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Utilizing *Saccharomyces* in the classroom: a versatile organism for teaching and learning

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ABSTRACT

In the world of higher education, one of the struggles instructors face in the classroom is engaging students in the material. A second discussion in higher education pedagogy is how to weigh content versus activity in the science classroom. How should college teaching be set up when students now have every fact ever found at their fingertips on a device no larger than a half sandwich? What is the correct balance in the classroom between content/knowledge and activity? As instructors grapple with these questions, a new type of learning experience called the Course Based Undergraduate Research Experience (CURE) has been developed, whereby students engage in an authentic research question in a classroom and laboratory setting. CUREs have been shown to be effective learning experiences for students but can be difficult to implement. *Saccharomyces cerevisiae* is a versatile and easy to use organism in the classroom that can be used for a wide variety of classroom activities. Described herein are a number of ways an instructor can use yeast in the classroom for authentic research experiences, especially focused towards a CURE.

KEYWORDS

Saccharomyces cerevisiae; undergraduate learning; laboratory; active learning; course based undergraduate research experiences

One of the most pressing issues in higher education today is how to stimulate students to apply what they have learned in class. Students are very good at memorizing and reciting random facts but often cannot connect the dots to apply information to new scenarios. As we train a new generation of scientists, professors are challenged to apply learning theory to our classroom activities. One way to stimulate students' interest and challenge their abilities is to engage them in the laboratory, either as an apprentice in a research lab or in a laboratory classroom setting. Engaging undergraduate students in the research lab can be highly effective, but is extremely time and resource consuming. An alternative way to engage students in authentic research experiences in a more efficient manner is to use a classroom based approach.

There are many ways to engage students in lab activities in the classroom, including traditional defined labs, more open ended labs, and student research projects. In each case, the instructor must weigh the pros and cons of the laboratory activities in order to maximize the learning and minimize the expense, hazards, and set up. One of the most cost effective and versatile organisms for the classroom is *Saccharomyces cerevisiae*. Summarized concisely in this comprehensive review are the many opportunities to use yeast as a research organism for classroom laboratories.

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Traditional classroom experiences

The simple activities that have a known outcome are called 'cookbook' or 'closed ended' labs and are often frowned upon as the students have little incentive to be creative or work hard, as they know that the results are simply a desired outcome, already known to the instructor. These types of labs can be very useful, however. Students who lack sophistication or experience can struggle when challenged to develop any type of protocol or hypothesis. A well-developed traditional type lab can walk students through the steps of the scientific method and model for them how to develop scientific and critical thinking skills.

Traditional type labs also can be effective to demonstrate to students how to perform science. The lab activity can be written in such a way that students follow a detailed protocol. This can help students to gain expertise and self-confidence in lab skills. Further if the students can lean the techniques in a cookbook lab and then apply them to a new question or extend them in some way, utilizing a lab with a known outcome can be an essential learning experience and a springboard for more creative activities. Further, there are ways to take a traditional lab experience and change them so that students can learn more than a traditional lab (Peters 2005).

Traditional labs can also be very useful to help students to gain skills in analyzing data. Experiments often fail in labs where the protocols are not well developed or guaranteed to work. In the teaching lab, it can be extremely difficult or impossible for students to repeat experiments as the lab set up varies from week to week and students who have to repeat experiments will also fall behind the remainder of the class. A well-developed lab with detailed protocols that guarantees results can generate data for students to then analyze themselves.

One of the cornerstones of the determination of effective learning is assessment. Aligning learning objectives with assessment (Dirks, Wenderoth, and Withers 2014) is the key in determining if the activity were an effective teaching and learning tool. As traditional labs generally are geared towards knowledge acquisition, low level problem solving, and skill acquisition, assessment should align with the activities. Examples of assessment of this type of activity include content exams, calculations, and lab practicals. To extend the learning from a traditional lab, instructors may develop a subsequent laboratory in which the student has to apply or extend what they learned in the closed ended lab. For example, students can learn how to perform a protein Bradford assay with the appropriate standard curve one class period and then the next class period students would bring in their own food samples, hypothesize how much protein it has based upon the package and/or outside of class research they have done, and then students would be challenged to perform another Bradford protein assay (with standard curve) on their samples to determine protein concentration.

Guided learning

A type of classroom lab that is more sophisticated that the traditional lab is often called guided learning. Generally students are given a set up and can work within a few parameters to develop a lab question. Students are not given free rein to ask and answer questions and develop independent protocols but do have a bit of a say in the development of the question or the protocol. An effective and easy to implement guided inquiry lab is Bio-Rad's GMO Investigator. Students are challenged to bring in a food and hypothesize based upon the packaging and their research whether the food is transgenic or not. Students then following the detailed instructions to extract DNA from the sample and use PCR to amplify a small region of the genome unique to transgenic crops. The amplification reactions are analysed using gel electrophoresis and tell-tale patterns of DNA fragments indicate whether the food was transgenic or not.

The advantages to a guided inquiry lab are many in the teaching lab. First of all, all students are given the same lab protocols and questions. Second of all, the lab set up for each lab group is similar. Third, the instructor can anticipate the equipment and supplies needed for the lab and the laboratory preparation is not onerous. Finally, as learning objectives in the lab should be the same across lab

groups, the guided inquiry lab activity allows creativity and flexibility on the students' part while still allowing the laboratory assignment to fulfill the same learning objectives across the class and lab sections. Further, if students are given a bit of creativity or choice in their activities, hopefully driven by student interest, student engagement should increase (Anderman and Leake 2005). Assessment of the guided inquiry activity is similar to assessment of the traditional labs. Guided inquiry is still generally bounded by a specific set of knowledge and skills that can be assessed using traditional exams, lab practicals, and lab reports (Dirks, Wenderoth, and Withers 2014).

Authentic research and course based undergraduate research experiences

Other lab activities in the classroom can include more authentic research including activities that are more open ended and/or allow for student creativity. Authentic research in the classroom entails having students perform activities or experiments in which the outcome is not known a priori and students have a hand in developing hypotheses. These authentic research activities can span one or a few classroom periods. The Course Based Undergraduate Research Experience (CURE) (Corwin, Graham, and Dolan 2015) is a more intensive (and usually longer) authentic research experience in which students themselves will develop all or part of the hypotheses and methods for the activity. CURES have been shown to stimulate interest in science (Auchincloss et al. 2014) and give more diverse students an authentic research experience than stand-alone undergraduate research in a lab (Bangera and Brownell 2014). There are many successful CURE examples that hope to enhance students' interest in science as well as their skill set and critical thinking abilities.

Assessing CUREs can be more difficult, depending on the scope of the experience. If students are given little guidance and supervision, then the experience can be uneven if some projects fail and some succeed. For this reason, CUREs should be standardized with explicit learning objectives and assignments/assessments matched to the learning objectives (Brownell et al. 2014; Shortlidge and Brownell 2016). Further assessment using instruments such as the CURE Survey (Lopatto and Tobias 2010).

