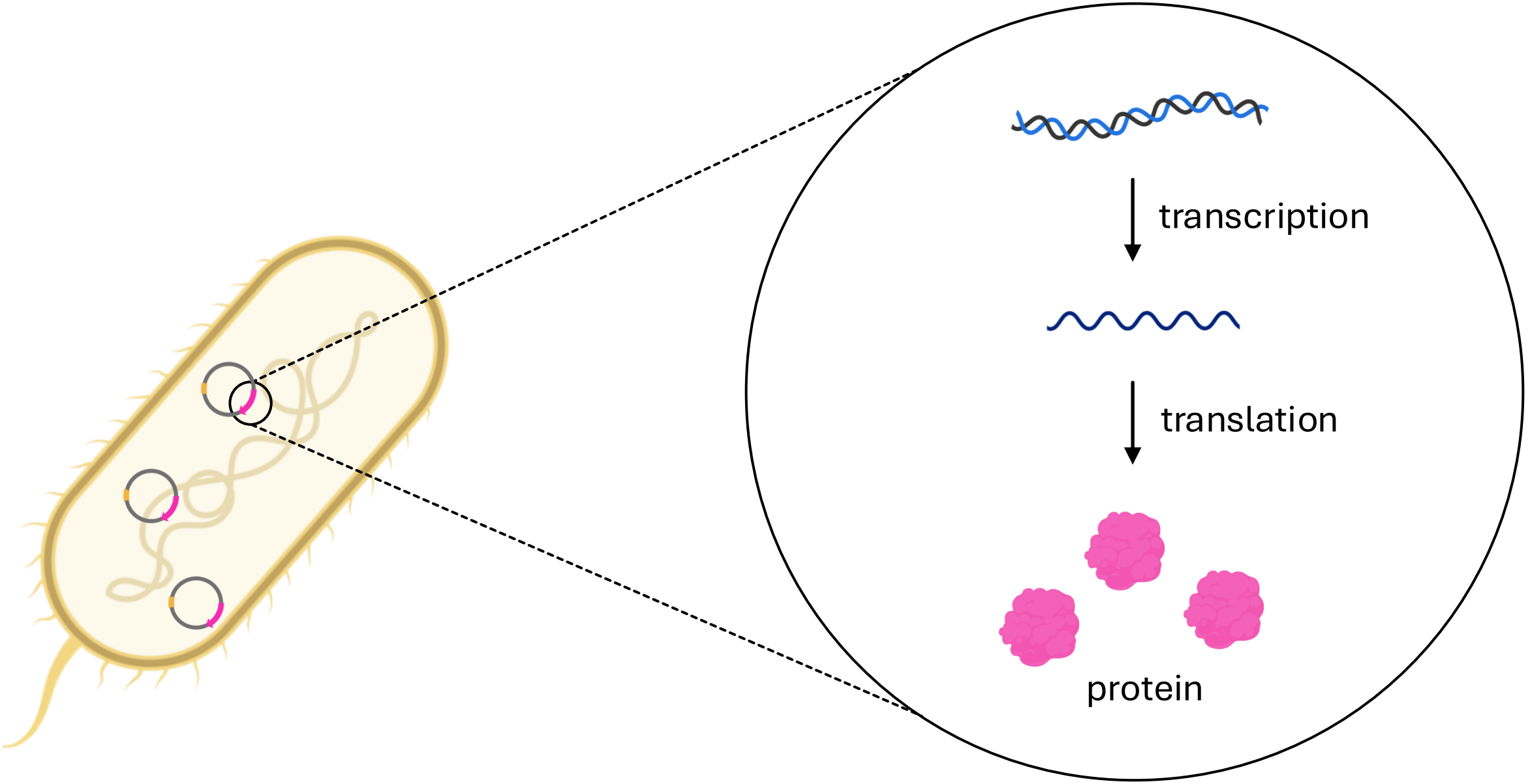


Genetic transformation occurs when a cell takes up DNA and expresses the genes on that DNA.

Many different types of cells can be transformed – plant, animal, human, bacterial.

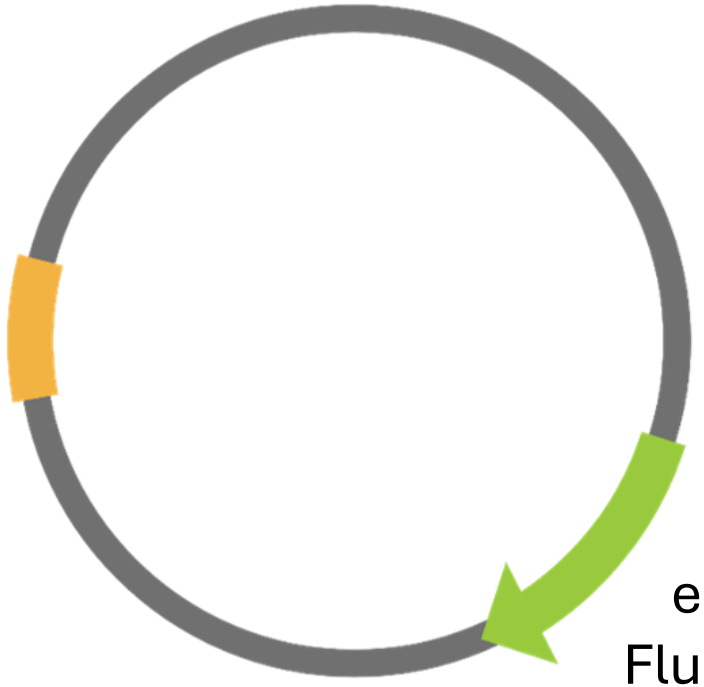
Bacteria divide, and new bacteria get plasmids

Genes are transcribed and translated



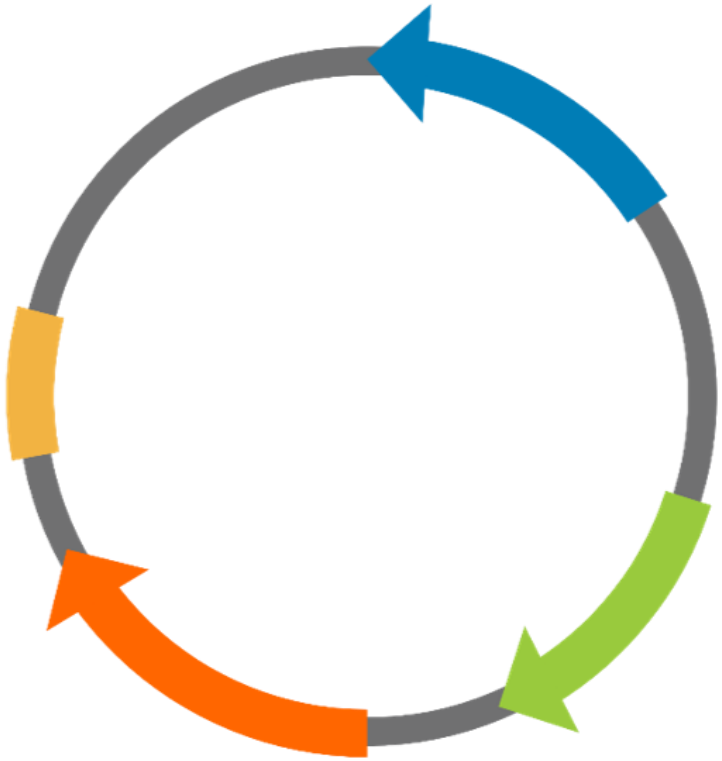
Plasmid with gene for GFP

ori
origin of replication
allows bacteria to
make copies of
plasmid



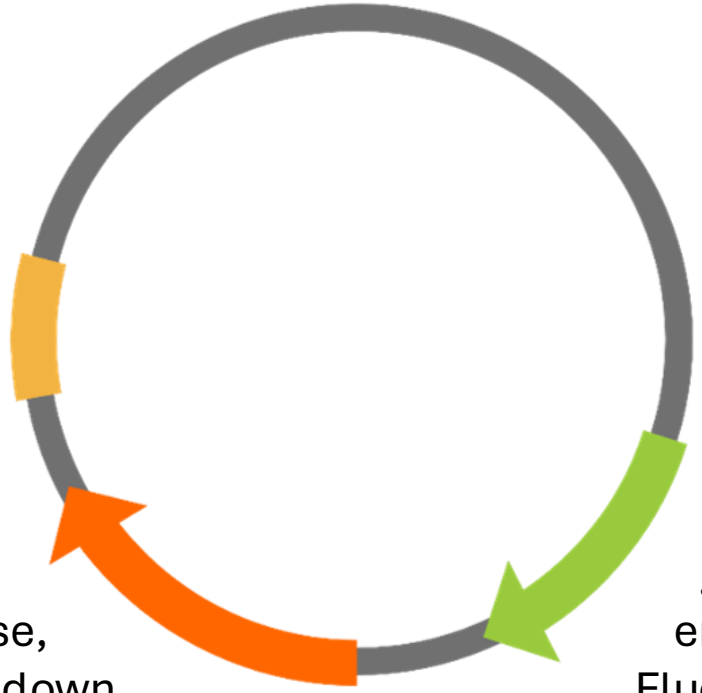
***gfp* gene**
encodes Green
Fluorescent Protein
(GFP)

