Media and Culture of Yeast

This unit describes basic aspects of preparing media for and growing *Saccharomyces cerevisiae* cells. It also covers some aspects of handling yeast strains, such as growth, storage, and shipment. *Saccharomyces* has been one of the most extensively studied organisms in the laboratory, and an extensive repertoire of genetic and molecular biology methods have been developed, as described in this volume and elsewhere (see Key References). A large number of yeast strains with useful characteristics have been documented, and many can be obtained for a nominal cost from public collections including the Yeast Genetics Stock Center (YGSC; Rebecca Contopoulou, Curator; e-mail: *ygsc305@violet.berkeley.edu*) or the American Type Culture Collection (ATCC). For contact information for these collections, see Internet Resources and *SUPPLIERS APPENDIX*.

Culturing *Saccharomyces* is relatively straightforward and can be accomplished with readily available and inexpensive materials. Wild-type yeast are prototrophic for most nutrients; that is, they are capable of synthesizing most metabolites from inorganic salts and a carbon source for energy. However most laboratory strains carry mutations that make them auxotrophic for one or more metabolites, such as amino acids or nucleotides, that must be supplied in the growth medium. The auxotrophic mutations, or *markers*, are used in the maintenance of plasmids and in other genetic experiments.

The first part of this unit will discuss the preparation of media. General aspects of media preparation will be described followed by specific recipes. The second part of the unit will discuss the growth and handling of yeast strains.

NOTE: All incubations of yeast cells are performed at 30°C unless otherwise noted.

PREPARATION OF MEDIA

Yeast media are referred to as either *liquid* or *solid*. Liquid medium is self-explanatory. A solid medium typically contains 2% (w/v) Bacto Agar. Other comparable gelling agents can be used in special circumstances, but they are not discussed in this unit.

Liquid Media

Liquid media are prepared in the same way as solid media (described below) except that agar is not added. As a result, liquid media can be sterilized either by autoclaving or by filter sterilizing with a 0.22-µm filter. Filter sterilization is faster and eliminates the risk of heat inactivation of any medium ingredients. Liquid medium is prepared in either bottles or growth flasks. In flasks, medium should never exceed one-fifth the flask volume, so that maximum aeration can be achieved during shaking.

Solid Media

Solid media can be prepared in Erlenmeyer or Fernbach flasks; however, the most convenient container for pouring plates is the Fleaker (Corning). Heat-stable ingredients are mixed in water until completely dissolved (with the exception of the agar, which is added but will not dissolve). It is convenient to add a magnetic stir bar to the flask or Fleaker prior to autoclaving. The medium is autoclaved at 121°C on the liquids or slow exhaust program/setting at 15 psi for 15 min, after which it is placed on a stir plate at room temperature and stirred as it is allowed to cool to 60° to 65°C. Alternatively, the flasks or Fleakers can be placed in a 55°C water bath. Filter-sterilized, heat-sensitive ingredients are added at this point, and the medium is mixed completely prior to plate pouring. At this point the agar should be fully suspended within the medium.

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Most yeast medium recipes, including those described in this unit, should result in a pH between 5.5 and 7.0. If a more acid medium is prepared, care should be taken to minimize or avoid autoclaving agar at pH ≤4.8, as the agar will hydrolyze and fail to gel when cooled.

A pouring area should be prepared in a quiet place with minimal dust and airflow, and the bench surface should be wiped with 70% ethanol or another sterilizing agent. The appropriate number of bags of petri dishes (typically 15 × 100–mm or 15 × 95–mm) should be opened by carefully cutting off the top of the bags. The bags are overturned, allowing the plates to slide out in a stack. The bags are saved for storing the filled plates. The bottom of the plates are labeled, and the plates overturned and divided into stacks of eight. When the medium has cooled to 55° to 60°C, it can be carefully poured into the plates. When pouring, the Fleaker or flask of medium should be held in one hand, allowing the other hand to grab the stack of plates. The top seven plates are lifted as a stack, the medium is poured into the eighth (bottom) plate, and the stack is lowered. This process is then repeated by picking up the top six plates, pouring the seventh plate, and so on. Although this system of pouring is awkward at first, it has the advantages of being faster and taking up less space than pouring plates in stacks of one.

