More Background on Yeast

Nomenclature

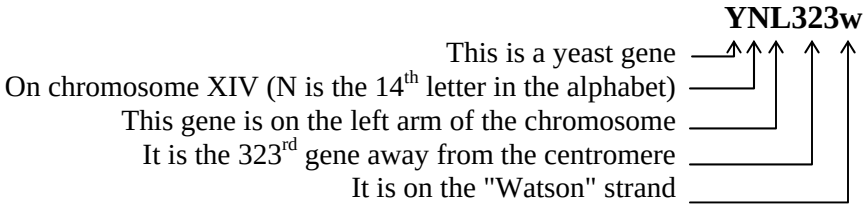
Naming Yeast Strains

Now that you've become (somewhat) comfortable working with yeast in the lab, it's time to master some of the jargon associated with yeast…

S. cerevisiae researchers have adopted a systematic approach for describing the genotypes of their yeast strains. Genes that have been studied or characterized in some way often have a "common" name ascribed to them, and this name generally consists of three letters and a number. For example, *LEU2* refers to a gene encoding an enzyme in the leucine biosynthetic pathway, and *PDR3* refers to a gene encoding a transcription factor involved in pleiotropic drug resistance. The table below describes the basics of yeast genetic nomenclature which is always typed up in *italics*.

Wild-type alleles are written in all caps	*LEU2*
And sometimes with a "plus" sign	*LEU2*$^{+}$
Recessive mutant alleles are written in lower case	*arg2*
And may include an allele number	*arg2-9*
Dominant mutant alleles are written in all caps	
And should include an allele number	*PDR3-11*
Or other notation to indicate it is not wild-type.	*OLI*^{r} (dominant mutation conferring oligomycin resistance)

Besides its "common" name, each gene has a systematic name that refers to its precise position in the genome…



In addition to talking about the genotype of yeast, you should also be able to use the appropriate jargon to discuss the phenotype of yeast. For example, auxotrophies (or nutrient requirements) are among the most common phenotypes used as a tool in yeast. In fact, later this semester we'll use auxotrophic markers to help us select diploid strains as well as strains that have taken up various plasmids. Thus understanding auxotrophies is an important and fundamental concept that you need to master. So let's get started…

Amino Acids, Nitrogenous Bases, and Auxotrophies

Organisms that need to obtain a particular amino acid or nitrogenous base from their environment in order to grow are called *auxotrophs*. On the other hand, the term *prototroph* refers to an organism that can make its own amino acids and nitrogenous bases and thus does not need to get them from its environment. When describing this aspect of a yeast strain's phenotype, we say it is "plus" or "minus" for a particular amino acid or nitrogenous base. For example,

Trp^+ strains can make their own tryptophan
Trp^- strains cannot make their own tryptophan

These phenotypes correspond with the strains' respective genotypes, because Trp^+ strains carry wild-type alleles of all the genetic loci encoding tryptophan biosynthetic enzymes. Therefore Trp^+ strains can grown on media lacking tryptophan (i.e.- can make their own tryptophan). On the other hand, Trp^- strains have at least one defective enzyme in the tryptophan biosynthetic pathway, and because they cannot make their own tryptophan, they must get this amino acid from their environment/media in order grow.

Now take a moment to make sure you understand everything above, because this scheme for describing microbes based on phenotype will get a little tricky when we add carbon source into the mix…

Carbon Source Utilization

So, most wild-type yeasts are pretty good at using just about any sugar you throw at them. For example, wild-type yeast are equipped to metabolize the six-carbon monosaccharide galactose and can therefore be described as Gal^+, because they can grow on media containing galactose as the sole carbon source. However, mutants carrying defects in one or more enzymes involved in galactose metabolism are no longer capable of using galactose. Such strains are called gal^-, because they would starve to death if grown on media containing galactose but lacking an alternative carbon source. In summary,

Gal^+ strains can use galactose as a carbon source
Gal^- strains cannot use galactose as a carbon source

See how this system is different from the one presented above for amino acids and nitrogenous bases?

With amino acids and nitrogenous bases, we focus on what a strain can make.

With sugars, we focus on what a strain can break down and use!

If no specific genotype or phenotype is mentioned, you can assume that the yeast strain is wild-type in that respect (i.e.- it can metabolize that specific sugar or make that particular amino acid or nitrogenous base).

Naming Yeast Media

Fortunately, the nomenclature for yeast media is less complicated than the system for describing the yeast themselves. Yeast media is simply named based on what's in it. As you read in the Introduction to *Saccharomyces cerevisiae* portion of the lab manual, YPD refers to rich media that contains **y**east extract, **p**eptone, and **d**extrose (a.k.a.-glucose), whereas **s**ynthetic media containing **d**extrose and a **c**omplete mix of other nutrients is called SDC.