Plasmids can carry multiple genes



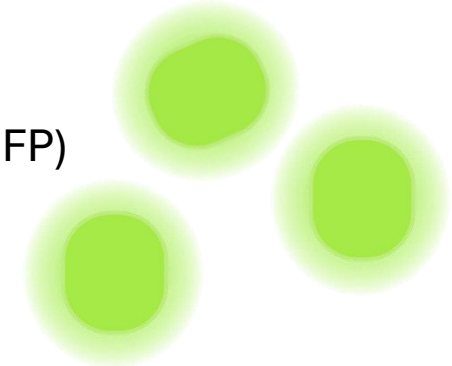
β-lactamase gene for antibiotic resistance

ori
origin of replication
allows bacteria to make
copies of plasmid

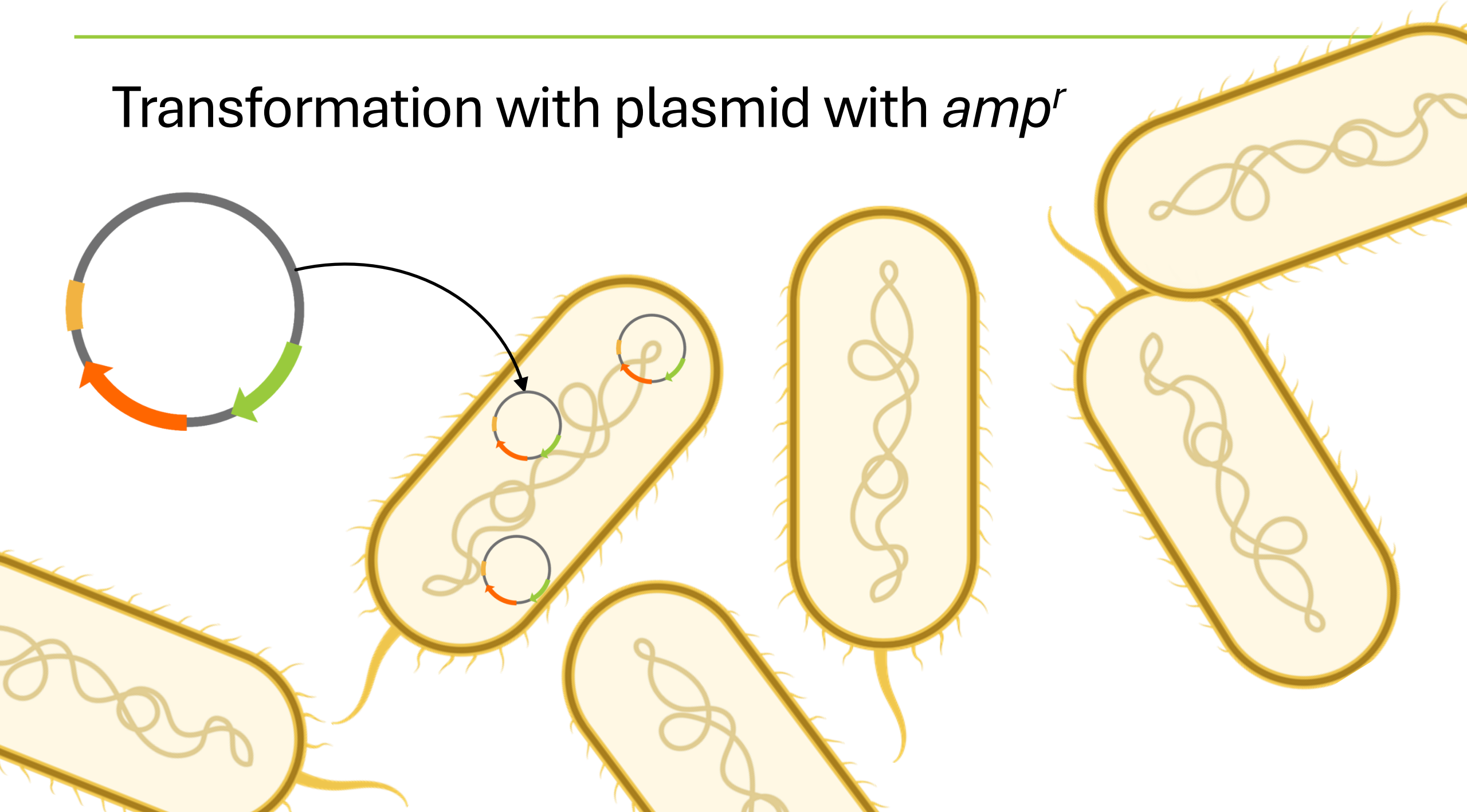


***amp*^{r} (or *bla*) gene**
encodes beta-lactamase,
an enzyme that breaks down
ampicillin (an antibiotic)

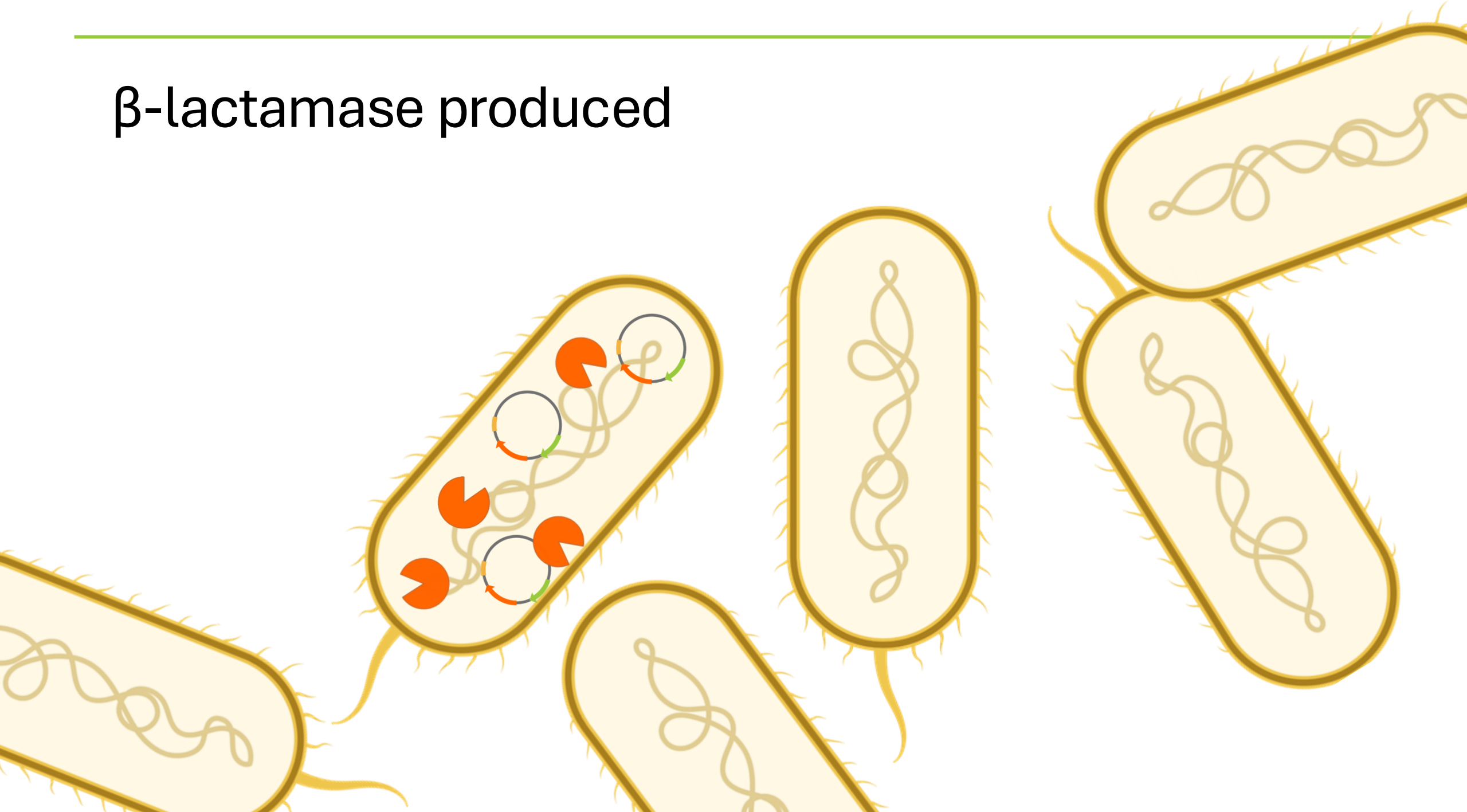
***gfp* gene**
encodes Green
Fluorescent Protein (GFP)