One liter of medium should generate 40 to 50 plates. Sometimes bubbles will be formed while pouring medium. These can be removed by quickly passing a bunsen burner flame over the surface of the molten agar. The lid should be placed on the plates as soon as possible to minimize the risk of contamination. After the agar hardens (20 to 45 min), the plates should be flipped so that the lids are on the bottom. Plates should be allowed to dry at room temperature for 2 to 3 days. Because they are more prone to collecting condensation, media for yeast are left to dry at room temperature for a longer time than are media for bacteria. Once dried, the plates are placed in plastic bags, and the bags are taped closed and stored at room temperature or at 4°C for extended periods of time. Most media will be stable for ≥3 months; if excessive drying and contamination can be avoided, most media are stable for up to one year. Sometimes it is helpful to make small slits in the plastic bags to let them "breathe." This reduces condensation, which in turn leads to the appearance of fewer contaminating organisms.

Defined Versus Complex Media

Yeast media are often referred to as either *defined* or *complex*. A defined medium is made with chemically defined components, such as salts, sugars, and amino acids. A complex, or rich, medium includes the addition of a complex lysate or hydrolysate, such as yeast extract or peptone. For the purpose of these definitions, agar-containing medium may still be referred to as defined. The following sections provide details about materials for media, and give recipes for specific complex and defined media. A number of special-purpose media are detailed.

Materials

Media should be prepared using distilled or deionized water. Most materials for media preparation should be of high grade, although the specific vendor is usually not important. However, yeast extract, peptone, agar, and yeast nitrogen base (YNB) should all be Bacto brand, sold by Difco. Difco products are carried by several scientific distributors.

Best results are obtained when the sugar component of a yeast medium (e.g., dextrose, sucrose, raffinose, galactose) is not autoclaved with the other components of the medium. If dextrose is included with other ingredients during the autoclaving process, it can caramelize. The standard alternative method is to autoclave or filter sterilize a concentrated stock solution of the sugar and add it to the medium after the other components have been autoclaved. For dextrose, a sterilized 40% (w/v) stock solution is added at 50

ml/liter to the autoclaved mixture of other medium components. Other sugars are typically made as 20% (w/v) stock solutions and added at 100 ml/liter.

Complex Media

Yeast extract/peptone/dextrose (YPD or YEPD) medium

This complex medium is the one most commonly used for the routine growth of yeast when no auxotrophic selection is required.

10 g Bacto yeast extract (1% w/v final)
20 g Bacto Peptone (2% w/v final)
20 g agar (2% w/v final)
950 ml H_2O
Autoclave
Add 50 ml 40% (w/v) dextrose (2% final; sterilized separately by autoclaving or filtering)

Many researchers prefer to add adenine to YPD medium, especially when working with adenine auxotrophs. Red-colored strains of Saccharomyces almost always lack either ade1 or ade2 gene function. To make YPAD, add 20 mg/liter adenine sulfate to YPD prior to autoclaving.

Similar media can be made by substituting other sugars for dextrose. Generally, Saccharomyces grows best on dextrose, but specific experiments may require replacement of dextrose with another sugar. The most common substitutes are YPF (2% w/v fructose), YPGal (2% w/v galactose), YPSuc (2% w/v sucrose), YPRaf (2% w/v raffinose), or YPMal (2% w/v maltose). Except for fructose, not all yeast strains are capable of growing on these alternative sugars. Growth on raffinose is sometimes facilitated by adding 0.05% (w/v) dextrose.

Yeast extract/peptone/glycerol (YPG) medium

Nonfermentable glycerol is the only defined carbon source in this complex medium. Because there is no fermentable carbon source, YPG does not support the growth of *petite* yeast strains (mutant strains that lack functional mitochondria).

10 g Bacto yeast extract (1% w/v final)
20 g Bacto Peptone (2% w/v final)
50 ml glycerol (5% v/v final)
20 g agar (2% w/v final)
950 ml H_2O
Autoclave

Some recipes call for 3% glycerol, but 5% gives better growth. Glycerol can be autoclaved with the medium. Other nonfermentable carbon sources can be used, including ethanol, acetate, or lactate, or combinations thereof. Glycerol is the most commonly used nonfermentable carbon source.