So what do you call media that contains a carbon source other than glucose? Easy. Just drop the "d" for dextrose and put in an abbreviation for the type of sugar that is present. For example, SGalC refers to synthetic complete media that has been made with galactose instead of glucose.

Another common variation you'll see is when a specific nutrient has been left out of media. For instance, SDC-ura (pronounced "ess dee see minus ura") is synthetic media made with glucose and a nearly complete mix of amino acids and nitrogenous bases… just uracil has been left out!

Now let's see if you've got the hang of these naming systems. Match the following yeast phenotypes with the media they *can* grow on:

Strain	**Media**
1. Ura^{-} Lys^{+} Leu^{+}	A. SDC-ura-lys
2. Ura^{+} Leu^{+} Lys^{-}	B. SDC-lys-leu
3. Gal^{-} Ura^{+} Lys^{+} Leu^{-}	C. SGalC-ura

Answer: 1. B 2. C 3. A

Yeast and Media Nomenclature Worksheet

(To be turned in at the beginning of lab!)

Name: _____

Lab Section (circle one): Monday Tuesday Thursday

1. Describe the precise genetic location of the gene YNL323w.

True/False:

____2. A strain that is Raf^+, where Raf refers to the carbon source raffinose, cannot grow on media where raffinose is the only carbon source.

____3. A strain that is ura^-, where ura refers to the nitrogenous base uracil, can synthesize its own uracil, and thus does not need uracil to be provided in the media on which it is growing.

4. On which of the following types of media can a $Man^- Trp^+ Arg^-$ strain grow? (man = mannose, trp = tryptophan, arg = arginine)

a. SManC-trp
b. SDC-arg
c. SGalC-trp

5. Which of the following strains can grow on SGalC-leu plates?

a. $Gal^- Leu^+$
b. $Leu^+ Trp^-$
c. $Leu^- Trp^+$

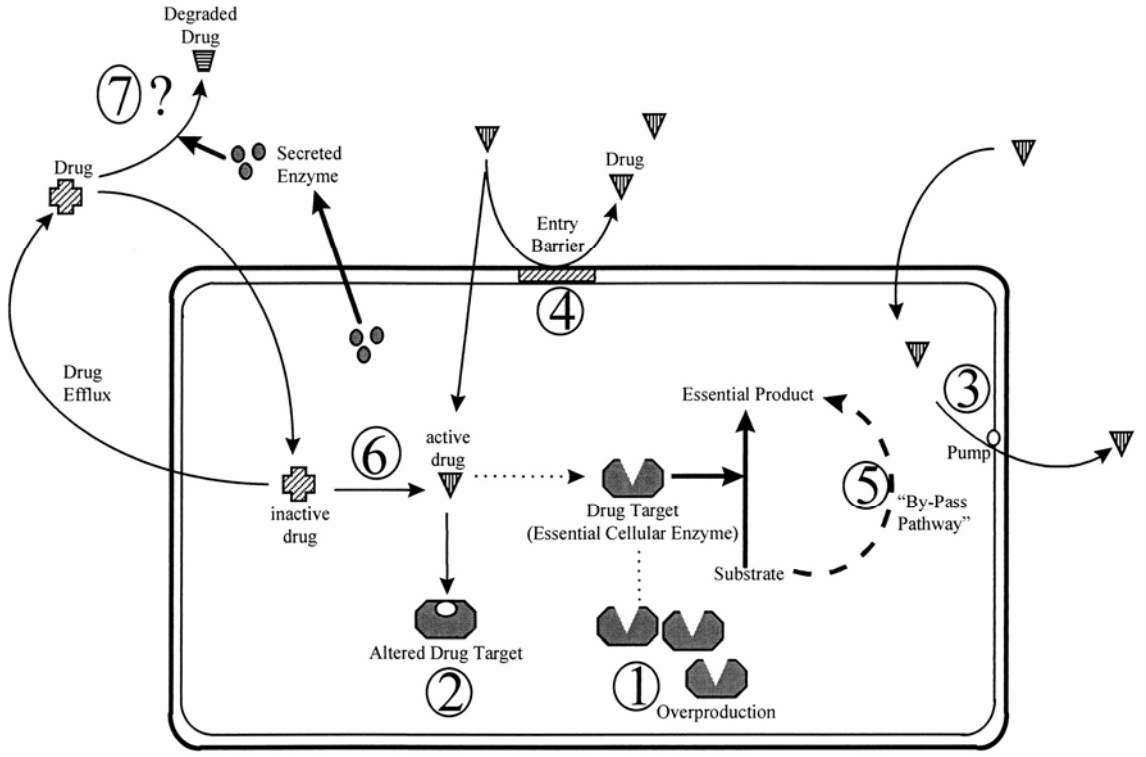
6. A strain with the genotype *cdc50 AFT1-1* has

a. a recessive mutation in the *CDC50* gene and a dominant mutation in the *AFT1* gene
b. a dominant mutation in the *CDC50* gene and a recessive mutation in the *AFT1* gene
c. a recessive mutation in the *CDC50* gene and no mutation in the *AFT1* gene

