Introduction to *Saccharomyces cerevisiae*

**The vegetative life cycle of yeast**

*Saccharomyces cerevisiae*, commonly known as Baker's yeast, is a unicellular fungus that reproduces by budding. During S phase of the cell cycle (while the replication machinery in the nucleus makes a second copy of the genome), a small bud emerges from the ovoid mother cell (Figure 1.1). This bud continues to grow as the cell prepares for cell division (mitosis in M phase). During mitosis, the nucleus divides and one full complement of DNA is packaged into the large bud before it is pinched off. Both the mother and daughter cells go on to G1 where they grow and subsequently embark on a new round of the cell cycle. Thus by observing the cellular morphology of *S. cerevisiae*, you can determine each cell's position in the cell cycle, as summarized below:

![Cell cycle stages](image)

**Figure 1.1.** Bud size reflects cell cycle status. The dark circle inside the cell represents the cell nucleus.

Using bud size as a morphological marker for position in the cell cycle, researchers in the 1970's began looking for mutants defective in their ability to progress through the cell cycle. Because proper cell cycle progression is essential for maintaining and propagating life, these mutant phenotypes were typically inducible under certain conditions such as high temperature. Mutant yeast identified in this manner grew normally at their permissive temperature, but could be arrested at various points in the cell cycle when incubated at the non-permissive temperature. For example, cell division cycle mutant *cdc37-1* behaves like wild-type yeast when grown at 23°C, but cells arrest as unbudded cells in G1 when incubated at 37°C. Interestingly, human homologues of many *cdc* genes have been identified, and these genes may play a role in cancer progression and/or prevention. In fact, Leland Hartwell, the lead author of the textbook we're using, won the 2001 Nobel Prize in Medicine for his work on yeast cell cycle genes.
**The sex life of yeast**

Okay, so calling it sex may be overstating things a bit, but yeast can (and do!) mate, and this mating is an incredibly useful tool for yeast geneticists. So, here's the scoop…

Laboratory yeast strains can be cultured and maintained as haploid cells (like eggs or sperm) or diploid cells (as in somatic tissues), carrying one or two versions of the yeast genome, respectively. Haploid *S. cerevisiae* come in two mating types, a and α, and each mating type secretes its corresponding pheromone, a small peptide that tells neighboring cells which mating type it is (this works much better than those pheromone colognes and perfumes that were so trendy a few years back!). When a particular haploid cell senses pheromone from the opposite mating type, it will prepare to mate by forming a mating projection (Figure 1.2). This mating projection is commonly called a "shmoo," because it causes the yeast cells to take on a shape similar to that of Al Capp's cartoon character, shmoo (Figure 1.3). If shmooed cells of opposite mating types meet, mating can occur, resulting in the fusion of the two cells and formation of a dumbbell-shaped zygote. Ultimately, the two nuclei in this zygote will fuse (a process called karyogamy) to create a diploid cell that can be propagated as such in the lab. During vegetative growth, diploid cells reproduce by budding as described above (Figure 1.1).

Just as our bodies use meiosis to create haploid eggs or sperm, yeast use meiosis to go from the diploid to the haploid state. A key trigger for initiating meiosis in yeast is nitrogen starvation. Upon completion of the meiosis program, a diploid yeast cell will have been converted to four haploid spores, two of mating type a and two of mating type α. These spores are surrounded by a tough coat called an ascus, and this packet of spores is extremely resistant to environmental stresses. In the wild, these spores would germinate when conditions improve, and the resulting yeast would mate with each other to give rise to diploid budding yeast. In the laboratory, however, scientists take advantage of spore formation by dissecting these siblings away from each other and tracking traits in the haploid progeny of diploid yeast. If a diploid yeast heterozygous for the trait "z" is sporulated, how many of the resulting spores would you expect to carry each allele (big Z vs. little z)?

