

Inducing Expression of Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP)

Module 2, Lesson 4

What is Inducible Protein Expression?

Inducible protein expression is the ability to ‘turn on’ and/or ‘turn off’ protein expression in a cell at a specific time.

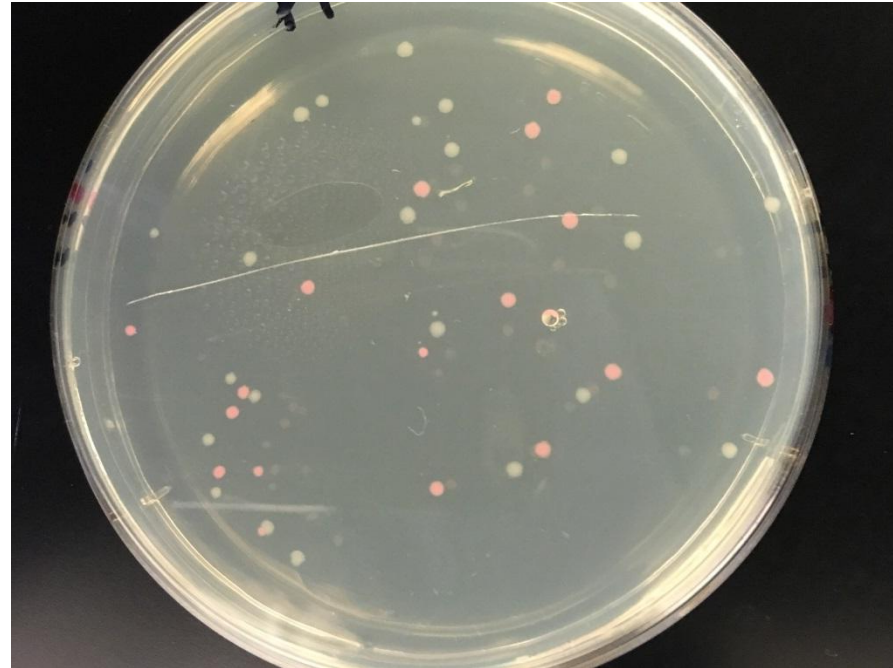
Reasons to use Inducible Protein Expression

- **To study the effect of the protein in the cell only at certain times.**

Example: You want to study the effect of a protein at a specific stage of cell development or cell growth.

- **The production of the protein is toxic to the cell. You want to turn on protein production only for a short time and then turn it off.**
- **The production of the protein impedes cell growth.**

How do you induce *E. coli* to produce RFP or GFP?



White colonies = bacteria that are not producing RFP

Pink colonies = bacterial that are producing RFP

What is the difference?

Plasmids Containing Inducible Markers

A plasmid containing RFP or GFP under the control of an inducible element allows you to turn on the expression of RFP or GFP.

