2

Laboratory Setup and Equipment

T FIRST, THERE WILL SEEM to be a bewildering amount of equipment on the benches, on the floors, sometimes even in the aisles. In most labs, this is very standard equipment, and after a while, all labs will seem to be very familiar places. In certain specialized labs, such as electron microscopy labs or electrophysiology labs, there will be a concentration of certain, atypical kinds of equipment, but a general lab ambiance will still be apparent.

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If you don't understand the equipment you are using for an experiment, you don't fully understand the experiment. Start immediately learning what each piece of equipment is, and what it is used for. Not only will this help you feel more at home, but it will also acquaint you with the experimental potential in your back yard. Notice who uses what, so you will know where to find an expert when you need one. When it comes time to use a particular apparatus, be sure to understand the rudiments of its operation: Only then can you properly manipulate conditions or know when an unexpected and apparently Nobel-quality result is merely the result of mechanical failure.

LAY OF THE LAND

Main Laboratory

Laboratory benches predominate, physically and psychologically: These are long or short peninsulas, with drawers underneath, bottles on shelves above, and small equipment and open working spaces on the surface. Often, there is a desk attached to one end. Each person usually has a bench, or part of a bench, and "the bench" is, to its owner, home. The person who shares your bench is your benchmate, observer of all your experimental and emotional ups and downs and thus, the laboratory equivalent of a spouse.

Some labs have nothing but "private" lab benches, and lab members are expected to perform all tasks on their own lab benches. Check it out, for the rules are usually very different for private vs public lab areas. On your lab bench, you can probably allow a used paper towel or pipet to remain, but you must be meticulous about removing your supplies and waste from a common area.

The typical lab bench

A slab of wood, slate, metal, or plastic—this will be the center of your lab life, your primary working area. On the bench are small pieces of equipment, such as a vortex and holders for various pipettors, and supplies such as pipettor tips.

Most lab benches are equipped with a vacuum line, an air line, a gas line, and sometimes, a water line. The *water line* is the most useful in theory but the least in practice: Using it often results in a puddle on the bench. The *air* can be used to blow obstructions from tubes, to dry glassware quickly, and for other brute purposes. But this is dusty air, and it shouldn't be used on glassware that will be used for experiments or buffer preparation. The *gas line* is used to fuel Bunsen burners, needed for aseptic technique at the bench. The *vacuum line* is extremely useful, especially for removing supernatants.

Above the bench are usually shelves. Here are stored personal buffers and reagents. Detergents and Tris buffers often comprise the bulk of the bottles. Pipet tips and contain-

ers of microfuge tubes also sit here.

If there are cabinets beneath the lab bench, acids and bases (not in the same cabinet, of course) and large bottles of buffer or solvents will be found. Odd, old, favorite, or infrequently used small equipment might also be here.

There are drawers beneath the lab bench. A casual peek might reveal a terrifying mix of old pH papers, pasteur pipet bulbs, and Sharpies, since these drawers tend to be where harried investigators throw odds and ends, to be organized later. Here might also be found boxes of tips, bags of tubes, and errant pieces of common lab equipment such as gel spacers and combs. These drawers are usually considered to be personal space, so don't just rummage through a neighbor's bench drawers without asking.

Common lab equipment may be found on the bench, usually at the end. Having a piece of common equipment on the bench does not entitle one to preferential use of the equipment! And using a piece of common equipment on someone's bench does not allow

you to use the pipets, tubes, or other supplies on the bench.

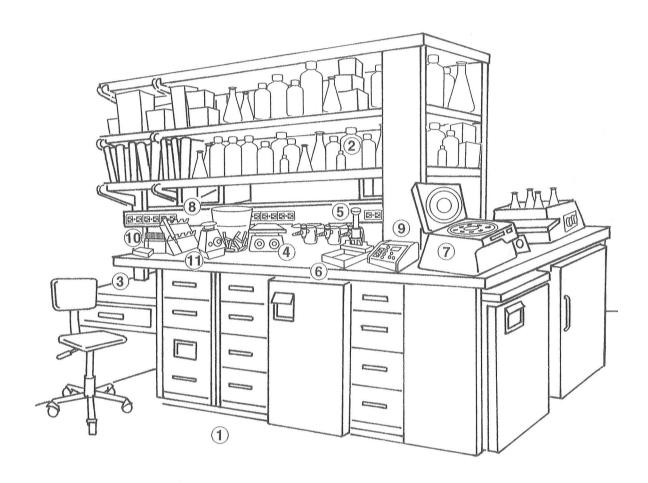


FIGURE 1.

The laboratory bench. Key: (1) Bays. The area between benches, and more a psychological than a physical entity; sharing a bay with someone is a close and somewhat intimate relationship. Equipment tends to be shared in bays, reagents borrowed and lent, favors asked, stories told. Be kind to your baymate. (2) Buffers and other reagents. After being autoclaved, most buffers can be stored at room temperature. These belong to the owner of the lab bench, and should not be touched without permission. (3) Desk. Desks, particularly in older labs, may not be part of the lab bench, but may be found wherever there is space. Very few desks are found in some labs, and there are instead rooms filled with desks for all the departmental students and/or postdocs. Sometimes, not everyone gets a desk but must use the department library or conference room to read or to make notebook entries. Usually, however, desks are found at the end of the lab bench and against the wall. (4) Hot plate. Used to heat liquids. Samples are usually boiled in a beaker on a hot plate. Hazards: Burns, liquids bubbling over. Alternatives: Water bath, microwave. (5) Flame burner (also known as a Bunsen burner). Vital to aseptic technique, for heating bottles and loops. Hooked up to house gas supply. Turn off after each use. Alternatives: Electric loop sterilizers and disposable plastic loops. (6) Gel box. Plastic containers used to run protein, DNA, or RNA gels. They range in size from mini-gels to sequencing gels. (7) Microfuge. A small, benchtop centrifuge that spins volumes up to 2 ml at approximately 12,000g. Used to pellet cells, precipitate DNA ... a workhorse. Some models are refrigerated, most are not. Some units are kept in a refrigerator or cold room. Alternatives: Adapters can be used in larger centrifuges and rotors. (8) Pipettors. Instruments used to measure and transfer small volumes of liquid. (9) Power supply. Used to run electrophoresis, perform transfers of gels to filters. Shocks can result from careless handling. Not all power supplies perform all tasks, so be sure you have the correct one for the job. (10) Tip boxes. Pipettors require the right size tips to dispense fluids accurately. These tips are usually autoclaved before use, and are disposable. (11) Vortex. Used to mix the contents of tubes. Can have an adapter for multiple tubes. Alternatives: Nutator, wheel, shaker.

