# *Working with DNA & Bacteria in Precollege Science Classrooms*

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Cover image is of cellular DNA, magnified one million times, taken with a scanning tunneling microscope. This "isometric" projection of DNA shows an isolated length of DNA making a loop and crossing over on itself. The average distance between the coils of the helix is about 35 angstroms. Courtesy of Lawrence Berkeley Laboratories, Berkeley, CA.

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These guidelines were developed to assist teachers in conducting DNA and bacterial manipulation activities in precollege classrooms. While every effort has been made to anticipate questions and situations that could arise, the safe implementation of these activities must depend on the good judgment of teachers and is the responsibility of the local school district/institution.

NABT recognizes the pervasive social phenomenon of litigation with respect to even the most unfounded claims. For that reason, NABT disclaims any legal liability for claims arising from use of these guidelines. This information has been provided to teachers and to schools as a service to the profession and we provide this material only on the basis that NABT has no liability with respect to its use. Responsibility for use of any of this information is assumed by the local district/institution.

NABT believes that under the guidance of a properly trained and responsible teacher work with rDNA can be safely conducted in high school classrooms and that biotechnology represents a critical topic for students in today's precollege curriculum.

# F O R E W O R D

School science curricula are changing. There are more hands-on and inquiry-oriented lab activities. Work with DNA is popular with students and feasible for both classroom teaching and student projects. There are many ways to study the chemical and biological properties of DNA, among them, construction and propagation of recombinant DNA (rDNA) molecules.

Scientists have been conducting rDNA research in accordance with safety guidelines issued by the federal government. The National Institutes of Health *Guidelines for Research Involving Recombinant DNA Molecules* provides a framework to ensure the safety of research projects. These procedures include establishing the safety of the DNA molecules and their host organisms, checking the facility in which work will be performed, training all involved and planning for the disposal of materials.

These guidelines concern working with vectors, rDNA and their bacterial hosts in precollege classes. Many of the procedures involved in construction and propagation of rDNA molecules are based on standard microbiology techniques that are also applicable to other types of DNA studies. Using these prudent methods of handling DNA, host organisms, associated chemicals and equipment will assist with the success and safety of DNA studies in school classrooms.

Regulators and scientists agree that the safety of certain types of work with rDNA has now been so well-established that they are "exempt" from the NIH guidelines. It is not surprising, then, that labs using plasmids, rDNA and host bacteria are being conducted in high school biology classes. What is missing, however, is a relevant set of guidelines for precollege teachers and students.

Safety is a key issue in all laboratories. The guidelines for working with DNA and host organisms outlined in this publication are similar to Good Laboratory Safety Practices (GLSPs) used by research scientists. Teachers can instill safe and responsible behavior in their students by example and by proper instruction in following safety guidelines. As students follow these guidelines, they will gain an appreciation for how scientists work in the laboratory and will develop valuable work skills. The guidelines outlined here address both microorganisms and chemicals used in the experimental procedures.

In essence, the key to safety in working with any microorganisms--whether or not they involve rDNA molecules--is to follow Standard Microbiological Practice, as defined in the Centers for Disease Control-National Institutes of Health (CDC-NIH) manual. *Biosafety in Microbiological and Biomedical Laboratories.* The supervising teacher should be trained in the practice of these techniques.

For those student experiments specifically involving recombinant DNA techniques, do the following:

- Maintain standards for good microbiological practice
- Use only organisms and DNAs that are exempt from the NIH guidelines
- Verify that the supervising teacher is trained in standards of good microbiological practices with a working knowledge of the organisms and materials involved
- Ensure that classroom labs have standard safety equipment such as a fire extinguisher, fire blanket, goggles, aprons, shower, first-aid kit and eyewash station, and have adequate work space per student to safely conduct experiments.

***Teachers can instill safe & responsible behavior in their students by example & by proper instruction in following safety guidelines.***

# INTRODUCTION

## *Why follow DNA safety guidelines in high school?*

Safe handling of DNA and its host organisms is not difficult. The key requirements are the proper handling of chemicals and the practice of good microbiological techniques. These standards should be maintained with microorganisms whether or not recombinant DNA experiments are involved. Following safety measures means proceeding with caution and following the proper steps every time.

In fact, NIH guidelines for research involving rDNA apply to anyone receiving federal funding for this type of work, and manufacturers voluntarily follow these guidelines. In addition, all professional facilities that do rDNA work conduct biosafety training programs for their employees. Simple instruction in biosafety is possible and desirable in high school classes as well.

In the early 1970s it became possible to construct recombinant DNA molecules. By using highly specific restriction and ligation enzymes, scientists could join different DNA molecules together and combine natural or synthetic DNA to DNA that could be replicated in a living cell. Almost immediately, scientists convened to discuss health and ethical implications. At a 1974 meeting, held at Asilomar Observatory, the participants decided there was a need for national oversight and guidelines. The National Institutes of Health Office of Recombinant DNA Activities was soon formed.

After almost 20 years of worldwide research experience with rDNA, it has become clear that it is safe to conduct research on certain DNA sequences and host organisms under appropriate conditions and with responsible handling. As a result, many types of experiments are now designated as "exempt" from the guidelines. The exempt cases involve special bacterial strains used for DNA work that are physiologically weak and dependent upon specific laboratory conditions for survival; they cannot survive in humans, animals, plants or the environment outside the laboratory. Exempt DNA molecules and hosts can be used without first having the research and working conditions approved by a review board. However, in all cases, even when working with exempt materials, the experimenter should use good microbiological practices.

The techniques involved are relatively simple, requiring little more than an understanding of how to handle common microbes and chemicals safely. Many of the procedures can be performed over a short period of time, using small and relatively inexpensive equipment.

The ease of conducting DNA work and related techniques has made these procedures ideal for high school classes. The technology is popular among biology and chemistry students. DNA technology has been incorporated into biology classes and is now a part of the Advanced Placement (AP) Biology program. The AP Biology course outline includes two DNA manipulation experiments: DNA restriction and bacterial transformation. DNA manipulation and rDNA technology also have potential for student science fair research projects. In the past few years, thousands of U.S. high school science teachers have been trained in DNA science, including the use of rDNA.

When students perform laboratory activities that involve construction of recombinant DNA molecules, these rDNA molecules should be compatible for propagation in *Escherichia coli* (*E. coli*) K-12 bacterial host strains. This system will be sufficient for all teaching purposes and more than adequate for science project experiments that are conducted in school with teacher supervision. These DNA sequences and organisms are exempt from NIH guidelines. Other kinds of studies that use DNA, such as polymerase chain reaction (PCR) analysis of buccal samples, do not involve construction of recombinant DNA.

### *Before you begin*

**These guidelines are intended to assist the teacher who already has training in working with microbes, DNA and associated chemicals.** There are many excellent courses with academic credit offered by universities and training centers that teach these methods. The teacher should be familiar with aseptic techniques, methods for sterilization and the safe handling of chemicals in the classroom. General laboratory safety guidelines should always be followed. Sources of existing district and state guidelines may be available from a science supervisor or from the teacher's state education department.

