

## Preparing an Overnight Culture of *E. coli* -UPDATE-

This is an update for those starting an overnight culture for mFP purification. This update describes what we believe is a more efficient method of producing overnight cultures expressing the mutant fluorescent protein, mFP.

### Materials/Reagents

Sterile flask containing LB/*amp* broth (volume will vary but should not exceed 75mL)  
Vented cap for sterile flasks (used for overnight shaking)  
Frozen cells (LMG 194) transformed with pARA-R  
Tube of sterile arabinose (500mg/mL) final concentration should be 5mg/mL of LB/*amp* broth

### Procedure

1. Aseptically, add 500 $\mu$ L of transformed cells into *each* flask containing LB/*amp* broth.
2. Secure the vented cap to the flask. This will allow the culture to aerate while the culture is growing.
3. Shake and incubate (35°C) the cells according to the directions on the incubator/shaker. Shake for 2-3 hours. Broth should become cloudy or there should be an indication that cells are growing.
4. Following the 2 to 3 hours of shaking, add the appropriate volume of sterile arabinose to *each* flask so the *final* concentration of arabinose is 5mg/mL. Continue to shake overnight.

### Rationale

The original protocol has been modified as a result of observing overnight cultures that were not resulting in strong protein expression. It was discovered that the bacteria were somehow restricting the expression of the *rfp* gene after several generations of growth. This drop in expression seems to be an intermittent problem, but negatively impacts mFP purification (Lab 7) when it does occur.

It has been demonstrated that this problem can be circumvented by growing up the culture initially in LB/*amp* broth before exposing the cells to arabinose. If cells are taken from a red colony directly from an LB/*amp*/*ara* plate and used for this overnight culture, the inoculum sometimes contain cells that have managed to repress *rfp* expression. These cells will grow faster than those who are expressing the gene and the overnight culture will contain a mixture of these cells, most of which are not producing mFP. It has also been discovered that if we inoculate LB/*amp*/*ara* broth with cells, never exposed to arabinose, but transformed with pARA-R, a portion of these cells will stop expressing *rfp* at a fairly early point in the growth. This, too, will yield a mix of cells and a poor yield of mFP. By allowing the transformed cells to grow in the absence of arabinose for 2-3 hours before exposing them to arabinose, a greater yield of mFP will result.