

## *Experiment 11*

# Heterologous Gene Expression in *Escherichia coli* and *Bacillus subtilis*

Plasmids pRIT4501 and pRIT4502 can replicate in both *E. coli* and *B. subtilis*. At this point you have demonstrated that the *E. coli* gene for ampicillin resistance can be expressed in *E. coli* and the *B. subtilis* gene for chloramphenicol resistance can be expressed in *B. subtilis*. You have not tried the kanamycin resistance gene yet, nor have you looked for heterologous expression, that is the expression of *B. subtilis* genes in *E. coli*, and the expression of *E. coli* genes in *B. subtilis*. Possible barriers to heterologous expression would be differences in promoters or ribosome binding sites between the two species. *B. subtilis* has an additional problem that could express heterologous expression. *B. subtilis* is notorious for reducing the sizes of plasmids that are inserted into it. The mechanism is unclear, but any DNA for which there is no specific selection is at risk of being spliced out. Thus, if a novel phenotype does not appear in a transformed *B. subtilis* strain, we cannot simply assume that the cells were unable to express the gene.

In this experiment, then, we will analyze the abilities of the *E. coli* and *B. subtilis* to express the three antibiotic resistance genes, and we will screen *B. subtilis* to make sure that the plasmid has not been altered. Upon completion of this experiment, you will be required to submit a lab report covering experiments 9 and 10.

### **The Day Before**

1. Prepare 12 tubes containing 2 ml LB broth + 10 µg/ml chloramphenicol.
2. Pick 4 colonies each from transformations #2, #3, and #4 and inoculate one into each of the tubes. Incubate standing over night at 37°

### Plasmid Analysis

1. Of the four cultures that you started for each *B. subtilis* transformation, discard one and use the remaining three each to perform a rapid plasmid prep according to the instructions in Experiment 5, *with the exception that you should use a higher concentration of lysozyme (120 mg/ml stock)*
2. Cut your rapid plasmid preps with a restriction enzyme of your choice and run them on a “piggy-back” gel. One row for cut, the other for uncut. As controls you should include your stock pRIT4501 and pRIT4502 preps, as well as molecular weight standards.

### Gene Expression

1. Divide your antibiotic plates into an appropriate number of pie-shaped sectors.
2. Streak each sector of the TBAB plates with a sample of the *B. subtilis* cultures that you used for the plasmid prep. Be careful to keep adequate records so that you can correlate each lane on the gel with each sector of the plate. Invert and incubate over night at 37°.
3. Retrieve your stock cultures of *E. coli* transformed with pUC9, pRIT4501, and pRIT4502 and streak each on the LB plates. Invert and incubate over night at 37°.

***It is important that you not mix up the two sets of plates!***

*E. coli* has a higher tolerance to antibiotics than does *B. subtilis*. Thus a sensitive *E. coli* strain can grow on 10 µg/m antibiotic while a resistant *B. subtilis* strain can be killed by 50 µg/ml.

4. On the following day, record growth vs. non-growth on each sector of each plate. Compare the growth patterns with the restriction digests and correlate gene expression and plasmid downsizing.