[2] How to Set up a Yeast Laboratory

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Introduction

The organizational strategies recommended in this chapter are biased in favor of a highly organized and cooperative laboratory community. The emphasis will be on efficient laboratory practices, pools of common supplies, and providing glasswashing and media preparation protocols for support personnel. Laboratory equipment, supplies, and experiment protocols constantly evolve and specific product recommendations soon become outdated. A helpful starting point is to see what other new researchers have bought recently to set up their yeast laboratories.

Role of Technician Manager

The work of organizing and maintaining pooled collections of chemicals, restriction enzymes, laboratory strains, antibodies, and radioactive chemicals for use by members of the laboratory community should be overseen by a long-term laboratory resident, a permanent research or technical associate. Laboratories that can retain a long-term employee in this role have the advantage of continuity in a community where others are transients. An effective laboratory manager should be perceived by laboratory members as having the full support of the principal investigator.

Orientation and Departure

A great time-saver is to develop written statements in the form of handouts that encapsulate information and state the practices to be followed by laboratory members. Three recommended handouts are the following.

Newcomer Orientation

This document should include personnel to contact in the administration, other staff to get acquainted with, and contact persons to make appointments with to receive radiation and safety training, which are necessary prerequisites to starting research work. A sample handout is shown in Fig. 1.

Laboratory Jobs

Each individual is assigned tasks and responsibilities for management, equipment care, and stocking common supplies. The manager should post current lists of laboratory job assignments. A list of job assignments is shown in Fig. 2. Ideally,

0076-6879/02 $35.00

Welcome to the Lab: Guide for Newcomers (Sample)

Preliminaries

Introduce yourself and learn the names of:

1. Office staff
2. Safety coordinator
3. Laboratory technician
4. See safety coordinator and the laboratory safety representative for training.
5. Radiation Office: Call and make appointment for training.
6. Glasswashers and media preparation staff.

Learn the location of necessary items:

1. Office supplies and Rolodex finder.
2. Orders are placed by computer requisition. Ask for instruction.
3. Reference books and common laboratory handbooks.

Common Items and Standard Practices

Distilled water: Produced by reverse osmosis through a resin bed (R/O water).
Melting agar in microwave: Get instruction on the microwave oven settings for melting agar. Loosen the bottle cap well. *Caution:* Superheated molten agar boils explosively when agitated. Handle with care.
Deionized (Milli-Q) water: The filter system is in Media Prep Room. Instructions are attached to the apparatus. There are reserve tanks of nonsterile water in carboys at the sink. Sterilized Milli-Q water in purple-cap bottles is on shelves in the hallway. These PURPLE-CAP bottles are for WATER ONLY. They are never washed. Always keep caps on bottles, even when empty. Place on sinkboards at designated sites.
Hoods: The working surface is for *experiments in progress* only. LABEL your experiment and clean up promptly. IDENTIFY all reagents used in hood. See the hood's manager for permission to store anything in the hood.

FIG. 1. A sample orientation handout for newcomers.

Laboratory agents: Animal agent, safety representative
Management and organization: Hoods and hazardous wastes, antibodies, balance room, coldroom benches, gel electrophoresis room, oligonucleotide library, curators of laboratory strains, chemicals databases, radiation materials, phages
Equipment care and maintenance: Shakers, roller drums, and water baths; centrifuges and microfuges; pH meter and test papers; gel dryers, vacuum pumps; Speed Vac, traps, and oil changes; computers; sonicator; spectrophotometer; PCR machine; electroporator; hybridizer machine; PhosphorImager plates; X-OMAT film processor; freezer −80° frost maintenance; electrophoresis equipment; microscopes; pipettors; scintillation counters and vials
Stock and preparation of common supplies and reagents: Chemicals, solvents, and ethanol; plastic goods and other disposables; films; hexamers; PCR kits, and restriction enzymes; 20× SSC; Southern base and neutralizer; TAE, TBE; protein gel buffer

FIG. 2. A list of laboratory job assignments.

each job holder will keep notes on newly accumulated information to add to the original laboratory job descriptions.

Departure Checklist

This checklist (Fig. 3) is a key handout facilitating turnover of laboratory space. The manager should give the checklist to researchers about a month before departure. The essential "tour" is a physical inspection by the manager together with the researcher of all laboratory sites occupied by the researcher. The manager verifies firsthand that all spaces are completely empty.

Management Strategies

Radioactivity

The principal investigator obtains federal and state licenses and has the ultimate responsibility for compliance. Regulations usually require that radioactive substances be stored in a separate secured place, such as a locked refrigerator–freezer. The ordering, stocking, posting of usage signup sheets, and arranging for waste pickup plus general oversight are best assigned to a single individual. One policy decision to make is whether to have a single location where all radioactive work is performed or to have individuals do radioactive work at their own benches. The former has the disadvantage that the individuals responsible for spills at a common site cannot be readily identified, and responsibility for cleaning up can become an issue.

Restriction Enzymes

The most cost-efficient method for managing restriction enzymes and other enzymes for manipulating DNA is to establish a common supply, stored alphabetically in labeled racks in the top freezer section of a household type refrigerator–freezer. To protect enzymes from inadvertent contamination, a dedicated 10-microliter pipettor and pipette tips with filters should be provided for exclusive use with the enzyme collection. For additional protection the freezer should be connected to an emergency power source.

Water

Two levels of purified water are needed in a yeast laboratory: (1) Water for preparing growth media requires that the chlorine, heavy metals, and other toxic impurities found in tap water be removed. Facilities designed for research work may have a separate plastic plumbing system which delivers water treated by reverse osmosis ("R/O"), a process which purifies tap water by passing it through a resin bed. (2) Deionized water is needed for biochemical work. Deionizing filtration

LABORATORY AND INSTITUTION DEPARTURE CHECKLIST (Sample)

People who are departing are expected to leave their desk and bench clean. It should be ready to be occupied by the next research worker. The preparation for departure and clean-up are also important so that no hazardous chemicals of unknown composition or origin remain.

ONE MONTH BEFORE LEAVING

___1. Notify the lab manager, the radiation officer and the secretary of your expected departure date. Be sure to:

- ___ Turn in your access/ID cards.
- ___ Empty out your clothing locker(s).
- ___ Turn in keys to your desk and other keys.
- ___ Turn in your telephone card.

___2. Provide Yeast Strain Curator and Bacterial Strain Curator with Xerox copies or a database file of all published strains or strains that will be published. Check the stock lists to be sure all of your strains are in the Laboratory Collection. It is helpful to provide the curator(s) with an annotated copy of published papers showing the names of all strains described.

