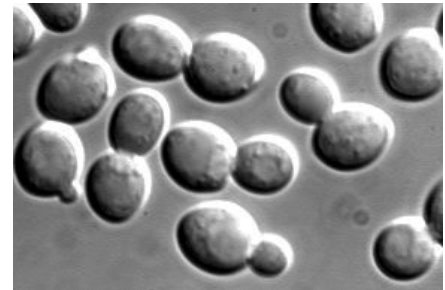


Lab # 12 Yeast Genetics

For the next 2 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 a 2 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; MAT_a or MAT_α. 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 below). The diploid state can also be maintained indefinitely as long as the culture has a good supply of nutrients. Under starvation conditions however, the diploid cell will sporulate (undergo meiosis) to produce an ascus with haploid 4 spores. The ascus is 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.

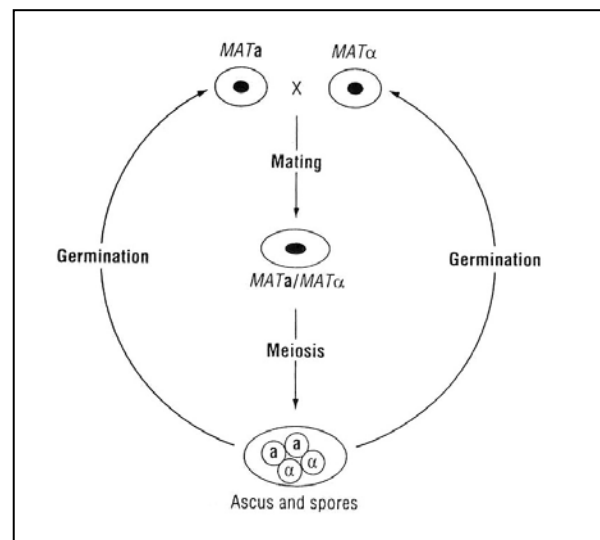


Figure 1. Yeast Life Cycle

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.

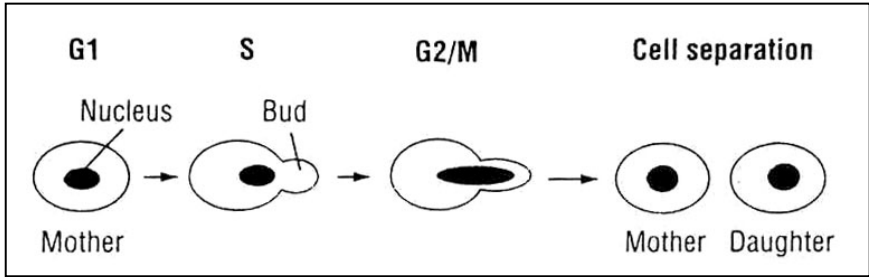


Figure 2. Cell division occurs by budding.

When haploid yeast cell of opposite mating types ($Mat\alpha$ and $Mat\alpha$) 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.

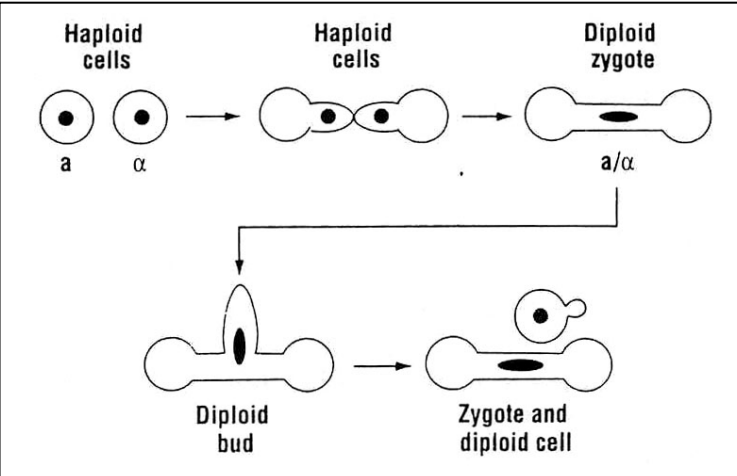


Figure 3. Mating behavior in budding yeast.

