Getting Started with Yeast

By Fred Sherman

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Yeast is a Model System

The yeast *Saccharomyces cerevisiae* is now recognized as a model system representing a simple eukaryote whose genome can be easily manipulated. Yeast has only a slightly greater genetic complexity than bacteria, and they share many of the technical advantages that permitted rapid progress in the molecular genetics of prokaryotes and their viruses. Some of the properties that make yeast particularly suitable for biological studies include rapid growth, dispersed cells, the ease of replica plating and mutant isolation, a well-defined genetic system, and most important, a highly versatile DNA transformation system.[1] Being nonpathogenic, yeast can be handled with little precautions. Large quantities of normal bakers' yeast are commercially available and can provide a cheap source for biochemical studies.

Strains of *S. cerevisiae*, unlike most other microorganisms, have both a stable haploid and diploid state, and are viable with a large number of markers. Thus, recessive mutations are conveniently manifested in haploid strains, whereas complementation tests can be carried out with diploid strains. The development of DNA transformation has made yeast particularly accessible to gene cloning and genetic engineering techniques. Structural genes corresponding to virtually any genetic trait can be identified by complementation from plasmid libraries. Plasmids can be introduced into yeast cells either as replicating molecules or by integration into the genome. In contrast to most other organisms, integrative recombination of transforming DNA in yeast proceeds exclusively via homologous recombination. Cloned yeast sequences, accompanied with foreign sequences on plasmids, can therefore be directed at will to specific locations in the genome.

In addition, homologous recombination coupled with high levels of gene conversion has led to the development of techniques for the direct replacement of genetically engineered DNA sequences into their normal chromosome locations. Thus, normal wild-type genes, even those having no previously known mutations, can be conveniently replaced with altered and disrupted alleles. The phenotypes arising after disruption of yeast genes has contributed significantly toward understanding of the function of certain proteins *in vivo*. Many investigators have been shocked to find viable mutants with little or no detrimental phenotypes after distrupting "essential" genes. Genes can be directly replaced at high efficiencies in yeasts and other fungi, but only with difficulty in other eukaryotic organisms. Also unique to yeast, transformation can be carried out directly with short single-stranded synthetic oligonucleotides, permitting the convenient productions of numerious altered forms of proteins. These techniques have been extensively exploited in the analysis of gene regulation, structure–function relationships of proteins, chromosome structure, and other general questions in cell biology.

S. cerevisiae was the first eukaryote whose genome was completely sequenced.[2] Subsequently, yeast became one of the key organisms for genomic research,[3] including extensive use of DNA microarrays for investigating the transcriptome[4-9]as well as genome-wide analysis of gene functions by gene disruption,[10] of serial analysis of gene expression (SAGE),[11] of protein localization,[12] of 2-D protein maps,[13,14] of enzymatic activities,[15,16] of protein-protein interactions by two-hybrid analysis,[17,18] and of functional analysis synthetic lethality.[19] Furthermore, the genomic sequences of related species improved the assignment of genes and regulatory motifs in *S. cerevisiae*.[20,21]

The overriding virtues of yeast are illustrated by the fact that mammalian genes are routinely being introduced into yeast for systematic analyses of the functions of the corresponding gene products. Many human genes related to disease have orthologues in yeast,[22] and the high conservation of metabolic and regulatory mechanisms has contributed to the wide-spread use of yeast to as a model eukaryotic system for diversed biological studies. Furthermore, the ability of yeast to replicate artificial

circular and linear chromosomes has allowed detailed studies of telomeres, centromeres, length dependencies, and origins of replication. Mitochondrial DNA can be altered in defined ways by transformation,[23] adding to the already impressive genetic and biochemical techniques that have allowed detailed analysis of this organelle. The ease with which the genome of yeast can be manipulated is truly unprecedented for any other eukaryote.

Information on Yeast

An elementary introduction to yeast genetics is available in a book chapter by Winston.[24]. A general introduction to a few selected topics on yeast can be found in the book chapters "Yeast as the *E. coli* of Eucaryotic Cells" and "Recombinant DNA at Work".[25] A popular introduction to the genetics and molecular biology of *S. cerevisiae* is presented on the Internet in a review by Sherman.[26] A series of introductory course lectures from Göteborg University is available on the Internet.[27] Comprehensive and excellent reviews of the genetics and molecular biology of *S. cerevisiae* are contained in three volumes entitled "Molecular Biology of the Yeast *Saccharomyces*".[28,29,30] An important source for methods used in genetics and molecular biology of yeast is contained in a two volume "Methods of Enzymology" series,[31,32] as well as in an earlier volume,[33] all edited by Guthrie and Fink. Overviews of numerous subjects are also covered in other sources,[28,29,30,34,35] including protocols applicable to yeasts[36] and introductory material.[37] The "Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual"[38] contains useful elementary material and protocols, and is frequently updated. The use of yeast as an instructional tool at the secondary school level has been provided by Manney and others,[39] including classroom guides and kits.[40] Information on yeast for the general public is available from several souces.[41-45] A more comprehensive listing of earlier reviews can be found in an article by Sherman.[46] Interesting and amusing accounts of developments in the field are covered in "The Early Days of Yeast Genetics".[47] A collection of landmark papers, along with historical commentaries, is scheduled to appear in December 2003.[48] The journal "Yeast" publishes original research articles, reviews, short communications, sequencing reports, and selective lists of current articles on all aspects of *Saccharomyces* and other yeast genera.[49] A journal, "FEMS Yeast Research", began publication in 2001.[50] Current, frequently-updated information and databases on yeast can be conveniently retrieved on the Internet, including the "*Saccharomyces* Genomic Information Resource" [51,52] and linked files containing DNA sequences, lists of genes, home pages of yeast workers, and other useful information concerning yeast. From the MIPS page[53] one can access the annotated sequence information of the genome of *Saccharomyces cerevisiae* and view the chromosomes graphically or as text, and more. The YPD page[54,55,56] contains a protein database with emphasis on the physical and functional properties of the yeast proteins.

Strains of *S. cerevisiae*

Although genetic analyses have been undertaken with a number of taxonomically distinct varieties of yeast, extensive studies have been restricted primarily to the many freely interbreeding species of the budding yeast *Saccharomyces* and to the fission yeast *Schizosaccharomyces pombe*. Although "*Saccharomyces cerevisiae*" is commonly used to designate many of the laboratory stocks of *Saccharomyces* used throughout the world, it should be pointed out that most of these strains originated from the interbred stocks of Winge, Lindegren, and others who employed fermentation markers not only from *S. cerevisiae* but also from *S. bayanus, S. carlsbergensis, S. chevalieri, S. chodati, S. diastaticus,* etc.[57,58] Nevertheless, it is still recommended that the interbreeding laboratory stocks of *Saccharomyces* be denoted as *S. cerevisiae,* in order to conveniently distinguish them from the more

distantly related species of *Saccharomyces*; these can be designated, for example, as *S. cerevisiae* var. *bayanus*, *S. cerevisiae* var. *carlsbergensis*, *S. cerevisiae* var. *chevalieri*, and *S. cerevisiae* var. *chodati.*

Care should be taken in choosing strains for genetic and biochemical studies. Unfortunately there are no truly wild-type *Saccharomyces* strains that are commonly employed in genetic studies. Also, most domesticated strains of brewers' yeast and probably many strains of bakers' yeast and true wild-type strains of *S. cerevisiae* are not genetically compatible with laboratory stocks. It is often not appreciated that many "normal" laboratory strains contain mutant characters. This condition arose because these laboratory strains were derived from pedigrees involving mutagenized strains, or strains that carry genetic markers. Many current genetic studies are carried out with one or another of the following strains or their derivatives, and these strains have different properties that can greatly influence experimental outcomes: S288C; W303; D273–10B; X2180; A364A; Σ1278B; AB972; SK1; and FL100. The haploid strain S288C (*MAT*α *SUC2 mal mel gal2 CUP1 flo1 flo8-1 hap1*) is often used as a normal standard because the sequence of its genome has been determined, because many isogenic mutant derivatives are available[58,59], and because it gives rise to well-dispersed cells. However, S288C contains a defective *HAP1* gene,[60] making it incompatible with studies of mitochondrial and related systems. Also, in contrast to Σ1278B, S288C does not form pseudohyae. While true wild-type and domesticated bakers' yeast give rise to less than 2% ρ^- colonies (see below), many laboratory strains produce high frequencies of ρ^- mutants. Another strain, D273–10B, has been extensively used as a typical normal yeast, especially for mitochondrial studies. One should examine the specific characters of interest before initiating a study with any strain. Also, there can be a high degree of inviability of the meiotic progeny from crosses among these "normal" strains.

Many strains containing characterized auxotrophic, temperature-sensitive, and other markers can be obtained from the Yeast Genetics Stock Culture Center of the American Type Culture Collection,[61] including an almost complete set of deletion strains.[62] Currently this set consists of 20,382 strains representing deletants of nearly all nonessential open-reading-frames (ORFs) in different genetic backgrounds. Deletion strains are also availabe from EUROSCARF[63] and Research Genetics.[64] Other sources of yeast strains include the National Collection of Yeast Cultures,[65] the Centraalbureau voor Schimmelcultures,[66] and the Culture Collection of *Saccharomyces cerevisiae* (DGUB, Bratislava, Slovak Republic). [67] Before using strains obtained from these sources or from any investigator, it is advisable to test the strains and verify their genotypes.

The Genome of *S. cerevisiae*

S. cerevisiae contains a haploid set of 16 well-characterized chromosomes, ranging in size from 200 to 2,200 kb. The total sequence of chromosomal DNA, constituting 12,052 kb, was released in 1996[2], from which a total of 6,183 ORFs of over 100 amino acids long were reported. Subsequently, comparions to sequences of related Saccharomyces species indicated that *S. cerevisiae* contains 5,773[20] or 5,726[21] protein-coding genes. In contrast to the genomes of

Table 1 Inheritable Systems of a Diploid *Saccharomyces cerevisiae* Cell

Inhertiance	Mendelian		Non-Mendelian						
System	Nucleic acid genomes							Prion	
Molecular basis	Double-stranded DNA			Double-stranded RNA				Protein	
Location	Nucleus			Cytoplasm					
Genetic determinant	Chromosomes	2-μm plasmid	Mitochondrial DNA	RNA virus					Prion
				L-A	M	L-BC	T	W	
Relative amount	85%	5%	10%	80%	10%	9%	0.5%	0.5%	ü ý þ Various
Number of copies	2 sets of 16	60 - 100	~50 (8 - 130)	103	170	150	10	10	
Size (kb)	13,500 (200 - 2,200)	6.318	70 - 76	4.576	1.8	4.6	2.7	2.25	
Deficiencies in mutants	All kinds	None	Cyto. $a \cdot a_3$, b	Killer toxin		None			All kinds
Wild-type	*YFG1*$^+$	cir$^+$	ρ^+	KIL-k_1					[*yfg1*$^-$]
Mutant or variant	*yfg1-1*	cir^o	ρ^-	KIL-o					[*YFG1*$^+$]

A wild-type chromosomal gene is designated as *YFG1*$^+$ (Your Favorite Gene) and the mutation as *yfg1-1*. Although cir$^+$ and cir^o strains are phenotypically the same in most strains, the presence and absence of the 2-μm plasmid are inherited in a non-Mendelian manner. T and W RNA viruses, also designated 20S and 23S dsRNAs, encode RNA polymerases.[68]. The Table does not include the Ty1-Ty5 retroviruses, which are generally inherited as integrated Mendelian elements, nor does it include extrachromosomal circular rDNA, which arise from chromosomal rDNA. Adapted from ref. [69-72]

multicellular organsims, the yeast genome is highly compact, with genes representing 72% of the total sequence. The average size of yeast genes is 1.45 kb, or 483 codons, with a range from 40 to 4,910 codons. A total of 3.8% of the ORFs contain introns. Approximately 30% of the genes already have been characterized experimentally. Of the remaining 70% with unknown function, approximately one half either contain a motif of a characterized class of proteins or correspond to genes encoding proteins that are structurally related to functionally characterized gene products from yeast or from other organisms.

Ribosomal RNA is coded by approximately 120 copies of a single tandem array on chromosome XII. The DNA sequence revealed that yeast contains 262 tRNA genes, of which 80 have introns. In addition, chromosomes contain movable DNA elements, retrotransposons, that vary in number and position in different strains of *S. cerevisiae*, with most laboratory strains having approximately 30.