Yeast extract/peptone/dextrose/glycerol (YPDG) medium

This complex medium is used to determine the proportions of wild-type and *petite* cells. The different cells form large and small colonies respectively.

10 g Bacto yeast extract (1% w/v final)
20 g Bacto peptone (2% w/v final)
30 ml glycerol (3% v/v final)
20 g agar (2% w/v final)
970 ml H_2O
Autoclave
Add 2.5 ml 40% (w/v) dextrose (0.1% final; sterilized separately by autoclaving or filtering)

Special-Purpose Complex Media

Galactose indicator medium

Several gene expression systems used in yeast research rely on a *GAL* (galactose) promoter for regulated expression. When such a system fails to work as expected, one source of trouble is the use of yeast strains that are unable to respond to galactose. Strains that respond to galactose will ferment it and release acid into the medium. Acidification causes the indicator dye to change color from blue to yellow.

10 g Bacto yeast extract (1% w/v final)
20 g Bacto Peptone (2% w/v final)
20 g agar (2% w/v final)
930 ml H_2O
Autoclave
Add 50 ml 40% (w/v) galactose (2% final; sterilized separately by autoclaving or filtering)
Add 20 ml 0.4% (w/v) bromthymol blue (0.08% final; sterilized separately by filtering)

Sporulation medium

Saccharomyces strains can be maintained as either haploids or diploids. Most diploid strains will sporulate when grown on a poor source of carbon and nitrogen.

10 g potassium acetate (1% w/v final)
1 g Bacto yeast extract (0.1% w/v final)
0.5 g dextrose (0.05% w/v final)
20 g agar (2% w/v final)
1 liter H_2O
Autoclave

For this recipe dextrose can be added prior to autoclaving.

Presporulation (PSP) medium

Sporulation of some *Saccharomyces* strains is facilitated by growth on a rich medium prior to plating on sporulation medium.

8 g Bacto yeast extract (0.8% w/v final)
3 g Bacto Peptone (0.3% w/v final)
20 g agar (2% w/v final)
750 ml H_2O
Autoclave
Add 250 ml warm 40% (w/v) dextrose (10% final; sterilized separately by autoclaving or filtering)

The dextrose solution should be warmed to ~40° to 55°C before it is added, so that the agar does not harden prematurely.

Dissection agar

Preparation of dissection agar is frequently tailored to the configuration of the dissection apparatus. In general, thin slabs of YPD medium (10 ml per standard 15 × 100–mm petri plate) are used. Best results are obtained with light-colored (most transparent) medium, which can be facilitated by minimizing the length of time it is heated and by autoclaving the dextrose separately.

Defined Media

It is generally best to add inositol to all synthetic media. Although wild-type yeast strains are prototrophic for inositol, some laboratory strains bear an *ino1* mutation. Unfortunately, this mutation is sometimes forgotten in reporting the genotype because yeast nitrogen base (YNB) contains enough inositol that the effect of the mutation is not easy to see. However, the amount of inositol in YNB is not enough to fully supplement the auxotrophy, and selection for compensating mutations can occur.

Salts and dextrose (SD) medium

This minimal medium contains enough nutrients to support growth of prototrophic yeast strains (strains having no nutritional requirements).

6.7 g Bacto yeast nitrogen base (YNB) without amino acids (0.67% w/v final)
20 g agar (2% w/v final)
950 ml H_2O
Autoclave
Add 50 ml 40% (w/v) dextrose (2% final; sterilized separately by autoclaving or filtering)

Similar media can be made by substituting other sugars for dextrose. Generally, Saccharomyces grows best on dextrose, but specific experiments may require replacement of dextrose with another sugar. The most common substitutes are SGal (2% galactose) and SRaf (2% raffinose). Not all yeast strains are capable of growing on these alternative sugars. Growth on raffinose is sometimes facilitated by adding 0.05% (w/v) dextrose.

Vendors carry several similar types of YNB, e.g., with or without amino acids or ammonium sulfate. YNB in this recipe includes ammonium sulfate. Although ammonium sulfate is the preferred nitrogen source, Saccharomyces will grow on other nitrogen sources, albeit more slowly. For these media, YNB without ammonium sulfate and without amino acids is used at 1.7 g/liter. Nitrogen sources include ammonium sulfate (0.5% w/v), arginine (0.1% w/v), asparagine (0.1% w/v), or proline (0.1% w/v).

