Mystery Yeast Mutation

Teachers Guide

Activity Overview

Abstract
Yeast strain X carries a mutation. What is it? And what does it do to the yeast? In this inquiry-based exploration students are asked to discover the nature of the mutation in a yeast strain by designing and carrying out their own experiments. Students are provided with a conundrum: the same mutant yeast strain can grow on one type of media plate but not another. What is wrong with it?

Suggested uses
Use this activity to:
- Help students conceptualize that 1) the cell needs building blocks to replicate its DNA and 2) where the cell obtains those building blocks.
- Help students verbalize for themselves how genetic mutations can cause disease and how these diseases can be treated through medical intervention.
- Build students' ability to form hypotheses, design experimental tests, and formulate conclusions based on their own data.
- Assess students' understanding of nucleic acids, cell division and mutation.

Prerequisites
Before beginning this experiment, students need to understand:
- The concept of diffusion.
- The general structure of DNA (a ladder-like structure with each rung composed of a pair of nucleic acids).
- The mechanics of DNA replication, cell division and mutation.

Grade level
Grades 7 – 14

Preparation time
Two hours to order materials, prepare nucleic acid disks, streak yeast plates, and set up student materials; an additional two hours if pouring your own plates of media.

Classroom time
Portions of class periods over approximately two weeks.

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National Science Education Standards

Grades 9-12:

- Content Standard A: Science as Inquiry – formulating and testing hypotheses; designing and conducting scientific investigations; formulating and revising scientific explanations using logic and evidence; recognizing and analyzing alternative explanations and models; communicating and defending a scientific argument.
- Content Standard C: Life Science, The Cell – cell functions involve chemical reactions; the genetic information stored in DNA is used to guide cell function. The Molecular Basis of Heredity – the structure of DNA; DNA mutations.
- Content Standard F: Science in Personal and Social Perspectives, Personal and Community Health – the severity of disease symptoms is dependent on many factors; many diseases can be controlled.
- Content Standard G: History and Nature of Science, Nature of Scientific Knowledge – scientific explanations must meet certain criteria.

Resources in this Teachers Guide

Equipment and Materials

- Yeast strain HA2 (*ade2*)
- YEAD (Yeast-Extract Adenine Dextrose) media plates – number depends on experiments
- MV (Minimal plus Vitamins) media plates – number depends on experiments
- Adenine paper disks
- Cytosine paper disks
- Guanine paper disks
- Thymine paper disks
- Baking yeast (from the grocery store)
- Sterile containers with lids
- Sterile pipettes to measure 1 ml
- Pipette bulb or pipette pump to use with the pipette, if needed
- Sterile distilled water
- 70% or 95% ethyl or isopropyl ("rubbing") alcohol
- Waterproof marking pens with fine tips - 1/student group
- Sterile, flat toothpicks
- Paper towels
- Self-sealing ("Zip-lock") plastic bags
- Place to incubate the yeast (30^{o}C incubator or room temperature)

Material sources

Carolina Math and Science, 1-800-334-5551, http://www.carolina.com
Fisher Education, 1-800-955-1177, http://fisheredu.com
Fisher Scientific, 1-800-766-7000, http://fishersci.com
Sigma, 1-800-325-3010, http://www.sigma-aldrich.com
Note: Catalog numbers and prices are from 2002-2003 catalogs

Item	Company	Catalog Number	Quantity	Price
HA2 yeast strain	Carolina	WW-17-3624		$7.50
YEAD media plates	Carolina	WW-17-3692	Sleeve of 10	$20.00
MV media plates	Carolina	WW-17-3698	Sleeve of 10	$20.00
YEAD medium (dry)	Carolina	WW-17-3652 WW-17-3653	For 500 ml For 2 liters	$3.40 $12.00
MV medium (dry)	Carolina	WW-17-3658 WW-17-3659	For 500 ml For 2 liters	$3.40 $12.00
Yeast extract	Fisher Education	S71605	100 g	$41.75
Dextrose, anhydrous (glucose)	Fisher Education, or any	S73415 S73418	100 g 500 g	$3.50 $5.95
Powdered agar	Fisher Education Carolina	S70210 S70213 WW-84-2131 WW-84-2133	100 g 500 g 120 g 500 g	$17.75 $63.00 $24.95 $59.95
Bacto-yeast nitrogen base, *without* amino acids, *with* ammonium sulfate	Made by Difco, supplied by Fisher	DF0919-15-3	100 g	$74.65
Adenine	Sigma	A 8626	1 g	$9.60
Cytosine	Sigma	C 3506	1 g	$8.40
Guanine	Sigma	G 0506	5 g	$5.00
Thymine	Sigma	T 0376	5 g	$8.40
Sterile, polystyrene Petri dishes, 100 x 15 mm	Carolina Fisher Education Fisher Scientific	WW-74-1250 S33580 08-757-12	Sleeve of 20 Sleeve of 20 Case of 500	$4.90 $4.25 $110.01
Sterile, polystyrene Petri dishes, 60 x 15 mm	Fisher Education Fisher Scientific	S43115 08-757-13A	Sleeve of 20 Case of 500	$3.85 $103.70
Sterile containers	Fisher Scientific	02-540-10	Case of 100	$132.23
Sterile, graduated, plastic pipets	Fisher Scientific	13-711-20	500, indiv. wrapped	$53.01
Sterile water	Carolina	WW-19-8697	1 liter bottle	$12.00

Preparation and Activity Timeline

Four or more weeks before beginning the experiments
- Order HA2yeast strain.
- Order YEAD and MV Media Plates or Dry Medium and Petri Dishes.
- Order any other needed supplies.