Overview of this Semester's Research Project

Drug resistance is a common obstacle encountered when attempting to treat a variety of diseases including cancer, bacterial infections, and infection by protozoal parasites. To be able to combat drug resistance we must first gain a better understanding of how cells become resistant to drugs. The figure below was taken from Ghannoum and Rice (1999) and illustrates some of the ways cells can become resistant to various drugs that kill cells by inhibiting one or more intracellular targets, such as an enzyme whose activity is required for successful cell growth and division.

1) Overproduction of the intracellular target.
2) Alteration of the target so the drug can no longer bind and inhibit it.
3) Reduction of intracellular accumulation by pumping the drug out of the cell.
4) Reduction of intracellular accumulation by preventing the drug from getting into the cell in the first place.
5) Compensation for any effects caused by the drug inhibiting the target enzyme.
6) Disruption of the ability of cellular enzymes to convert an inactive drug precursor into an active form of the drug.
7) Degradation of the drug by enzymes outside the cell.



The goal of your work this semester is to identify and characterize mutant strains of yeast that are resistant to the anti-cancer drug hexadecylphosphocholine (HePC). In your mutant strain, one or more of the pathways above may be working to make the cells resistant to the toxic effects of HePC.

Please note that throughout the semester we will be discussing papers related to this topic. This week you will attend a journal club presentation given by your instructor. Afterwards, you will be assigned to a small group and will choose which date you would like to present a paper to the rest of your lab section.

Journal Club

Because the amount of published biological and biomedical research is increasing at an almost exponential rate, scientists often help each other keep up with the most exciting developments by participating in journal clubs where they present the results from an interesting new paper. To assist each other in learning about some of the previous research conducted on HePC and on drug resistance, you will work in groups and present assigned papers to the rest of your laboratory section. You and your partners will be responsible for doing additional library research to develop a better understanding of the context and significance of this work. As a team you will then (1) present appropriate background material (beyond what is presented in the article's introduction), (2) walk the class through the paper, figure by figure, and (3) relate the key points the authors make in the discussion. In addition to understanding the data you are presenting, you should also be prepared to lead a discussion on the strengths and weaknesses of each experiment as well as the paper overall. When preparing your presentation, you should use PowerPoint, and be sure to keep the slides clear, simple and easy to read. As presenter, you will be expected to explain new techniques and any pertinent background not provided in the paper (*yes, this will mean extra trips to the library!*). Furthermore, since your labmates will be grading you according to the criteria described on subsequent pages of the lab manual, you should refer to these guidelines when preparing your presentation.

During the journal clubs when you are not presenting, you are still expected to read the primary article being discussed, and submit a corresponding write-up using the worksheet that's coming up in just a few more pages (additional copies may be obtained on Blackboard). These write-ups are due at the *beginning* of the appropriate lab session.

Tips for Reading Scientific Papers

Adapted (some portions verbatim) from the Amherst Quantitative Skills Center web site:
http://www.amherst.edu/~qcenter/scipaper.html

- Read the easy parts (abstract, introduction, conclusion) first.
- Then read the entire paper, marking confusing sections with question marks.
- Reread the entire paper again, and then (but probably not until then) will it start to make sense. Know that even scientists have to reread papers many times.
- Don't get too frustrated, and don't give up!
- As you read, write down important points and lingering questions.
- Once you feel you have a pretty good grasp of the paper, summarize it. Your summary will help you talk to others about the paper and will help you if you have to read the paper again a week, two months, or four years later.
- If the paper is understandable and well-written, ask yourself:
  - What makes this paper easy to read? (easy is relative)
  - How detailed is the paper?
  - What questions remain unanswered?
  - How did the author link his/her work to previous research?
  - What does this paper contribute to the field?
- Your answers to these questions will help you write lab reports that are supposed to read like scientific papers.
- If you find yourself cursing the author, ask yourself what makes the paper so impenetrable or otherwise maddening. Research scientists are notoriously bad writers, and an understanding of bad scientific writing will help you avoid the same mistakes as you write lab reports that read like good scientific papers.