# INTRODUCTION

New kinds of work with DNA include:

- Construction of recombinants and their propagation in host organisms
- Use of commercially prepared rDNAs containing safe DNA sequences
- Classical bacterial transformation labs
- Restriction enzyme analysis and gel electrophoresis
- PCR amplification

Following the general practices described here will help promote success in all types of DNA studies.

## *Contents of the guidelines*



## *Who helped formulate these guidelines?*

These guidelines have been compiled with input from experts in industry and academia in the areas of rDNA technology, biosafety and risk assessment; school district science supervisors; science researchers and trainers; and high school teachers who conduct rDNA labs in their classrooms. This document describes ways to work with DNA and host organisms in precollege classrooms. We are confident that the information provided will be useful for school administrators, teachers, students and members of the community.

Judy Grumbacher

*Figure 1: Student loading a sample of DNA for analysis by agarose gel electrophoresis.*

# I. Permitted DNA Molecules, Vectors & Recommended Host Organisms for Constructing rDNA

***E. coli K-12 & its compatible cloning vectors are recommended.***

Experiments that use *E. coli* K-12 host-vector systems are exempt from the NIH guidelines (Federal Register, 51, 16957). This means that any piece of DNA that has been previously established to code for a safe compound can be propagated in *E. coli* K-12 using a plasmid vector that is compatible with *E. coli* K-12.

For precollege teaching and classroom research purposes, *E. coli* K-12 and its indigenous plasmid derivatives, chromosomal or phage DNAs and safe insert DNA segments from other organisms, are recommended in rDNA lab activities and related experiments.

*Insert DNA molecules*

DNA molecules used as inserts in recombinant DNA should be well-characterized and known to be nontoxic to humans, animals and plants, based on analyses by scientists.

*Recommended vector DNA molecules*

Exempt vectors for *E. coli* K-12 contain *E. coli* K-12 DNA and DNA originating in viruses and plasmids that normally infect *E. coli* K-12. The plasmids, pAMP, pKAN, pUC and pBR 322 and the bacterial viruses, bacteriophage M13 and lambda, are examples of vectors.

*Recommended hosts*

Although exempt host organisms include the bacteria *Bacillus subtilis* and the yeast *Saccharomyces cerevisiae*, the genetic components of *E. coli* K-12 bacteria are understood far better than those of the other two host organisms. Therefore, NABT recommends the use of *E. coli* K-12 for classroom rDNA procedures.

**Table 1: Summary of Recommended DNA Molecules & Host Organisms for Student Constructions**

| DNA Molecules | Host Bacteria |
|---|---|
| *vectors* | *E. coli K-12 strains* |
| pAMP<br>pKAN<br>pUC<br>pBR322 | MM294<br>or HB101 |
| M13 | JM series |
| *DNA inserts* | |
| Bacteriophage lambda<br>Bacteriophage T4<br>*E. coli* sequences<br>Any of the above listed plasmids, viruses or parts thereof<br>Any well-characterized, nontoxic DNA segment | |

*What cannot be done*

Recombinants containing DNA coding for oncogenes, or other human, plant or animal toxins (including viruses) cannot be made and/or propagated in *E. coli* K-12, yeast or *B. subtilis* vector DNA molecules in precollege classrooms.

# II. Preparation of Materials & the Work Area

Adherence to good microbiological and chemical safety practices and aseptic techniques protects the individuals working with bacteria, DNA and chemicals. But there is another equally important safety practice--keeping the cells, DNA and chemicals free of contaminants. Using sterile bottles, tubes, tools and solutions helps to minimize contamination of the experiment. Various sterilization methods are available, including steam sterilization in an autoclave or pressure cooker, dry heat, filtration through a membrane with pore size of 0.45 micron or below, flaming or purchase of presterilized materials.

The method you choose will depend on the particular materials used and on your school's facilities or the availability of facilities at a nearby laboratory or hospital. Certain chemicals remain stable during steam sterilization (see page 7, *Table 2: Sterilization of Liquids*).

## *MATERIALS*

When working with plasmids or microorganisms such as *E. coli* K-12, reagents, media and glassware must be sterilized to minimize the risk of contamination. Depending upon the material, supplies can be sterilized by a pressure cooker, autoclave, dry heat or filtering through a 0.45 micron pore-size filter. Aseptic handling of sterilized materials is also essential to the success of the experiment.

Do not sterilize DNA or bacteria by heating or autoclaving, as this destroys the biological activity. Filter sterilization of pure bacterial cultures or DNA is unnecessary.

### *Glassware*

Autoclave glass for 15 minutes at 15 pounds per square inch of pressure and 121°C. To help ensure that the inside of the vessel is sterilized, rinse it with distilled water, leaving a few drops of water inside. Be sure the vessel opening is loose enough so that the steam that forms inside can escape. There are many alternatives for covering the vessel: screw caps (loosen half a turn), culture caps or aluminum foil for test tubes, aluminum foil for beakers, and gauze-covered cotton stoppers for large test tubes or Erlenmeyer flasks. Lay empty glassware on its side in the autoclave to help the steam circulate. Wrap other glassware in heavy brown paper or aluminum foil. Aluminum foil can be reused several times, but be sure to check for holes or tears. To distinguish between sterilized and unsterilized items, tag with a small piece of autoclave indicator tape. Autoclave tape has marks that change color when heated (see insert, *Figure F*). In addition, mark the date in pencil on the tape.

Although autoclaving is preferable, glassware may be sterilized by baking at a temperature of 160°C for two hours. Another alternative is to use a pressure cooker for 15 minutes at 15 pounds per square inch of pressure.

### *Sterile plasticware*

Sterile plastic disposable pipettes and test tubes are used in many laboratories and are available from most science lab suppliers. Presterilized materials may be used effectively without flaming. These materials may melt or burn if flamed. Steam autoclave for 30 minutes or soak for one hour in 10 percent bleach (1 volume of bleach plus 9 volumes of water) before disposal.

***Using sterile bottles, tubes, tools & solutions helps to minimize contamination of the experiment.***

# *Preparation of Materials & the Work Area*

*Check the lab activity manual for detailed preparation instructions. Ask for assistance from the technical service department of the company where the material was purchased.*

*Tools*

Sterilize metal tools by autoclaving for 15 minutes at 15 pounds per square inch of pressure and 121°C. Flame-sterilize wire inoculating loops as well as glass and metal spreaders since they are used for transferring and spreading cells. Forceps and spreaders can be baked at 160°C for two hours, or flame-sterilized by dipping in 70 percent ethanol or isopropyl alcohol, and allowing the alcohol to burn off. Keep the alcohol soak beaker away from the flame.

*Media*

Most culture media, such as nutrient agar and broth, and Luria agar and broth, can be autoclaved. For volumes of 1 liter or less per container, 15 minutes at 15 pounds per square inch of pressure and 121°C is sufficient. If the medium is sterilized too long, the sugar caramelizes and turns dark brown. Strive for a deep golden color with the standard medium, Luria Bertani (LB) broth, but do not autoclave for less than 15 minutes at the pressure and temperature indicated. Most media can be stored at room temperature after sterilization. Store poured plates upside down in plastic sleeves to prevent them from drying out. Keep at room temperature so you can detect contamination before beginning an experiment. If you prepare the plates aseptically, they will last at least a week at room temperature. Store plates with antibiotic in the refrigerator to maintain activity. Some components of the media must be autoclaved separately or filter-sterilized. Check the lab activity manual for detailed preparation instructions. Ask for assistance from the technical service department of the company where the material was purchased.