Most yeast strains require one or more nutrients that are not included in SD medium. These nutrients can be added in various ways (see supplemented SD medium and SC medium).

Supplemented SD medium

Add supplements (see Table 1.6.1) individually or in groups to SD medium. Alternatively, add supplements to individual plates by spreading, and allow one day for supplement(s) to permeate the medium. Add most supplements before autoclaving; add tryptophan and histidine, which are sensitive to heat, after autoclaving. Store tryptophan in the dark, as it is also sensitive to prolonged exposure to light.

Synthetic complete (SC) medium

As an alternative to adding individual nutrients to SD medium, add a dry mixture of the most common supplements. Combine the dry reagents listed in Table 1.6.1 and mix thoroughly using a coffee grinder or a mortar and pestle. Then add the mixture before sterilizing the medium, without tryptophan and histidine, which should be added after autoclaving.

Alternative mixtures can be constructed (e.g., SC minus tryptophan) by omitting specific ingredients. These mixtures are sometimes referred to as "dropout" media—e.g., SC –Trp dropout medium.

5-Fluoroorotic acid (5-FOA) medium

Selection for *URA3* function is used for the stable maintenance of many yeast plasmids. For some experiments, however, loss of the *URA3* gene is desired. The *URA3* gene product converts 5-FOA into a toxin. Therefore, strains that have lost the *URA3* gene can be identified by their resistance to 5-FOA.

continued

Solution 1:
1 g 5-FOA (0.1% w/v final)
500 ml H_2O
6.7 g yeast nitrogen base (YNB) – amino acids (0.67% w/v final)
50 mg uracil (50 µg/ml final)
20 g dextrose (2% w/v final)
Appropriate supplements (see Table 1.6.1)

Add 5-FOA, H_2O, and a stir bar to a 1-liter flask. Autoclave or stir over a low to medium heat ~1 hr to dissolve completely. Add YNB, uracil, glucose, and supplements. Choose supplements from Table 1.6.1 based on the genotype of the strain, but always add uracil. If supplements are added in liquid form, be sure to adjust the volume of H_2O accordingly. Dissolve completely (warming to 55°C is useful) and filter sterilize.

Solution 2:
20 g agar (2% w/v final)
500 ml H_2O

Mix agar and H_2O in a 2-liter flask, autoclave, and cool to ≤80°C.

5-FOA medium:
Mix solution 1 into cooled solution 2
Cool to 55°C
Pour into petri dishes

Table 1.6.1 Common Supplements Used In Defined Media

Nutrient	Stock concentration (g/100 ml)	Stock volume per liter medium (ml)	Final concentration (mg/liter)[a]	Stock volume to spread on plate (ml)
Adenine sulfate[b]	0.2	10	20	0.2
L-Arginine·Cl	1	2	20	0.1
L-Aspartic acid	1	10	100	0.2
L-Glutamic acid	1	10	100	0.2
L-Histidine·Cl	1	2	20	0.1
L-Isoleucine	1	3	30	0.1
L-Leucine	1	3	30	0.1
L-Lysine·Cl	1	3	30	0.1
L-Methionine	1	2	20	0.1
Myo-inositol[c]	3.6	1	36	0.02
L-Phenylalanine	1	5	50	0.1
L-Serine	8	5	400	0.1
L-Threonine	4	5	200	0.1
L-Tryptophan	1	2	20	0.1
L-Tyrosine	0.2	15	30	0.2
Uracil	0.2	10	20	0.2
L-Valine	3	5	150	0.1

[a]Mixtures of supplements can be made in advance by weighing out individual components in the ratio indicated by this column; mixing the dry reagents in a coffee grinder, mill, or mortar and pestle; and then weighing out the appropriate amount of the mixture.

[b]When working with *ade1* or *ade2* mutants, the amount of adenine should be increased three fold to prevent accumulation of red color.

