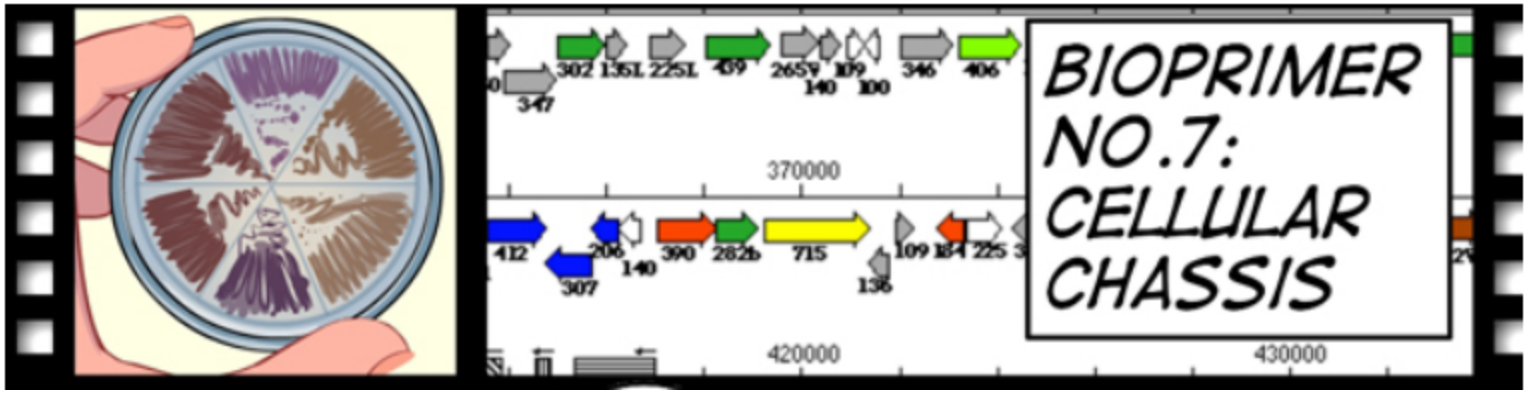
BioBuilding: What a Colorful World



BioBuilder

Lab 4: What a Colorful World

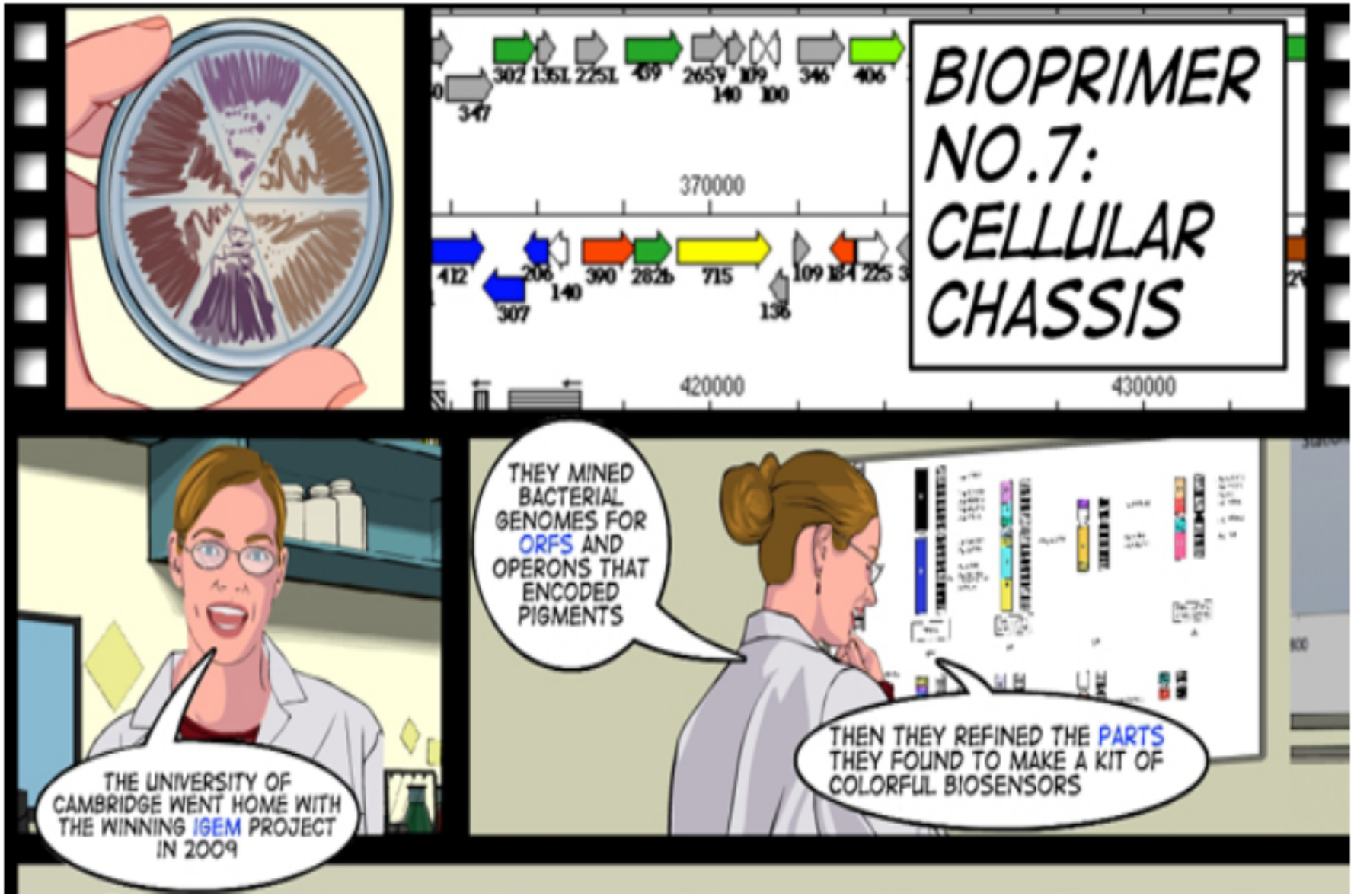
- Simplifying assumptions about "the cell" are brought into question when different strains are transformed with DNA that makes them grow in colorful ways.

Acknowledgments: This lab was developed with materials from the University of Cambridge 2009 iGEM team, as well as guidance and technical insights from Drew Endy and his BIOE.44 class at Stanford University