**Figure 1.3.**

**ANSWER:** two big Z and two little z!
The life of yeast in culture

Wild-type cells in a liquid culture of yeast will continue dividing as long as conditions are favorable and nutrients plentiful, completing a round of the cell cycle approximately every 90 minutes. Growth of yeast cultures can be monitored by using a Spec20 or similar device to measure the cell suspension's optical density (or absorbance) at 600nm. An optical density of 1.0 correlates with approximately $1 \times 10^7$ cells/ml (this number varies somewhat depending on the specific strain used). When tracking the growth of yeast, researchers take samples of a liquid culture and measure its optical density as a function of time. This data is then used to construct a growth curve like the one depicted in Figure 1.4 and described below.

Early in the time-course, there is a slight lag as the cells become accustomed to their new environment and "realize" that nutrients are available. Then the culture density increases exponentially as the cells rapidly divide. As nutrients become scarce, the growth rate slows, and the yeast adjust their metabolic machinery. They must switch from fermenting the now scarce sugars and begin respiratory metabolism in order to take advantage of the ethanol produced during fermentation. This portion of the growth curve is referred to as the diauxic shift. Eventually, when all the nutrients (sugar and ethanol) are exhausted, the culture enters stationary phase where a significant number of the cells in the culture have exited the cell cycle thus entering Go. Cells can survive in stationary phase for extended periods of time due to molecular changes that have occurred, including modifications to the cell wall, filling the cytosol with the special storage sugar trehalose, and dramatic slowing of many basic cellular processes including transcription and translation. The return of a plentiful carbon source will trigger cells to re-enter the cell cycle and to begin dividing.

Figure 1.4. Typical growth curve for yeast.
Yeast Media

So, what does it take to grow yeast? One of the most common types of media used by yeast geneticists is YPD, a type of rich media that contains all the nutrients that yeast need to grow and be “happy.” The name simply refers to the fact that this media contains yeast extract (a complex mixture of peptides and amino acids), peptone (a water soluble protein derivative), and glucose (also known as dextrose) dissolved in water.

When scientists want tighter control of the nutrients available to their yeast strains, they use synthetic dextrose-containing media or SD. In addition to glucose, synthetic media also contains a nitrogen source and some combination of amino acids (the building blocks for proteins) and nitrogenous bases (the building blocks for nucleic acids). Synthetic media that contains a complete mix of amino acids and nitrogenous bases is called SDC (synthetic dextrose complete). Although this contains all the nutrients yeast need, it is not as "rich" as YPD, and thus yeast grow more slowly in SDC.

In addition to being cultured in liquid media, yeast can also be cultured on media that has been solidified by the addition of agar. One advantage of streaking cells onto solid media in a Petri dish is that this technique allows you to study yeast that are all the progeny of a single cell. When cells are spread far enough apart on a plate of solid media, you will see well-defined, single colonies. All of the millions of cells making up this colony arose when a single cell deposited on the plate divided to form two cells, then these two cells each divided to form two cells, for a total of four, and so on… Being able to streak yeast onto media and obtain single colonies is also an important skill, because colony size can be a key indicator of genetic defects.
Microbiological Technique

Whether working with yeast in liquid media or on plates, it is critical that you maintain pure cultures and avoid contamination. **Good sterile technique is a must!**

For starters, when working with microbes (yeast or bacteria, for example), you should begin by creating a sterile working environment. Start off by squirting 70% ethanol on your bench top and then wiping it off with a paper towel. Next connect your Bunsen burner to a nearby gas valve, turn the valve to the on position, and use a striker to light the Bunsen burner. Keeping the Bunsen burner on while you're working will help keep air circulating in an upward direction so that dust and mold particles in the air won't settle onto your cultures as depicted below.

To keep all of your supplies sterile, *never ever* leave Petri dishes, tip boxes, or containers of media sitting around uncovered. Always put the lid back on when not using something, even if it is only for a minute (or less!).

When working with sterile solutions, always run the mouth of the bottle through the flame of your bunsen burner before removing what you need. Also be sure to flame the mouth of all flasks and culture tubes after removing and before replacing the cap or foil covering.

If a living culture is spilled, cover it with disinfectant (bleach or ethanol) and report it to your instructor.