Other nucleic acid entities, presented in Table I, also can be considered part of the yeast genome. Mitochondrial DNA encodes components of the mitochondrial translational machinery and approximately 15% of the mitochondrial proteins. ρ^o mutants completely lack mitochondrial DNA and are deficient in the respiratory polypeptides synthesized on mitochondrial ribosomes, i.e., cytochrome *b* and subunits of cytochrome oxidase and ATPase complexes. Even though ρ^o mutants are respiratory deficient, they are viable and still retain mitochondria, although the mitochondria are morphologically abnormal.

The 2-μm circle plasmids, present in most strains of *S. cerevisiae*, apparently function solely for their own replication. Generally *cir*^{o} strains, which lack 2-μm DNA, have no observable phenotype. However, a certain chromosomal mutation, *nib1*, causes a reduction in growth of *cir*$^+$ strains, due to an abnormally high copy number 2-μm DNA.[73,74]

Similarly, almost all *S. cerevisiae* strains contain intracellular dsRNA viruses that constitutes approximately 0.1% of total nucleic acid. RNA viruses include three families with dsRNA genomes, L-A, L-BC, and M. Two other families of dsRNA, T and W, replicate in yeast but so far have not been shown to be viral. M dsRNA encodes a toxin, and L-A encodes the major coat protein and components required for the viral replication and maintenance of M. The two dsRNA, M and L-A, are packaged separately with the common capsid protein encoded by L-A, resulting in virus-like particles that are transmitted cytoplasmically during vegetative growth and conjugation. L-B and L-C (collectively denoted L-BC), similar to L-A, have a RNA-dependent RNA polymerase and are present in intracellular particles. *KIL*-o mutants, lacking M dsRNA and consequently the killer toxin, are readily induced by growth at elevated temperatures, and chemical and physical agents.

Yeast also contains a 20S circular single-stranded RNA (not shown in Table I) that appears to encode an RNA-dependent RNA polymerase, that acts as an independent replicon, and that is inherited as a non-Mendelian genetic element.

Only mutations of chromosomal genes exhibit Mendelian 2:2 segregation in tetrads after sporulation of heterozygous diploids; this property is dependent on the disjunction of chromosomal centromeres. In contrast, non-Mendelian inheritance is observed for the phenotypes associated with the absence or alteration of other nucleic acids or prions described in Table I.

Genetic Nomenclature

Chromosomal Genes

The accepted genetic nomenclature for chromosomal genes of the yeast *S. cerevisiae* is illustrated in Table II, using *ARG2* as an example. Whenever possible, each gene, allele, or locus is designated by three italicized letters, e.g., *ARG*, which is usually a describer, followed by a number, e.g., *ARG2*.

Unlike most other systems of genetic nomenclature, dominant alleles are denoted by using uppercase italics for all letters of the gene symbol, e.g., *ARG2*, whereas lowercase letters denote the recessive allele, e.g., the auxotrophic marker *arg2*. Wild-type genes are designated with a superscript "plus" (*sup6*$^+$ or *ARG2*$^+$). Alleles are designated by a number separated from the locus number by a hyphen, e.g., *arg2-9*. The symbol Δ can denote complete or partial deletions, e.g., *arg2*-Δ*1*. (Do not use the symbols Δ*arg2* or *arg2*Δ for deletions.) Insertion of genes follow the bacterial nomenclature by using the symbol :: . For example, *arg2*::*LEU2* denotes the insertion of the *LEU2* gene at the *ARG2* locus, in which *LEU2* is dominant (and functional), and *arg2* is recessive (and defective).

Phenotypes are denoted by cognate symbols in Roman type and by the superscripts + and –. For example, the independence and requirement for arginine can be denoted by Arg$^+$ and Arg$^-$, respectively. Proteins encoded by *ARG2*, for example, can be denoted Arg2p, or simply Arg2 protein. However, gene symbols are generally used as adjectives for other nouns, for example, *ARG2* mRNA, *ARG2* strains, etc. Resistance and sensitivity phenotypes are designated by superscript R and S, respectively. For example, resistance and sensitivity to canavanine sulphate are designated Can^R and Can^S, respectively.

Although most alleles can be unambiguously assigned as dominant or recessive by examining the phenotype of the heterozygous diploid crosses, dominant and recessive traits are defined only with pairs, and a single allele can be both dominant and recessive. For example, because the alleles *CYC1*$^+$, *cyc1-717* and *cyc1*-Δ*1* produce, respectively, 100%, 5% and 0% of the gene product, the *cyc1-717* allele can be considered recessive in the *cyc1-717*/*CYC1*$^+$ cross and dominant in the *CYC1-717*/*cyc1*-Δ*1* cross. Thus, it is less confusing to denote all mutant alleles in lower case letters, especially when considering a series of mutations having a range of activities.

Wild-type and mutant alleles of the mating-type locus and related loci do not follow the standard rules. The two wild-type alleles of the mating-type locus are designated *MAT***a** and *MAT*α. The wild-type homothallic alleles at the *HMR* and *HML* loci are denoted, *HMR***a**, *HMR*α, *HML***a** and *HML*α. The mating phenotypes of *MAT***a** and *MAT*α cells are denoted simply **a** and α, respectively. The two letters *HO* denote the gene encoding the endonuclease required for homothallic switching.

Auxiliary gene symbols can be used to further describe the corresponding phenotypes, including the use of superscript R and S to distinguish genes conferring resistance and sensitivity, respectively. For example, the genes controlling resistance to canavanine sulphate (*can1*), copper sulphate (*CUP1*) and their sensitive alleles could be denoted, respectively, as *can*^{R}*1*, *CUP*^{R}*1*, *CAN*^{S}*1*, and *cup*^{S}*1*.

Dominant and recessive suppressors are designated, respectively, by three uppercase or three lowercase letters, followed by a locus designation, e.g., *SUP4, SUF1, sup35, suf11,* etc. In some instances UAA ochre suppressors and UAG amber suppressors are further designated, respectively, **o** and **a** following the locus. For example, *SUP4*-**o** refers to suppressors of the *SUP4* locus that insert tyrosine residues at UAA sites; *SUP4*-**a** refers to suppressors of the same *SUP4* locus that insert tyrosine residues at UAG sites. The corresponding wild-type locus that encodes the normal tyrosine tRNA and that lacks suppressor activity can be referred to as *sup4*$^+$. Intragenic mutations that inactivate suppressors can be denoted, for example, *sup4*$^-$ or *sup4-**o**-1*. Frameshift suppressors are denoted as *suf* (or *SUF*), whereas metabolic suppressors are denoted with a variety of specialized symbols, such as *ssn* (suppressor of *snf1*), *srn* (suppressor of *rna1-1*), and *suh* (suppressor of *his2-1*).Capital letters are also used to designate certain DNA segments whose locations have been determined by a combination of recombinant DNA techniques and classical mapping procedures, e.g., *RDN1*, the segment encoding ribosomal RNA.

The general form YCRXXw is used to designate genes deduced from the sequence of the yeast genome, where Y designates yeast; C (or A, B, *etc*.) designates the chromosome III (or I, II, *etc*.); R (or L) designates the right (or left) arm of the chromosome; XX designates the relative position of the start of the open-reading frame from the centromere; and w (or c) designates the Watson (or Crick) strand. For example, YCR5c denotes *CIT2*, a previously known but unmapped gene situated on the right arm of chromosome III, fifth open reading-frame from the centromere on the Crick strand.

E. coli genes inserted into yeast are usually denoted by the prokaryotic nomenclature, e. g., *lacZ*.

A current list of gene symbols can be found on the Internet.[75]

TABLE II

GENETIC NOMENCLATURE, USING *ARG2* AS AN EXAMPLE

Gene symbol	Definition
ARG$^+$	All wild-type alleles controlling arginine requirement
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
arg2$^-$	Any *arg2* allele confering an arginine requirement
ARG2$^+$	The wild-type allele
arg2-9	A specific allele or mutation
Arg$^+$	A strain not requiring arginine
Arg$^-$	A strain requiring arginine
Arg2p	The protein encoded by *ARG2*
Arg2 protein	The protein encoded by *ARG2*
ARG2 mRNA	The mRNA transcribed from *ARG2*
arg2-Δ1	A specific complete or partial deletion of *ARG2*
ARG2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *ARG2* remains functional and dominant
arg2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *arg2* is or became nonfunctional
arg2-10::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and the specified *arg2-10* allele which is nonfunctional
cyc1-arg2	A fusion between the *CYC1* and *ARG2* genes, where both are nonfunctional
P_{CYC1}*-ARG2*	A fusion between the *CYC1* promoter and *ARG2*, where the *ARG2* gene is functional

TABLE III

MITOCHONDRIAL GENES AND MUTATIONS WITH EXAMPLES

Wild-type	Mutation (with examples)	Mutant phenotype or gene product
Nuclear genes		
PET^{+}	*pet* $^{-}$	Nfs^{-}
	pet1	Unknown function
	cox4	Cytochrome *c* oxidase subunit IV
	hem1	δ-Aminolevulinate synthase
	cyc3	Cytochrome *c* heme lyase
Mitochondrial DNA		
	Gross aberrations	
ρ^{+}	ρ^{-}	Nfs^{-}
	ρ^{o}	ρ^{-} mutants lacking mitochondrial DNA
	Single-site mutations	
ρ^{+}	*mit* $^{-}$	Nfs^{-}, but capable of mitochondrial translation
[*COX1*]	[*cox1*]	Cytochrome *c* oxidase subunit I
[*COX2*]	[*cox2*]	Cytochrome *c* oxidase subunit II
[*COX3*]	[*cox3*]	Cytochrome *c* oxidase subunit III
[*COB1*]	[*cob1*] or [*box*]	Cytochrome *b*
[*ATP6*]	[*atp6*]	ATPase subunit 6
[*ATP8*]	[*atp8*]	ATPase subunit 8
[*ATP9*]	[*atp9*] or [*pho2*]	ATPase subunit 9
[*VAR1*]		Mitochondrial ribosomal subunit
ρ^{+}	*syn*$^{-}$	Nfs^{-}, deficient in mitochondrial translation
	tRNA^{Asp} or M7-37	Mitochondrial tRNA^{Asp} (CUG)
	ant^{R}	Resistant to inhibitors
[*ery*^{S}]	*ery*^{R} or [*rib1*]	Resistant to erythromycin, 21S rRNA
[*cap*^{S}]	*cap*^{R} or [*rib3*]	Resistant to chloramphenical, 21S rRNA
[*par*^{S}]	*par*^{R} or [*par1*]	Resistant to paromomycin, 16S rRNA
[*oli*^{S}]	*oli*^{R} or [*oli1*]	Resistant to oligomycin, ATPase subunit 9

Nfs^{-} denotes lack of growth on nonfermentable substrates.

Mitochondrial Genes

Special consideration should be made of the nomenclature describing mutations of mitochondrial components and function that are determined by both nuclear and mitochondrial DNA genes. The growth on media containing nonfermentable substrates (Nfs) as the sole energy and carbon source (such as glycerol or ethanol) is the most convenient operational procedure for testing mitochondrial function. Lack of growth on nonfermentable media (Nfs^{-} mutants), as well as other mitochondrial alterations, can be due to either nuclear or mitochondrial mutations as outlined in Table III. Nfs^{-} nuclear mutations are

generally denoted by the symbol *pet*; however, more specific designations have been used instead of *pet* when the gene products were known, such as *cox4*, *hem1*, etc.

The complexity of nomenclatures for mitochondrial DNA genes, outlined in Table III, is due in part to complexity of the system, polymorphic differences of mitochondrial DNA, complementation between exon and intron mutations, the presence of intron-encoded maturases, diversed phenotypes of mutations within the same gene, and the lack of agreement between various workers. Unfortunately, the nomenclature for most mitochondrial mutations do not follow the rules outlined for nuclear mutations. Furthermore, confusion can occur between phenotypic designations, mutant isolation number, allelic designations, loci, and cistrons (complementation groups).

Non-Mendelian Determinants

Where necessary, non-Mendelian genotypes can be distinguished from chromosomal genotypes by enclosure in brackets, e.g., [*KIL*–o] *MAT***a** *trp1–1*. Although it is advisable to employ the above rules for designating non-Mendelian genes and to avoid using Greek letters, the use of well-known and generally accepted Greek symbols should be continued; thus, the original symbols ρ^+, ρ^-, ψ^+, and ψ^- or their transliterations, rho^+, rho^-, [*PSI*$^+$] and [*psi*$^-$], respectively, have been retained.

