国外大学生物学优秀教材（影印版）

Methods in Yeast Genetics

D. Burke, D. Dawson, T. Stearns

酵母遗传实验方法

冷泉港实验课手册

清华大学出版社　　冷泉港实验室出版社

METHODS
IN
YEAST GENETICS

A Cold Spring Harbor Laboratory Course Manual

2000 Edition

酵母遗传实验方法

冷泉港实验课手册

2000年版

Dan Burke
Dean Dawson
Tim Stearns

清华大学出版社
冷泉港实验室出版社

（京）新登字 158 号

Methods in Yeast Genetics

A Cold Spring Harbor Laboratory Course Manual

D. Burke, D. Dawson, T. Stearns

北京市版权局著作权合同登记号： 图字 01-2001-4567

书　名：酵母遗传实验方法　　冷泉港实验课手册
作　者：D. Burke, D. Dawson, T. Stearns
出版者：清华大学出版社（北京清华大学学研大厦，邮编 100084）
　　http:// www.tup.tsinghua.edu.cn
印刷者：北京市清华园胶印厂
发行者：新华书店总店北京发行所
开　本：850×1168 1/16　印张：14
版　次：2002 年 1 月第 1 版　2002 年 1 月第 1 次印刷
书　号：ISBN 7-302-05096-1/Q・20
印　数：0001～3000
定　价：26.00 元

出 版 前 言

为了使生物学教学适应21世纪生命科学发展的需要，同时也为了提高学生阅读专业文献和获取信息的能力，结合当前生物学在高等院校中教学的实际情况，我们精选了一些国外优秀的生物学教材，组织专家进行了评阅和审核，组成国外大学生物学优秀教材系列（影印版）。该系列反映了国外大学生物学教材的最新内容和编写特色，多数教材经过教学实践，被国外很多大学广泛采用，并获得好评，因而不断再版。本书即是其中的一册。

希望这套教材能对高等院校师生和广大科技人员有所帮助，同时对我国的生命科学赶超世界先进水平起到一定的推动作用。

欢迎广大读者将使用本系列教材后的意见反馈给我们，更欢迎国内外专家、教授积极向我社推荐国外的优秀生物学教材，以便我们将国外大学生物学优秀教材系列做得更好。

清华大学出版社

2001年8月

Preface

This laboratory course manual incorporates significant portions of the manuals used in previous Cold Spring Harbor Yeast Genetics Courses. Although most of the experiments have now been revised and several new techniques have been added, the basic structure of this course is the same as in those that were taught in the Cold Spring Harbor Yeast Genetics Course for the past 30 years. We are indebted to our predecessors, Fred Sherman, Gerry Fink, Jim Hicks, Mark Rose, Fred Winston, Phil Hieter, Susan Michaelis, Aaron Mitchell, Alison Adams, Chris Kaiser, and Dan Gottschling, for their teaching and for making this course an important part of the yeast community. We also thank Mike Cherry for his invaluable assistance with the genetic and physical maps.

Dan Burke
Dean Dawson
Tim Stearns

Introduction

Genetic investigations of yeast were essentially initiated by Winge and his co-workers in the mid-1930s. Approximately ten years later, Lindegren and his colleagues also began extensive studies. These two groups are responsible for uncovering the general principles and much of the basic methodology of yeast genetics. Today, yeast is widely recognized as an ideal eukaryotic microorganism for biochemical and genetic studies. Although yeasts have greater genetic complexity than bacteria, they still 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 genetic studies include the existence of both stable haploid and diploid cells, rapid growth, clonability, the ease of replica plating, mutant isolation, and ability to isolate each haploid product of meiosis by microdissection of a tetrad ascus. Yeast has been successfully employed for the study of all areas of genetics, such as mutagenesis, recombination, chromosome segregation, gene action and regulation, as well as aspects distinct to eukaryotic systems, such as mitochondrial genetics.