Using *Saccharomyces cerevisiae* in the classroom lab

The ease of use of yeast in the lab is one of the myriad of positives going for it. In additional to well-developed protocols and readily available strains and knockouts, yeast is classified as BSL1 and requires low level PPE. Yeast can be grown on the bench if necessary and plates and cultures need minimal supervision between classroom activities. Media is inexpensive and yeast grow robustly with little care. For more information on how to get started with yeast in the laboratory classroom, novice instructors are directed to read (Manney et al. 1997) and more experienced microbiologists should refer to (Dunham, Gartenberg, and Brown 2015). Because of the ease of use, the panoply of strains and plasmid available, and the added bonus that *S. cerevisiae* is a eukaryote, there have been a plethora of labs developed over the years that use yeast as a model organism for study. As an additional positive, there are yeast lab exercises to fit different topics, instructor expertise, and activity type (summarized in Table 1 and described below).

The GENE project

Early on in the evolution of active, problem based learning, a group of innovators at Kansas State University developed a series of activities exploring the lifecycle and genetics of *S. cerevisiae* (Manney and Manney 1992, 1993; Manney et al. 1997; https://ww.k-state.edu/gene/). The experiments described can either be done as a stand along module or as a series of interlinked research into the powerful tools of yeast genetics. The manual is complete with recipes, detailed descriptions of the pathways studied, and also complete student handouts. Experiments begin with basic genetic experiments, covering life cycle (haploid to diploid than back to haploid and response to mating pheromone), crosses and epistasis, environmental effects on phenotype, and transformation and plasmid loss. The next section

Table 1. Analysis of Lab Exercise by Instructor Expertise and Experiment Type. Experiments are classified to aid instructors who wish to incorporate yeast into their classrooms. Exercises are classified by instructor level (novice to expert) as well as by exercise type (traditional to CURE). Novice instructors need to have general lab skills and other skills as indicated. Intermediate and expert instructors should have some familiarity with microbiology, sterile technique, yeast cultivation, microscopy and additional techniques as indicated.

Activity	Instructor Level	Skills Necessary	Experiment Classification	References
Antimicrobials	Novice	Microbiology	Traditional or guided inquiry	Marsh and Arriola 2009
Fermentation, bioethanol formation, and brewing	Novice	None	Traditional	Bonner 2009; Chan 2016; Epstein et al. 2010; van Seters et al. 2011; Gillespie and Deutschman 2010; Reinking, Reinking, and Miller 1994
Mutagen testing in yeast (UV)	Novice	Microbiology	Guided inquiry	D'Costa and Santoro 2009; Manney et al. 1997
Fungal-pathogen interaction	Novice	None	Traditional	Chanchaichaovivat, A., B. Panijpan, and P. Ruenwongsa 2008
Adenine pathway mutants	Intermediate	Genetics	Guided inquiry	Aronson and Silveira 2009; Manney et al. 1997; Williamson, 1999
Brewing with student hypothesis testing	Intermediate	Chemistry and biochemistry	Authentic research	Hooker, Deutschman, and Avery 2014; Sato et al. 2015
Cell respiration	Intermediate	Cell biology	Guided inquiry	Sanchez and Konigsberg 2006
Chromosome transmission mutants	Intermediate	Cell biology	Authentic research or CURE	Sleister 2007
Enzyme analysis	Intermediate	Enzymology, cell biology, and biochemistry	Traditional	Bryer 2016; Heinzerling, Schrader, and Schanze 2012; Hunnes 2003; Miranda et al. 2008; Monti-Hughes, Alonso, Garófalo, Burgos, and Stella 2007; Raghevendran 2005; Stambuk 2002; Miller 1992; Tatina, Mansor, and Maierin 2001; Taipa et al. 2015; Timerman, Fenrick, and Zamis 2010;
Enzymology with students developed questions	Intermediate	Chemistry and mathematics	Guided inquiry	Silverstein 2016
Evolution	Intermediate	Cell biology	Guided inquiry	Agren et al. 2017; Ratcliff et al. 2014; Smith et al. 2015
Fermentation with additional variables	Intermediate	Biochemistry	Guided inquiry	Collins and Bell 2004; Grammer 2012; Keller and Gilliam 2010; Kosinski 2010
Fungal unknown	Intermediate	Molecular biology and bioinformatics	Traditional or guided inquiry	Safranek 2014
Isolating mutants in lysine pathway	Intermediate	Biochemistry	Authentic research or CURE	Keeney and Reed 2000
Mating	Intermediate	Cell biology	Guided inquiry	Hoopes et al. 1998; Hutchins 2012
Mutagen testing in yeast (D7)	Intermediate	Genetics	Guided inquiry or authentic research	Marshall 2007
Protein Analysis	Intermediate	Cell biology	Guided inquiry	Belanger 2009; Connerly; Parra-Belky 2002; Parra-Belky et al. 2005
RNA analysis	Intermediate	Cell biology	Traditional	Deutch and Marshall 2008

Cell biology and genomics	Expert	Cell biology, genomics, and molecular biology	Guided inquiry	Dymond et al. 2009; Hutchins and Key 2008; Menella 2015; Will, McWatters, and McQuade 2006; Willhite and Wright 2009; Wolyniak 2013
Proteomics	Expert	Cell biology, genetics, and proteomics	CURE	Teixeira et al. 2009
Reverse genetics	Expert	Cell biology, bioinformatics, and molecular biology	Authentic Research or CURE	Brame, Pruitt, and Robinson 2008; Brownell et al. 2015; Hekmat-Scafe et al. 2016; Gammie and Erdeniz 2004; Parra, Osgood, and Pappas 2010
Secretion pathway	Expert	Cell biology and genetics	Guided inquiry or authentic research	Hood-DeGrenier 2008; Vallen 2002
Transcriptional regulation and noncoding RNAs	Expert	Genetics, genomics, and molecular biology	Authentic research and CURE	Kushner 2007; Luo et al. 2007; Oelkers, 2017; Walker, Lutz and Alvarez 2008
Yeast two hybrid	Expert	Cell biology, genetics, biochemistry, and bioinformatics	Guided learning, authentic research, or CURE	Gammie and Erdeniz, 2004 Mäkinen et al. 2005; Mordacq and Ellington 2008; Odom and Grossel, 2002; Witherow and Carson, 2011

covers yeast response to UV radiation, outlined below in ***Mutagen Testing in Yeast***. The next section utilizes the ADE pathway to isolate mutants. In this activity student take the red *ade2* mutant and look for spontaneous or induced white mutants to study the adenine biosynthesis pathway, including complementation analysis and identifying allelism. Red mutants can also be isolated as outlined below in ***Making and Isolating Mutants***. Finally, yeast crosses, complementation analysis, sporulation, and spore analysis are used to study independent assortment. Through the activities in Manney et al. 1997, students received a complete grounding in classical yeast genetics, meiosis, and yeast life cycle. These is enough detail in this manual for even a novice to perform the experiments, and enough flexibility that students can also extend the work and design their own experiments. For first time users of yeast in a lab, this is the recommended manual to use to integrate yeast project based learning into a lab.