A week or more before beginning the experiments
- Prepare media plates if you are pouring them yourself.

Two-four days before beginning the experiments
- Prepare a fresh culture of HA2 yeast. You may choose to prepare one media plate for each group or several groups may share a plate. For each plate:
  - Use the rounded end of a sterile toothpick to collect a very small amount of yeast from the culture you ordered.
  - Gently streak the yeast in several lines across the surface of a YEAD media plate. Yeast cells will be spread on the media even if you cannot see them.
  - Turn the plate upside down and incubate it at 30^{o}C (1-2 days) or room temperature (several days) until the yeast has grown.
  - Store the plate upside down in a sealed plastic bag in a refrigerator.

One day or more before beginning the experiments
- Engage in learning experiences with students to prepare them to carry out the experiment.

Immediately before beginning the experiments
- Prepare a visibly turbid suspension of yeast cells in sterile water (see Techniques for Working with Yeast).

Full or partial class periods for two weeks
- Students plan, carry out and discuss their experiments.
- After completing their experiments, students present their work in a poster session.

Conditional Genetic Disorders

If an organism carries a harmful genetic mutation, it can still survive and sometimes even function normally if something in the organism's environment can compensate for the genetic defect. In this situation, the organism may not appear to have the mutation at all (in other words, though its genotype contains the mutation, its phenotype is normal. This type of mutation is termed a "conditional" mutation, meaning that the phenotype of the mutation is only observable under certain conditions.

The *ade2* yeast strain has a conditional mutation that prevents it from growing in the absence of added adenine. This yeast, in other words, has a nutritional deficiency. To compensate for conditional mutations, missing substances normally made by the

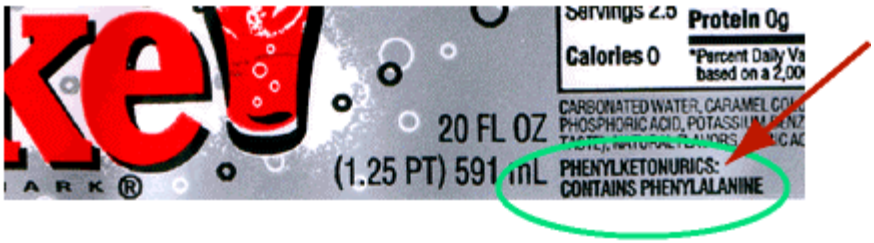
product of the mutated gene can sometimes be provided, or substances normally removed by the product of the mutated gene can be limited in the diet.

Other types of conditional mutations (seen in organisms such as fruit flies, yeast and bacteria) include temperature-sensitive mutations, which can be either heat or cold sensitive. Individuals with these types of mutations grow normally at one temperature but not at another.

Conditional mutations are very valuable to scientists, because they allow researchers to carefully manipulate their experiments. They can decide for themselves when the organism should display the effects of a mutation by controlling the environment of the organism. For example, with a conditional mutation, the scientist can learn when in an organism's life a particular protein is needed. A series of experiments is performed; in each, the organism is grown at the "good" (permissive) temperature for a while and then shifted to the "bad" (restrictive) temperature for a time. If the mutation has no phenotypic effect, then the scientist has learned that the protein is not needed during the time of the temperature shift. In this way, scientists can map a window of time in which the protein that would be made by normal copies of the gene is needed. Further experiments would focus on finding out what that protein is doing at that defined time.

Conditional mutations in humans - Phenylketonuria (PKU)
The genetic disorder phenylketonuria, or PKU, is an example of a disorder whose negative effects can be lessened by changing the diet of the individual with the mutation. In a normal person, the PKU gene product changes the amino acid phenylalanine into another chemical. People with PKU mutations do not make this product and are therefore unable to remove phenylalanine from the body. As a result, unwanted chemicals build up in the body. If the condition is untreated, it leads to severe mental retardation.

If the individual is identified at birth as having the PKU mutation, simple dietary changes can help to overcome its effects. Infants are put on a very low phenylalanine diet. Because the cause of this genetic defect is now understood, foods containing phenylalanine are labeled by manufacturers to help people with PKU avoid eating them and endangering their health.



To identify people with the PKU gene mutation early enough to help them avoid the serious effects of the disorder, all newborn babies are tested for PKU deficiency using a simple blood test.

What Students Will Learn

This experiment demonstrates how the effects of a mutation can sometimes be hidden by the environment in which an organism lives. By doing this experiment students should learn that:

- Environmental conditions can have an effect on the severity of a mutant phenotype.
- Adjusting the type and amount of nutrients an organism receives can sometimes help overcome the negative consequences of a mutation.

Student Misconceptions to Watch For

Students' prior understanding of the term "division" can sometime hinder their understanding about how cells divide. To divide in math means to cut things into equal pieces; each division product will have only a fraction of the original material. When cells divide, they first must replicate, or copy, all of their DNA completely, so that each daughter cell will have a complete set of genetic instructions (not just half). In addition, cells often produce new cellular organelles before cell division so that each daughter cell will have a sufficient amount. After cell division, two cells are produced, usually of equal size and shape, and generally about half the size of the original cell.