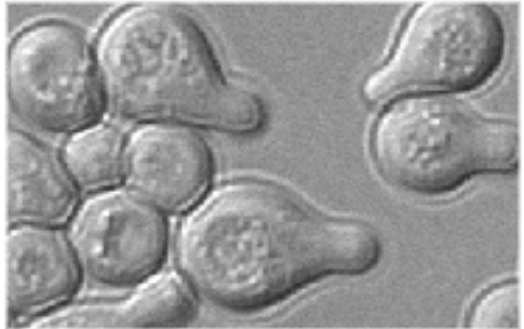


Figure 4. Schmoos

Part 1

Observation of a Simple Cross

Day 1 (4-17-12)

Today's experiment demonstrates the phenomenon of complementation through mating. You will be given two haploid strains of *Saccharomyces cerevisiae*, HBT and HA2. The characteristics of these mutants are listed below:

HBT

- Haploid
- Mating type α
- Deficient for tryptophan synthesis
- White colony color on all media

HA2

- Haploid
- Mating type a
- A mutation in the ADE2 gene makes this mutant deficient for adenine biosynthesis
- White or red colony color depending on the presence or absence of adenine in the media

The red colony color of the HA2 strain 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 HA2 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 HA2 cultures at a high frequency.

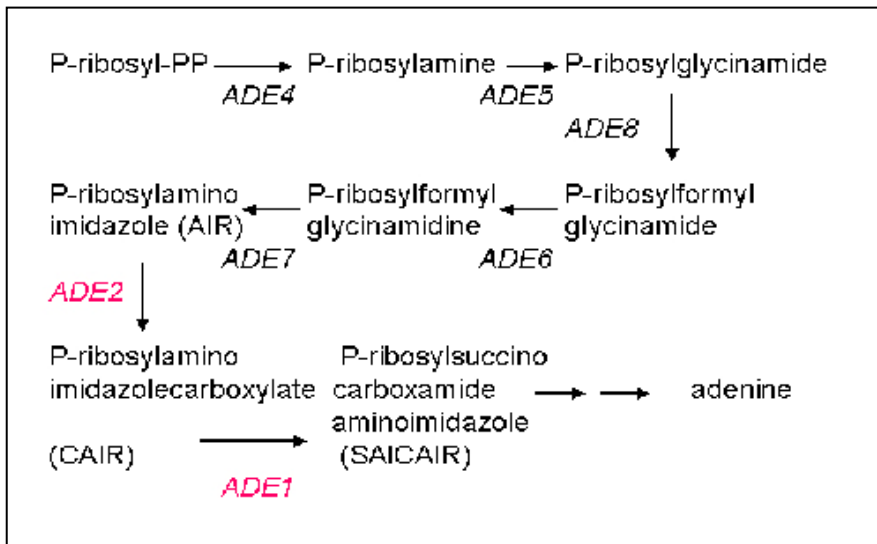


Figure 5. Adenine Biosynthesis Pathway (ADE 1-8 are enzymes)

The growth of HBT and HA2 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 media that contains all the nutrients yeast need to grow. It contains a small amount of adenine and tryptophan therefore it supports the growth of HA2 and HBT 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 and tryptophan, therefore HA2 and HBT 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

This experiment will be carried out over the course of 3 weeks. The timeline of the experiment is listed below:

Tuesday 4-17-12, Day 1. Mate haploid strains HBT and HA2

Thursday 4-19-12, Day 2. Select for diploids on MV plates.

Tuesday 4-24-12, Day 3. Transfer Diploids to YED plates for presporulation.

Thursday 4-26-12, Day 4. Transfer diploids to Yekac plates for sporulation.

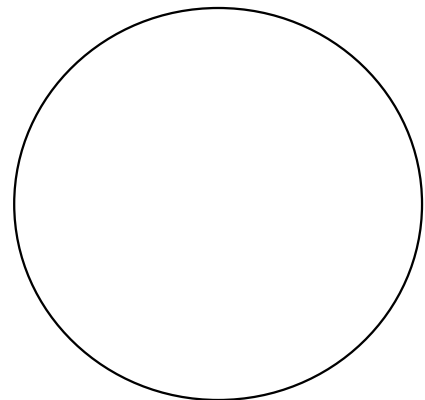
Tuesday 5-1-12, Day 5. Observe asci and streak for single colonies.