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. DNA is 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 primarily via homologous recombination. This permits efficient targeted integration of DNA sequences into the genome. Homologous recombination coupled with high levels of gene conversion has led to the development of techniques for the direct replacement of normal chromosomal loci with genetically engineered DNA sequences. This ease of performing direct gene replacement is unique among eukaryotic organisms and has been extensively exploited in every aspect of yeast genetics, cell biology, physiology, and biochemistry. Many of these modern genetic techniques are reviewed in Volume 194 of *Methods in Enzymology* (Guthrie and Fink 1991) and by Rose (1995).

Recent advances in modern yeast genetics have come from determining the complete DNA sequence of the *Saccharomyces cerevisiae* genome. The assembly of this sequence into a public sequence database has made yeast one of the premier organisms for detailed analysis of eukaryotic cellular function and genome organization. Several Internet web sites provide convenient and organized access to the sequence database.

Two of the more popular sites are the Saccharomyces Genome Database (SDB) and the Munich Information Center for Protein Sequences (MIPS). Each of these sources augments the sequence database by providing powerful search functions for analyzing the genome, as well as helpful links to relational databases, such as literature cross-references or protein structure of yeast gene products. In addition, the Yeast Protein Database (YPD) has each yeast protein and predicted open reading frame compiled with information about its structure and the phenotypes associated with mutations in its gene.

One of the most useful collections of information on *Saccharomyces cerevisiae* biology is found in two companion series of reviews entitled *The Molecular Biology of the Yeast* Saccharomyces (Strathern et al. 1981 and 1982) and *The Molecular and Cellular Biology of the Yeast* Saccharomyces (Broach et al. 1991; Jones et al. 1992; Pringle et al. 1997). While a few chapters in the later series update progress made since the first series was published, each volume has detailed reviews that synthesize vast literatures of yeast biology that are not available anywhere else. All five of these books are essential for the library of a yeast biologist. *The Early Days of Yeast Genetics* is also a book to consider reading, for it provides a historical perspective on why yeast became a model organism and traces the development of the yeast community's influence on modern genetics and eukaryotic molecular biology.

This course will focus exclusively on the baker's yeast, *Saccharomyces cerevisiae*. After completing the course, you should be able to carry out all of the techniques commonly employed by yeast geneticists and be able to follow the literature with greater ease. Except for the dissection of asci, most of the methods do not differ significantly from the methods employed with other microorganisms, and the skills should be rapidly acquired with little practice. Experiments will be conducted in pairs, and whenever possible, an investigator more familiar with microbiological techniques will be assigned to a less-experienced partner. Since most of the experiments will be initiated at the outset of the course, it is advisable to read the entire manual thoroughly. Please note that some experiments span many days due to extended periods of incubation.

We wish to emphasize that some of the procedures in this manual have been condensed in order to save time and are not necessarily standard for research purposes. For example, mutants are usually purified by subcloning the initial isolates. Also, some of the techniques may not be directly applicable to your research problems, but they have been included to illustrate general principles and methods.

HIGHLY RECOMMENDED BOOKS

Broach J.R., Jones E.W., and Pringle J.R. 1991. *The molecular and cellular biology of the yeast* Saccharomyces. I. *Genome dynamics, protein synthesis, and energetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

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

Jones, E.W., Pringle J.R., and Broach J.R. 1992. *The molecular and cellular biology of the yeast* Saccharomyces. II. *Gene expression*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Pringle J.R., Broach J.R., and Jones E.W. 1997. *The molecular and cellular biology of the yeast* Saccharomyces. III. *Cell cycle and cell biology*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Strathern, J.N., E.W. Jones, and J.R. Broach, eds. 1981. *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

———. 1982. *The molecular biology of the yeast* Saccharomyces: *Metabolism and gene expression*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

OTHER BOOKS ABOUT YEAST

Fincham J.R.S., Day P.R., and Radford A. 1979. *Fungal genetics*. University of California Press, Berkeley and Los Angeles.

