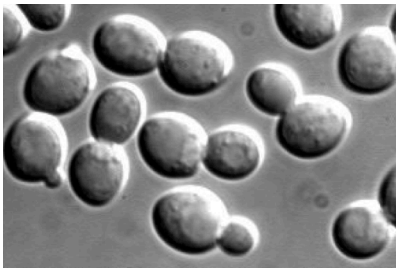
# Introduction to Yeast Genetics

For the next two weeks we will be studying the life-cycle and genetics of the budding yeast, *Saccharomyces cerevisiae.* We will carry out many different, but related experiments. In one experiment we will follow yeast through its entire life-cycle; from haploid to diploid to haploid again. We will demonstrate the process of gene complementation through genetic crosses. We will examine the effect of the environment on the phenotype of yeast. Some of these experiments will require only a few days, while others will go for our full two weeks. To avoid confusion, it is essential that you **label plates very well** and come to lab well prepared.



The budding yeast, *Saccharomyces cerevisiae,* is a unicellular, eukaryotic fungi. It can grow aerobically or anaerobically in simple media and has a doubling time of 1.5 hours under ideal conditions. It is used in the baking industry to make bread rise and in the beer and wine industry to produce alcohol by fermentation. *Saccharomyces cerevisiae,* is a popular research organism because it is the simplest of all the eukaryotes. Our understanding of many essential cell processes, such as cell-cycle control and the secretion pathway, has been gained through studying *Saccharomyces cerevisiae.*

Yeast can exist in either a diploid or haploid state and have the ability to reproduce either sexually or asexually. Haploid yeast exists as one of 2 mating types; MATa or MATα. If kept separate from each other, these mating types can be maintained in a haploid state indefinitely, however, when these 2 mating types encounter each other they fuse to become a diploid cell (see figure 1). The diploid state can also be maintained indefinitely as long as the culture has a good a supply of nutrients. Under starvation conditions however, the diploid cell will sporulate (undergo meiosis) to produce an ascus with four haploid spores. The ascus has a strong shell that protects the spores from environmental conditions. Germination of the ascus and its spores occurs when a nutrient supply is restored. Otherwise the ascus may remain intact for long periods of time, awaiting better environmental conditions.

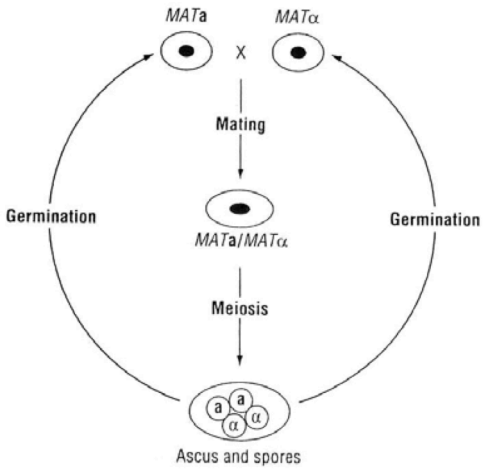



Figure 1. Yeast Life Cycle

Budding yeast radically change their morphology as they progress through their life cycle (refer to figure 2 below). Cells undergoing cell division by mitosis form a small bud during S phase. The bud enlarges as the cell progresses through G2 and Mitosis. Finally the bud separates from the mother cell. During this process the mother cell does not change size. The budding process is the same in both haploid and diploid yeast cells.

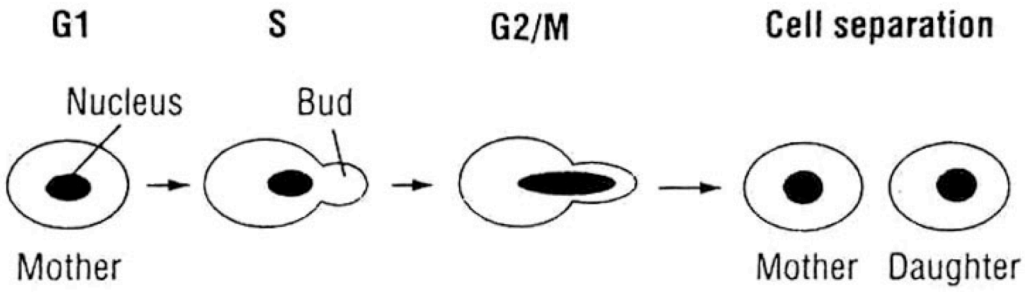
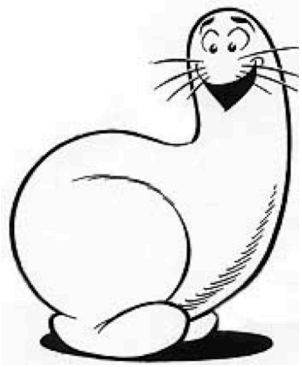



Figure 2. Cell division occurs by budding.