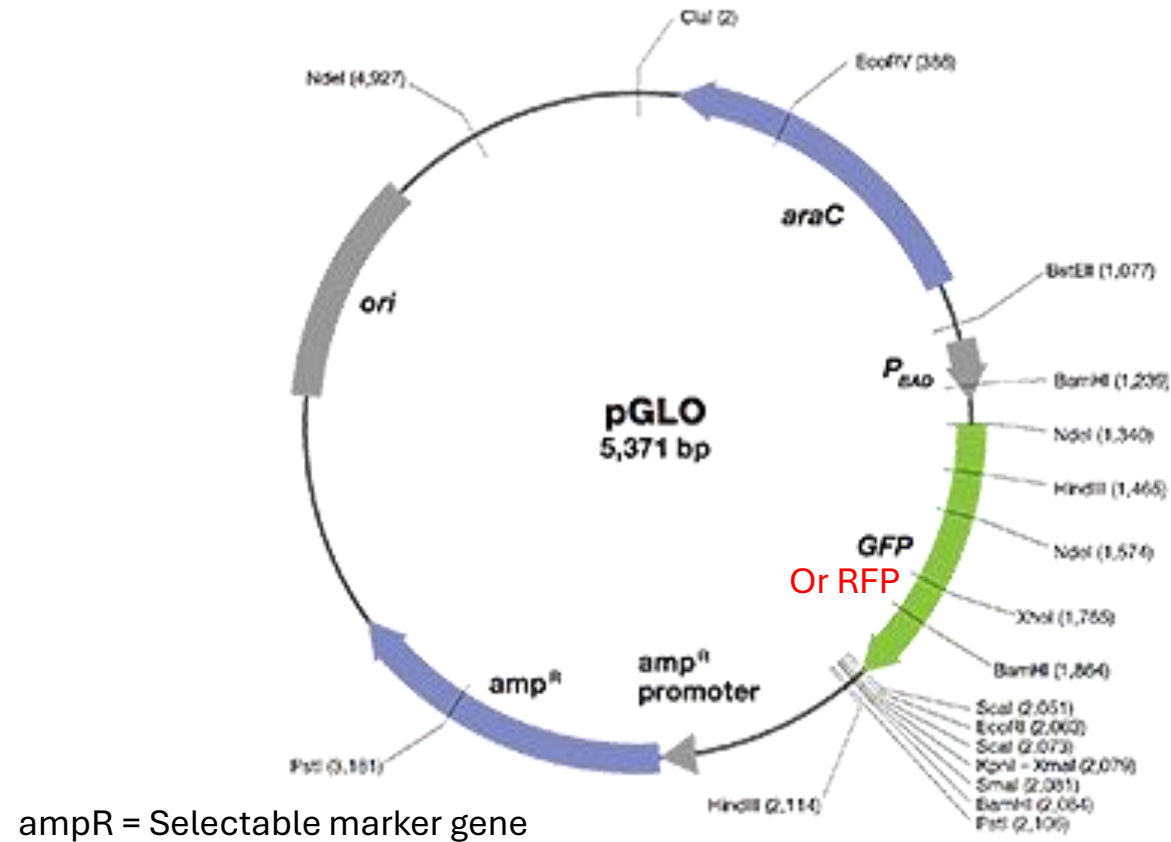
The L-arabinose or *ara* operon is used in the ABE RFP+ plasmid and in the BioRad pGLO (GFP+) plasmid.

This system can control gene production through an inducible promoter regulated by the *araC* gene product (the *araC* protein) and the sugar arabinose.

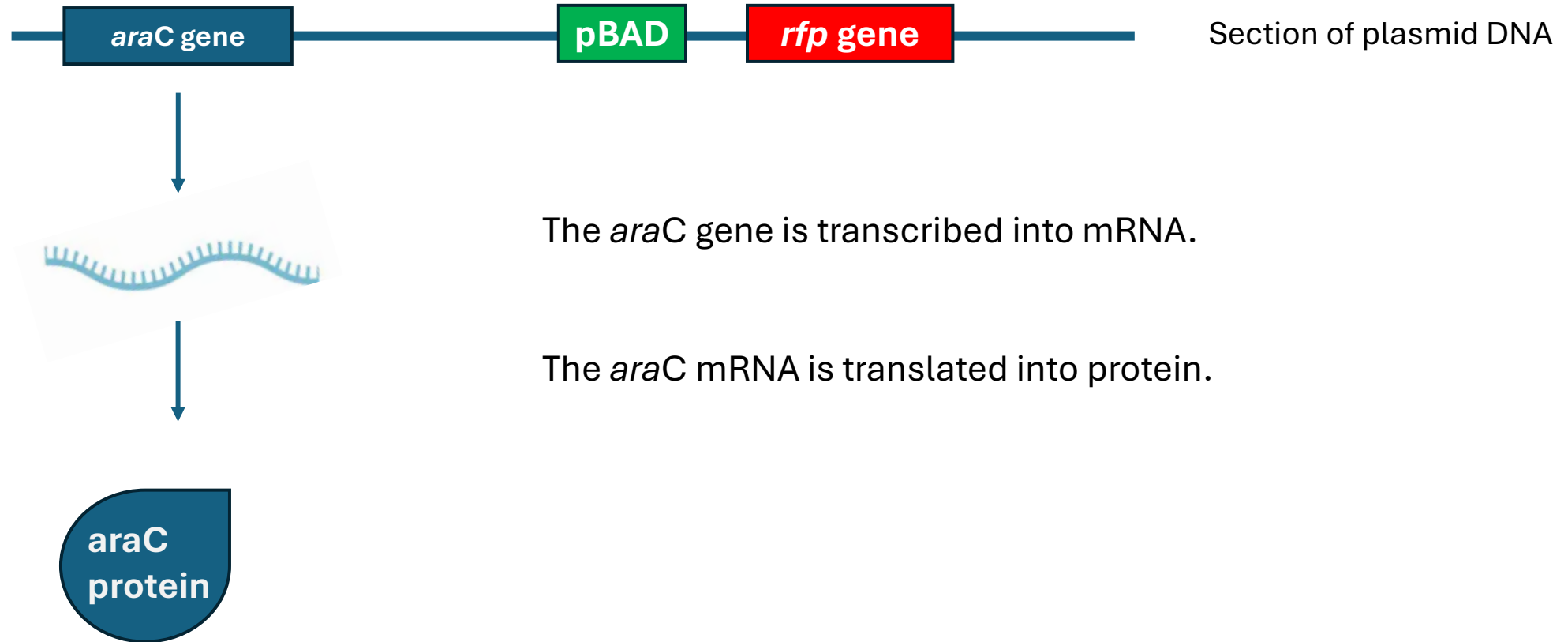
In the presence of arabinose, the AraC protein helps initiate gene transcription and protein production.