Stress that before cells divide, the cellular DNA must be completely copied, or replicated. Therefore, in order to complete DNA replication, cells need building materials (sugars, phosphates, and the four types of nucleic acids, A, G, C, and T). Just as we cannot build a house without bricks and wood, cells must have the appropriate building blocks to build a new strand of DNA.

Teaching Strategies

This activity is designed to be carried out as an inquiry-based investigation. Students are challenged to resolve a discrepancy – a strain of yeast will grow on one type of media but not on another.

Divide students into groups of four. Tell them that their task is to figure out what mutation is carried by the strain of yeast that you will provide to them.

Your role is to act as a facilitator to help students to develop focused questions they can test by designing and carrying out experiments. Ask leading questions to get students started and to focus their thinking, but do not answer these questions for them. At first the students may be stuck, waiting to be told what to do. Explain that it is up to them to choose the course of what they want to learn. Remind them that each question or hypothesis they decide to test is only a small step toward answering the larger question. Explain that each group will carry out an experiment and then report on their results to

the whole class. Impress upon the students that careful note taking is critical to understanding their results later.

You may limit the number of media plates that students use by allowing each group to use only a certain number of each type. This forces students to think carefully about their experiments. To carry out all of the experiments listed in the POSSIBLE COURSES OF INVESTIGATION section below, each group of students would need 15 YEAD media plates and 8 MV plates.

After the students have conducted several experiments, summarize on the board the conclusions each group has made. All pieces of data should be acknowledged in a positive manner. Any experimental result arrived at through a well-designed experiment is a success. Let the class as a group consider the data that have been gathered so far. Each piece of information will help the whole class on the quest.

Have the students return to their groups to come up with the next experiment they would like to try, based on the data of the entire class. Let the students carry out 2 or 3 more experiments.

To conclude the project, have student groups prepare posters describing their work and present them to the class. The posters should explain the questions they chose to ask, the experimental designs they developed to ask those questions, the results they obtained, and the group's conclusions and interpretations based on those results. Have the students identify where the work of others in the class formed the basis for their own experimental designs. (Part of learning about the Nature of Science is understanding that most advances in science are made through collaborations with other scientists.)

At the close of the poster session, have the class discuss the experiments and work together to synthesize the class results into a theory regarding the mutation carried by the yeast strain. There need not be a "right" answer or ultimate conclusion here. (Science is a never-ending process; no matter where you stop, there will always be more questions to ask.)

This inquiry project will take portions of class periods over approximately two weeks. The efficiency with which this work can be done depends on:

- The temperature at which the yeast is incubated.
  - Yeast will grow overnight in a 30^{o}C incubator and take several days to grow at room temperature.
- The availability of materials when they are needed.
  - Whether media plates, chemicals, etc. are available when students think of the experiment they would like to do.
- Whether students are able to think about more than one experiment at a time.
  - It may be that progress can be made on more than one front by carrying out two experiments at once.

Assessment

Students should not be judged on whether they discover the exact nature of the yeast mutation, but instead on whether they asked questions about the yeast, formed hypotheses about what they thought might be happening, and designed cogent experiments to test those hypotheses. Students should also be assessed on how well they worked together - entertained the ideas of all members of the group, listened to each other, divided tasks and shared responsibility. To make these kinds of assessments, it will be important for you to circulate among the groups, listening to their discussions and observing their experiments. Resist the temptation to answer their questions for them as you do this. Instead, turn their questions around, asking "How would you find the answer to that question?" Students should be given credit at the end of the project for being able to identify flaws in their experimental procedures or for thinking that occurred during the course of their work. Each member of the student groups is responsible for understanding all aspects of the work of the group. As part of their assessment, ask each student to assess their own success in the project as well as to assess the contributions of the other members of their group.

Remember, whether the students actually figure out the nature of the yeast mutation is less important than whether they design experiments that give answers to hypotheses they formulate and choose to pursue.

Possible courses of investigation

Following is an example of a possible course of investigation. This is not comprehensive, but it should help you get a feel for the types of questions students might ask, the hypotheses they might form, and the types of experiments they might carry out to gain information relevant to their hypotheses. Remind the students that there is no one right way to figure out this (or almost any) question. Some of the experiments can be used to answer more than one question. When the students are formulating conclusions from their data, encourage them to try to derive everything they can from an experiment - sometimes an experiment tells you something you didn't realize you were asking!

What do yeast look like? How can you work with them?
Before beginning the experiments, teach students general techniques for working with yeast, including:

- Clean hands and work area before working with the yeast.
  - Wash hands with soap and water.
  - Wipe hands and work area with 70% or 95% alcohol and a paper towel.
- Label media plates with waterproof markers, writing in small letters around the outside edge of the bottom of the plate (small letters do not obscure the yeast growing on the plate; the bottom always stays with the media and the yeast).
- Keep the yeast and sterile things sterile.
  - Do not laugh, sing, cough or sneeze while working with the yeast or sterile items.

  - Only open media plates as much as needed to work, holding the lid over the plate.
  - Only touch sterile items, yeast or media with sterile items.
- Using sterile tootpicks.
  - Tap the end of the box opposite the hole cut in the corner to get toothpicks out enough for you to grasp them.
  - Only hold the end of a toothpick when you remove it from the corner cut in the box; do not touch the sterile end to anything except the yeast.
  - Use toothpicks that you grasp by the pointed end, leaving the rounded end sterile. The rounded end is less likely to gouge the agar. Discard toothpicks that you grasp by the rounded end.
- Incubate plates of yeast in an incubator or at room temperature.
  - Keep media plates upside down except when they are being used.