Making and isolating mutants

The ease of making mutants in yeast has been exploited by researchers for decades to look at a myriad of phenotypes and pathways (Duina, Miller, and Keeney 2014). The adenine biosynthetic pathway includes several intermediate metabolites that will build up when a gene is mutated. It is serendipity that when the *ADE1* or *ADE2* genes are mutated that the buildup product is red and can been seen by the naked eye. In one effective activity, students are challenged to mutagenize yeast and screen for red colonies, indicating loss of function in either the *ADE1* or the *ADE2* gene when cells are grown on media with limited adenine (Aronson and Silveira 2009). Students are then challenged to analyze the red mutants they find. They perform a nutrient screening to determine phenotype changes and then also amplify both the *ADE1* and *ADE2* genes and perform subsequent DNA analysis, then analyze the DNA sequences to determine where the mutation lies, and apply their knowledge of the Central Dogma of Molecular Biology to their understanding of the laboratory exercises. A similar activity, entitled 'The Red Mutant Hunt,' (Manney et al. 1997 and Williamson 1999) guides students to make yeast mutants that also lack one of these two functional genes, hunting for a red mutant. Then the students use yeast mating and complementation analysis to characterize their mutants. In this case, when two *ADE2* mutants, for example, are crossed, the resulting diploid remains red, but if an *ADE1* mutant is crossed to an *ADE2* mutant the resultant diploid strain through complementation becomes white again. This activity integrates a variety of protocols, including sterile technique, careful dilution, and strain analysis. Depending on the interest of the instructor, the mutants can then be analysed by sequencing and/or complementation analysis for further understanding of genetics, proteins, and the Central Dogma.

Lysine mutants are the subject of a project outlined in (Keeney and Reed 2000). Yeast mutants with spontaneous mutations in the lysine biosynthetic pathway are selected for on media containing alpha-aminoadipate and lysine. Yeast require a nitrogen source in order to grow, generally supplied by ammonium sulfate. In the absence of ammonium sulfate, yeast cannot grow. However, when alpha-amnioadipate is supplied, mutants that lack *LYS2*, *LYS5*, or *LYS14* gene function can utilize that alternative nitrogen source. Spontaneous mutations are positively selected for and can be tested for complementation analysis to determine the loci of mutation. Because this method of selecting for mutants requires knowledge of enzyme activity and kinetics, as well as an understanding of the lysine biosynthesis pathway, this activity can be couple with a lesson on biochemistry.

A project was also developed to isolate mutants defective in chromosome transmission (Sleister 2007). The yeast strain carries an artificial yeast chromosome (YAC) with the *ADE2* gene, complementing an *ade2* mutation in the yeast genome. Cells are mutated and red sectored colonies are selected for, with the intent of identifying strains that have lost the YAC, not mutated the *ADE2* gene. These mutants were analyzed to determine if the mutation was dominant or recessive and by complementation, to determine if the mutation was already isolated by another student. They were also analyzed for temperature sensitivity and rate of loss of the YAC. Students also as part of this project designed their own assays to test their mutants for additional phenotypes, such as to analyze morphology and test for defects in sporulation.

Reverse genetics

Laboratories have also been developed in which the protein and DNA sequence are well characterized and mutagenesis is used to determine structure/function relationships of the gene and protein. The V-ATPase1 proton pump complex, subunit d, is a subunit of the Vo domain of the V-ATPase (*VMA6*) has been extensively characterized and a semester-long course has been designed to link bioinformatics, site directed mutagenesis, and protein structure/function of this important subunit (Parra, Osgood, and Pappas 2010). Students design their own mutagenic primers for the subunit, based upon protein sequence alignment with homologues and rational design that focused on hypothesized conserved changes. After performing mutagenesis, molecular biology and DNA sequencing; students use Ras Mol to predict how their mutations will map onto the subunit and affect its function. Students use cell lysis, SDS-PAGE, and immunoblotting to determine the effects of the mutations.

S. cerevisiae casein kinase is another malleable protein that can be studied in the classroom laboratory (Brame, Pruitt, and Robinson 2008). Students first use bioinformatics to analyze the structure of the *YCK2* gene product to develop hypotheses surrounding conserved amino acid sequences in casein kinases and develop primers to mutate their chosen sequence. After mutation, sequencing confirmation, and subsequent cloning; students study their gene product through complementation analysis, cell morphology and localization, using a GFP tagged protein in a strain with one of the two redundant but essential casein kinases knocked out (*yck1*). The complementation analysis uses a temperature sensitive allele of the remaining casein kinase (Yck2p ts) and students study complementation in both the permissive and nonpermissive temperatures. This analysis should lead to student understanding of the concepts of temperature sensitivity, including permissive and restrictive temperature, as well as complementation analysis, protein structure and protein folding.

Yeast can also be used to assay human protein structure and function, including mutant proteins. A human gene encoding for p53 has been used as a model for students to study the activity, structure and function of this tumor suppressor gene (Brownell et al. 2015; Hekmat-Scafe et al. 2016). Known p53 missense mutations are cloned into yeast and are tested for their ability to transactivate response elements driving *ADE2* and lacZ. Students also test their mutants for protein levels via western blot and DNA binding ability via a colorimetric assay. The mutants are also analyzed via bioinformatics to determine the location and putative extend of the mutation. Finally students assay a GFP-p53 fusion for subcellular localization. This activity, performed in yeast, can help to connect the Central Dogma to protein function as well as human disease.

In an innovative project described by (Gammie and Erdeniz 2004), students analyse the human tumor suppressor gene *MSH2*, which maintains genomic stability by functioning in mismatch repair, and apply their research to the yeast *MSH2* homologue. Students research and identify common missense mutations in the human *MSH2* gene, which can lead to cancer. The yeast *MSH2* gene is then mutated at homologous sites and cells are assayed for their ability to perform mismatch repair. These mutants are further characterized as to their protein stability, localization, and ability to interact with other subunits of the yeast mismatch repair system (interaction is performed using the yeast two hybrid system). With this projects students are able to discern the utility of using yeast as a model organism for many processes in humans, and are exposed to important techniques in molecular and cellular biology.

Enzymology, protein isolation and analysis, protein expression, and proteomics

A variety of straightforward enzyme activity laboratories using yeast have been developed. Instructors who have knowledge of microbiology and cell biology can perform these activities easily. One can study catabolite repression by analyzing yeast maltose permease induction and repression by carbon sources, as it is induced by maltose and repressed by glucose (Stambuk 2002), or in batch cultures (Raghevendran, Nielsen, and Olsson 2005). Lactose metabolism between yeast and lactobacillus can be compared (Monti-Hughes et al. 2007). Catalase (Miller 1992), invertase (Miller 1992; Timerman,

Fenrick, and Zamis 2009; Heinzerling, Schrader, and Schanze 2012), immobilized cell-based invertase (Taipa et al. 2015), alcohol dehydrogenase (Silverstein 2016), and phosphate uptake (Tatina, Mansor, and Maierin 2001) can also be assayed in yeast. Yeast can also be trapped in sodium alginate spheres for enzyme analysis (Bryer 2016). Bioethanol can also be produced in the classroom from several sources (Epstein et al. 2010; van Seters et al. 2011). Cytochrome c oxidase can be purified from both beef heart and yeast and can be compared to determine conserved similarities and differences in the enzymes (Hunnes 2003). The correlation between oxidative stress and glyoxalase I expression can be determined biochemically, as this enzyme is induced under conditions of oxidative stress (Miranda et al. 2008).

