Bacterial DNA as a Teaching Tool

VERNE M. MILLS
BRENDA GAUNT

STUDENTS WITH LITTLE or no background in chemistry often find the concepts of biochemical genetics virtually incomprehensible. Despite the availability of models, diagrams, text materials, and competent instructors, students seldom demonstrate an understanding of this part of biology that is adequate for people who may one day be asked to make far-reaching decisions about the funding of research on molecular genetics or the clinical applications of research findings.

One solution to this education gap is to provide students with opportunities for hands-on investigations with real DNA. We have developed a simple method of extracting bacterial DNA for use in laboratory investigations. Our work is based principally upon previously published procedures (Holt and Choe 1968; Biological Sciences Curriculum Study 1970) and involves combining, modifying, and simplifying these methods into a technique that is both reliable and adaptable to most teaching laboratories. This paper describes the extraction procedure and the use of DNA so obtained to investigate genetic transformation in bacteria.

Extraction of Bacterial DNA

Isolate a pure culture of *Escherichia coli* from sewage-polluted water, using standard isolation procedures and identification tests (Seely and Vandemark 1962). If necessary, a culture of *E. coli* can be readily obtained from most university microbiology departments and biological supply companies.

Inoculate a tube of sterile nutrient broth with *E. coli* and incubate for 24 hours at 37 °C. Aseptically transfer 1 ml of this broth culture to a 500-ml Erlenmeyer flask containing 100 ml of sterile nutrient broth. Incubate this secondary culture at 37 °C. After 24 hours transfer the culture to centrifuge tubes (these need not be sterile) and centrifuge them for 10 minutes at 1550 x g to sediment the bacterial cells. We have found that the highest speed of most laboratory centrifuges is adequate for this purpose, although required centrifuge times will vary.

Pour off the clear or slightly cloudy supernatant and resuspend the cells in 5 ml of saline-citrate buffer, pH 7 (prepare by dissolving 0.6 g sodium chloride and 3.0 g sodium citrate in 50 ml of distilled water). To inhibit DNAse activity, add 0.5 ml of 0.1*M* EDTA to the cell suspension.

Lysis of the bacterial cells is accomplished with a 20% aqueous solution of sodium lauryl sulfate. Place 0.5 ml of this solution in a clean test-tube, and quickly add and mix in the cell suspension. Place the test-tube in a water bath at 45–50 °C, and swirl the mixture gently at frequent intervals. After 10–15 minutes the cloudy suspension becomes clear and very viscous, indicating cell disruption and the release of DNA.

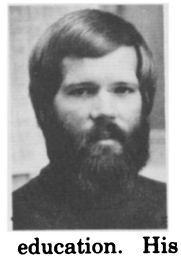
To denature the protein that contaminates the released DNA, add 6 ml of chloroform to the lysate, shake vigorously for 2 minutes, and centrifuge for 10 minutes at 1550 x g. The turbid upper layer in the centrifuge tubes, which contains the purified DNA, should then be transferred with a Pasteur pipette to a clean test-tube.

Slowly add approximately 6 ml of 95% ethanol to the DNA solution, holding the test-tube at a 45° angle. As the alcohol is stirred into the DNA solution with a glass rod, long strands of high molecular weight DNA precipitate and adhere to the stirring rod. When precipitation of DNA is complete, the DNA collected on the glass rod may be redissolved in 2 ml of sterile 10% sodium chloride and stored under refrigeration for at least one week without loss of biological activity.

We have consistently obtained good yields of DNA from *E. coli* using this method; however if DNA is to be extracted from gram-positive species such as *Bacillus mycoides,* it is necessary to treat the cell suspension with 0.5 ml of an aqueous solution of lysozyme (10 mg/ml) for 15 minutes prior to the sodium lauryl sulfate treatment.

Investigating Genetic Transformation

In order to study genetic transformation, it is necessary to isolate a strain of bacteria having a distinctive genetic trait—in this case, resistance to penicillin. Penicillin-resistant *E. coli* are easily isolated by spreading cells from the stock culture on a plate of nutrient agar that contains



Verne M. Mills is chairman of the science department at Wayne Community School, 611 West 7th St., Wayne, Neb. 68787. He received his B.S. in biology from Wayne State College and his M.S. in molecular biology from Ball State University in 1970. He is currently researching enzyme systems in plants and is also interested in the development of an improved interdisciplinary approach to environmental education. His hobbies include mountain camping and backpacking. **Brenda Gaunt** is a first-year prepharmacy major at Wayne State College, Wayne Neb. 68787. She received first and second awards for research in human physiology and bacterial genetics in the 1974 Northeast Nebraska Junior Academy of Science student research competition and recognition from the American Society of Microbiology for research in bacterial genetics during her participation in the 1974 Greater Nebraska Science and Engineering Fair.