**Do not discard living cultures down the drain or in the trash.** Add a squirt of bleach, and place it in the designated dirty dishes area.

Finally, be sure to wipe down your work area with 70% ethanol and **wash your hands** before you leave the lab!

As the hot air created by the Bunsen burner rises, it creates upward air currents that help carry away germs and other contaminants.

To prevent contamination, flame the openings of bottles, culture tubes, etc immediately after opening and immediately before closing.
Streaking Yeast

Today we will begin by practicing sterile technique and learning how to properly streak yeast to obtain single colonies. Each single colony you see on a plate arose from the repeated divisions of a single cell and its offspring. Thus, all the cells in a colony are genetically identical. Streaking for single colonies also helps you evaluate the purity of the culture you are working with. If your streak contains colonies of different sizes, shapes or colors, you know that what you started with was not pure.

In order to obtain single colonies when streaking yeast, you must spread the cells out… *A LOT!* In order to accomplish this, follow the protocol below.

Begin by wiping off your working space with 70% alcohol and then turn on your bunsen burner. Next, while being careful to keep the Petri dish closed, use a Sharpie marker to divide a fresh YPD plate into 4 sections. Now is the time to get in the habit of writing on the bottom of the plate (the half with the media in it). Also be sure to write your name on the plate. Now set your plate on the bench upside-down (the side you just wrote on should be facing up). Keeping your plate upside down as much as possible has many advantages which are described in the box on page 16.

Now find the foil-wrapped packet of sterile toothpicks on your lab bench. Carefully open the packet, remove a single toothpick, and immediately fold the packet to close it and keep the remaining toothpicks sterile. When you remove this toothpick from the packet, be sure to hold the pointed end of the toothpick and use the rounded end to manipulate the yeast. Using the rounded end of the toothpick to spread the yeast onto solid media will minimize the odds of you digging into and damaging the agar (the goal here is to gently spread cells along the surface!).

**Why Toothpicks and Sticks?**

- *They're inexpensive.*
- *When streaking cells onto solid media, toothpicks are much easier to work with than long, unwieldy inoculating loops.*
- *Since you can have hundreds sterile and ready-to-use, you don't have to waste time sterilizing them every time you want to pick a colony. This minimizes the risk that you'll fry the poor little yeast or bacteria (a common problem when using freshly flamed/sterilized inoculating loops!).*
With the sterile toothpick you just removed from the packet, pick a single colony from a plate labeled "wild-type yeast." To do this, gently touch the colony with the rounded end of the toothpick to obtain a barely visible glob of cells. Now pick up just the bottom half of your Petri dish (the half with the media in it), and begin the streaking process in one quadrant of your fresh YPD plate as depicted in Figure 1.5 below.

**Step 1**: Gently "draw" a line of yeast at the outer edge of the quadrant, and close your Petri dish (it should be upside-down!). Now put your used toothpick into a "dirty toothpick" beaker. Don't worry if you can't see big globs of yeast on your plate (in fact, big globs are a bad thing!). Baker's yeast is a microbe after all, and it doesn't take much to get plenty of growth if you just give 'em a day or two.

**Step 2**: Obtain a new sterile toothpick, open your Petri dish, and use one gentle swipe to gently pull some cells from your initial streak towards the center of the plate by a centimeter or two. Close your Petri dish (it should be upside-down!), and put this used toothpick into a "dirty toothpick" beaker.

**Step 3**: With another new toothpick, gently swipe through the streak from step 2 one time. Then without lifting your toothpick from the surface of the agar, swiftly and gently sweep across the quadrant in a zig-zag motion to spread the cells out without passing through any other streaks (remember: you're trying to deposit single cells on the media!). As you streak the cells out across the media, be sure to move them from the perimeter towards the center of the plate. Now close your Petri dish (it should be upside-down!), and place your last toothpick into a "dirty toothpick" beaker.