*Additives*

Many additives can be autoclaved, but some need to be filter-sterilized because the heat will inactivate the substance. Check the preparation instructions; call the supplier of the additive if specific instructions cannot be found. Some vitamins, amino acids and antibiotics should not be autoclaved, but can be filter-sterilized. Use filters with pore sizes of less than 0.45 micron, preferably 0.2 micron, and filter into a sterile container. Most additives need to be kept frozen or stored at refrigerator temperature (4-8°C).

*Antibiotics*

Most antibiotics are heat sensitive. Prepare the required concentrate using distilled water or alcohol per instructions. Alcohol solutions can be considered sterile and need no further treatment. Filter-sterilize water solutions and store small portions in the freezer in sterile plastic or glass culture tubes. It is best to prepare a 100X stock solution, so that 0.01 volume of the antibiotic can be added per volume of culture medium (e.g. 1 ml of 100X stock antibiotic per 100 ml of medium). When adding an antibiotic to a medium containing agar, first cool the agar medium to 50-60°C, the temperature of hot tap water. Mix well by swirling, then aseptically pour into the plates.

## *WORK AREA*

*Planning*

Think through what is planned. Have students read the "Student's Working Checklist"

# PREPARATION OF MATERIALS & THE WORK AREA

below before beginning any lab. Make sure no students are allergic to materials used in the lab. Prepare the materials, pour the plates and transfer the cells ahead of time. Keep cold materials on ice. Have only the needed materials in the immediate area.

## STUDENT'S WORKING CHECKLIST

Before you begin, carefully read the instructions. Keep these instructions visible as you work.

1. Wash hands with antibacterial soap.
2. Keep reagents, biologicals and hands away from mouth, face and hair.
3. Have disinfectant and paper towels nearby for spill cleanup.
4. Make sure all papers, plastics and alcohol beakers are away from the Bunsen burner.
5. Report spills immediately to the teacher/supervisor.
6. Keep hands, hair, arms and sleeves away from the openings of all vessels and tools.
7. Dispose of all materials properly by putting bacterial and recombinant DNA waste into a biohazard bag or basin for decontamination.
8. Clean up the work area when finished.
9. Wash hands again with antibacterial soap.
10. Make sure you are aware of the location of the nearest fire extinguisher and eyewash station.

I have read and understand each of these instructions before beginning the lab. As I work, I will comply with these instructions. signed _____ _____

*Disinfection*

Before you begin, wipe down the work bench with a solution of 10 percent bleach (1 volume of bleach plus 9 volumes of tap water). After using microbes or DNA, wipe down the work area with the 10 percent bleach solution. **Prepare the bleach solution fresh each day.** Exposure to light degrades bleach, and it will break down rapidly if the water is acidic.

**Table 2: Sterilization of Liquids**

| Solution | Sterilization | Storage |
|---|---|---|
| Agar | Autoclave* | Room temperature |
| Amino acids | Filter** | 4°C |
| Antibiotics | Filter | -20°C |
| Bacto-peptone | Autoclave | Room temperature |
| EDTA | Autoclave | Room temperature |
| Glucose—20% | Autoclave | Room temperature |
| Glucose—1-2% | Filter (low concentrations caramelize if autoclaved) | Room temperature |
| Salt solutions(w/out glucose) | Autoclave | Room temperature |
| Vitamins | Filter | -20°C |
| Water | Autoclave | Room temperature |

*Autoclave—(15lb/in$^2$) 121°C for 20 min.
**Filter—0.45 µm pore size
Source: Table adapted from *Culture of Animal Cells* by R. Ian Freshney, with permission.

*Procedures for isolating DNA have been developed that minimize use of toxic & flammable chemicals, while yielding material suitable for restriction enzyme analysis & rDNA production.*

***...choose DNA isolation procedures that do not require use of phenol or chloroform.***

Wear gloves when using bleach. You may substitute Lysol or some other hospital disinfectant (Roccal, Wescodyne) diluted according to the manufacturer's instructions. Keep all the reagents tightly covered when not in use.

*Fire safety*

Exercise extreme caution when working around an open flame. Do not allow students to wear hats or dangling earrings. Long hair should be pulled back securely. Keep papers, plastic and especially alcohol as far from the flame as possible. Shake off any tools that were soaking in alcohol before passing them through the flame. Allow tools to cool to room temperature before returning them to the alcohol. Keep alcohol containers covered when not in use.

*Organic solvents*

Procedures for isolating DNA have been developed that minimize use of toxic and flammable chemicals, while yielding material suitable for restriction enzyme analysis and rDNA production. These microscale methods help to further minimize chemical hazards.

Important Notes: Ethyl alcohol is flammable.
Methanol is both extremely flammable and toxic.
Chloroform is toxic.
Phenol burns skin on contact.

Use a chemical fume hood when handling methanol, chloroform or phenol, and wear goggles, gloves and a lab coat or apron. When possible, choose DNA isolation procedures

**Table 3: Sterilization of Equipment & Apparatus**

| Item | Sterilization |
|---|---|
| Apparatus containing glass & silicone tubing | Autoclave* |
| Disposable tips for micropipettes | Autoclave |
| Filters—Millipore, Sartorius | Autoclave |
| Glassware | Dry heat** |
| Glass bottles with screw caps | Autoclave |
| Glass coverslips | Dry heat |
| Glass slides | Dry heat |
| Instruments | Dry heat |
| Magnetic stirrer bars | Autoclave |
| Pasteur pipettes—glass | Dry heat |
| Pipettes—glass | Dry heat |
| Screw caps | Autoclave |
| Silicone tubing | Autoclave |
| Stoppers—rubber, silicone | Autoclave |
| Test tubes | Dry heat |

* Autoclave—(15lb/in $^2$) 121°C for 20 min. ** Dry Heat—160°C/2 hrs.
Source: Table adapted from *Culture of Animal Cells* by R. Ian Freshney, with permission.

that do not require use of phenol or chloroform. To minimize student exposure, the teacher can dispense these chemicals at the hood.

*Waste containers*

Do not mix acids, bases and organics in the same container. Discard razor blades in a secure, well-marked container, such as a coffee can with a slit in the lid. Keep bleach containers well-marked.

*E. coli* K-12 cultures, Petri plates and biological waste solutions can be disinfected by either of two ways before discarding:

- Put bacterial waste into a specially marked biohazard bag and autoclave at 15 pounds per square inch of pressure for 30 minutes at 121°C. Biohazard bags are made of a plastic that will not melt away during autoclaving.
- Place in a plastic or enamel bin and soak one hour in 10 percent bleach.