___3. Turn over antibodies and antisera to the Lab Curator, and provide details in writing.

___4. Transmit information about your lab job(s) in writing to the next person.

___5. If you plan to continue to working with yeast, make copies of the strains that you want to take. Requests for additional strains after you leave will have to be approved by the Principal Investigator, as are all requests from outside labs.

TWO WEEKS BEFORE LEAVING

___1. Clean out your radioactive probes and check out with the lab Radiation Officer.

___2. Return all small bench hand tools to the technician.

___3. Clear desk and drawers of all contents. Return materials to office supplies.

___4. Return all borrowed books and journals.

___5. Reagents: Donate them to people who will put their own names on them. Place reagents on display for people to scavenge. You dispose of the remainders. Dispose of the contents in a NON-HAZARDOUS, SAFE MANNER, following advice from the Safety Coordinator. Rinse bottles clean and remove all adhesives and labels except washable labels.

___6. Place unwanted X-ray film in the silver recovery box in the XOMAT darkroom.

___7. Give away unused media to other lab members.

___8. Remove all your personal reagents from, the hood cold room, and elsewhere.

___9. Clear out all of your frozen strains, competent cells, and cassettes.

___10. Make arrangements if you wish to transport frozen material from the laboratory.

___11. Equipment purchased with your grant money for your research is the property of the laboratory, and cannot be removed without the Principal Investigator's consent.

___12. Chemicals at your bench which are still in the original container need to be integrated into the laboratory collection. Provide the technician with a list of the new locations.

___13. Leave your new address and phone number with the secretary. Notify the post office of your address change. Your mail will not be forwarded.

___14. Give computer-related information, especially passwords to the secretary.

___15 Check with the Principal Investigator concerning the disposition of your notebooks. There may be institutional and legal restrictions that apply.

___16. Goods may be left behind to be reclaimed later. Make arrangements with another person to take charge of your goods during the interim to make sure they are not discarded.

THE TOUR: Show the technician these cleaned areas:

Your clean and bare bench, shelves and desk with nothing left for the next person.
Your –20° shelf The –80° freezer space Radioactive storage areas
A check of the hoods Your cold room shelf
Labware and equipment which will become laboratory property
Any materials and goods you are leaving for the general public
The list of new locations of your bench chemicals.

FIG. 3. A sample checklist for researchers leaving a yeast laboratory.

systems designed by Millipore Corporation (Bedford, MA)[1] work well. The choice of purification system depends on the purity of the incoming water supply and the degree of "polishing" needed. It is not necessary to remove endotoxins and pyrogens for yeast work.

Bottles of sterile, deionized water for making biochemical reagents and for mixing with sterile media components are a great convenience. To keep this water supply pristine, it is advisable to maintain a separate set of bottles solely for deionized sterile water. The glass bottles can be marked with a paint pen, and caps of an identifying color should be purchased for them. Nothing foreign should be introduced into the bottles and caps should be kept on them at all times. The bottles are never washed, only refilled and autoclaved.

Glasswash Procedures

Glassware to be recycled needs three sites: (a) a collection site for soiled labware, (b) a washing and processing site, and (c) a storage site for the cleaned and sometimes sterilized labware. Laboratory rules regarding the condition of labware that researchers place at the collection site should be formulated to shield laboratory aides from harm and to make their work easier. Agar-containing bottles and flasks should be left standing filled with water to prevent the agar residue from drying into a tough film. No agar in any form should be allowed to enter sink drains. Generally, labware used for cultures must be decontaminated with Lysol or Clorox and rinsed with tap water. It must be free of toxic chemicals and radioactivity. Broken glassware should be discarded. Recyclable glass serological pipettes need separate plastic collection containers 1/3 full of water with 10% Clorox. Used velveteens need their own collection container 1/2 full of water with a capful of Lysol. Detailed procedures for processing labware are shown in Table I.

Design for Media Preparation by Support Staff

The basic media for culturing yeast are "complete" (YPD), "minimal" (SD), and "synthetic complete" (SC). Bacteria are cultured in "LB medium" (LB) and, occasionally, in "minimal" medium (M9). The media are used as liquid cultures or combined with agar to make petri plates. For a discussion of media and formulas, see Sherman.[2] Medium is more efficiently prepared in batches by support staff. Here is a description of one system which is designed to serve about 15 users. Some media products are made for immediate use (Table II). Other products are designed to be mixed together by the researcher, usually to make agar plates (Table III). Media cannot be made too far in advance because some types of media deteriorate with time. Table IV shows a list of titles of protocols for preparing

[1] http://www.millipore.com/catalogue.nsf/docs/C1756

[2] F. Sherman, *Methods Enzymol.* **350,** [1], 2002 (this volume).

TABLE I
GLASSWASH PROCEDURES

Product and process
Cleaned sterile glass pipettes
(1) Collect soiled pipettes. Bring back to glasswash facility.
(2) Pour diluted (2%) Micro cleaning agent over pipettes to cover. Autoclave for 10 min.
(3) Separate pipettes from Micro and load pipettes into rinsing baskets. Lower baskets into siphon rinsing units and rinse in tap water followed by water (R/O).
(4) Place pipettes in drying baskets and dry in a hot oven.
(5) Sort cooled pipettes into cans and bake at high temperature for several hours.
Cleaned glass and plastic labware
(1) Collect soiled labware and deliver to glasswash facility.
(2) Hand-brush soiled labware and visually inspect. Separate by type (plastic, glass, large, small) and load glasswasher. Add minimum amount of detergent and wash.
(3) Nonsterile glass and plastic nonsterile beakers are dried in oven. Sterile labware is capped and autoclaved.
Sterile velveteens
(1) Collect used velveteens from buckets, place them in clothes washer, but do not add detergent (the liquid in the buckets already contains Lysol.). Fluff dry in dryer.
(2) Flatten and brush squares and wrap nap-side down 10 each in aluminum foil.
(3) Autoclave sterilize on the dry cycle for 35 min. Then bake to dry in a low oven (160°F).

media. See Appendix for protocols. Some of the formulas are alternatives to the ones described by Sherman.[2] Table V provides additional information about media components, naming, and sources.