Plate the yeast on YEAD media plates.
- Plate yeast in two different ways. Gather observations of how cells grow each way.
  - Streaking
  - Lawns
- What is happening when a lawn of cells is formed? Hypothesis: Many colonies begin to grow, and since there are so many, they all overlap. Is this true?
  - Make serial dilutions (1:100, 1:1000, 1:10,000. 1:100,000) of a solution of yeast cells like the one you used to plate a lawn of cells; plate several of these dilutions. At lower concentrations of cells, the lawn will eventually break down into individual colonies.
- When you streak cells and individual colonies are formed, how are these cells related to each other?
  - The cells in a colony are clones of each other, in other words, identical cells.
- Use what you know so far to figure out how to determine the concentration of yeast cells in a yeast cell solution. Remember that a colony is the progeny of a single yeast cell.
  - Using serial dilutions you can produce a plate with a countable number of yeast colonies on it no matter how concentrated the solution originally was (just keep diluting!). Aim for about 50-100 colonies per plate. Make duplicate plates if you can, so you can be sure your experiment is reproducible. Remember that different dilutions should give you different numbers of colonies. In other words, if a 1:100 dilution gives you 150 colonies, then a 1:10 dilution should give you about 1500, and a 1:1000 dilution should give you about 15.
  - What dilutions of the solution did you do? How much of each solution did you put on the plate? How many colonies grew on each? Make a table to keep track of your data.
  - Calculate from these data the concentration of yeast cells in the original solution.

Do these yeast contain a mutation?
Tell the students you are going to demonstrate that the yeast contain a mutation - that even though they behaved perfectly well in the experiments above, there is actually something wrong with them.

- Streak yeast on YEAD and MV media. (The cells will only grow on YEAD.)
- Have students work in their groups to form hypotheses about what is going on and plan an experiment to help them find out what it is.

Why don't the yeast always grow?

1. Maybe the yeast strains on the two plates are not really the same (or maybe they were the same to begin with but being on the two types of plates has changed them somehow).
   - Take some of the yeast from the plate that is growing well and streak it on the two types of plates. Use the same toothpick so you are using a single sample of yeast. (The yeast will grow well on the YEAD plate and will not grow on the MV plate).
2. Maybe the yeast strain is mutant and the yeast cells really are present on the plate that appears to be not growing.
   - Move a sterile toothpick across the surface of the non-growing (MV) plate. Streak it on a YEAD plate. (The yeast that was not growing at all will now grow well on the YEAD plate. Therefore something the yeast needs to grow is missing from the MV media.)
3. Maybe something on the non-growing plate is poisoning the yeast. Are they dead? Or just not growing?
   - Move a sterile toothpick across the surface of the non-growing (MV) plate. Streak it on a YEAD plate. (The yeast that was not growing at all will now grow well on the YEAD plate. This tells you two things: 1) the plates are somehow bad, or not good enough for the cells, and 2) the plates do not kill the cells, they just do not support their growth.)
4. Maybe something in the "good" plates (the ones that support yeast growth) is critical and provides something the yeast need. (From a practical standpoint, this is a more difficult question to address. You could make media plates without some of the ingredients in them, streak out the yeast and observe on which plates the yeast will grow. However, the YEAD media uses ground up yeast as a source of nutrients, not a chemically-defined set of ingredients. Another way would be to use MV media plates and add back some ingredients that you think might be important for cell growth. However, since you do not know the nature of the mutation, the cells might need anything. Therefore, this would likely be an open-ended nightmare.)

How does the information that the yeast are mutants fit in?

1. Maybe the yeast are not mutants and it is the media plates that are at fault. Maybe they were not made right, or have some poison, or do not have enough nutrients.
   - Try growing some other yeast, such as baking yeast, on both types of media plates. Prepare a suspension of these cells using warm, sterile water. Plate some of these cells on both YEAD and MV media plates. (The cells should grow on both types of media.)
2. We know that in some conditions this yeast strain does not grow, but from one of the experiments above, we know that the cells are not killed - they are just not dividing. What might be wrong in the mutant strain that would prevent cells from dividing and that could be fixed by putting them on a different kind of media?

- Have students brainstorm a list of all the reasons they can think of that would prevent the yeast cells from growing on one medium when they do grow on another medium.

Will adding DNA building blocks enable the yeast cells to grow on MV media?

1. Perhaps the yeast cells are surviving, but are not growing because for some reason they can not copy their DNA. Will supplying them with some of the building blocks needed for DNA synthesis help?
   - Plate out lawns of yeast and then place paper disks soaked in the DNA building blocks - adenine, guanine, cytosine and thymine - on the plates. (The nucleic acids in the disk diffuse into the media. Yeast within the range of diffusion will absorb the nutrients; yeast outside the area of diffusion will not. If there is an effect, cells will grow in a halo around the paper disk. Have the students describe what they see and present possible explanations. Encourage the students in coming up with alternative explanations. This is a good example of a situation where the results could be explained in several different ways.)

What is the ade2 mutation? What effect does it have on the yeast cells?

Yeast carrying the *ade2* mutation have a defect in their ability to utilize adenine that they have stored in their cells.

