Laboratory Protocol

INTRODUCTION

Transformation, the uptake of DNA by a bacterium, is crucial to genetic research because large amounts of a particular DNA sequence can be produced. The following elements are required to make a useful transformation system: (1) a self-replicating plasmid to carry a gene of interest into the host bacterium; (2) a suitable host bacterium in which to replicate the plasmid; and (3) a means of selecting for host bacterium that have taken up the plasmid.

In this experiment the host organism is *Escherichia coli* (strain MM294). Two samples of *E. coli* bacterium are rendered "competent" to take up plasmid DNA by suspension in a solution of calcium chloride. The phosphates of the DNA and the phospholipids of the cell membrane are negatively charged, causing an electrostatic repulsion that works against movement of the DNA through the adhesion zones in the cell membrane. The Ca++ cations help to shield these negative charges. One sample of *E. coli* cells is exposed to the plasmid, pGFP, which contains a gene for resistance to the antibiotic ampicillin. A heat shock is used to create a thermal imbalance on either side of the *E. coli* membrane that helps to physically pump DNA through adhesion zones.

Transformed *E. coli* are plated onto culture medium with and without ampicillin. Only the small fraction of cells that take up the pGFP plasmid and express the antibiotic resistance gene can grow on the culture plate containing ampicillin; untransformed cells fail to grow. Subsequent division of a single antibiotic-resistant cell produces a colony of resistant clones. Thus, each colony seen on an ampicillin plate represents a single transformation event.

PROCEDURE

CAUTION: This entire experiment must be performed under sterile conditions.

1. Use permanent marker to label one sterile 15-ml tube "+". Label another 15-ml tube "–". Plasmid DNA (pGFP) will be added to the +tube; none will be added to –tube.

2. Use a 100-1000 µl micropipet with a sterile tip to add 250 µl of $CaCl_2$ solution to each tube.

3. Place both tubes on ice.

4. Use sterile inoculating loop to transfer a visible mass of *E. coli* from starter plate to +tube:

   a. Sterilize loop in Bunsen burner flame until it glows red hot. Then pass the lower one-half of shaft through flame.

   b. Stab loop into agar to cool.

   c. Scrape up a visible mass of *E. coli*, but be careful not to transfer any agar. (Impurities in agar can inhibit transformation.)

   d. Immerse loop tip in $CaCl_2$ solution and *vigorously* tap against wall of tube to dislodge bacteria. Hold tube up to light to observe the bacteria drop off into the calcium chloride solution. Make sure cell mass is not left on loop or on side of tube.

   e. Reflame loop before setting it on lab bench.

5. Immediately resuspend cells in +tube by repeatedly pipetting in and out, using a 100-1000-µl micropipet with a fresh sterile tip.

   a. Pipet carefully to avoid making bubbles in suspension or splashing suspension far up sides of tube.

   b. Hold tube up to light to check that suspension is homogeneous. No visible clumps of cells should remain.

6. Return +tube to ice.

7. Transfer a second mass of cells to –tube as described in Step 4 above, and resuspend cells as described in Step 5 above.

8. Return –tube to ice. Both tubes should be on ice.

9. Use a 0.5-10 µl micropipet to add 10 µl of 0.005 µg/µl pGFP solution *directly into cell suspension in + tube.* Tap tube with finger to mix. Avoid making bubbles in suspension or splashing suspension up sides of tube.

10. Return +tube to ice. Incubate both tubes on ice for 15 minutes.

11. While cells are incubating, use permanent marker to label two LB plates and two LB/amp plates with your name and the date.

    Label one LB/amp plate "+". This is the experimental plate.
    Label the other LB/amp plate "–". This is a negative control.
    Label one LB plate "+". This is a positive control.
    Label the other LB plate "–". This is a positive control.

12. Following 15-minute incubation on ice, heat shock the cells in the "+" and "–" tubes. *It is critical that cells receive a sharp and distinct shock.*

    a. Carry ice beaker to water bath. Remove tubes from ice, and *immediately* immerse in 42°C water bath for 90 seconds.

    b. Immediately *return both tubes to ice*, and let stand on ice for at least 1 additional minute.

13. Place + and –tubes in test tube rack at room temperature.

14. Use a 100-1000 µl micropipet with a fresh sterile tip to add 250 µl of sterile LB medium to each tube. Gently tap tubes to mix.

15. Use matrix below as a checklist as + and – cells are spread on each type of plate:

	Transformed cells +tube	Nontransformed cells –tube
LB/amp	100 µl	100 µl
LB	100 µl	100 µl

16. Use a 100-1000-µl micropipet with a fresh sterile tip to add 100 µl of cell suspension from the –tube onto the –LB plate and another 100 µl onto the –LB/amp plate. *Do not let suspensions sit on plates too long before proceeding to Step 17.*

17. Use sterilized glass beads to spread cells over surface of each –plate.

    a. Obtain two 1.5 ml tubes containing at least five sterilized glass beads.

    b. Lift lid of one –plate, only enough to allow pouring of the beads from one of the 1.5 ml tubes onto the surface of the agar. Replace plate lid; do not set the lid down on lab bench. Repeat for second –plate.

    c. Use beads to spread bacteria evenly on plates by moving plates side to side several times. *Do not move plates in a circular motion.*

    d. Rotate plates ¼ turn, and repeat spreading motion. Repeat two more times. *The object is to separate cells on agar so that each gives rise to a distinct colony of clones.*

18. Use a 100-1000-µl micropipet with a fresh sterile tip to add 100 µl of cell suspension from +tube onto +LB plate and another 100 µl of cell suspension onto +LB/amp plate.

19. Repeat Step 17a-d to spread cell suspension on +LB and +LB/amp plates.

20. Let plates set for several minutes to allow suspension to become absorbed into agar. Then wrap together with tape.

21. Place plates upside down in 37° C incubator, and incubate for 12-24 hours.

RESULTS AND DISCUSSION

1. Observe the plates, and record number of colonies on each in the matrix below. If cell growth is too dense to count individual colonies, record "lawn." Were results as expected? Explain possible reasons for variations from expected results.

	Transformed cells +plasmid	Nontransformed cells –plasmid
LB/amp	experiment	negative control
LB	positive control	positive control

2. 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 and –LB

   b. –LB/amp and –LB

   c. +LB/amp and –LB/amp

   d. +LB/amp and +LB

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

   a. Determine total mass (in µg) of pGFP used in Step 9.
   Concentration x Volume = Mass.

   b. Determine fraction of cell suspension spread onto +LB/amp plate (Step 18).
   Volume Suspension Spread/Total Volume Suspension = Fraction Spread.

   c. Determine mass of pGFP in cell suspension spread onto +LB/amp plate.
   Total Mass of pGFP (a) x Fraction Spread (b) = Mass of pGFP Spread.

   d. Determine number of colonies per µg of pGFP. Express answer in scientific notation.
   Colonies Observed / Mass of pGFP Spread (c) = Transformation Efficiency.

4. What factors might influence transformation efficiency?