Tuesday 5-8-12, Day 6. Observe restoration of red phenotype.

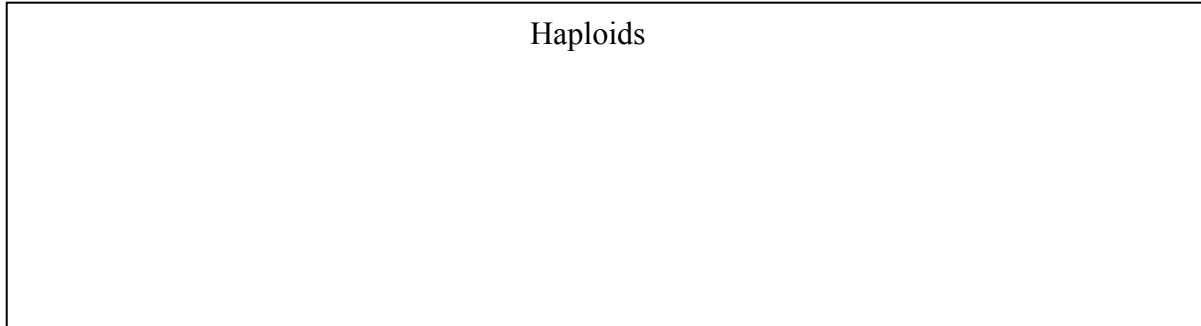
Observation of a Simple Cross

Day 1 (4-17-12)

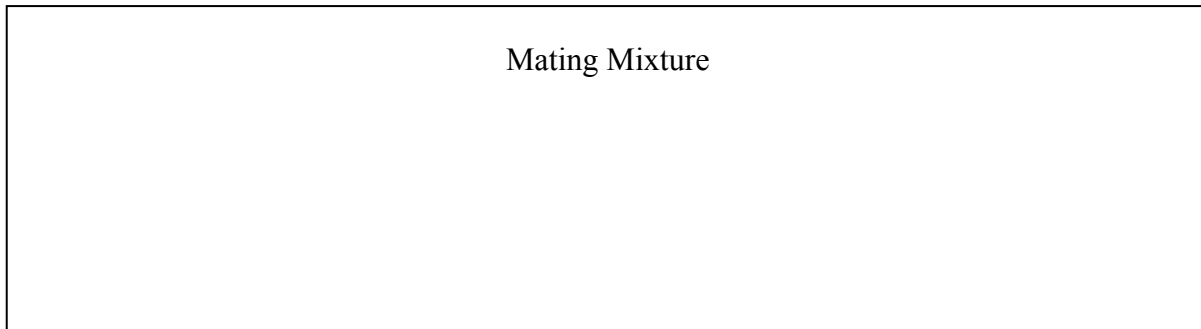
1. You will be provided with a YED plate containing a culture of both HA2 and HBT strains. Label this plate as “Mating”, with your initials, and the date. Label with a permanent marker on the bottom edge of the plate.
2. Touch a sterile toothpick to the HBT streak on the YED plate and then touch it to the agar at the center of this same plate. Discard the toothpick.
3. Touch another sterile toothpick to the HA2 streak and touch it to a spot close to the spot you just made at the center of the plate. Discard the toothpick.
4. Then use a third sterile toothpick to mix the two spots of yeast together. Be gentle, don't tear up the surface of the agar.
5. Draw a sketch of the yeast cultures in the empty circle to the right. Label the yeast strains, the streak color, the kind of medium, and the date of the procedure.
6. Incubate your culture at 30⁰C for 3 hours.



7. After 3 hours, make a wet-mount slide from either one of the parent strains (HA2 or HBT) 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. Draw a sketch of about 10 of these cells in the rectangle below. Draw cells in various stages of the cell cycle.



8. Make a wet-mount slide of the mating mixture 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 the rectangle below. Label unbudded zygotes with "Z," budded zygotes with "BZ," and shmoos with "S."

