Teacher Prep & Follow-up

STUDENT LAB BRIEFING

To get the most out of the laboratory, we suggest that you prepare your class prior to coming to the DNA Learning Center (DNALC). Make sure your students have a good understanding of the structure and function of DNA. They should also read the enclosed **Carolina Tips** article "DNA Transformation of *Escherichia coli*." After reading this article, students should be able to discuss:

- The historical background of bacterial transformation.
- The host cell, *Escherichia coli*.
- The plasmid vector.
- Induced transformation, including the four major steps:
  - → PRE-INCUBATION – Cells are suspended in solution of positive ions and incubated at 0°C.
  - → INCUBATION – DNA is added, and cell suspension is further incubated at 0°C.
  - → HEAT SHOCK – Cell/DNA suspension is briefly incubated at 42°C, and then returned to 0°C.
  - → POST-INCUBATION/RECOVERY – Luria broth is added to cell suspension and incubated at 37°C, prior to plating on selective media.
- Mechanism for DNA uptake.
- Selection of transformants.

You may also wish to have the students read over the enclosed lab protocol.

AT THE DNA LEARNING CENTER

Before starting the experiment, the instructor will briefly review the purpose of the laboratory and the uses of bacterial transformation. Students will be introduced to the lab equipment and given an opportunity to practice using micropipettes. The lab protocol will be discussed step by step. Teachers must supervise students in the lunchroom, or students may choose to eat on the school bus, if available. Students will not be permitted to eat in the laboratories or in the hallways of the DNALC.

RESPONSIBLE HANDLING AND DISPOSAL OF *E. coli*

A commensal organism of *Homo sapiens, Escherichia coli* is a normal part of the bacterial fauna of the human gut. It is not considered pathogenic and is rarely associated with any illness in healthy individuals. Adherence to simple guidelines for handling and disposal makes work with *E. coli* a nonthreatening experience for instructor and students alike.

- Avoid over-incubating plates. Because a large number of cells are inoculated, E. coli is generally the only organism that will appear on plates incubated for 12 to 24 hours. However, with longer incubation, contaminating bacteria and slower-growing fungi may arise. If students will not be able to observe plates following initial incubation, refrigerate plates to retard growth of contaminants.
- Wipe down lab bench with 10% bleach solution, soapy water, or disinfectant (such as Lysol) after observing plates.
- Wash hands after working with bacterial cultures.
- Disinfect all agar plates as soon as possible after use. Contaminants, often smelly and sometimes potentially pathogenic, can be cultured over a period of several days at room temperature. Disinfect plates in one of two ways:
  - → Treat with 10% bleach solution (5,000 ppm available chlorine). Plates should be placed in sink or tub and flooded with bleach solution. Let materials stand in bleach solution for 15 minutes or more. Then drain excess bleach solution, seal materials in plastic bag, and dispose in regular garbage.
  - → Autoclave at 121°C for 15 minutes. Collect contaminated materials in an autoclavable, disposable bag; seal bag before autoclaving. Dispose in regular garbage.

RESULTS AND DISCUSSION

- Have students count the number of individual colonies on the +LB/amp plate. Observe colonies through bottom of culture plate, using a permanent marker to mark each colony as it is counted.
- If plates have been over-incubated or left at room temperature for several days, tiny "satellite" colonies may be observed that radiate from the edges of large, well-established colonies. Nonresistant satellite colonies grow in an "antibiotic shadow" where ampicillin has been broken down by the large resistant colony. Do not include satellite colonies in count of transformants.
- A "lawn" should be observed on positive controls, where the bacteria cover nearly the entire agar surface and individual colonies cannot be discerned.

In addition to the questions in the lab protocol, you can discuss the ethical ramifications of recombinant DNA in agriculture, industry and medicine, the Human Genome Project, human gene therapy, and genetic engineering. There are often articles in journals on teaching bioethics, for example "Genetic Engineering—A Lesson on Bioethics for the Classroom," by Kerri Armstrong and Kurt Weber (*The American Biology Teacher*, May 1991, Volume 53(5)). There is also a very good section on societal issues in *A Sourcebook of Biotechnology Activities* from the National Association of Biology Teachers and the North Carolina Biotechnology Center.

Answers to student questions are in bold.

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	Non-transformed cells –plasmid
LB/amp	Experiment **5-500 colonies**	Negative Control **No Growth**
LB	Positive Control **Lawn**	Positive Control **Lawn**

Variations in results are due primarily to technique. Sources of error include: not taking large enough cell mass, not resuspending cells, failing to put plasmid in +LB/amp tube, putting plasmid in both +LB/amp and –LB/amp tubes, missing or indistinct heat shock, and mixing up plates or tubes during plating step.

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
   Cells are viable. In absence of antibiotic, both wild-type cells and transformed cells grow equally well.

   b. –LB/amp and –LB
   Wild-type cells fail to grow in the presence of ampicillin. Antibiotic is active.

   c. +LB/amp and –LB/amp
   Only transformed cells grow in the presence of ampicillin.

   d. +LB/amp and +LB
   Transformation is a rare event; only about 1 per 1 million cells is transformed.

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, therefore, responsible for the transformants observed.

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

      0.005 µg/µl x 10 µl = 0.05 µg

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

      100 µl / (250 µl + 250 µl) = 0.2

   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.

      0.05 µg x 0.2 = 0.01 µl

   d.

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

      100 colonies / 0.01 µg = 10,000 = 1 x 10^4 transformants/µg

4. What factors might influence transformation efficiency?

   Technique errors mentioned in Question 1: Temperature and length of incubation periods; growth stage of cells — healthy, rapidly dividing cells are more likely to uptake DNA; mass of DNA used — once cell solution becomes saturated with DNA, additional plasmid decreases transformation efficiency.

FURTHER SUGGESTIONS for experiments and questions can be found in *DNA Science: A First Course in Recombinant DNA Technology* by David Micklos and Greg Freyer.