[c]The traditional recipe for SC medium does not include inositol. Inositol is added as a precaution because some common laboratory strains contain the *ino1* mutation, which is not always documented. YNB contains enough inositol for significant, albeit incomplete, supplementation, which makes recognizing the presence of an *ino1* mutation difficult.

Xgal medium

Wild-type *Saccharomyces* strains lack endogenous β-galactosidase activity, which facilitates use of the *E. coli lacZ*-encoded β-galactosidase enzyme as a reporter activity. This plate assay provides a semi-quantitative assay for activity.

6.7 g yeast nitrogen base (YNB) − amino acids (0.67% w/v final)
Appropriate supplements (see Table 1.6.1)
20 g agar (2% w/v final)
850 ml H_2O
Autoclave
Cool to 65°C
Add 100 ml 0.7 M potassium phosphate, pH 7.0 (70 mM final; best if warmed prior to adding to agar)
Add 50 ml 40% (w/v) dextrose (2% final; sterilized separately by autoclaving or filtering)
Add 2 ml 20 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal) in 100% *N*,*N*-dimethylformamide (DMF)

Xgal can be prepared in advance, aliquoted, and stored as a frozen stock. Supplements are chosen from Table 1.6.1 based on the genotype of the strain. If supplements are added in liquid form, the volume of H_2O should be adjusted accordingly.

Xgal plates are frequently made as above, but without the substrate Xgal. Approximately 1 to 2 days prior to using the plates, 0.1 ml of 20 mg/ml Xgal stock is spread across the surface of the plate and allowed to diffuse through the agar.

GENERAL CONSIDERATIONS FOR CULTURING YEAST

For the most part, culturing *Saccharomyces* is fairly straightforward. Wild-type strains are typically grown at 30°C, although they are capable of growing at a wide range of temperatures. Many strains will grow to some extent at temperatures as low as 4°C and at least as high as 37°C. Liquid or solid media can be used. Liquid cultures grow better with aeration, but this is not required. For the most consistent results, it is important to maintain a reproducible level of aeration with each experiment by marking or taping the speed dial of the shaker.

Culturing Yeast in Liquid Media

Yeast strains are readily cultured in liquid media. For most experiments it is best to grow them with agitation, as this prevents them from settling to the bottom of the liquid. When cultures are grown in flasks, the liquid volume should be limited to 20% of the flask volume, although this guideline is sometimes exceeded. Another standard method is to grow 1- to 5-ml cultures in 18 × 150–mm glass tubes that are rotated on a tube roller (e.g., New Brunswick). Metal or plastic caps are readily available for these tubes. Many brands of tabletop or preparative centrifuges accommodate these tubes directly.

Yeast cells can be transferred from liquid or solid cultures to a new liquid culture by many methods. One popular method is to use sterile disposable 15-cm-long applicator sticks. The sticks can be autoclaved in capped culture tubes. Another popular method is to use nonflavored, noncolored wooden toothpicks, which can be autoclaved in their original boxes.

For any given set of conditions—namely, yeast strain, medium, temperature, and agitation—a generation time and saturation density can be readily determined. Most strains grown with agitation in YPD medium at 30°C will double every 80 to 100 min. An overnight culture seeded with ~3×10^4 to 1×10^5 cells or a ~1-mm colony will typically

grow to ~3×10^7 cells/ml. After two days the culture should reach ~5×10^8 cells/ml. When grown in SD or SC medium, the generation time will be on the order of 120 to 150 min. An overnight culture will typically grow to ~5×10^6 cells/ml.

The number of cells per milliliter of culture can be estimated using a spectrophotometer. With a standard 1-cm-path-length cuvette at 600 nm, an optical density (OD_{600}) of 1.0 is equal to ~2×10^6 cells/ml. Because this method relies on measuring light scattering and not true absorbance of the culture, this rule of thumb should be confirmed for each specific spectrophotometer. The conversion factor can vary widely depending on the configuration of the instrument. The spectrophotometer can be calibrated by comparing the OD_{600} reading with an accurate cell count obtained with a hemacytometer or by counting the number of colonies that can be grown from a diluted sample. Once an instrument is calibrated, however, the value should not change from day to day.