Objectives

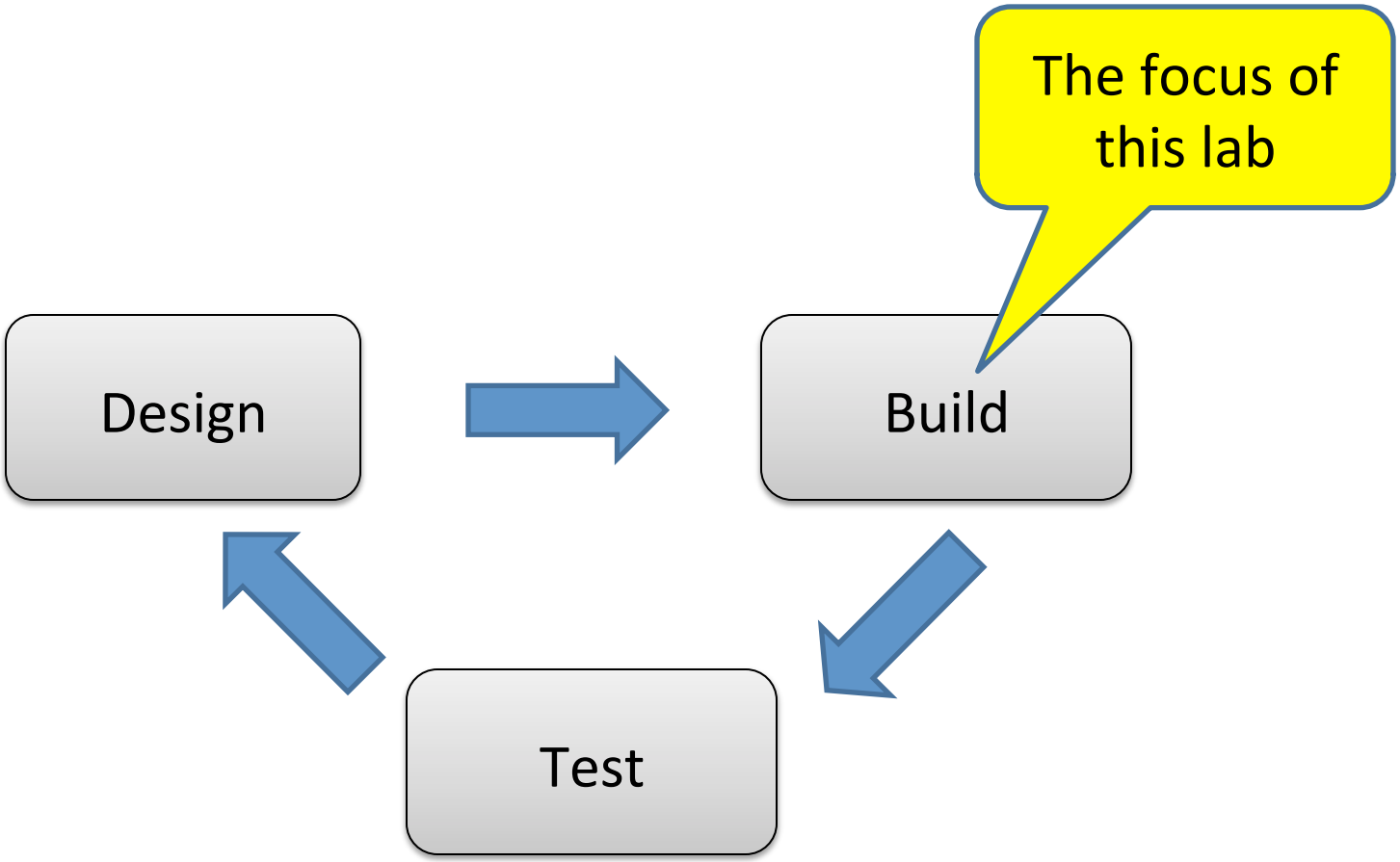
By the conclusion of this laboratory investigation, the student will be able to:

- Define and properly use synthetic biology terms: chassis, system, device, minimal cell, sensor, color generator.
- Define and properly use molecular genetics terms: operon, gene expression, bacterial transformation.
- Explain the role of chassis in synthetic biology and engineering.
- Conduct and interpret the results of a bacterial transformation.


BIOPRIMER NO.7: CELLULAR CHASSIS
370000
420000
430000
THE UNIVERSITY OF CAMBRIDGE WENT HOME WITH THE WINNING IGEM PROJECT IN 2009
THEY MINED BACTERIAL GENOMES FOR ORFS AND OPERONS THAT ENCODED PIGMENTS
THEN THEY REFINED THE PARTS THEY FOUND TO MAKE A KIT OF COLORFUL BIOSENSORS

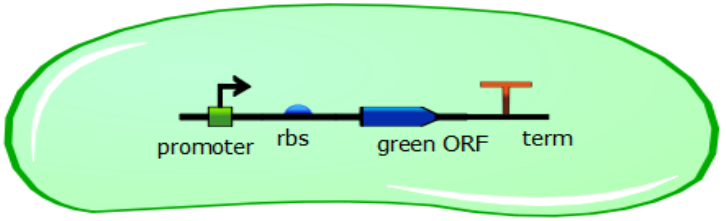

ONE THING THAT PUZZLED THEM, THOUGH, IS HOW DIFFERENT SOME OF THE COLORS LOOKED, DEPENDING ON THE TYPE OF E. COLI THEY TRANSFORMED
I'D REALLY LIKE TO KNOW IF THE CELLULAR "CHASSIS" ALWAYS MAKES SUCH A DIFFERENCE!
AND IF IT DOES MAKE A DIFFERENCE, I'D LIKE TO KNOW WHY.
SAVE/PRINT AS PDF
YOUR TURN: COMPARE THE COLORS YOU GET WHEN YOU TRANSFORM SOME COLOR GENERATORS