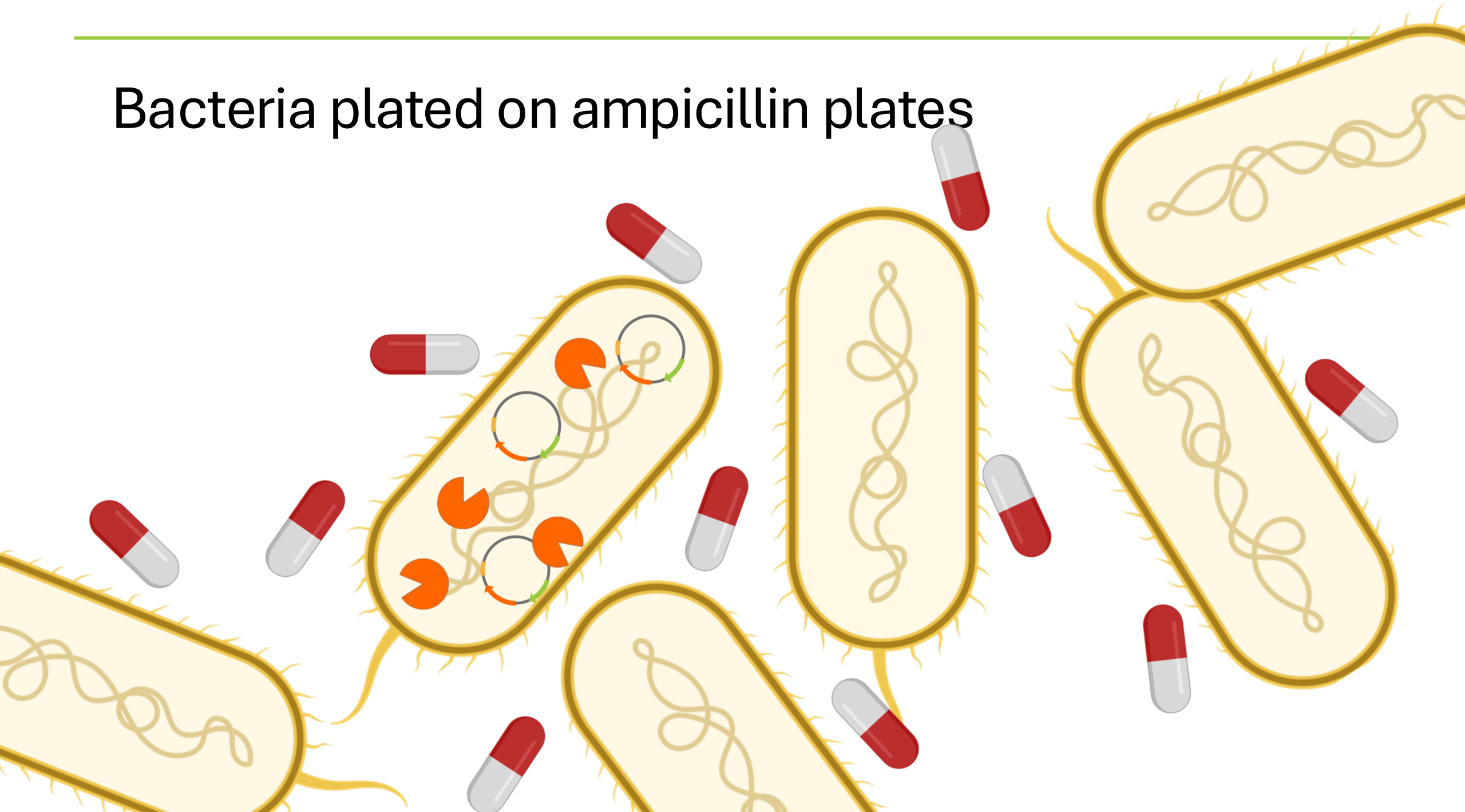
Transformation with plasmid with *amp*^{r}



