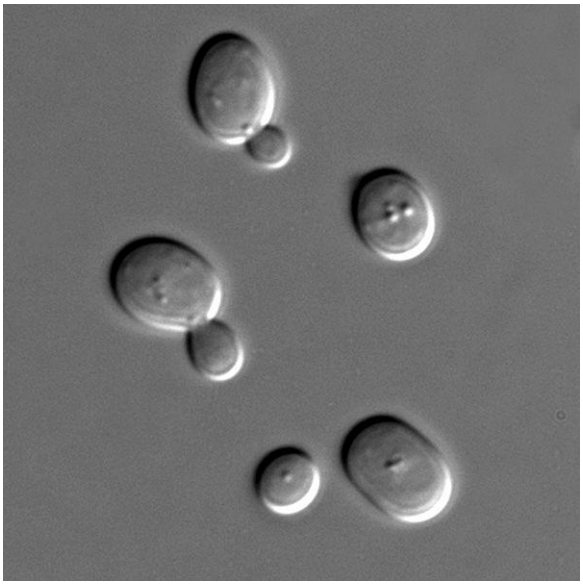


Learning Objectives for the From Genes to Proteins to Behavior (FGPB) Project

Over the next five weeks, you will conduct a series of related experiments in the model organism *S. cerevisiae* (baker's yeast) that mimics the process of investigational science and is aimed at accomplishing the following learning objectives.

(1) To understand that cellular compounds (such as adenine) are made by a series of biochemical reactions, and each of the reactions are catalyzed by an enzyme (metabolic pathway objective)

(2) To understand that enzymes are composed of amino acids, the order of which is specified by nucleotides in genes (genetic code objective)

(3) To understand how enzymes arise via transcription and translation of genes (central dogma objective)

(4) To understand that a heritable change in a gene's nucleotide sequence (i.e. mutation) can lead to a change in the amino acid sequence of the encoded enzyme (mutation objective).

(5) To understand that changes in the amino acid sequence of an enzyme can lead to changes in the shape of the enzyme, and changes in the shape can lead to an inability of the enzyme to function (protein structure/function objective).

A flowchart of the From Genes to Proteins to Behavior Project

Week 1 Objective: Determine if treating a white version of yeast with a DNA damaging agent will give rise to variant forms (e.g. a red version).

→

Week 2 Objective: Observe colonies and the effect of UV mutagenesis. Test the nutritional requirements of the red and white types of yeast.

→

Week 3 Objective: To test the Hypothesis that The difference between red and white yeast is a difference in their DNA, we will purify DNA from the red version of yeast. The DNA sequence from the white version (wild--- type) has already been characterized and will be used for comparison.

→

Week 4 Objective: Isolate and amplify ADE1 and ADE2 genes to allow for the determination of the sequence of bases that make up these genes in the red version.

→

Week 5 Objective: Analyze the DNA sequence of the ADE1 and ADE2 genes from the red yeast and compare to the white.

This will allow a direct test of the hypothesis that the difference between red and white yeast is a difference in one of their genes.

From Genes to Proteins to Behavior

CAN UV LIGHT CHANGE THE APPEARANCE OF YEAST?

In the **From Genes to Proteins to Behavior** Project, you will be studying baker's yeast strains that differ in appearance. Yeast, schmeast, you may say, why should I care about different kinds of yeast? However, the presence of different types of yeast leads to a fundamental question of biology: **What are the underlying causes of biological variation? Why are some yeast red and others white? Why do some of us have black hair and others blonde or red or brown?** In the laboratory sessions over the next 5 weeks, our overall goal will be to determine the genetic cause of variation we observe between white and red yeast. The insights obtained by this study of yeast can be applied to understanding variation in other organisms, such as humans.

Specifically, in this week's laboratory you will test whether one type of yeast can be changed into another by exposure to UV light. You will examine the effect of different doses of ultraviolet (UV) light on *S. cerevisiae* (yeast) cells and the resulting colonies. One macromolecule that is known to undergo change after UV light exposure is DNA. Can change in the DNA result in a change in the appearance of the resulting yeast colony? That is, starting with a yeast that forms white, smooth, round colonies and exposing it to UV light, will we see any colonies with altered color or morphology?

To most fully measure the effects of UV light you will subject the yeast to increasing doses of UV light. Not only can we answer the question of whether one type of yeast can be generated from another, but we can answer more general questions as well. For example: What happens when macromolecules in a cell are subjected to chemical change beyond what normally occurs inside of the cell? Does the cell grow faster than in the absence of the chemical change? Does the cell die as the result of the chemical change? Does the cell acquire a different property as the result of the chemical change? Do all cells react the same way?