In the absence of arabinose gene transcription and protein production is halted.

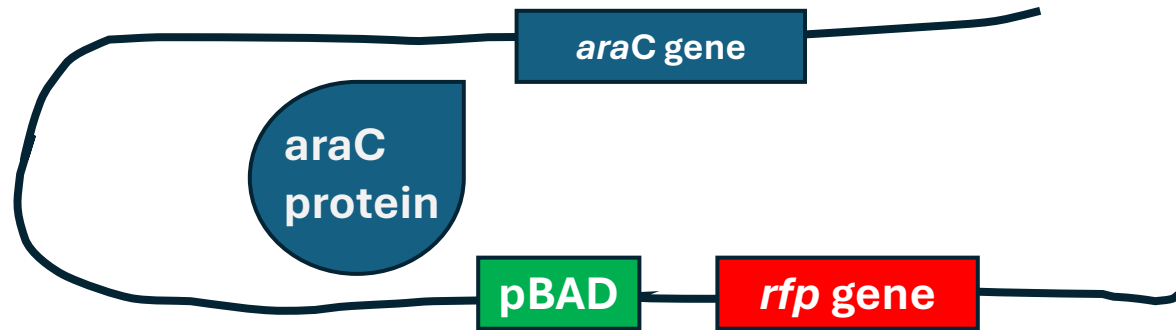
Example Plasmid Diagram



Mechanism of Protein Induction with araC



Mechanism of Protein Induction with araC

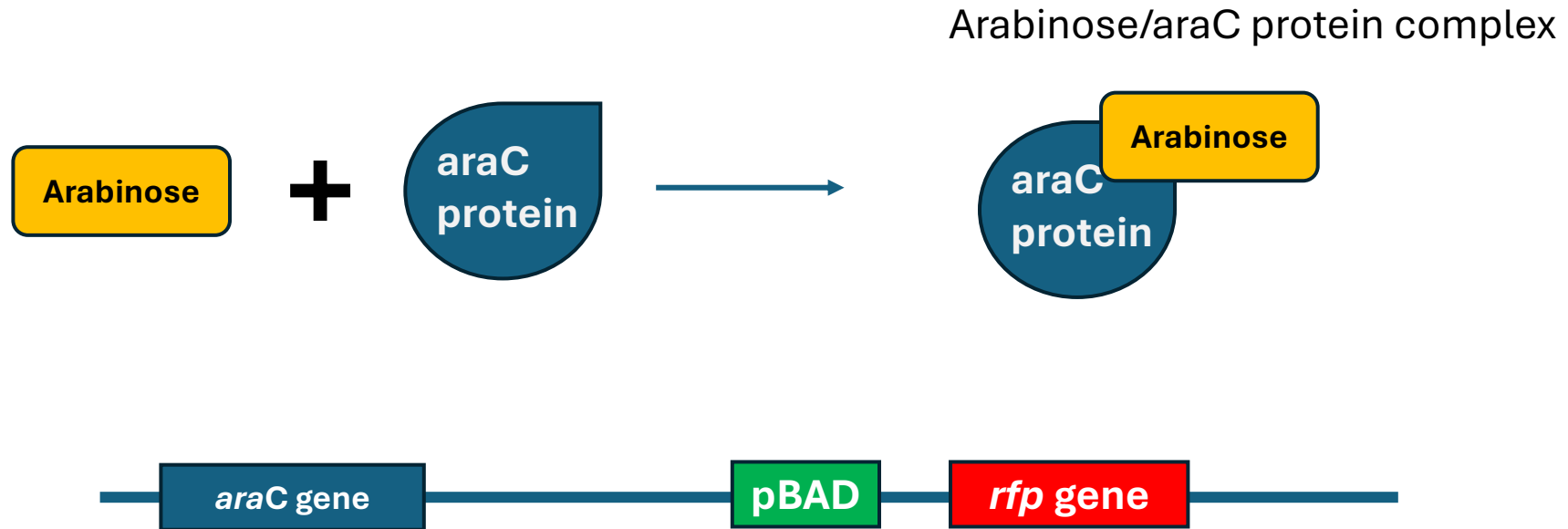


The araC protein causes a loop to form in the region of the DNA close to the gene of interest (in this case *rfp*) that is under the control of the pBAD promoter.