Now use fresh toothpicks to pick another colony of wild-type yeast and streak it in another quadrant on your YPD plate, and then repeat the process two more times so that you have streaked yeast onto each of the four quadrants on your YPD plate. Now place your plate (upside-down, of course!) in the 30°C incubator. In two days, your instructor will examine the plates and subsequently notify you if further streaking tutorials are necessary.

![Figure 1.5. Streaking for single colonies. Use a new sterile toothpick for each step.](image-url)
Some common mistakes to avoid when streaking for single colonies…

• Starting with a big glob of yeast. If you start with too many cells, it will be virtually impossible to spread them out enough to get single colonies.

• Not switching toothpicks. It is exceptionally difficult to get single colonies if you do not switch toothpicks between the different parts of the streak.

• Pulling through the top part of the streak during step 3. The point of using three steps when streaking is to “dilute” the cells as much as possible. If you keep going back to the dense part of the streak you’ll be hard-pressed to spread the cells out enough to get single colonies.

• Holding the toothpick too firmly. Although you need to hold the toothpick firmly enough that you won’t drop it, you also need to hold it loosely enough that when you gently press it against the agar, it has some give and doesn’t jab into and damage the media.

• Thinking too hard. If you think too hard you’ll probably streak slowly, thereby increasing the time your plate is open to contamination. Excessive thinking while streaking may also increase your likelihood of damaging the agar.

Why is it important to incubate and store plates upside-down?

Quick FAQ:
Spreading Yeast

In addition to streaking for single colonies, every good yeast geneticist should know how to spread a cell suspension (cells in liquid) onto solid media. Just like when you were streaking cells, you should adhere to good sterile technique throughout this procedure. So, if you haven’t done so already, wipe off your work space with 70% ethanol and turn on your bunsen burner.

Next, while being careful to keep the Petri dish closed, use a Sharpie to write your name and the phrase “wild-type cell spread” onto the bottom of a Petri dish containing SDC.

Now turn the plate right-side up (this is one of the rare exceptions to the “always upside-down” rule!), and take a moment to locate the liquid culture of wild-type yeast that is in a microfuge tube labeled “wt”.

Vortex the culture briefly to ensure that the cells haven’t settled to the bottom of the tube, then use a pipetman to transfer 200 μl of this culture to the solid SDC plate, lifting the lid off the plate for as short a time as possible. If you don’t remember how to use a pipetman, refer to the “Pipetman Cheat Sheet” on the last page of your lab manual.

In order to spread these cells evenly on the surface of the SDC agar, you’ll need to sterilize a cell spreader that is currently resting in a beaker full of 70% ethanol. To sterilize the spreader, slowly pull the wet spreader through the flame of your Bunsen burner, leaving it in the flame just long enough to catch the alcohol on fire. While holding the spreader away from the Bunsen burner, watch while the ethanol burns off, sterilizing the spreader.

Now use your free hand to lift the lid off your Petri dish. While holding the lid with one hand and the spreader in the other, touch the spreader to a portion of the plate where there are no cells. As long as you don’t here any sizzling (which you shouldn’t!), it’s safe to gently spread the cell suspension evenly across the surface of the agar.

After you are finished spreading the cells, close your Petri dish and let it rest right-side up for a few minutes while the liquid is absorbed.

Also, dip the cell spreader in 70% ethanol, pull it through the flame of your Bunsen burner, and wait for all the ethanol to burn off. Then return the spreader to the beaker containing ethanol.

Once all the liquid has been absorbed into the solid media, flip the Petri dish upside down and place it in the 30°C incubator. Then repeat this procedure, spreading 200 μl of wild-type yeast culture on an SDC plate containing the anti-cancer drug HePC (we’ll talk more about this drug next week!).
Looking at Yeast

Next we will examine yeast at different stages in their life cycle. You have been provided with samples of exponentially growing yeast (microfuge tube labeled "EXP"), mating yeast (Petri dish labeled "MATING"), and spores (microfuge tube labeled "SPO").