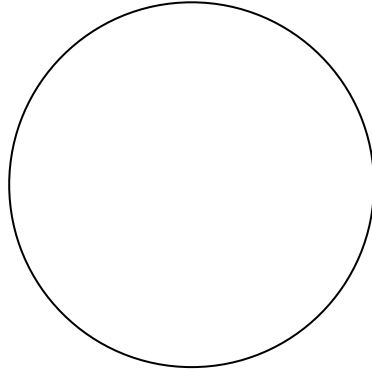


9. Incubate your mating mixture at 30°C for 2 days.

Observation of a Simple Cross

Day 2 (4-19-12)

1. Your mating mixture has now grown into a large colony. Record the appearance of your plate by making a drawing of it in the circle below. Label the parents and the mixture 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.



What is the color of the mating mixture colony? Provide a rationale for this color phenotype.

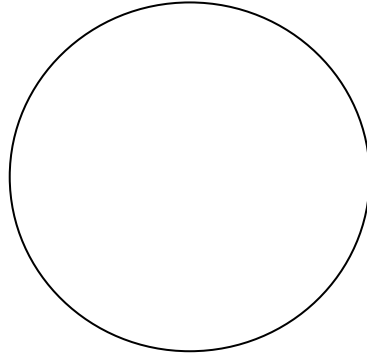
On the basis of this observation, is the red phenotype dominant or recessive? Explain.

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 these two haploid strains can grow on MV. By putting the cells on MV you can select for diploid cells.

2. Make a copy (replica) of the YED plate by transferring the original haploids and the mating mixture onto an MV plate. Make the streaks the same size and shape as those on the original plate.
 - a) With a sterile toothpick pick up some HBT cells streak from your YED plate. Then make a streak on the MV plate. Discard the toothpick.
 - b) With another sterile toothpick pick up some HA2 cells from YED plate and make a streak of HA2 cells on the MV plate. Discard the toothpick.
 - c) With a third sterile toothpick pick up some of the mating mixture in the middle of the YED and then streak cells in the center of the MV plate. Discard the toothpick.
 - d) Label this MV plate with your name, the date and "Selecting Diploids".
 - e) Incubate the plate at room temperature until the next lab period on tuesday.

Observation of a Simple Cross
Day 3 (4-24-12)

1. Obtain your “Selecting Diploids” plate from the instructor. The cultures on this plate have been growing on MV media for 5 days. In the empty circle below, 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.

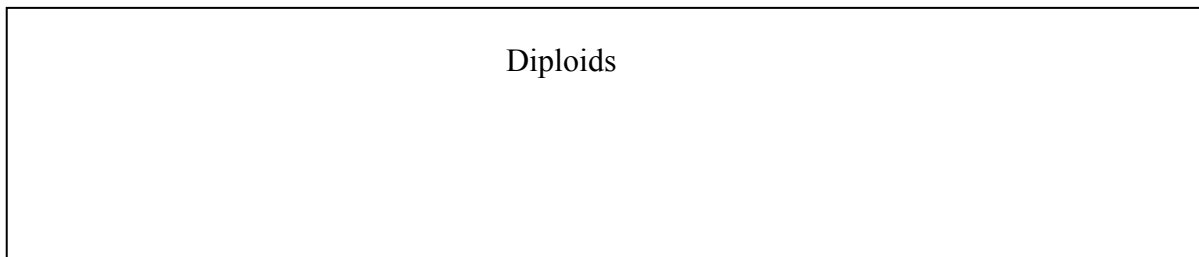


Questions:

The mating mixture is predicted to grow well on the MV plate, while the parent haploid strains grow poorly or not at all. Do your observations match this prediction?

Why does this prediction 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 the rectangle below.



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. Using a sterile toothpick, transfer some of the diploids from your MV plate to a YED plate. Label this YED plate as “Presporulation”. Incubate the “Presporulation” plate at 30⁰C for 2 days.