This loop prevents the binding of RNA polymerase to the pBAD promoter so the gene cannot be transcribed into mRNA.

If the *rfp* gene cannot be transcribed, then it cannot be translated into rfp protein.

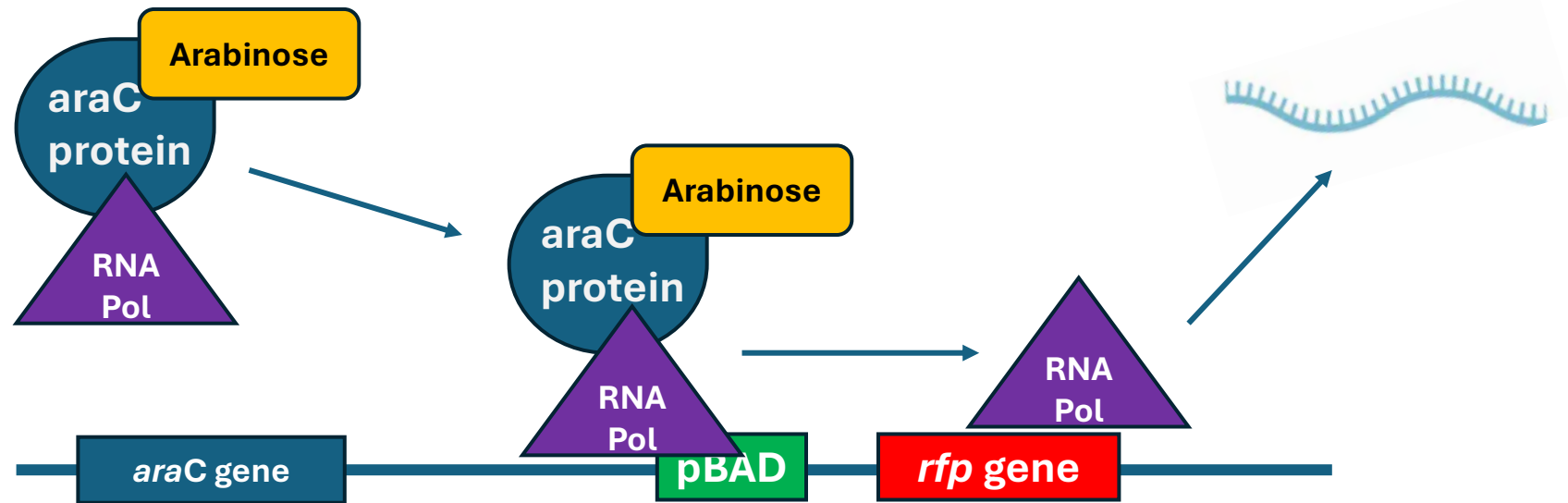
Mechanism of Protein Induction with araC



In the presence of arabinose, an arabinose/araC protein complex forms.

The formation of this complex inhibits the ability of the araC protein to cause the formation of the DNA loop.