Rose M.D. 1995. Modern and post-modern genetics in *Saccharomyces cerevisiae*. In *The yeasts*, 2nd edition (ed. A.E. Wheals et al.), vol. 6, pp. 69–120. Academic Press, New York.

Hall M.N. and Linder P. 1993. *The early days of yeast genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

RECOMMENDED YEAST WEB SITES

Munich Information Center for Protein Sequences
http://speedy.mips.biochem.mpg.de/mips/yeast/

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

Yeast Protein Database
http://www.proteome.com/YPDhome.html

Genetic Nomenclature

CHROMOSOMAL GENES

Early recommendations for the nomenclature and conventions used in yeast genetics have been summarized by Sherman and Lawrence (1974) and Sherman (1981). Whenever possible, gene symbols are consistent with the proposals of Demerec et al. (1966) and are designated by three italicized letters (e.g., *arg*). Contrary to the proposals of Demerec et al. (1966), the genetic locus is identified by a number (not a letter) following the gene symbol (e.g., *arg*2). Dominant alleles are denoted by using uppercase italics for all three letters of the gene symbol (e.g., *ARG2*). Lowercase letters symbolize the recessive allele (e.g., the auxotroph *arg*2). Wild-type genes are designated with a superscript plus sign (*sup6*$^+$ or *ARG2*$^+$). Alleles are designated with a number separated from the locus number by a hyphen (e.g., *arg2-14*). Locus numbers are consistent with the original assignments; however, allele numbers may be specific to a particular laboratory.

Phenotypic designations are sometimes denoted by cognate symbols in Roman type followed by a superscript plus or minus sign. For example, the independence from and requirement for arginine can be denoted by Arg$^+$ and Arg$^-$, respectively.

The following examples illustrate the conventions used in the genetic nomenclature for *S. cerevisiae:*

ARG2	A locus or dominant allele
arg2	A locus or recessive allele that produces a requirement for arginine as the phenotype
ARG2$^+$	The wild-type allele of this gene
arg2-9	A specific allele or mutation at the *ARG2* locus
Arg$^+$	A strain that does not require arginine
Arg$^-$	A strain that requires arginine
Arg2p	Designation for the protein product of the *ARG2* gene

There are a number of exceptions to these general rules. Gene clusters, complementation groups within a gene, or domains within a gene that have different properties can be designated by capital letters following the locus number (e.g., *his4A, his4B*).

The extensive use in yeast of recombinant DNA techniques has introduced a nomenclature that pertains to gene insertions, gene fusions, and plasmids:

ARG2::LEU2	An insertion of the *LEU2* gene at the *ARG2* locus where the insertion does not disrupt *ARG2* function
arg2::LEU2	An insertion of the *LEU2* gene at the *ARG2* locus where the insertion disrupts *ARG2* function
arg2-101::LEU2	An insertion of the *LEU2* gene at the *ARG2* locus where the insertion disrupts *ARG2* function and the disruption allele is specified
cyc1–arg2	A gene fusion between the *CYC1* gene and ARG2 where neither gene is functional
P_{cyc1}–*ARG2*	A gene fusion between the *CYC1* gene promoter and *ARG2* where the *ARG2* gene is functional
[YCp–*ARG2*]	A centromere plasmid carrying a functional *ARG2* locus
[pCK101]	Designation for a specific plasmid whose structure is given elsewhere

Although superscripts should be avoided, it is sometimes expedient to distinguish genes conferring resistance or sensitivity by a superscript R or S, respectively. For example, the genes controlling resistance to canavanine sulfate (*can1*) and copper sulfate (*CUP1*) and their sensitive alleles can be denoted, respectively, as can^R1, CUP^R1, CAN^S1, and cup^S1.