First prepare your slide of exponentially growing, wild-type yeast. Briefly vortex the EXP tube and then pipette 5 μl of culture onto the center of a slide (If you've forgotten how to use a pipetman, check out the last page of your lab manual for a cheat sheet!). Place a coverslip on top of the sample by first resting one edge of the coverslip on the slide adjacent to the droplet, then gently lower the opposite edge of the coverslip (Figure 1.6). This approach minimizes the formation of bubbles.

Now you're ready to look at your sample of yeast. Turn on your microscope and place the slide (coverslip up) on the microscope stage, using the clip to keep it firmly in place. Rotate the objectives so that the lowest power (4x) is pointed toward the slide. Focus up and down until you can see the yeast cells (they will still be very tiny at this magnification!). Then and only then switch to the 10x objective. Refocus before moving on to the 40x. At this level of magnification you can easily visualize the cells and determine whether they are budding, mating, etc. **DO NOT SWITCH TO THE 100X OBJECTIVE**, the highest magnification requires immersion oil, and you can break the microscope if you do not use oil with this lens! And besides, 40x magnification is more than adequate for today's exercise! If you're having trouble seeing the cells, consult the trouble-shooting guide on the next page.

**Figure 1.6. Applying a coverslip**

Anatomy of a Microscope

1 = ocular  
2 = body tube  
3 = arm  
4 = mechanical stage knob  
5 = coarse adjustment knob  
6 = fine adjustment knob  
7 = base  
8 = substage lamp  
9 = diaphragm  
10 = condenser  
11 = stage clip  
12 = stage objective  
13 = objectives  
14 = revolving nose piece  
15 = nose piece
**Microscopy Trouble Shooting Guide**

Having trouble finding your cells? Let's start from the beginning…

1. Using the Coarse Adjustment Knob, move the stage all the way down. (This prevents damage to the objectives.)
2. Using lens paper ONLY, clean all pieces of glass: the oculars, objectives, condenser lens, and light source. (Be especially careful not to get oil from your fingers onto any of these pieces!!)
3. Using the condenser knob, move the condenser lens upward until it is almost flush (at the same level) with the stage.
4. Now make sure your scope is plugged in and the light is turned on.
5. Using the diaphragm lever or diaphragm dial (located under the stage), open the diaphragm.
6. Using the rotating nosepiece, move the 4X objective into place.
7. Place the microscope slide FLAT on the stage with the coverslip on top.
8. Holding the mechanical stage clip open, SLIDE the microscope slide into the mechanical stage holder.
9. Looking directly at the stage (DO NOT look through the oculars!) , center the specimen or cover slip directly over the brightly-lit condenser lens. Use your mechanical stage knobs to do this.
10. Using the course adjustment knob, move the stage all the way up until it comes to the Automatic Stop. (Only the 4X lens has this Auto Stop! Do not try this with other objectives. You will damage the scope!)
11. NOW (But not before now), look in the oculars.
12. SLOWLY move the course adjustment knob downward until you come into focus.
13. Once you are in focus on 4X, YOU ARE AUTOMATICALLY IN FOCUS ON ALL OTHER POWERS! (This is called Parfocalization.)
14. DO NOT touch the course adjustment knob anymore, or you will lose your focus. Simply dial-in the objective you need.
15. If you need to re-focus, use ONLY the fine adjustment knob.

*LAST RESORT: If you still can't see 'em ask your T.A. or instructor for help.*
Now that you have the yeast cells in focus, it's time to begin characterizing this population of exponentially growing yeast. Working with your partner, you must determine what fraction of cells are in each phase of the cell cycle. The best (and least biased) way to do this is NOT to count all the cells in G₁ then all the cells in S phase, then all the cells in G₂/M. Instead, try this…

In your notebook, set up three columns, the first labeled G₁, the second labeled S, and the third labeled G₂/M. Now one of you should look through the oculars at the field of yeast cells, and working systematically (left to right, for example) describe each cell your eyes encounter and call out to your partner whether it has a small bud, a large bud (looks almost like two full-size cells stuck together) or no bud. For each cell you describe, your partner will record a tick in the column for its respective cell cycle position (see page 9 of the lab manual if you need a refresher!). After you've counted all the cells in that field of view, move to a different part of the slide and score the cells in that region. Repeat this procedure until you have counted 50 cells, then make a new slide, swap places with your partner, and working together, count 50 more. Once you have finished counting 100 cells, rotate the objectives to the 4X power and dispose of the slides in the beaker of bleach provided on your lab bench. Then tally your results in your notebook. Now it's time to take a look at mating yeast.

