From Genes to Proteins to Behavior

CAN RED YEAST BE OBTAINED BY TREATMENT OF WHITE YEAST WITH UV LIGHT?

DO RED YEAST HAVE SPECIAL NUTRITIONAL REQUIREMENTS?

Last week you treated white yeast cells with UV light. It was suggested that UV light can alter DNA. This week you will examine your UV-treated plates to see if any of the yeast cells exhibit altered characteristics as a result of the UV treatment. We are particularly interested in any yeast that changed in color from white to red. *Our working hypothesis will be that these and other changes occurred because UV light altered the DNA of the white yeast.* The rest of the experiments in the From Genes to Proteins to Behavior project are designed to test this hypothesis.

Our focus this week will be in identifying and purifying colonies that are altered in appearance by the UV treatment. You should also notice more general effects of UV light on the yeast culture – do you notice any overall trends that are related to the dose of UV light received? If so, what might be the explanation for these trends?

Some students in the class will probably obtain red-colored colonies on their UV-treated plates. We are going to focus our future studies on these red yeast. Therefore, our second mission this week will be to begin studying previously identified strains of red yeast. You will use the streaking technique you learned last week investigate the growth characteristics of red and white yeasts. This plate test will address the question of whether red yeast and white yeast have different nutritional requirements. To generalize, we can ask whether there is a single difference between the two types of yeast (the color difference) or whether other differences exist, and if so, whether these differences are correlated. We will provide you with two yeast (*Saccharomyces cerevisiae*) strains (HAO and HB2) that are growing on rich medium, medium that has all the nutrients that the cells could possibly need. You will test whether these strains can grow on minimal medium, or medium that has limited types of nutrients. In particular, you need to determine if the strains can grow without added adenine, a component of DNA. Some strains can make their own adenine, but others cannot and therefore need to "eat" it from their environment before they can make their DNA.

METHODS:

A. Evaluating the effects of UV light on white yeast:

1) Examine your petri plates from last week. Record in your lab notebook the number of colonies on each UV-treated plate and on the untreated control plates.

In some cases the number of colonies will be so great that you can only estimate the number. To do this, count the number of colonies in one square centimeter of the plate and multiply by 63.6 (this is the total surface area of the plate in cm^2). Alternatively, you may divide the plate into sections (e.g. 2 halves, 4 fourths), count the number of colonies in one section, and account for the fraction of that amount relative to the total plate. To keep track of which colonies have been counted, it is helpful to put a small dot of sharpie marker on the bottom of the plate over each colony as you count it.

> *Later in the semester, you will need these numbers, as well as notes about the other observations you will make regarding this project, for your lab report. Make sure you write your notes in a safe place and that EACH person in the lab group has ALL of the information every week.*

Your undiluted negative control plate (minus UV light) should have several thousands of colonies, probably too many to estimate accurately. Remember, you also included a negative control plate that was diluted prior to plating. It will be easier to count the number of colonies on your dilution plate and then use the dilution factor to make a correction when comparing the number of colonies on your UV-treated plates to that of the untreated plates.

> *Here's an example of how dilution factor is calculated: if you had diluted 50 ul into 950 uL of water, then your dilution factor would have been 1000 ÷ 50 = 20 fold dilution. Calculate the dilution factor for the dilution <u>you used</u> on your control plates. How do you think you can use the dilution factor to calculate the number of colonies on the undiluted control plate?*

2) Record any differences you observe between colonies on UV-treated plates and colonies on untreated plates. Are there any examples of colonies that look unlike all other colonies (for example, red rather than the typical white color, have strange colony morphology, etc.)?

B. Evaluation of practice streak plate:

3) Last week you used a sterile technique called a streak plate to try to separate a mixed culture of yeast. Look at your streak plate from last week. Did you succeed in separating the two types of yeast into single colonies of each?

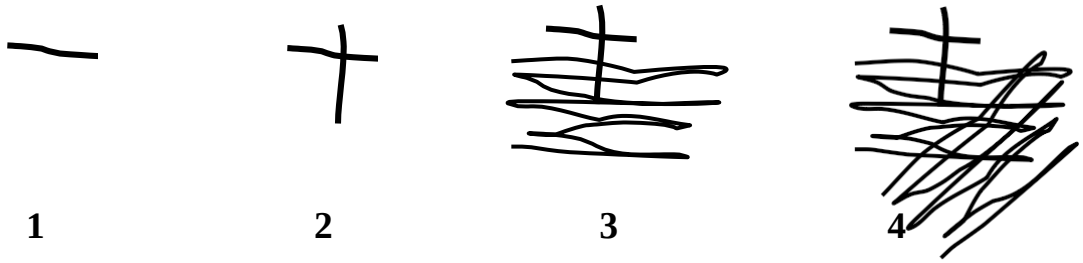
C. Streak plates to test nutritional requirements of red vs. white yeast:

4) Obtain a "minimal medium" plate, a "mimimal plus adenine" plate, and a YPD plate. Use a marker to draw a line on the **bottom** of the plate to divide each plate into 2 halves. Label the two areas HAO and HB2.

In addition to using the streak plate technique to purify colonies, it can also be used to place yeast on different growth media in testing their nutritional requirements. You are going to streak two strains onto each of the three plates. In order to make the streaks fit into their sections of the plate, you will use a slightly different streaking pattern than you did last week.