When haploid yeast cell of opposite mating types (Matα and Mat**a**) encounter one another they produce pheromones that induce mating behavior (see figure 3 below). Initially they adopt a pear-like shape. They are now called schmoos (after a cartoon character from the 1940s). Two schmoos fuse together at their tips to become a diploid zygote (a/α). Soon after fusion, the diploid begins cell division by budding. The culture will remain in a diploid state indefinitely until harsh environmental conditions (such as starvation) induce meiosis and formation of asci.



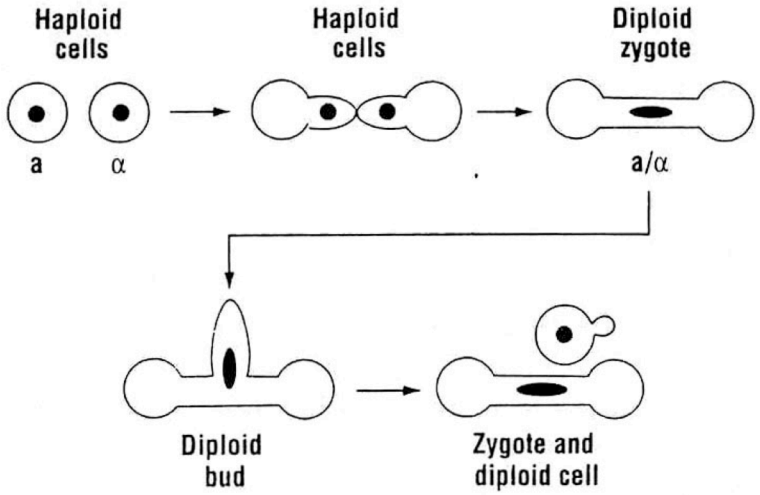



Figure 3. Mating Behavior in Budding Yeast

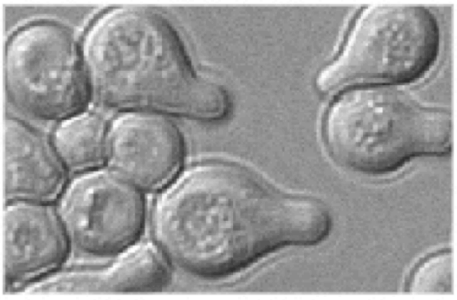


Figure 4. Schmoos

## Observation of a Simple Cross Day 1

Today's experiment demonstrates the phenomenon of complementation through mating. You will be given four haploid strains of *Saccharomyces cerevisiae,* HA1, HAR, HB1, HBR. The "A" strains are mating type "a" while the "B" stratins are mating type "α". The "1" mutants have mutations in ADE1, while the "R" strains have a mutation in ADE2. The strain colony color will be white or red depending on the presence or absence of adenine in the media.

The red colony color of the ADE mutatnts is due to the accumulation of an intermediate, P-ribosylamino imidazole (AIR), from the adenine biosynthesis pathway (see figure 5 below). P-ribosylamino imidazole is not a red molecule initially, but is oxidized to become a red pigment in an aerobic environment. The accumulation of this pigment decreases the growth rate of ADE mutant cells and thus provides a strong selection for additional mutations that reduce the production of the pigment. Consequently, new white mutants arise in otherwise red ADE mutant cultures at a high frequency.

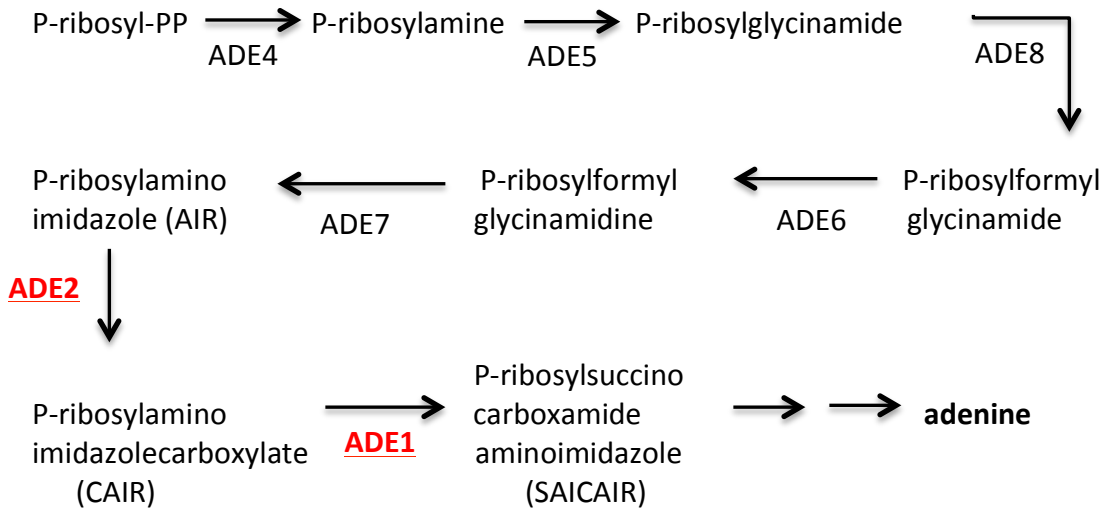



Figure 5. Adenine Biosynthesis Pathway (ADE 1-8 are different enzymes coded for by different genes)

The growth of ADE mutant yeast strains varies depending on the type of agar media on which they are grown. The characteristics of the media used in the experiment are listed below.