TABLE IV

SOME NON-MENDELIAN DETERMINANTS OF YEAST

Wild-type	Mutant or polymorphic variant	Genetic element	Mutant phenotype
ρ^+	ρ^-	Mitochondrial DNA	Deficiency of cytochromes $a{\cdot}a_3$, *b*, and respiration
KIL-k_1	*KIL*-o	RNA plasmid	Sensitive to killer toxin
cir^+	cir^o	2-μm circle plasmid	None
[*psi*$^-$]	[*PSI*$^+$]	Sup35p prion	Decreased efficiency of certain suppression
[*ure3*$^-$]	[*URE3*]	Ure2p prion	Ureidosuccinate uptake not repressible
[*PIN*$^-$]	[*PIN*$^+$]	Rnq1p prion	Required for [*PSI*$^+$] induction

Adapted from ref. [70,71,72,76,77,78,79] Other non-Mendelian determinants have been reported.[74]

In addition to the non-Mendelian determinants described in Table I (2 μm plasmid, mitochondrial genes, and RNA viruses), yeast contains prions, i.e., infectious proteins. The nomenclature of these prions, representing alternative protein states, are presented in Table IV, along with other non-Mendelian determinants.

Growth and Size

"Normal" laboratory haploid strains have a doubling time of approximately 90 min in YPD medium (see below) and approximately 140 min in synthetic media during the exponential phase of growth. However, strains with greatly reduced growth rates in synthetic media are often encountered. Usually strains reach a maximum density of 2×10^8 cells/ml in YPD medium. Titers 10 times this value can be

achieved with special conditions, such as pH control, continous additions of balanced nutrients, filtered-sterilized media and extreme aeration that can be delivered in fermentors.

The sizes of haploid and diploid cells vary with the phase of growth[80] and from strain to strain. Typically, diploid cells are 5 x 6 µm ellipsoids and haploid cells are 4 µm diameter spheroids.[81] The volumes and gross composition of yeast cells are listed in Table V. During exponential growth, haploid cultures tend to have higher numbers of cells per cluster compared to diploid cultures. Also haploid cells have buds that appear adjacent to the previous one; whereas diploid cells have buds that appear at the opposite pole.[82]

TABLE V
SIZE AND COMPOSITION OF YEAST CELLS

Characteristic	Haploid cell	Diploid cell
Volume (μm^3)	70	120
Composition (10^{-12} g)		
Wet weight	60	80
Dry weight	15	20
DNA	0.017	0.034
RNA	1.2	1.9
Protein	6	8

Growth and Testing Media

For experimental purposes, yeast are usually grown at 30°C on the complete medium, YPD (Table VI), or on synthetic media, SD and SC (Tables VII[83]and VIII). For industrial or certain special purposes when large amounts of high titers are desirable, yeast can be grown in cheaper media with high aeration and pH control.[84] The ingredients of standard laboratory media are presented in Tables VII-VIII. Synthetic media[83] are conveniently prepared with Bacto-yeast nitrogen base without amino acids (Difco Laboratories, Detroit, MI), containing the constituents presented in Table VIII. Nutritional requirements of mutants are supplied with the nutrients listed in Table VI. Growth on nonfermentable carbon sources can be tested on YPG medium (Table VI), and fermentation markers can be determined with indicator media (Table IX), on which acid production induces color changes.

S. cerevisiae strains can be sporulated at 30°C on the media listed in Table X ; most strains will readily sporulate on the surface of sporulation medium after replica plating fresh cultures from a YPD plate.[85]

Media for petri plates are prepared in 2-liter flasks, with each flask containing no more than 1 liter of medium, which is sufficient for approximately 40 standard plates. Unless stated otherwise, all components are autoclaved together for 15 minutes at 250°F (120°C) and 15 pounds pressure. The plates should be allowed to dry at room temperature for 2-3 days after pouring. The plates can be stored in sealed plastic bags for over three months at room temperature. The agar is omitted for liquid media.

Different types of synthetic media, especially omission media, can be prepared by mixing and grinding dry components in a ball-mill.

TABLE VI
COMPLEX MEDIA

Medium	Components	Composition
YPD (for routine growth)	1% Bacto-yeast extract	10 g
	2% Bacto-peptone	20 g
	2% Dextrose	20 g
	2% Bacto-agar	20 g
	Distilled water	1000 ml
YPG [containing a nonfermentable carbon source (glycerol) that does not support the growth of ρ^- or *pet* mutants]	1% Bacto-yeast extract	10 g
	2% Bacto-peptone	20 g
	3% (v/v) Glycerol	30 ml
	2% Bacto-agar	20 g
	Distilled water	970 ml
YPDG (used to determine the proportion of ρ^- cells; ρ^+ and ρ^- colonies, appear, respectively, large and small on this medium)	1% Bacto-yeast extract	10 g
	2% Bacto-peptone	20 g
	3% (v/v) Glycerol	30 ml
	0.1% Dextrose	1 g
	2% Bacto-agar	20 g
	Distilled water	970 ml
YPAD (used for the preparation of slants; adenine is added to inhibit the reversion of *ade1* and *ade2* mutations)[a]	1% Bacto-yeast extract	10 g
	2% Bacto-peptone	20 g
	2% Dextrose	20 g
	0.003% Adenine sulfate	40 mg
	Distilled water	1000 ml
	2% Bacto-agar	20 g

[a] The medium is dissolved in a boiling water bath and 1.5 ml portions are dispensed with an automatic pipetter into 1-dram (3-ml) vials. The caps are screwed on loosely, and the vials are autoclaved. After autoclaving, the rack is inclined so that the agar is just below the neck of the vial. The caps are tightened after 1 to 2 days.

TABLE VII
SYNTHETIC MINIMAL GLUCOSE MEDIUM (SD) [a]

Component	Composition
0.67% Bacto-yeast nitrogen base (without amino acids)	6.7 g
2% Dextrose	20 g
2% Bacto-agar	20 g
Distilled water	1000 ml

	Amount per liter
Carbon source:	
Dextrose	20 g
Nitrogen source:	
Ammonium sulfate	5 g
Vitamins:	
Biotin	20 µg
Calcium pantothenate	2 mg
Folic acid	2 µg
Inositol	10 mg
Niacin	400 µg
p-Aminobenzoic acid	200 µg
Pyridoxine hydrochloride	400 µg
Riboflavin	200 µg
Thiamine hydrochloride	400 µg
Compounds supplying trace elements:	
Boric acid	500 µg
Copper sulphate	40 µg
Potassium iodide	100 µg
Ferric chloride	200 µg
Manganese sulphate	400 µg
Sodium molybdate	200 µg
Zinc sulphate	400 µg
Salts:	
Potassium phosphate monobasic	850 mg
Potassium phosphate dibasic	150 mg
Magnesium sulphate	500 mg
Sodium chloride	100 mg
Calcium chloride	100 mg

[a] This synthetic medium is based on media described by Wickerham[83] and is marketed, without dextrose, by Difco Laboratories (Detroit, MI) as "Yeast nitrogen base without

TABLE VIII

SYNTHETIC COMPLETE MEDIA (SC)[a]

Constituent	Final concentration (mg/L)	Stock per 100 ml	Amount of stock (ml) for 1 liter
Adenine sulfate	20	200 mg[b]	10
Uracil	20	200 mg[b]	10
L-Tryptophan	20	1 g	2
L-Histidine-HC1	20	1 g	2
L-Arginine-HC1	20	1 g	2
L-Methionine	20	1 g	2
L-Tyrosine	30	200 mg	15
L-Leucine	60	1 g	6
L-Isoleucine	30	1 g	3
L-Lysine-HC1	30	1 g	3
L-Phenylalanine	50	1 g[b]	5
L-Glutamic acid	100	1 g[b]	10
L-Aspartic acid	100	1 g[b,c]	10
L-Valine	150	3 g	5
L-Threonine	200	4 g[b,c]	5
L-Serine	400	8 g	5

[a]SC contains synthetic minimal medium (SD) with various additions. It is convenient to prepare sterile stock solutions which can be stored for extensive periods. All stock solutions can be autoclaved for 15 min at 250°F. The appropriate volume of the stock solutions (see below) is added to the ingredients of SD medium and sufficient distilled water is added so that the total volume is one liter. The threonine and aspartic acid solutions should be added separately after autoclaving. Given above are the concentrations of the stock solutions (amount per 100 ml). Some stock solutions should be stored at room temperature in order to prevent precipitation, whereas the other solutions may be refrigerated. It is best to use HCl salts of amino acids wherever applicable.

[b]Store at room temperature.

[c]Add after autoclaving the media.

TABLE IX
INDICATOR MEDIA

Indicator medium	Components	Composition
MAL[a]	1% Bacto-yeast extract	10 g
	2% Bacto-peptone	20 g
	2% Maltose	20 g
	Brom-cresol purple solution (0.4% stock)	9 ml
	2% Agar	20 g
	Distilled water	1000 ml
GAL[b]	1% Yeast extract	10 g
	2% Peptone	20 g
	2% Galactose	20 g
	2% Agar	20 g
	Brom-thymol blue (4 mg/ml stock)	20 ml
	Distilled water	880 ml

[a]Maltose Indicator Medium (MAL) is a fermentation indicator medium used to distinguish strains which ferment or do not ferment maltose. Owing to the pH change, the maltose-fermenting strains will produce a yellow halo on a purple background. A 0.4% brom-cresol purple solution is prepared by dissolving 20 mg of the indicator in 50 ml of ethanol.

[b]Galactose Indicator Medium (GAL) is a fermentation indicator medium used to distinguish strains which ferment or do not ferment. The galactose-fermenting strains will produce a yellow halo on a blue background. After autoclaving, add 100 ml of a filter-sterilized 20% galactose solution.

TABLE X
SPORULATION MEDIA

Sporulation medium	Components	Composition
Presporulation[a]	0.8% Bacto-yeast extract	0.8 g
	0.3% Bacto-peptone	0.3 g
	10% Dextrose	10 g
	2% Bacto-agar	2 g
	Distilled water	100 ml
Sporulation[b]	1% Potassium acetate	10 g
	0.1% Bacto-yeast extract	1 g
	0.05% Dextrose	0.5 g
	2% Bacto-agar	20 g
	Distilled water	1000 ml
Minimal sporulation[c]	1% Potassium acetate	10 g
	2% Bacto-agar	20 g
	Distilled water	1000 ml

[a] Strains are grown one or two days on Presporulation Medium before transferring to sporulation medium. This is only necessary for strains that do not sporulate well when incubated on sporulation medium directly.

[b] Strains will undergo several divisions on Sporulation Medium and then sporulate after 3 to 5 days incubation. Sporulation of auxotrophic diploids is usually increased by adding the nutritional requirements to the sporulation medium at 25% of the levels given above for SD complete meduim.

[c] Diploid cells will sporulate on Minimal Sporulation Medium after 18-24 hrs without vegetative growth. Nutritional requirements are added as needed for auxotrophic diploids as for sporulation medium described above (25% of level for SD complete meduim).

Practical information on the preparation of media has been presented by Styles.[86]

Small batches of liquid cultures can be grown in shake flasks using standard bacteriological techniques with high aeration. High aeration can be achieved by vigorously shaking cultures having liquid volumes less than 20% of the flask volume.

Testing of Phenotypes and Gene Functions

Hampsey[87] has compiled a useful list of phenotypes that can be conveniently scored or selected, including the use media for testing a sensitivity and resistance to a large number of different chemical and physical agents. Furthermore, known mutant genes corresponding to each of the phenotypes have been tabulated.[88] An international project, designated EUROFAN 2 (European Network for the Functional Analysis of Yeast Genes Discovered by Systematic DNA Sequencing), is dedicated to the the functional analysis of all 6000 yeast ORFs by using gene disruptants.[89] The phenotypic analysis includes the testing for the sensitivity and resistance to toxic compounds on 300 different types of growth conditions.[90]

Strain Preservation

Yeast strains can be stored for short periods of time at 4°C on YPD medium in petri dishes or in closed vials (slants). Although most strains remain viable at 4°C for at least one year, many strains fail to survive even for a few months.