Using the Media System

This system affords versatility and economy. As soon as a need for media is perceived, a researcher can quickly prepare the media he or she wants from premade ingredients. To prepare to pour plates, bottles with agar are heated in a microwave oven or in an autoclave to melt the agar. The molten agar may become superheated, making the opening of the bottles hazardous. It is advisable to let the

TABLE II
READY-TO-USE MEDIA

Container size	Quantity
500-ml media bottle	300 ml 1× LB with 2% agar
500-ml flask	100 ml LB liquid
2-liter flask	500 ml LB liquid
500-ml media bottle	300 ml 1× YPD

TABLE III
PREMADE MEDIA COMPONENTS[a]

Media bottle size	Component
1 liter	300 ml 4% (w/v) Agar
500 ml	300 ml 2× LB
500 ml	300 ml 2× YEP
500 ml	300 ml 2× Minimal
500 ml	300 ml 2× Minimal + amino acid mixes
500 ml	200–300 ml 40% (w/v) Glucose
160 ml (rectangular)	100 ml Single amino acid stock solutions

[a] The amounts and concentrations are based on an approximate final volume of 600 ml.

bottles stand for a minute before agitation. The worker then combines 300 ml of nutrient medium with a carbon source, e.g., 30 ml of 40% (w/v) glucose, and cautiously adds this mixture to the molten agar. The contents need to be thoroughly mixed. After mixing standard media for yeast, the bubbles can be safely dispersed with a short spray of ethanol. If YPD or YNB agar media should solidify in the bottle before plates can be poured, these can be remelted. Note that SC agar media cannot be remelted. The amino acid mix hydrolyzes the agar while it is remelting, and it will no longer solidify.

The site for plate pouring may be on individual benches or at a common site. In buildings with poor air quality control, plate pouring will need to be done in a sterile hood. Agar plates work best for micromanipulation and replica plating if allowed to stand for 2 to 3 days before being wrapped in a tightly closed bag. Plates standing unwrapped for more than 7 days dry up and are of little use. The shelf-life of well-wrapped media plates can be about 6 months, except for Synthetic Complete media. Light and heat lead to media deterioration.

Laboratory Databases

A technician with good organizational skills should be encouraged to develop and maintain laboratory database systems.

Laboratory Storage System

An efficient approach to codifying locations of stored items is to assign a sequence of permanent numbers to all cupboards, shelf sets, and drawers within a given room. The location of the item then is simply stated as: Room number, site number. The names of stored items may be listed on Rolodex cards or in a computer database.

TABLE IV
MEDIA PREPARATION PROTOCOLS[a]

LB liquid medium
LB 2×
LB 2% agar
2×-YT
Agar 4%
YNB 2×
Yep 2×
YPD 1×
YNB 2× + AA dropout
Glucose 40%
Histidine 100 m*M*
Adenine 30 m*M*
Uracil 20 m*M*
Lysine 100 m*M*
Leucine 100 m*M*
Tryptophan 40 m*M*
Inositol 200 m*M*
M9A 20×
SOB
LB top agar
Soft agar
T agar
Casamino acids
Sodium hydroxide 1 *N*
Amino acid powder mix, complete
Presporulation GNA
Sporulation Agar NGS
Sporulation Agar SPOR
Terrific broth
Fortified broth
T broth
SOB from capsules
Inositol-free minimal

[a] See Appendix for protocols.

Placing Orders in a Database

Regardless of differences among institutional ordering systems, it is invaluable to maintain a database within the laboratory of all orders placed. The database design (Fig. 4) should be useful to both laboratory workers and office staff. Computerized ordering has the advantage of being legible, using less paper, and providing a permanent searchable file of past orders. Records can be duplicated and edited to place repeat orders. A categories field is useful for inventorying chemicals and for creating summaries for accounting purposes. All computer databases need to

TABLE V
ADDITIONAL INFORMATION ON MEDIA

Medium	Comments
Agar	Comercially available in various degrees of purity. For yeast media, the same level of concern paid to laboratory water should also apply to agar. Neither product needs to be completely pure, but the level of purity needs to be unvarying. For yeast laboratories, we prefer Difco Bacto-agar.
YEP	Represents the ingredients, yeast extract (Difco) and Bacto-peptone (Difco). 2× YEP is made without a carbon source to allow the researcher to add one of choice, usually glucose.
2× Minimal medium	Also provided without a carbon source. We call this YNB, naming it after the sole ingredient, Yeast Nitrogen Base (Difco) (without amino acids).
2× Minimal + amino acid mixes	Refers to SC (without glucose) and is called 2× YNB + all amino acids (AA). The amino acid mixes usually have one or more of the amino acids omitted, hence the name "drop-out" medium. This so-called amino acid mix also contains adenine, uracil, and *p*-aminobenzoic acid (PABA). Dry powder mixtures of amino acids can be stored almost indefinitely. Once mixed with water, however, they have a limited shelf-life of about 1 month. The composition of Synthetic Complete medium varies as it is modified for particular strains or mutants. We have settled on a formulation that uses equal amounts by weight of each ingredient, except 1/10 that amount of *p*-aminobenzoic acid with two adjustments: (1) to support wild-type growth of leucine auxotrophs, the amount of L-leucine is doubled, and (2) if one wishes *ade1* and *ade2* mutants to turn red, the amount of adenine is reduced. A variety of SC drop-out powder mixes are commerically available.[a]
Minimal medium	Made from Difco Yeast Nitrogen Base supports weak growth of *ino*-strains. For research not involving inositol mutants the presence or absence of inositol probably can be ignored. Problems arise when wanting to distinguish inositol auxotrophs from wild type. To produce inositol-free medium,[b,c] minimal medium must be made from scratch (Table II).
LB Medium	Made from yeast extract, Bacto-tryptone, and sodium chloride and pH-adjusted with sodium hydroxide. Handling sodium hydroxide can be eliminated by purchasing LB medium as a complete powder or liquid concentrate, already pH-adjusted.[a]

[a] http://www.bio101.com/

[b] F. Sherman, G. R. Fink, and J. Hicks, "Cold Spring Harbor Laboratory Course Manual for Methods in Yeast Genetics," p. 165. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986.

[c] C. W. Lawrence, *Methods Enzymol.* **194,** 280 (1991).