- If there is plenty of free adenine in the medium these cells grow well.
- If no adenine is present in the medium they do not grow at all.
- If there is some adenine in medium, the cells start to grow and try to activate the biochemical pathway for using the adenine that they have stored. Because of the mutation they carry, these yeast get stuck before this process is completed and an unwanted intermediate product piles up. This product changes the yeast colonies from a cream to a red color.

A striking result will be seen when placing adenine-impregnated paper disks on MV media. A halo of cream-colored colonies grows around the disk, surrounded by a halo of red colonies, surrounded by an area where the yeast does not grow at all. (A photograph of these results is available at http://www.phys.ksu.edu/gene/photos/ag.html). Why does this occur? Remember that the media in the Petri dishes contains a lot of water. When the disk is placed on the media, the adenine in the disk diffuses into the water of the media and spreads away from the disk in a gradient fashion – the highest concentration is closest to the disk, with lower and lower concentrations further away from the disk. The cream colored yeast cells closest to the disk have sufficient adenine to grow. At intermediate concentrations of adenine, the cells start to grow and turn on their adenine -utilization mechanisms. However, the *ade2* mutation blocks their ability to use their stored adenine. A chemical intermediate builds up and causes the yeast cells to look red, forming a red halo around the cream colored cells. Outside the red halo, the yeast cells do not receive enough

adenine from the disk to even begin growing. Guanine, thymine or cytosine disks should have no effect on yeast growth; i.e., the cells should not grow on MV media.

This experiment is a demonstration of a conditional mutation in action; the yeast behaves three different ways, depending on the environmental conditions in which it finds itself. The explanation for the different colors of concentric halos is very subtle. It will be difficult at first for the students to grasp that so many processes are going on in the cells at once (DNA synthesis, adenine uptake, initiation of cell division, utilization of stored building materials, etc.). However, cells are complicated, amazing places, which is a worthwhile lesson in itself.

Techniques for Working with Yeast

Keeping sterile things sterile
Dust is the most common carrier of contamination (bacteria and molds) to yeast cultures and media. It can come from people, from the air or from the bench or table top. To minimize contamination, do the following:

- When you are streaking or pouring plates choose a time (usually after classes end for the day) and place where you will not be interrupted by students, colleagues or janitors. Lock the door to your work area.
- If possible, choose a work area that is far from plants, animals, *Drosophila* (fruit fly) cultures, and other biological materials that are sources of mold.
- Choose a work area with the least amount of air turbulence possible. Do not talk, sing, whistle, cough or sneeze in the direction of sterile items or your work area.
- Before beginning to work, clean your work area with soap and water. Then wipe it down with 70% or 95% ethyl or isopropyl (rubbing) alcohol (70% is actually more effective). Wash your hands with soap and water and then wipe them with alcohol.
- Keep sterile media, plates, toothpicks, etc. covered as much as possible.
- Only touch the yeast and sterile things with other sterile things or surfaces.

Streaking yeast cells on a media plate

1. Use the rounded end of a sterile toothpick to collect a very small amount of yeast.
2. Gently streak the yeast in a zig-zag pattern all across the surface of the media plate. Yeast cells will be spread on the media even if you cannot see them.

Making a suspension of yeast cells

1. Use the rounded end of a sterile toothpick to collect a small amount of yeast.
2. Place the yeast on the side of a sterile container and add sterile water; add enough water for each media plate on which you will place the yeast. You will need 1 ml for 100 x 15 mm Petri dishes and 0.3 ml for 60 x 15 ml dishes.
3. Replace the lid on the container and swirl it to mix the yeast and water. Add more yeast cells if necessary to make a visibly cloudy suspension of cells.
4. You may choose to aliquot the yeast solution for each media plate into sterile, capped test tubes or dispense the yeast yourself.

Yeast cells do not survive long in water; it is best to prepare a fresh yeast suspension immediately before each use.

Plating a lawn of yeast cells

Plating a lawn of yeast cells means to spread a solution of yeast cells over the surface of a media plate so densely that when colonies grow up from each cell they will touch each other and form a solid layer of cells.

1. Swirl the solution of yeast cells to get the cells into suspension.
2. Place 1 ml of yeast solution on 100 x 15 ml media plates and 0.35 ml of solution on 60 x 15 ml plates.
3. Gently tilt and rotate the Petri dish to spread the liquid.
4. If there are places the liquid does not cover, use the rounded end of a sterile toothpick to move the liquid over them.
5. Let the Petri dish sit for 5-10 minutes to allow the water to soak into the media.

Providing extra nutrients to the environment in which the yeast will grow

Nutrients impregnated into paper disks will diffuse into media after being placed on the surface of the plates. By adding nutrients in this way, you can test whether the substance you have chosen affects the growth of the yeast on the plate. Remember that the greatest concentration of the substance will be near the disk, with less and less of it present in concentric circles out from the disk. To add nutrients in this way:

1. Begin the experiment early in the week rather than the end, to allow adequate opportunity for daily observations of changes in the yeast growth.
2. Plate a lawn of yeast cells, as described above. Allow all of the water to soak into the media.
3. Use sterile forceps to place the paper disk on the surface of the media.
4. Incubate the media plate with the lid side of the dish **up** (note that this is contrary to the usual practice for growing yeast).
5. Record observations about yeast growth on the plate for several days.

