Chapter 6

Red Mutant Hunt with *Saccharomyces cerevisiae*

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Brad Williamson received his MA in Systematics and Ecology from the University of Kansas (1977). Currently he teaches a dual-credit biology class at Olathe East High school (students earn both high school and community college credit for this class). His interests include the development of improved, laboratory-based curriculum for students at all levels. He was an integral part of the NSF-funded GENE (Genetics Education Network) program at Kansas State University. This program developed educational applications of common research organisms in the classroom for genetics instruction. He also is a co-founder of the NSF-funded Monarch Watch project, a collaborative project to study monarch butterfly migration phenomena, at the University of Kansas.

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Abstract

Red Mutant Hunt with *Saccharomyces cerevisiae*.

Mutant hunts are the epitome of open-ended investigations. Every hunt can lead to potentially unknown mutants. In this investigation mutations are induced in haploid, yeast (*Saccharomyces cerevisiae)* with ultraviolet radiation (UV-C). The survivors are screened for red, adenine mutants which are then characterized for nutritional requirements. Mutants produced in both mating types of yeast are crossed to determine patterns of inheritance. Since two, separate adenine mutants express the red phenotype the inheritance pattern is unexpected.

Introduction

This laboratory exercise was developed as part of the GENE project at Kansas State University. The GENE project adapted yeast genetic research techniques to the educational laboratory. For the past eight years the Mutant Hunt laboratory has been used in a dual-credit introductory biology class. Students enrolled in this class can opt for four hours of credit from the local community college. The students are comparable to typical, university non-major biology students.

The pivotal work that linked classical genetics and molecular genetics was Beadle and Tatum's work characterizing the gene. This laboratory provides a similar link for students trying to develop an overall understanding of genetics. Though, developed as an introductory laboratory the very nature of a mutant hunt provides a rich potential for advanced work.

Though the laboratory requires a number of days to complete, the timing can be adjusted for typical laboratory schedules with refrigeration to retard yeast culture growth. Yeast strains, growth media and a reference manual, A Classroom Guide to Yeast Experiments, Manney (1996), can be purchased from Carolina Biological Supply Company. Additional information can be found on the GENE project's web site at: http://www.phys.ksu.edu/gene. A critical component of this laboratory is a UV-C irradiator. Plans for construction of an irradiator are available in the manual and on the web site.

Materials

1. Haploid yeast strains of opposite mating type for each class--HA and HB strains.
2. Sterile water (about 500 *ml*) for serial dilutions.
3. Sterile 1-*ml* pipettes for serial dilutions.
4. 10 sterile 13 x 100 *mm* culture or test tubes with caps per class for serial dilutions.
5. 100 microliter (&*l*) pipettor with sterile tips for serial dilutions and cell plating.
6. Metal spreaders
7. 40 to 50 YED agar plates for each class for initial radiation. Divide this number by the student teams.
8. At least one UV-C irradiator per class.
9. Sterile toothpicks .
10. One marker pen per team for marking and counting colonies and mutants.
11. 3 YED agar plates, 1 MV agar plate and 1 MV + adenine agar plate per student team for mutant isolation and test crossing.
12. 1 Replica plating device per class and 1 sterile velveteen cloth per student team.

Student Background

A mutation is a random change in the base code of a DNA molecule. Sometimes a mutation results in a change in the phenotype of an organism. Mutations are the ultimate source of genetic variability and without genetic variability populations of organisms would have difficulty adapting to ever-changing environments.