Culturing Yeast on Solid Media

Yeast strains are readily cultured on solid media. For most experiments it is best to use a 30°C incubator. While growth rates are hard to establish on solid media, colonies formed from single cells are generally visible to the unaided eye after 2 or 3 days and patches of cells are visible after one day. There are five common methods for transferring yeast cells to solid media: (1) spread plating a suspension of cells, (2) streaking cells with a toothpick or inoculating loop, (3) patching cells with a toothpick or inoculating loop, (4) replica plating from an existing plate, and (5) suspending cells in top agar and pouring them over an agar plate. Each of these is described below.

Spread plating cells

In this method, an appropriate number of yeast cells are calculated and suspended in a small volume of liquid, typically 0.05 to 0.25 ml. The appropriate cell number will depend on the nature of the experiment. It is generally desirable to produce 20 to 200 colonies per plate.

A glass rod can be fashioned into a spreader as follows. A roughly 30-cm length of 3-mm glass rod is cut and both ends are smoothed in a flame. Hold the rod over a bunsen burner flame ~5 cm from one end. When the glass is pliable, bend it ~45°. Hold the rod over the flame ~10 cm from the same end and bend it another 45°, so that one end has now formed a triangle. Optionally, the point at which the triangle section meets the shaft can be bent 30° from the plane of the triangle to make the spreader more ergonomic. Prior to use the spreader is sterilized by dipping the triangle end in 70% to 95% ethanol and igniting it in a flame. Before applying to a cell suspension, the spreader should be cooled by touching it onto the surface of the agar plate or onto the condensation that frequently collects on the lid of the plate. Alternatively, an unmodified sterile glass pipet can be used to spread cells, but this is more difficult. Plastic pipets are not recommended as they tear the agar.

Although not required, spread plating works better if an inoculating turntable (available from Fisher Scientific and other laboratory suppliers) is used. An inoculation turntable consists of a solid base and a freely rotating platform that is the approximate size of a petri plate.

The cells should be spread across the plate immediately after pipetting them onto the agar. If a turntable is used, give it a gentle push. If it is not used, the plate can be rotated frequently by hand. The flat part of the triangle is used to push the cells back and forth across the agar. The technique of spread plating yeast cells, especially with a turntable, differs from spread plating bacteria in one notable respect: yeast cells are more likely to be pushed to the periphery of the plate. To avoid this problem, gently push the "elbow" of the spreader into edge of the plate for a few rotations and then, while the plate is still

rotating, push the elbow back and forth across the plate in a chord that is roughly half way between the center and the edge of the plate.

Streaking cells

The streaking method is used for isolating single colonies of yeast. Single isolated colonies are desirable because, when the procedure is done properly, each colony is derived from a single cell, so that every cell in the colony is genetically identical.

In this method, the plate is divided into four pie-shaped sections and one sample of yeast cells is streaked for single colonies in each section. With practice and increasing skill, the plate can be divided into six or more sections. This method is best performed with sterile toothpicks. A minimal amount of yeast cells are picked with the toothpick and placed in a spot near the edge of the plate. A clean toothpick is used to smear the cells back and forth in a 4×10–mm patch with the long axis of the patch running roughly parallel to the lip of the plate. A third toothpick is drawn once across the short axis of the patch and for another ~15 mm towards the center of the plate. A fourth toothpick is dragged on the agar in an arc that parallels the plate edge and crosses the line made by the third toothpick; the toothpick is then drawn in a series of shorter and shorter arcs that parallel the plate edge and move consecutively towards the center of the plate. The plate is then incubated for 2 to 4 days until individual colonies are visible.

Patching cells

Once pure strains of yeast have been isolated, it is frequently useful to compile a set of the strains on a single agar plate, either for short-term storage or in preparation for testing them in parallel by replica plating. Between 30 and 100 patches can be arranged on a standard 100-mm petri plate.

Before patching yeast from one plate to another, an ordered grid is prepared on a piece of paper and is inserted in a clear plastic sleeve. A standard sheet of paper (A4 or 8×10–in.) can accommodate four identical grids for 100-mm petri plates. The agar plates are set on the grid with the lids facing up. The plates can be held in place with a piece of double-sided tape or with tape that has been rolled into a circle. Two pieces of tape are much more effective than one in holding the plates in place. Either sterile toothpicks or inoculating loops can be used for the patch technique, but toothpicks work better. The patches are made by barely touching a source colony with the end of the toothpick and then gently dragging the toothpick over a ~2-mm diameter spot on the recipient plate.