- Try reading difficult articles with other students. You might move more slowly through the article, but when you reach the end, you might find that you more thoroughly understand the article than you would have otherwise.

Journal Club Write-up

Your Name_____ Author(s)_____

Title_____

Complete this worksheet ***before*** class. Answers may be written or typed, and feel free to use additional pages as needed. During the journal club you should take additional notes (in a different color of ink or pencil) without scratching out any of your previous answers and comments.

1. List key terms and concepts, defining those you don't already know. Circle those that you feel need to be discussed. (Even if you know all the terms and concepts presented, you still need to list the most important ones… if you leave this question blank, you will not earn full credit on this assignment!)

2. Summarize the author's general point in 3 or 4 sentences (kind of like an abstract).

3. Critically evaluate the data. Which experiments were especially convincing? Which weren't? Were there any controls missing? Do you agree with the author's interpretation of the data?

4. What, if any, are the implications of this work for basic science? science education? medicine?

5. Evaluate the style of this article. Was it well written and easy to understand? Are the figures presented neatly and interpreted readily?

Evaluation of Journal Club Presentations

Your name _____ Presenters _____

Author_____

Title _____

Rate the quality of the following aspects of the presentation with 5 being the best and 1 being the worst. When assigning each score, be sure to take into account whether this aspect of the presentation ***improved your understanding*** of the paper.

1. Use of text on the slides (not too much or too little)	1	2	3	4	5
2. Use of graphics and animation (improved presentation without being distracting)	1	2	3	4	5
3. Background information (sufficient, not excessive)	1	2	3	4	5
4. Explanation of techniques	1	2	3	4	5
5. Accurate representation of data and the author's interpretation	1	2	3	4	5
6. Ability to answer questions	1	2	3	4	5
7. Presentation improved my understanding of the paper	1	2	3	4	5

Individual Performance

8. Presentation skills (did the presenter… speak loudly and clearly? make eye contact with the audience? appear calm and confident?)

Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5

9. Did the presenter do his/her "fair share" in presenting the material?

Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5
Name_____	1	2	3	4	5

Evaluation of Journal Club Presentations

Your name _____

Author_____

Title _____

Rank your partners' contributions to the preparation for this journal club as well as their performance during the presentation itself. If you all contributed equally to the presentation give your partners 5s, but if one or more of your partners slacked off, decrease his/her score accordingly.

Partner _____ 1 2 3 4 5

Partner _____ 1 2 3 4 5

Partner _____ 1 2 3 4 5

Partner _____ 1 2 3 4 5

Partner _____ 1 2 3 4 5

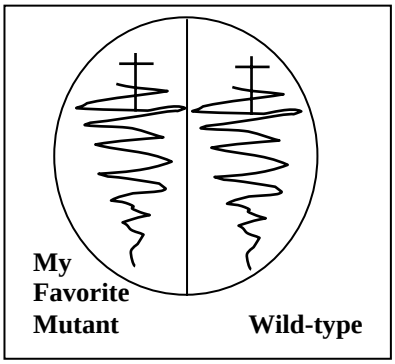
Isolation of HePC Resistant Mutants

As explained earlier the lab manual, the goal of your labwork this semester is to identify and characterize mutant strains of yeast that are resistant to the anti-cancer drug hexadecylphosphocholine (HePC).

Now you may remember that during last week's lab you spread a sample of wild-type yeast onto an SDC plate and an SDC plate containing the drug HePC. When you compare these two plates, what do you see?

- ✓ Which plate has more growth? What do you think accounts for this difference?
- ✓ How many colonies are growing on your SDC+HePC plate? If wild-type yeast are sensitive to the toxic effects of HePC, what might account for these colonies?
- ✓ If you were to take the yeast on your SDC plate and replica plate them onto SDC+HePC, would you expect to see a lawn of growth? Why or why not?

After recording your thoughts and observations in your lab notebook, you'll need to select your favorite colony of drug resistant yeast from the SDC+HePC plate. To ensure that you'll have plenty of mutant yeast to work with, each student should use good sterile technique to pick a single colony off of your SDC+HePC plate and streak it out onto half of a new, well-labeled SDC+HePC plate. When picking a colony, be sure to select a relatively large colony that is well isolated on the plate. If other colonies are nearby, you might accidentally pick a mixture of strains which will likely give you uninterpretable results (not to mention a serious headache when you're trying to write up your lab report at the end of the semester!). On the other half of the plate, streak a wild-type strain so that you can confirm the HePC resistance phenotype of the mutant. Place this plate in the 30^{o}C incubator. Next week you should have lots of growth of the drug resistant strain. You will keep this plate as a stock of mutant yeast that can be used for the rest of the semester.



References

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