Very early in the study of genetics, geneticists realized that mutations could valuable tools for investigating how genes are transmitted or how genes work. One such mutation occurred in a particular male fruit fly (*Drosophila melanogaster*) that was discovered in 1909. Normal Drosophila have red eyes. The story is that, while washing bottles of fly cultures, Calvin Bridges happened to notice a white-eyed male. This exceptional fruit fly was rescued and mated to normal red-eyed females. From these matings geneticists learned about sex chromosomes, sex-linked inheritance and were able to eventually establish the chromosomal basis of inheritance. This work was done by Thomas Hunt Morgan and his students: Alfred Sturtevant, Calvin Bridges and Herman Muller. Based on their success with the original white-eyed male this group continued to search for and collect mutant fruit flies. They identified over 85 individual mutations of Drosophila melanogaster. With these mutations this group was able to determine much of what is known today about how genes are physically transmitted to the next generation.

Mutations are very rare events. The ability of DNA to replicate without mistakes is very good but occasionally there are random errors in the replication. Mutations like the white-eyed fruit fly are called spontaneous mutations. The chances of finding a specific spontaneous mutation are very slim. To speed up the mutation process various mutagens such as chemicals, x-rays, and UV-radiation are used to induce mutations in experimental organisms.

Experiment

Three steps are involved in a mutant hunt: 1) Induce mutations, 2) Screen for mutants, and 3) categorize the mutants by complementation testing. For this experiment we will use exposure to UV-C radiation as a method to produce mutations in haploid yeast cells. Even with the radiation, mutation is still a random event and so you will need to look at a lot of yeast colonies to find specific mutations. Each class will need to pool all of their results to get just a few candidate mutants. For this experiment screening will be done by visual examination, looking for red or pink yeast. Yeast strains of opposite mating type will be exposed to UV-C radiation so that resulting mutants can be crossed. There is a certain level of excitement involved in a mutant hunt--you never know what you are going to get. It could be that you will find a new mutation.

Time Line

Day 0:	15' to subculture the strains for the next day (instructor)
Day 1:	50' for serial dilution, plating, and UV-C exposure
Day 2-3:	Incubation (weekend is a good time)
Day 4:	50' to count survivors, screen for red mutants, pick mutants, compile class data
Day 5:	Incubation
Day 6:	30' to prepare confrontation test plates
Day 7-8:	30 ' to perform mating on confrontation plates
Day 9:	30' to score mating results, prepare confrontation plates to known mutants
Day 10:	15' to mate confrontation plates
Day 11-12	30' to score results of confrontation tests.

Day zero: This step is usually done ahead of time by the laboratory instructor.
Procedure:

1. Streak about 4 or 5 streaks of HA0 or HB0 onto YED media with a sterile toothpick. A different mating type is used for each class. For the overall experiment both mating types will be used. It simplifies matters if each class is responsible for only one mating type.
2. Incubate plates at 30 %C overnight.

Day one: Inducing mutations.
Overview: Using serial dilution approximately one thousand (10^3) HA0 or HB0 yeast cells are plated on YED media. Normally these yeast strains can actually synthesize most of the important molecules for life--molecules such as amino acids, vitamins, and nitrogen bases--from raw materials found in minimal media (MV). The mutations that you will be hunting for will not be able to grow on MV media so you will plate the original cells on YED media that has these molecules available. Each plate is exposed to ultraviolet (UV-C) radiation. UV-C radiation damages cells in a number of ways. Some are actually killed but most simply can no longer divide because of damage to the DNA in the chromosomes. Those that survive the UV-C exposure and can still reproduce often have new errors or mutations in the DNA code. Some of

these mutants can only reproduce because they are growing on YED media. As the UV-C exposure goes up the mutation rate goes up. This is what you want. However as the UV-C exposure goes up the survival goes down so now you have fewer cells. An optimal exposure will yield about 20% survival. That means that for each plate of 1000 cells before radiation you should have about 200 surviving yeast colonies after incubation. Each of the surviving colonies might be a potential new mutation. So that you can be sure of finding specific mutants that express a red color you need about 10,000 surviving yeast cells for each mating type.

Procedure:

USE sterile technique throughout this procedure.

1. Pipette 0.9 *ml* of sterile water into each of 10 sterile test tubes. Clearly label and arrange these tubes in a rack so that you can keep them in order. (Fig. 6.1) This will make it easier to keep track of the dilution steps.

