Transformations

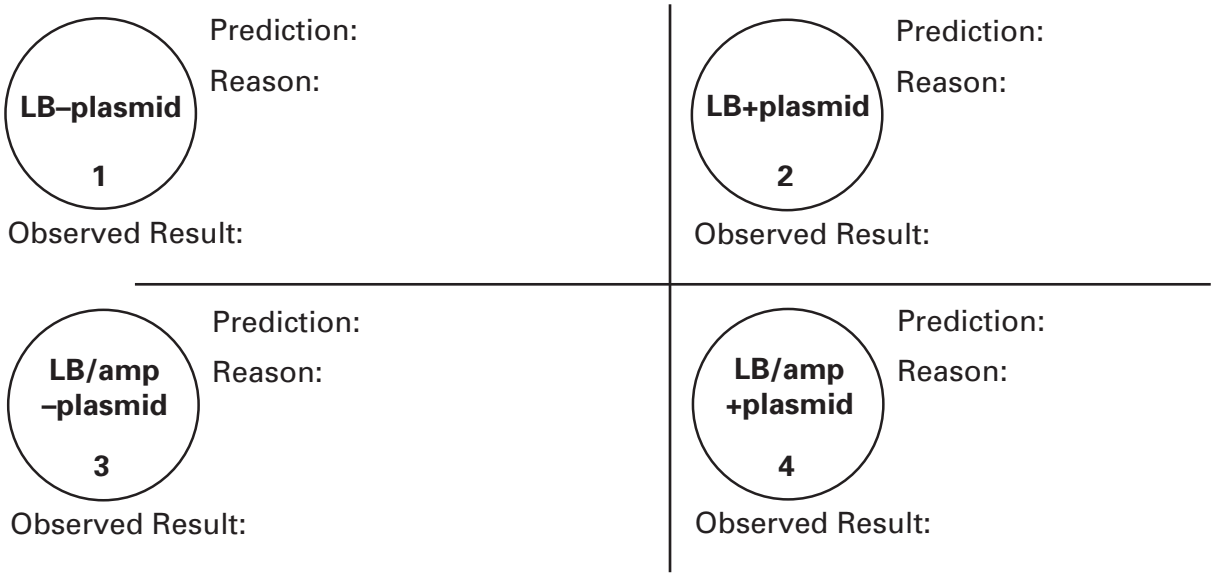
Student Sheet

Name:

Date:

Data and Analysis for pGREEN

1. Predict your results. Write "yes" or "no," depending on whether you think the plate will show growth. Give the reason(s) for your predictions.
2. Observe the colonies through the petri plate lids. Do not open the plates.



3. Record your observed results in the spaces above. If your observed results differed from your predictions, explain what you think may have occurred.

4. Count the number of individual colonies and, using a permanent marker, mark each colony as it is counted. If the cell growth is too dense to count individual colonies, record "lawn."

LB+plasmid (Positive Control)	LB–plasmid (Positive Control)
LB/Amp+plasmid (Experimental)	LB/Amp–plasmid (Negative Control)

5. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?

   a. LB+plasmid and LB–plasmid

   b. LB/Amp–plasmid and LB–plasmid

   c. LB/Amp+plasmid and LB/Amp–plasmid

   d. LB/Amp+plasmid and LB+plasmid

6. What are you selecting for in this experiment? (i.e., what allows you to identify which bacteria have taken up the plasmid?)

7. What does the phenotype of the transformed colonies tell you?

8. What one plate would you first inspect to conclude that the transformation occurred successfully? Why?

9. Transformation efficiency is expressed as the number of antibiotic-resistant colonies per μg of plasmid DNA. The object is to determine the mass of plasmid that was spread on the experimental plate and that was, therefore, responsible for the transformants (the number of colonies) observed.

Because transformation is limited to only those cells that are competent, increasing the amount of plasmid used does not necessarily increase the probability that a cell will be transformed. A sample of competent cells is usually saturated with the addition of a small amount of plasmid, and excess DNA may actually interfere with the transformation process.

a. Determine the total mass (in μg) of plasmid used. Remember, you used 10 μL of plasmid at a concentration of 0.005 μg/μL.

total mass = volume × concentration

b. Calculate the total volume of cell suspension prepared.

c. Now calculate the fraction of the total cell suspension that was spread on the plate.

volume suspension spread/total volume suspension = fraction spread

d. Determine the mass of plasmid in the cell suspension spread.

total mass plasmid (a) × fraction spread (c) = mass plasmid DNA spread

e. Determine the number of colonies per μg plasmid DNA. Express your answer in scientific notation.

colonies observed/mass plasmid spread (d) = transformation efficiency

10. What factors might influence transformation efficiency? Explain the effect of each factor you mention.