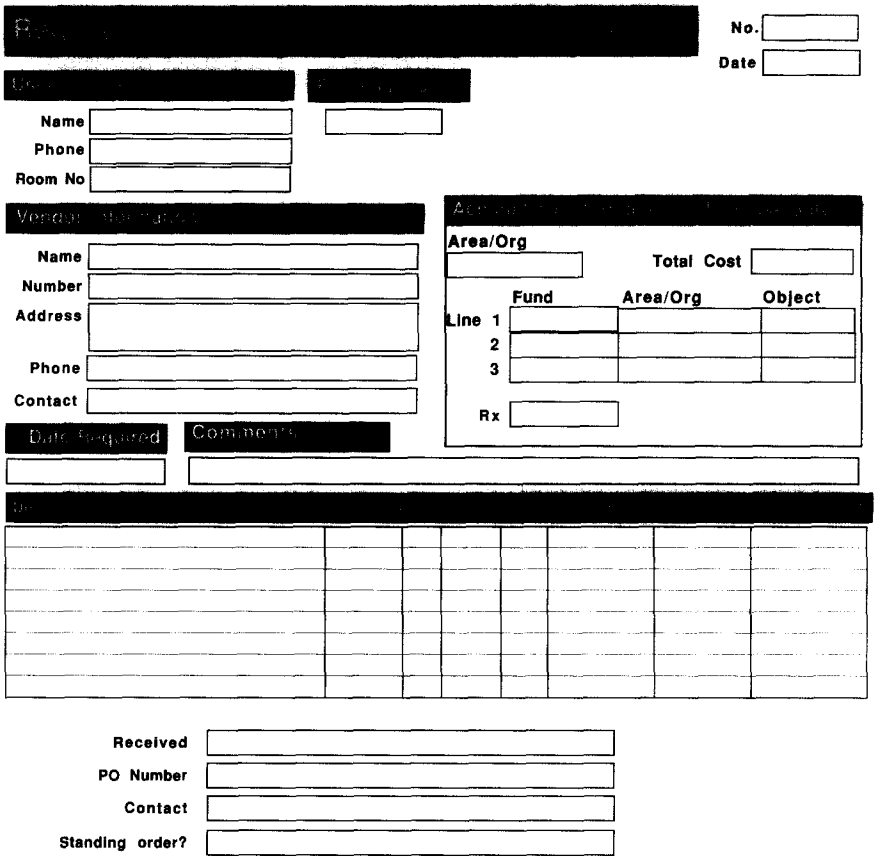

FIG. 4. A sample record in one layout of an orders database based on FilemakerPro 4.1. Two fields have automatic functions: Record Number, a "number field," automatically enters serial numbers in increments of one, and Date, a "date field," enters the current date. Several fields have drop lists from which to choose an entry: User Name, Accounting information fields, Type, and Unit fields. Other fields are designated "Look-up" fields. When an entry is made in User Name, the application locates a second FilemakerPro 4.1 database which has User Names, User Phones, and User Room Numbers. The information found in the second database is placed in the appropriate fields of the Orders database record. The same is true for Vendor Name, which supplies the Vendor Number from a third database. A fourth database holds Descriptions and looks up Type, Quantity, Unit, Code, Catalog No., and Unit Cost. The Look-up databases need to be kept up to date. The Description field in this design needs to contain information clarifying the Quantity entry. For example, if an item is sold "10 per case," that information must be entered in the description. Under Quantity enter "1" and in Unit enter "case." The Description field is a repeating field. If one searches for an item in a repeating field, all Description fields will be searched. It is convenient to duplicate a previous order and edit it to create a new order. If the secretarial staff has access to laboratory computers via a network, paper requisitions may be eliminated altogether.

be safeguarded with backups and password protection. Password protection should allow laboratory users all activities except deleting files. That function should be reserved under password for the database manager.

Chemicals Inventory

Federal law requires research laboratories to maintain an inventory of all chemicals on the premises. Careful maintenance of a laboratory chemicals database can reduce the labor of taking a physical inventory. The database can also serve to inform workers not only whether a chemical exists in the laboratory, but also where it is located. All chemicals, even those kept at workers' benches which are still in the manufacturer's bottle, are included in the chemicals database. For keeping track of chemicals, an ideal system would be to assign an identification number to each chemical unit coming into the laboratory. The simplest way to track chemicals with precision is to assign a new identification number to each bottle. The system is amenable to barcoding, which would facilitate taking inventory. Essential information to include in the database is (1) the ID number, (2) the year ordered, (3) the volume in the original container, (4) the person who ordered it, (5) the type of chemical or the purpose for which it was bought, and (6) the storage location, such as room number, site in room, and detail within the site if appropriate, e.g., "Room 557, Chemicals Freezer, Shelf 3, Storage jar #2." From the database the manager can extract and can post alphabetized lists of chemicals stored at various locations, such as the contents of a chemical storage freezer.

The entire database, write-protected, should be available to all laboratory members. A sample layout of a chemicals database is shown in Fig. 5. The database should be managed by a laboratory manager working together with the laboratory member in charge of chemicals. The manager should monitor the Orders database regularly to be alert to incoming chemicals. Incidentally, purchasers of toxic chemicals should be made aware of the high cost of disposing of unused and expired toxic chemicals.

When chemicals are used up or a bottle discarded, the bottle identification number is reported to the database manager, who will delete it from the file. When laboratory personnel leave, one of their departure duties is to integrate their bench collection into the common collection. They must report to the chemicals database manager where each bottle in their personal collection has been relocated in detail.

Yeast and Plasmid Laboratory Strain Collections

The yeast strain collection belonging to the principal investigator will form the starting point for a permanent collection unique to his or her laboratory. Researchers in the laboratory are expected to contribute strains produced in the laboratory to the permanent collection. A well-managed, carefully documented collection will grow into a powerful asset.

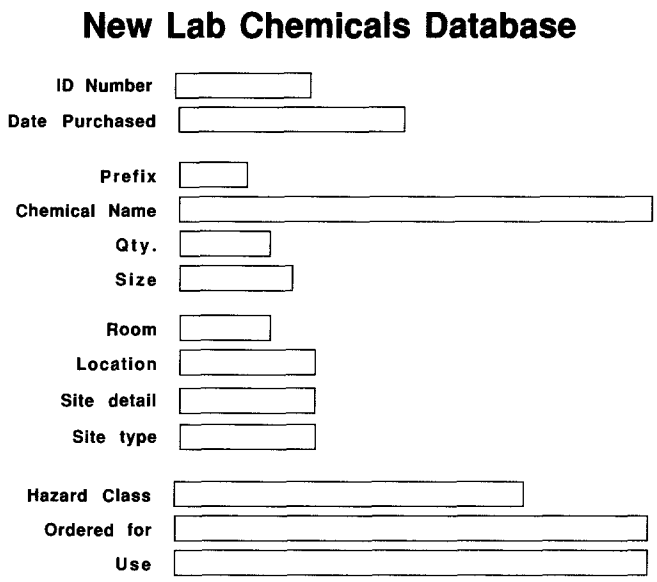

FIG. 5. A sample record in one layout of a Chemicals Database based on FilemakerPro 4.1. This Chemicals Database has additional layouts for producing special-purpose lists to post at storage sites and for laboratory members to use when relocating their bench chemicals collection.

The simplest method for organizing strains is to keep all of them in a single collection where each new entry receives the next number in a series. Strain numbers are conventionally given one or more prefixed letters, such as one's initials followed by Y for yeast. Naming all yeast strains in a single numerical sequence simplifies freezer space organization. Establish one database for yeast (*Saccharomyces cerevisiae*) and another for bacteria with plasmids.

