

Biology 423L

Sept. 8/9

**Genes and Alleles in yeast.**

Report due Sept 22/23.

**Readings:** Genes and alleles: Hartwell Chapter 7 pp225-226.  
see web site for the following PDFs:  
Bakers Yeast and its Life Cycle  
A Closer Look at Adenine-requiring Mutants in Yeast-  
A Classroom Guide to Yeast Experiments:  
Genetics of Baker's Yeast.

**PART 1 Complementation test using yeast**

**Objectives:** This experiment would be done after a scientist has performed a genetic screen to identify new mutations. Each mutant will be crossed to the parental strain (wild type) to test whether the mutations are dominant or recessive to the wild type alleles. In addition, yeast mutants that have the same phenotype will be mated together to see if the mutations are in the same gene or in different genes. If the mutations can complement each other when they are combined in diploid yeast, they are mutations in different genes. If they cannot, the mutations are alleles of the same gene..

**Introduction:** Yeast has proven to be an indispensable model organism for the study of biochemical pathways, eukaryotic gene regulation, principles of cell division and replication. Yeast can be grown in large numbers quickly in the laboratory. It grows on defined media which allows selection of mutants with nutritional requirements. The mutants we will examine today have a deficiency in the ability to synthesize their own adenine. However, they will grow when they can get adenine from the medium. These mutants also have a red color when grown on adenine supplemented medium.

One advantage of yeast is its ability to form colonies as haploid cells and as diploid cells. This allows us to characterize mutations in haploids. Because each cell has only one copy of each gene, mutations that lead to loss of function can be seen as easily as mutations that lead to a gain of function. However when haploid cells of opposite mating types are grown together, they mate and form colonies of diploid cells with two alleles of every gene. Traits like the inability to synthesize adenine will affect the growth of both haploid and diploid colonies. This backcross allows us to test if the mutations are dominant or recessive to alleles in a reference strain, the normal or wild type. In addition, we can mate two mutants with similar phenotypes to test if the mutations are in alleles of the same gene or if they are changes in two different genes. This is called a complementation test.

Mating requires combining haploid cells of opposite mating type. There are two mating types in yeast,  $a$  and  $\alpha$ . They are alternate alleles at the mating type locus  $MATa$  or  $MAT\alpha$ . The control of yeast mating type is well characterized. You can find some additional information in the Classroom Guide to Yeast Experiments pp. A6-8 and A11.

Many proteins are required to produce adenine from amino acids and sugars. (see a A Closer Look at Adenine-requiring Mutants in Yeast F1-5 on web site).

Therefore, when mutants are identified based on the inability to synthesize adenine, any one of several genes could be mutated. The mutations could be recessive. For example, if a necessary enzyme catalytic function was lost in the protein sequence change. However other mutations can be dominant. For example, a transcription activating protein that regulates production of an enzyme needed for adenine synthesis can be mutated so that it no longer promotes transcription but instead represses it. If the mutant protein binds its target promoter irreversibly, functional transcription activators cannot bind and the adenine enzyme will not be made even if the cell has a functional allele for the transcription factor in addition to the mutant allele.

In this experiment, we will mate several haploid strains representing eight different genotypes. They differ in mating type alleles, and in two independently selected adenine requiring mutations, *ade1* and *ade2*. Two different media will be used to distinguish the mutant strains from the normal phenotype, YED a medium supplemented with yeast extract which contains adenine and many other essential nutrients, and MV a defined minimal medium that does not contain adenine.

### **Materials:**

Mating type a strains:  
A1, A2, A3, A4.

Mating type  $\alpha$  strains  
 $\alpha$ 1,  $\alpha$ 2,  $\alpha$ 3,  $\alpha$ 4

Some strains may have a mutation (variant allele) in more than one gene required for adenine biosynthesis.

2 Petri plates of YED medium **Be sure plates are dry when lab begins**  
Petri plate of MV medium

Sterile Toothpicks  
Mating grid  
Tape

Recipes: YED medium for 1 liter (Yeast Extract Dextrose)  
10 g Yeast extract  
20 g anhydrous dextrose  
20 g Agar  
water to 1 liter.

Autoclave 20 minutes on liquid cycle.

MV medium: (Minimal with Vitamins)  
1.5 g Yeast Nitrogen bases without amino acids and ammonium sulfate  
5.2 g ammonium sulfate  
20 g anhydrous dextrose  
20 g agar  
water to 1 liter  
Autoclave 20 minutes on liquid cycle.