Yeast strains can be stored indefinitely in 15% (v/v) glycerol at -60°C or lower temperature. (Yeast tend to die after several years if stored at temperatures above –55°C.[91]

Many workers use 2-ml vials (35 x 12 mm) containing 1 ml of sterile 15% (v/v) glycerol. The strains are first grown on the surfaces of YPD plates; the yeast is then scraped-up with sterile applicator sticks and suspended in the glycerol solution. The caps are tightened and the vials shaken before freezing. The yeast can be revived by transferring a small portion of the frozen sample to a YPD plate.

Micromanipulation and Micromanipulators

The separation of the four ascospores from individual asci by micromanipulation is required for meiotic genetic analyses and for the construction of strains with specific markers. In addition, micromanipulation is used to separate zygotes from mass-mating mixtures and, less routinely, for positioning of vegetative cells and spores for mating purposes and for single-cell analyses, such as used in aging studies. The relocation and transfer of ascospores, zygotes and vegetative cells are almost exclusively carried out on agar surfaces with a fine glass microneedle mounted in the path of a microscope objective and controlled by a micromanipulator. Although specialized equipment and some experience is required to carry out these procedures, most workers can acquire proficiency within a few days of practice.

Micromanipulators used for yeast studies operate with control levers or joysticks that can translate hand movements into synchronously reduced movements of microtools.[92,93,94] Most of the instruments were designed so that movement of the tool in the horizontal (x and y) plane is directly related to the movement of the control handle, whereas the vertical (z plane) tool movement is controlled by rotating a knob, located either on or near the horizontal control handle. Other designs have other combinations in which the joystick controls the x and z planes, a screw controls the z plane. The main commercially available micromanipulators that have single control levers and that are commonly used for yeast studies are listed in Table XI, along with the distributors.

Transmission of hand motions to the tool with the de Fonbrune micromanipulator is based on pneumatic principles, whereas the other units rely on several ingenious mechanical principles involving direct coupling to sliding components.

The Zeiss Tetrad "Advanced Yeast Dissection Microscope" (Table XI, Fig. 1),[95] and the TDM 400 E "Tetrad Dissection System" (Table XI),[96] are based on the design described by Sherman[98], and are primarily intended for dissection of asci.

The Zeiss Tetrad Microscope incorporates a modified Zeiss Axioskop fixed-stage microscope and a stable stage-mounted micromanipulator, with joystick control and adjustable y-z movement ranging from 0.1 to 5 mm. Stage movement incorporates clickstops at 5 mm intervals in both x and y directions, and an engraved x-scale on the

TABLE XI

COMMERCIALLY AVAILABLE MICROMANIPULATORS WITH SINGLE-LEVER CONTROLS

Distributor and Micromanipulator	Ref.
Carl Zeiss, Inc. (Thornwood, NY)	
Tetrad™ Microscope (Cat. No. 4509079902K)	95
Schütt Labortechnik GmbH (Göttingen, Germany)	
Tetrad Dissection Microscope, TDM 400 E, Type I	96, 97
Tetrad Dissection Microscope, TDM 400 E, Type II	
Singer Instrument Co. Ltd. (Watchet, Somerset, U. K.)	
Singer MSM System Series 200	99
Singer MSM Manual	
Technical Products International (St. Louis, MO)	
TPI de Fonbrune-type micromanipulator (with or without Olympus microscope)	101

holder for an inverted petri dish facilitates the systematic relocation of spores. The stage assembly for tetrad dissection is easily removed, allowing the microscope to be used for general purposes. Also, the manipulator can mounted on either the left or right hand side of the stage.

Similarly, the TDM 400 E system incorporates the Nikon Eclipse E 400 microscope and includes a joystick micromanipulator, a holder for 100 mm petri dishes, and a calibrated stage. The microscope is complete with long working distance optics for viewing through the inverted dish containing spores or vegetative cells. The micromanipulator is normally mounted on the left side of the stage and both move in concert when the microscope is focussed, thus eliminating the need for a fixed stage. A joystick controls the y and z motion, whereas a knurled knob is rotated for movement along the x-axis. The mechanical stage has coaxial control knobs, which are tension adjustable, and the stage is indexed with click-stops every 5 mm on the x and y-axes. Ten cm petri dishes can be accommodated on the stage. The Nikon Eclipse E 400 Microscope is designed with a 25 degree binocular body, and options are available to adjust the height of the eyepieces for abnormally tall operators. Focussing and micromanipulation can be done with both arms resting on the benchtop. The one-side fine focus allows for the operation of the stage and the fine focus with one hand. The TDM 400 E is offered in two versions, type I and type II, having, respectively, 150x and 200x maximum magnification. Both types come with an Abbe condenser which has been modified so that it will provide proper Köhler illumination over the extended distance to the specimen. Stand and stage are built for right-handed operation but left-handed models are optional.

The Singer MSM System series 200 (Table XI, Fig. 2)[99] is a complete, computer-controlled workstation for micromanipulation in yeast genetics, including tetrad dissection, pedigree analysis, cell and zygote isolation. Repetitive movements can be automated with a resolution of 4 µm, a repeatability of 2 µm, and an overall movement of 15 cm x 10 cm, using a computer-controlled motorized stage that accepts standard Petri dishes. The workstation includes a integral trinocular microscope having 15x widefield eyepieces, 4x and 20x XLWD objectives, electronically controlled fine focusing from the joystick, and a

FIG. 1. The Zeiss Tetrad "Advanced Yeast Dissection Microscope", showing the micromanipulator mounted on a Zeiss Axioskop fixed-stage microscope modified for tetrad dissection

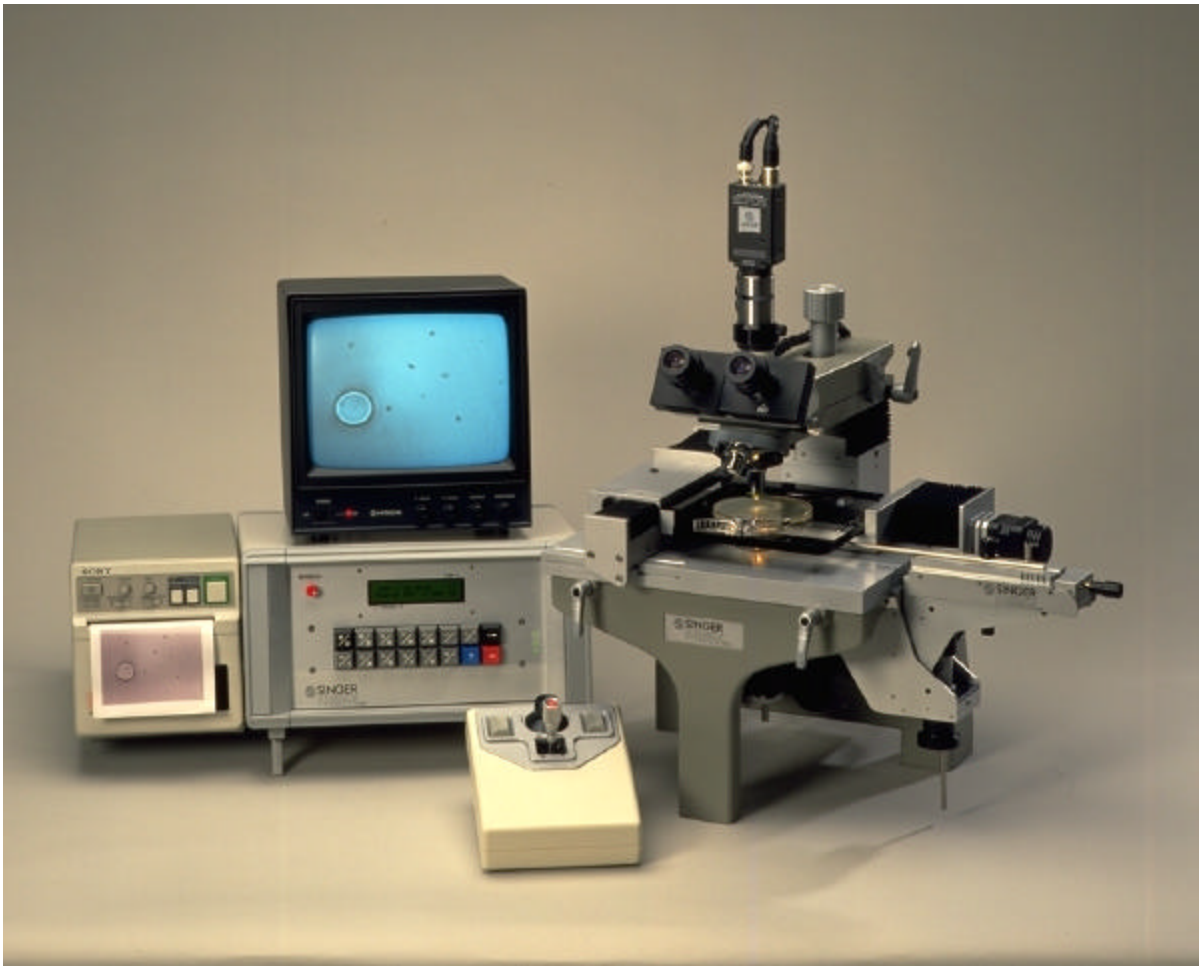

FIG. 2. The Singer MSM series 200 System, showing the microscope with a motor-driven stage, the micromanipulator, the control console, the joystickand, and the optional accessories, a CCD CCTV camera and monitor, and video printer.

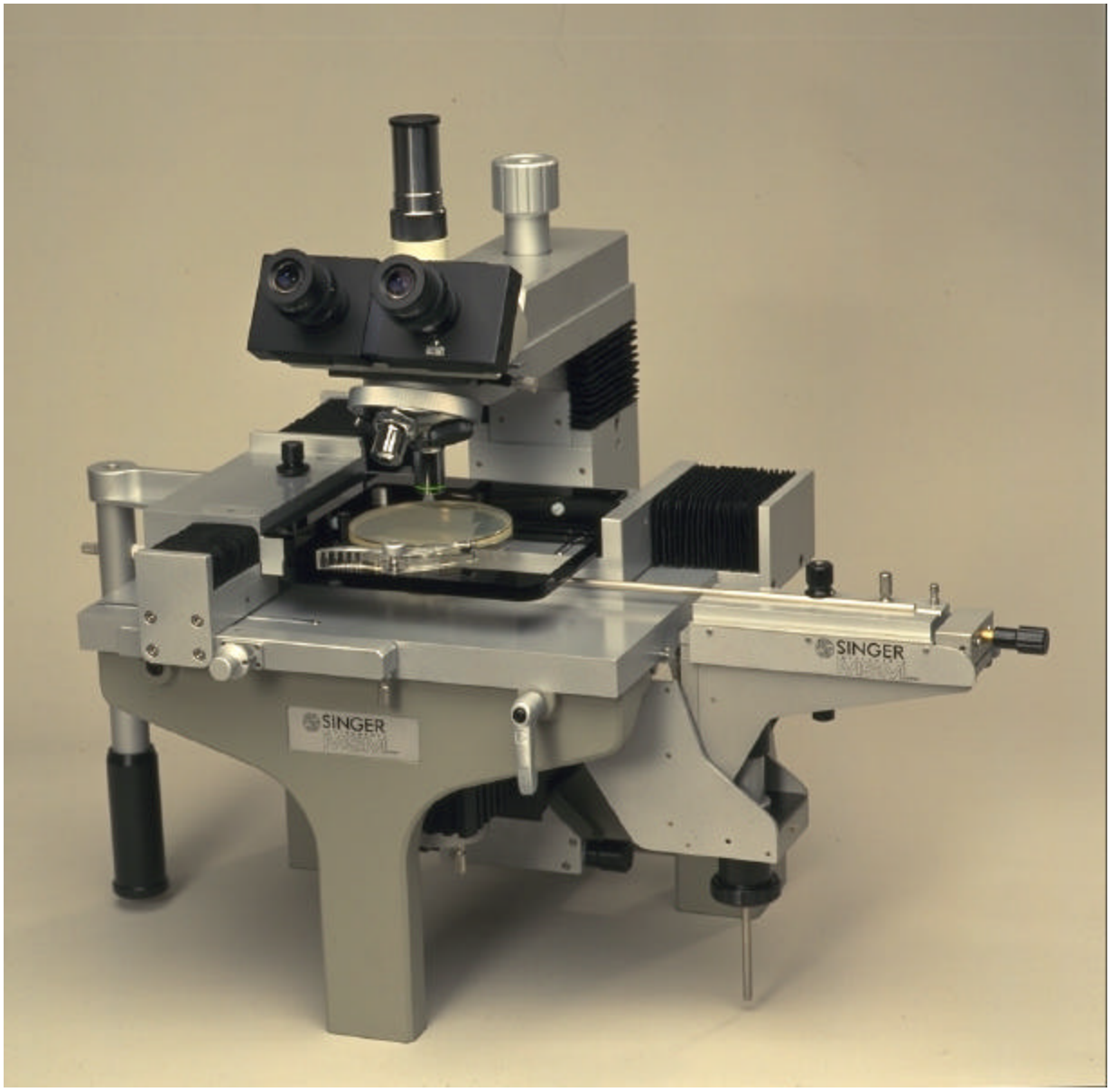

FIG. 3. Singer MSM Manual, which incorporates a stage-mounted micromanipulator and a manually operated stage and microscope. The fine focus is operated by knobs positioned on each side, underneath the stage at the bench height, whereas a pendant stage handle controls the x-y movement of the petri dish.