Observation of a Simple Cross

Day 4 (4-26-12)

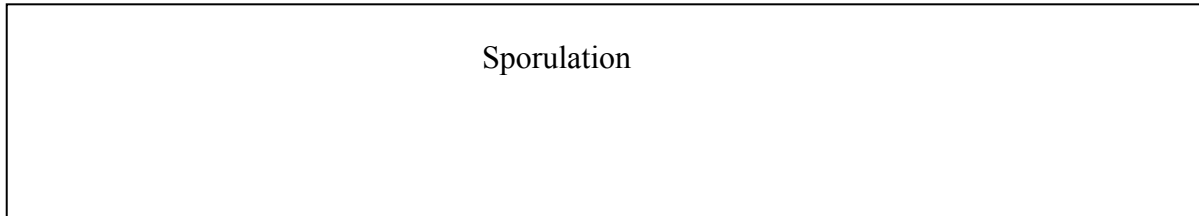
By this point you have seen half of the yeast life cycle; mating between two haploids to form a stable diploid cell. You can now see the other half of the life cycle by sporulating the diploid, to obtain four haploid spores. To do this you need to transfer the diploid cells to sporulation medium (YEKAC). 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, tryptophan 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 room temperature until the next lab period on Tuesday.

Observation of a Simple Cross

Day 5 (5-1-12)

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 the rectangle below.



Questions:

What characteristic of YEKAC plates induces diploid yeast to sporulate?

What advantage is gained when yeast sporulate?

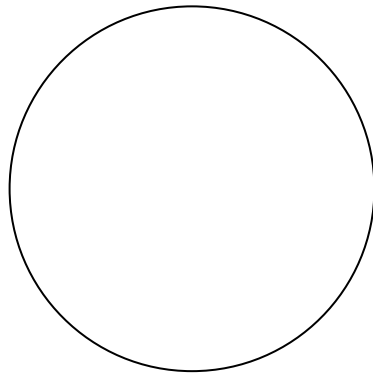
2. Now you will try to isolate and grow individual haploid colonies from the “Sporulation” culture, completing the yeast life cycle from haploid to diploid and back to haploid. Obtain a wire loop and sterilize it in the flame of a Bunsen burner. Scoop up some cells from the YEKAC “Sporulation” plate and streak for single colonies on a YED plate.
3. Label the YED plate as “Restoration of Haploids”.
4. Incubate the YED plate at room temperature for 7 days

Observation of a Simple Cross

Day 6 (5-8-12)

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 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 the circle below.



Questions

- 1) Neither HBT nor HA2 strains can grow on minimal media, yet the diploid produced by mating these strains can grow on minimal media. Explain why this is so.
- 2) Briefly describe the life-cycle of budding yeast.

Part 2: Environmental Effects on Colony Color

Day 1. (4-17-12)

The traits that we observe in an organism are determined not only by the genetics of the organism, but also by the environment. The red colony color of the HA2 strain of *S. cerevisiae* is due to a mutation in an enzyme in the adenine biosynthetic pathway. The absence of this enzyme results in the build-up of a colorless intermediate that becomes oxidized to form a red pigment. The production of this red pigment can be dramatically affected by the presence or absence of oxygen and adenine in the environment.

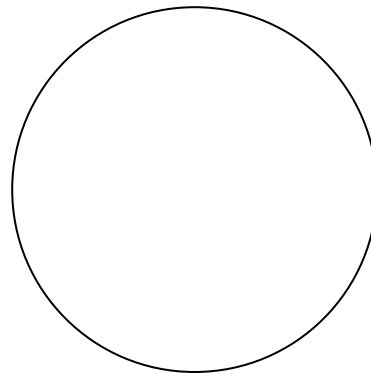
A. Effect of Adenine Concentration on Pigment Formation

1. Work as a group for this experiment.
2. Make a visibly turbid suspension of the HA2 cells: Scrape some yeast cells from an overnight culture with a sterile wire loop. Resuspend the cells in 1 ml of sterile water. Pipet the cells up and down until the suspension is uniform. If the suspension is not visibly cloudy, add more yeast cells.
3. Obtain a new MV plate and label it as **adenine gradient** and with your initials and the date.
4. Using a sterile glass spreader, spread 100 μ l of the HA2 suspension on the MV plate
5. Allow the agar to absorb the liquid for about 10 minutes, or until the liquid disappears. Do not move on to the next step until the liquid is absorbed.
6. Using sterile forceps, put an adenine-impregnated filter paper disk in the middle on the surface of the agar. Adenine disks are found at the teacher's bench.
7. Incubate the plate at room temperature for 7 days. Incubate right-side-up with the agar on the bottom.