**Method:**

Each student will set up their own mating experiment. Media in plates will be provided.

**Day 1: Set up mating plate**

You can use the mating grid at the end of the protocol to align your patches for mating. Tape one to the bottom of a YED plate

Using a fresh sterile toothpick for each strain, make streaks of the haploid strains across the top and down the side of the plate.

Use the grid as a guide.

Incubate the plate overnight at 30 degrees.

**Day 2: Make crosses**

Using a fresh, sterile toothpicks for each transfer, transfer and mix the haploid strains in all possible combinations at the intersections of the grid.

Remember to use a new toothpick for each different strain. The best results are obtained when only small amounts of yeast are used to make each mating mixture. Just touch the tip of your toothpick lightly in the yeast. It will pick up 1000s of cells.

Incubate the plate overnight at 30 degrees.

**Day 3: Replica plate to MV minimal medium and a fresh YED plate**

Use toothpicks to replica plate the YED plate onto MV and a fresh YED plate. It is very important to transfer a tiny bit of yeast. If there are large numbers of yeast on the minimal plate the transferred colonies will have enough adenine in them to allow a bit of growth on the MV plate even if the mutations have not been complemented. Just touch your toothpick into the mating mixture and then spread a small square of cells on the MV plate. You can use the same toothpick for transfer to both the MV and the YED plates. Do the YED plate first. Use a fresh toothpick for every mating combination.

Incubate the plates overnight at 30 degrees.

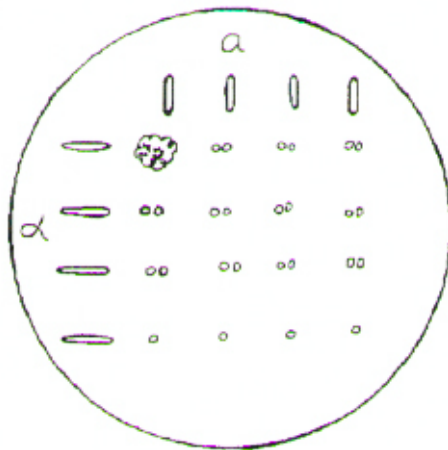
**Day 4:** Read the colors of the mixtures from the second YED plate and the growth pattern on the MV plate. If the mixtures are not mixed thoroughly there may be pink areas in otherwise cream-colored (white) mixtures. These mixtures should be scored as cream-colored (white).

Record your results in a table (results section).

**Questions for discussion of lab report:** 1. Why is yeast an ideal organism to use for this allelism test? Give me two reasons.

2. When several mutations are identified in haploid colonies, you must introduce the mutations into two mating types in order to intercross the mutants for the complementation test. Assuming you mutated a strain of mating type  $\alpha$  in order to select your mutations, what crossing strategy do you need to use in order to get your mutation into mating type  $\alpha$ ? Assume that *MAT $\alpha$*  and your mutation are not closely linked.

Example of how YED plate should be set up:



Mating grids:

	A1	A2	A3	A4
alpha1				
alpha2				
alpha3				
alpha4				

	A1	A2	A3	A4
alpha1				
alpha2				
alpha3				
alpha4				

	A1	A2	A3	A4
alpha1				
alpha2				
alpha3				
alpha4				

	A1	A2	A3	A4
alpha1				
alpha2				
alpha3				
alpha4				

**Report:** You will find information on the format of lab report on the course information page on the lab web site. For this report write an abstract that summarizes what you did and the results you obtained. Write a short introduction stating the purpose of the experiment and introducing the organism and genes involved.

For the materials and methods section: refer to the lab exercise unless you made any changes to the protocol.

Results: make a table of the results. Describe the results briefly and refer to your table. It is there to make your written description easier and shorter.

Discussion: Explain which strains have allelic mutations and how you know they are allelic. Answer the questions in the lab protocol. Finish with a concluding statement summarizing the results of the lab. Refer back to the purpose of the lab exercise you presented in the introduction.

**Note for TA:**

Demonstrate how to use toothpick to streak yeast colonies.

Emphasize how important it is to use a small amount of yeast. It should not be visible on the toothpick or the plate.

Show where the minimal and YED plates can be found for the final plating and where to incubate the yeast.

Arrange to have the lab room open at times when students can finish the experiment.