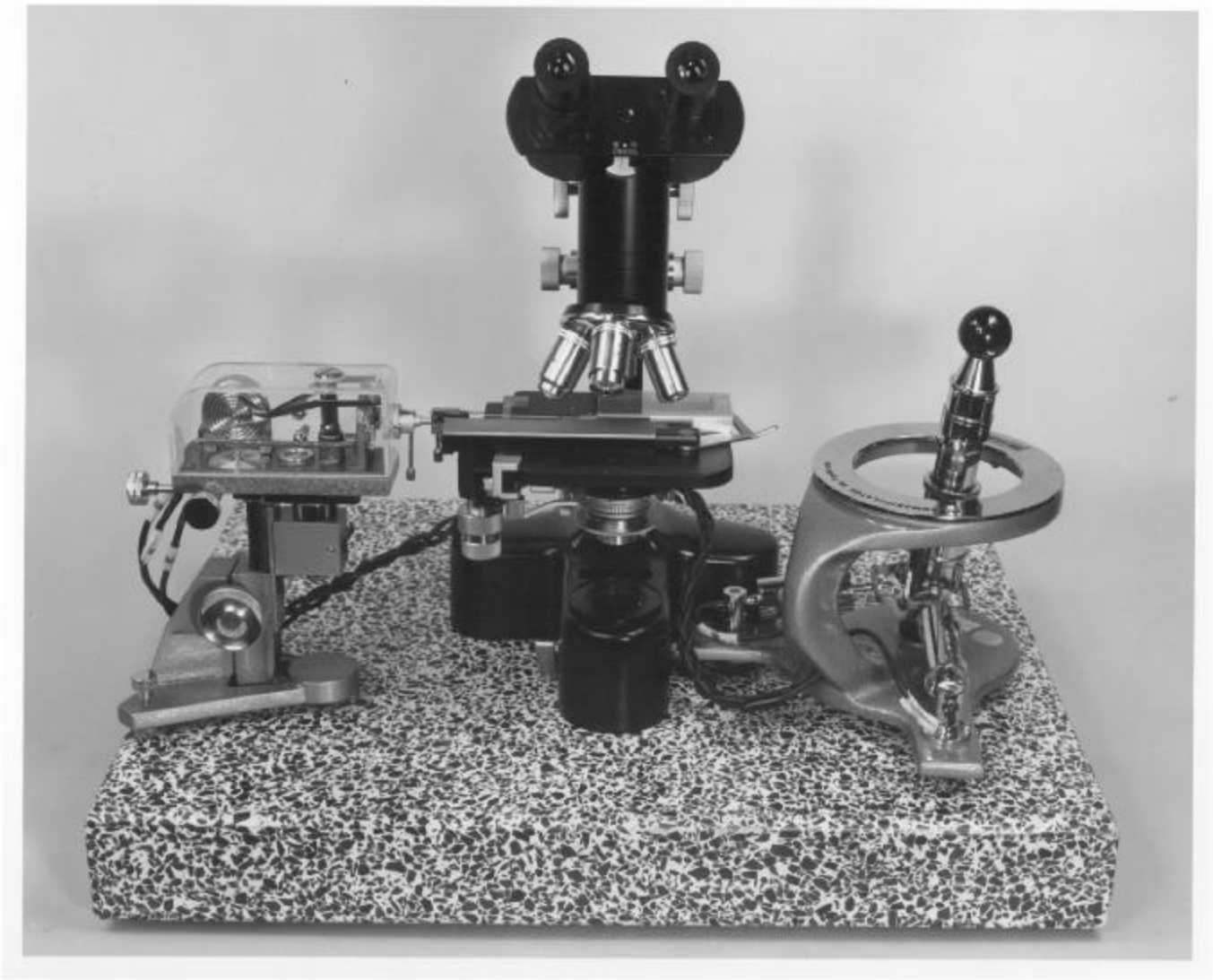

FIG. 4. The de Fonbrune-type micromanipulator. The control unit (right) and the receiver (left) are interconnected by flexible tubing on opposite sides of a Leitz Laborlux II microscope. The complete assembly is on a vibration eliminator (Vibration Damping Mount, Vibrasorb® , Cat. No. 67120-01, Electron Microscopy Sciences, Fort Washington, PA).

hinged overarm to conveniently clear petri dishes. A television camera can be conveniently attached to the unit. The MSM Micromanipulator can be locked by a single handle to either side of the sub-stage for right or left hand operation. Horizontal movements (x and y) of the needle are controlled by a pendant joystick, whereas the the vertical (z) drive is controlled by a coaxial ring. Coarse adjustments of the needle are also convenient. The Singer MSM System series 200 is supplied with a needle holder and needles.

The manually operated Singer MSM Manual (Table XI, Fig. 3)[99] includes the same microscope and stage-mounted manipulator as the Singer MSM System series 200 unit. Spring loaded stops allow the detection of matrix grid points along the y axis, whereas a incrementing stop contols stage movement along the y axis, thus allowing the rapid positioning of the petri dish in a 6 mm grid, which is ideally suited for asci dissection. In addition, the y axis in the area of the inoculum streak is resticted, and a trim screw enables the operator to return to certain positions in the x axis, making the search for asci convenient. 4

The de Fonbrune micromanipulator, shown in Fig. 4, pneumatically transmits a fine degree of motion from a single joystick.[100,101] The micromanipulator consists of two free-standing units: (1) a joystick controlling three piston pumps that is connected by tubing to (2) three diaphragms or aneroids that actuates a lever holding the micro-tool. Lateral movement of the joystick controls the x and y horizontal movements, whereas rotation of the joystick controls vertical z movement. A moveable collar on the joystick provides simple ratio adjustment control that can be varied from 1:50 to 1:2500. Thus, the movement of the microtool can be adjusted to correspond to the magnification of the optical system or to increase or decrease the control sensitivity. In addition, mechanical controls on the receiver unit provide fast and coarse adjustments. The de Fonbrune micromanipulator, which is not directly attached to the stage, should be used in conjunction with various microscopes having fixed stages and tube focusing, Fine mechanical stages with graduations are essential with all micromanipulators. It is convenient to have long working distance objectives for magnifications in the range of 150-300x. Long working distances can be achieved with 10x and 15x objectives, and the appropriate magnifications with 20x or 25x eye pieces.

Because of the low cost and compactness, the Tetrad Microscopes, or Tetrad Dissection Microscopes, are the most commonly used models and are highly recommended. Although it has been our experience that the skill of asci dissection can be taught more quickly with the de Fonbrune-type micromanipulator, this and other micromanipulators not attached directly to the microscope stage require more space, and in some instances heavy base plates or vibration eliminators (see Fig. 4). The Singer MSM System series 2000, although expensive, is the ultimate apparatus for dissection of asci.

Microneedles

The separation of ascospores, zygotes, and vegetative yeast cells can be carried out with simple glass microneedles attached to any one of the micromanipulators described above. Microneedles can be made from a stock of commercial glass fibers[102,103] or glass fiber strands with polished ends,[104,105] they can be made individually from glass rods,[94] or they can be obtained from the Singer Instrument Co.[99]

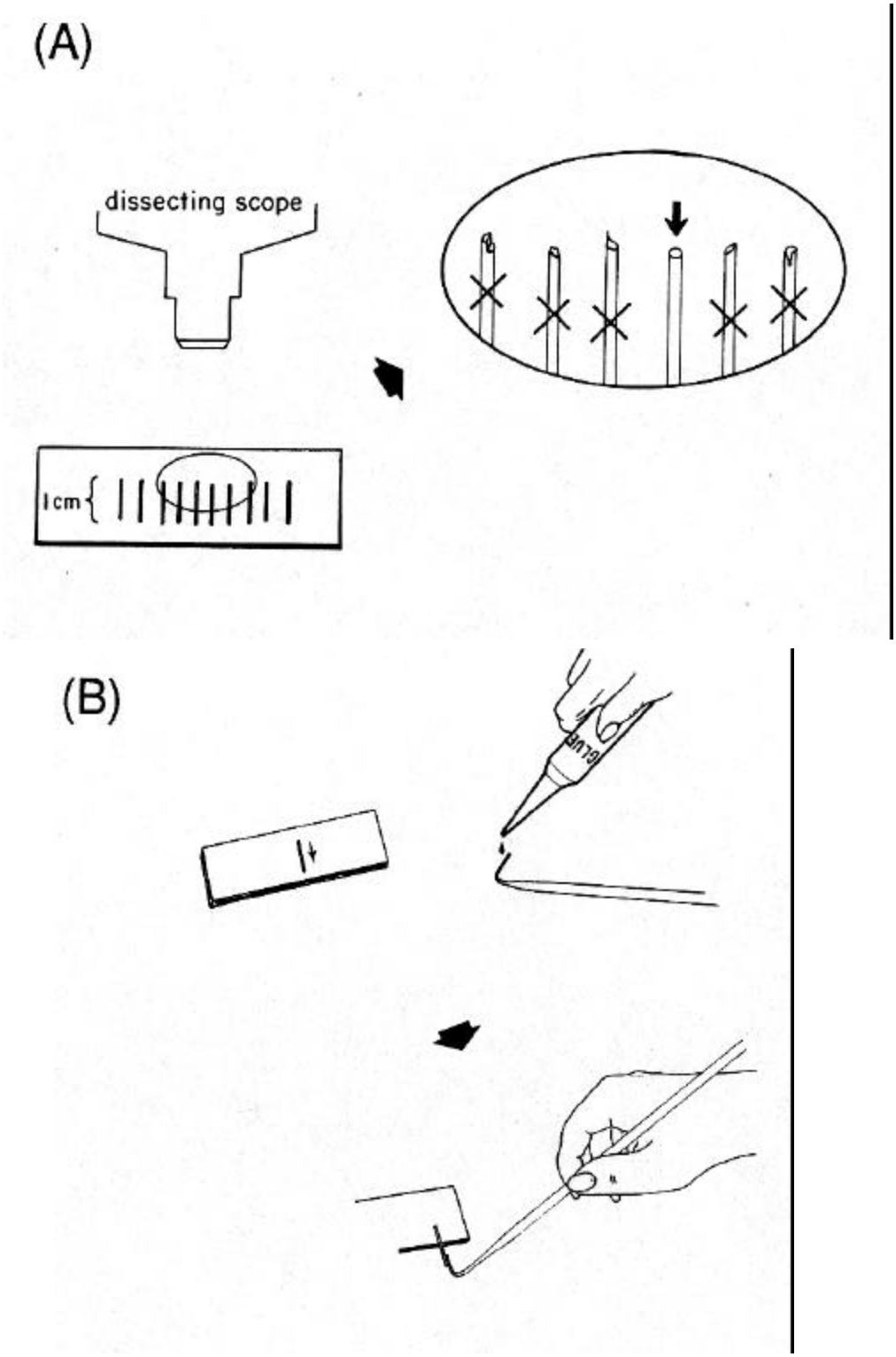

FIG. 5. Use of glass fibers for constructing microneedles. (A) a glass fiber approximately 40 mm in diameter is broken into segments approximately 1 cm long with a razor blade and examined with a microscope. (B) The segments containing flat ends are attached at a right angle to a mounting rod with cyanoacrylic "Super-Glue".[94]