Protein analysis is also very facile in *Saccharomyces cereviaise*, as evidenced by a wide variety of lab activities developed for undergraduate teaching and learning. These range from protein analysis, to protein-protein interactions to more complicated proteomics. Proteins from the vacuolar ATPase can be analyzed by both western blotting (Parra-Belky 2002) and immunoprecipitation (Parra-Belky et al. 2005). The environment of cells can be altered and the growth rate and protein composition can be analyzed (Connerly 2007). Protein interactions can be probed using affinity chromatography (Belanger 2009). Finally, a proteomics analysis of yeast treated with the agricultural fungicide mancozeb compared to untreated cells can reveal gene expression differences at the protein level (Teixeira et al. 2009).

Fermentation, respiration, and brewing

The original harnessing of yeast for human use revolved around its ability to ferment to produce alcohol and respire to cause bread to rise. It is easy to take advantage of these characteristics of yeast in a wide variety of laboratory settings. After learning about fermentation (Freeland 1973), students can propose their own experiments, varying items such as substrate type and concentration, temperature, and yeast amount and analyse CO_2 production by bubble formation (Collins and Bell 2004), including addition of acetic acid in a fictitious bread making scenario (Keller and Gilliam 2010); in fermentation tubes (Bonner 2009; Reinking, Reinking, and Miller 2004); by simple tube mass calculations (Grammer 2012); or by a color change with universal indicator (Chan 2016). They can extend these taking fermentation exercises by taking into account the Pasteur effect (aerobic respiration will occur more readily in low populations) as well (Kosinski 2010).

Engaging students in topics they are interested in can increase student commitment and interest. The process of brewing can be used to develop students' abilities in scientific method and experimentation. Lee et al. (2015) used the process of brewing and varying one ingredient to challenge the students to use the scientific method to hypothesize the outcome of their beer. In an intensive 4 week interdisciplinary biology/chemistry lab course (Hooker, Deutschman, and Avery 2014), students do both chemistry based labs on components of beer ingredients, beer pH analysis, calcium and ethanol content of beer and measurement of bitterness and biology based labs on yeast flocculation, genotyping, and barley amylase activity. Gillespie and Deutschman (2010) takes a decidedly chemistry-oriented spin on the process of brewing by having students follow sugar consumption by TLC and refractive index and fermentation by CO_2 evolution. Adapting an intrinsically interesting process such as brewing to teaching and learning can increase students' engagement by giving them a reason to care about the outcome of the activities.

Mutagen testing in yeast

Students are intrigued by the health implications of their choices, including the food they eat and the personal care products they use. Yeast can easily be adapted as a model organism for testing of household chemicals. I have developed a lab in which the D7 diploid strain of *Saccharomyces cerevisiae* (Zimmermann 1975 and Zimmermann, Kern, and Rasenberger 1975) was used to stimulate students to research potential mutagens and bring them from home for testing (Marshall 2007). This strain has been modified to demonstrate three different types of mutations with three different types

of growth on petri dishes. Red or sectored colonies on YPD plates demonstrate mitotic crossing over (recombinational repair). Growth on plates lacking isoleucine show reverse point mutation. Growth on plates lacking tryptophan occur after Mitotic gene conversion (gap repair synthesis followed by mismatch repair). Although there are many household compounds that are mutagens (most notably hair coloring and tobacco), most of the compounds students brought in were determined to be toxic, as seen by a lowering of the number of colonies on YPD plates of cells treated with the compounds compared to buffer only control.

Students can also use yeast as a model for testing the effects of UV irradiation. This type of experiment is interesting to students and is easy to implement for first time instructors. Cells can be exposed to artificial UV sources or sunlight and can be assessed for viability in a time and dose dependent manner (D'Costa and Santoro 2009; Manney et al. 1997). Adding to the interest of this assay, students can propose their own experimentation, trying to block the UV from the yeast, using items such as sunscreen, cloth, and sunglasses. Extensions of the experiment include comparing a wild type strain to a radiation sensitive strain (*rad1* is suggested in this case). D'Costa and Santoro also add a case study linking UV mutagenesis to the human disorder XP, in order to demonstrate the utility of yeast as a model system.

Yeast secretion pathway

The yeast secretory pathway has been a model for understanding human secretion, organelle biogenesis and function, and protein sorting (Feyder et al. 2015). The class project Vallen (2002) developed integrates the study of the yeast secretory system with isolation of yeast mutants in a clever selection scheme. Yeast deficient for histidinol dehydrogenase (*his-*) are transformed with a chimeric plasmid with the histidinol dehydrogenase gene (*HIS4*) fused to a secretion sequence. In wild type cells, this protein is secreted and the cells remain his-. However, in *sec61* mutants (temperature sensitive secretory translocation mutants that are inhibited in their ability to translocate proteins into the early ER, the first step of the secretion pathway), the His4p chimera builds up enough in the cytosol for the cells to grow on media lacking histidine. Students compare growth of *sec61* and *sec18* (blocked in their ability to transport vesicles from ER to Golgi) cells to analyse the yeast secretory pathway. In a cell biology approach to studying the yeast secretory pathway, students analyse pre-pro-alpha-factor and invertase via western blotting to discern where the blocks occur in these two mutants (Hood-DeGrenier 2008). By these two analyses of mutants in the yeast secretory pathway, students can investigate yeast mutants and their effects on cellular physiology.

Transcriptional regulation and RNA analysis

Yeast contain a wide variety of gene expression responses induced or repressed in accordance to environmental cues. A semester long lab course was developed to probe yeast transcriptional regulation at a subset of student chosen promoters (Oelkers 2017). Students first research genes of interest and identify their putative promoters. The yeast genome is easy to analyze for cis acting factors as genes are tightly packed and intergenic regions are small, unlike mammalian genomes. Students design primers to the promoter regions, amplify these regions, and then clone them promoters 5' to beta-galactosidase genes. Transcriptional regulation is probed by treating cells with a wide variety of chemicals and under several environmental conditions and then beta-galactosidase activity is assayed.

Early on in the evolution of the research-based program active learning movement of this century, the Genome Consortium for Active Teaching (GCAT) was developed (Campbell et al. 2006, 2007). This grass roots conglomeration of faculty at primarily undergraduate institutions was dedicated to infusing research projects into the curriculum and focused on microarray technology to analyse gene expression to do so (Kushner 2007; Walker, Lutz, and Alvarez 2008). Microarray technology is easily accessible by undergraduates and the data can be analysed by the open source and free Magic Tool (Heyer et al. 2005). Although microarray technology has recently been outpaced by transcriptome

analysis, it is a more cost effective method of transcriptional profiling; and data analysis is easier on microarrays than on raw RNAseq reads.