Replica plating cells

Replica plating is used for testing multiple strains or isolates in parallel. This technique requires three specialized pieces of equipment: a replica block, a locking ring, and velveteen cloth squares. All three are available commercially from Fisher Scientific, Cora Styles, and other suppliers. The diameter of the block must be designed for the diameter of the petri plates being used; 95-mm diameter plates will not work with a block designed for 100-mm plates. Having the correct type of cloth is also essential for replica plating to work.

Prior to the first use and after each subsequent use, the cloth squares are laundered in any standard washer and dryer with standard laundry detergent. If recombinant organisms are used, it is prudent to soak the contaminated squares in a disinfectant prior to laundering them. Care should be taken to monitor the lint screen during drying because a significant amount of lint is produced; failure to do this can damage the clothes dryer. After the squares are dried, excess lint can be removed with a standard lint brush, although this is not always necessary. The squares are sterilized by wrapping them in heavy-gage aluminum foil and autoclaving them. Depending on usage patterns, 5 to 50 squares can be stacked, soft-side down, on the center of a large piece of foil. The foil is then folded

around the stack to make a securely closed package that is held with a piece of tape. The package is then autoclaved on the dry cycle.

A *master* plate is made by spread plating, streaking, or patching cells. Typically, the master plate is incubated for 1 to 3 days before use. The master plate works best if the colonies are not extremely large and overgrown. Each good-quality master can be used to produce up to eight replicas.

The replica block and locking ring can be cleaned by squirting them with 70% ethanol and drying them with a paper towel. The package of velvets is carefully opened with the soft side of the cloth squares facing down. One velvet is picked up by the corner, turned over, and placed on the block. The package should then be partially closed to prevent contamination. The locking ring is pushed down so that the velvet is drawn tight. The master plate is gently but firmly pushed onto the surface of the velvet and then lifted up again. Each test plate is then pushed onto the velvet in the same manner.

Suspending cells in top agar

This is probably the least preferred method unless the yeast cell wall has been compromised and an osmotic support is needed. A stock of sterile Bacto agar is prepared in water or medium. The agar should be prepared so that the final concentration is between 0.5% and 1.0% after adding the yeast suspension. Once it has been sterilized, the agar stock solution can be melted any number of times in a boiling water bath. For a 100-mm plate, 3 to 10 ml of the melted agar is transferred to a sterile tube and cooled to 37°C. A small volume of yeast cells are added, and the suspension is mixed and poured over the surface of an agar plate. After a few minutes, the plates can be inverted and incubated for 2 to 4 days at 30°C.

Storage of Yeast Strains

Short-term storage of yeast strains

Most yeast strains will survive extended periods of storage at room temperature or 4°C; however, this type of storage should not be relied upon. Storage at these temperatures may result in loss of viability, or worse, selection of uncharacterized mutants that survive better under these conditions.

Long-term storage in glycerol

Two methods are commonly used for long-term storage of yeast strains: glycerol/water vials at −80°C and agar slants at room temperature or 4°C. The glycerol vials are the preferred method of storage.

Yeast strains can be stored indefinitely at −80°C in 15% (v/v) glycerol in water. Any glass vial may be used; however, 2-ml Wheaton vials (in a lab file) are an ideal size. These vials can be stored in standard freezer storage boxes (100 per box).

To prepare the vials, 1 ml of 15% glycerol solution is dispensed per vial, lids are placed loosely on the vials, and the vials are autoclaved. As soon as the vials are cool enough to handle, the lids are tightened. Prior to use, the vials may be stored at room temperature indefinitely.

To store the strain, the vials are labeled and a single colony is transferred using a sterile toothpick or inoculating loop from a fresh plate into each vial. Each vial is shaken until the colony is evenly distributed in the liquid. The vials are then transferred to a −80°C freezer. The best colonies are 1 to 3 days old, although older ones will work.