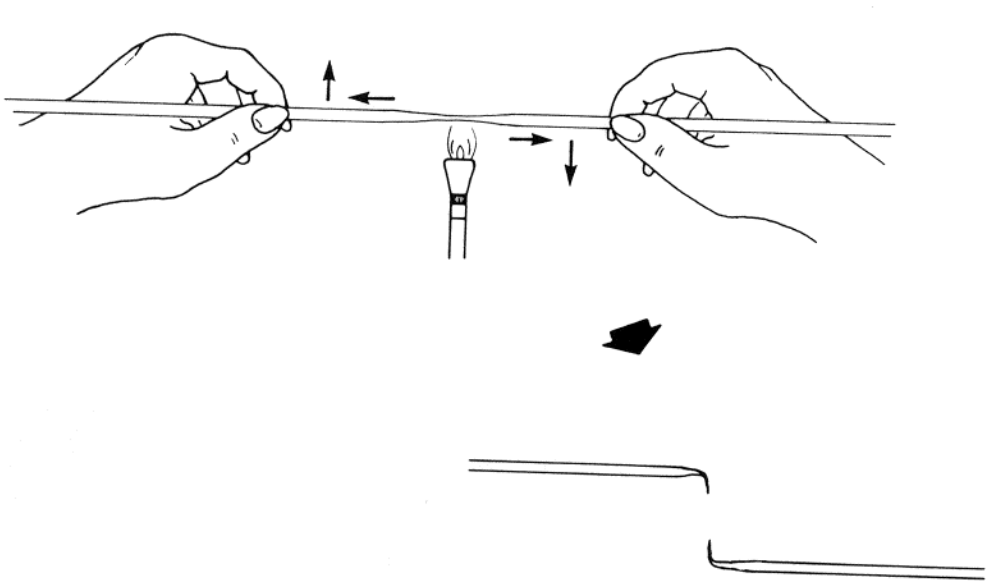

FIG. 6. Construction of microneedles. Microneedles required for the separation of ascospores can be made by first drawing out a 2-mm glass rod to a fine tip and then drawing out the end to an even finer tip at a right angle.[94]

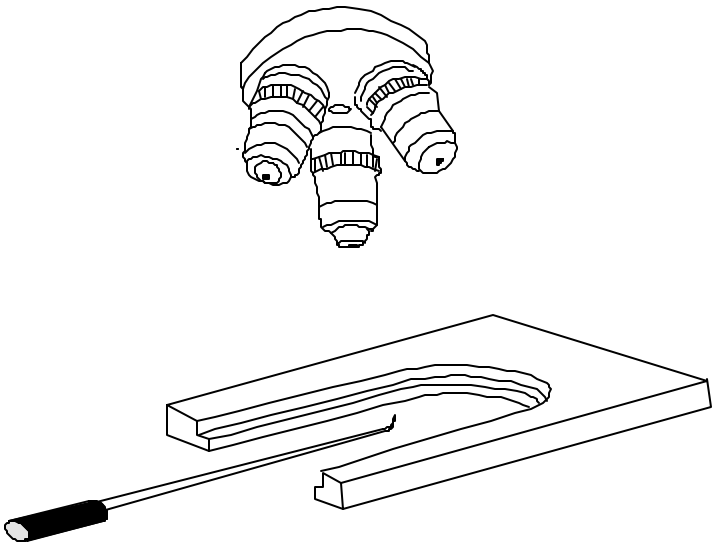

FIG. 7. The relative positions of the microneedle, a rig for holding a petri dish, and the microscope objectives.[94]

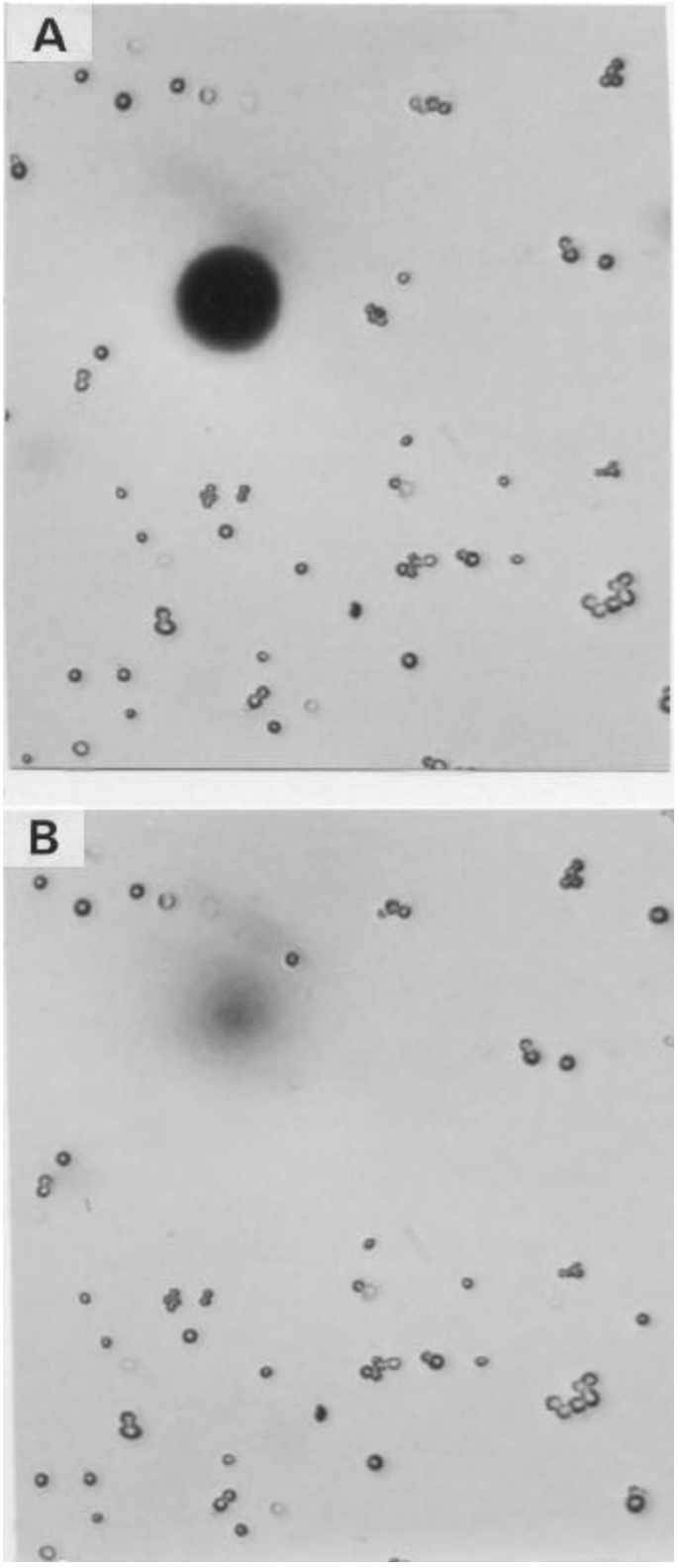

FIG. 8. A field of sporulated culture. (A) A four-spored cluster is seen at the right of the microneedle tip. (B) The cluster was picked up on the microneedle, which was lowered beneath the focal plane. The ascospores and the tip of the microneedle are, respectively, approximately 5 and 50 mm in diameter.[94]

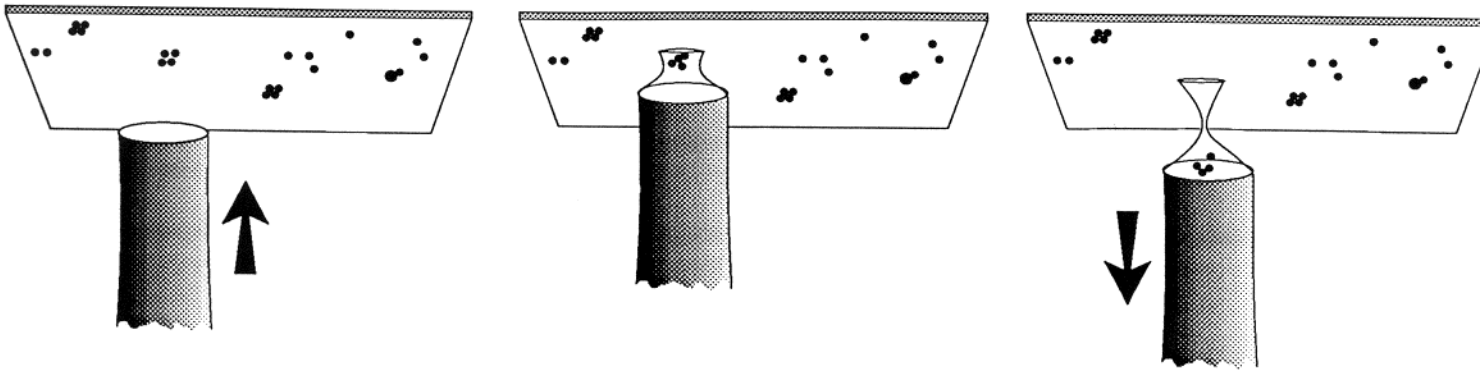

FIG. 9. The transfer of four spores from the surface of agar to the platform of a microneedle, by way of a water miniscus.[94]

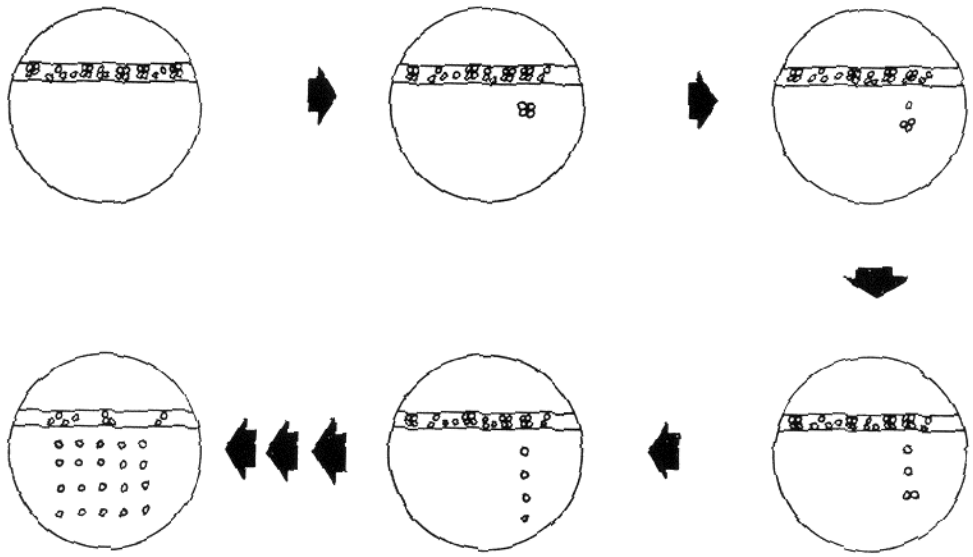

FIG. 10. The steps for sequentially separating the cluster of four ascospores approximately 5 mm apart on petri dishes.[94]

Microneedles are commonly constructed from glass fibers by a procedure that involves two steps: (1) the preparation of a stock of glass fibers; and (2) the gluing of a short segment of glass fiber perpendicular to a glass or metal mounting rod (Fig. 5). The glass mounting rod is made by first heating a 2 mm rod in a burner and pulling slowly to form a taper. When the rod has sufficient taper, the end is pulled quickly at right angles, similar to the procedure shown in Fig. 6; the end is broken so that the right angle projection is approximately 2 mm. The mounting rod should be cut with a file to approximately the size required to fit on the microscope stage, taking into account the distance from the manipulator to the center of the microscope field of view. The microneedle is attached to the micromanipulator and positioned under the microscope objective as shown in Fig. 7.

Most researchers use optical glass fibers,[103] which are commercially-available (0.002 inch diameter, cat. no. F31.735, Edmund Scientific [101 East Gloucester Pike, Barrington, NJ 08007]), and which have a uniform size. However, glass fibers can be made by drawing thin filaments from a 2 mm glass rod.[102] The glass fibers can be broken with the fingers or cut with scissors or cover slips. The segments are placed on a microscope slide for examination under a dissecting microscope (Fig. 5). Segments of about 1 cm are usually desired for dissection on a petri dish with a standard micromanipulator. The exact length is not too important at this point, because the microneedle eventually can be cut to size with a cover slip.

The segments of glass fibers are examined under a low-power dissecting microscope to determine which of them will make a good needle, i.e., which have tips with a flat surface perpendicular to the long axis of the needle and no burrs or cracks in the tip (Fig. 5). However, a needle with minor imperfections, (e.g., a half circle) sometimes will work if it has a flat working surface.