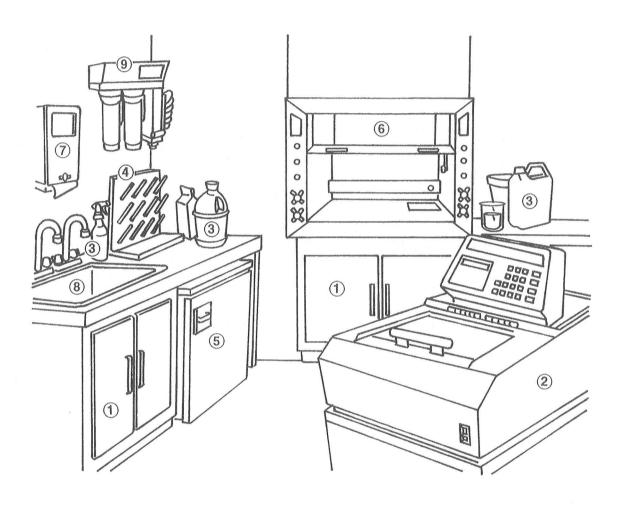


FIGURE 2.

Sink area, centrifuge, fume hood. Key: (1) Cabinets. Acids or bases or organic solvents are stored in common areas throughout the lab. (2) Centrifuge. Spins tubes filled with a liquid/solid mix, separating the mix into (hopefully) distinct phases and concentrating solid phases. There are several kinds of centrifuges, categorized by speed and tube size capabilities. Alternatives: No practical alternative. Filtration can remove the media and trap solids for some material. Hazards: Generation of aerosols and mechanical failure. Aerosols of biohazard or toxic materials can be produced if good laboratory technique and centrifuge safety and containment equipment aren't used when centrifuging such substances. A mechanical failure can produce fragments moving at great velocity, and if such fragments escape the protective bowl of the centrifuge, they can cause traumatic injury to personnel. (3) Detergents. There may be several kinds of detergents and cleaning agents here: for hands, for glassware, and for radioactivity. (4) Drying rack. After handwashing, beakers and other labware are placed here. (5) $-20^{\circ}C$ Freezer. Used to store serum, most enzymes, reagents. There are often several freezers in the lab. (6) Chemical fume hood. Air is vented out of a chemical hood, making this the place of choice for working with volatile substances such as chloroform (and phenolchloroform). Volatile radioactive labeling is done in some hoods, which are certified for this purpose. If this is true of the hood you are using, check for radioactivity with a Geiger counter. (7) Paper towels. Used for wiping hands and lab benches, and sometimes, for recording data. Replenish the stock if you use the last one. (8) Sink. Keep the sink clear for disposal and work. Be careful what you pour down the sink. Untreated supernatants from cells and bacteria should not be disposed of here, nor should hazardous chemicals. (9) Water purification unit. Tap water cannot be used for most laboratory applications. By distillation, or by reverse osmosis and ion exchange, the unit removes particles and other impurities from water. Alternatives: Purified water can be purchased in small quantities of 500 or 1000 ml.

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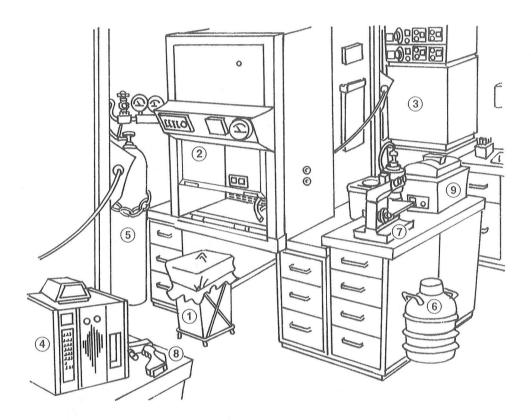


FIGURE 3.

Tissue culture area. Key: (1) Biohazard waste disposal. Anything living or used to hold anything living must be autoclaved before disposal. (2) Biosafety cabinet. Sometimes casually referred to as a hood or a laminar flow hood, this has a forced airflow to minimize the entrance of any dust or organisms into the working area. Laminar flow hoods should always be left on. (See Chapter 8.) Alternatives: If a biosafety cabinet is not required—if there is not a biohazard associated with the work material—a still-air box or traffic-less and draft-free place can be used for tissue culture. (3) CO2 incubator. Used primarily for tissue culture, CO2 is piped into an incubator for CO2-requiring organisms or to maintain pH in the culture medium. Remarks: Buzzing may indicate a need to fill the water jacket, or a lack of CO₂. Alternatives: CO₂ can be pumped or generated in a container and incubated at the correct temperature. A buffer such as HEPES in a closed system can be used instead of CO₂ to maintain the pH of some cultures. (4) Coulter counter (also known as a cell counter). Electronically counts cells or particles. Alternatives: A counting chamber can be used with a microscope to manually count cells. (5) Gas cylinders. Pressurized gases have many uses in the lab, such as CO2 for incubators, or nitrogen for disrupting cells. Most gas cylinders in use have a regulator attached to the valve, which is used to close the tank and regulate the outward flow of gas. The tank should always be roped or chained to a wall when standing and should be manipulated carefully when being moved. There is a danger of explosion or fire with oxygen and hydrogen, and you should get instruction from the EHS about the use of these gases. Valves open by turning counterclockwise. (6) Liquid nitrogen tank. A metal container filled with liquid nitrogen, it is used for the long-term storage of cells, viruses, and microorganisms. (7) Microscope. Used to magnify and observe tissues, cells, and microorganisms; there are two designs found in the lab. A standard compound microscope is used to observe samples that have been removed from culture medium and placed on a slide. An inverted microscope (the objective lens is situated below, not above, the sample) can magnify cells and organisms while they are still in the culture container. Microscopes often have attachments for fluorescence, and a camera. (8) Pipet aids. Liquids (volumes over 1 ml) are measured and transferred with pipets. Since no mouth pipetting is allowed, pipet aids such as automatic pipettors or bulbs are used to provide controlled suction. (9) Water bath. Used to thaw serum and do enzyme reactions. The contents of test tubes will reach the desired temperature much more quickly in water than in the air of an incubator. Alternatives: An insulated ice bucket filled with water at a moderate temperature will maintain a stable temperature for a while.