*Preparing to work*

- Students should conduct work with microorganisms only under the direct supervision of a trained teacher or scientist.
- Keep nearby: 10 percent bleach, Lysol or standard hospital disinfectant for use in case of a spill.
- Have sterile materials available including culture media, glass or plastic Petri plates, culture tubes or flasks, and pipettes.
- Keep paper towels nearby in case of spills.
- Caution students not to chew gum or apply lipstick, gloss or lip cream. Caution them to keep all pens and pencils, hair, jewelry, paper, cough drops, fingers and hands out of their mouths whenever they or others are working with microorganisms. This is standard safe behavior in any biology or chemistry classroom lab.
- Have students keep long hair tied back and remove hats or dangling earrings.
- Wash hands before and after doing any work.
- Encourage students to check with the teacher if they have any questions.

***Students should conduct work with microorganisms only under the direct supervision of a trained teacher or scientist.***

# III. Storage of DNA & Bacteria

***Due to health & safety hazards, do not store DNA, microbes or chemicals in refrigerators that are used for food or beverages.***

*Storing DNA*

Most DNA can be stored in a refrigerator or freezer. Do not store DNA, microbes or chemicals in refrigerators that are used for food or beverages. Read the supplier's instructions to determine whether DNA is to be refrigerated or frozen. If the DNA is to be used several times during the year, divide it into smaller portions (aliquots) before freezing. If DNA is subjected to repeated freeze-thaw cycles, the long DNA molecules begin to break down. For this reason, DNA should not be stored for long periods in a freezer with an automatic defrost cycle. It is good practice to handle DNA molecules aseptically, so that bacterial contaminants do not enter the experiment through this route.

*Storing host bacteria*

Bacteria can be stored as slants, stabs, plates, frozen cultures at -70°C or in liquid nitrogen, but in most schools only slants, stabs or plates will be used. Store slants or stabs at room temperature; store plates in the refrigerator and seal with Parafilm or masking tape to prevent drying. Due to health and safety hazards, do not store in a refrigerator used for food or beverages.

Propagate bacteria from plate cultures at least monthly to continue to have live cells. Be sure these are pure culture plates so that a contaminated colony of cells that cannot support growth of the rDNA is not inadvertently selected. Contaminants can be harmful organisms and probably cannot be transformed by DNA. Observe stock cultures for uniformity of colonies. Avoid picking a contaminant.

When preparing for a transformation experiment, always begin with a single colony from a streak plate. Prepare this the day before growing a liquid culture. Check that the culture remains antibiotic-sensitive. Do this by streaking on a fresh nutrient agar plate containing the appropriate antibiotic the day before conducting the experiment. Include this as a control when conducting a transformation experiment.

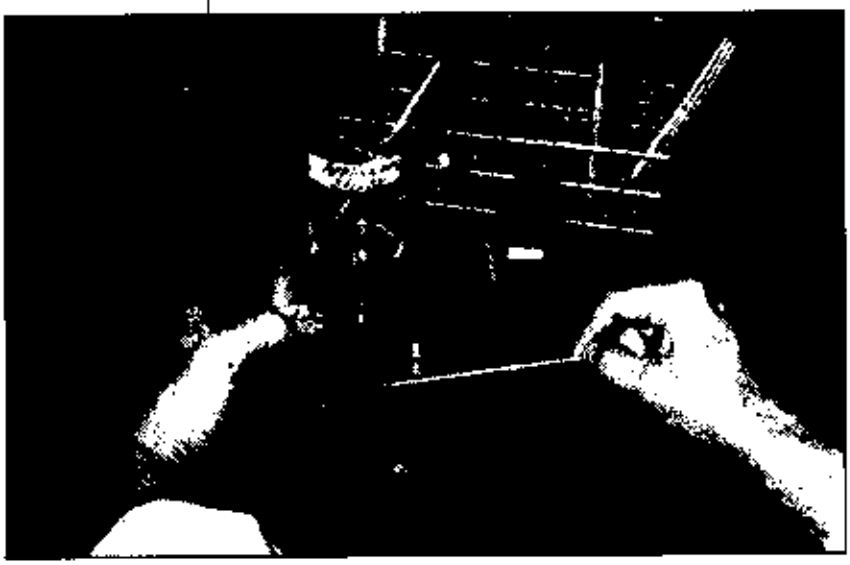

*Figure 2: Streaking the sterile agar surface of a Petri dish to obtain isolated colonies.*

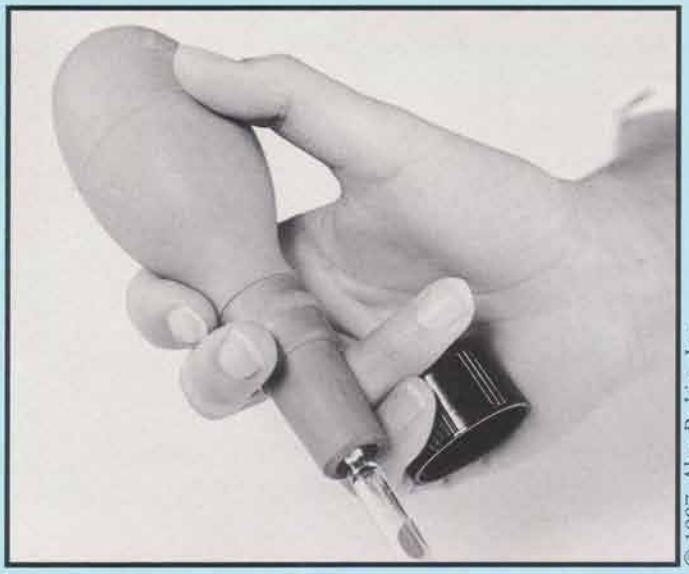

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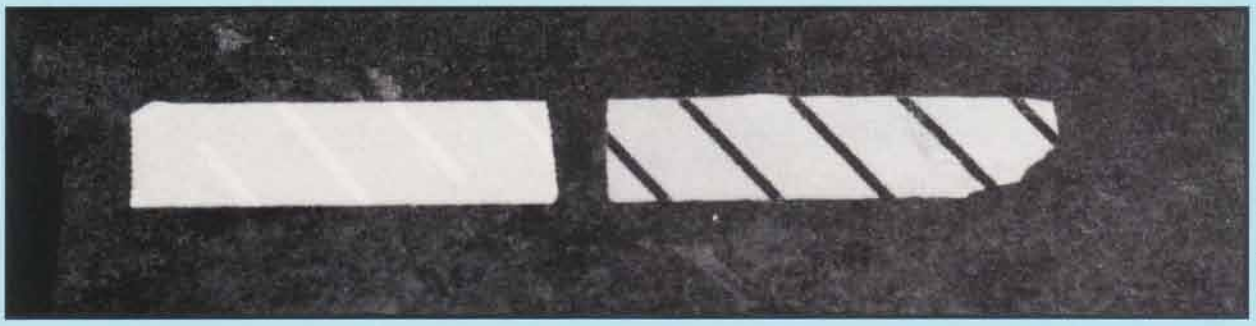
*Figure D (top left): Proper technique for holding cap and pipette. Figure E (top right): This photomicrograph shows the copious production of aerosols and droplets when the last drop in a pipette is blown out. Figure F (middle): Autoclave tape: invisible lines to black. Figure G (bottom left): Holding vessels horizontally. Figure H (bottom right): Notice neat work area.*