Lab projects can also be developed to study RNA in yeast cells. We developed a three part lab investigation in which student compare and contrast baking (such as Fleischmann's and Red Star) and lab strains of yeast to analyse the consequences of polyploidy (Deutch and Marshall 2008). Students use microscopy to study yeast morphology and size, extract RNA from cells to determine concentration and analyse the RNA by gel electrophoresis. The investigations culminate in students' determination of RNA per cell to compare and contrast the different cells they were studying. Yeast can also be used as a model for more complicated RNA analysis, such as noncoding RNAs. Students also studied noncoding RNAs in additional strains of fungi by identifying putative box C/D snoRNAs bioinformatics; and then they analyzed their expression by extracting RNA and performing RT-PCR (Luo et al. 2007).

Cell biology and genomics

There are a wide variety of cell biology and genomic experiments students can perform in the teaching lab. Mitochondrial function can be assayed by the MTT assay after a variety of cellular treatments (Sánchez and Königsberg 2006). Students can use synthetic biology to build a chromosome or genome in *S. cerevisiae* (Dymond et al. 2009). Students can also study the ubiquitin degradation pathway and the N-end rule (Will, McWatters, and McQuade 2006) as well as the extraction and components of lipids rafts in yeast (Willhite and Wright 2009).

Students can apply human gene therapy ideas to homologous recombination in yeast (Hutchins and Key 2008). Students use long hybrid oligos to amplify a chimera DNA construct of the *TRP1* gene with *ADE2* ends. They then transform a *trp1* strain with the construct. The construct should target to the *ADE2* gene, knocking out *ADE2* function and creating a red colony. Since the chimera had a functional *TRP1* gene, homologous recombination should change the *trp1* cells to *TRP1* cells and they should be selected for on drop out plates lacking tryptophan. Students often struggle with the Central Dogma of Molecular Biology and how to connect genotype to phenotype. Layered on top of this genetic complexity, students also must grapple with the concepts of protein structure, function, and localization.

In a semester long lab project (Mennella 2015), students first extract yeast genomic DNA, then can amplify the tubulin gene (*TUB1*), clone it into GFP fusion expression vectors, and then use sequencing to check for the correct DNA constructs. Once the plasmids have been verified, students transform them into yeast, confirm expression via western blotting, and then analyze tubulin localization using fluorescent microscopy to connect DNA to protein, using both western blotting and microscopy.

A unique aspect of yeast is their strain-specific ability to vary in regards to both ploidy and zygosity. Students in (Wolyniak 2013) use PCR to determine ploidy and zygosity of unknown strains and correlate these genotypes to phenotypes they score, such as growth, sporulation, and cell morphology. Students can then derive conclusions about the influence of genome structure on cellular biology using this series of laboratory experiments.

Yeast mating

The yeast mating pathway and signal transduction cascade have been studied in a wide variety of contexts and can be applied to several class room questions. Yeast respond in specific ways to mating factors in the media. Students can correlate cell physiology, cell cycle arrest, and transcriptional response to alpha factor (Hoopes et al. 1998). They are also give mutants in the pathway and have to determine where in the pathway the missing protein lies. Students can also study the *bar1* mutant, which lacks a protease that degrades mating factor, to analyze cell physiology and use the system to propose experiments to perform to test hypotheses about yeast mating physiology and signal transduction (Hutchins 2012).

Evolution

Due to the quick doubling time and ease of growth and manipulation, students can use yeast as a model system to study evolution. Although generally a unicellular organism, *S. cerevisiae* can mutate to demonstrate certain characteristics similar to multicellularity (Ratcliff et al. 2014). Students can place selective pressure on a culture of yeast, selecting each time for faster settling cells. Over a series three weeks with daily transfer, students are able to isolate yeast that demonstrate a marked change in phenotype to a snowflake type multicellularity modality. Students can extend this work with discussion and research on multicellularity and discuss yeast as a model system for evolution and complexity. Alternatively, students can mix strains of yeast that demonstrate both flocculation characteristics and nonflocculating characteristics and can study the nature of these to look at kin selection and the greenbeard phenomenon (Ågren et al. 2016).

Studying spontaneous mutations and how they arise can be useful for students to understand Darwinian evolution. Students can utilize serial dilutions and replica plating to determine the frequency of mutations that allow copper tolerance in yeast (Dennison and Goldman 1994). As part of this activity, students determine if the mutations are induced or are pre-existing in a yeast population in order to study evolution and spontaneous mutation. A similar activity, more modelled on the Luria-Delbrück Fluctuation Test, studies yeast spontaneous mutation of resistance to canavanine, as well as sequencing the arising red-colored mutations (Smith et al. 2015).

Yeast in the microbiology lab

Yeast is such a versatile organism it can be used for many different activities including a model organism in a microbiology class. It can be used as a model microorganism to test antimicrobials (Arriola and Marsh 2009). As part of an unknown lab in a microbiology class, a yeast or fungi can be isolated and PCR of ribosomal rRNA genes can be used to attempt to identify the unknown (Safranek 2014). Yeast can be used to control a fungal plant disease as well (Chanchaichaovivat, Panijpan, and Ruenwongsa 2008).

Yeast two hybrid system

Protein-protein interactions turn out to be a very important motif in cell biology, and scientists have long been looking for proteins that interact. Yeast two hybrid analysis began as a way to use a known protein as a bait and fish for prey proteins that interact with them (Fields and Song 1989). Once scientists understood that proteins contained domains and could be somewhat modular, they began genetically engineering hybrid polypeptides with different sections of genes. Yeast two hybrid takes advantage of recombinant DNA technology and the understanding of transcription factor modularity and developed a genetic method to have a 'biological read-out' if two proteins interact in specifically engineered *Saccharomyces cerevisiae* genetic backgrounds. Fields and Song engineered a plasmid to contain a gene for protein of interest fused in frame to a transcription factor DNA binding domain. This fusion protein would be translated and generally cytosolic (usually known as bait). A second plasmid would contain a gene for another protein of interest also fused in frame to a transcription factor activation domain (usually known as prey). This fusion gene would be transcribed and translated and has a nuclear localization signal. If the bait and prey interact, they would bind to each other and move into the nucleus. Since the bait houses a DNA binding domain, it would sit down on a promoter region. Since the prey has an activation domain, this protein would recruit transcriptional machinery. Together the bait-prey complex would then stimulate transcription of the reporter gene. The reporter gene is something that can easily be assayed, such as the ability of the cells to grow on plates lacking a required nutrient or an enzyme whose level can be determined with a simple assay.

Yeast two hybrid was first used widely as a 'fishing' or probing research tool with a known bait and a 'library' of prey, screening for proteins that interact with the bait of interest. Generally an entire

genome or cellular cDNA was cloned into the prey vector. These plasmids would be transformed into yeast and each cell would get the same bait but a different prey. The thousands of combinations would get plated on petri dishes where the reporter was easily seen. Often the reporters have been two fold: a gene for histidine biosynthesis that was lacking in the two hybrid strain and then LacZ. We would do a first selection whereby the cells would only grow on plates lacking histidine if the bait and prey interacted and then those colonies would be tested for Beta-galactosidase activity using X-Gal.