2 With a sterile toothpick remove a small sample of yeast from one of the plates that were streaked yesterday. This small sample should be about the size of the period at the end of this sentence. Now carefully transfer the yeast to the first test tube by wiping it on the inside of the tube. Ever so gently, suspend the yeast by tipping the tube and thumping the end of the tube. Take care not to spill any over the top of the tube. Visually examine the turbidity (cloudiness) of the yeast suspension. You should just barely see that the yeast + water is slightly cloudy compared to the other tubes that have just water in them. If it is too turbid (easily seen to be cloudy) then add some more sterile water with a sterile pipette. If the yeast + water is still clear then add very small amount of yeast and resuspend. This is a critical step, so be careful. If all goes well this starting tube has a concentration of about one million (10^6) yeast cells per ml of water.

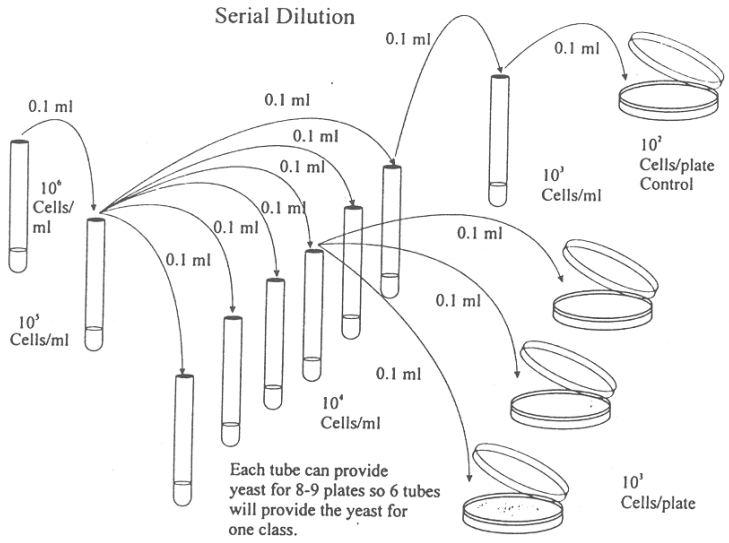

Figure 6.1.

3. Gently thump the end of the 10^6 cells /*ml* tube to suspend the yeast. Pipette 0.1 *ml* or 100&*l* of the yeast solution from the 10^6 cells/*ml* tube into the next tube. This is a 0.1 dilution so this tube now has about 100,000 (10^5)cells per *ml*. The yeast will no longer be visible so the suspension will be clear.
4. From the 10^5 dilution pipette 0.1 *ml* into each of 6 different 10^4 cells/*ml* tubes. Be sure to suspend the yeast each time with the thumping technique. In order to plate approximately 1000 yeast cells on 50 different YED plates we need this much of the 10^4 cells/*ml* dilution.
5. To provide a control and to determine the actual number of yeast cells in the dilution one more dilution needs to be made. From one of the 10^4 cells/*ml* tubes pipette 0.1 *ml* or 100&*l* into a separate 10^3 cells/*ml* tube.
6. Distribute a 10^4 dilution tube to student lab teams. About 8 or 9 YED plates can be plated with each 10^4 dilution tube. Each student is responsible for 2-3 plates in the mutant hunt. Label the bottom of the plate, neatly along the edge, with a marking pen. Include in your label: your name, the date, 10^3 cells, 20 seconds UV exposure. Each group should have pipettors and spreading equipment.
7. Pipette 0.1 *ml* or 100 &*l* of 10^4 cells/ml dilution onto a fresh YED plate. Don't forget to suspend the yeast with the thumping technique each time. This is again a 0.1 dilution so you are plating about 1000 (10^3) cells onto the media. Using sterile "hockey sticks" or metal spreaders spread the 0.1 ml of yeast about the surface of the plate. Try to avoid spreading too near the edge of the plate. Repeat this for each of your plates.
8. Expose your plates in the UV irradiator for 20 seconds. Follow procedures outlined by your instructor. After placing your plates (up to 2 plates at a time) in the box, remove the lids before exposing to UV. The plastic in the Petri dish lid will act as a filter block the UV from the yeast on the plate surface. If that happens then there are no mutants. Exposure of 20 seconds should result in about 20% survival. Since about 1000 cells were on the plate before the UV exposure about 200 should survive per plate. Several of the survivors will have suffered mutagenic damage.