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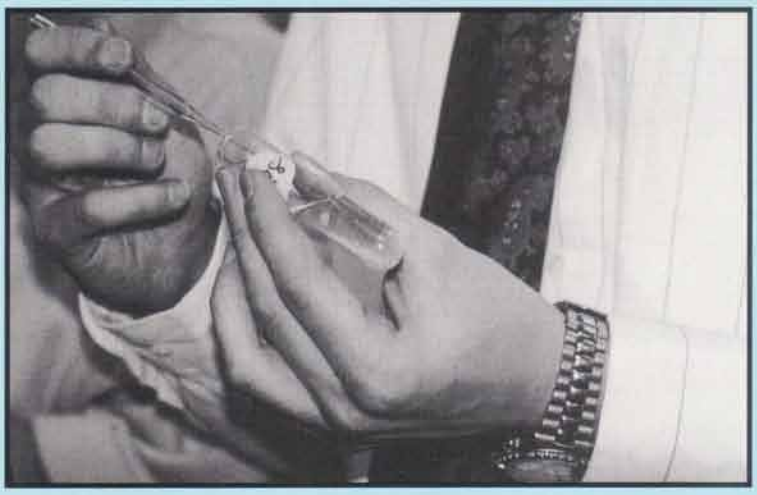

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# *Standard Microbiological Practices*

*Handle all bacteria and DNA carefully. Treat them as if they were infectious.*

1. **Keep classroom doors closed during lab periods.**
2. **Do not eat, drink, smoke or apply cosmetics in the work area.**
   Store food in marked cabinets or refrigerators outside the work area.
3. **Wash your hands both before and after handling viable materials and before leaving the laboratory.**
4. **If you have cuts on your hands or arms, wear latex gloves for protection.**
5. **Wear a laboratory coat to prevent contamination or soiling of your clothes.**
   Launder in hot soapy water with bleach.
6. **Do not mouth pipette anything.**
   Use mechanical pipetting devices.
7. **Perform all procedures carefully to minimize the creation of aerosols.**
   Do not force the last drop of liquid from a pipette. First, place the pipette tip close to the liquid layer, then discharge the fluids down the inner wall of the tube or bottle.
8. **Decontaminate work surfaces once a day and after any spill of viable material.**
   At the beginning and end of each class or session, have student assistants wipe the work areas down with a 10 percent bleach solution or appropriately diluted disinfectant.
9. **Decontaminate all contaminated liquid and solid wastes before disposal.**
   Loosen bag seals, caps, lids, etc., so steam or disinfectant can circulate. Steam-autoclave or sterilize in a pressure cooker for 30 minutes at a pressure of 15 pounds per square inch and temperature of 121° C. When cool, dispose of materials according to local and government regulations. Alternatively, place waste materials in a plastic or enamel bin and flood with a 10 percent bleach solution. Soak for one hour, then rinse well before disposal according to local and government regulations.

(Adapted from CDC-NIH *Biosafety in Microbiological and Biomedical Laboratories,* 2nd ed., May 1988, pp.11-12.)

# *Aseptic Techniques*

## Remember! YOU may be the major source of contamination.

Hands, face, hair, clothing, the outsides of all objects -- even the air -- contain microbes that can enter and infect the experimental culture. Aseptic handling helps minimize exposing the culture or DNA to microbes that naturally and normally are on the body or in the air, reducing the probability of contaminating experiments. These methods apply to teachers and students doing lab work:

### 1. Use sterile media, vessels & tools.

Purchase or prepare sterile culture media. You may also need to have sterile additives, water for dilutions, pipettes, cylinders, flasks and bottles. Media poured into non-sterile vessels will become contaminated by unwanted organisms.

### 2. Keep your hands far from the working ends of tools & vessels.

Keep hands from the rims or necks of bottles. Hold forceps, spreaders and loops far from their working ends. Handle pipettes only upstream of their safety lines. If the tip of any tool may have touched a non-sterile place, use a fresh, sterile tool.

### 3. Keep your work area neat.

A properly organized work area helps to keep the process aseptic. Place items near the work area. This procedure helps prevent contamination and allows items to be moved into and out of the work area as needed.

### 4. Keep vessels covered when not in use.

Keep loosened caps on the bottle or tube. Remove caps only when transfering in or out of a tube or bottle. Streak or spread a plate while holding the lid at an angle. This will minimize the entry of unwanted microbes into the culture or media from the air.

### 5. Hold vessels as close to horizontal as possible when making a transfer.

This will minimize entry of unwanted microbes.

### 6. Do not pass your hands or arms over tools or open vessels.

Hands, face, hair, arms and sleeves naturally and normally harbor microbes, which can contaminate the experimental culture. Keep hands out of pockets and away from face and hair while working.

### 7. Practice aseptic handling BEFORE conducting the actual experiment.

# IV. Standard Microbiological Practices & Aseptic Techniques

At this time, only Biosafety Level 1 work is suggested for high schools. This is defined as being "suitable for work involving organisms of no known or of minimal potential hazard to laboratory personnel and the environment." (CDC-NIH *Biosafety in Microbiological and Biomedical Laboratories*, 2nd ed., May 1988, pp. 7, 11.) Level 1 studies can be performed on a regular lab bench using standard microbiological practices. No special containment hood is necessary.

***At this time, only Biosafety Level 1 work is suggested for high schools.***

## *STANDARD MICROBIOLOGICAL PRACTICES*

(Adapted from CDC-NIH *Biosafety in Microbiological and Biomedical Laboratories*, 2nd ed., May 1988, pp.11-12.)

1. Access to the laboratory should be limited or restricted at the laboratory director's discretion when experiments are in progress.
2. Decontaminate work surfaces once a day and after any spill of viable material. It is prudent to have students wipe the benches down with 10 percent bleach (1 volume of bleach plus 9 volumes of water from the tap) or disinfectant at the beginning and end of each class or session. Make sure no student is allergic to bleach before using and that gloves are worn when using the bleach.
3. Decontaminate all contaminated liquid or solid wastes before disposal. This is done by steam-sterilizing in an autoclave for 30 minutes at 15 pounds per square inch of pressure at 121°C. When cool, the wastes are ready for disposal. An alternate method of sterilization is to soak wastes in 10 percent bleach for one hour, then rinse before disposal.
4. Mouth pipetting is prohibited (see insert, *Figures A-D*).
5. If you have any cuts on exposed hands or arms, be sure to wear gloves or do not handle the DNAs or cells.
6. Do not eat, drink, smoke or apply cosmetics in the work area. Food may be stored only in designated cabinets or refrigerators located outside the work area.
7. Lab participants should wash their hands both before and after handling viable materials and before leaving the laboratory.
8. Perform all procedures carefully to minimize the danger of aerosols (see insert, *Figure E*). For example, do not force the last drop of liquid from a pipette. Place the pipette tip in the receiving vessel close to the liquid layer, then release the last drop.
9. Wear laboratory coats, gowns or uniforms to prevent contamination or soiling of street clothes. These can be laundered in hot water with soap and bleach.

