

YEAST COMPLEMENTATION LABORATORY

INTRODUCTION

Because yeast is small, reproduces quickly, can reproduce both asexually and sexually, and is easily grown in the laboratory, it is a very useful organism for studies of genetics. In addition, the fact that yeast is eukaryotic, like humans, allow for experiments more applicable to humans than experiments performed using bacteria (which are prokaryotic). Because yeast is unicellular and can be grown as a haploid, mutant yeast can be easily isolated. Because the haploid has only one set of genes, the allele it carries determines the phenotype. By mating mutants, genetic analyses of yeast can be carried out.

We will be studying the common baker and brewer's yeast, *Saccharomyces cerevisiae*, one of the most commonly studied strains of yeast. Yeast have two separate mating types that are alike in structure and can only be differentiated by allowing them to mate. The two mating types are designated α and *a*. Cells of type α can only mate with cells of type *a*.

When an *a* cell meets an α cell, the two cells fuse. The result is a diploid zygote. Through mitosis and cell division, this diploid cell can form a population of genetically identical diploid cells. Under certain conditions, diploid cells can undergo meiosis and form haploid gametes. Two distinct and opposite mating types (*a* & α) of gametes are formed. The life cycle of *S. cerevisiae* is shown in Fig. 1a.

**key words: eukaryotic, haploid, diploid, allele, phenotype

Figure 1:

(a) *Life cycle*¹: The life cycle of *Saccharomyces cerevisiae* is a complete sexual cycle, in which the major stages are represented by cells of distinctively different shapes. Both the haploid and diploid phases are stable, and the transitions from one to the other can be controlled by environmental conditions. The major stages and cell shapes are shown.

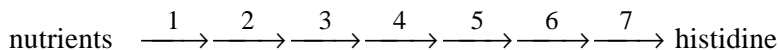
(b) A "shmoo"²: Named after this character from the "Li'l Abner" comic strip drawn by cartoonist Al Capp.

¹Manney, Thomas R. and Monta L. Manney. Yeast: A Research Organism for Teaching Genetics. *The American Biology Teacher*, vol. 54, no. 7, October 1992, pp.426-431.

²<http://sun10.vsz.bme.hu/~s6071far/pic/shmoo.gif>

Yeast can usually make many of the chemicals they need to live. Examples of these chemicals are histidine and tryptophan (amino acids) and adenine (part of DNA, ATP and other molecules). If yeast are missing the genes to make these chemicals (or if these genes are mutant), they will not grow unless they can get these chemicals from their food.

In this experiment, you will examine the histidine biosynthetic pathway. Histidine is an amino acid that can be made by the yeast by a series of enzymes if given the proper starting nutrients. There are seven genes (each coding for an enzyme) that are necessary to make histidine. Each enzyme is represented by a number in the diagram below:



Because a haploid yeast has only one set of genes, if it is mutant in any one of these enzymes, it cannot make histidine. Thus, it cannot grow if histidine is not present in the medium (i.e., it can grow only if histidine is present).

A diploid must have at least one normal copy of each gene to make histidine. Two mutant copies will result in a yeast which cannot make histidine. What mode of inheritance is this called?

THE CHALLENGE:

You will be given 8 different strains of yeast, four of each mating type.

Genetic notation in yeast:

1. 'his1' indicates a mutation involving enzyme 1 of the histidine biosynthetic pathway.
 2. 'trp' indicates a mutation in the ability to make tryptophan, another amino acid. Similarly, 'ade' indicates a mutation in the ability to make adenine.
 3. To indicate "normal" functioning genes, one would use 'HIS1,' 'TRP' and 'ADE.'
- In other words, capital letters = "normal" or wildtype while lowercase letters indicate a mutant. For a diploid made from crossing strain A and strain 1, one would describe its genotype (with respect to adenine and tryptophan) as: $\frac{a \text{ trp} \text{ ADE}}{\alpha \text{ TRP} \text{ ade}}$, where the genotypes above the line come from strain A and the genotypes below the line come from strain 1.