An engineering paradigm

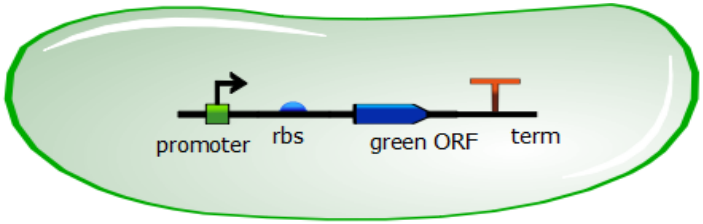


There are two major laboratory strains of E. coli:

The K 12 strain



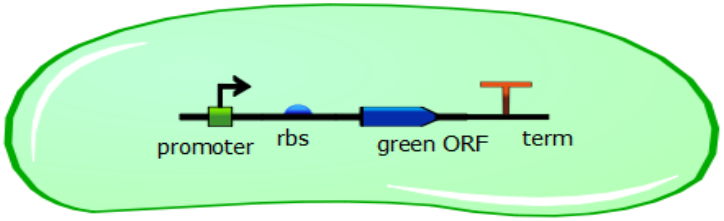
The B strain



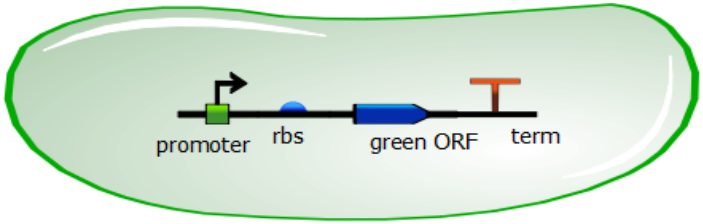
A biology question: Do they behave the same?

In Synthetic Biology, the cell into which the genetic device is inserted is known as the ***Chassis***

The K 12 strain



The B strain

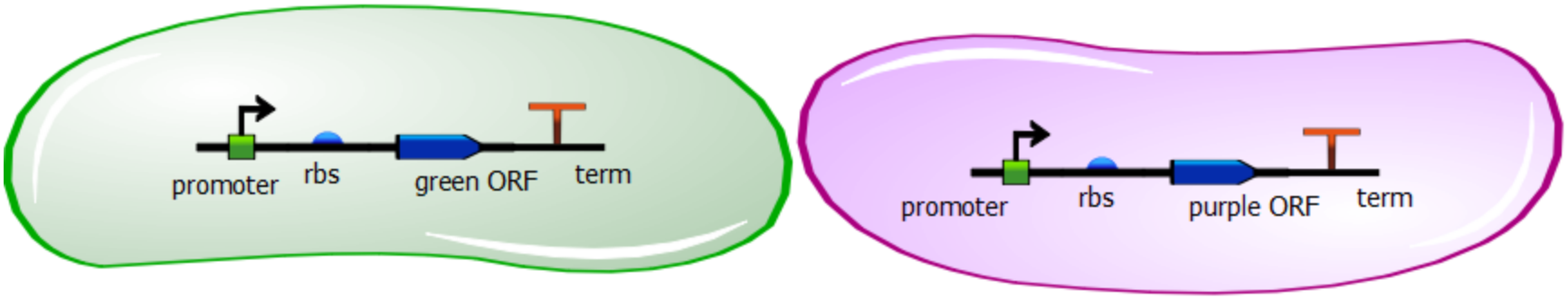


An engineering question:
If we insert the same device into each chassis, will it behave the same?