Because mating yeast have trouble finding a partner in liquid media, you will need to prepare your slide from the mating reaction occurring in the Petri dish labeled "mating." To do this, pipette 5μl of sterile water onto a slide. Then use a sterile toothpick to obtain a tiny glob of cells from the mating reaction. Mix these cells into the water on the slide. Top off the cell suspension with a cover slip and take a look at your cells, focusing first at the 4X magnification and gradually working up to 40X. Work with your partner to find yeast that have shmooed (put out a mating projection), yeast that have fused to form a dumbbell shaped zygote, and a zygote that has progressed to the three-lobed stage. When you find each of these cell types/shapes, show your T.A. or your instructor to confirm your observation and initial your Introduction to Yeast Worksheet. Once you are through observing the mating yeast, rotate the objectives to the 4X power and dispose of the slide in the beaker of bleach provided on your lab bench.

Next you will examine yeast that have sporulated in response to nitrogen starvation. Begin by vortexing the microfuge tube labeled "SPO." Then pipette 5μl of cells onto a slide and top off this cell suspension with a coverslip. Now take a look at your cells, focusing first at the 4X magnification and gradually working up to 40X. After you and your partner find an ascus containing four spores, show your T.A. or your instructor to confirm your observation and initial your Introduction to Yeast Worksheet. Once you are through observing the sporulated yeast, rotate the objectives to the 4X power and dispose of the slide in the beaker of bleach provided on your lab bench.
Your final microscopic analyses for today involve characterizing a temperature-sensitive cell cycle mutant. First go to the 37°C incubator and obtain a microcentrifuge tube labeled “cdc”. Vortex the mixture and prepare a slide by pipetting 5μl onto a slide and topping off the cell suspension with a coverslip. Using the same protocol you employed to characterize the exponentially growing yeast, score this mutant population by looking at 100 cells and determining which fraction are in G1, which fraction are in S phase and which fraction are in G2/M. Once you have finished counting 100 cells, rotate the objectives to the 4X power and dispose of the slides in the beaker of bleach provided on your lab bench. Be sure to record your results in your notebook.

Now that you have completed the data acquisition, it’s time to interpret your results and record your conclusions on the Introduction to Yeast Worksheet. When finished, turn in this worksheet as well as the carbon copy of your lab notebook entries for this week. Now pat yourself on the back. You just survived your first Genetics lab and are officially on your way to becoming a yeast geneticist!

References


Introduction to Yeast Worksheet

Name: _____________________________________________

Lab Section (circle one): Monday      Tuesday     Thursday

Exponentially Growing Yeast
   Number of cells in G1 (unbudded):
   Number of cells in S phase (small bud):
   Number of cells in G2/M (large bud):

Mating Yeast  (must have T.A. or instructor initials for each category)
   _______shmoo _______dumbbell-shaped zygote _______three-lobed zygote

Sporulated Yeast  (must have T.A. or instructor initials)
   _______spore/tetrad

Temperature-Sensitive Cell Cycle Mutant
   Number of cells in G1 (unbudded):
   Number of cells in S phase (small bud):
   Number of cells in G2/M (large bud):

1. Based on your data, which phase of the cell cycle seems shortest in wild-type yeast? How did you arrive at this conclusion?

2. When you compare the wild-type and cell cycle mutant, what differences, if any, do you notice?

3. In the mutant strain you studied, the cells “arrest” or get stuck in a specific phase of the cell cycle and cannot progress on to subsequent phases once arrested. Based on your results, in which phase of the cell cycle does this mutant arrest? (circle one).
   
   G1      S      G2/M      random

How did you arrive at this conclusion?