The genotypes of the mating type a are given below:

- strain A: MAT a his1, trp, ADE [HIS4, HIS5, HIS7]
- strain B: MAT a his4, trp, ADE [HIS1, HIS5, HIS7]
- strain C: MAT a his5, trp, ADE [HIS1, HIS4, HIS7]
- strain D: MAT a his7, trp, ADE [HIS1, HIS4, HIS5]

Strains of mating type α :

- strain 1: MAT α his?, ade, TRP
- strain 2: MAT α his?, ade, TRP
- strain 3: MAT α his?, ade, TRP
- strain 4: MAT α his?, ade, TRP

The genotypes of the mating type α are "unknown." You must determine which enzyme in the histidine pathway is defective for each strain. Each MAT α strain has a mutation corresponding to a mutation in the MAT a yeast. In other words, each strain of mating type α (strains 1, 2, 3 and 4) will either have mutation his1, his4, his5, or his7. **EACH α STRAIN HAS ONLY ONE MUTANT HISTIDINE GENE.**

STRATEGY:

You will learn how to identify these unknown mutants through **complementation tests**. This identification test is based on the idea of "simple dominance," which states: Only one dominant allele is required. That is, only one "good" copy of each gene is required to produce enough of each enzyme that is needed to make histidine. (In this case, all mutations are recessive.)

For example:

Mate:

MAT *a*: mutant in his1 and normal for HIS2 x MAT α : normal for HIS1 and mutant in his2

The diploid from this mating will be $\frac{a \text{ his1 } \text{HIS2}}{\alpha \text{ HIS1 } \text{his2}}$.

Thus, it will be able to synthesize histidine and will grow normally because it has a "normal" copy of both HIS1 and HIS2. In this case, the MAT *a* and MAT α strains "complement" each other--they compensate (make up for) each others' defects.

How to select for diploid cells: The MAT *a* types have a deficiency in an enzyme to synthesize tryptophan (trp) while the MAT α types have a deficiency in an enzyme to synthesize adenine (ade). This allows you, the student, to easily distinguish between haploid and diploid yeast (again, the concept of **complementation**). On plates that supply no additional nutrients (minimal media plates), the haploids cannot make either trp or ade and therefore will not grow. The diploid has the ability to make both; therefore it will grow on such plates.

<i>a</i> trp ADE → needs tryptophan	α TRP ade → needs adenine	$\frac{a \text{ trp } \text{ADE}}{\alpha \text{ TRP } \text{ade}} \rightarrow$ can grow on minimal media
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You will be using three different types of plates throughout this experiment:

YPD plate - contains all amino acids

SD plate - minimal media: only the essential nutrients (i.e., no amino acids)

SD + histidine - minimal media + amino acid histidine

Think about the consequences of plating on each plate:

Will strain A grow on a YPD plate? on a SD plate? on a SD + histidine plate? Why/why not?

DAY ONE

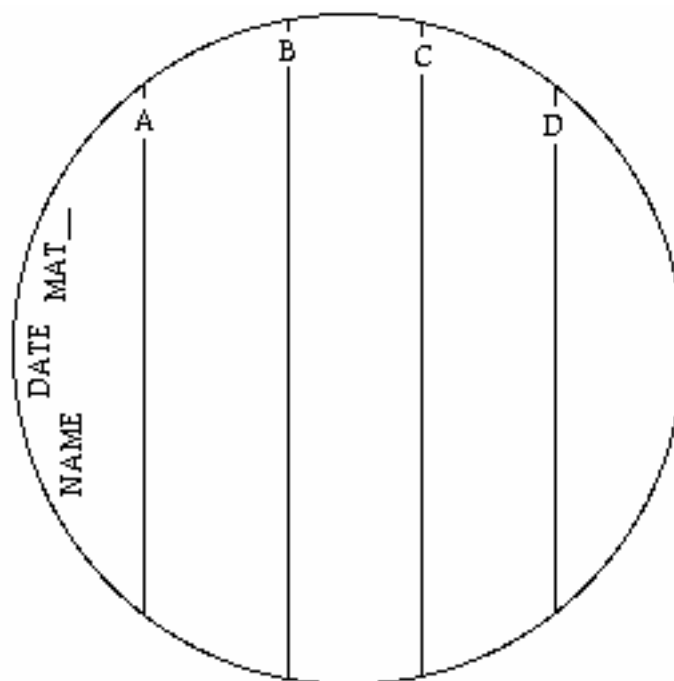
MATERIALS:

- 2 YPD plates
- four mutant strains (A, B, C, D) of mating type a
- four mutant strains (1, 2, 3, 4) of mating type α
- sterile toothpicks
- 30°C incubator: the cells may also be grown at room temperature but they will take longer to grow

PROCEDURE:

1. Obtain all necessary materials.
2. Label your YPD plates with name, date, mating type and strain number/letter. See Figure 1 as an example.
3. Place a YPD plate over the striped pattern below and streak the four mutant strains of the same mating type along the lines across the plate. **Be sure to use a different sterile toothpick for each strain.**

Figure 1: Sample Plate



4. Prepare a similar plate with the second group of four mutants.
5. Incubate both plates at 30°C for one day (or room temperature for 2 days - your teacher will specify).