USEFUL VOCABULARY:

sterile: adj. Containing no living organisms.

medium: n. What bacteria or yeast grow in or on. Can be solid or liquid.

culture: n. Yeast (or bacteria or other microorganism) growing in or on a medium.

colony: n. Usually found on a petri plate. A visible "pile" of yeast (or other microorganism) that are all descendants of a single yeast cell.

strain: n. A particular variety of a microorganism. Different strains are more similar than are different species. This term is functionally equivalent with the term "breed" (used in animal husbandry) or "variety" (used in agriculture). In this lab you will be working with a white strain of yeast to see if you can alter it to make a different strain with a new appearance. Do not confuse this term with the word "strand" which is a long thin object such as a hair or one half of a DNA helix.

aliquot: v. To subdivide a liquid sample into smaller portions. n. A portion of a larger sample.

METHODS:

- Pipetting: This lab will require precise measurement of small volumes so we'll be using micropipettes. Remember that each micropipette has a limited range, and you must only depress the plunger to the first stop position before dipping the tip into the desired solution.

- Sterile technique: You are testing whether you can change one type of yeast into another by using UV light. If you observe any colonies next week that appear different from the colonies you started with, you want to be assured that these colonies were derived from the yeast in your original culture and are not some random cells from the air that just happened to land in the tubes or on your plates. In other words, it is essential that your yeast cultures be kept free from other microorganisms that are found in the air, on your hands, or elsewhere in environment. You must use **sterile technique** to keep the cultures from becoming contaminated. There are several different components to sterile technique; your instructor will demonstrate those that are necessary for today's lab.

- Streak plates: Next week you will want to see individual colonies of the yeast in order to tell if any of them look different from those of the original strain. A streak plate is often used to separate the cells in a liquid culture by spreading them evenly over the surface of a petri plate so that each cell can give rise to a separate colony. This technique will be demonstrated by the instructor and instructions are also provided below.

A. Plating yeast cells

1) Obtain **8** YPD plates/group. These YPD plates are a nutrient-rich medium for growing yeast.

2) Obtain a liquid culture of yeast cells marked "HAO". HAO is a strain of white-colored yeast. Check the label carefully – it is critical to use the correct culture! **Just before using the culture, invert the tube a few times to mix.**

*The yeast cells are dense and will settle to the bottom of the tube. Thus, to ensure pipetting a homogeneous solution of cells, invert the tube immediately prior to **each** use.*

3) Take a 100 uL ml aliquot of the culture using a sterile pipet and *sterile technique*. Dispense it into the center of a YPD plate. Work near a Bunsen burner and don't touch the pipet tip to anything that is not sterile, such as your fingers or the benchtop.

Use a sterile plastic spreader to gently push around the drop of culture on the dish (be careful not to dig into agar). Repeat the identical procedure for 5 more YPD plates, mixing the culture before each aliquot. Allow the plates to dry for a few minutes on your lab bench, agar side down.

Try to spread the culture as evenly as possible across the plate, spreading until the plate is dry. You should have 6 spread plates in total.

B. UV mutagenesis of yeast cells

You will expose **6** yeast plates to ultraviolet light for differing lengths of time: 0 seconds (negative control), 10 seconds, 20 seconds, 20 seconds, 40 seconds, and 80 seconds. **Note: As indicated, the 20 second exposure will be repeated on 2 different plates.**

4) Label the **bottom** of each of the plates with a marker to indicate the amount of UV exposure the plate will receive (e.g. 0 seconds, 10 seconds, etc.) and the lab group's initials. Expose the yeast to UV for the appropriate length of time. The "0 seconds" plate serves as a negative control and will not be exposed to UV.

> *Exposure to the ultraviolet light will be done in the hood, which is equipped with a 30W germicidal UV lamp. The instructor will provide the operating instructions and help coordinate group sharing of the hood space.* ***UV light will not penetrate the plastic lid, so remove the lid immediately before exposure.***

C. Preparing dilution for untreated control plate

As a control for your experiment, you already prepared one plate that is not exposed to UV light (i.e. 0 seconds). While this will be a useful standard from which to compare the effect of the UV treatment, a limitation of this plate is that it will likely yield too many colonies to count. To circumvent this problem, you will make a 1:10 dilution of the original culture as an additional control plate.

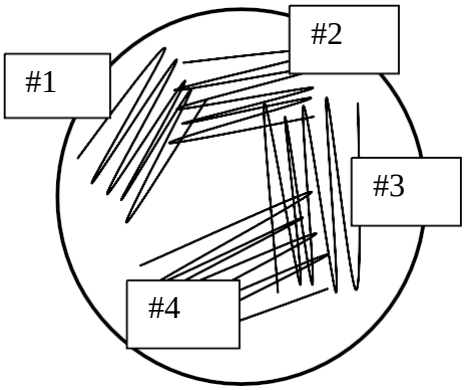
5) Dilute the HAO yeast culture as follows: Invert the original culture tube thoroughly to resuspend the cells. Using sterile technique, add 100 uL of culture to a separate microfuge tube that has 900 uL of sterile water. Mix thoroughly by inverting the tube. Spread 100 uL of the diluted cells on a YPD plate using sterile technique, as done above.