2000 units of procaine penicillin G. (This form of penicillin is readily available without prescription at pharmacies that sell veterinarian supplies.) Upon incubation, resistant cells produce colonies which may be isolated and maintained on nutrient agar slants.

Prepare an enriched broth by dissolving 0.3 g peptone, 1.0 g yeast extract, and 3.7 g brain-heart infusion (Difco) in 100 ml of distilled water. After sterilization, add 5 ml of the enriched broth to a saline solution of DNA extracted from penicillin-resistant *E. coli* cells. Prepare a control tube by adding 5 ml of sterile enriched broth to a culture tube containing 2 ml of sterile 10% sodium chloride solution. Each tube should then be inoculated with 1 ml of a 24-hour broth culture of nonresistant *E. coli* and incubated for 24 hours at 37 °C. It is desirable to prepare a third culture tube containing enriched broth and extracted DNA only. This preparation allows the investigator to determine whether the extracted DNA is contaminated with viable bacterial cells from the resistant culture.

After incubation, prepare serial dilutions ranging from 1:10 to 1:1,000,000 from each culture, using sterile distilled water. Spread 0.1 ml aliquot of each dilution on a nutrient agar plate containing 2,000 units of penicillin, and incubate the plates for 24 hours at 37°C. By counting colonies on plates containing between 30 and 200 colonies, determine the number of resistant cells in the transformed and control cultures.

If a simpler, less quantitative method of detecting transformation is preferred, a sterile cotton swab may be dipped into the transformed culture and used to trace a letter or other figure on the surface of a penicillin agar plate. This should be repeated with the control cultures. After incubation, amounts of bacterial growth on the plates can be compared visually.

Results and Discussion

The results of the genetic transformation investigation are summarized in the table. Control cultures of *E. coli* typically contain significant numbers of penicillin-resistant cells; however, the transforming influence of the extracted DNA in the experimental cultures is readily apparent even with casual observation.

The procedures described here provide numerous opportunities for creative investigation. Once a supply of biologically active DNA is available, students will often propose their own ideas for its study. Some students may be content to study the strands of DNA under a microscope. Others may wish to hydrolyze the DNA and analyze its components with paper chromatography. A simple technique for DNA hydrolysis and chromatography is outlined in the student laboratory guide of the BSCS "yellow version" (Biological Sciences Curriculum Study 1968). Problems for investigation that were generated by our genetic transformation experiments include the following: (i) Can DNA from *E. coli* transform another bacterial species? (ii) Does the transformed strain of *E. coli* differ from the parent strain in more than one characteristic? (iii) Is the penicillin-resistant strain of *E. coli* also resistant to other antibiotics?

Data from investigation of bacterial genetic transformation.

A. Average number of penicillin-resistant cells per ml in control cultures	8.7×10^5
B. Average number of penicillin-resistant cells per ml in experimentally transformed cultures	3.1×10^6
C. Ratio of A:B	1:3.5

The technique of extracting bacterial DNA is well known to microbiologists (Marmur 1961); however, it has not been widely used in teaching biochemical genetics to beginning students. The procedures described here should enable students to conduct laboratory investigations that will lead them to a deeper understanding of the activity, properties, and significance of deoxyribonucleic acid.

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Highly Questionable Dining Habits

The highly questionable dining habits of a shark are noted in Thomas Helm's book *Shark.* Helm reveals that the stomach of one blue shark contained (in addition to the usual garbage and fish) two soft-drink bottles, an aluminum soup kettle, a carpenter's square, a plastic cigar-box, a screw-top jar partly filled with nails, a two-cell flashlight, several yards of quarter-inch nylon rope, a rubber raincoat, a tennis shoe, and a 3-foot roll of tarpaper with 27 feet of it still wound on the spool.

Saltwater Aquaria

Marine Hobbyist News, edited by Charles Wonderlin, 205 Orr Dr., Normal, Ill. 61761, is a monthly publication devoted to the scientific maintenance of saltwater aquaria, large and small. The newspaperlike *News* is full of goodies; for example: "Does your octopus want out? The Shedd Aquarium in Chicago keeps Octavia the octopus in her tank by placing Astroturf (artificial grass) on the top edge of the tank. Once the tentacles come in contact with this, she immediately withdraws her arms into the tank."