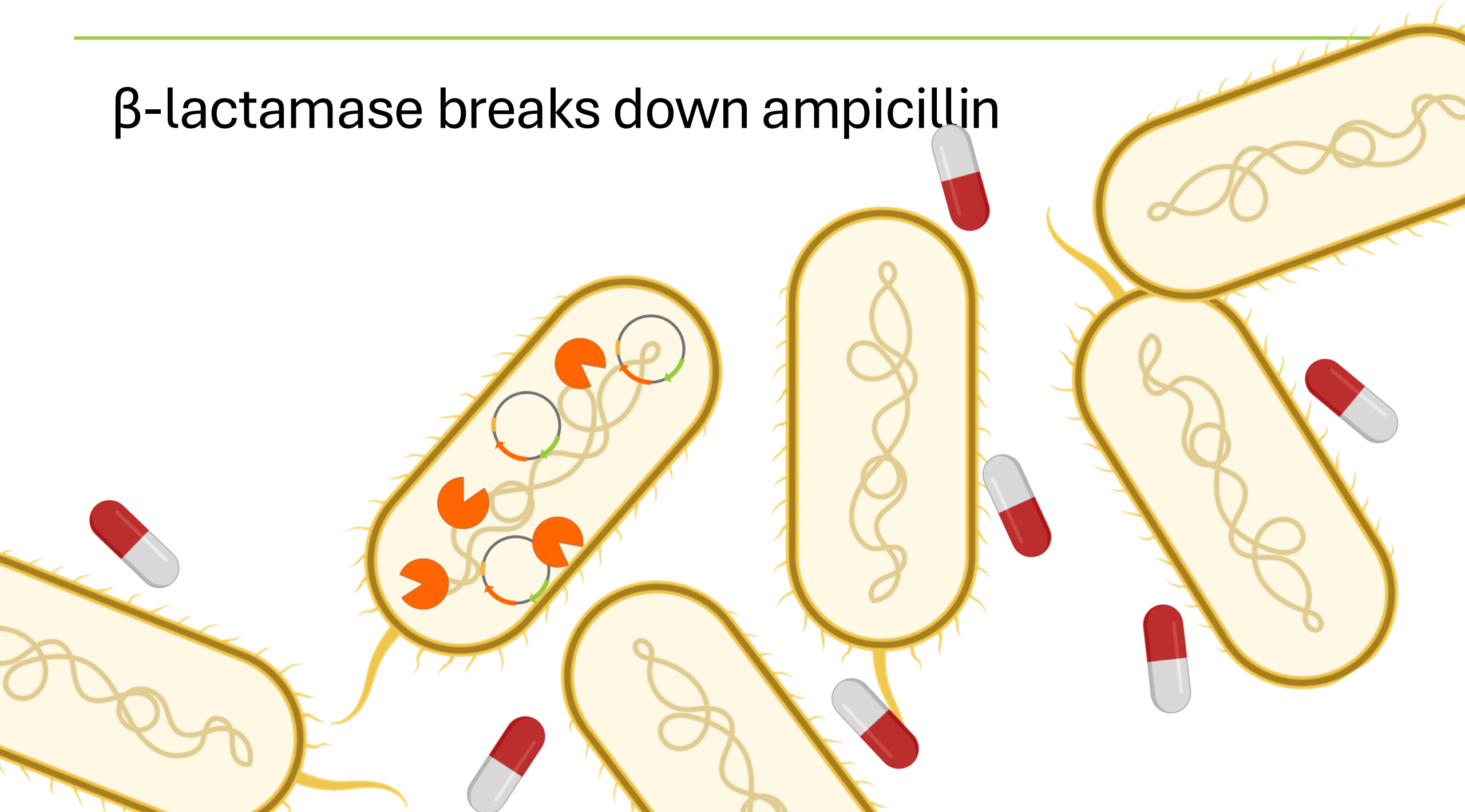
β-lactamase produced



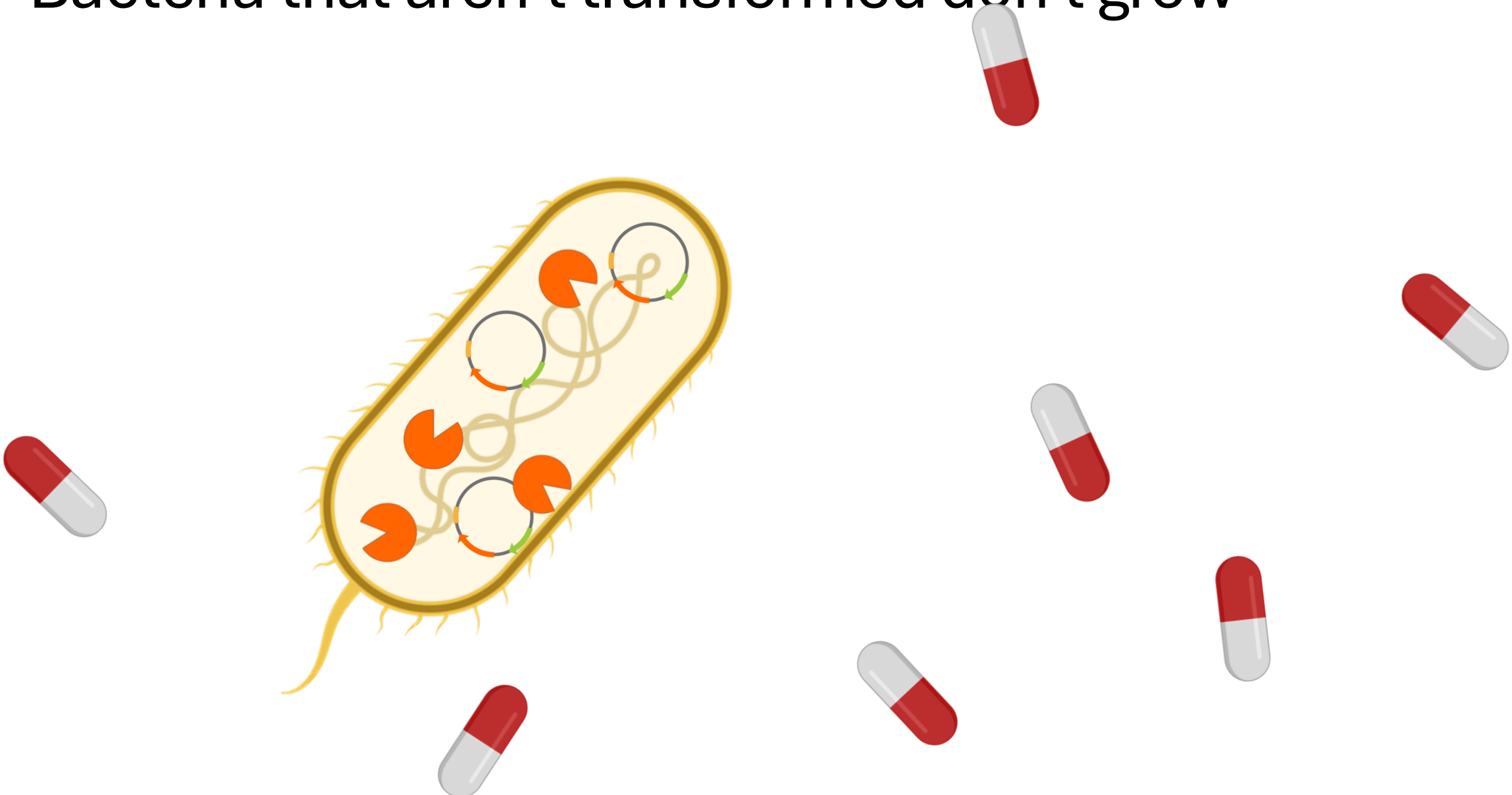
Bacteria plated on ampicillin plates



β-lactamase breaks down ampicillin

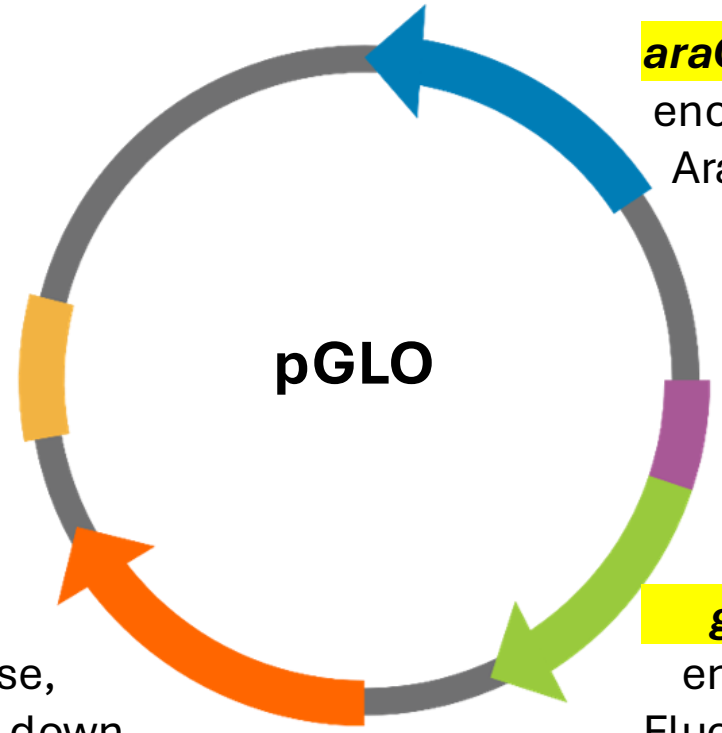


Bacteria that aren’t transformed don’t grow



Transformed bacteria grow into colonies

pGLO plasmid – regulation of GFP expression



araC gene
encodes the
AraC repressor protein

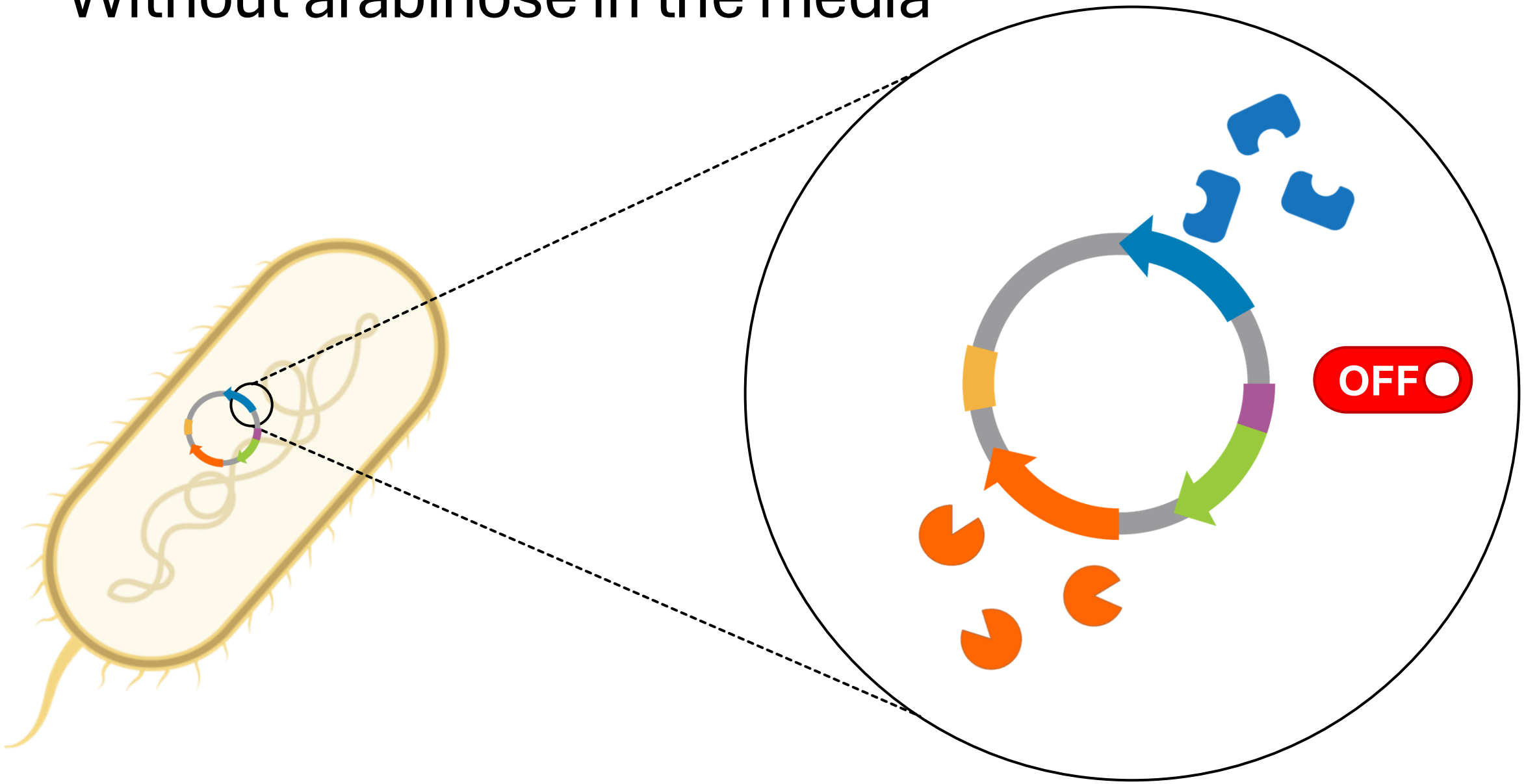
pBAD promoter
landing site for RNA polymerase
to transcribe downstream genes

***gfp* gene**
encodes Green
Fluorescent Protein (GFP)

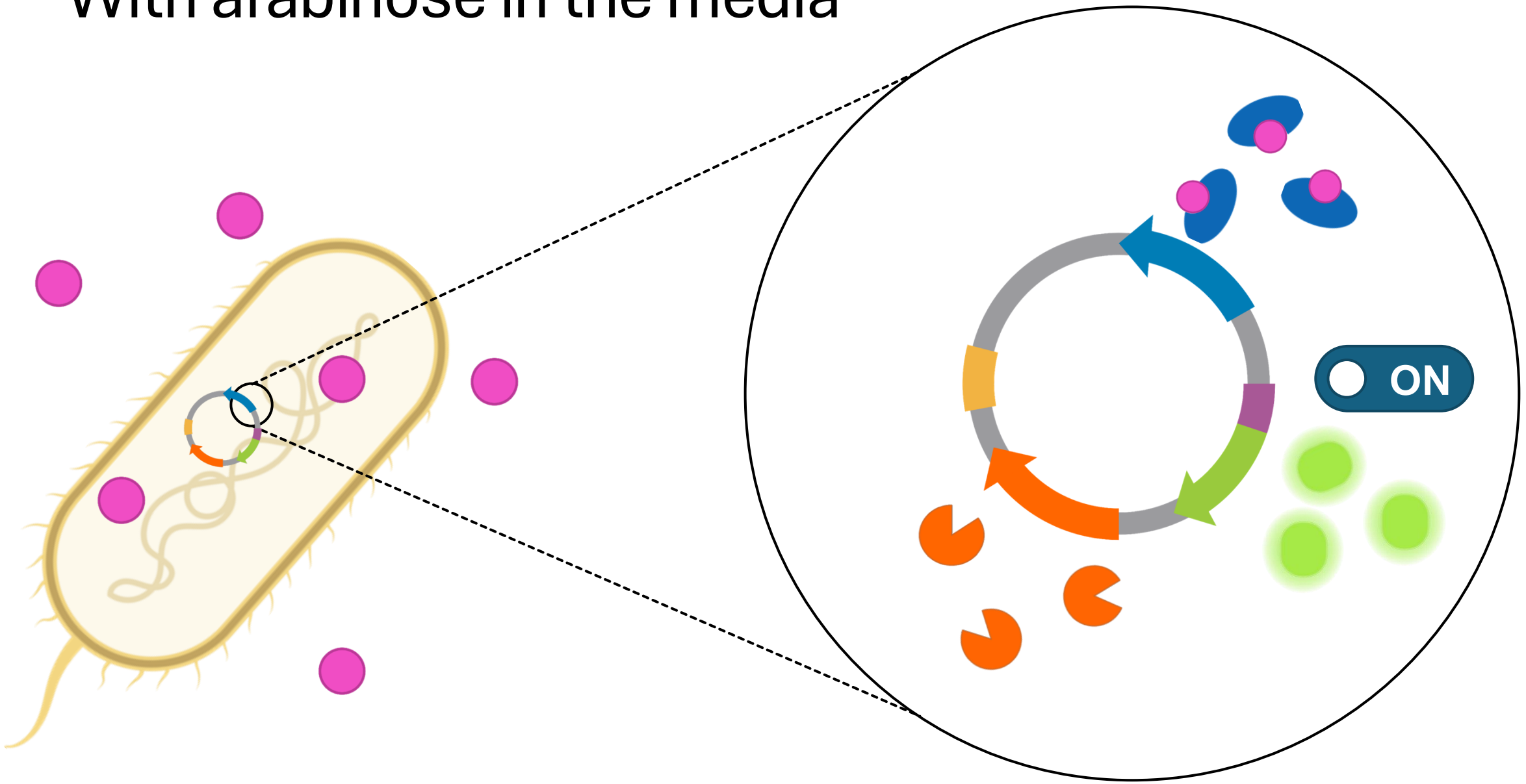
ori
origin of replication
allows bacteria to make
copies of plasmid