A water source and sink area are needed in every lab, and some equipment will be placed with access to the sink in mind. Large equipment, such as the fume hood, will be placed wherever there is space, often squeezing into seemingly impossible places.

Many labs are organized into functional working areas, and each area may then have its own set of rules. Often there may be a tissue culture area, where only cell culture work is done, and where bacterial or yeast work is passionately prohibited. The biosafety cabinet is the center of such a workspace. In such an area, you might



FIGURE 4.

pH and weighing area. Key: (1) Acids and bases. Concentrated and dilute acids and bases are used to adjust the pH of solutions. (2) Balance. A scale used for weighing. There are several kinds, with the top-loading balance being the most useful for weighing lab amounts of solids (and liquids). A two-pan balance is usually used to weigh tubes for centrifugation, and an analytical balance is used for accurately weighing small amounts, usually under a gram. (3) Hot plate stirrer. When making solutions, a little bit of heat with mixing is needed to get some materials into solution. Alternatives: Hot plate with occasional hand stirring. (4) pH meter. Used to measure and adjust the H+ concentration in a solution. Alternatives: pH paper, acid and base addition determined by calculation, but no practical alternative. (5) Spatulas, scoopulas. Metal or plastic instruments used to transfer solids from a container to a weighing vessel. These are usually stored in a drawer with weigh boats and stir bars. (6) Stock reagents. Supplies of chemicals to be used to make up solutions are kept near the prep area for convenience. (7) Wash bottle. A plastic bottle with a spout that delivers distilled water to wash the electrode of the pH unit. (8) Weigh boats, weigh paper. Solids must be placed on a support, such as weigh boats and weigh paper, before being placed on the balance to be weighed.

also find an inverted and an upright microscope, ${\rm CO_2}$ incubators, a slow-speed centrifuge and microfuge, a refrigerator, and storage for centrifuge tubes, pipets, and tissue culture flasks and plates.

Most laboratories have an **area for preparing reagents**. Here are found the supplies and equipment to weigh chemicals and pH solutions for the buffers needed for experiments.

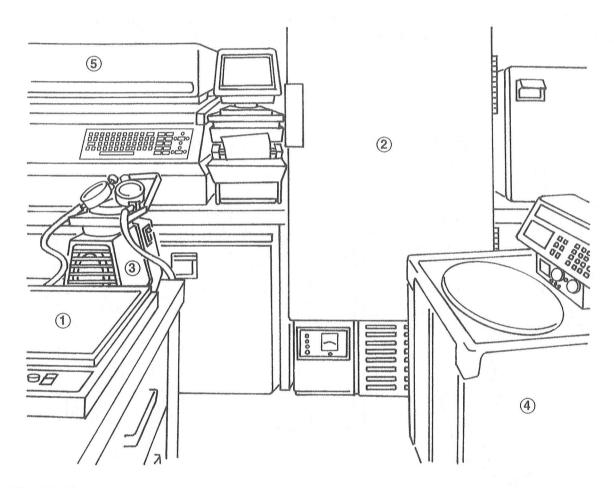


FIGURE 5.

Equipment room. Key: (1) Gel dryer. After electrophoresis, gels are dried under vacuum to enable autoradiography. Alternative: Drying film or vacuum alone. (2) Low-temperature (usually –70°C) freezer. The freezer can be upright or horizontal. Used to store bacterial stocks, reagents, samples. Hazards: Cold burn. Use at least latex gloves when manipulating samples from a –70°C freezer. Never walk away from an alarm on a freezer, as a meltdown could not only destroy years of work, but could also become a biohazard. Alternatives: Liquid nitrogen for cell and bacterial cultures. (3) Pump. The vacuum pump is the most commonly found pump in the lab, and is used to drive equipment such as lyophilizers and gel dryers. Remarks: Oil-requiring pumps must be tended carefully, especially to avoid uptake of fluid into the pump. To prevent volatile substances from a reaction or distillation from getting into the pump and then the lab atmosphere, a cold trap (filled with liquid nitrogen or dry ice) may be installed between the equipment and the pump. Newer pumps don't require oil. (4) Ultracentrifuge. A centrifuge that can achieve speeds of over 100,000 rpm; used to separate or pellet small molecules such as viruses and organelles. Since such high speeds are attained, this centrifuge must be used with caution to avoid accidents. (5) Scintillation counter. Beta radiation in samples is quantitated. Isotopes commonly counted are ³H, ³²P, and ¹⁴C.

Other functional working units often found are for microscopy, electrophoresis, radioactivity, bacterial culture, or medium and plate preparation.