A computerized strains database performs best as a search tool rather than as an archive of all information. Information not in the database can be organized in binders with numbered pages which the database records can reference. An example of a record in a yeast database is shown in Fig. 6a. An important feature is that mutant genes are listed individually in a repeating field. The advantage of using a repeating field is that a FIND request will search all entries within the field. This type of field also enables the database manager to produce a summary report of all mutations in the collection, which is useful for editing purposes and as a tool to maintain uniformity in naming. In other fields where entries are limited to only a small number of options, such as "Mating Type," uniformity can be ensured by attaching a drop-list to the field.

The plasmid database (Fig. 6b) is similar to the yeast database. In addition to the computer records, a hard copy of the entire database is handy.

(a) **New Lab Yeast Strains**

Lab Strain Number
Mating Type
Plasmid
Donor
Former Name
Parents
Strain Background
Comments

Genotype

Creation Date
card number

(b) **New Lab Plasmid Strains**

Lab Strain Name PIB0001
Plasmid name
Plasmid markers

Bacterial host
Insert Organism
Insert

Type
Map available O yes O no

Source
via
Reference

Comments

It is essential that researchers contribute a select number of their strains to the permanent laboratory collection. Recommended guidelines for contributions are the following: All strains received from other laboratories should be deposited promptly, together with the sender's accompanying documentation. The database record should state clearly all limits on the use and distribution of strains received. Strains received through Material Transfer Agreements need great attention to compliance, since they are covered by legal agreements. All strains that researchers produce in the laboratory which are generally useful should be added, including transformants that are hard to reproduce and strains obtained from other laboratory members, who may not have submitted them. Finally, all strains likely to appear in publications, together with the strains from which they were derived, must be submitted to the laboratory collection. Adding strains to the collection should be overseen by a single individual, usually a technician in the role of curator. Strains in the permanent frozen (−80°) collection are stored in sterilized 2-ml polyethylene vials with 1 to 1.5 ml glycerol (15% v/v glycerol/water) for yeast and 50% (v/v) glycerol for bacteria carrying plasmids.

Laboratory Equipment

Basic Small Equipment

A microwave oven needs an interior chamber height to accommodate 1-liter media bottles. When choosing roller drum racks and other tube-holding equipment, make sure the size of holes matches the choice of culture tube size (see Glass Culture tubes below).

Bench Tools

Multiprong blocks ("froggers") are useful for transferring cultures from solid medium to solid or liquid medium. For transferring liquid samples from 96-well

FIG. 6. Sample records of strains databases for yeast and for plasmids based on FilemakerPro 4.1. Both databases are password protected. The password "master" allows complete access. The password "user" allows all functions except editing, deleting, and creating, which are reserved for the Strains Curator, who knows the master password. (a) In Yeast Strains, Lab Strain Number is a "number" field which automatically assigns each new record a serial number increasing in increments of one. The number may be preceded by the Principal Investigator's initials. Card Number is another automatic numbering field. Creation Date is a "date" field, which automatically fills in the current date. Other fields are "text" fields. Mating Type and Strain Background have drop lists which limit the variety of entry notations. Genotype is a "repeating (text) field," which allows multiple fields to be included in a single search. (b) In the Plasmid Strains database, the strain name and creation date fields are like those in Yeast Strains. Marker List is a repeating field. Type has a drop list limiting the choice of entries. Both databases have additional layouts useful for summary purposes. The "Hard Copy" layout in Plasmid Strains is used to print out paper forms to store in notebook binders.

plates to solid medium or to other plates, multichannel mechanical or electronic pipettors are recommended to minimize cross-contamination of adjacent wells.

Velveteen cloth (all cotton) for replica plating can be purchased from major fabric stores. Hemming is optional. Unhemmed velveteen squares when first washed and fluff dried produce a large ball of waste threads. On subsequent washing the fringed velveteens are relatively stable.

Glass Bottles and Accessories

Media and reagent bottles should be heat-resistant glass. Glass culture tubes are sold both as disposable and reuseable. One problem with reusing glass culture tubes is their fragility. The turbulent spray in some glasswashing machines may break culture tubes and clog the water lines with shards. Glass culture tube sizes are 13 mm diameter and 16 or 18 mm diameter. One should decide early on whether to use 16- or 18-mm diameter tubes and choose other equipment, such as tube racks and roller drum tube trays, to match. Rolls of labels can be purchased with water-soluble adhesive[3] for easy removal. These can be hung on a toilet paper roll holder at convenient locations.

Microscopes and Accessories

Three leading manufacturers are Zeiss, Olympus, and Nikon. Finding a reliable, knowledgeable sales representative is as important as selecting the brand. A low-power microscope with a reflective adjustable mirror or mirror slit is convenient for viewing small yeast colonies. A higher power inverted microscope is a convenient option for viewing yeast cells and liquid well cultures. A high-power fluorescence microscope is needed to observe antibody staining. Ideally, each microscope should have a camera port (see Refs. 4 and 5). Dissection microscopes are fully discussed by Sherman.[2]

Refrigeration Units

A 20-cubic-foot household refrigerator–freezer with a top freezer cabinet is useful for a restriction enzyme collection. The lower section may be a storage site for refrigerated chemicals. As the laboratory grows, an additional small chest freezer is recommended for storing backup supplies of restriction enzymes and kits. At least one $-20°$ freezer, such as an undercounter model, should have external contacts to permit safe storage of flammables. Full-size upright household freezers work well for storing frozen chemicals and provide shelves for researchers to store

[3] Shamrock Scientific, Bellwood, IL, 1 (800) 323-0249. Blank labels 1 × 2.5 in. × 1000, wash-away adhesive.

[4] S. J. Kron, *Methods Enzymol.* **351,** in preparation (2002).

[5] D. R. Rines, X. He, and P. K. Sorger, *Methods Enzymol.* **351,** in preparation (2002).

plasmids and reagents. Ultracold freezers (−80°), upright models with shelves and drawers with compartments to hold boxes, work well for storing strain collections, plasmids, and antibodies.

All freezers should be connected to the emergency power system of the building.

Labware

Standard 100-mm diameter plastic petri plates vary in size and design. Test sample plates with equipment designed to hold plates, e.g., the dissection microscope stage plate holder and plate holder accessories for an inverted microscope stage. If the plates are too big for this equipment, the recommendation is to find a competent machinist to enlarge the plate holders to fit the plates rather than buying expensive plates to fit the equipment.

Appendix: Protocols[6,7] for Preparing Media

LB Liquid Medium

Prepare in 20-liter carboy with spigot.

Ingredients	For 20 liters
LB Medium powder (BIO-101 low salt, from Q Biogene) (or [small] capsules)	500 g
Water (R/O)	20 liters

Dissolve with stir bar in 3–5 liters water. Transfer to more convenient height. Add remaining water.