***amp*^{r} (or *bla*) gene**
encodes beta-lactamase,
an enzyme that breaks down
ampicillin (an antibiotic)

Without arabinose in the media

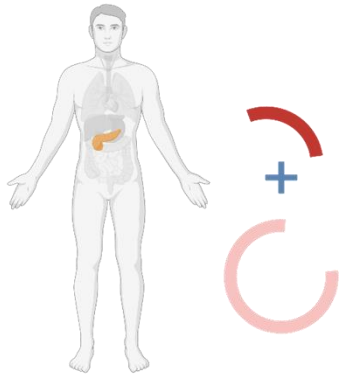


With arabinose in the media

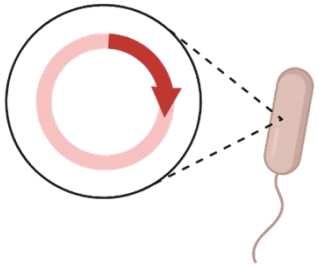


How do we produce drugs like insulin?

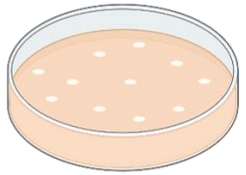
1. Clone human insulin gene into plasmid



2. Transform bacteria with plasmid

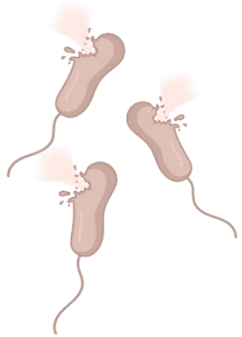


3. Select transformed bacteria

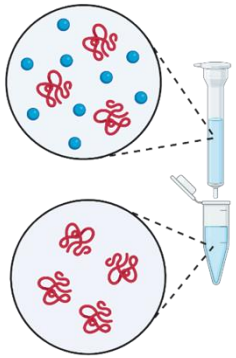


4. Culture bacteria and induce gene expression

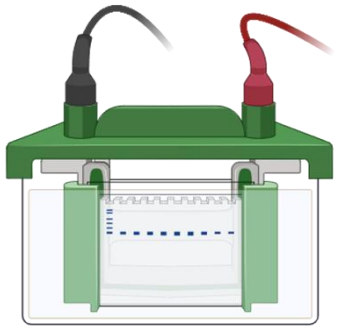
5. Lyse cells



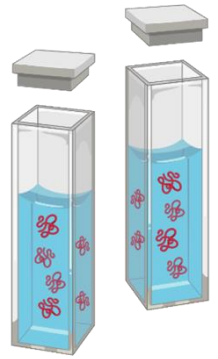
6. Purify protein with chromatography



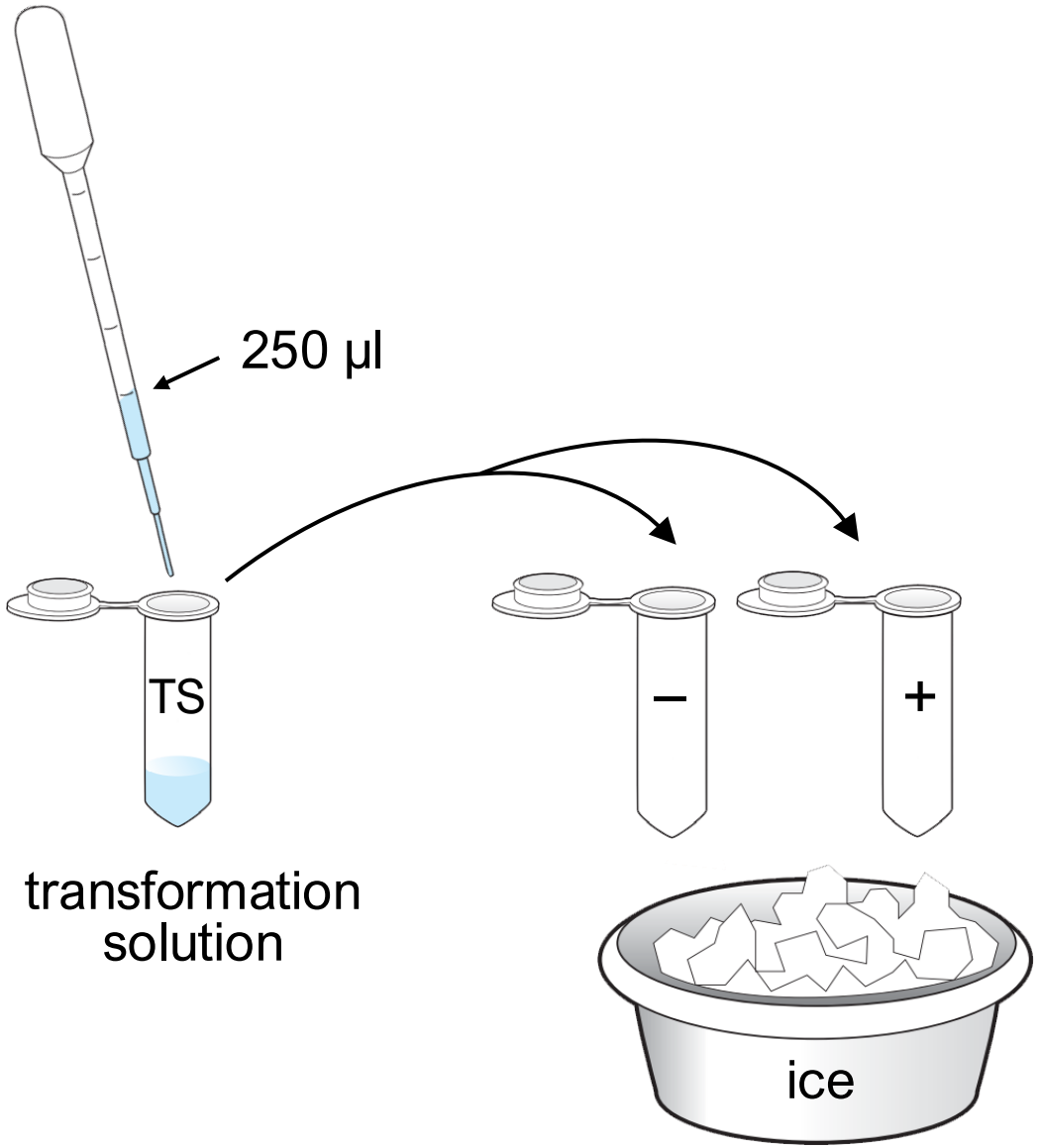
7. Analyze purification with gels



8. Assay protein activity



Lab protocol

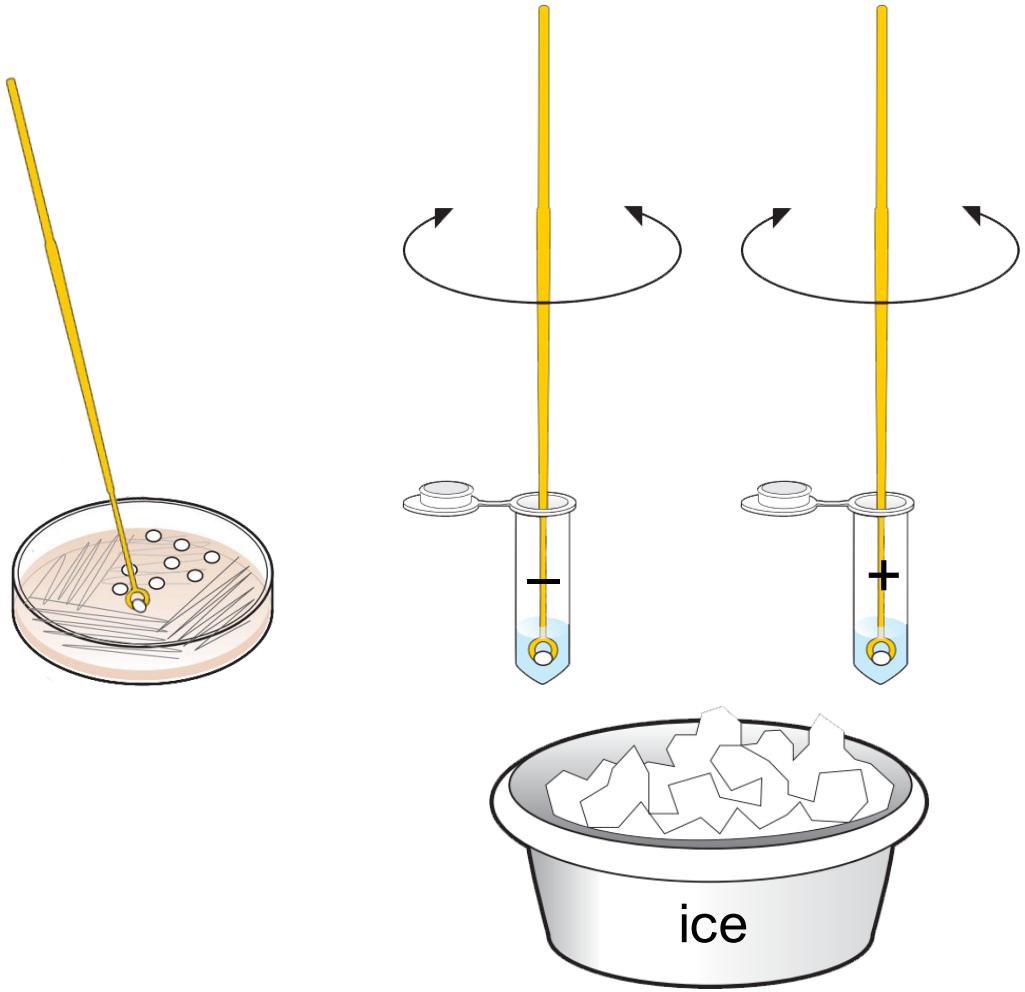


1. Label 1 empty tube – and the other +. Place tubes in foam rack.