Yeast incubation temperature and growth rates

- The optimum growth temperature for yeast is 30^{o}C, but it will grow satisfactorily at room temperature; the cooler the room, the slower it will grow.
- Incubate yeast in the dark as much as possible.
- For optimal growth, the plates must be aerobic. Do not tightly seal them in plastic bags while they are incubating. You may find it helpful to incubate them in open food-storage bags, which keep them from drying out too quickly and protects them from contamination.
- If the yeast has grown enough to show differences among the treatments and students are not able to immediately observe the plates, place the plates upside down in the refrigerator in a sealed plastic bag. This will stop the yeast from growing. If the yeast grows too much it may obscure differences among the treatment areas on the dish.
- Yeast strains can be stored on media plates in the refrigerator for up to six months or a year.

Material Preparation Guide

HA2 yeast strain
The HA2 (*ade2*) yeast strain has a mutation in the cellular pathway required to utilize stored adenine, a DNA building block. The ade 2 cells need added adenine to grow.

see "Part F.6. A Closer Look at… Repair of DNA" in *A Classroom Guide to Yeast Experiments* by Tom Manney, et. al., available at http://www.phys.ksu.edu/gene/chapters.html

YEAD (Yeast-Extract Adenine Dextrose) and MV (Minimal plus vitamins) media plates
Pre-poured plates can be ordered from Carolina Math and Science – see Material Sources. Instructions for pouring plates yourself are listed below.

Petri dish size
The pre-poured plates available from Carolina Math and Science are prepared in 100 x 15 mm Petri dishes. If you pour plates yourself, it is more economical to use smaller, 60 x 15 mm Petri dishes. If you are unable to use the smaller dishes, you may choose to have groups of students divide and share the larger dishes.

Amount of media needed
- 100 x 15 ml Petri dish – approximately 25 ml of media for each dish
- 60 x 15 ml Petri dish – approximately 10 ml of media for each dish

Equipment and Materials
- Dry YEAD or MV media
- Sterile, polystyrene Petri dishes
- Deionized or distilled water
- 70% or 95% ethyl or isopropyl (rubbing) alcohol
- Glass flasks with a capacity of about twice the amount of liquid media you will place in them
- Graduated cylinder
- Balance, weighing paper, and scoopulas (if not using pre-weighed media)
- Autoclave, canning pressure cooker or microwave
- Aluminum foil (autoclave or pressure cooker) or plastic wrap (microwave)
- Sterile facial tissues (from a freshly-opened box, or several tissues down in an opened box)
- Heat-resistant gloves or potholders (for handling hot flasks)
- Waterproof, broad-tipped markers in two colors
- Rubber bands or masking tape

Dry media
Packets of pre-measured dry media can be ordered from Carolina Math and Science – see Material Sources.

YEAD media – 500 ml
- 5 grams Yeast Extract
- 10 grams Dextrose, anhydrous (glucose)
- 10 grams Agar
- 500 ml deionized or distilled water

MV media – 500 ml
- 3.35 grams Difco Bacto-Yeast Nitrogen Base *without* amino acids and *with* ammonium sulfate
- 10 grams Dextrose, anhydrous (glucose)
- 10 grams Agar
- 500 ml deionized or distilled water

Autoclave or pressure cooker preparation of media
1. Use a flask with a capacity of about twice the amount of liquid media you plan to place in it; flasks larger than 2 liters are difficult to handle.
2. Pour the dry media into the flask, weighing chemicals if needed.
3. Add the appropriate amount of deionized or distilled water to the dry media.
   - Pour about one-fourth of the water into the flask.
   - Swirl the flask until most of the lumps are dissolved and the media is thoroughly wet.
   - Use the rest of the water to wash any dry media on the sides of the flask down into the solution; avoid further mixing.
4. Cover the flask with aluminum foil.
5. Sterilize the media in an autoclave or canning pressure cooker for 15 minutes at 15 pounds per square inch of pressure. Allow the pressure to return to zero.
6. Swirl the hot flask vigorously after sterilization; this eliminates layering of the ingredients and ensures a uniform distribution of nutrients.
7. Cool the media to 55^{o}C – the flask will be warm, but not too hot to touch comfortably (do not place a thermometer in the sterile media). It will take about 30 minutes at room temperature for the media to cool. If you prepare several flasks at the same time, you may place them in a 55^{o}C water bath to keep them at pouring temperature. If you allow the media to become too cool, it will begin to “gel” in the bottom of the flask. Cooling the media to 55^{o}C before pouring prevents excess condensation from forming on the Petri dish lids.
8. While the media cools, prepare to pour the plates.
   - Wash your hands with soap and water.
   - Wipe down your work space and hands with alcohol to remove possible contaminants.
   - Remove the Petri dishes from their plastic bag and align them in a stack.
   - To easily distinguish between the YEAD and MV plates, choose a different color of broad-tipped marker to identify each type of plate. Run the appropriate marker down the stack of Petri dishes 1-3 times, marking the edges of the lids.
   - Arrange the Petri dishes along the edge of your working surface so they will not need to be moved after you pour in the liquid media. You may lay them out singly or in stacks of 3-5.