Labeling the vials can be difficult because some brands of label fall off at such cold temperatures and some inks run when they come in contact with freezer frost. One method

that works is to use good quality office labels and to cover the labels with one wrap of cellophane tape. The tape sticks to itself very well and protects the writing.

A sample of a liquid culture may be preserved by this method, although it is not recommended. If liquid cultures are to be preserved, the concentration of glycerol should be adjusted accordingly to allow for dilution by the liquid culture.

Long-term storage in YPAD slants

YPAD medium is prepared and the agar is dissolved into the medium in a boiling water bath. Portions of 1.5 ml are dispensed into 3-ml vials, screw cap lids are applied loosely, and the medium is autoclaved. After autoclaving, the vials are inclined so that the agar is just below the neck of the vial. After drying for 1 to 2 days, the lids are tightened.

A small sample of a fresh culture is transferred to the agar slant using a sterile toothpick or inoculating loop. The culture may be spread across the agar surface or it may be pushed into the agar. The screw-cap lid is tightened. Parafilm can be wrapped around the lid to improve the seal. The slants are stored in a cool (23°C) dark place. Viable yeasts can be recovered from the slants for ≥ 3 years.

Shipping Yeast Strains

Yeast strains can be shipped by any of several different methods. The shipped cultures may be dried, on agar, or in liquid as long as sterility is maintained. The simplest method is to use sterile swabs. Other methods generally involve more preparation time or are more vulnerable to damage in the mail.

Shipping yeast strains in sterile swabs

One convenient and safe method is to use sterile swabs for taking throat cultures, such as the Culturette system from Becton Dickinson. These systems contain a sterile swab and a saline solution housed in a leak-proof container that is resistant to rough handling. To use this system, the package is opened, a colony is swabbed from a fresh culture plate, the swab is inserted back into the package, and the package is labeled and taped or stapled shut. To revive the strain, the swab is removed and dabbed on a small area of a petri plate containing YPD or another appropriate medium. Toothpicks are then used to streak for single colonies and the plate is incubated.

Shipping yeast strains on filter paper

Yeast cultures can be dried onto filter paper and stored or transported. This method was developed by at the YGSC by John Bassel, and is best used for strains that are frequently sent out, because multiple filters may be prepared once and stored up to several years at 4°C. Sterile filter paper squares (Whatman no. 4, 1 × 1 cm) are prepared by packaging them in a sheet of heavy-duty aluminum foil (7 × 6 cm) and autoclaving the packages. The strain to be shipped is grown exponentially in YEPD and a 5-µl sample of culture is added to 0.2 ml evaporated milk. The milk is handled aseptically but is not sterilized. The filter paper squares are immersed in the milk suspension of cells and returned to the aluminum foil packet. The packets are folded only once and stored in a desiccator at 4°C for 2 to 3 weeks or until the liquid congeals into a hard, dry lump. Following desiccation, the packets are folded tightly and stored at 4°C in plastic boxes. To revive the strain, the filter paper is swiped on a petri plate of YPD or other appropriate medium and returned to its packet. Toothpicks are used to streak for single colonies and the plate is incubated.

Shipping yeast strains by other methods

Other methods include sending agar slants (described above) or petri plates. Care should be taken in packaging petri plates because they are easily broken in the mail.

KEY REFERENCES

Guthrie, C. and Fink, G.R. (eds.) 1991. Guide to yeast genetics and molecular biology. *Methods Enzymol.* vol. 194.

A compendium of technical methods in yeast cell culture, genetics, biochemistry, molecular biology, and cell biology.

Rose, M.D, Winston, F., and Hieter, P. 1990. Methods in Yeast Genetics, A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

The laboratory manual from a Cold Spring Harbor laboratory source on yeast molecular biology.

INTERNET RESOURCES

http://www.atcc.org/

Web site for the ATCC.

http://www.atcc.org/hilights/sc_info.html

Web site for the ATCC Saccharomyces page.

http://dgm2ibm.nihs.go.jp/ygsc.htm

This Web page provides access to YGSC catalogs, though it is not official.

http://genome-www.stanford.edu/Saccharomyces/

This Web site contains an extensive amount of information and links related to Saccharomyces.

http://www.tiac.net/users/cstyles/

This Web site contains specific information about Saccharomyces and is a source for some harder-to-find equipment.

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