CAUTION: The UV light used in this box is hazardous--particularly to your eyes. Do not circumvent the safety features of the box.

9. Don't forget the controls. One group can be responsible for the controls. Label three YED plates on the bottom: Control, Your Name, 10^2 cells, no UV and the date. From the 10^3 cells/*ml* dilution tube pipette 0.1 *ml* or 100 &*l* onto each of the 3 YED control plates. This should result in about 100 cells per plate. DO NOT expose these plates to UV. The actual number of colonies that grow on the plate after incubation will provide your basis for determining mutation rate and per cent survivorship from the UV exposure.
10. Incubate all plates for 3-5 days at 30%C.

Day five or six: Collecting Data and Isolating Mutants

1. After the individual, surviving cells have grown to visible colonies examine your plates for possible red mutant colonies. If you find one, circle it and tell the instructor. Look closely, it's amazing how easy it is to overlook one of these red mutants when they are surrounded be hundreds of white colonies. Don't be too disappointed if you do not find one on your plate but don't be too surprised either. Whether you find visible mutants or

not, it is almost certain that some of the surviving colonies on your plates are auxotrophic mutants that now require the addition of nutrients to their media for survival. Later, you can work on designing an experiment to discover these mutants. Count the surviving colonies on each plate. Working on the bottom of the plate use a marker pen to mark each colony as you count it. Calculate a per cent survival from the data from the control plates. Enter your data in a clear table and also compile data for the entire class. After compiling the data for the class, calculate the number of surviving yeast colonies per red mutation. To do this total the number of surviving yeast colonies on all of the plates from your class. Also sum the number of red mutants found in your class. Divide the number of surviving yeast by the number of red mutants. Compare your class results to other classes.

Sample Data:

Plate 1 Survival	Plate 2 Survival	Plate 3 Survival	Total Survival	Average Survival	Expected Survival* (control 10X)	Per Cent Survival	Red Mutants
265	302	247	814	271.3	1240	21.9%	2

Remember that the UV exposed plates were plated originally with approximately 1000 cells. The control plates were plated with a different dilution so that the colony numbers would not be too great to count on a single plate. The controls were plated with about 100 cells each. Since all of the dilutions started from the same source they should be different by factors of 10. For this reason we multiply the average number of colonies on the control plates by 10 to estimated the actual number of colonies that were initially on the UV exposed plates.

2. This next step requires someone with steady hands and a light touch. One person can do this for the entire class. On a fresh YED plate collect all of the red mutants that were found in this class. Label the bottom of the plate with: the class, the mating type of the mutations (important), and number each mutant. To collect the mutants use the sharp end of a sterile toothpick to just touch the suspected red mutant colony. Gently drag the end of the toothpick on the fresh YED to streak the red mutant yeast.. Keep the streaks on this plate orderly and separate. Other classes will be doing the same thing with the other mating type. If your class has more than 8 or 10 mutants then use two plates for isolating the mutants. Incubate for 1 to 2 days.

Day seven: Prepare Test Plates

Overview: Test plates will be prepared from all or most of the red mutants produced by all classes. The mutations can be used as tools to investigate how a yeast cell works. Our first step in this process is to determine the inheritance patterns of these red mutants.