E.chromi

In 2009, the Cambridge iGEM team altered an operon found in *Chromobacterium* and inserted it into *E. coli*.

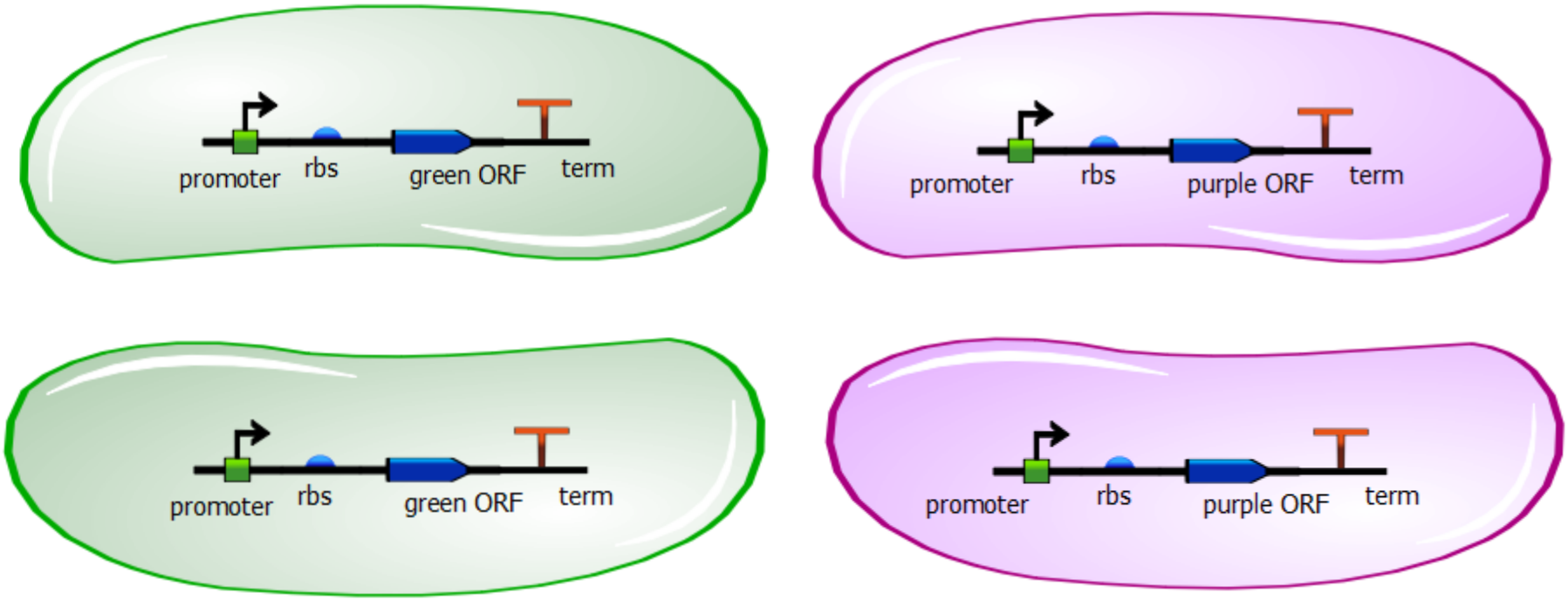
They found that they could organize the operon in one way to get a green color and in another way to get a purple color.