2. Add 250 µl of transformation solution (TS) into each tube.

3. Place both tubes on ice.

Lab protocol

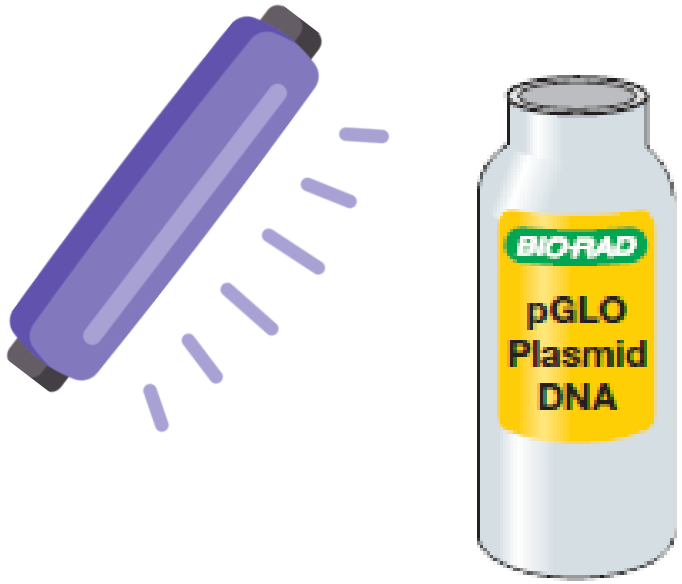


4. Use a loop to pick up 2-4 large colonies of bacteria from the starter plate.

5. Put the loop into the transformation solution in the – tube. Spin the loop between your fingers about 30 seconds, until the bacteria is completely distributed (no chunks). Close the tube and put it back on ice.

6. Using a new loop, repeat step 5 for the + tube.

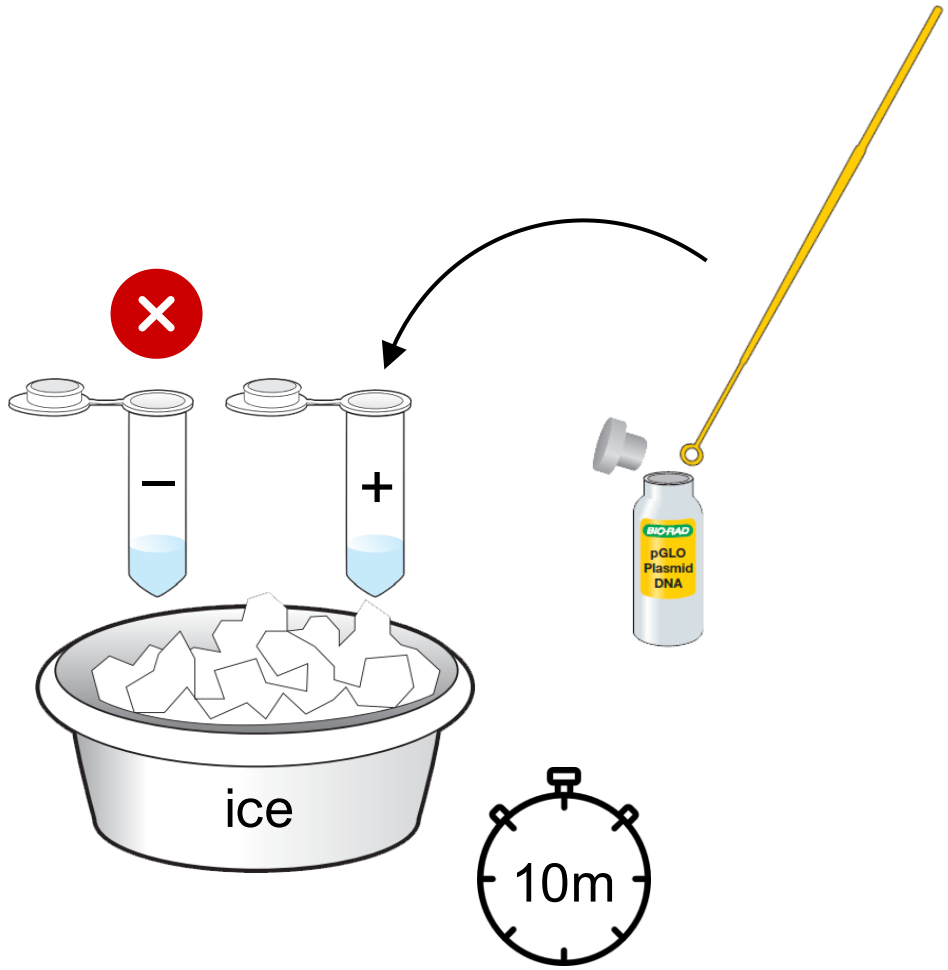
Look at the plasmid under UV light



What color is the DNA?

Can you see any green?

Lab protocol



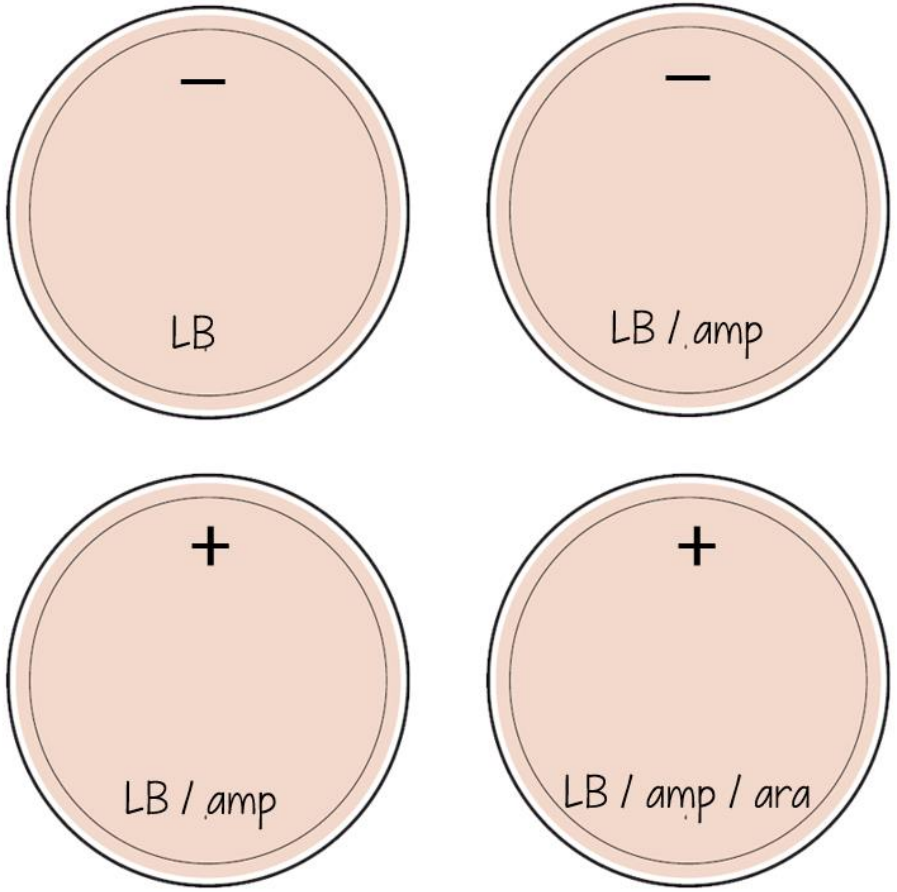
7. Use a new loop to transfer a loopful (~10 µl) of pGLO plasmid to the + tube. Swirl the loop in the tube to mix. Close the tube and put back on ice.

   Important! Do NOT put pGLO plasmid into (**–**) tube!

   The (–) means no DNA!

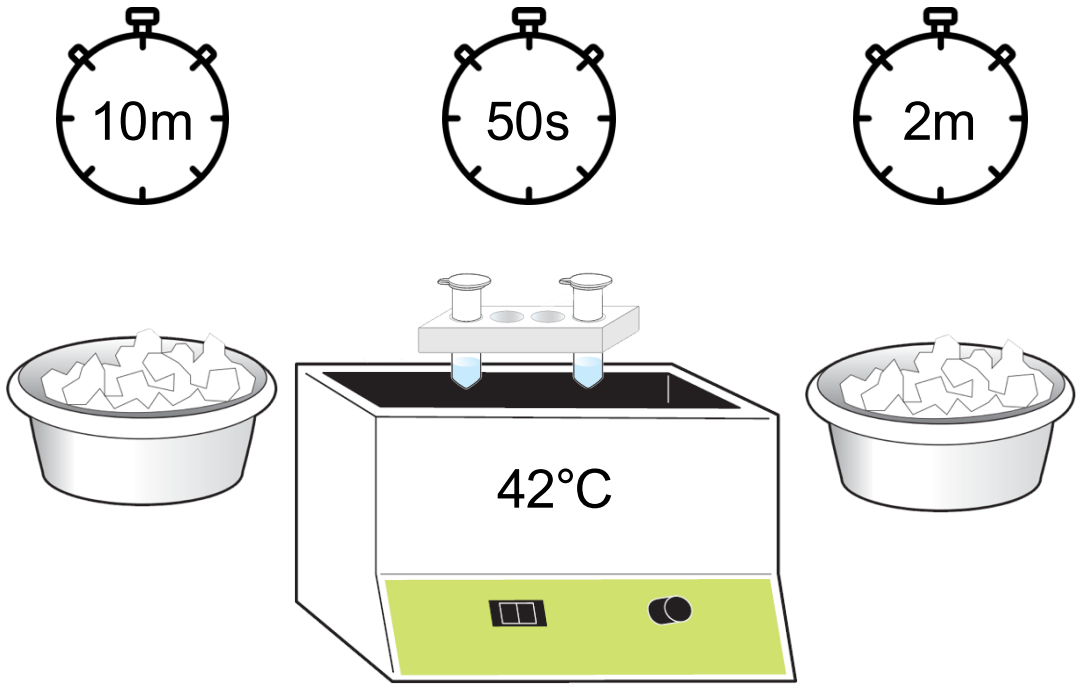
8. Incubate both tubes on ice for 10 minutes.