Other Rooms and Places

The lab extends beyond the doors of the main lab or labs. An **equipment room** for centrifuges and other large equipment such as freezers and scintillation counters is usually located quite near the laboratory. The room may be dedicated to one type of

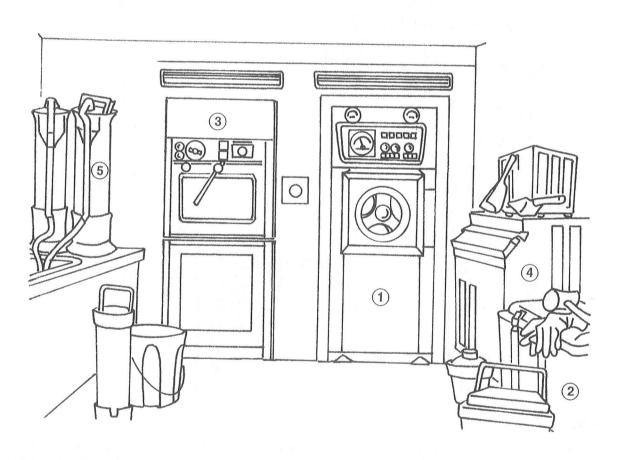


FIGURE 6.

The kitchen. Key: (1) Autoclave. Sterilizes by subjecting material to saturated steam under pressure. It is used to render glassware, media, and buffers sterile before use, as well as to sterilize biohazard waste before disposal. Hazards: Scalding. Wait until all steam has been released from the chamber before taking anything out or looking into the autoclave. Alternatives: Liquids can be filter-sterilized. Glass and plastic ware can be radiation-treated, but few places have this capability. (2) Dry ice storage chest. Dry ice is delivered once or twice a week and is kept in a chest where pieces can be broken off as needed. A mallet and gloves should be beside the chest: Always use the gloves to transfer pieces of dry ice. (3) Glassware washer. Washes and dries lab glassware. (4) Ice maker. Ice is made constantly as the level of ice goes down. Remove the ice with a scoop, not with your ice bucket. Never eat this ice! People might use ice buckets or other contaminated labware to remove it, and there could easily be hazardous substances in the ice. (5) Pipet washer. Water is circulated to wash reusable glass pipets. The pipets may be plugged with cotton and loaded onto canisters at a station in the kitchen.

equipment, such as centrifuges, but most institutions (and particularly, older institutions) have a medley of machines in the equipment room. In some places, safety laws allow big equipment such as freezers to be kept in the hallways. Some of the equipment, such as gel dryers and scintillation counters, handle radioactive samples. Always wear gloves in the equipment room.

The autoclave is usually found in a separate room, a room sometimes known as the kitchen. Here may be a glassware washer, a glass-baking machine, storage for glassware, and perhaps an area for medium and plate preparation.

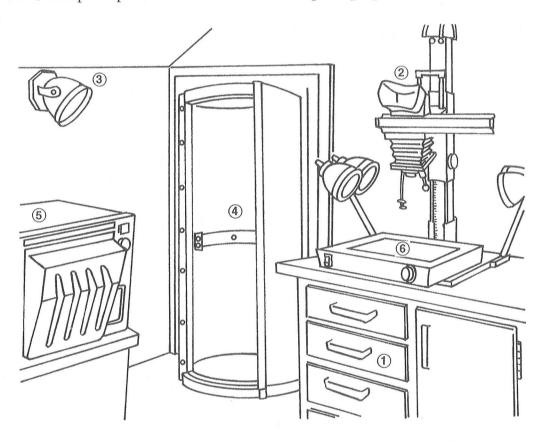


FIGURE 7.

The darkroom. Key: (1) Drawers. X-ray and Polaroid film are stored in the drawers. Even though the X-ray film is packaged, do not open the drawers unless the room is dark or is lit only by a safelight. (2) Polaroid camera. Polaroid film allows one picture at a time to be taken and developed immediately. The most common use is documenting gels whose bands have been stained with ethidium bromide and are seen on a light box. Alternatives: Digital imaging systems. (3) Safelight. A red light that does not expose film but provides enough light to see by. It is usually operated by a switch as you enter the darkroom. Be sure you know which switch is for the safelight, and which is for the regular light. (4) Revolving door. This round door permits entry and exit without allowing light into the room. Step into the opening and slowly push the door around until you come to the entrance or exit. Be sure no one is in the darkroom before you turn on the light. (5) X-OMAT. Develops X-ray film used for autoradiograms. Alternatives: Manual development of the exposed film. The phosphorimager can be used to document radioactivity without exposure to film. (6) UV transilluminator or light box. Ethidium bromide-stained nucleic acid can be visualized; bands on gels can be manipulated. Hazards: Eyes and skin can be burned. Always wear glasses or goggles, unless the light box is shielded. Use a shield if you will be manipulating the gel, as your face can easily get burned. When cutting out bands, the wrist area between gloves and lab coat is particularly vulnerable to a burn. Alternatives: A handheld UV light.

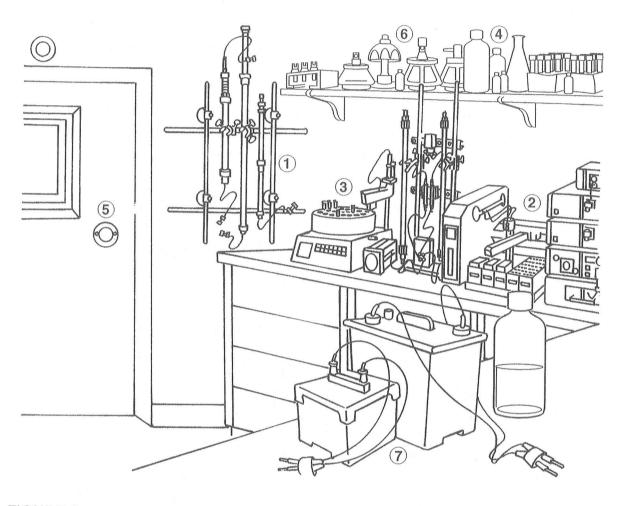


FIGURE 8.

