

### Equipment (continued)

	Per Lab Group	Per Class
500-ml beaker with disinfectant	1	
L-shaped bent glass rod (optional)	1	
Beaker with 95% ethyl alcohol (optional)	1	
Turntable, if using spread-plate method (optional)	1	
Wire inoculating loop (optional)	1	
Quebec colony counter	1	

### Procedural Points to Emphasize

1. Some strains of *E. coli* have been associated with disease outbreaks, and students may be concerned about individual safety. The strains of *E. coli* used in molecular biology laboratories do not contain disease-producing genes and are harmless under normal conditions.
2. At the end of the experiment, students must collect all culture materials and equipment that have come in contact with the culture and autoclave at 121°C for 15 minutes before disposal.
3. Spilled cultures may be disinfected with a 10% bleach solution or the disinfectant used in the laboratory.
4. Scraping the agar must be avoided when transferring the large cell mass, and the cell mass must be deposited directly into the  $\text{CaCl}_2$  solution and not left on the loop or the side of the tube.
5. Cells should not be allowed to clump. Be certain to suspend the cells rapidly once they are placed in the  $\text{CaCl}_2$ . Clumped cells are difficult to resuspend.
6. The DNA plasmid must be placed directly into the cell suspension.

7. Abrupt heat shock (Step 10) is a critical step. Transformation tubes should be taken from the ice bucket and transferred to the 42°C waterbath and then quickly back to the ice.
8. Transformed cells should be spread rapidly. If the cell suspension sits too long on the surface of the agar plate, too much of the suspension will be absorbed in one spot, and the cells will not spread evenly on the plates.

### Optional Procedural Additions or Modifications

Rather than use the glass-bead technique as described in the procedure, the instructor may opt to use the spread-plate technique shown in Figure 2.3 for plating the cultures.

### Tip

- The molarity and pH of  $\text{CaCl}_2$  is critical for the production of competent cells.

### Additional Reading

- Martinez, J. L. (2011). Bottlenecks in the transferability of antibiotic resistance from natural ecosystems to human bacterial pathogens. *Frontiers in Microbiology*, 2:265.

### Answers to Review Questions

1. Considering that a DNA plasmid is used in this experiment, care must be taken to prevent it from being introduced into other competent laboratory stock cultures of *E. coli*. Immediate autoclaving of all experimental materials and cultures will prevent the possibility of cross contamination.
2. Scientists have the ability to alter the genome of an organism by transferring the genetic material DNA from one cell to another. There are several methods, which include transformation, transduction, and conjugation. Bacterial cells can be genetically engineered today to produce insulin by inserting genes for insulin production into the organism's genome. Similarly, interferons can be used in cancer chemotherapy, in the treatment of multiple sclerosis, and in the production of human growth hormone.

# Microbiology Laboratory Manual 104

**Annelies Wilder-Smith**



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