### *Special practices*

1. When autoclaving materials for disposal, loosen bottle caps and open the bags so steam can circulate--to prevent the buildup of steam pressure and the possibility of explosion. If decontamination is to take place away from the laboratory, tightly seal contaminated materials in a durable, leak-proof container for transport. Autoclave for 30 minutes to be sure that all materials have been heated long enough to destroy spores and other contaminants.
2. Check with the school's engineer concerning the building's insect and rodent control program. Do not conduct any rDNA experiments without such a program. Put away all materials when not in use; insects and rodents may be attracted to spillage from contaminated materials.

# *Standard Microbiological Practices & Aseptic Techniques*

***Remember! YOU are the major source of contamination.***

*Containment equipment*

Special containment equipment generally is not required for manipulations. A biosafety cabinet is not necessary.

*Laboratory facilities*

1. The laboratory should be designed so that it can be cleaned easily.
2. Bench tops should be impervious to water and resistant to acids, alkalis, organic solvents and moderate heat.
3. Laboratory furniture should be sturdy with the spaces between benches, cabinets and equipment accessible for cleaning.
4. Each laboratory should have a sink for handwashing.
5. If the laboratory has windows that open, they should be fitted with screens.
6. Safety equipment should include a first-aid kit, fire blanket, all-purpose fire extinguisher, eyewash station, shower and lab aprons for each student.
7. Each student should have his/her own safety goggles. Safety goggles should not be shared.
8. The laboratory must provide adequate work space for each student. Space should be available for all students to conduct the experiment simultaneously.

*ASEPTIC TECHNIQUES*

**Remember! YOU are the major source of contamination.**

Hands, face, hair, clothing, the outsides of all objects -- and even the air -- contain microbes that can grow on the culture media. The following rules apply to teachers and students who work in the lab:

1. **Use sterile media, vessels & tools.**
   Purchase or prepare sterile culture media. You may also need to have sterile additives (see page 7, *Table 2: Sterilization of Liquids* and page 8, *Table 3: Sterilization of Equipment & Apparatus*), pipettes, cylinders, flasks or bottles and sterile deionized or distilled water for diluting. Sterile media poured into non-sterile vessels will be contaminated. Check the material to determine the best sterilization method.
2. **Keep hands far from the working ends of tools & vessels.**
   Most pipettes have a "double line" of safety (see insert, *Figure B*). You can easily hold and operate a pipette upstream of this marking.
   Do not touch the tip or column of the pipette with any object, even hands or the bench top. If something may have touched the pipette, use a fresh, sterile one. Many suppliers package pipettes individually.
3. **Keep the work area neat.**
   A properly organized work area helps to keep the process aseptic. Items can be moved so that they are nearby when needed or out of the way so they do not accidentally become contaminated (see insert, *Figure H*).
4. **Only uncover vessels when conducting a transfer.**
   Flame caps and necks of vessels before and after conducting a transfer. Lift the lid of the

Petri plate as seldom and at as shallow an angle as possible. Bacteria and mold spores are in the air. Culture media are a source of nutrition for these airborne contaminants. After some practice, pipetting can be done easily while holding the vessel cap open end down (see insert, *Figure D*).

5. **Hold vessels as horizontally as possible when making a transfer.**
   (See insert, *Figure G.*)
6. **Do not pass hands or arms over open vessels, caps or tools.**
   Hands, face, hair, arms and sleeves naturally and normally harbor microbes, which can contaminate the experimental culture. Keep hands out of pockets and away from face and hair while working.
7. **Do not pipette out the last drop.**
   When the last drop in a pipette is blown out, large amounts of aerosols and droplets are produced (see insert, *Figure F*).

**Table 4: Chemical Decontamination**

| | Quaternary Ammonium Compounds | Phenolic Compounds | Chlorine Compounds | Iodophor Compounds | Alcohol (ethyl or isopropyl) |
|---|---|---|---|---|---|
| USE OF PARAMETERS | | | | | |
| Concentration of active ingredient | 0.1-2% | 0.2-3% | 0.01-5% | 0.47% | 70-85% |
| Contact time (minutes) | 10-30 | 10-30 | 10-30 | 10-30 | 10-30 |
| EFFECTIVE AGAINST* | | | | | |
| Vegetative bacteria | + | + | + | + | + |
| Bacterial spores | | | ± | | |
| HIV | + | + | + | + | + |
| HBV | | ± | + | ± | ± |
| APPLICATIONS* | | | | | |
| Contaminated liquid discard | | | + | | |
| Contaminated glassware | + | + | + | | + |
| Contaminated instruments | | + | | | |

*A + denotes very positive response; a ±, a less positive response; and a blank, a negative response or not applicable.
(Note to teachers regarding chemical compounds: Prepare fresh daily. Consult with suppliers regarding chemical composition of brand name products.)
Source: Table adapted from *Biosafety in the Laboratory*, with permission.

# V. Handling of Chemicals, Cleanup & Disposal

## HAZARDOUS CHEMICALS

*When working with bacteria:*

1. The culture media are safe to work with in powdered form, although some people may be allergic to tryptone dust or to agar, which are components of LB and many other standard bacterial culture media. Use an inexpensive dust mask when weighing out the components.
2. Use caution when weighing out and preparing antibiotic solutions. A dust mask and disposable gloves are recommended.
3. Treat all plates that have bacteria as biohazard waste. Plates often become contaminated with common molds that may cause an allergic reaction, or *Staphylococcus aureus*, which can cause skin infections. Immersing the plates, with covers removed, in 10 percent bleach for an hour should kill bacteria and mold.
4. Keep alcohol soak beakers and flames as far from each other as possible. In the classroom, establish alcohol soak and flame stations that are far apart.

*When isolating DNA:*

1. Sodium Dodecyl Sulfate (SDS) is a powerful irritant. Prepare solutions using a dust mask. Avoid creating dust.
2. Ethanol and isopropyl alcohol, which are used for DNA precipitation, are somewhat toxic. Avoid skin contact.

*When conducting gel electrophoresis:*

1. Most gels in class are run with 50-100 volts of direct electric current which can cause a severe jolt. Gel boxes should have built-in safety features, such as covers that incorporate the electrode leads so people cannot stick their fingers in the running buffer. When building gel boxes, design them with this safety feature.
2. More than 100 volts can generate enough heat to melt some agarose gel media which can change the sieving properties.

***Due to the mutagenic & possible carcinogenic effects of ethidium bromide, NABT strongly suggests teachers use methylene blue.***

## DNA STAINING SOLUTIONS

Methylene blue and ethidium bromide are common stains for visualizing DNA bands after gel electrophoresis. Although more sensitive, ethidium bromide is a mutagen based on the Ames test and a possible carcinogen. Most research laboratories use ethidium bromide and train their workers in safe handling methods. In many instances, the classroom teacher may have to decide whether to use methylene blue or ethidium bromide after consulting with the local and state regulations regarding the use of DNA staining solutions in the classroom. Due to the mutagenic and possible carcinogenic effects of ethidium bromide and the possible harm to the teacher and/or student, **NABT strongly suggests the use of methylene blue.** (Note: Students should never use ethidium bromide or handle gels stained with ethidium bromide.) Since the teacher ultimately has the responsibility for which staining solution to use, a comparison of ethidium bromide and methylene blue is provided on the opposite page to help make the decision.