Cold room. Key: (1) Columns. Contain the solid matrix used for chromatography. They may be smaller than a finger, or almost as tall as the cold room itself. (2) FPLC. Automated and pressurized chromatography: High performance liquid chromatography (HPLC) and fast pressure liquid chromatography (FPLC) are used for the separation and analysis of molecules and compounds. Columns, which are the soul of the system and must be carefully tended before, during, and after use, are available for dozens of specific applications. Pump, detector, autosampler, injector, and computer facilitate sample handling and analysis. Remarks: The HPLC is kept at room temperature, and the FPLC is usually found in a cold room or a chromatography refrigerator. Alternatives: Gravity chromatography with a fraction collector is an alternative for some applications. (3) Fraction collector. The fraction collector allows the sequential sampling of the fluid emerging from a column. As the effluent drips from a column, the drops are collected at a preset volume/time into a tube: When that volume has been collected, the old tube is moved and replaced by an empty tube. Alternatives: Manual collection. (4) Prepared media. Media for cells or bacteria can be purchased ready-made, and must usually be stored cold. Some buffers and reagents are also kept in the cold room. (5) Release knob for door. When someone is working inside the cold room, the door should be pulled almost closed to maintain the temperature. To open the door, hit the button or switch hard with the palm of your hand or the side of your fisted hand. (6) Rotors. Used to hold tubes in a centrifuge. Rotors are often kept in a cold room, to keep the samples cool before the run is begun. (7) Transfer chambers. DNA, RNA, or protein can be transferred electrophoretically from a gel to a membrane. During this high amperage transfer, the transfer buffer can heat considerably, and the entire process is often performed in the cold room to reduce the heat. Alternatives: Dry or semi-dry transfer apparatuses.

Much of the recording of data is done on film, making access to a darkroom indispensable. Darkrooms have a multitude of uses. Of course, they are used to develop and print film, but that may be the least of the uses in a time when most labs send their film out for processing. Instead, the main use of darkrooms is to provide a safe and dark haven for autoradiography and chemiluminescent film loading. A reddish safelight (Make sure you know which light is the safelight, since regular light will ruin the film!) gives enough light that one can take film in and out of boxes and cassettes without risking unwanted film exposure. Some labs keep fluorescent microscopes in the room, even though total darkness is not absolutely required.

Cold rooms are walk-in refrigerators, and are kept at approximately 4°C. Cold rooms are for both working and storage: Many of the shelves will hold plates, film, old bacterial cultures, and bottles of serum, but much of the space will be given over to microfuges, gel boxes, and transfer units, for procedures that sometimes must be performed in the cold. Don't worry if the door closes, there is always a release knob on the inside of the cold room and the warm room.

Warm rooms are kept at 37°C, or at the temperature of the organism that the lab works on. They physically resemble cold rooms, but contain equipment designed to enhance growth and reactions. They are filled with shakers and rollers for aerating growing bacteria, shelves for stacking plates of semi-solid media, and perhaps roller bottle setups for growing hybridomas and other cell lines.

Some departments cluster desks together in a room, called a desk bay, instead of scattering them throughout the working lab. Typically, graduate students—and especially, rotation students—and postdocs are given desks here. General use computers might be found and a microwave used for food only. Desk bays are supposed to be working places but tend to be quite social, and desk-mates need to be adaptable but considerate of each other.

The departmental or unit library has the current and back issues of especially relevant publications, as well as background and how-to books. The library may double as a conference room, and it may also be the only room in the department in which you can make or drink coffee. The photocopy machine is likely to be here.

The coffeepot can be the most controversial piece of equipment in a lab! Find out and follow the rules, which are often extensive and cover perennially touchy topics such as payment, materials replacement, and cleanup. Basically, make a new pot when you have finished the old, clean up after yourself, and don't leave the pot on all night.

Other Equipment

Much of bench work consists of taking a substance or organism, changing it by heating, mixing, and disruption, or adding chemicals, and analyzing the change in the original material. All the changes that are experimentally induced must be quantitated: A signal—often, the signal is light—is measured and transformed into a number.

The machines that quantitate are usually the most complex equipment in the lab. Most of the equipment you see is for specialized variations of measuring and mixing. Equipment will not be grouped as shown in the following figures: It will be scattered throughout the lab and department.

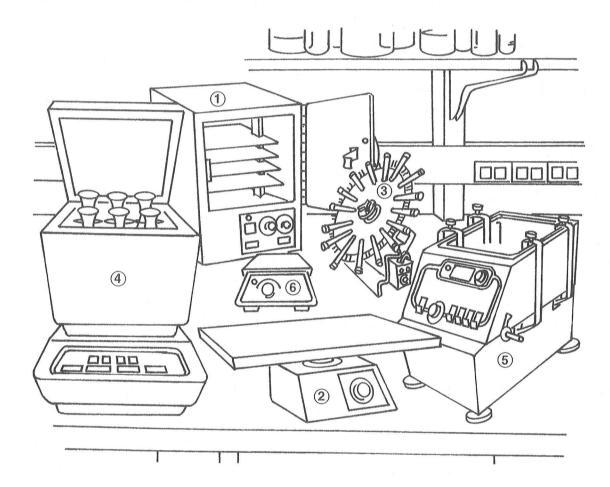


FIGURE 9.

Things that mix and shake. Key: (1) *Incubator shaker*. Used for hybridizations of transfer membranes; accommodates trays and has adjustable speed and strokes. (2) *Nutator*. A motorized platform that twists for gentle mixing. Good for microtiter plates. (3) *Roller wheel*. A rotating wheel that is especially good for bacterial cultures. (4) *Shaking incubator*. Used primarily for growing bacteria, shaking incubators agitate the flasks as well as maintain the set temperature. Alternatives: A shaker in a warm room. (5) *Shaking water bath*. Usually used for hybridizations, can also be used for microorganisms. Temperature and shaking speed can be controlled. (6) *Stir plate*. These plates can be hot plates only, magnetic stir plates only, or, more often, a plate that can be used as a hot plate and a magnetic stirrer. They are used to boil liquids and mix liquids; the mostly likely place to find one is next to the pH meter.

	Vibrating. For vigorous mixing of small vessels such as test tubes or microtiter plates.
	Orbital. Swirling action needed for cultivation of microorganisms and cell and tissue cultures.
\longleftrightarrow	Reciprocal. Back and forth motion of vigorous mixing of flasks and separatory funnels in chemical applications.
	Rocking. Gentle up and down motion for cultivating cell cultures and staining/destaining gels.
	Wave. Three-dimensional motion is ideal for mixing round culture plates, preparing slides and electrophoresis gels.