Dispense as needed:

(a) 100 ml in 500-ml flasks
(b) 300 ml in 500-ml bottles
(c) 500 ml in 2-liter flasks
(d) 100 ml in 500-ml flask

Cover flasks with foil or paper towel and 2 paper cups.

Autoclave 35 min or less.

Let hot media rest 10–15 min. Place in front of fan to let cool.

Apply labels with date. Record batch with lot numbers in daily log. When no longer warm, tighten caps. Stock shelves.

[6] D. Burke, D. Dawson, and T. Stearns, "Cold Spring Harbor Laboratory Course Manual for Methods in Yeast Genetics," Appendix A—Media. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2000.

[7] J. Miller, "Experiments in Molecular Genetics," p. 431. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1972.

LB 2×

Prepare in 20-liter carboy with spigot.

Ingredients	For 10 liters
LB Medium powder (BIO-101 low salt) (or [small] capsules)	500 g
Water (R/O)	10 liters

Dissolve with stir bar in 3–5 liters water. Transfer to more convenient height. Add remaining water.
Dispense 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Let hot media rest 10–15 min. Place in front of fan to let cool.
Apply labels with date. Record batch with lot numbers in daily log. When no longer warm, tighten caps. Stock shelves.

LB 2% Agar

This product is ready-made LB agar in 300 ml quantity. Convenient for melting, adding antibiotic, and pouring about 10 to 12 plates. Prepare in two 6-liter flasks.

Ingredients	For each flask
LB Medium powder or capsules (BIO-101 low salt)	75 g
Agar (Difco)	60 g
Water (R/O)	3 liters

Dissolve in small autoclave 20–25 min to melt agar. Add stir bar afterward and mix thoroughly until "lines" are gone (1 min). (Apply additional heat and stir longer if agar was not completely melted.) Keep hot so agar does not thicken and solidify.
Dispense 300 ml in 500-ml bottles. Be careful of this HOT liquid. You can wait till it cools down to a more comfortable temperature. Place the flasks on an insulated surface while cooling (paper towel).
Loosely cap bottles.
Autoclave 15 min or more.
Apply labels with date. Record batch with lot numbers in daily log. When no longer warm, tighten down caps. Stock shelves.

2×-YT

See comments for LB media. Prepare in 20-liter carboy with spigot.

Ingredients	For 10 liters
2×-YT Medium powder (BIO-101 Low Salt)	310 g
Water (R/O)	10 liters

Dissolve with stir bar in 3–5 liters water. Transfer to more convenient height. Add remaining water.
Dispense 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Let hot media rest 10–15 min. Place in front of fan to let cool.
Apply labels with date. When no longer warm, tighten down caps. Stock shelves.

Agar 4%

Prepare in any number of 1-liter bottles.

Ingredients	For each bottle
Agar (Difco)	12 g
Water (R/O)	300 ml

Label 1-liter bottles with permanent marker ("4%").
Weigh 12 g—or simply fill a cut-away coffee scoop (practice weighing to get right amount) with agar.
Pour agar into 1-liter bottle with aid of large powder funnel.
Add 300 ml water with aid of large powder funnel.
Loosely cap bottles.
Autoclave 35 min
When no longer warm, tighten down caps. Stock shelves.

YNB 2×

YNB (yeast nitrogen base) is minimal medium lacking glucose. Difco's YNB without ammonium sulfate is less expensive than the same product with ammonium sulfate. Inositol is added to YNB so that the medium will support wild-type growth of inositol-requiring strains. Inositol-free medium for detecting inositol auxotrophs must be made from scratch. Prepare in two 6-liter flasks.

Ingredients	In each flask
Difco Yeast Nitrogen Base w/o AA, AS[a]	9 g
Ammonium sulfate	30 g
Inositol (200 m*M*) stock (*myo*-inositol)	6 ml
Water (R/O)	3 liters

[a] With and without amino acids (AA) ammonium sulfate (AS). Record lot number in log.

Dissolve with stir bar. Heat may be applied.
Dispense 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Let hot media rest 10–15 min. Place in front of fan to let cool.
Apply labels with date. When no longer warm, tighten caps. Stock shelves.

YEP 2×

YEP is yeast extract and [Bacto] peptone with no carbon source. Prepare in two 6-liter flasks.

Ingredients	In each flask
Bacto-peptone	120 g
Yeast extract	60 g
L-Tryptophan	0.9 g
Water (R/O)	3 liters

Dissolve with stir bar. Heat may be applied.
Dispense 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Let hot media rest 10–15 min. Place in front of fan to let cool.
Apply labels with date. Record lot numbers. When no longer warm, tighten down caps. Stock shelves.

YPD 1×

This product is ready-made for users to aliquot directly into culture tubes. Here the glucose is autoclaved with the media. Watch out for over-autoclaving as indicated by a dark brown color indicating that the glucose has caramelized. The medium is supplemented with additional tryptophan. Prepare in two 6-liter flasks.

Ingredients	In each flask
Bacto-peptone	60 g
Yeast extract	30 g
L-Tryptophan	0.45 g
D-Glucose (dextrose)	60 g
Water (R/O)	3 liters

Dissolve with stir bar. Heat may be applied.
Dispense 300 ml in 500-ml bottles and 100 ml in 200-ml square bottles.
Autoclave 35 min or less.
Let hot media rest 10–15 min. Place in front of fan to let cool.
Apply labels with date. When no longer warm, tighten down caps. Stock shelves.

YNB 2× + AA Drop-Out: Generic Protocol

Prepare in two 6-liter flasks.

Ingredients	In each flask
Difco Yeast Nitrogen Base w/o AA, AS[a]	9 g
Ammonium sulfate	30 g
Inositol (200 m*M*) stock	6 ml
"All Amino Acids" Powder mix drop-out	12 g
Water (R/O)	3 liters

[a] Record lot number in log.

Dissolve with stir bar. Heat may be applied.
Dispense 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Apply labels with date. When no longer warm, tighten down caps. Stock shelves.

Glucose 40%

Prepare in large Nalgene beakers (must fit into the microwave).

Ingredients	Per 1 liter final volume
D-Glucose (dextrose)	400 g
Water (R/O)	780 ml (to make final volume 1 liter)

Dissolve in Nalgene beaker (see www.nalgenunc.com) by covering with Saran wrap and microwaving on high for 17 min (microwave ovens vary). Handle the hot liquid carefully. Add a stir bar *after* sugar is dissolved and mix thoroughly.
Dispense approx. 300 ml in 500-ml bottles.
Autoclave 35 min or less.
Apply labels with date. When no longer warm, tighten down caps. Stock shelves.