Wild-type and mutant alleles of the mating-type and related loci do not follow the standard rules. The two wild-type alleles of the mating-type locus are designated *MAT***a** and *MATα*. The two complementation groups of the *MATα* locus are denoted *MATα1* and *MATα2*. Mutations of the *MAT* genes are denoted, e.g., *mat***a***-1*, *matα1-1*. The wild-type homothallic alleles at the HMR and HML loci are denoted *HMR***a**, *HMRα*, *HML***a**, and *HMLα*. Mutations at these loci are denoted, e.g., *hmr***a***-1*, *hmlα-1*. The mating phenotypes of *MAT***a** and *MATα* cells are denoted simply **a** and α, respectively.

Dominant and recessive suppressors should be denoted, respectively, by three uppercase or three lowercase letters followed by a locus designation (e.g., *SUP4*, *SUF1*, *sup35*, *suf11*). In some instances, UAA suppressors and UAG suppressors are further designated o and a, respectively, 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 coding for the normal tyrosine tRNA and lacking suppressor activity can be referred to as $sup4^+$. Thus, the nomenclature describing suppressor and wild-type alleles in yeast is unrelated to the bacterial nomenclature. For example, an ochre *E. coli* suppressor that inserts tyrosine residues at both UAA and UAG sites is denoted as su_4^+, and the wild-type locus coding for the normal tyrosine tRNA and lacking suppressor activity can be referred to as Su_4, su_4^-, or *sup*C.

For most structural genes that code for proteins, the functional wild-type allele is usually dominant to the mutant form of a gene. In yeast, the convention for dominant genes utilizes italic symbols such as *HIS4* and *LEU2*. Because the sites of recessive mutations are usually used for genetic mapping, published chromosome maps usually contain the mutant form of the gene. For example, chromosome III contains *his4* and *leu2*, whereas chromosome IX contains *SUP22* and *FLD1*. Because capital letters are used to represent dominant wild-type genes that control the same character (e.g., *SUC1, SUC2*), and because the dominant forms are used in genetic mapping, such chromosomal loci are denoted in capital letters on genetic maps. In addition, capital letters are 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).

NON-MENDELIAN DETERMINANTS

Where necessary, non-Mendelian genotypes can be distinguished from chromosomal genotypes by enclosure in brackets. Whenever applicable, it is advisable to employ the above rules for designating non-Mendelian genes and to avoid the use of Greek letters. However, when referring to an entire non-Mendelian element, it is best to either retain the original symbols [ρ⁺], [ρ⁻], [ψ⁺], and [ψ⁻] or use their transliteration, [*rho*⁺], [*rho*⁻], [*PSI*⁺], and [*psi*⁻], respectively. Detailed designations for mitochondrial mutants have been presented by Dujon (1981) and Grivell (1984, 1990) and for killer strains by Wickner (1981). Unlike the other non-Mendelian determinants, [*PSI*⁺] and [*URE3*] are not based on different states of a nucleic acid; rather, the [*PSI*⁺] and [*URE3*] traits result from heritable conformational states of proteins. The unusual behavior of these traits can be explained by the prion hypothesis, which has been used to explain infectious neurodegenerative diseases such as scrapie in mammals (Lindquist 1997). [*PSI*⁺] corresponds to a heritable conformational state of the translation termination factor Sup35p, and [*URE3*] corresponds to a heritable inactive state of *URE2*, a gene whose product is involved in nitrogen regulation. The known non-Mendelian determinants in yeast are listed in Table 1.

Table 1. *Non-Mendelian determinants of yeast*

Wild type	Mutant variant	Element	Mutant trait
[ρ⁺]	[ρ⁻]	Mitochondrial DNA	Respiration deficiency
[*KIL*-k_1]	[*KIL*-o]	RNA plasmid	Sensitive to killer toxin
[*cir*⁺]	[*cir*°]	2μ plasmid	None
[*psi*⁻]	[*PSI*⁺]	Prion form of Sup35p	Enhanced suppression of nonsense codons
[*ure3*⁻]	[*URE3*]	Prion form of Ure2p	Unregulated ureidosuccinate uptake