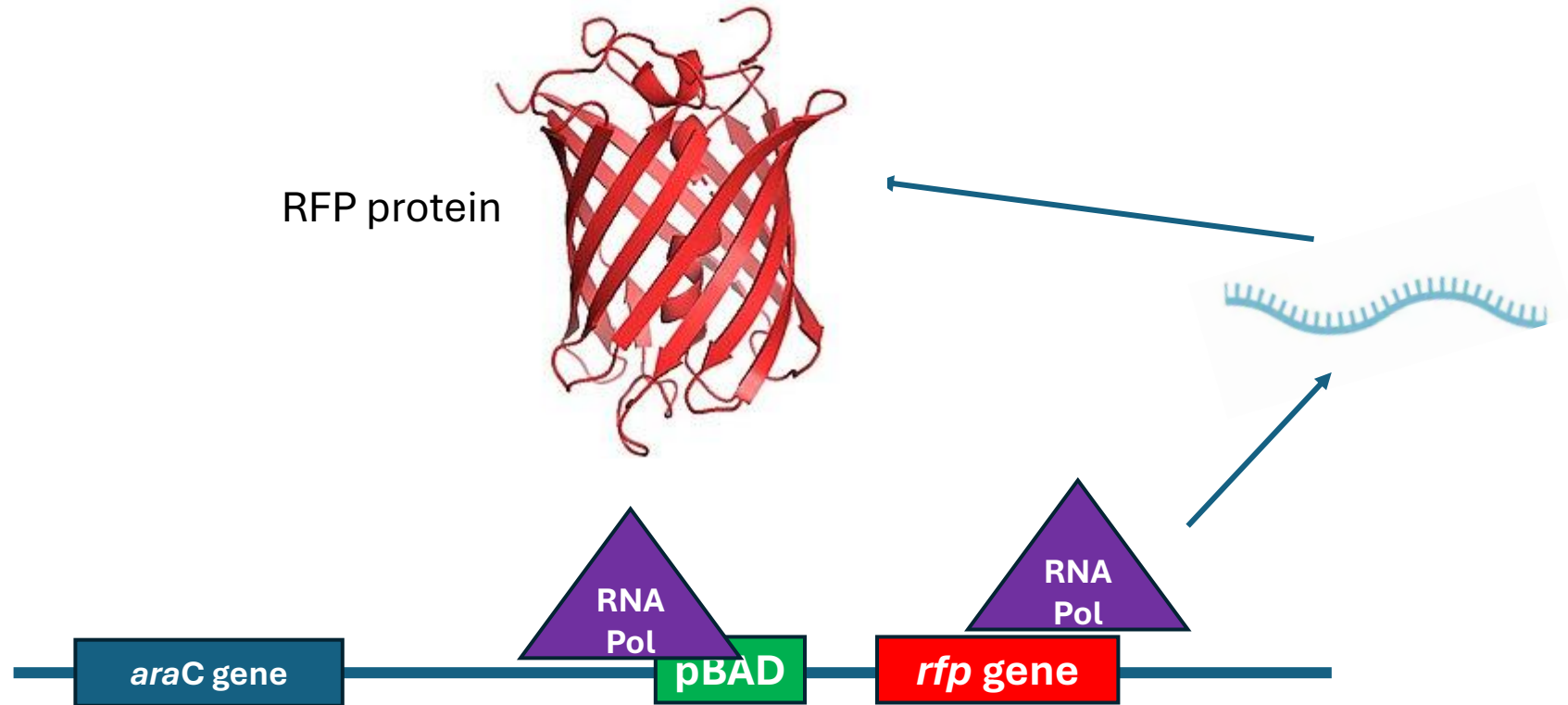
Mechanism of Protein Induction with araC



The arabinose/araC protein complex also helps the RNA polymerase bind properly to pBAD.

Now transcription can take place, resulting in production of rfp mRNA.

Mechanism of Protein Induction with araC



The *rfp* mRNA can then be transcribed into *rfp* protein.

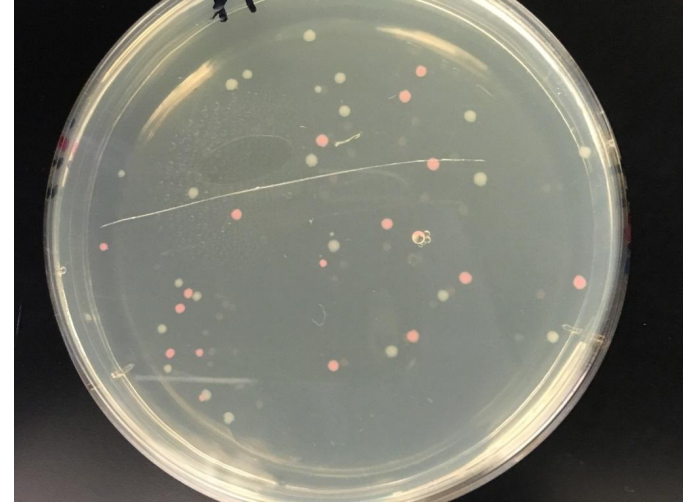
Both RFP and GFP proteins have similar barrel-like structures.