FIGURE 9B.

Motions of shakers and incubators. Some motions are better than others for particular applications, but most equipment can be adapted to suit your need.

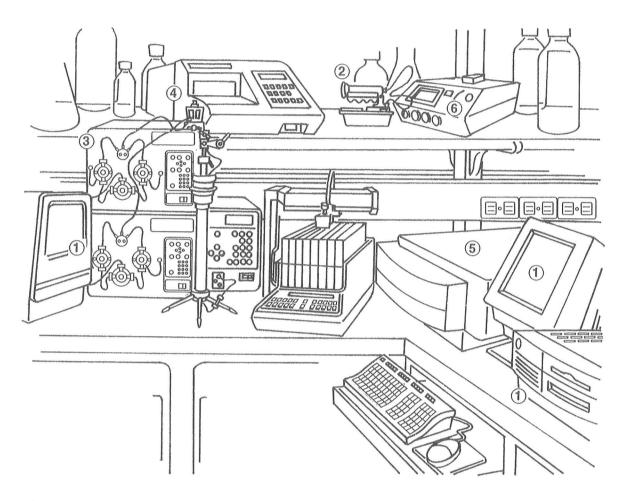


FIGURE 10.

Things that quantitate. Key: (1) Computer. Computers are part of most kinds of new quantitative equipment and allow fine-tuning of the experiment and thorough analysis of the data. They are indispensable for manuscript generation, data management and storage, and electronic communication. (2) Geiger counter (ionization chamber). Hazards: Make sure the probe is clean (not radioactive), or the dial may frighten you into thinking you and everything else are contaminated when the counter itself is. Remarks: There is a sensitivity dial, which, if turned high, may give such high apparent readings that it appears to be the China Syndrome all over. Alternatives: Although a gamma or beta counter can read a wipe test, there is no practical alternative for monitoring a working area on the spot. (3) HPLC. Automated and pressurized chromatography: High performance liquid chromatography (HPLC) is used for the separation and analysis of molecules and compounds. Columns, which are the soul of the system and must be carefully tended before, during, and after use, are available for dozens of specific applications. Pump, detector, autosampler, injector, and computer facilitate sample handling and analysis. The HPLC is kept at room temperature, whereas the FPLC is usually found in a cold room or a chromatography refrigerator. Alternatives: Gravity chromatography. (4) Microplate reader, or plate reader. Basically a spectrophotometer that can test the light emitted through or from samples on plates (usually 96-well plates), and is used for colorimetric assays such as ELISA, cytotoxicity, cell proliferation, and protein determinations. Many have adapters for plates other than 96-well plates. Alternatives: Sample-by-sample reading in a spectrophotometer. (5) Phosphorimager. Autoradiography is performed by exposure of the radioactive gel, membrane, TLC plates, or tissues to a storage phosphor screen, inducing an image that is collected and digitized. This image is then quantitated and analyzed by computer. Exposure times are much less than for standard film autoradiography. A department may share a phosphorimager, but each lab or investigator usually purchases his own storage phosphor screen. Alternatives: X-ray film autoradiography. (6) Spectrophotometer ("spec"). Measures the transmittance of light through solutions. Used for growth curves, determining the concentration of DNA and RNA, colorimetric assays. Spectrophotometers can vary drastically in size, shape, and complexity. Readings at visible light and UV light require different kinds of cuvets. Alternatives: Klett tubes and reader for bacterial cultures.

M.

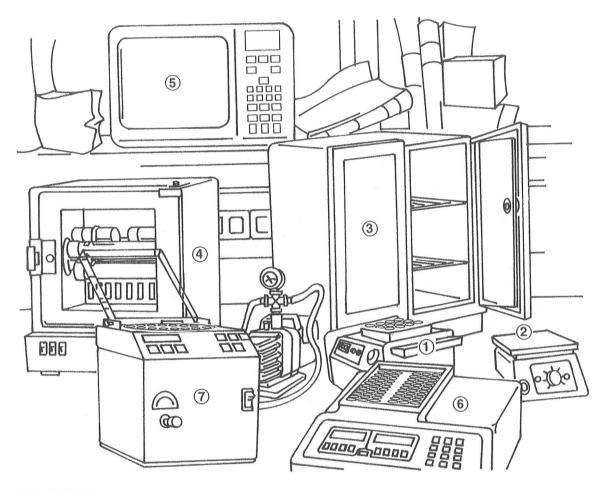


FIGURE 11.

Things that maintain or change temperature. Key: (1) Dry bath. Samples (tubes) in metal heating blocks, in modules, can be heated to over 100°C. (2) Hot plate. Used to heat small volumes of liquids. (3) Incubator. Maintains a chosen temperature. Incubators are used for cell and bacterial culture, and may be hooked up to a gas supply. Other incubators are used for sample preparation, such as filter hybridizations. Most incubators are dedicated to a certain temperature. Never change the temperature setting on a laboratory incubator without consulting the other personnel, or leaving a note, if that is the custom. Alternatives: Warm room. (4) Hybridizer incubator. A set temperature is maintained, and large tubes are rotated to gently agitate samples, usually filters for colony lifts, Northerns, Southerns, and Westerns. Remarks: Be sure the tubes have been cleaned before use. Alternatives: Filters can be placed in dishes and rocked in a shaking incubator or water bath. (5) Microwave. Melting agarose used for pouring electrophoresis gels is the main use of the microwave in the lab. Hazards: Agarose gel solutions often contain ethidium bromide, a mutagen. Always use gloves when manipulating objects in the microwave. Never cap bottles tightly, as they can explode. Never use a lab microwave for heating food! Remarks: Many eccentric uses, such as lysing cells, drying membranes, and fixing bacteria to membranes. Alternatives: Agarose solutions can be melted with a heating plate. (6) Temperature cycler. An automated heating block used for the rapid temperature changes of the polymerase chain reaction (PCR). Some models change temperatures, some physically move the sample to a new heating area with a robotic arm. Hazards: PCR machines have a high "melting" temperature, which could burn a hand. Remarks: Contamination is a major problem, so avoid all pipetting or sample preparation anywhere near the machine. Alternatives: None. It is a fancy heating block, but can achieve rapid changes of temperature that an ordinary heating block can't come close to. (7) Vacuum dryer (speedy vac). By centrifugation under a vacuum, water or other solvents are removed from a sample. Typical samples are post-ethanol nucleic acid pellets. Alternatives: House or simple pump vacuum on a chamber, large unit which may also be used for drying gels and lyophilizing large quantities of material.