1. Prepare a test plate of the suspected mutants from both mating types on YED media. Use the master plates that your instructor has made from the original mutants that your class prepared earlier. Use a separate, sterile toothpicks to set up the your plate as in Figure 6.2. Your number of mutants will probably vary but don't put more than 3 or 4 unknowns

for either mating type. There probably isn't room on the plate for all of the mating mixes. Incubate 1-2 days.

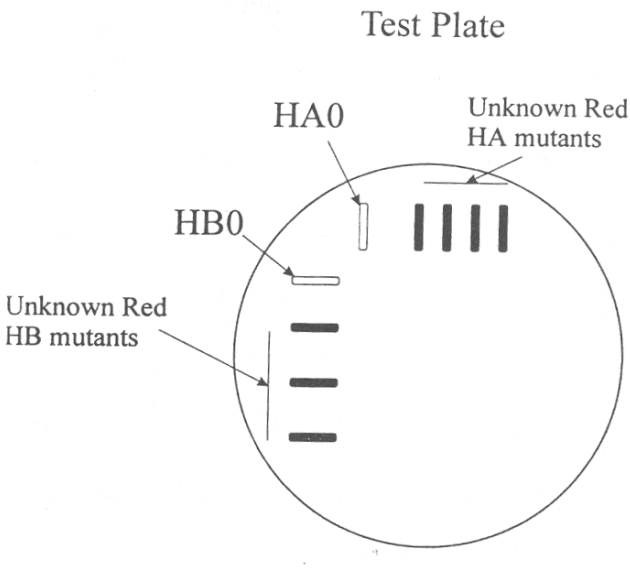

Figure 6.2.

Day eight: Isolating Mutants

1. Make all of the possible crosses among the unknowns and the known haploid stains. Use a fresh, sterile toothpick to transfer a small amount of yeast from each of the HA0 and the HA unknowns to positions marked in the Figure 6.3.

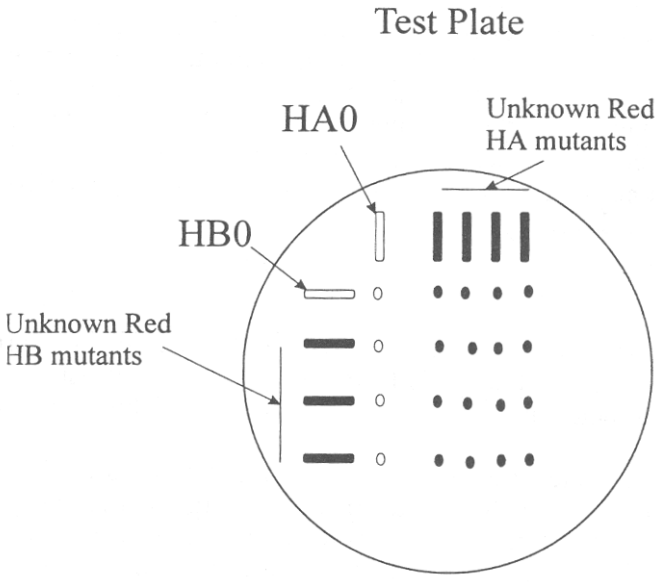

Figure 6.3.

2. Repeat step one for the HB0 and the HB unknowns. Do not mix or touch the HA samples. If you do, use a fresh toothpick. Your plate should now look something like Figure 6.4. (The number of mutants may vary.)