GENETIC BACKGROUNDS

The genetic background from which a *S. cerevisiae* strain is derived is an often hidden aspect of the genotype that should be taken into account when designing experiments. Most strains used in modern genetic studies come from one of a small set of genetic backgrounds, including S288C, X2180, A364A, W303, Σ1278b, AB972, SK1, and FL100. The genealogies of some of these backgrounds have recently been reconstructed from records of crosses that were carried out in the 1940s between wild yeasts and brewing strains (Mortimer and Johnston 1986). This analysis shows that although most backgrounds share a common ancestry, a significant degree of genetic heterogeneity has been introduced by outcrossing. In practice, crosses between distantly related strains often give inviable combinations of alleles leading to many inviable spores, whereas crosses between strains from the same background usually give >95% viable spores. The S288C and A364A genetic backgrounds have similar genealogies and in crosses give a high frequency of spore viability, but an analysis of genomic sequences from these strains reveals an average of 3.4 nucleotide sequence differences per kilobase of genomic DNA. Thus, even apparently closely related strains can differ at a very large number of sites.

Allelic differences between strain backgrounds can seriously influence the outcome of many different kinds of experiments, and it is best to avoid genetic heterogeneity as much as possible by using a single genetic background. At the beginning of a new mutant hunt, it is worth considering which strain background to use—usually the background used by most investigators in the same field is the best choice. S288C is probably the most commonly used background; however, other backgrounds offer distinct advantages for particular types of experiments. For example, Σ1278b will form pseudohyphae whereas S288C will not, and SK1 sporulates much more rapidly than S288C. It is often necessary to move a desired mutation from one background into another. Ideally, this can be done using recombinant plasmids and the methods for gene replacement described in Experiment VII. Mutations that have not been cloned can be moved by backcrossing to the desired strain background (usually successive backcrosses are carried out until a clear 2:2 pattern of segregation for the desired trait has been achieved).

REFERENCES

Demerec M., Adelberg E.A., Clark A.J., and Hartman P.E. 1966. A proposal for a uniform nomenclature in bacterial genetics. *Genetics* **54:** 61–76.

Dujon B. 1981. Mitochondrial genetics and functions. In *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance* (ed. J.N. Strathern et al.), pp. 505–635. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Grivell L.A. 1984. Restriction and genetic maps of yeast mitochondrial DNA. In *Genetic maps*, 3rd edition (ed. S.J. O'Brien), pp. 234–247. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

———. 1990. Mitochondrial DNA in the yeast *Saccharomyces cerevisiae*. In *Genetic maps*, 5th edition (ed. S.J. O'Brien), pp. 3.50–3.57. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Lindquist S. 1997. Mad cows meet psi-chotic yeast: The expansion of the prion hypothesis. *Cell* **89:** 495–498.

Mortimer R.K. and Johnston J.R. 1986. Genealogy of principal strains of the yeast genetic stock center. *Genetics* **113:** 35–43.

Sherman F. 1981. Genetic nomenclature. In *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance* (ed. J.N. Strathern et al.), pp. 639–640. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sherman F. and Lawrence C.W. 1974. *Saccharomyces*. In *Handbook of genetics: Bacteria, bacteriophages, and fungi* (ed. R.C. King), vol. 1, pp. 359–393. Plenum Press, New York.

Wickner R.B. 1981. Killer systems in *Saccharomyces cerevisiae*. In *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance* (ed. J.N. Strathern et al.), pp. 415–444. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Contents

EXPERIMENT I

Looking at Yeast Cells

Yeast cells are approximately 5 μm in diameter, and many of their important features can be seen in the light microscope. It is good laboratory practice to routinely examine cultures under phase microscopy for indications of the physiological state of the cells, and for evidence of contamination. Much of modern yeast cell biological work involves more sophisticated examination of yeast cells stained with protein-specific antibodies, or with fluorescent dyes that specifically associate with certain organelles. This experiment will provide examples of the standard types of light microscopy that are used in the examination of yeast cells.

