

Laboratory 6

Preparing an Overnight Culture of *Escherichia coli* for Plasmid Miniprep

INTRODUCTION

Before we can confirm that the red colonies on the LB/amp/ara plates contain pARA-R, it will be necessary to purify the plasmid from the colony and analyze it using gel electrophoresis. In order to obtain enough of this plasmid to run a restriction digest, we will need to grow one of these colonies overnight in LB/amp broth. The addition of ampicillin in the LB broth maintains antibiotic selection. By culturing a colony overnight, sufficient numbers of bacteria, along with their transforming plasmids, can be produced for restriction analysis. Analysis of plasmid restriction fragments, resulting from the *Bam*H I/*Hind* III digest, will provide visual data to confirm the construct of the transforming plasmid.

In addition to culturing a glowing colony, you will select a non-red colony to culture as a control. This will give you a visual comparison with a plasmid construct that does not express mFP but still provides ampicillin resistance for the bacterium.

MATERIALS

Reagents

10 mL of LB/amp broth
LB/amp/ara plate (from transformation lab)

Equipment and supplies

Transfer loop
Sterile 15-mL culture tubes (2)
10-mL sterile serological transfer pipette
Pipette helper

PROCEDURES

1. Obtain two sterile 15-mL culture tubes. Label one tube "**R**" (red) and the other "**W**" (white). Mark both tubes with ***your name*** so that you can locate it later.
2. Sterilely transfer 5 mL of LB/amp broth to each tube using the following procedures.
 - a. Obtain 10 mL of sterile LB/amp broth and a sterile serological pipette.
 - b. Attach the pipette helper to the serological pipette being careful to avoid touching the pipette tip.
 - c. Light the propane burner.
 - d. Flaming the tube can be omitted if you work quickly. Remove the cap from the tube containing the LB/amp broth and quickly flame the opening of the tube. Be careful not to melt the tube. Transfer 5 mL of LB/amp broth to the tubes labeled R and W. Cap the

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R and W tubes.

3. Take a clean yellow pipette tip or sterile toothpick and gently scrape-up a single red colony from the LB/amp/ara plate but avoid transferring agar from the plate.
4. Drop the pipette tip (or toothpick) into the **R** tube.
5. Lift the lid of the plate and transfer a well-isolated, non-red colony to the **W** tube using a clean yellow pipette tip or sterile toothpick.
6. Vortex the W and W tubes to mix the cells with the LB/amp broth.
7. Incubate 24 hours at 37°C while vigorously shaking.
8. Return the LB/amp/ara plates to the instructor for proper disposal.

CONCLUSIONS

1. What is the purpose of culturing these colonies- i.e., what is the purpose of the lab?
2. What is the purpose of selecting a non-red colony to inoculate an overnight culture?
3. What two restriction fragments must be present to produce a red colony?