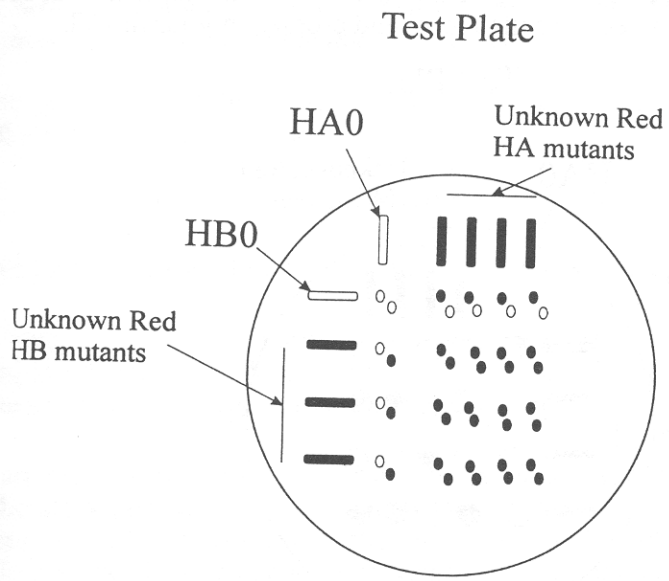

Figure 6.4.

3. Use a fresh sterile toothpick to mix each pair of strains together, being careful not to tear the surface of the agar. Mix thoroughly but don't overlap into other mating mixes. The shaded circles in the Figure 6.5 are to indicate mating mixtures and do not imply results. Incubate for 1-2 days.

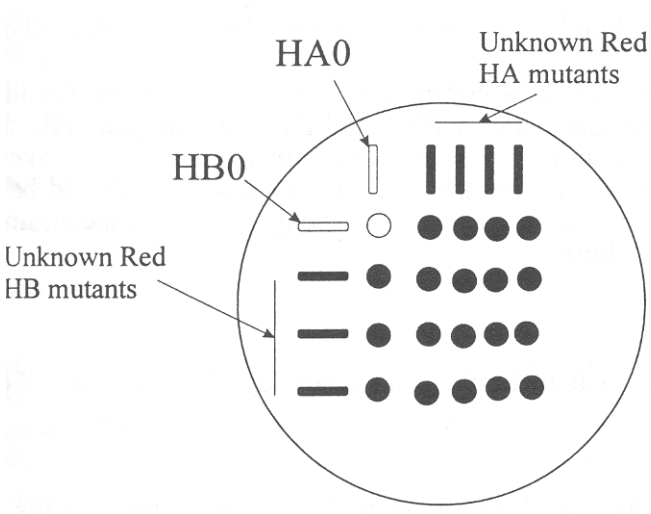

Figure 6.5.

Day 10: Looking at the results:

1. Sketch a diagram of your results. The results may have looked something like Figure 6.6. This time the shaded circles represent red diploid yeast. The unshaded circles represent white diploids. Describe a model of gene interaction that can explain your results. Include the white x white, white x red, and the red x read crosses in your model.

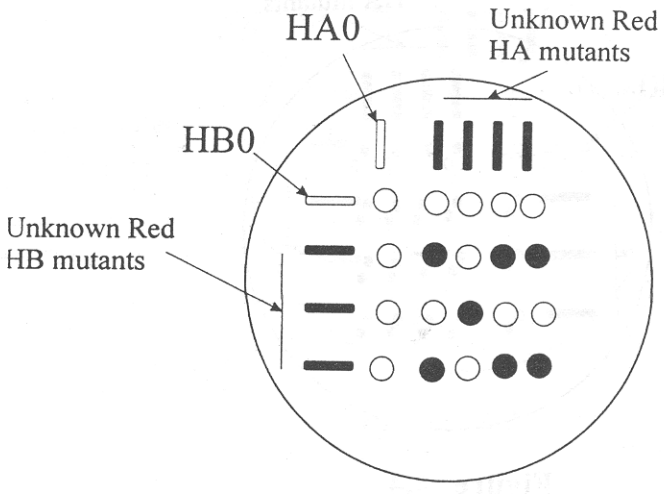

Figure 6.6.

2. Typical red mutant yeast are unable to grow if the chemical adenine is missing from the media. What about your red mutants? Do they also require adenine for growth? To test whether your mutants require adenine you will need to make a copy or replica of the confrontation test plate onto MV media and onto MV+adenine media. This is easily accomplished using the replica plating technique. If adenine is required growth will not occur on MV media but will on MV+adenine. Incubate both plates for 1-3 days.