DAY TWO

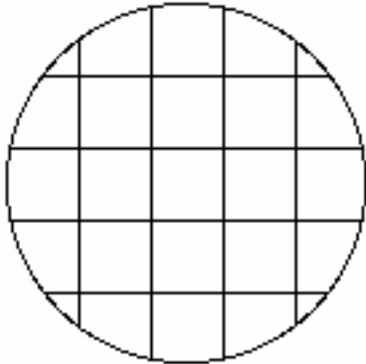
MATERIALS:

- 1 velveteen
- replica plating block
- 1 YPD plate

PROCEDURE:

1. Obtain your plates from the incubator.
2. Make an impression of one of the plates on a velveteen pad using the replica block. **Note the orientation of your strains.** This will be demonstrated by your teacher.
3. Using the **same** velveteen, press the second plate containing mutants of the opposite mating type so that the streaks are **perpendicular** to the first set. Again, **note the orientation of your strains.**

4. Imprint the checkerboard pattern from the velveteen to a new YPD plate. Be sure to label each line with the correct strain number/letter. Where do you think mating will occur? Where will you see growth?



Use this diagram to indicate where you predict growth to occur:
 - Label each line with the strain number/letter that corresponds to it.
 - Draw circles where mating will occur.

Check this diagram tomorrow (DAY 3) to see if your predictions are correct.

5. Incubate this plate at 30°C.
 6. Dispose of your 2 DAY ONE plates as directed by your teacher.

DAY THREE

MATERIALS:

- 1 velveteen
- 1 SD plate
- 1 SD + histidine plate

PROCEDURE:

1. Obtain your DAY TWO plate from the incubator. Check your predictions for growth from DAY TWO.
2. Obtain one SD plate and one SD + his plate. Label each with your name and the date. Make a mark to indicate the top of the plate.
3. Replica plate the YPD pattern to a SD plate **being sure to keep the orientation the same.**
4. Using the same velveteen, replica plate to a SD + histidine plate.
5. Again, label the lines on both plates with the correct strain number/letter.
6. Incubate at 30°C for one day.

DAY FOUR

1. Score the growth at the intersections. Record in Table 2 using a '+' to indicate growth and a '-' to indicate no growth.

Table 2: Growth on SD and SD + His plates

α a	1	2	3	4
A				
B				
C				
D				

α a	1	2	3	4
A				
B				
C				
D				

ANALYSIS

1. Fill out the chart below, indicating the complete genotype of the diploid:

	1	2	3	4
A	$\frac{a}{\alpha} \frac{\text{trp}}{\text{TRP}} \frac{\text{ADE}}{\text{ade}}$			
B				
C				
D				

- Using the information in Table 2 and the table above, determine which strains **complement** one another and which strains don't complement. Support your answer with your data.
- What are the genotypes of the α strains?
- Explain the differences in growth along the lines and at the intersections on the YPD plate from DAY TWO, the SD plate from DAY THREE, and the SD + His plate from DAY THREE.
- What is the purpose of the SD + His plate? What would you conclude if an intersection did not grow on the SD plate and the SD + His plate?
- Go back to the prediction of growth of haploids on Table 1. Were your predictions correct? Use your observations throughout this lab to either justify or correct your predictions.

Pre-Lab Questions

1. Why are the MAT α strains trp mutants, and the MAT a strains ade mutants? What will that allow you to do in this lab?
2. Write out the genotype of a strain that is mating type α , mutant in histidine enzyme 3, normal for tryptophan, and mutant for adenine.
3. Fill out Table 1, predicting whether each different strain would grow on each type of plate.

Table 1: Predicted Growth on Various Types of Plates

(Use a '+' to indicate growth and a '-' to indicate the absence of growth)

strain	YPD	SD	SD + His
A			
B			
C			
D			
1			
2			
3			
4			

Be sure to come back to this chart after you have completed your experiment to check your predictions.

4. Explain why you filled in the table the way you did for strain A? For strain 3?
5. What are you trying to find out in this experiment?