9. Remove the aluminum foil from the neck of the flask. Wrap several layers of sterile facial tissues around the neck of the flask to protect your hand while pouring (the media is still hot).
10. Pour liquid media into the Petri dishes:
    - Lift the lid of each Petri dish only enough to pour in the media; this helps prevent contamination of the media by mold spores, etc. Replace the lid as soon as you finish pouring.
    - If you have arranged the Petri dishes in stacks, first lift the lid of the bottom dish, balancing the other dishes on top of it; continue upward through the stack, pouring each dish in sequence.
    - Fill each Petri dish about one-half full. This can be estimated by pouring the media on one side of the dish and stopping when the flow of media reaches the other side.
    - Once you begin pouring, keep the flask in pouring position until it is empty; this keeps contaminants from falling into the media. Since most contamination is airborne, do not talk, sing or whistle while pouring plates.
11. When the flask is empty, immediately rinse it with very hot tap water to remove any remaining media.
12. When the media in the Petri dishes has cooled and solidified, invert them so that condensation does not drip onto the media surface.
13. To store the plates, return them to the bags they came in (keep them inverted) and tightly seal the bag tops with rubber bands or tape. The plates can be stored for several months at room temperature, or in the refrigerator, which inhibits the grown of contaminants.

<u>Microwave preparation of media</u>

Media prepared in a microwave will not be as sterile as media that is autoclaved. However, microwave preparation is a viable alternative if you do not have an autoclave or canning pressure cooker. It is also useful for preparing small amounts of media for emergency use. Because media prepared in this way is not as sterile, plates usually can not be stored for long periods of time without growing molds or other contaminants. To prepare media in the microwave, use the procedure below:

Follow Steps 1-3 in the autoclave procedure above.

4. Cover the flask with a piece of plastic wrap. Punch a hole in the plastic wrap to allow steam to escape.
5. Dissolve the media in the microwave.
    - Microwave the flask on HIGH for several minutes. Watch the media carefully through the window in the door.
    - When the media boils, stop the microwave and swirl the media; use a folded paper towel or hot pad to protect your hand. Continue to do this until the media dissolves.

Continue with Steps 6-13 in the autoclave procedure.

Removing water condensation on the lids of media plates

Before turning media plates right side up to use them, look to see if there are droplets of water on the inside of the lid. If there are, do the following:

- Hold the plate in the upside down position.
- Grasp the bottom (which is facing up) with one hand.
- Remove the lid with the other hand, turn it over, and briskly flick it to remove the water droplets.
- Replace the lid on the plate and turn it over.

If there is moisture condensation in the lid that cannot be removed by flicking, pull a sterile facial tissue out of the box and use a part of it that you have not touched to wipe the condensation from the lid.

Resource on preparing yeast media plates

Yeast Experiments Video 3: Methods and Materials - "Taking the Magic out of Making Media" demonstrates the complete media preparation and plate pouring process. Available from Carolina Math and Science, catalog # WW-17-3682, $25.75.

Nucleic Acid Blotter Paper Disks

Only the adenine disks are absolutely needed for this experiment. In addition to disks containing each of the separate nucleic acids, you may also wish to prepare ones containing all four together.

Equipment and Materials

- 1.25 grams of nucleic acid (Adenine, Cytosine, Guanine, or Thymine)
- 150 ml distilled water
- 300 ml beaker
- Hot plate or Bunsen burner
- Art blotter paper
- Hole punch
- Sterile forceps (see instructions for sterilizing a container below)
- Sterile Petri dish

1. Cut disks from the art blotter paper using a hole punch.
2. Pour the distilled water into the beaker and add the nucleic acid.
3. Boil five minutes.
4. Add blotter paper disks and boil five minutes more.
5. Remove the disks with sterile forceps to a sterile, covered container such as a Petri dish.
6. Place the disks in a single layer and allow them to dry.
7. Store in the Petri dish or another sterile container.

Sterile container with lid
Use a beaker covered with aluminum foil, a baby food jar, or any other container that you can sterilize and cover. If you can get a donation from a doctor's office, clinic or hospital, sterile, plastic specimen containers with lids are easy to use.

- The container needs to hold at least twice the volume of the yeast solution you will need (1 ml/group for 100 x 15 ml Petri dishes and 0.35 ml for 60 x 15 ml dishes).
- To reduce contamination, use a different container for each class.

To sterilize a container, place the cover or aluminum foil loosely over the mouth and use one of the following sterilization methods:

- Autoclave or pressure cook for 15 minutes at 15 pounds of pressure.
- Bake at 320^{o}F for 2 hours.

Sterile distilled water
Sterile distilled water can be purchased, or prepared in either of the following ways:

- Autoclave or pressure cook a loosely covered bottle of water for 15 minutes at 15 pounds of pressure.
- Place a loosely covered bottle of water in a pan of boiling water for 15 minutes.

After the water and container cool, tighten the lid.

Sterile, flat toothpicks
Toothpicks in unopened boxes are sterile and can be used directly from the box. Use scissors to cut a small hole (about 1/4 inch across) in one corner of the box to serve as a dispenser. The toothpicks in the box are pointing in both directions; use only the ones you grasp by the pointed end (leaving the rounded end sterile, for use).

Additional Resources

A Classroom Guide to Yeast Experiments. Kansas State University.
http://www.phys.ksu.edu/gene/chapters.html
Part A. 2. Genetics of Bakers Yeast – provides an introduction to yeast genetics.
Part F. 1. A Closer Look At … Adenine-requiring Mutants – provides in-depth information about these mutants, including a diagram of the biochemical pathway.