Lab protocol



9. While the tubes are on ice, label agar plates:
   - LB plate: **–**
   - LB/amp plate: **–**
   - LB/amp plate: **+**
   - LB/amp/ara plate: **+**

Lab protocol

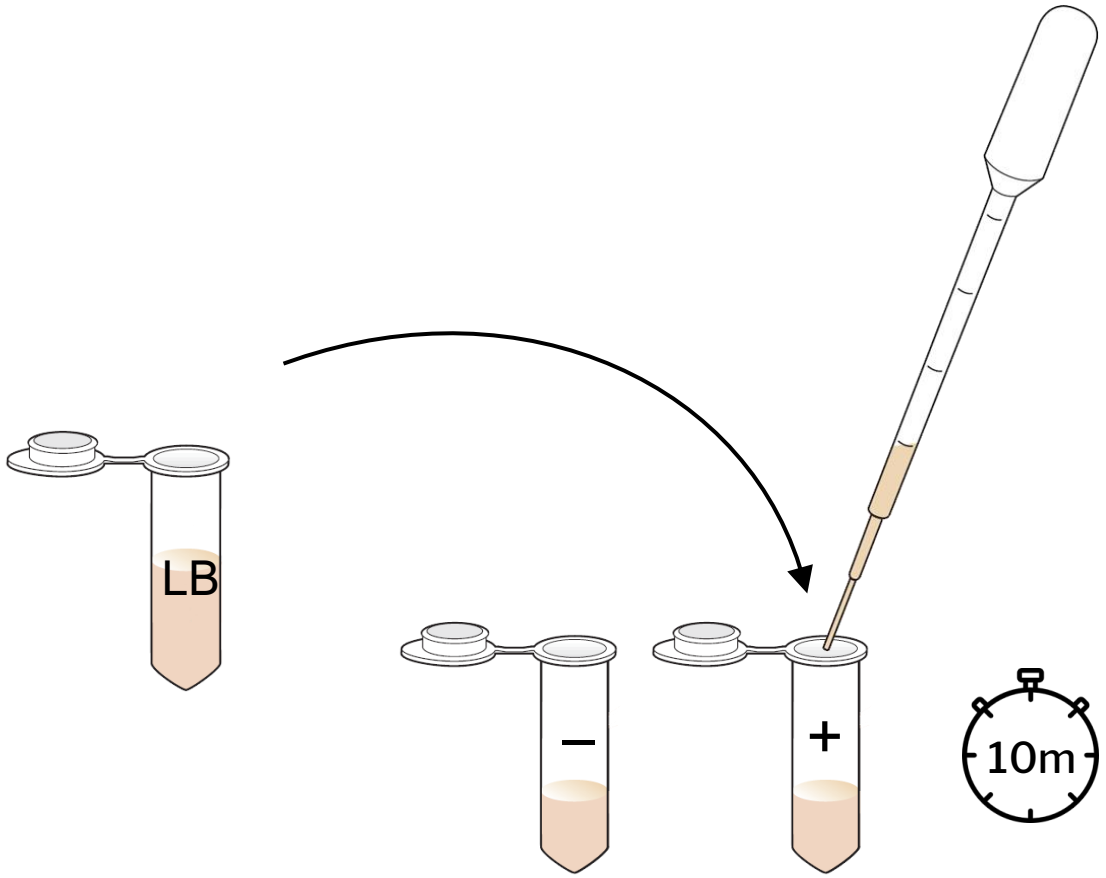


10. Heat shock. Bring your tubes on ice to the water bath. Set time for 50 seconds. Place the rack with the tubes in the 42°C water bath for exactly 50 seconds.

This is the transformation step – from being exposed to heat, the bacteria will take up the DNA