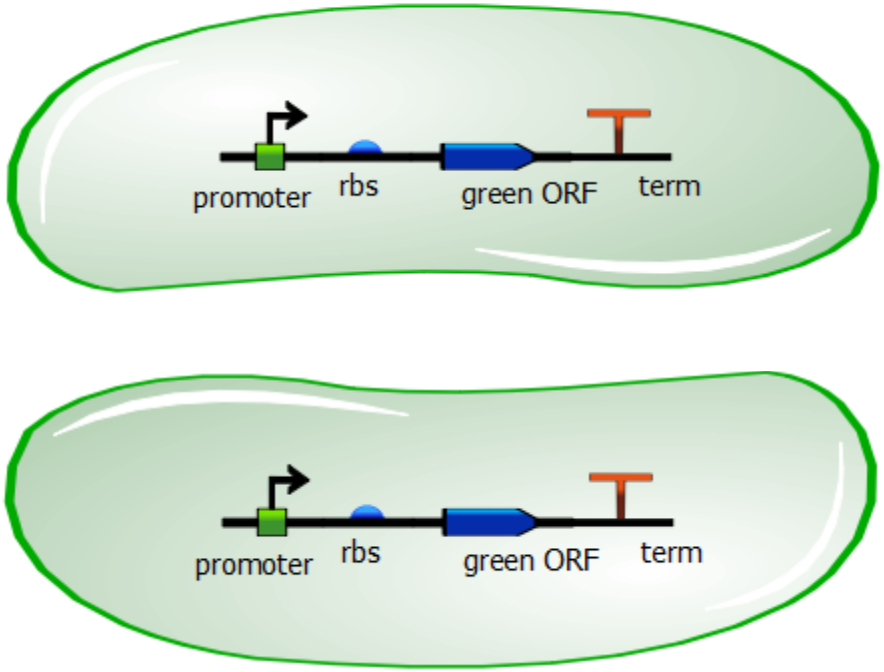
They moved these devices into *E. coli* because, well, everyone loves working with *E. coli*…

E.chromi

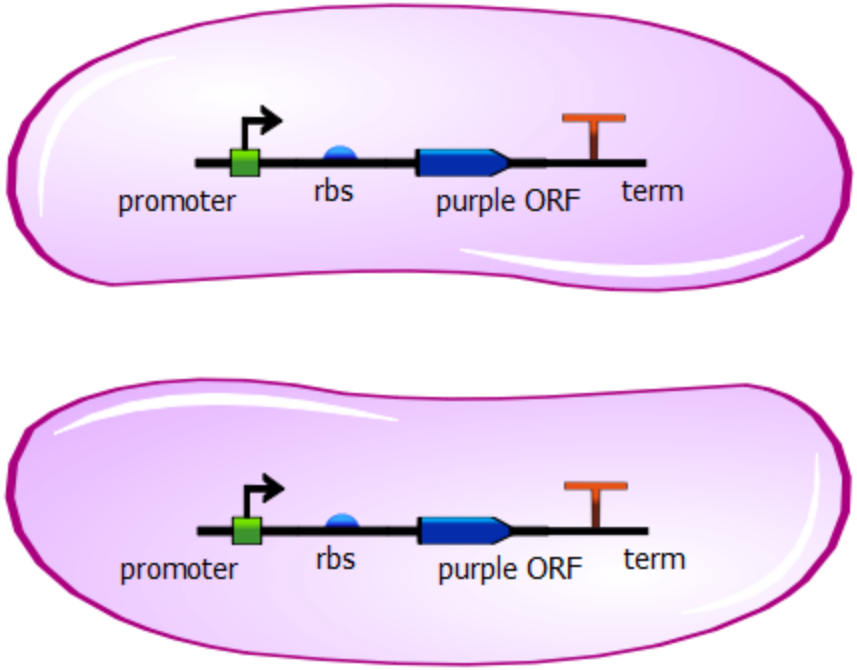
But will these devices work the same in the different strains of *E. coli*?



We will insert the green color generating device into each strain. Will we get the same colors and appearance of the bacteria?



We will insert the purple color generating device into each strain. Will we get the same colors and appearance of the bacteria?