HIS 100 m*M*

The following applies to all six stock solutions listed here: The recommended amount to add per 600 ml is appropriate for supplementing minimal medium. It is not equivalent to the amount of amino acid in the dry powder mix used for making SC medium. Prepare in a 2-liter flask.

Ingredients	For 1 liter
L-Histidine hydrochloride (Sigma, St. Louis, MO)	20.9 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 1.8 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

ADE 30 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
Adenine hemisulfate (molecular weight 184.2; Sigma)	5.5 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 6 ml/600 ml."
When no longer warm, tighten caps. Stock shelves.

URA 20 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
Uracil (Sigma)	2.24 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 6 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

LYS 100 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
L-Lysine (Sigma)	18.3 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 6 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

LEU 100 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
L-Leucine (Sigma L-8000)	13.1 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 10 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

TRP 40 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
L-Tryptophan (formula weight 204.2; Sigma)	8.0 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar. Use heat if necessary.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Cool bottles in dark. Wrap in foil.
Apply labels with date. Add instruction: "Use 6 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

Inositol 200 m*M*

Prepare in a 2-liter flask.

Ingredients	For 1 liter
myo-Inositol	36 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 1 ml/600 ml."
When no longer warm, tighten down caps. Stock shelves.

M9A 20×

Prepare in 2-liter flask.

Ingredients	For 1 liter
Na_2HPO_4 (disodium phosphate, dibasic, anhydrous)	116 g
KH_2PO_4 (potassium phosphate, monobasic)	60 g
NaCl (sodium chloride)	10 g
NH_4Cl (ammonium chloride)	20 g
Water (R/O)	900 ml

Start with 900 ml water + stir bar in 2-liter flask. Add and dissolve each ingredient, one after the other.
Pour solution into 1000 ml graduated cylinder and add water, if needed to bring volume to 1 liter.
Dispense 100 ml each into square bottles. Cap loosely.
Autoclave 18 min (small autoclave).
Apply labels with date.
When no longer warm, tighten down caps. Stock shelves.

SOB

Prepare in two 6-liter flasks.

Ingredients	For each flask
Bacto-tryptone	60 g
Yeast extract	15 g
NaCl (sodium chloride) (10 m*M*)	1.74 g
KCl (potassium chloride) (25 m*M*)	0.57 g
NaOH 1 *N* solution (wear goggles and gloves)	4 ml
Water (R/O)	3000 ml

Dissolve with stir bar. Heat may be applied.
Dispense 300 ml each into 500 ml bottles.
Autoclave 30 min.
Remove and let cool.
Label and store.

LB Top Agar

Prepare in two 6-liter flasks.

Ingredients	For each flask
LB Medium powder or capsules (BIO-101 low salt)	75 g
Agar (Difco)	18 g
Water (R/O)	3 liters

Dissolve in small autoclave 13 min. Add stir bar afterward and mix thoroughly until "mixing lines" are gone. (Apply additional heat if needed.)
Dispense 300 ml in 500-ml bottles. Be careful of this HOT liquid.
You can wait till it cools down to a more comfortable temperature. Place the flasks on an insulated surface (paper towel).
Loosely cap bottles.
Autoclave 15 min or more.
Apply labels with date. When no longer warm, tighten caps. Stock shelves.

Soft Agar

Prepare in two 6-liter flasks.

Ingredients	For each flask
Nutrient broth powder	8 g
NaCl (sodium chloride)	15 g
Agar (Difco)	19.5 g
Water (R/O)	3 liters

Dissolve in small autoclave 13 min. Add stir bar afterward and mix thoroughly until "lines" are gone. (Apply additional heat if needed.)
Dispense 300 ml in 500-ml bottles. Be careful of this HOT liquid.
You can wait till it cools down to a more comfortable temperature. Place the flasks on an insulated surface (paper towel).
Loosely cap bottles.
Autoclave 15 min or more.
Apply labels with date. When no longer warm, tighten caps. Stock shelves.

T Agar

Prepare in two 6-liter flasks.

Ingredients	For each flask
Bacto-tryptone	30 g
NaCl (sodium chloride)	7.5 g
Agar (Difco)	19.5 g
Water (R/O)	3 liters

Dissolve in small autoclave 13 min. Add stir bar afterwards and mix thoroughly until "lines" are gone. (Apply additional heat if needed.)
Dispense 300 ml in 500-ml bottles. Be careful of this HOT liquid.
You can wait till it cools down to a more comfortable temperature. Place the flasks on an insulated surface (paper towel).
Loosely cap bottles.
Autoclave 15 min or more.
Apply labels with date. When no longer warm, tighten caps. Stock shelves.

Casamino Acids

Prepare in a 2-liter flask.

Ingredients	For 1 liter
Casamino acids	100 g
Water (R/O)	1 liter

Dissolve in 2-liter flask with stir bar.
Dispense 100 ml in 200-ml square bottles.
Autoclave 18 min (small autoclave).
Apply labels with date. Add instruction: "Use 1 ml/600 ml."
When no longer warm, tighten caps. Stock shelves.

NaOH 1 *N*

Prepare in 1-liter flask.

Ingredients	Per 100 ml final volume[a]
NaOH (sodium hydroxide)	4 g
Water (R/O)	100 ml

[a] Or a multiple of 100 ml up to 500 ml.

HAZARD: Pellets and solution are strong base. Wear goggles and gloves.
If contact, flush 15 min in water and report accident.
Add pellets to water with stir bar and mix.
Dispense 100 ml in 200-ml square bottles. Tighten caps.
Apply labels with date.

AA Powder Mix Complete

Make 10×

Amino acid amount	Amino acid amount	Amino acid amount
Ade 2 g	Gly 2 g	Ser 2 g
Ala 2 g	Leu 4 g	Thr 2 g
Arg 2 g	Ile 2 g	Trp 2 g
Asn 2 g	Lys 2 g	Tyr 2 g
Asp 2 g	His 2 g	Ura 2 g
Cys 2 g	Met 2 g	Val 2 g
Gln 2 g	Phe 2 g	PABA[a] 0.2 g
Glu 2 g	Pro 2 g	

[a] *p*-Aminobenzoic acid.

Weigh each and add to plastic jar. Check off each item as it is added.
Include several steel balls to facilitate mixing powder (or use a powder grinding mill).
Close jar and shake to mix, longer than you think necessary.

GNA (Presporulation Media)

This medium, when autoclaved all together, regularly boils over. Here parts are autoclaved separately and then combined. Prepare in 3 flasks.