11. Put tubes on ice and incubate for 2 minutes.

Lab protocol

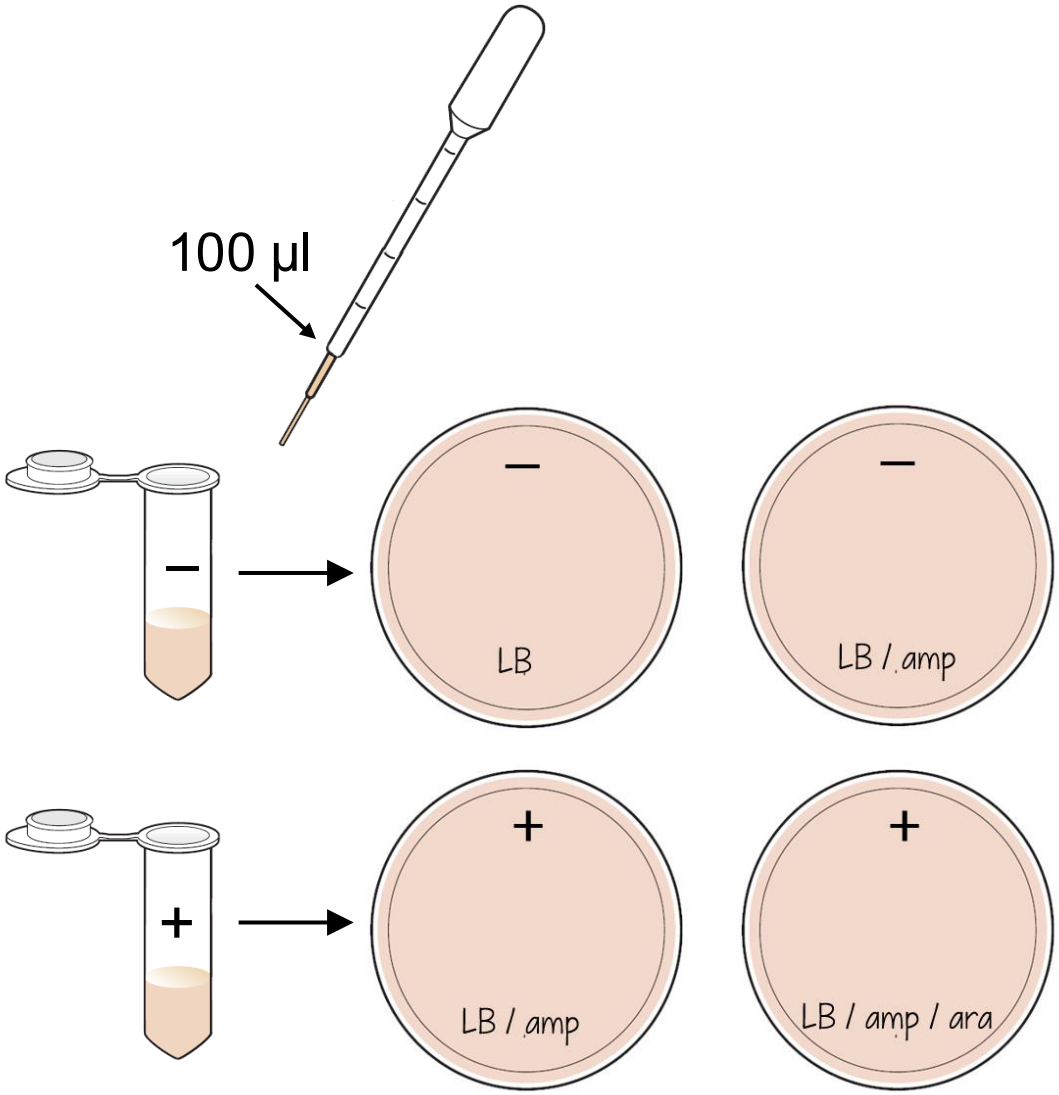


12. Move the tubes out of the ice and onto the benchtop.

13. Add 250 µl of LB broth (LB) to each tube. Use a new pipet for each tube.

14. Close the tubes and incubate at room temperature for 10 minutes.

Lab protocol



15. Flick the tubes a few times to mix up the bacteria.

16. Add 100 µl (about 1 drop) of bacteria from each tube to the appropriate plates. Use a new pipet for each tube.

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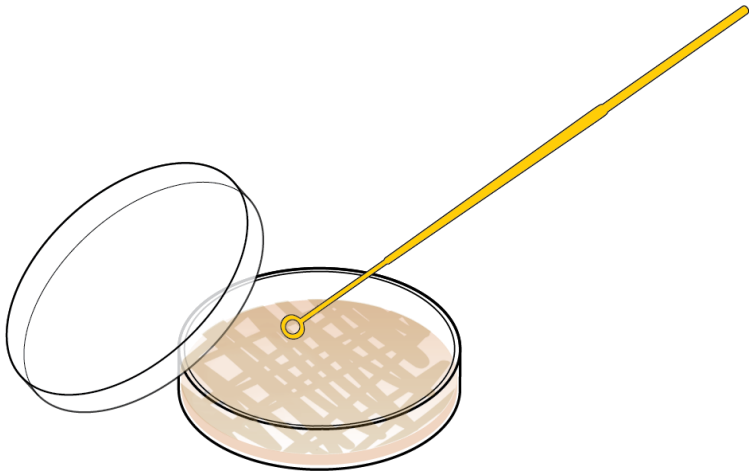
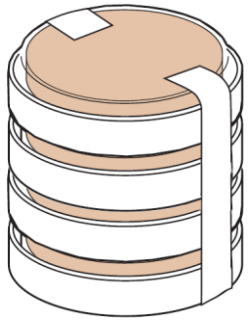
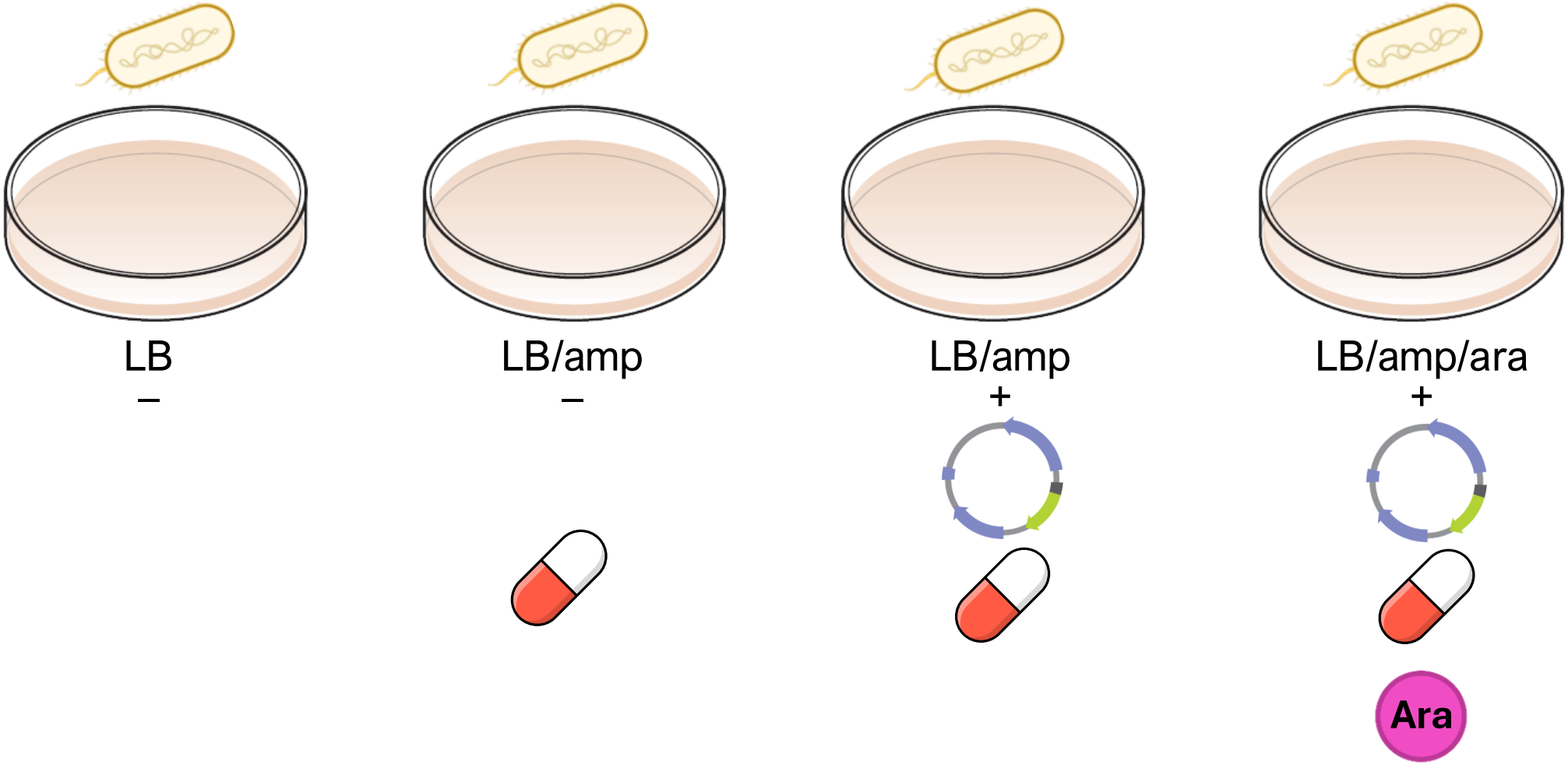
Lab protocol



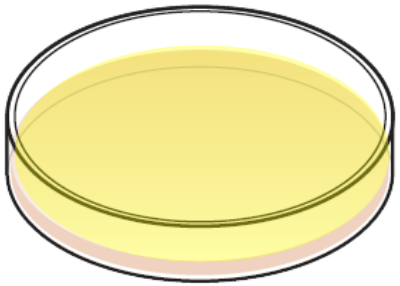


17. Use a loop to spread the bacteria all over the agar. Use a new loop for each plate.

18. Stack plates upside down, tape together, and add lab group or initials. Incubate at 37°C overnight, or at room temperature for 2 days.

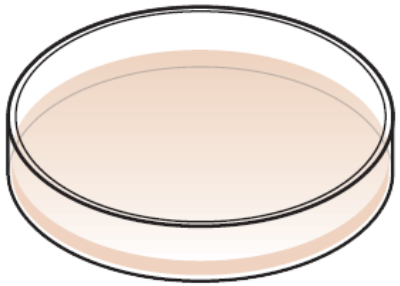

LB
–
LB/amp
–
LB/amp
+
LB/amp/ara
+
Ara

Expected results



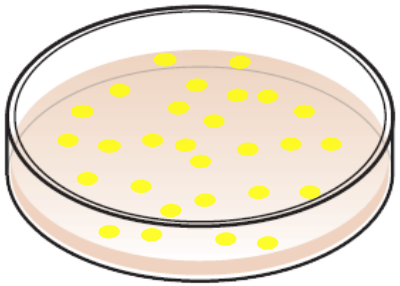
LB
–

Off-white lawn of bacteria covering plate



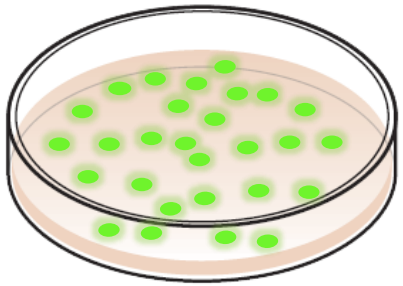
LB/amp
–

No bacterial growth



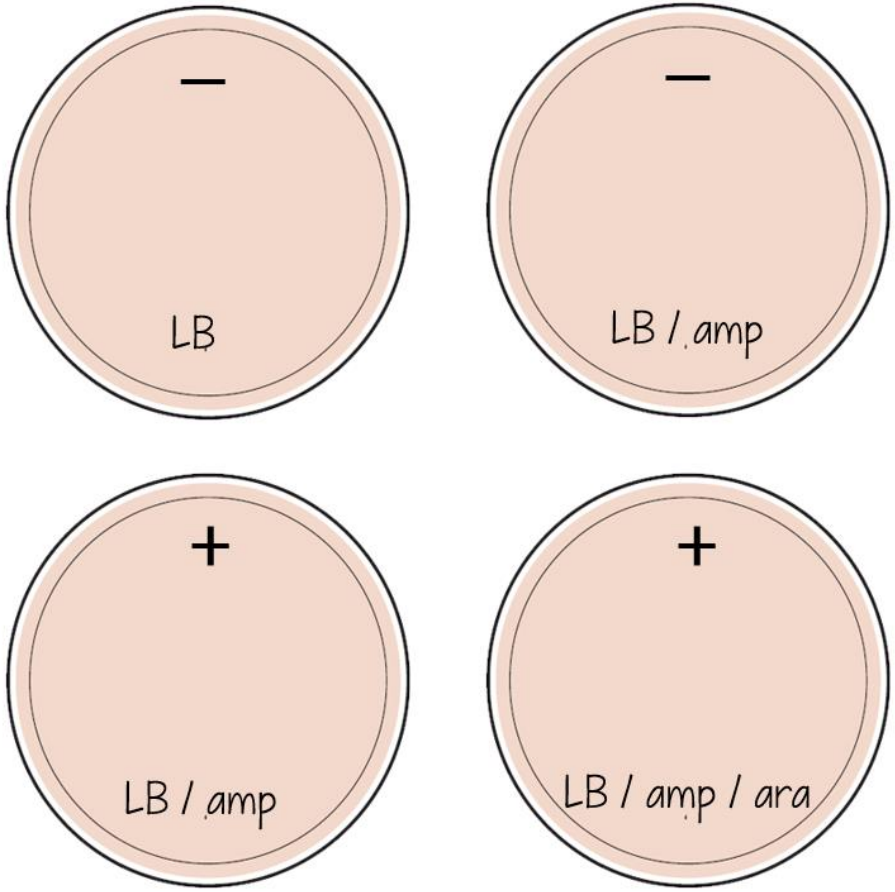
LB/amp
+

Many off-white colonies (~75)



LB/amp/ara
+

Many off-white colonies that glow green under UV light



I will upload photos of your plates to Canvas tomorrow – you will use this to do the calculations on page 19

Make sure you write your names on your plates.

Do not answer the questions! That is what the conclusion & summary assignment on Canvas is for