The glass-fiber segment with the best tip is moved down on the slide so that the good end is on the slide and the end to be glued is hanging off the edge. A small drop of Super Glue (cyanoacrylic) (Pacer Technology, Rancho Cucamonga, CA) is applied to the mounting rod and the glass fiber is glued to the end as shown in Fig. 6. The easiest way to apply the glue is to place a drop on a microscope slide and then to dip the whisker of the mounting rod into the drop. After contact, the glass fiber will usually come off the slide and stick to the mounting rod without any coaxing. If the glass fiber is not perpendicular to the stock, one may quickly adjust the angle before the glue sets.

Microneedles can be more conveniently prepared from commercially-available glass fiber segments with polished ends.[104,105] The use of these polished glass fibers is highly recommended for the preparation of glass microneedle.

The preparation of individual glass microneedle from glass rods requires more skill and patience, but allows the construction of microneedle with different diameters. The individually-prepared glass microneedle can be made with the small flame from the pilot light of an ordinary bunsen burner. A 2-mm-diameter glass rod is drawn out to a fine tip with the bunsen burner; by using the pilot flame, an even finer tip is drawn out at a right angle with an auxiliary piece of glass rod as illustrated in Fig. 6. The end is broken off so that the tip has a diameter of 10 to 100 μm and a length of a few millimeters. The drawn-out tip can be cut with a razor blade or broken between the surface and edge of two glass slides. It is critical that the microneedles have a flat end, which sometimes requires several attempts. The exact diameter is not critical, and various investigators have different preferences. Spores are more readily picked up and transferred with microneedles having tips of larger diameters, whereas manipulations in crowded areas having high densities of cells are more manageable with microneedles having smaller diameters. An approximately 40-μm-diameter microneedle is an acceptable compromise. Some investigators prefer larger diameters for picking up zygotes. The length of the perpendicular end should be compatible with the height of the petri dish or chamber; too short an end may result in optical

distortions from the main shank of the microneedle. Longer microneedles are required for manipulations on the surfaces of petri dishes.

The needle is mounted into the micromanipulator and centered in the field. The adjustment of the needle is made most easily first at low magnification and then at higher magnification. As recommended above, asci are usually dissected at 150x or greater magnification.

Dissection of Asci[94]

Digestion of the Ascus Sac

Sporulated cultures usually consist of unsporulated vegetative cells, four-spored asci, three-spored asci, etc. Dissection of asci requires the identification of four-spored asci and the relocation of each of the four ascopores to separate positions where they will form isolated spore colonies. The procedure requires the digestion of the ascus wall with Zymolyase, or another enzyme, without dissociating the four spores from the ascus.[106] (With very unusual strains that are particularly sensitive to enzyme treatments, the separation of ascospores can be carried out by rupturing the ascus wall with a microneedle.)[57,107]

Sporulated cells from the surface of sporulation medium are suspended in 50 µl of a stock solution of Zymolyase T100 (ICN) (50 µg/ml in 1 M sorbitol), and the suspension is incubated at 30ºC for approximately 10 minutes. The exact time of incubation is strain dependent and the progress of the digestion can be followed by removing a loopful of the digest to a glass slide and examining it under phase contrast at 400x magnification. The sample is ready for dissection when the spores in most of the asci are visible as discrete spheres, arranged in a diamond shape. Typical digestsed asci are seen in Fig. 8A. If a majority of the asci are still arranged in tightly-packed tetrahedrons or diamond shapes in which the spores are not easily resolved, digestion is incomplete and the spores will not be easily separated by micro dissection. It is convenient to use a Zymolyase concentration that will digest the ascus wall in approximately 10 minutes. The digestion is terminated by placing the tube on ice and gently adding 150 µl of sterile water. Extensive treatment sometimes can decrease the viability and dissociate the clusters of four spores. The culture is suspended by gently rotating the tube; an aliquot is transferred with a wire loop to the surface of a petri plate or agar slab. It is important not to agitate the spores once they have been treated. If the treated spores are vortexed or shaken, the integrity of the ascus cannot be assured since the contents of one ascus may disperse and reassemble with the contents of another.

The digestion can also be carried out with snail juice, which can be obtained commercially as Glusulase (NEN Research Proucts, catalogue no. NEE-154) or Suc d'*Helix pomatia* (L'Industrie Biologique Francaise, Genevilliers, France), or which can be prepared from snails, *Helix pomatia* or *Helix aspersa*.[106]

Separation of Ascospores

Micromanipulation can be implemented directly on the surfaces of ordinary petri dishes filled with nutrient medium or in special chambers on thin agar slabs. The petri dish (or chamber) is positioned so that the inoculum is in the microscope field over the microneedle. Examination of the streak should reveal the presence of the desired four-spored clusters as well as smaller clusters and vegetative cells. A typical preparation is shown in Fig. 8. A cluster of four spores is picked up on the microneedle by positioning microneedle tip next to the four-spored cluster on the surface of the agar. The microneedle is moved in a sweeping action, first touching the agar surface and then lowering the microneedle with a single motion. The absence of the four spores from the agar surface indicates that they have been transferred to the microneedle. Several attempts may be required to pick up all four ascospores. The

microneedle can be considered a platform to which the spores are transferred. It is obvious from the relative sizes of the microneedle and spores (Fig. 8) that the microneedle does not "poke" the tetrad of spores to pick them up. The flat surface of the microneedle does not interact with the spores themselves, but rather with the water layer on the surface of the agar. When the microneedle approaches the surface of the agar, a miniscus forms and often a halo of refracted light can be seen around the shadow of the microneedle. At this time a column of water connects the microneedle and the agar (Fig. 9). The spores disappear from view into the miniscus. The combined sideways and downward sweeping motion is an attempt to coax the spores into the half of the miniscus that remains on the microneedle surface as it breaks away. Success in this endeavor is assayed by the disappearance of the spores from the visual field in the microscope. At the new position the process is repeated, this time with the hope that spores go from the microneedle miniscus to the surface of the agar.

Once the four spores have been transferred to the first position, it is necessary to separate at least one spore from the rest so that it can be left behind. A simple technique for achieving this goal is to move the microneedle onto the surface of the agar, forming the crisp image and halo, directly next to the cluster of spores and to vibrate the microneedle by gently tapping on the table near the microscope or on the microscope stage. The spores will often be separated by several microneedle diameters by this action. Three spores can be collected by sweeping the surface of the agar with the needle tip, and the process is repeated at the next three stops.

Note the position on the mechanical stage and place the four spores on the surface of the agar at least 5 mm from the streak. Pick up three spores and move the dish away from the streak another 5 mm (Fig. 10). Deposit the three spores and pick up two spores. Move the chamber an additional 5 mm; deposit the two spores and pick up one spore. Move the chamber 5 mm more and plant the remaining spore. Move the chamber 5 mm from the line of the four spores and select another four-spore cluster. Separate the spores as before at 5 mm intervals. Continue until a sufficient number of asci is dissected or until the entire dish is covered.

After picking up the four spores from an ascus, it is often convenient to set the stage micrometer so that each group of four spore colonies falls on cardinal points such as 15, 20, 25, etc. This makes it easier to keep track of progress and prevents the spore colonies from growing too close together. Likewise, positions on the y axis can be marked on the stage micrometer so that the four spore colonies from each ascus are evenly spaced. Take care are not to break the microneedle when removing the dish or chamber from the stage. The thin agar slab is transferred from the chamber to the surface of a nutrient plate, which is then incubated for three days until the spore colonies are formed. Petri dishes containing separated spores are similarily incubated. As shown in Fig. 11, colonies derived from ascospores that were separated directly on a petri dish can be replica plated directly to media for testing nutritional requirements.

Although considerable patience is required to master ascus dissection, most workers are able to carry out this procedure after a few days of practice.

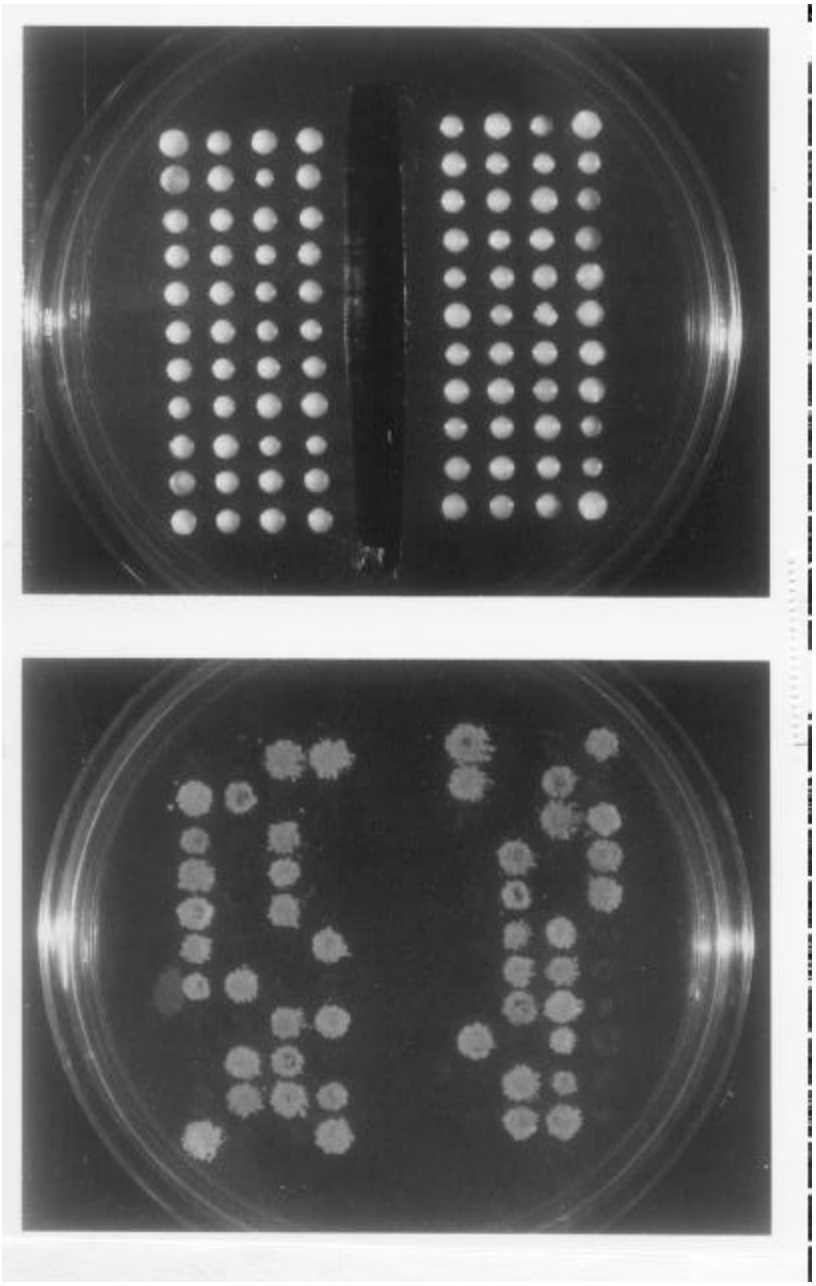

FIG. 11. Spore colonies derived from asci separated on the surface of a petri dish (Top). The central area of the dish, containing a streak of the sporulated culture, was cut out and removed after dissection. The spore colonies were replica plated to a synthetic medium lacking a nutrient (Bottom). The 2:2 segregation of a heterozygous marker is revealed by the growth pattern on the seletivemedium. The complete viability and uniform colony size shown in this figure is not typical of the meiotic progeny from most diploids; however, these properties can be choosen during the course of strain construction.[94]

Isolation of Cells

In addition to ascus dissection, micromanipulation is occasionally required for separating zygotes from mating mixtures, for pairing vegetative cells and spores for mating, and for separating mother cells and daughter cells during vegetative growth. Zygotes usually can be picked up on microneedles, although vegetative cells usually cannot. However, vegetative cells can be separated simply by dragging them across the agar surface with microneedles. The use of microneedles is rather effective, since the cells usually follow closely in the wake of the microneedle as it is moved along the liquid surface film of the agar.

Tetrad Analysis

Meiotic analysis is the traditional method for genetically determining the order and distances between genes of organisms having well-defined genetics systems. Yeast is especially suited for meiotic mapping because the four spores in an ascus are the products of a single meiotic event, and the genetic analysis of these tetrads provides a sensitive means for determining linkage relationships of genes present in the heterozygous condition. It is also possible to map a gene relative to its centromere if known centromere-linked genes are present in the cross. Although the isolation of the four spores from an ascus is one of the more difficult techniques in yeast genetics, requiring a micromanipulator and practice, tetrad analysis is routinely carried out in most laboratories working primarily with yeast. Even though linkage relationships are no longer required for most studies, tetrad analysis is necessary for determining a mutation corresponds to an alteration at a single locus, for constructing strains with new arrays of markers, and for investigating the interaction of genes.