- YED media is a complete medium that contains all the nutrients yeast need to grow. It contains a small amount of adenine and therefore it supports the growth of HA2 and HB1 strains. Although adenine-requiring mutants grow well on this media, they do accumulate the characteristic red pigment.
- MV media is a minimal media that contains the minimum amount of pure chemicals required to support the growth of wild-type yeast. MV lacks adenine, therefore HA2 and HB1 mutants are unable to grow on this media.
- YEKAC media is a nutritionally unbalanced starvation media that induces sporulation of diploid yeast to form asci containing 4 haploid spores.

## Experimental Timeline for Simple Cross

The timeline of the experiment is listed below:
Day 1. Mate haploid strains (Monday July 9th)
Day 2. Select for diploids on MV plates (Wednesday July 11th)
Day 3. Transfer diploids to YEKAC plates for sporulation (Friday July 13th)
Day 4. Observe asci and streak for single colonies. (Monday July 16th)
Day 5. Observe restoration of red phenotype (Wednesday July 18st)

**Observation of a Simple Cross Day 1**

The adenine biosynthesis pathway requires several enzyme-catalyzed steps (refer to figure 5 on page 3 above). Mutations in any of the genes encoding these enzymes could potentially inhibit the synthesis of adenine and result in the conversion of cream-colored cells to red cells. Assume a scientist has introduced random mutations into a wild-type, cream-colored population of yeast cells, then screened the mutant population for red colonies, and found several red mutant colonies. Will all of those red colonies have mutations in the same gene or might they have mutations in different genes within the adenine biosynthetic pathway. This question can be answered with the following complementation test. In this experiment you will cross four haploid red strains. HA1 and HAR are both mating type a, while HB1 and HBR are both mating type α. You will perform the following crosses: HA1 with HB1, HAR with HBR, HAR with HB1, and HA1 with HBR.

1. Obtain a YED plate that has all 4 haploid, red mutants. Label it as "mating mixtures" and with your initials.
2. Using sterile toothpicks transfer a small amount of each mutant to the positions shown in figure 6.
3. Using new sterile toothpicks, mix each pair of strains together so they can mate. Your plate should now look like figure 7.

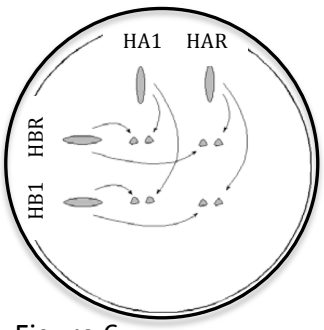


Figure 6.

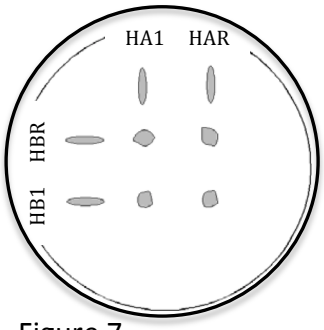


Figure 7.

4. Draw a sketch of the yeast cultures in your lab notebooks. Label the yeast strains, the streak color, the kind of medium, and the date of the procedure.
5. Incubate your culture at 30° Celsius until our next class meeting

**Observation of a Simple Cross Day 2**

1. Your mating mixtures have now grown into large colonies. Record the appearance of your plate by making a drawing of it in your lab notebooks. Label the parents and

the mixtures and describe their colors. Be sure to write down what kind of medium (YED or MV) cells are growing in, the color and appearance of the yeast, and the date.

Answer the following questions in your lab notebook:

a. What is the color of the mating mixture colony? Are the mating mixtures consistent in their appearance? Provide a rationale for this color phenotype.
b. On the basis of this observation, is the red phenotype dominant or recessive? Explain.

Your mating mixture is likely a combination of haploids and diploids. Both of the haploid strains can grow on the rich YED medium, but neither can grow on the nutritionally poor MV medium. However, the diploid cells formed from the right combination of two haploid strains can grow on MV. By putting the cells on MV you can select for diploid cells that have complemented one another, allowing for the completion of the adenine pathway.

c. Hypothesize about how you think your colonies will look after growing on the MV medium. Explain.