EXAMINATION OF GROWING CULTURES

Growth Properties

Saccharomyces cerevisiae cells grow by budding. A cell that gives rise to a bud is called a mother cell, and the bud is sometimes referred to as the daughter cell. A new bud emerges from a mother cell close to the beginning of the cell cycle and continues to grow throughout the cell cycle until it separates from the mother cell at the end of the cell cycle. Because all of the growth of a yeast cell is concentrated in the bud, and because this growth is essentially continuous throughout the cell cycle, the size of the bud gives an approximate indication of the position of a given cell in the cell cycle. An exponentially growing culture of yeast cells has approximately one-third unbudded cells, one-third cells with a small bud, and one-third cells with a large bud. When cells in a growing culture use up the available nutrients, they stop growing by arresting in the cell cycle as unbudded cells. Thus, a simple way of determining the growth state of a culture is to determine the frequency of budded cells in the microscope. Note that for some strains, the mother and daughter cells remain stuck together even though they have completed cytokinesis. In these cases, it is necessary to vortex or sonicate the culture to separate cells prior to microscopy. Many kinds of mutants also arrest in the cell cycle in a way that is diagnostic of their phenotype. For example, cells in which there is a defect in the mitotic spindle arrest as large budded cells, a point in the cycle that would normally correspond to mitosis. It is important to note that the arrest point, or terminal phenotype, of mutant cells can be morphologically distinct from any cell type

seen in a normal culture. In the mitotic mutant above, the mother and daughter cells continue to grow at the arrest point until both are much larger than normal yeast cells.

Haploids vs. Diploids

Haploid and diploid yeast cells are morphologically similar but differ in several important ways. First, diploid cells are larger than haploid cells. Cytoplasmic volume increases with ploidy, and the diameter of a diploid cell is roughly 1.3 times that of a haploid cell. This difference can be readily seen when haploids and diploids are compared side by side. Because they are larger, diploid cells (or even tetraploids in some cases) are often used for fluorescence microscopy where the larger size helps in being able to resolve small cellular structures. Second, diploid cells tend to have a more elongated, or ovoid, shape than haploid cells, which are often almost round. Third, diploids and haploids have a different budding pattern. Yeast cells generally bud about 20 times before becoming senescent. Successive buds emerge from the surface of the mother cell in stereotyped patterns. Haploid cells bud in an axial pattern wherein each bud emerges adjacent to the site of the previous bud. Diploid cells bud in a polar pattern wherein successive buds can emerge from either end of the elongated mother cell. The history of a cell's budding pattern can be visualized by staining cells with Calcofluor, a fluorescent compound that binds to the rings of chitin that remain at old bud sites. These chitin rings are called bud scars, and we will use Calcofluor staining of haploid and diploid cells to visualize the axial and polar bud scar patterns.

Mating Cells

Yeast cells come in three mating types: *MAT***a** and *MAT*α; these two are able to mate with each other to yield a *MAT***a**/α. *MAT***a**/α cells cannot mate with cells of either mating type. Generally, *MAT***a** and *MAT*α strains will be haploids and MAT**a**/α strains will be diploid, although this is not always the case, and one should be careful to consider mating type independent of ploidy. The mating process between two cells begins with an exchange of pheromones that causes each of the cells to arrest in the cell cycle as unbudded cells and to induce the expression of proteins required for mating. The pheromone also causes the cells to make a projection of new cell surface specialized for cell fusion. This projection is usually oriented toward the mating partner. Cells with a mating projection are called "shmoos" because of their resemblance to an Al Capp cartoon character from the 1940s. The shmooing cells join at the tips of their projections, their cytoplasms fuse, and then their nuclei fuse to form a diploid *MAT***a**/α nucleus. The process of nuclear fusion is termed karyogamy. The newly formed diploid is termed a zygote and has a characteristic appearance that is particularly easy to identify when the first bud emerges. It is possible to isolate zygotes by micromanipulation, allowing for the isolation of diploid cells even in situations where there is no genetic selection for diploid formation. We will look at a population of mating cells to identify shmoos and zygotes.