There are three classes of tetrads from a hybrid which is heterozygous for two markers, *AB* x *ab*: PD (parental ditype), NPD (non-parental ditype) and T (tetratype) as shown in Fig. 12. The following ratios of these tetrads can be used to deduce gene and centromere linkage:

	PD	NPD	T
	AB	*aB*	*AB*
	AB	*aB*	*Ab*
	ab	*Ab*	*ab*
	ab	*Ab*	*aB*
Random assortment	1 :	1 :	4
Linkage	>1 :	<1	
Centromere linkage	1 :	1 :	<4

There is an excess of PD to NPD asci if two genes are linked. If two genes are on different chromosomes and are linked to their respective centromeres, there is a reduction of the proportion of T asci. If two genes are on different chromosomes and at least one gene is not centromere-linked, or if two genes are widely separated on the same chromosome, there is independent assortment and the PD : NPD : T ratio is 1 : 1 : 4. The origin of different tetrad types are illustrated in Fig. 12.

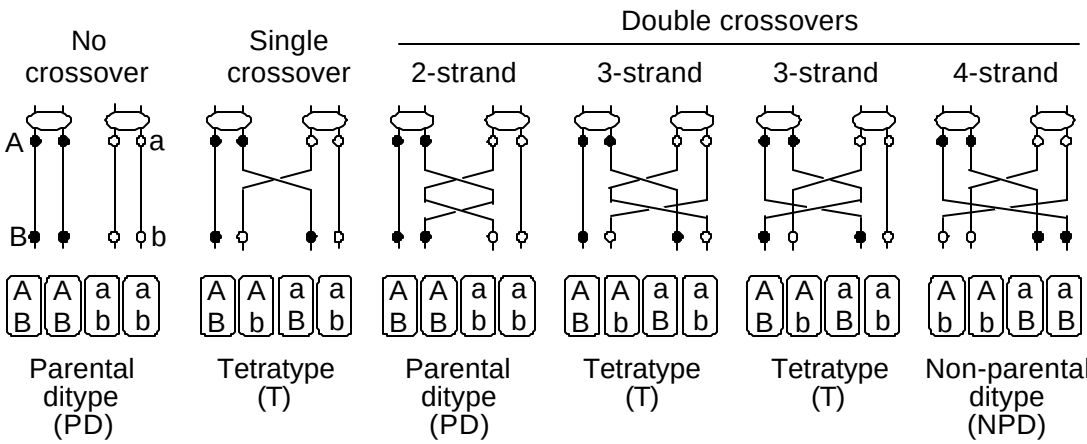

FIG. 12. Different tetrad types originating from a *AB* x *ab* heterozygous cross after either no crossover or single or double crossovers between the *A*-*B* interval. The fractions of the different tetrad types reveal whether A and B are linked and the values can be used to calculate the map distance.

The frequencies of PD, NPD, and T tetrads can be used to determine the map distance in cM (centimorgans) between two genes if there are two or lesser exchanges within the interval:[108]

$$\text{cM} = \frac{100}{2} \quad \frac{\text{T} + 6\text{NPD}}{\text{PD} + \text{NPD} + \text{T}}$$

The equation for deducing map distances, cM, is accurate for distances up to approximately 35 cM. For larger distances up to approximately 75 cM, the value can be corrected by the following empirically-derived equation:[109]

$$\text{cM (corrected)} = \frac{(80.7)(\text{cM}) - (0.883)(\text{cM})^2}{83.3 - \text{cM}}$$

Similarly, the distance between a marker and its centromere cM′, can be approximated from the percentage of T tetrads with a tightly-linked centromere marker, such as *trp1*:

$$\text{cM}' = \frac{100}{2} \quad \frac{\text{T}}{\text{PD} + \text{NPD} + \text{T}}$$

Gene Mapping

Recombinant DNA procedures have by-and-large replaced traditional genetic methods for determining the chromosomal positions of genes and subsequently their identification. The cloning of a DNA segment corresponding to a mutation, and the sequence of the complementing fragment is a rapid method for identifying the mutant gene. The chromosomal position can be easily determined from the database.[51]

However, there are rare occasions that a mutation can not be identified by complementation with plasmid libraries. A new recessive mutation should be first tested by genetic complementation, involving crossing the unknown mutant to known, characterized mutants and examining the phenotype of the diploid. New mutants should be crossed to a series of known mutants having the same or similar phenotypes. Lack of complementation of two recessive mutations is almost always indicative of allelism. Meiotic analysis of the presumed homozygous diploid should reveal complete linkage and therefore identity. However, complementation of recessive mutations does not establish that they correspond to different genes. A meiotic analysis could be carried out when allelic complementation is suspected, especially when the diploid appears to have a partial mutant phenotype.

The second step in characterizing an unknown mutation should involve a meiotic analysis to determine if the mutant phenotype is controlled by a single gene. This is particularly critical when the mutant was derived from heavily mutagenized cells, such as those commonly used to obtain temperature-sensitive mutations and related defects. There have been numerous examples where temperature-sensitive growth and a particular enzyme deficiency segregated independently from each other, indicating mutations of two separate genes.

The mutant haploid strain should be crossed to a strain carrying at least one centromere-linked marker, such as *trp1*. Thus, a meiotic analysis would reveal both single-gene segregation and centromere linkage. The diploid should then be sporulated, the asci dissected and the haploid segregants tested according to the methods outlined above A 2:2 segregation of the mutant phenotype is indicative of a single-gene mutation. Less than $^2/_3$ second division segregation (less than $^2/_3$ tetratype asci relative to *trp1*) is indicative of centromere linkage of the unmapped gene. If centromere linkage is suspected, the mutant should be crossed to a set of centromere-tester strains that have markers near the centromeres of each of the 16 chromosomes.[61] The unmapped gene should exhibit linkage to one of the centromere-linked markers and should be further analyzed with additional markers on the assigned chromosome.

If the mutant gene is not centromere-linked, it is advisable to next determine on which chromosome it resides, using the 2 μm mapping or other procedures.[110]

Other Techniques for Genetic Analysis

In addition to the major techniques used for genetic analysis that are covered above in this chapter and elsewhere in this and the previous[33] volume, there are other simple procedures worthy of mention.

Replica Plating

Testing of strains on numerous media can be carried out by the standard procedure of replicaplating with velveteen.[111] However, subtle differences in growth are better revealed by transferring diluted suspensions of cells with specially–constructed spotting apparatuses. An array of inoculating rods, fastened on a metal plate, is dipped into microtiter or other compartmentalized dishes, containing yeast suspensions. Small and uniform aliquotes can be repetively tranferred to different types of media. Furthermore $^1/_{10}$ serial dilutions of cell suspensions are often tested to better reveal differences

Mating and Complementation

A few crosses can be simply carried out by mixing equal amounts of the *MAT***a** and *MAT*α strains on a YPD plate and incubating at 30°C for at least 6 hours and preferably overnight. Prototrophic diploid colonies can then be selected on appropriate synthetic media if the haploid strains contain

complementing auxotrophic markers. Similarily, testing mating types or other markers of meiotic progenies, requiring the selection of numerous diploid hybrids, can be carried out by replicaplating, using any one of a number techniques such as cross-steaking, spotters, etc. Prototrophic diploids also can be selected by overlaying a mixture of the two haploid strains directly on minimal plates, although the frequencies of matings may be slightly reduced. If the diploid strain cannot be selected, zygotes can be isolated from the mating mixture with a micromanipulator. Zygotes, which can be identified by their characteristic thick zygotic neck, are best isolated 4-6 hrs after mixing, when the mating process has just been completed;[112] diploids isolated by micromanipulation should be verified by sporulation and the lack of mating.

Formation of prototrophic diploids, indicative of complementation, is used to test *MAT***a** and *MAT*α mating types and to determine unknown markers in new mutants and meiotic segregants. Mating type tests are best carried out with *MAT***a** and *MAT*α tester strains, each containing markers not in the strains to be tested.

Complementation analysis consists of testing diploid strains that were constructed from two haploid mutants which have the same mutant phenotype, such as a specific amino acid requirement, sensitivity to UV, etc. If the mutant character is found in the diploid and if the two mutant genes are recessive, it can be concluded that the two mutant genes are allelic, i.e., the lesions are in genes controlling the same function, or in most cases, the same polypeptide chain. In rare and special instances, a double heterozygous diploid strain may exhibit the phenotype of the recessive marker, confusing this test of complementation.

However, because of allelic (or intragenic) complementation, the growth of double heterozygous diploids does not always indicate that the two mutations are in different genes. Some cases of allelic complementation occur when the normal enzyme is composed of two or more identical subunits. The enzymes formed by allelic complementation are mutant proteins containing two different altered polypeptides, in which each of the mutant polypeptides compensates for each other's defects to produce a catalytically active protein. Allelic complementation can be pronounced when the enzyme contains separate domains carrying out different catalytic functions, such as the *HIS4A*, *HIS4B* and *HIS4C* regions. Allelic complementation is frequent in yeast. For example, mutations in five of the ten genes controlling histidine biosynthesis show extensive allelic complementation. Because of allelic complementation, frequencies of meiotic recombination are required to determine if two complementing mutants are alleles of the same gene. The frequencies of recombination are extremely low if the mutations are in the same gene, while the frequencies of normal meiotic segregants can be as high as 25% if the mutations are in different genes.

Complementations test are required for scoring meiotic progeny from hybrids heterozygous for two or more markers controlling the same character. For example, a $HIS3^+$ $his4^-$ X $his3^-$ $HIS4^+$ diploid will produce tetratype tetrades having the following genotypes:

$HIS3^+$ $HIS4^+$
$HIS3^+$ $his4^-$
$his3^-$ $HIS4^+$
$his3^-$ $his4^-$

Intergenic complementation tests are required to determine the segregation of the his alleles in the $HIS3^+$ $his4^-$, $his3^-$ $HIS4^+$, and $his3^-$ $his4^-$ segregants. These tests are carried out with *MAT***a** and *MAT*α tester strains having either $HIS3^+$ $his4^-$ or $his3^-$ $HIS4^+$ markers. Diploids homozygous for either $his3^-$ or $his4^-$ will not grow on histidine deficient medium, as indicated below:

		X *HIS3*$^+$ *his4*$^-$	X *his3*$^-$ *HIS4*$^+$
HIS3$^+$ *HIS4*$^+$	+	+	+
HIS3$^+$ *his4*$^-$	–	–	+
his3$^-$ *HIS4*$^+$	–	+	–
his3$^-$ *his4*$^-$	–	–	–

Random Spores

Although dissection of asci and recovery of all four ascospores is the preferred procedure for obtaining meiotic progeny, random spores can be used when isolating rare recombinants, or when analyzing a large number of crosses. Several techniques have been devised for eliminating or reducing unsporulated diploid cells from the culture. The proportion of random spores can be increased by sporulating the diploid strain on a medium containing a high concentration of potassium acetate (2%), which kills vegetative cells of many sporulated strains.[113] The sporulated culture is treated with Zymolyase, as described above, the spores separated by a minimal level of sonication, and various dilutions are plated for single colonies. Alternatively, the spores of Zymolyase treated culture can be dispersed vortexing with 0.5 mm glass beads. Furthermore, vegetative cells can be preferentially killed by treating a sporulated culture with an equal volume of diethyl ether.[114] Another convenient method for producing random spores relies on the selection against vegetative diploid cells that are heterozygous for *can*^{R}*1*, the recessive marker confirming resistance to canavanine sulfate.[115] If the *can*^{R}*1*/+ diploid is sporulated and the spores are separated and plated on canavanine medium, one–half of the haploid spores will germinate and grow, while the other half of the haploids and all of the diploids will not.

Tong *et al*.[19] developed a series of robotic procedures that allowed large numbers of matings and recovery of meiotic recombinants for generating haploid double mutants in a genome-wide synthetic lethal analysis. This procedure included the use of the P_{MFA1}-*HIS3* reporter, that is only expressed in *MAT***a** cells and allows for germination of *MAT***a** meiotic progeny, but that prevents the growth on meduim lacking histidine of the *his3*/*his3* diploid parental strain and of diploid strains arising by mating of cells decending from meiosis.

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