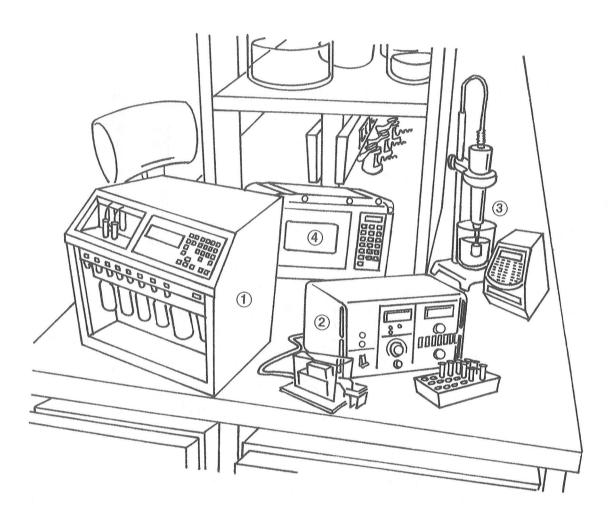


FIGURE 12.

Things that change things. Key: (1) DNA synthesizer. Oligonucleotides for sequencing and mutagenesis may be made for a lab or department, usually with a dedicated person to run the machine. Alternatives: Ready-made nucleotides can be ordered from many companies. Hazards: The solvents used are powerful, and empty bottles should be discarded with care. (2) Electroporator: The electroporator looks like a power supply and, basically, that is just what it is. Electroporation is a process of applying an electric field to a cell for a brief period of time, temporarily causing small openings to appear in cell membranes, through which molecules can be introduced into cells. Its main use is to transform bacteria and transfect eukaryotic cells with foreign DNA. Hazards: This is a high-voltage machine; do not use it without instructions. Alternatives: Several chemical and physical methods for transformation and transfections. (3) Sonicator (also known as an ultrasonic processor). Sonicators are used to disrupt and fragment biological substances by the emission of high-pitched and powerful sound waves. For example, they can be used to lyse cells or shear DNA. There are two basic types, bath or probe; probe sonicators are generally the most powerful. Hazards: Ear damage can result from probe sonicators, so always use with ear protection. Remarks: The smaller the tip diameter, the more concentrated the intensity. Keep the tip clean, or it will become damaged. Alternatives: Pushing material in and out of a small syringe is not as effective, but can work somewhat. Nitrogen cavitation bombs, detergents, chaotropic agents, and enzymes can be used to lyse cells. (4) UV crosslinker. Main use is to covalently bind nucleic acid to a membrane. Also can be used for mutagenesis and to eliminate PCR contamination. Remarks: Looks like a microwave. Alternatives: Membranes can be baked.

USING THE EQUIPMENT

Basic Rules

- Get a demonstration of its use from a lab member, even for a piece of equipment as mundane as the pH meter. At the very least, watch carefully while a lab member uses it, or ask if there are particular rules about that equipment. And write down the procedure! You may have been able to pH in your sleep with the pH meter in your old lab (which happens to be the same model as the one in your new lab), but you don't know whether this lab prefers to keep the electrode in buffer or water, whether people take turns making the acids and bases used for pH-ing, where the stir bars go when you are finished... details which, if missed, could drive other lab members quite mad.
- Wash, return, clean up, turn off appropriately each piece of equipment you use. Don't change settings. Don't force knobs or levers that don't want to move. Don't ignore alarms or flashing lights.
- Don't order equipment without consulting with the head of the laboratory. If your lab doesn't have a piece of equipment you need, he or she can suggest (or you can find) a lab which does have it and will let you use it.
- Be extraordinarily cooperative when using equipment in another lab. Check with people there to find the best time for them to demonstrate the use of the equipment and the most convenient time for you to use it.
- For each piece of equipment in the lab (even the ones you are not using), you should know (1) what it is, what it does; (2) who is in charge of it, whom to approach if there is a problem.
- For each piece of equipment you use, you should also know (1) how to operate it; (2) where the manual, instruction booklet, or protocol is kept. Either there is a central location for all instructions or manuals, or the manual will be in a drawer
 - near the equipment. (3) Is it turned off after use, or left on all day? (4) Must it be warmed up before use? Not warming up a machine before use can lead to erratic readings and a decreased work life for equipment components. (5) Is there is a sign-up sheet? If there is a sign-up sheet, sign up every time, even if you only use the equipment for 5 minutes.

Don't use a piece of equipment without the instructions. If they have been lost, call the manufacturer to obtain another copy.

• Respond to all equipment alarms immediately. Ignoring an alarm can have catastrophic consequences. An alarm on a shaking incubator might merely be a timer: Ignoring this might cause a bacterial culture to overgrow and ruin an exper-

iment. Ignoring an alarm on a CO_2 incubator might allow the complete depletion of CO_2 , and all the cells in the incubator might die as the pH rises. But the worst-case scenario might be what happens to the whole lab if the alarm on a $-70^{\circ}\mathrm{C}$ freezer or liquid nitrogen tank is ignored. An alarm on a freezer usually indicates a rising temperature, one on a liquid nitrogen tank a lowering of the liquid N_2 level: Eventually, the contents of the freezer or tank would be thawed and the lab's entire stocks of cell lines, viruses, and recombinant bacteria would be ruined. Years of work can be lost.