Over the course of the last 20 + years the yeast two hybrid system has been used in a variety of ways and modified for different researchers' needs, including developing a similar system in mammalian cells (mammalian two hybrid). People have also used the ideas and general methods to 'split the hybrid' (aka reverse two hybrid) by using known interacting proteins and looking for gene products or small molecules that interrupt this interaction (Huang and Schreiber 1997). Researcher have modified the yeast two hybrid methods to ask and answer other questions, such as looking for small molecules that induce ligand interactions (Lin and Cornish 2001).

Many papers have been written about the use of yeast two hybrid in the classroom. These activities fall into general categories:

(1) Yeast Two Hybrid Screen – Fishing for 'Prey' with a known bait (Mordacq and Ellington 2008; Odom and Grossel 2002; Witherow and Carson 2011).
(2) Ligand Binding Assay – Protein-protein interactions are facilitated by adding exogenous ligand in a dose dependent manner (Mäkinen, Petersen, and Honkakoski 2005).
(3) Analyzing Mutants of Known Interaction Partners for Altered Binding – A known protein interaction partner is mutated and yeast two hybrid is used to assess binding ability of mutant (Gammie and Erdeniz 2004).

Each yeast two hybrid exercise is unique in its question and opportunities and can be utilized as written in the literature or modified for a particular instructor's course. The yeast two hybrid system is a versatile method for students to identify molecular interactions within the context of a cellular environment. As easy as it is adaptable, students can either plug in to existing questions in the lab or can develop their own.

Limitations to using yeast in the classroom

For all the myriad of activities described above, there are certain drawbacks to using a single celled organism. The chief negative to using yeast in the classroom is the lack of tie in to the day to day life of many students. Students will generally be more interested in topics that they can personally relate to, such as a disease a friend or family member may have or an identifiable macroscopic organism (like a pet or a plant), than a small esoteric 'germ' not seen with the naked eye. Getting students to see the utility of yeast and getting them to be interested in a phenotype on a petri dish can be difficult. As outlined in Table 1, some of the experiments described herein require a high level of expertise. Furthermore, many yeast experiments require specific strains that must be obtained from a commercial supplier or the authors of papers. A final additional limitation of using yeast for research based projects is that in certain instances yeast is not a good model organism, chiefly in certain pathways (Karathia et al. 2011) and for questions on a multicellular scale (Mohammadi et al. 2015).

Conclusions

Saccharomyces cerevisiae is a robust model system for many cellular pathways and its ease of use and simple nutrient requirements lend itself to classroom use. It can be stored on plates for a month or more and can be experimented on in a weekly manner, similar to laboratory meeting times. It is easy to work with and almost impossible to kill under standard laboratory conditions. Even a terribly

contaminated plate can be carefully subcultured to yield pure colonies. Yeast can also be stored at -80 °C for years or on slants or petri dishes at 4 °C for up to 6 months.

As a true eukaryote, yeast contains all of the organelles of higher eukaryotes and has been a model system for many cellular processes and pathways and is increasingly moving into the forefront in systems biology and functional genomics (Botstein and Fink 2011). Its genome is available for mining on the Saccharomyces Genome Database (www.yeastgenome.org) (Cherry et al. 2012) and students and researchers alike can probe this website for protein function, genetic and physical interactions, subcellular localization, and more. Ther organism's versatility can be seen is the variety of laboratories that have been developed using it as a focal organism. From enzymology to cell biology, evolution to genetics, there is a lab that can be done in class utilizing the multifaceted yeast.

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References

Ågren, J. A., R. J. Williamson, B. E. Campitelli, and J. Wheeler. 2017. "Greenbeards in Yeast: An Undergraduate Laboratory Exercise to Teach the Genetics of Cooperation." *Journal of Biological Education* 51 (3): 228–236. doi:10.1080/00219266.2016.1217903.

Anderman, L. H., and V. S. Leake. 2005. "The ABCs of Motivation: An Alternative Framework for Teaching Preservice Teachers about Motivation." *The Relevance of Educational Psychology to Teacher Education* 78 (5): 192–196.

Aronson, and B. D., L. A. Silveira. 2009. "From Genes to Proteins to Behavior: A Laboratory Project That Enhances Student Understanding in Cell and Molecular Biology." *CBE LSE* 8 (4): 291–308. doi:10.1187/cbe.09-07-0048.

Marsh, T. L., and P. E. Arriola. 2009. "The Science of Salsa: Antimicrobial Properties of Salsa Components to Learn Scientific Methodology." *Journal of Microbiology and Biology Education* 10 (1): 3–8.

Auchincloss, L. C., S. L. Laursen, J. L. Branchaw, K. Eagan, M. Graham, D. I. Hanauer, G. Lawrie, et al. 2014. "Assessment of Course-Based Undergraduate Research Experiences: A Meeting Report." *Cell Biology Education* 13 (1): 29–40. doi:10.1187/cbe.14-01-0004.

Bangera, G., and S. E. Brownell. 2014. "Course-Based Undergraduate Research Experiences Can Make Scientific Research More Inclusive." *Cell Biology Education* 13 (4): 602–606. doi:10.1187/cbe.14-06-0099.

Belanger, K. B. 2009. "Using Affinity Chromatography to Investigate Novel Protein-Protein Interactions in an Undergraduate Cell and Molecular Biology Lab Course." *CBE LSE* 8 (3): 214–225. doi:10.1187/cbe.09-03-0019.

Bonner, J. M. 2009. "A Study of Fermentation by *Saccharomyces cerevisiae*." In *Tested Studies for Laboratory Teaching*, Vol. 30, edited by K. L. Clase, 25–40. Proceedings of the 30th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 403 pages.

Botstein, D., and G. R. Fink. 2011. "Yeast: An Experimental Organism for 21st Century Biology." *Genetics* 189 (3): 695–704. doi:10.1534/genetics.111.130765.

Brame, C. J., W. M. Pruitt, and L. C. Robinson. 2008. "A Molecular Genetics Laboratory Course Applying Bioinformatics and Cell Biology in the Context of Original Research." *CBE-LSE* 7 (4): 410–421. doi:10.1187/cbe.08-07-0036.

Brownell, S., D. S. Hekmat-Scafe, V. Singla, P. C. Chandler Seawell, J. F. Conklin Imam, S. L. Eddy, T. Stearns, and M. S. Cyert. 2015. "A High-Enrollment Course-Based Undergraduate Research Experience Improves Student Conceptions of Scientific Thinking and Ability to Interpret Data." *Cell Biology Education* 14 (2): ar21. doi:10.1187/cbe.14-05-0092.

Brownell, S. E., M. P. Wenderoth, R. Theobald, N. Okoroafor, M. Koval, S. Freeman, C. L. Walcher-Chevillet, and A. J. Crowe. 2014. "How Students Think about Experimental Design: Novel Conceptions Revealed by in-Class Activities." *BioScience* 64 (2): 125–137. doi:10.1093/biosci/bit016.

Bryer, P. J. 2016. "Exploring Catalase and Invertase Activity Using Sodium Alginate-Encapsulated Yeast (Yeast Spheres)." *Journal of Microbiology and Biology Education.* 17 (3): 490–491. doi:10.1128/jmbe.v17i3.1180.