# HANDLING OF CHEMICALS, CLEANUP & DISPOSAL

**Table 5: Benefits & Risks of Using Methylene Blue or Ethidium Bromide**

| | Methylene Blue | Ethidium Bromide |
|---|---|---|
| Health concerns | Moderately toxic | Mutagen and suspected carcinogen; UV light source damage to unprotected eyes |
| Relative amount of DNA required | Large | Small |
| DNA sensitivity | Low: Interacts with DNA | High: Tight complex with DNA |
| Light source required to see banding | Fluorescent light box | Ultraviolet |
| Bands after staining | Blue bands visible with light source from beneath | Intense with UV light |
| Staining time | Variable (30-60 minutes for major bands) | 15 minutes |
| Destaining time | 2-24 hours | 2 minutes |
| Stability | Shelf life of up to 6 months | Light sensitive, store in the dark |
| Gloves required for handling | Yes | Yes |
| Relative expense | Low | High |
| Relative preparation/disposal time involved | Less | More |

## Methylene Blue

*Staining DNA with methylene blue*

Greater amounts of DNA are required when staining with methylene blue. When substituting methylene blue in lab activities that call for ethidium bromide as the staining solution, increase the DNA concentration 4- to 5-fold and plasmid DNA, 2-fold. Only the DNA and plasmid concentrations need to be increased. The amounts of other components remain the same. **Wear gloves at all times when handling methylene blue.**

*Viewing DNA gels stained with methylene blue*

Gels stained with methylene blue may be viewed using a light source from beneath. The light source may be a fluorescent light box used for slides or negatives, an overhead projector, or a window. Protect the surface of the light box or overhead projector with a layer of plastic wrap. Put the gel in a clear plastic tray or plastic storage bag.

***Methylene blue is moderately toxic... Wear gloves.***

*Disposal of methylene blue*

Always check first with the local waste management authority before disposing of any chemicals, including methylene blue. The teacher has the ultimate responsibility to know

# HANDLING OF CHEMICALS, CLEANUP & DISPOSAL

***Students must not use ethidium bromide or handle gels stained with ethidium bromide.***

and precisely follow the method of disposal required by local waste management authorities for each chemical used in the laboratory. Chemicals should never just be put down the drain.

If the school drains are connected to a sanitary sewer system with a water treatment plant that handles the effluent from the drains, the municipality may permit the disposal of methylene blue down the drain with copious amounts of water. Methylene blue should not be combined with any other chemicals during the disposal procedure. A reminder: **Always check with local authorities first.**

Under no circumstance should methylene blue be disposed of down the drain if the school's drain system is not connected to a wastewater treatment plant that specifically handles the school drains' effluent. In this instance, check local regulations as to how to properly dispose of methylene blue.

For further information consult the current *Flinn Chemical Catalog/Reference Manual*, Flinn Scientific, Inc., Batavia, Illinois.

## Ethidium Bromide

(Adapted from *DNA Science: A First Course in Recombinant DNA Technology*, 1990, by D.A. Micklos & G.A. Freyer.)

**Gloves must be worn in all situations that involve the use of ethidium bromide.** When handling the dry solid form of ethidium bromide, a respirator--a special mask with absorptive charcoal--must be used. Remember: When wearing gloves, refrain from touching objects or surfaces that must be kept free of contamination, such as doorknobs, drawer pulls, telephones, record books, pens, hair, face or clothing. After completing procedures and upon removing gloves, persons handling carcinogens, suspected carcinogens or toxic substances such as ethidium bromide should immediately wash their hands in warm water with soap. **Students must not use ethidium bromide or handle gels stained with ethidium bromide.**

*Responsible use of ethidium bromide*

Ethidium bromide, like many natural and man-made substances, is classified as a mutagen by the Ames microsome assay and is a suspected carcinogen. With responsible handling, the dilute solution (1 µg/ml) used for gel staining poses less risk to the user than the 5mg/ml stock solution, or the powder form of ethidium bromide used to make the 5mg/ml stock solution. To avoid handling the dry solid form, ready-mixed stock solutions may be purchased from many molecular biology product suppliers. The stock solution is diluted to make a staining solution with a final concentration of 1 µg/ml. Procedures using ethidium bromide must be performed by a qualified instructor in a controlled area.

*Caution/handling & decontamination of ethidium bromide:*

1. **Always wear gloves when working with ethidium bromide solutions or stained gels.**
2. Limit ethidium bromide use to a restricted sink area.
3. Following gel staining, use a funnel to decant as much as possible of the ethidium bromide solution into a storage container for reuse or decontamination and disposal.
4. Disable (i.e. inactivate ethidium bromide in) stained gels and used staining solution according to accepted laboratory procedure. The method given below is from Quillardet and Hofnung (1988):

a. If necessary, add sufficient water to reduce the concentration of ethidium bromide to less than 0.5 mg/ml. (Note: The working concentration for staining gels is 500 times more dilute.)
b. Add 1 volume of 2.5 M $KMnO_4$, and mix carefully. **Caution: $KMnO_4$ is an irritant and is explosive. Solutions containing $KMnO_4$ must be handled in a chemical hood.**
c. Add 1 volume of 2.5 N HCl, and mix carefully.
d. Let stand at room temperature for several hours.
e. Add 1 volume of 2.5 N NaOH, and mix carefully.
f. Although the ethidium bromide is decontaminated, studies suggest that the decontamination by-product is mutagenic. As a result, some institutions use a solid waste disposal program for the by-product of the decontamination process.

Regulations for mutagenic waste vary. Check with your local and state waste management authorities for the proper method of disposal. It may be possible in your area to make arrangements with a solid waste disposal program at a local university or other institution.

*Viewing stained gels*

Transillumination, where light passes up through the gel, gives superior viewing of gels stained with either ethidium bromide or methylene blue. A mid-wavelength (260-360 nm) ultraviolet (UV) lamp emits in the optimum range for illuminating ethidium bromide-stained gels. Avoid short wavelength lamps, whose radiation is most dangerous. Long wavelength ("black light") lamps, although safe, give less-intense illumination.
**Caution: Ultraviolet light can damage the retina of the eye. Never look at an unshielded UV light source with naked eyes. View only through filter or safety glasses that absorb harmful wavelengths.**

## *SPILLS*

If bacteria or DNA are spilled, wear gloves and absorb the spill with paper towels or with plastic-backed bench protector, "diaper" paper. Then, working from the outside of the spill, pour on properly diluted disinfectant, such as Lysol, Zephiran or Wescodyne, or 10 percent bleach. Wipe toward the center of the spill. If a chemical is spilled, use a chemical spill clean-up kit.

## *CLEANUP & DISPOSAL*

Clean the work area by swabbing down with 10 percent bleach, Lysol or Wescodyne diluted according to the manufacturer's instructions.