Day 2 (4-24-12) Effect of Adenine: Score results

1. Make a sketch of the MV plate with adenine disc in the circle below. Indicate the color and quality of growth.

How do you explain the white phenotype near the center?



Describe the nutritional conditions in the red zone.

Is there any growth at the edges of the plate? If not then why not?

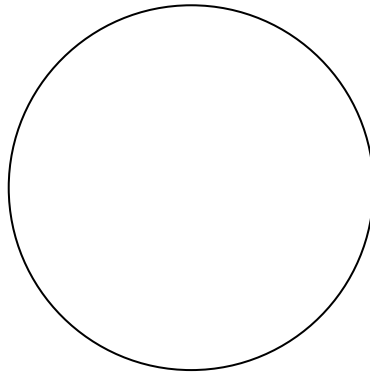
B. Effect of Anaerobic Growth on Pigment Formation

Day 1 (4-17-12)

1. Work as a group for this experiment.
2. Obtain a new YED plate and label it as **Anaerobic** and with your initials and the date.
3. Using a sterile loop heavily inoculate the entire surface of the plate with haploid HA2 yeast
4. Using a small, sterile metal spatula, cut the agar across the plate such that one side is about a quarter inch smaller than the other.
5. Fold the smaller portion over the larger portion to form an agar sandwich with the cell in the middle. Press the smaller portion down to seal the sandwich. The sealed portion will become an anaerobic environment.
6. Incubate the plate agar side up at room temperature for 7 days.

Day 2 (4-24-12) Effect of anaerobic growth: Observations

1. Make a sketch of the anaerobic plate and the aerobic control plate in the circles below. Indicate the color and quality of growth.



Is there any region on the agar sandwich that has not turned pink or red?
Provide a rationale for color differences observed on the plate.

2. Cut the top layer of the folded agar in half with a sterile spatula. Lift one of the new quarters and flip it over so that the cells are facing up.
3. Watch for a change in the color of the cells over the next few hours.

Did the pink phenotype return to those cells that reentered an aerobic environment?

Was the change from a white to a red phenotype after oxygenation a genetic or an environmental effect? Explain.

Part 3: Two Genes / One Trait
Day 1 (4-24-12)

The adenine biosynthesis pathway requires several enzyme-catalyzed steps (refer to figure 5 on page 3). 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 4 haploid red strains. HA1 and HA2 are both mating type α , while HB1 and HB2 are both mating type α . You will perform the following crosses: HA1 with HB1, HA1 with HB2, HA2 with HB1, and HA2 with HB2. After making these crosses and observing their growth on YED and MV plates, you should be able to determine how many genes are involved in these mutant phenotypes.

Procedure

1. Obtain a YED plate that has all 4 haploid, red mutants. Label it as “2 genes/1 trait” and with your initials.
2. Using sterile toothpicks transfer a small amount of each mutant to the positions shown below 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.

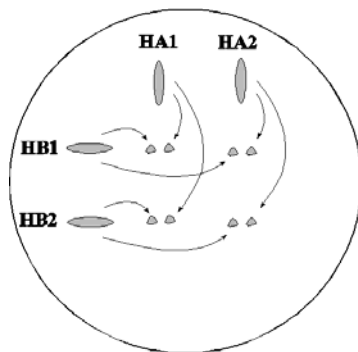


Figure 6

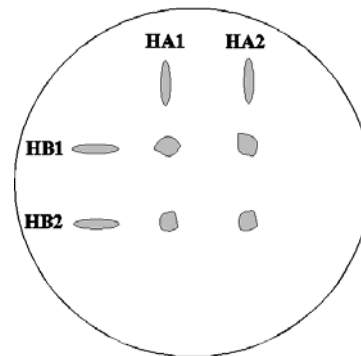


Figure 7

4. Incubate your cultures at 30°C for 2 days