Campbell, A. M., T. T. Eckdahl, E. Fowlks, L. J. Heyer, L. L. M. Hoopes, M. L. Ledbetter, and A. G. Rosenwald. 2006. "COLLABORATIVE PROGRAMS: Genome Consortium for Active Teaching (GCAT)." *Science* 311 (5764): 1103–1104. doi:10.1126/science.1121955.

Campbell, A. M., M. L. Ledbetter, L. L. M. Hoopes, T. T. Eckdahl, L. J. Heyer, A. G. Rosenwald, E. Fowlks, S. Tonidandel, B. Bucholtz, and G. Gottfried. 2007. "Genome Consortium for Active Teaching: Meeting the Goals of BIO2010." *Cell Biology Education* 6 (2): 109–118. doi:10.1187/cbe.06-10-0196.

Chan, K. H. 2016. "A Simple Microscale Setup for Investigating Yeast Fermentation in High School Biology Classrooms." *The American Biology Teacher* 78 (8): 669–675. doi:10.1525/abt.2016.78.8.669.

Chanchaichaovivat, A., B. Panijpan, and P. Ruenwongsa. 2008. "Yeast Biocontrol of a Fungal Plant Disease: A Model for Studying Organism Interrelationships." *Journal of Biological Education* 43 (1): 36–40. doi:10.1080/00219266.2008.9656147.

Cherry, J. M., et al. 2012. "Saccharomyces Genome Database: The Genomics Resource of Budding Yeast." *Nucleic Acids Research*. 40 (Database issue): D700-5. doi:10.1093/nar/gkr1029.

Collins, L. T., and R. P. Bell. 2004. "How to Generate Understanding of the Scientific Process in Introductory Biology." *The American Biology Teacher* 66 (1): 51–53. doi:10.2307/4451617.

Connerly, P. L. 2007. "How to Perturb Yeast: A Series of Experiments Investigating Yeast Growth and Protein Composition." In *Tested Studies for Laboratory Teaching*, Vol. 28, edited by M. A. O'Donnell, 382–384. Proceedings of the 28th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 403 pages.

Corwin, L. A., M. J. Graham, and E. L. Dolan. 2015. "Modeling Course-Based Undergraduate Research Experiences: An Agenda for Future Research and Evaluation." *Cell Biology Education* 14 (1): es1. doi:10.1187/cbe.14-10-0167.

D'Costa, A. R., and I. Santoro. 2009. "The Effect of UV Radiation on the Survival of Yeast and Its Implication to a Real-Life Situation." In *Tested Studies for Laboratory Teaching*, Vol. 30. edited by K. L. Clase, 371–382. Proceedings of the 30th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 403 pages

Dennison, M. D., and C. A. Goldman. 1994. "When Do Adaptive Mutants Arise in Yeast?" In *Tested Studies for Laboratory Teaching*, Vol. 15, edited by C. A. Goldman, 277–291. Proceedings of the 15th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 390 pages.

Deutch, C. E., and P. A. Marshall. 2008. "Analysis of the RNA Content of the Yeast *Saccharomyces cerevisiae*." *The American Biology Teacher.* 70 (9): 537–545. doi:10.1662/0002-7685-70.9.537.

Dirks, C., M. P. Wenderoth, and M. Withers. 2014. *Assessment in the College Classroom*. New York, NY: W.H. Freeman.

Duina, A. A., M. E. Miller, and J. B. Keeney. 2014. "Budding Yeast for Budding Geneticists: A Primer on the *Saccharomyces cerevisiae* Model System." *Genetics* 197 (1): 33–48. doi:10.1534/genetics.114.163188.

Dunham, M., M. Gartenberg, and G. W. Brown. 2015. *Methods in Yeast Genetics and Genomics, 2015 Edition: A CSHL Course Manual*. Cold Spring Harbor Laboratory Course Manual.

Dymond, J. S., L. Z. Scheifele, S. Richardson, P. Lee, S. Chandrasegaran, J. S. Bader, and J. D. Boeke. 2009. "Teaching Synthetic Biology, Bioinformatics and Engineering to Undergraduates: The Interdisciplinary Build-a-Genome Course." *Genetics.* 181 (1): 13–21. doi:10.1534/genetics.108.096784.

Epstein, J. L., M. Vieira, B. Aryal, N. Vera, and M. Solis. 2010. "Developing Biofuel in the Teaching Laboratory: Ethanol from Various Sources." *Journal of Chemical Education* 87 (7): 708–710. doi:10.1021/ed100260 g.

Feyder, S., J.-O. De Craene, S. Bär, D. L. Bertazzi, and S. Friant. 2015. "Membrane Trafficking in the Yeast *Saccharomyces cerevisiae* Model." *International Journal of Molecular Sciences* 16 (1): 1509–1525. doi:10.3390/ijms16011509.

Fields, S., and O. Song. 1989. "A Novel Genetic System to Detect Protein-Protein Interactions." *Nature* 340 (6230): 245–246. doi:10.1038/340245a0.

Freeland, P. W. 1973. "Some Practical Aspects of Sugar Fermentation by Baker's Yeast (*Saccharomyces cerevisiae*)." *Journal of Biological Education* 7 (5): 14–22.

Gammie, A. E., and N. Erdeniz. 2004. "Characterization of Pathogenic Human MSH2 Missense Mutations Using Yeast as a Model System: A Laboratory Course in Molecular Biology." *Cell Biology Education* 3 (1): 31–48. doi:10.1187/cbe.03-08-0006.

Gillespie, B., and W. A. Deutschman. 2010. "Brewing Beer in the Laboratory: Grain Amylases and Yeast's Sweet Tooth." *Journal of Chemical Education* 87 (11): 1244–1247. doi:10.1021/ed100442b.

Grammer, R. T. 2012. "Quantitation & Case-Study-Driven Inquiry to Enhance Yeast Fermentation Studies." *The American Biology Teacher* 74 (6): 414–420. doi:10.1525/abt.2012.74.6.10.

Heinzerling, P., F. Schrader, and S. Schanze. 2012. "Measurement of Enzyme Kinetics by Use of a Blood Glucometer: Hydrolysis of Sucrose and Lactose." *Journal of Chemical Education* 89 (12): 1582–1586. doi:10.1021/ed200735f.

Hekmat-Scafe, D. S., S. E. Brownell, P. C. Seawell, S. Malladi, J. F. C. Imam, V. Singla, N. Bradon, M. S. Cyert, and T. Stearns. 2016. "Using Yeast to Determine the Functional Consequences of Mutations in the Human P53 Tumor Suppressor Gene: An Introductory Course-Based Undergraduate Research Experience in Molecular and Cell Biology." *Biochemistry and Molecular Biology Education*, 161–178. doi:10.1002/bmb.21024.

Heyer, L. J., D. Z. Moskowitz, J. A. Abele, P. Karnik, D. Choi, A. M. Campbell, E. E. Oldham, and B. K. Akin. 2005. "MAGIC Tool: Integrated Microarray Data Analysis." *Bioinformatics.* 21 (9): 2114–2115. doi:10.1093/bioinformatics/bti247.

