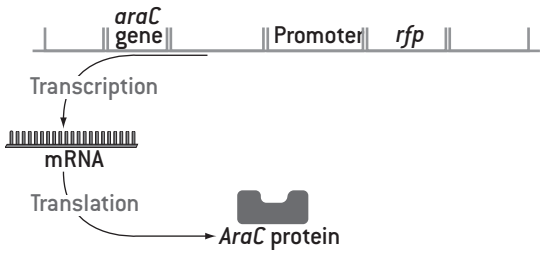
Preparing an Overnight Culture of *Escherichia coli*

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.

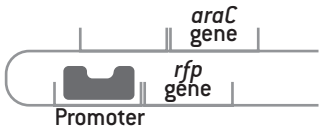
The gene for mFP was originally isolated from a sea anemone. The mFP is used extensively in research as the protein can be fused to other proteins and then followed through the cell using fluorescent microscopy. The original fluorescent protein gene was mutated to produce a molecule that fluoresces many 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_{BAD}); 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.

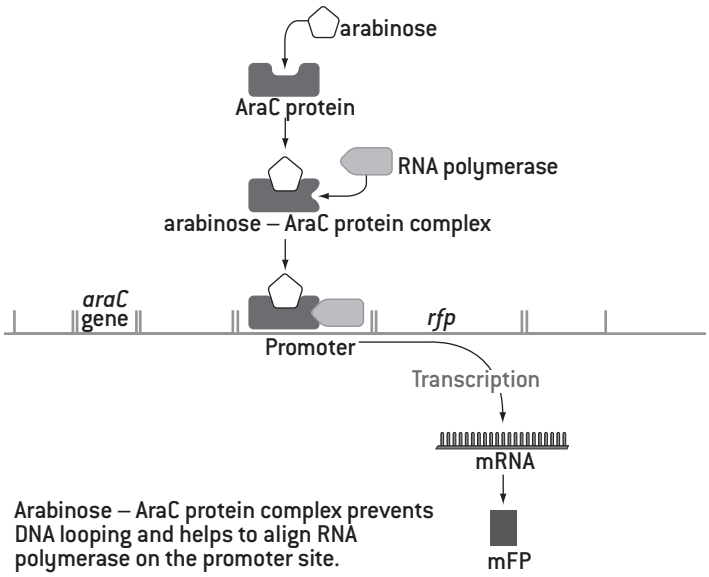
The **promoter site** is that portion of DNA where *regulation* of *rfp* expression occurs. When there is no arabinose in the bacterium's environment, the AraC protein will physically bind to two regions of plasmid, the promoter site and a region near the *araC* gene. This causes the DNA molecule to 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.



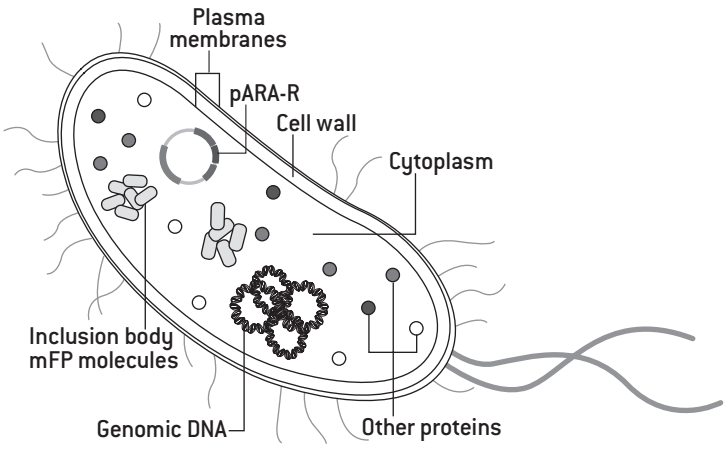
AraC protein prevents *rfp* transcription by causing a loop to form in the region of the *rfp* gene.

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 duel 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.



Arabinose – AraC protein complex prevents DNA looping and helps to align RNA polymerase on the promoter site.



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

Method 1

Sterile flask with LB/amp broth (volume of broth not to exceed 75mL)
Vented cap for sterile flask
Tube of sterile arabinose (500mg/mL)
Frozen cells transformed with pARA-R

Method 2

Lb/amp/ara plate (from Lab5 or 5a)
Sterile flask with LB/amp/ara broth

Methods

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

Method 1 (Teacher demonstration)

1. Aseptically, add 500μL of transformed cells to each flask containing LB/amp broth. Cells should be thawed in wet ice just prior to inoculating the broth.
2. Secure the vented cap to the flask. The cap will allow the culture to aerate during shaking.
3. Shake and incubate (@35°C) the culture following the directions included with the incubator/shaker.
4. Shake the culture until it is obviously cloudy, approximately 3 hours. Add the sterile arabinose solution (500mg/mL) to the flask so the final concentration of arabinose is 5mg/mL. The addition of the arabinose will induce rfp expression.
5. Continue shaking overnight. Full expression will take 24-36 hours.

Method 2 (Teacher demonstration)

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?

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?