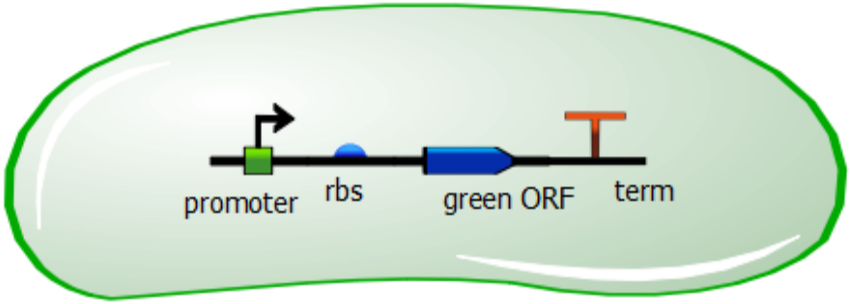


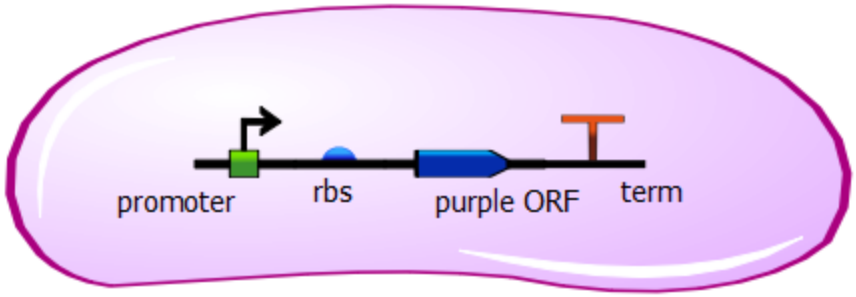
Inserting genes into a cell is known as **Transformation**:

We have the operons on plasmids that have been made for us.

We need to make the bacterial cells **competent** to take up the plasmids.

Transformation is tough on the bacteria so we need to be very careful.





E.chromi

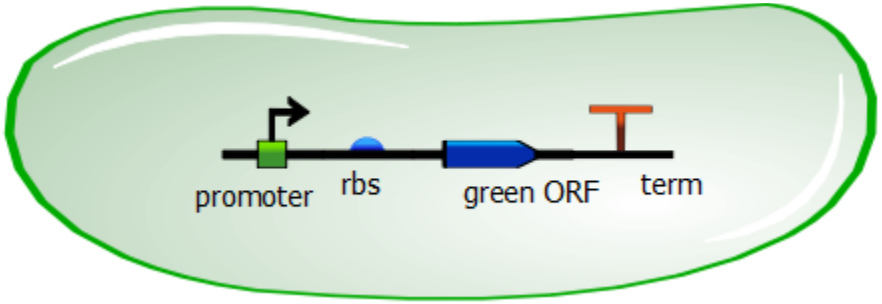
The procedure (summarized):

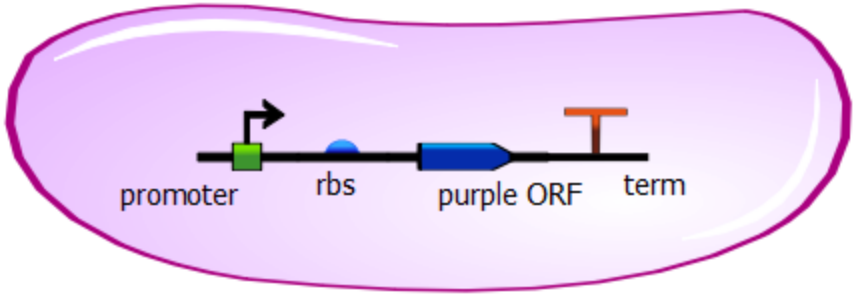
Scrape up a patch of cells of each strain.

Add $CaCl_2$ transformation buffer and keep the cells on ice.

Divide the cells of each strain into three vials.

One will get the purple DNA plus a gene for ampicillin resistance
One will get the green DNA plus a gene for ampicillin resistance
One will get no DNA. Why?