Mystery Yeast Mutation

Your challenge: Yeast Strain X Carries a Mutation – What is it?
And what does it do to the yeast?

Your teacher will provide you with the yeast, two types of media plates on which the yeast grow - or don't grow, several chemicals that might be of use to you, equipment you'll need to do experiments, plus some general instructions on how to work with yeast.

Your teacher will help you with practical details, but it is up to you to figure out what you want to know and how to do the experiments to find that out. You'll be working in groups of four to design and carry out experiments.

Remember to include control experiments so that the results you obtain will be believable. Only change one variable at a time so that you'll always be able to identify what important feature of your experimental design caused the results you observe. Take careful notes as you go, so you'll be able to report on what you did.

After the first few experiments your teacher will convene the class so that each group can report on its work. Be prepared to present the questions you asked, what experiments you carried out to answer them, what your results were, and what you think your experiments tell you. Remember that even if your experiment did not turn out as you had hoped, learning that you should have designed an experiment differently, and understanding why, is valuable, too. Help your classmates learn from your experiences - this yeast project isn't over yet. After the class shares all of its results and conclusions, discuss in your group what you think is known so far about the yeast strain. Think about what you need to examine next to learn the nature of the yeast mutation and plan the next set of experiments.

When this second set of experiments is finished, prepare a poster for presentation to the class that summarizes your best understanding of the work your group has completed. Include the perspective you have gained from listening to the results of the other students in your class. When a result from another group forms the basis for an experiment or conclusion of your own group, be sure to give the other group credit.

GOOD LUCK!

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Mystery Yeast Mutation

Relating experiments with *ade2* yeast to human genetic disorders

Some human genetic mutations function in a manner similar to that of the yeast carrying the *ade2* mutation. Like the yeast, under certain environmental conditions, humans carrying such a mutation may appear totally normal (be asymptomatic). Under another set of environmental conditions, the same person may show symptoms of the genetic disorder. For example, a person's body may not make enough of a nutrient, or too much of a chemical accumulates that their body would normally eliminate. These types of problems can sometimes be treated by changes in the environment of the individual (as was done with *ade2* yeast); that is, by providing the nutrient or by restricting the intake of certain chemicals. Much more often, however, the effects of genetic disorders can not be medically treated.

PKU, a Treatable Genetic Disorder

Phenylketonuria (PKU) is an example of a genetic disorder whose effects can be prevented or controlled by manipulating the environment of the individual. In this case, the diet of an individual with the PKU mutation is altered to prevent buildup of a toxic chemical.

PKU is a recessively inherited genetic disorder; this means that a person must have two copies of the PKU mutation to show symptoms of it. PKU occurs in approximately one of every 10,000-25,000 births. The normal PKU gene codes for a metabolic enzyme, phenylalanine hydroxylase. People who are homozygous for PKU mutations do not make this enzyme and are therefore unable to break down, or metabolize, the amino acid phenylalanine.

Although PKU babies appear normal at birth, the metabolic defect they carry results in a buildup of phenylalanine and other toxic metabolites in their systems after birth. These compounds are highly destructive to the central nervous system, and if the condition is untreated, result in severe mental retardation. It has been estimated that untreated PKU babies lose, on average, one to two IQ points per week during the first year of life. Thus, a PKU genotype can produce a severe disease phenotype.

Fortunately, it is easy to screen for PKU at birth using a simple blood test. The disorder can be treated in individuals homozygous for PKU mutations by starting a diet low in phenylalanine within one month of birth. Treated individuals still carry the PKU genotype. However, by modifying the environment – in this case, their diet – they do not develop the phenotype of mental retardation. Because the cause of this genetic defect is now understood, foods containing phenylalanine are labeled by manufacturers to help people with PKU avoid eating them and endangering their health.

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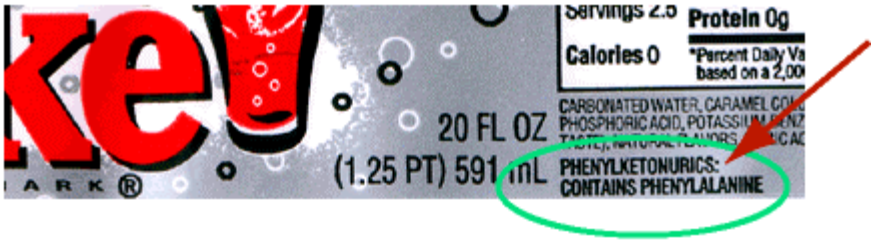

To identify people with the PKU gene mutation early enough to help them avoid the serious effects of the disorder, all newborn babies are tested for PKU deficiency using a simple blood test.

The example of PKU shows how phenotype is a combination of an interaction between genotype and environmental factors. These environmental factors can include not only a person's internal and external environment, but also their genetic environment – that is, other genes, whose products may interact with a specific gene or its product.

Learn more about PKU

University of Utah Genetic Science Learning Center
http://gslc.genetics.utah.edu/thematic/newborn/activity/infosheets/PKU.html
Provides a summary of the genetics, incidence, clinical outcome and testing for PKU.

National PKU News
http://205.178.182.34/
Site contains diet information, politics and legislation concerning PKU and PKU research.

PKU Resource Booklet for Families. The Montreal Children's Hospital.
http://www.mcgill.ca/pahdb/handout/handout.htm
Includes information about the genetics of PKU, consequences of the disorder, treatment through diet and a glossary.