5) Obtain plates (previously prepared by the instructors) with the yeast strains HAO and HB2. Use a sterile loop to touch a single white colony of the HAO strain. Don't scrape up a large amount of the colony – a barely visible or invisible amount is what you want. Remember cells are too small to see, so there are thousands of cells clinging to your loop, even though you can't see them. If you pick up too many cells it will be difficult to interpret the experiment later.

6) Touch the loop containing the cells to the minimal medium plate and make a small horizontal streak (**see diagram below, 1**) near the edge, on the half of the plate that you marked "HAO." Rotate the loop 180° to the opposite clean surface and make another small streak, this time vertically, that crosses the first streak (**2**). Use a second sterile loop to streak back and forth across the last streak to spread cells over about half of the area of the HAO section (**3**). Then, rotate the second loop 180° to the opposite clean surface and streak slantwise across those streaks to spread the cells out further still (**4**). Try to use most of the room available to you in that half of the plate.



1 **2** **3** **4**

7) Repeat this process for the red HB2 strain on the other half of the minimal media plate, making sure use a new loop before touching the strain. Using the same procedure, have your lab partner streak both strains onto the "minimal plus adenine" plate. Now fight over which one of you will repeat the process for the YPD plate.

D. Streak plates to isolate red colonies from UV-treated plates:

8) If you obtained any red colonies on your UV-treated plates, choose one to purify and obtain a YPD plate for the transfer. Use a sterile loop and touch it *lightly* to your red colony. Use the streak plate technique and the new YPD plate to purify the colony. Use the whole area of the plate and four streaks in a big square, as you did last week, to purify one red colony. If you found more than one red colony, please pass the extras on to lab groups that did not obtain a red colony.

> *Don't worry if you did not get to do this step; we will give you a red strain to work with in future weeks.*

E. Inoculating liquid medium:

Next week we will give you liquid yeast cultures that you will use in isolating DNA. This week you will practice preparing such a culture, just so you can see how it is done and to practice your sterile technique.

9) For each group, obtain two sterile test tubes containing YPD and label them: "control" and "yeast culture". Obtain a petri plate containing yeast colonies (use a plate prepared earlier by the instructors). Touch a sterile loop to a yeast colony to obtain some cells, and dip/shake the loop containing the yeast into the tube of YPD broth (labeled "yeast culture"), flaming the lip of the test tube **before** and **after** inoculation.

> *The flame not only kills bacteria and fungus on the lip of the tube, but also causes air to move out of the tube, so that microorganisms floating in the air do not enter the tube. Handling the tube cap with your pinkie frees up the rest of your hand for other jobs.*

10) For the "control" tube, do not touch a sterile loop to any yeast cells, but go through the same motions to dip/shake the loop into the YPD broth, flaming the lip of the test tube before and after inoculation. This "control" will verify whether or not you are implementing an effective sterile technique.

11) Put your tubes in the rack at the front of the room for 30˚ incubation.

Pre-Lab Assignment # 3 (12 points)

Using a word processor, answer the following questions.

1) Which two sterile techniques allow you to separate cells in a culture into individual colonies? Explain why these techniques can accomplish this separation. (2 points)

2) In the inoculation experiment, do you expect growth in the control tube? Explain your answer. In your answer, include what it would mean if there were growth in the control tube. (1 point)

3) Consider the following results:

Strain	Minimal medium	Minimal medium + adenine
1	no growth	no growth
2	growth	growth
3	no growth	growth

(a) Can strain 2 make its own adenine? Explain your answer. (1 point)

(b) Can strain 3 make its own adenine? Explain your answer (1 point)

(c) What situations might explain the results obtained with strain 1? The best answer will provide two different hypotheses. Consider both possible technical problems and possible nutritional requirements of the strain in formulating your hypotheses. Propose a nutritional test/experiment that could differentiate between the two possibilities. (2 points)

4) In your lab report, you will be asked to compare the number of colonies on your UV-treated plates to your control plates. How do you think you can use the dilution factor in making this comparison? (1 point)

5) In the following weeks you may be asked to interconvert milliliters (ml = 10^{-3} liters) and microliters (μl = 10^{-6} liters). (please show your work)

(a) How many microliters are in 0.2 ml? (1 point)

(b) How many milliliters are in 473 μl? (1 point))

6) Look at the flowchart entitled "Three steps in the making of adenine inside a yeast cell" (included with this handout). The flowchart shows a small portion of the steps needed to make adenine. Three genes encode three proteins (enzymes) – for example, gene A contains a code telling the cell how to build protein A. Each protein does a job. For example, protein A converts a colorless chemical, represented by the open shape, into a red chemical, represented by the filled-in bullet shape. The proteins must do their jobs in the order indicated – protein B cannot do its work until protein A has done its job.

(a) A student from last year's class claims that if a cell contains an altered version of gene A that encodes a non-functional version of protein A, then the cell will turn red. Similarly, the student claims that if a cell contains an altered version of gene B that encodes a non-functional version of protein B, then the cell would also turn red. Given the information in the diagram on page 2 of the lab manual, does this seem correct? Explain your answer. (1 point)

(b) Would an alteration in gene C leading to a non-functional protein C result in red or colorless/white yeast? Briefly explain. (1 point)