2. Make a copy (replica) of the YED plate by transferring the original haploids and the mating mixture onto an MV plate.
    a. View the video "Replica Plating" found on our Moodle page.
    b. Assemble your replica-plating tool according to the instructions in the video tutorial.
    c. Press the replica tool onto your YED yeast plate from day one.
    d. Stamp the cells onto your MV plate.
    e. Label this MV plate with your name, the date and "Selecting Diploids".
    f. Incubate the plate at 30°C for two days.

3. Make a wet-mount slide from any one of the parent strains provided and look at it through the microscope. Place a small drop of water on a glass slide, transfer a very small amount of yeast to the water with a sterile wire loop, mix the yeast to obtain a uniform suspension, and then place a cover slip over the dilute culture.

4. Draw a sketch of about 10 of these cells in your lab notebooks. Draw cells in various stages of the cell cycle (budding or not budding) and label appropriately.

5. Make a wet-mount slide of the mating mixture provided for you by Dr. Loomis and look at it through the microscope. Attempt to find and draw schmoos, diploid zygotes, budding zygotes and any other cell types you observe. Make your drawing in your lab notebooks. Label unbudded zygotes with "Z," budded zygotes with "BZ," and shmoos with "S."

**Observation of a Simple Cross Day 3**

1. Obtain your “Selecting Diploids” plate from the incubator. In your lab notebooks,

make a sketch of the yeast cultures seen on the plate. Label the yeast strains, the culture color, the quantity of growth (robust or poor) and the kind of medium.

**Questions:**

a. Some mating mixtures are predicted to grow well on the MV plate, while the parent haploid strains and other mixtures grow poorly or not at all. Do your observations match these predictions?
b. Why do these predictions make sense? Explain.

2. Make a wet mount of cells from the mating mixture on the "Selecting Diploids" plate and observe them in the microscope. Draw several cells in your lab notebooks.

3. Diploid cells can be induced to undergo meiosis and sporulation. Before inducing the yeast to sporulate, it is better to transfer the diploid cells back to a YED plate. Cells on YED medium will grow faster than on MV medium and rapidly growing cells will sporulate better. Dr. Loomis transferred some of the diploids from the MV plates to a YED plate and labeled this YED plate as "Prespor". The "Presporulation" plate was incubated at 30° C for 1day. Record these steps in your notebooks

By this point you have seen half of the yeast life cycle; mating between two haploids to form a stable diploid cell. You can now see the other half of the life cycle by sporulating the diploid, to obtain four haploid spores. To do this you need to transfer the diploid cells to sporulation medium (YEKAC). YEKAC contains no nitrogen source and only a nonfermentable carbon source (acetate). When diploid cells try to grow on YEKAC, they sporulate and go through meiosis. Meiosis produces two important results: the chromosome number is reduced from diploid to haploid, and the resulting haploid cells have all possible combinations of the adenine and mating-type genes

1. Using a sterile toothpick, transfer diploid cells from your "Presporulation" plate to a YEKAC plate. Label the YEKAC plate as "Sporulation" and with your initials and the date.
2. Incubate the plate at 30°C until Monday.

## Observation of a Simple Cross Day 4

1. Make a wet-mount slide of a sample from the "Sporulation" plate and examine it with a microscope. Look for lumpy cells that appear to have two, three, or four round spores inside a membrane. These are the asci. They should all have four haploid spores, but sometimes some of the spores don't develop. Draw a sketch of several asci in your lab notebook.

Questions:

a. What characteristic of YEKAC plates induces diploid yeast to sporulate?
b. What advantage is gained when yeast sporulate?

2. Now you will try to isolate and grow individual haploid colonies from the "Sporulation" culture, completing the yeast life cycle from haploid to diploid and back to haploid. Obtain a sterile toothpick. Scoop up some cells from the YEKAC "Sporulation" plate and streak for single colonies on a YED plate.
3. Label the YED plate as "Restoration of Haploids".
4. Incubate the YED plate in incubator for until Wednesday.

## Observation of a Simple Cross Day 5 (Friday July 21st)

When you put spores back onto YED growth medium, they germinate, begin budding, and grow into colonies. Since some will be mating type **a** and some mating type α, they may also mate. Therefore, the colonies that grow may be both haploid or diploid cells and either pink or cream-colored.

1. Look for different colors among the colonies. Did you or anyone in the class observe the reemergence of the red colony color in some colonies? Explain why this result is likely.
2. Draw and label a sketch of this plate in your lab notebooks.

**Questions**

a. None of the haploid ADE mutant strains can grow on minimal media, yet the diploid produced by some matings can grow on minimal media while others can't. Explain why this is so.
b. Based on your results, do the various red mutants have mutations in the same gene or different genes?
c. Briefly describe the life-cycle of budding yeast.