Ingredients	For 3 liters
Flask 1 (4-liter)	
Yeast extract (a very fine powder; wear mask)	30 g
Agar (Difco-Bacto)	60 g
Water (R/O)	1500 ml
Flask 2 (2-liter)	
Nutrient broth (a very fine powder; wear mask)	90 g
Water (R/O)	1125 ml
Flask 3 (1-liter)	
D-Glucose (dextrose)	150 g
Water (R/O)	375 ml

No stir bar and no premixing are necessary.
Cover with foil or paper towel and cups.
Autoclave 20 min (small autoclave).
After autoclaving, let the flasks stand 5 min to cool (so they are no longer superheated). But keep the agar flask warm as in a water bath so the agar does not solidify on the bottom. Swirl the flask to make the agar uniformly distributed. Then, using *sterile technique,* pour Flask 2 and

Flask 3 into Flask 1. *Swirl* Flask 1 until mixing is complete (schlieren lines are gone). Do not worry about bubbles.

Deliver flask to plate pouring room. Place flask in the water bath and notify the requester that the medium is ready. Note the time of day.

NGS Agar (for Sporulation)

(No Glucose Sporulation.) This medium works successfully with most laboratory strains. Prepare in 6-liter flask (A) and in a 1-liter flask (B).

Ingredients	For 3 liters total
(A) For 6-liter flask	
Potassium acetate	30 g
Agar (Difco)	60 g
Water (R/O)	2500 ml
(B) For 1-liter flask	
Amino acid mix (AA – URA – TRP)	1.5 g
Uracil stock solution	7.5 ml
Tryptophan stock solution	7.5 ml
Water (R/O)	500 ml

No stir bar and no premixing are necessary.

Cover with foil or paper towel and cups.

Autoclave 35 min (small autoclave).

After autoclaving, let flasks stand 3 min to cool (so they are no longer superheated). Then pour Flask B into Flask A, without touching the sterile areas. *Swirl* by hand (wear gloves) until schlieren lines are gone (mixing is complete).

Place flask on a paper towel on a dry cart and deliver to bay of requester.

SPOR(ulation) Agar

Prepare in 6-liter flask.

Ingredients	For 3 liters
Potassium acetate	30 g
D-Glucose (dextrose)	3 g
Yeast extract	3.75 g
Agar (Difco)	60 g
Water (R/O)	3000 ml

Autoclave 35 min (small autoclave).

After autoclaving, let flask stand 3 min to cool (so it is no longer superheated). Then *swirl* by hand (wear gloves) until schlieren lines are gone (mixing is complete).

Place flask on a paper towel on a dry cart and deliver to bay of requester.

TB (Terrific Broth)

Prepare on request for individual users. Prepare in 6-liter flask.

Ingredients	For 3 liters
Bacto-tryptone	36 g
Yeast extract	72 g
Glycerol	12 ml
KH_2PO_4 (potassium phosphate, monobasic)	3.9 g
K_2HPO_4 (potassium phosphate, dibasic, anhydrous)	37.5 g
Water (R/O)	3000 ml

No stir bar and no premixing are necessary.
Cover with foil or paper towel and cups.
Autoclave 35 min (small autoclave).
Remove and let cool.
Deliver to bay of requester.

FB (Fortified Broth)

Prepare on request for individual users. Prepare in 6-liter flask.

Ingredients	For 3 liters
Bacto-tryptone	75 g
Yeast extract	22.5 g
D-Glucose (dextrose)	3 g
NaCl (sodium chloride)	18 g
2 *M* Tris-Cl, pH 7.6	75 ml
Water (R/O)	2925 ml

No stir bar and no premixing are necessary.
Cover with foil or paper towel and cups.
Autoclave 35 min (small autoclave).
Remove and let cool.
Deliver to bay of requester.

T Broth

Prepare on request for individual users. Prepare in 6-liter flask.

Ingredients	For 3 liters
Bacto-tryptone	30 g
NaCl (sodium chloride)	7.5 g
Water (R/O)	3000 ml

No stir bar, no premixing necessary.
Cover with foil or paper towel and cups.
Autoclave 35 min (small autoclave).
Remove and let cool.
Deliver to bay of requester.

SOB Capsules

To make 20 × 100-ml media bottles of 50 ml each by filter sterilization. Prepare in one 2-liter flask.

Ingredients	
SOB powder (in capsules) (BIO 101)	31 g
Water (R/O)	1000 ml

Dissolve with stir bar. Use heat sparingly.
Gather materials: 20 sterile 100-ml media bottles, one sterile1-liter media bottle, one disposable 500-ml sterilizing filter cup, one vacuum flask liquid trap.
Using sterile technique, remove the cap from the 1-liter media bottle. Open the package and screw on the sterilizing filter firmly. Insert the white filter unit tip into the vacuum trap tube.
Pour the media into the cup and turn on the vacuum. Continue adding media to the cup until it has all been filtered through. Remove the tube from the white tip and turn off the vacuum.
Arrange the 100-ml bottles in a row with the caps very loose. Remove the white tip from the filter unit. With one hand, lift the cap off the bottle and with the other hand, pour about 50 ml into the first bottle. Replace the cap and go on to the next bottle.
When finished, screw down all the caps and keep the bottles 2–3 days (one at 36°) to see if they remain clear. Then stock them on the shelves.

The filter sterilizer is disposable, and it is not reusable.

Inositol-Free Minimal Medium[4,5]

Make stock solutions/suspensions in 1-liter flasks.

Ingredients: (A) trace elements stock mix	For 1 liter
Boric acid	50 mg
Copper sulfate	4 mg
Potassium iodide	10 mg
Ferric chloride	20 mg
Manganese sulfate	40 mg
Sodium molybdate	20 mg
Zinc sulfate	40 mg
Water (R/O)	100 ml

Ingredients: (B) vitamin stock mix	For 1 liter
Biotin	2 mg
Calcium pantothenate	400 mg
Folic acid	2 mg
Niacin	400 mg
p-Aminobenzoic acid	200 mg
Pyridoxine hydrochloride	400 mg
Riboflavin	200 mg
Thiamin hydrochloride	400 mg
Water (R/O)	1000 ml

Ingredients: (C) salts and nitrogen source solution	For 1 liter
Potassium phosphate monobasic	10 g
Magnesium sulfate	5 g
Sodium chloride	1 g
Calcium chloride	1 g
Ammonium sulfate	50 g
Water (R/O)	1000 ml

Transfer media A, B, and C to bottles with tight caps. B should be stored in brown glass. A and B will be suspensions; they will not dissolve.

Add 50 ml chloroform to prevent growth of contaminants. Store in the cold.

To make medium use per liter 1 ml of trace elements, 1 ml of vitamins, 100 ml of salts plus nitrogen, and 20 g of glucose. Add 20 g of agar for solid medium.

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