Discard all biologicals (cells, culture media, DNA) in clearly marked biohazard bags or trays. Pipettes, Petri plates and other materials that have been exposed to bacteria or DNA should be treated as biohazard waste.

If an autoclave is available, place all disposable materials in a biohazard bag or autoclavable steel or plastic tray. Autoclave for 30 minutes at 15 pounds of pressure per square inch and 121°C. If the glassware is to be reused, pour out any agar residues while still liquid.

***Pipettes, Petri plates and other materials that have been exposed to bacteria or DNA should be treated as biohazard waste.***

# HANDLING OF CHEMICALS, CLEANUP & DISPOSAL

***Check with the school district's risk assessment or waste management office, your district science supervisor, or city or county government for safe disposal information.***

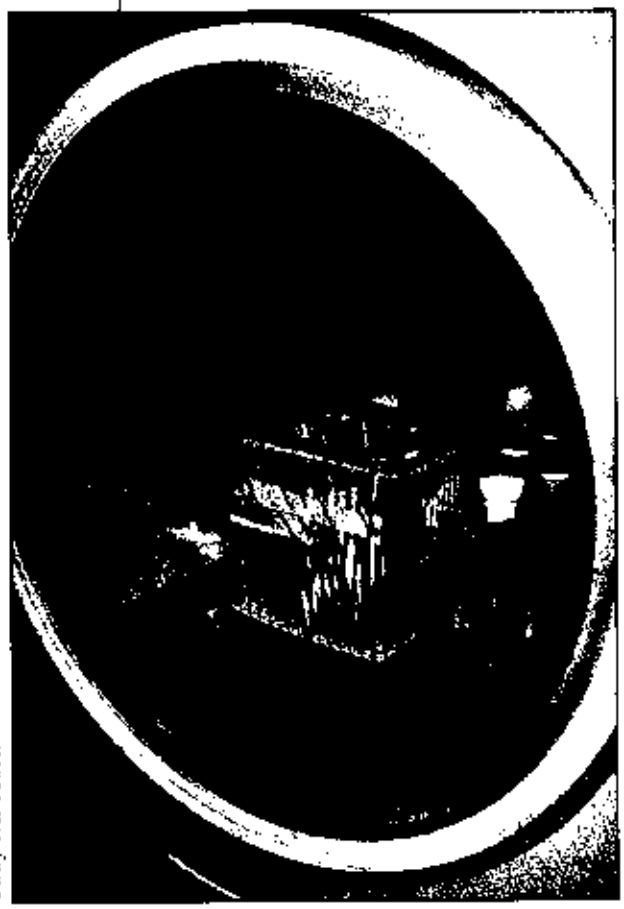
As an alternative to autoclaving, flood the materials with a 10 percent bleach solution in a tray or bucket made of heavy-duty plastic or rust-free enamel. Soak for one hour.

Check with the school district's risk assessment or waste management office, your district science supervisor, or city or county government for safe disposal information. If permitted by the local authorities:

- Dispose of liquids down the sink drain and flush with running water for five minutes.
- Put all disposables in the trash.
- Discard autoclaved materials.

## *SUPERVISION*

The teacher is responsible for making sure that students understand safety instructions at all times; students are responsible for obeying these instructions. Teachers should check with their science supervisors and/or their district or state education offices for general lab safety guidelines.



Toby M. Horn

*Figure 3: Shown here are autoclaved materials ready for disposal. Notice black lines have formed from invisible tape.*

# VI. APPENDIX

## *EXAMPLES OF EXPERIMENTS*

The following is a list of inquiry-based labs to teach DNA science:

A. Culturing *E. coli* K-12

Students can test:

- the effects of temperature of incubation on the growth rate
- the effect of agitation of the culture (whether it is shaken or kept stationary)
- what happens when a component of the medium is omitted.

B. Transforming *E. coli* K-12 with plasmid DNA

Students can test how transformation efficiency is affected by:

- the incubation time of plasmids with bacteria
- the concentration of calcium chloride in the transformation prep
- the use of magnesium in place of calcium
- the necessity for heat shock
- the length of the recovery period.

C. Restriction enzyme analysis

Students can:

- explore the time relationship at one enzyme concentration, checking the restriction pattern at various times up to completion (one hour)
- examine what happens when different buffers are used for the endonuclease treatment.

D. Making a pAMP-pKAN recombinant

Students can ask the following questions:

- What happens when different regions of one vector are inserted into the other?
- Where is the resistance gene located?
- What happens when there are two replication origins?

E. Studying the pUC LAC system

Students can study the effect of inserting other permitted DNA into different sites of the pUC vector.

F. Isolating mutants of *E. coli* K-12

Students can generate modified *E. coli* K-12 seeking higher transformation efficiencies, for instance by mutagenizing the bacteria with an ultraviolet lamp.

G. DNA fingerprinting

H. Identifying protein of bacteria

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# About the Author

This monograph reflects the author's personal commitment to enable science teachers to be as comfortable in a research laboratory as in the classroom. In *Working with DNA & Bacteria in Precollege Science Classrooms*, Toby Mogollon Horn, Ph.D., has developed safety guidelines to help teachers in precollege science classrooms conduct safe and successful exercises using DNA and bacteria.

Working with safety experts from across the nation, lab researchers, teachers and science supervisors, Toby has compiled detailed safety procedures, including:

- Permitted DNA molecules, vectors and recommended host organisms for constructing recombinant DNA.
- Procedures for preparing materials and assuring a safe classroom work area.
- Proper storage requirements for DNA and related materials.
- Standard microbiological practices and aseptic techniques.
- Proper handling of hazardous chemicals and DNA stains.
- Discussion of cleanup and disposal of lab materials.

"Dr. Toby" shares her love of science with students and faculty at Thomas Jefferson High School for Science and Technology, in Alexandria, Virginia, where she teaches Biotechnology Research and is the Director of the Life Sciences and Biotechnology Laboratory.

She has received several honors for her dedication to the teaching profession and to science education, including the Fairfax County Public School's Superintendent's Award; Science Teacher of the Year from the Falls Church Chapter of the American Association of University Women; and a Summer Teacher Research Fellowship from the Foundation for Advanced Education in the Sciences.

Toby has had extensive experience in laboratory research as Staff Fellow at the National Institutes of Health. Among her professional affiliations are: membership in the Chesapeake Area Chapter of the American Biological Safety Association (President, 1992-1993); Fellow of the NIH Science Education Academy; Founder of Science by Women at TJHSST; and member of the Board of Directors of The Roothbert Fund. Also, she is a member of the American Association for the Advancement of Science, American Chemical Society, Association for Women in Science, Fairfax County Federation of Teachers and the National Association of Biology Teachers.

Toby earned an A.B. in chemistry from Bryn Mawr College in Bryn Mawr, Pennsylvania; and a Ph.D. in molecular, cellular and developmental biology from the University of Colorado, in Boulder. She holds a professional teaching certificate in the Commonwealth of Virginia.

Toby wishes to thank the staff at NABT, the corporate sponsors, her husband Gustavo and her colleagues around the country for their support and encouragement. She hopes teachers find this document useful in working with their students and school district and looks forward to any and all comments.