Protein structure image: <https://en.wikipedia.org/wiki/MCherry>

Bacterial Transformation and Selection

Transform bacteria with a plasmid that contains:

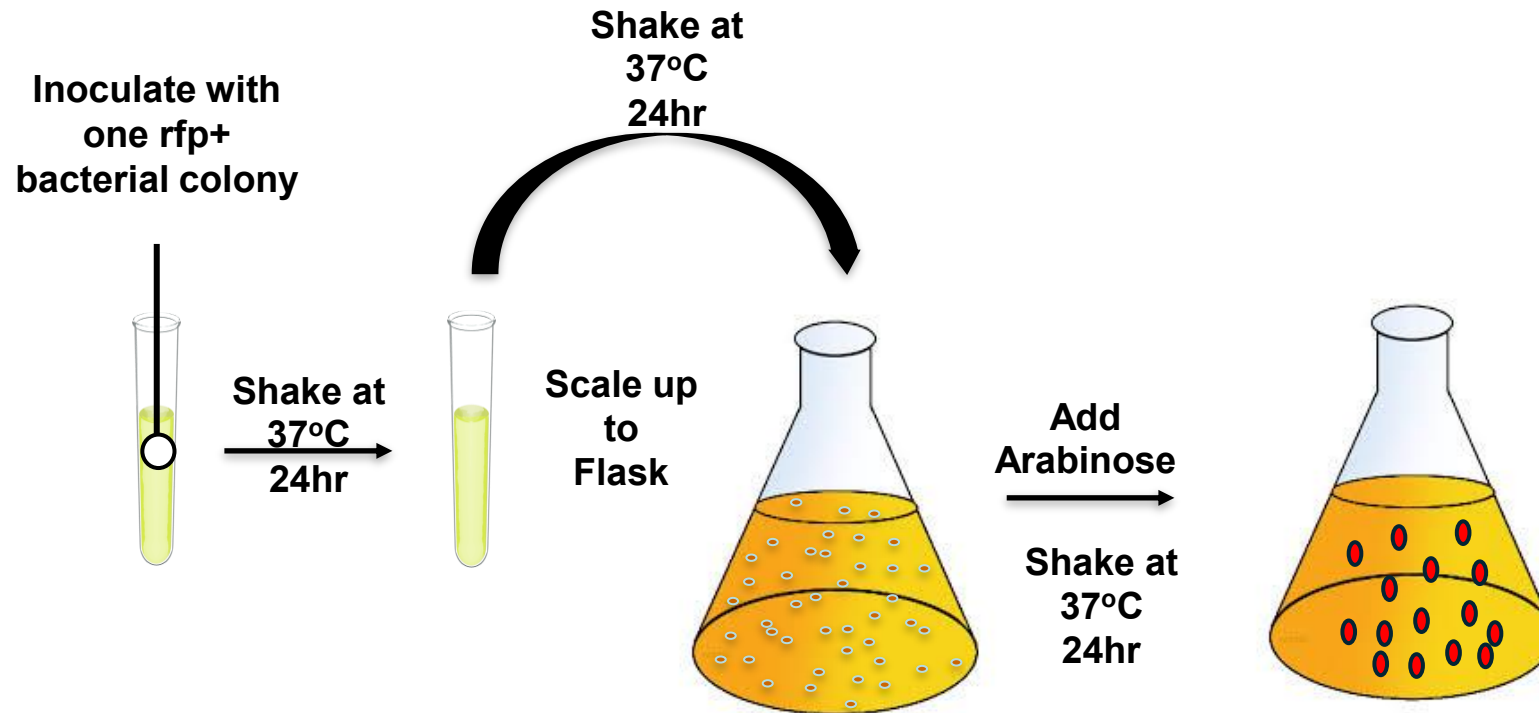
- *rfp* or *gfp* gene under the control of pBAD
- *araC* gene
- selectable marker gene like ampicillin resistance



Grow the transformed bacteria in the presence of the selectable marker (ampicillin) and arabinose.

Bacteria containing the plasmid and making rfp protein will be pink in color.

Scaling up Production of RFP (or GFP)



The production of RFP (or GFP) protein inhibits bacterial growth.

So, when scaling up protein production, you grow a large amount of bacteria before inducing rfp protein expression by adding arabinose.