3. Use a new YED plate and set up a confrontation test to determine the identity of your mutants. Test your HA mutants against HB1 and HB2 or test your HB mutants against HA1 and HA2. Either way will work. Label your plates accurately. Sketch your plates and predict the possible results before the mating. By now you should be able to design this test yourself. Remember to set up the plate today, but mate the strains tomorrow or the next day using sterile technique.

Day 11: Mating

1. Mate the confrontation test plate that you set up yesterday. Incubate for 1-2 days.

Day 12: A Final Look

1. Examine the MV and the MV + adenine replica plates. Sketch the results. Do your red mutants require adenine for growth?

- *An Extension:*
  This should give you an idea on how to design a screening technique to search through your original mutant plates for other adenine mutations. There are several other mutations that are not red colored so will not be visible among all of the surviving colonies. Design such an experiment. Using complementation tests see how many white adenine mutants you can isolate.

2. Examine your confrontation plate results. After analyzing these results go back and now label all red mutants as either *ade1* or *ade2*.

Notes for the Instructor

1. Suggested schedule for laboratories scheduled once a week:

2. **Week one**. Laboratory instructors would have the cultures of HA0 and HB0 yeast prepared in advance. Students would carry out serial dilution and UV radiation. About one hour of laboratory time is required for this step.

3. **Between week one and two**. Laboratory instructors would refrigerate plates until the next laboratory period to retard the growth of colonies. Two days before the next period the plates should be removed from the refrigerator and incubated at room temperature. This schedule may need to be revised depending on the local conditions. Plates ready for the second laboratory period should have colonies that are about two or three millimeters in diameter.

4. **Week two.** Students take data on survival and examine their plates for red mutants. All of the red mutants are collected and combined with other classes to make test plates for individual students. First the suspected mutants must be cultured. About one hour of laboratory time is required for this step.

5. **Between week two and three**. Laboratory instructors will need to create a Test plate master (Figure 2) from the grown strains of suspected mutants. Master plates can be copied by replica plating so that every student on week three has a prepared Test plate for crossing. The number plates required is determined by the number of students.

6. **Week three.** Students perform the crosses on the test plate. About an hour of laboratory time is required for this step.

7. **Week four**. Students observe the results of the test plate crosses.

8. A critical step for success in this investigation is establishing a starting concentration of 10^6 yeast cells per ml for the serial dilution. Standard counting chamber techniques can be use but the visual determination of the threshold of turbidity is very reliable with practice. The laboratory instructors should supervise this portion of the laboratory carefully.

9. Yeast strains and media can be purchased from Carolina Biological Supply Company. The following table includes the Carolina stock numbers as well as the strains and media required for this investigation.

Yeast Strains	
HA0	CB-17-3620
HB0	CB-17-3621
HA1	CB-17-3622
HB1	CB-17-3623
HA2	CB-17-3624
HB2	CB-17-3626
Media	
YED	CB-17-3650
MV	CB-17-3658
MV + adenine	CB-17-3660

1. Develop proper safety procedures for the UV irradiator used by your laboratories. Caution students about the risks associated with UV-C radiation. Have students where UV shielding eye goggles.

2. Usually this lab generates about six red mutants per 10,000 survivors. There are two mutant expressing the red phenotype. Usually both mutants will occur. There is a slight color difference between them. Both mutants require adenine for growth and are in the adenine biosynthetic pathway. When the two red mutants are crossed they complement each other and a white diploid is expressed. This is usually a surprise to the students–one that requires them to confront their usual models of simple dominance and recessive inheritance.

References

Manney, Tom, et al. 1996. A Classroom Guide to Yeast Experiments, a Research Approach to Mendelian and Molecular Genetics and Genetic-Environmental Interactions. Carolina Biological Supply Company.

Acknowledgments:

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