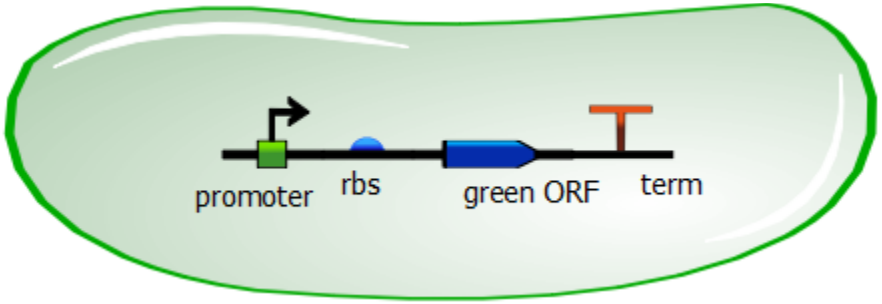
The procedure (summarized):

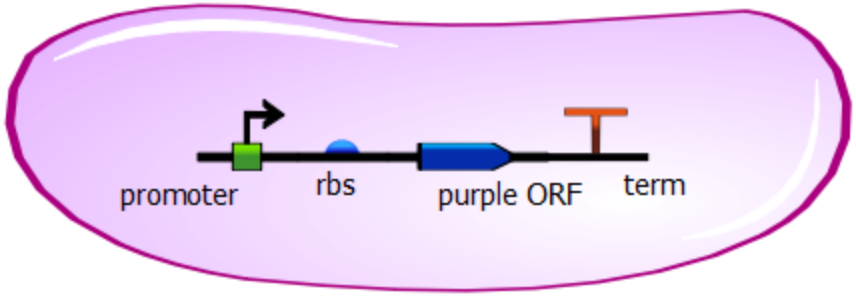
Heat the cells for exactly 90 seconds.

Add LB media to help the cells recover.

Plate the cells on LB plates containing ampicillin.

Why the ampicillin?





The procedure (summarized):

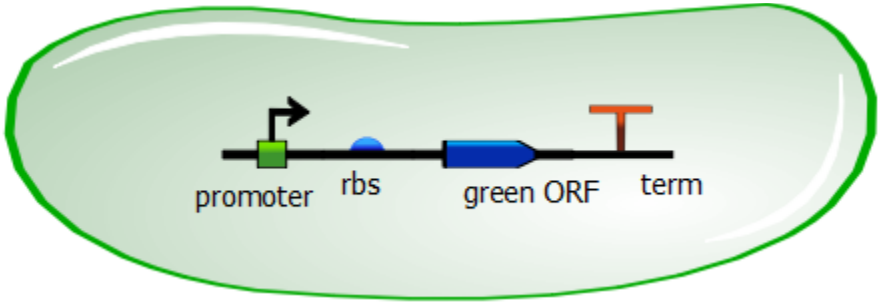
Then we incubate the cells overnight.

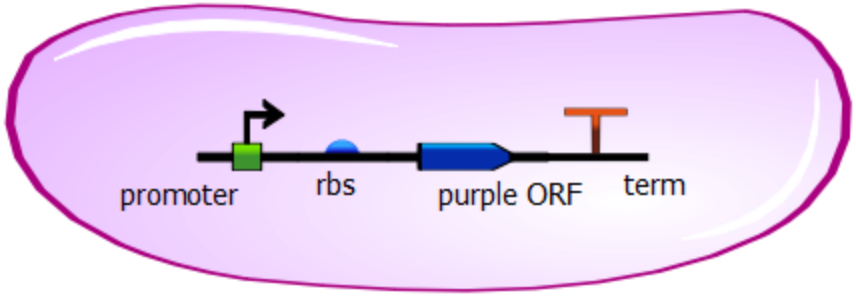
Tomorrow we will look for purple and green colonies.

Any guesses as to what we will see?

We will also calculate how efficient the transformation is.

Report your data.





You will need to set up a data table like this:

Strain	Plasmid	Colony Number on LB (if used)	Colony Number on LB + Amp	Transformation Efficiency (colonies/microgram DNA)	Color/shape/size on LB (if used)	Color/shape/size on LB + Amp
4-1	no DNA					
	pPRL					
	pGRN					
4-2	no DNA					
	pPRL					
	pGRN					