D. Streaking for single yeast colonies

Next week you will be asked to separate cells from any interesting colonies you obtain from the other colonies surrounding them on the plate. To do this, a technique called a streak plate will be used. This week you'll do a practice streak plate so you can see how this technique is done.

6) Obtain a liquid yeast culture that contains two types of yeast (it should be labeled "mixed yeast culture"). Open the microfuge tube using sterile technique and use a sterile loop to remove some of the liquid culture. Streak out on a YPD plate in the four streak pattern (see example image on next page), using a new loop between streaks 1 and 2, 2 and 3. Streak gently-- try not to break through the surface of the agar with the loop.

You can separate the two types of yeast by streaking them around the plate.



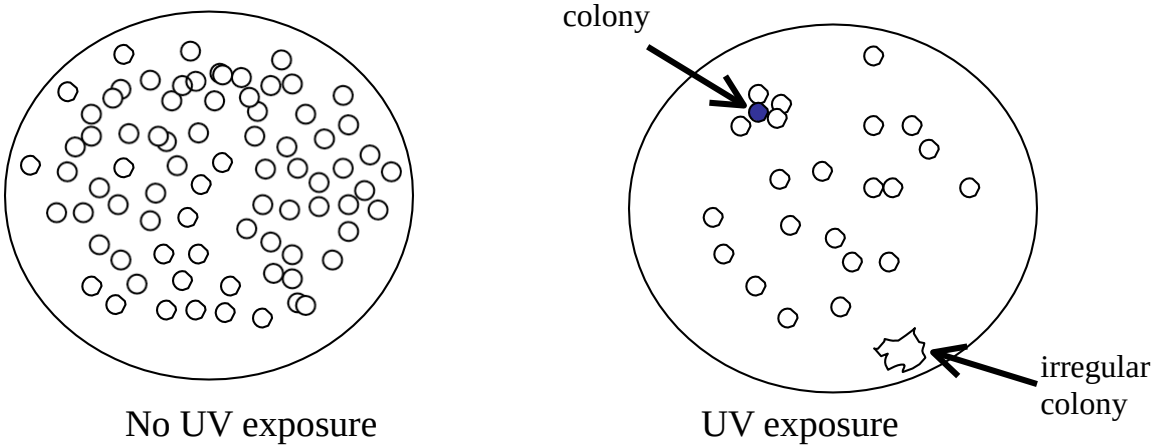
E. Yeast plate incubation @ 30°

7) Place your 8 YPD plates lid-side down in the bin at the front of the classroom.

It will be convenient if you bundle the plates together with saran wrap. The instructor will put the plates at 30°C, an optimal temperature for the growth of this organism.

Pre-Lab Assignment #2 (10 points)

(2 pts) **1.** The following observation is made in comparing a yeast plate without UV treatment vs. a UV-treated plate.



(a) Based on these observations, name several effects of UV light on this yeast.

1.

2.

3.

(b) How can UV light cause more than one type of effect?

(8 pts) **2.** A microbiologist wanted to understand the quantitative relationship between the amount of UV light used to expose a culture of yeast cells and the percentage of yeast cells that survived the treatment (where 100% is the number of cells surviving after no UV treatment). She obtained the following result.

Length of UV light exposure (seconds)	Number of cells surviving	% of cells surviving
0	1,540	100
10		90
20	890	
30	540	35
40	139	
60		0.4

(a) Fill in the blank spaces in the table. (2 points)

(b) Use computer graphing software (e.g. Microsoft Excel) or graph paper to plot the above results in a scatter graph (attach to this worksheet). The plot should show the percentage of cells surviving as a function of the length of UV exposure (time).

Label the X and Y axes and place a title at the top of the graph. The title should be meaningful but not verbose. For instance, a title of "Yeast experiment" will not have any meaning for anybody who is not in BIO208. On the other hand, "A study to determine the effects that ultraviolet light has on the ability of a fungus known as *Saccharomyces cerevisiae* to survive on YPD growth medium when incubated at 30°C" is an example of a title that is not succinct enough to be most effective. (4 points)

(c) What percentage of surviving cells would be expected if they were exposed to UV for 25 seconds? Use your graph to estimate the answer---show your use of the graph by plotting the datapoint that you would expect to be associated with the 25 second dose. (2 points)