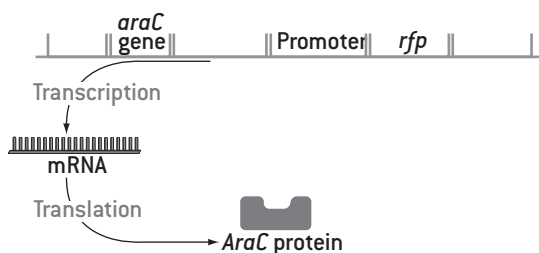


# Preparing an Overnight Culture of *Escherichia coli*

The purpose of this lab is to start a bacterial culture that will produce a sufficient quantity of mutant fluorescent protein to enable you to isolate and purify the protein. From your LB/amp/ara plate, your teacher will select one of the red colonies transformed with the pARA-R plasmid and use it to inoculate an overnight culture.

The gene for mFP was originally isolated from a sea anemone. The mFP is used extensively in research as the protein can be fused to other proteins and then followed through the cell using fluorescent microscopy. The original fluorescent protein gene was mutated to produce a molecule that fluoresces many times brighter. The plasmid pARA-R was then engineered for gene expression.

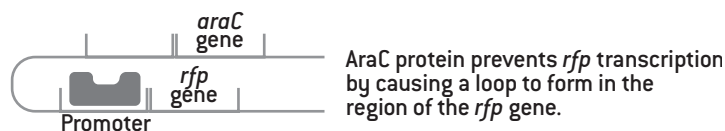
The diagram below depicts the region of pARA-R containing the major control elements required to express the *rfp* gene. It's important for you to note that only a small portion of the pARA-R plasmid is represented in this diagram and the DNA is depicted as a straight line rather than a circle. The diagram identifies three important regions: 1) *araC* gene; 2) the promoter region (*P<sub>BAD</sub>*); and 3) the location of the *rfp* gene relative to the other control elements in the plasmid.



The ***araC* gene** codes for a regulatory protein known as the “AraC protein.” The **AraC protein** is involved with turning the *rfp* gene off and on. The above diagram summarizes the relationship between the *AraC* gene, transcription, messenger RNA, translation and the *araC* protein.

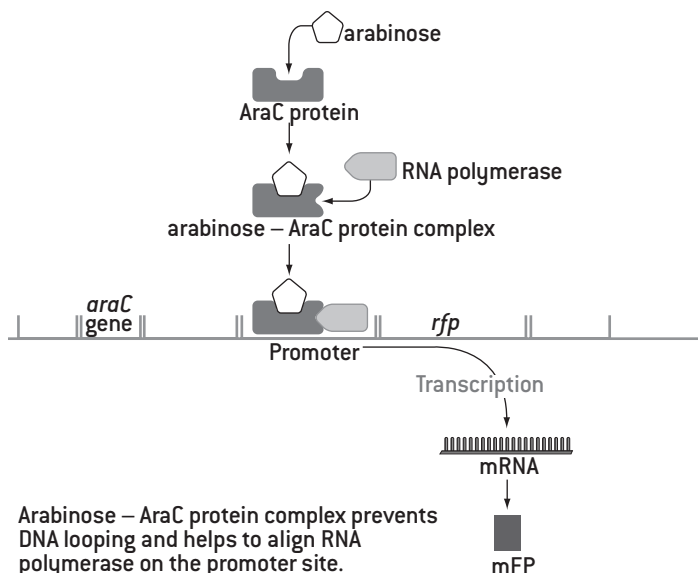
The **promoter site** is that portion of DNA where *regulation of rfp* expression occurs. When there is no arabinose in the bacterium's environment, the AraC protein will physically bind to two regions of

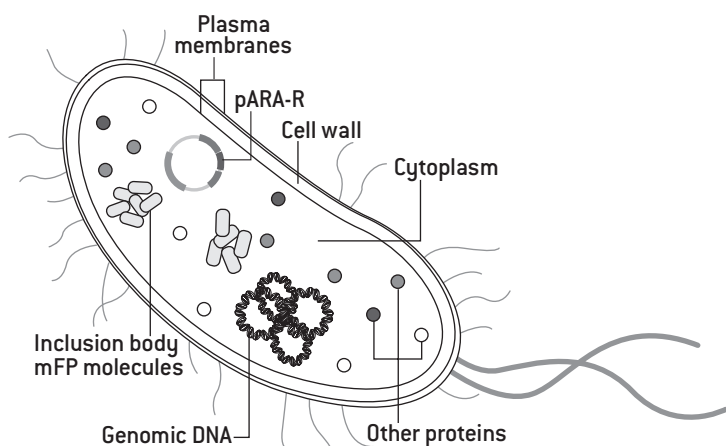
plasmid, the promoter site and a region near the *araC* gene. This causes the DNA molecule to bend around, forming a loop. When the DNA is in this configuration, mRNA transcription cannot occur as it prevents RNA polymerase from binding to the promoter site. Without mRNA, the bacterium cannot produce mFP.



When arabinose is present in the bacterium's environment, arabinose binds with the AraC protein, forming a complex. This prevents the DNA loop from forming. The binding of arabinose also causes a change in the protein's conformation (shape) resulting in the formation of a small pocket that will help a third molecule, RNA polymerase, to join the complex.

This complex of three molecules binds to the promoter site, and RNA polymerase is aligned on the DNA molecule in a way that it can transcribe the *rfp* gene. This transcription produces mRNA, which is translated into mFP. The AraC protein, then, serves a dual function: It can inhibit mFP synthesis by looping the DNA and preventing RNA polymerase from binding to the promoter region, and it can turn on the *rfp* gene transcription and, therefore, mFP production, if it binds to arabinose.





When the bacterium expresses the *rfp* gene and produces mutant fluorescent protein, the cell takes these mFP molecules and concentrates them into inclusion bodies. Inclusion bodies are concentrated granules of mFP molecules and are not bound by a membrane.

## Materials

### REAGENT

LB/amp/ara plate (from Lab 5 or 5a)  
Sterile flask with LB/amp/ara broth

### EQUIPMENT & SUPPLIES

Shaker  
Clean toothpick

## Methods

**Because you will be working with bacteria, it will be important that you work quickly to avoid contamination.**

- 1** Your instructor will use a clean toothpick to transfer a few cells from an mFP-expressing colony (red) into a flask containing LB/amp/ara broth.
- 2** The flask will be placed on a shaker and shaken overnight to encourage both cell division and *rfp* expression.

## Conclusions

- 1** Although your instructor worked quickly to transfer a sample of bacteria expressing *rfp*, there is a good chance that some non-*rfp*-expressing bacteria were transferred as well. What would prevent the growth of these bacteria in the LB/amp/ara broth?

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- 2** The purpose of this overnight culture is to clone the bacteria expressing the *rfp* gene and to have them produce sufficient mFP to purify the protein from the other proteins in the cell. As the cells are cultured, would you expect to find the mFP within the bacterial cells or in the nutrient broth surrounding the cells?

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