You must THINK about the equipment you use. The equipment is an important part of your experiments, and its misuse will affect your data in a sometimes unobvious way. Without some thought about the function of the equipment you are using, you won't have a clue why an alarm is suddenly buzzing, or how to know when the bulb has burned out. Some of the most common problems that a moment of thought can help include:

O.D. readings are zero on the spectrophotometer. The bulb may not have been turned on, something that often has to be done in addition to turning on the machine. You may not have chosen the right wavelength. You may have inappropriate cuvettes (not all cuvettes transmit all wavelengths of light). You may have put the cuvette in the wrong sample holder.

The medium covering the cells in the CO_2 incubator has become purplish, indicating that the pH has increased drastically. The CO_2 might have run out. The pan providing humidity may be empty (where did you think the humidity came from?), and without humidity, the CO_2 readings may be aberrant.

You cannot see any light on a Gram-stained slide of a sample taken from a patient's wound. The light source may have blown, or the diaphragm controlling the light may be turned down. The potentiometer controlling the light intensity may also be turned down. The objective lens may be turned so that no light is transmitted to the eyepiece. A camera may be attached, and the light set to be transmitted to the camera and not to the eyepiece.

What to Do if You Hear an Alarm

- 1. Identify the source of the alarm. Do this even if you must go into another department or lab. Don't turn it off until you know that it will be dealt with.
- 2. Notify the person in charge of the piece of equipment. Ask around the lab, or consult a list (if there is one that lists lab responsibilities) to find out. If the responsible person is home, call, even if it is 3 A.M. Once the person has been found, you can relax: Just stand by in case your help is needed.
- 3. If you can't find the person in charge, find someone who knows more than you. Believe it or not, this may not be possible! But try.

Unless you are immediately

checking out the situation,

leave temperature and atmos-

phere manipulating devices

4. If you are left to deal with the alarm:

- First, decide whether there is a *safety* issue. An example of this would be any equipment breakdown that has resulted in a spill of radioactivity. Call the EHS and the Laboratory Safety officer. Another example would be an obviously off-balance ultracentrifuge, for which you should call Laboratory Safety. Do not attempt to deal with a dangerous piece of equipment yourself.
- Decide whether there is a *lab emergency*. An example of this would be a rising temperature on a -70°C freezer, which could destroy the research of an entire department. It is not likely that you can deal with this alone. Call the Laboratory Safety officer, and, if you can't reach him/her, call Laboratory Safety.
- closed. This will keep the See whether there is an *experimental emergency*. Will temperature or gas mixture someone's results be ruined? Examine the material stable for hours, perhaps overnight. involved to find the name of the researcher and call if you can identify the owner. If not, check the experiment to decide where to place any experimental material (see Chapter 8, Storage and Disposal). The equipment can be dealt with later.
- If there is no crisis, turn off the alarm, place a note on the equipment so no one counts on it for an experiment, and leave a message for the responsible person.

HOW TO BUY NEW EQUIPMENT

- Decide carefully if you need the equipment. Can you use equipment you already have? Can you borrow it long-term? Will it be used a lot, or will it only be used for one set of experiments? Even if money is not a factor, equipment should never be purchased on a whim.
- 2. Check out the options of style and manufacturer.
 - Go to a comprehensive directory of suppliers of medical equipment, such as BioSupplyNet, and comparison shop.
 - Call colleagues to see whether they have used a particular model and can recommend it.

- Browse among the vendors at meetings to see what is available. But unless you have already researched the topic, don't buy yet, even if you are offered (as is the custom at meetings) a sizable discount.
- Post a question about the equipment on an on-line bulletin board.
- Ask the companies for the names and phone numbers of people who have purchased the equipment. Call a few to see if they have been satisfied with their purchase.

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II

- **3.** Decide which of two or three models you like. Ask the finalist for a demonstration or trial use of the equipment. Try to use the same conditions you will use for your experiments. Find out how much technical support and advice each company will offer.
- 4. Go into final arrangements with your top choices. Find out what will come with the machine, what you can arrange to have added. Some companies will agree to free software, lessons, supplies, or maintenance contracts to induce you to buy their product. Don't be shy about negotiating the best deal. If price is no object, you probably needn't bother with this.
- 5. Purchase the equipment on trial, if possible. Your purchasing department will handle this.
- 6. Try the equipment out, and stay closely in touch with the company. Ask questions and use their expertise; it should be one reason you chose that particular piece of equipment. Some companies will even provide protocols, and all should troubleshoot with you over the phone.

RESOURCES

Biochemical Resources

Phone: (517) 381-8269

http://biores.com (This web site is a database of chemicals, biochemicals, laboratory products, and services that can be searched.)

BioSupplyNet Source Book, BioSupplyNet, Inc., 10 Skyline Drive, Plainview, New York 11803.

Phone: (516) 349-5595

Fax: (516) 349-5598

http://www.biosupplynet.com (The BioSupplyNet Source Book is a comprehensive directory of biomedical research supplies and equipment. The web site allows you to search by key words for product names or categories.)

Chen T. 1997. Glossary of microbiology.

http://www.hardlink.com/~tsute/glossary/index.html/

Lackie, J.M. and Dow J.A.T., eds. 1995. *The dictionary of cell biology,* 2nd edition. Academic Press, London. Also available in interactive form at: http://www.mblab.glac.ac.uk/~Julian/Dict.html/

SciQuest, Research Triangle Park, North Carolina 27709-2156

Phone: (919) 786-1770

Fax: (919) 782-3128

http://www.sciquest.com/catalyst/welcome.cgi (This web site also allows searches by key words, and automatic requests of chosen vendors.)

UW GenChem Pages, University of Wisconsin-Madison, Department of Chemistry: http://genchem.chem.wisc.edu/labdocs/labdrwr/labequip.htm/

UW GenChem Pages Dictionary, University of Wisconsin-Madison, Department of Chemistry: http://genchem.chem.wisc.edu/labdocs/mainmenu.htm/