Hood-DeGrenier, J. K. 2008. "A Western Blot-Based Investigation of the Yeast Secretory Pathway Designed for an Intermediate-Level Undergraduate Cell Biology Laboratory." *Cell Biology Education* 7 (1): 107–117. doi:10.1187/cbe.07-07-0047.

Hooker, P. D., W. A. Deutschman, and B. J. Avery. 2014. "The Biology and Chemistry of Brewing: An Interdisciplinary Course." *Journal of Chemical Education* 91 (3): 336–339. doi:10.1021/ed400523 m.

Hoopes, B., N. L. Pruitt, K. Baier, and S. Brooks. 1998. "Signal Transduction and Control of the Cell Cycle in Yeast, *Saccharomyces cerevisiae* a Collaborative Laboratory Exercise." In *Tested Studies for Laboratory Teaching*, Vol. 19, edited by S. J. Karcher, 61–80. Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 365 pages.

Huang, J., and S. L. Schreiber. 1997. "A Yeast Genetic System for Selecting Small Molecule Inhibitors of Protein–Protein Interactions in Nanodroplets." *Proceedings of the National Academy of Sciences* 94 (25): 13396–13401.

Hunnes, C. H. 2003. "Isolation, Purification, and Characterization of Bovine Heart and Yeast Cytochromes c: An Integrated Biochemistry Laboratory Experience." *Biochemistry and Molecular Biology Education.* 31 (4): 242–248. doi:10.1002/bmb.2003.494031040248.

Hutchins, G. G. 2012. "Counting "Shmoos:" Identifying the Missing Yeast Mating Gene." In *Tested Studies for Laboratory Teaching*, Vol. 33, edited by K. McMahon. Proceedings of the 33rd Conference of the Association for Biology Laboratory Education (ABLE). 390 pages.

Hutchins, G. G., and S. C. S. Key. 2008. "Gene Knockout/Gene Therapy in Yeast Using Homologous Recombination." In *Tested Studies for Laboratory Teaching*, Vol. 30, edited by Kari L. Clase, 49–62. Proceedings of the 30th Conference of the Association for Biology Laboratory Education (ABLE). 403 pages.

Karathia, H., E. Vilaprinyo, A. Sorribas, and R. Alves. 2011. "*Saccharomyces cerevisiae* as a Model Organism: A Comparative Study." *PLoS ONE* 6 (2): e16015. doi:10.1371/journal.pone.0016015.

Keeney, J. B., and R. Reed. 2000. "A Genetics Laboratory Module Involving Selection and Identification of Lysine Synthesis Mutants in the Yeast *Saccharomyces cerevisiae*." *Journal of Microbiology and Biology Education* 1: 26–30.

Keller, M. J., and C. Gilliam. 2010. "Manipulation of Yeast Respiration Using Acetic Acid to Demonstrate the Scientific Method." In *Tested Studies for Laboratory Teaching*, Vol. 31, edited by K. L. Clase, 485–494. Proceedings of the 31st Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 534 pages.

Kosinski, R. J. 2010. "Using Yeast Fermentation to Suggest and Then Challenge a Model." In *Tested Studies for Laboratory Teaching*, Vol 31, edited by K. L. Clase, 162–186. Proceedings of the 31st Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 534 pages.

Kushner, D. B. 2007. "DNA Microarrays in the Undergraduate Microbiology Lab: Experimentation and Handling Large Datasets in as Few as Six Weeks." *Journal of Microbiology & Biology Education* 8 (1): 3–12.

Lee, Amanda K., Brian K. Sato, S. J. Dacanay, Usman Alam, and J. F. Shaffer. 2015. "Brewing for Students: An Inquiry-Based Microbiology Lab." *Journal of Microbiology and Biology Education* 16 (2): 223–229. doi:10.1128/jmbe.v16i2.914.

Lin, H., and V. W. Cornish. 2001. "In Vivo Protein-Protein Interaction Assays: Beyond Proteins." *Angewandte Chemie International Edition* 40 (5): 871–875. doi:10.1002/1521-3773(20010302)40:5<871::AID-ANIE871>3.0.CO;2-S.

Lopatto, D., and S. Tobias. 2010. *Science in Solution: The Impact of Undergraduate Research on Student Learning*. Washington, DC: Council on Undergraduate Research.

Luo, Y., X. Gong, L. Xu, and S. Li. 2007. "Isolation of RNA and RT-PCR, Cloning, and Sequencing of Noncoding RNAs from Fungi." *Biochemistry and Molecular Biology Education.* 35 (5): 355–358. doi:10.1002/bmb.76.

Mäkinen, J., S. Petersen, and P. Honkakoski. 2005. "Teaching the Basics of Nuclear Receptor Action: A Simple Laboratory Exercise Using the Yeast Two-Hybrid Method." *American Journal of Pharmaceutical Education* 69 (2): 176–183. Article 26.

Manney, T., L. David, B. Johnson, M. Manney, B. Montelone, L. Weaver, and B. Williamson. 1997. *A Classroom Guide to Yeast Experiments, a Research Approach to Mendelian and Molecular Genetics and Genetic-Environmental Interactions*. 2nd ed. Carolina Biological Supply Company.

Manney, T. R., and M. L. Manney. 1992. "Yeast: A Research Organism for Teaching Genetics." *The American Biology Teacher* 54 (7): 426–431. doi:10.2307/4449533.

Manney, T. R., and M. L. Manney. 1993. "Using Yeast Genetics to Generate a Research Environment." *Genetics* 134 (1): 387–391.

Marshall, P. A. 2007. "Using *Saccharomyces cerevisiae* to Test the Mutagenicity of Household Compounds: An Open Ended Hypothesis- Driven Teaching Lab." *CBE-LSE* 6 (4): 307–315. doi:10.1187/cbe.06-12-0204.

Mennella, T. A. 2015. "Designing Authentic Undergraduate Research Experiences in a Single-Semester Lab Course." *The American Biology Teacher* 77 (7): 526–531. doi:10.1525/abt.2015.77.7.7.

Miller, S. B. 1992. "Simple Enzyme Experiments." In *Tested Studies for Laboratory Teaching*, Vol. 6, edited by C. A. Goldman, S. E. Andrews, P. L. Hauta and R. Ketchum, 153–161. Proceedings of the 6th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 161 pages.

Miranda, H. V., A. E. N. Ferreira, A. Quintas, C. Cordeiro, and A. P. Freire. 2008. "Measuring Intracellular Enzyme Concentrations." *Biochemistry and Molecular Biology Education* 36 (2): 135–138. doi:10.1002/bmb.20166.

Mohammadi, S., B. Saberidokht, S. Subramaniam, and A. Grama. 2015. "Scope and Limitations of Yeast as a Model Organism for Studying Human Tissue-Specific Pathways." *BMC Systems Biology* 9: 695. doi:10.1186/s12918-015-0253-0.

Monti-Hughes, A., M. Alonso, J. Garófalo, H. I. Burgos, and C. A. Stella. 2007. "Teaching Lactose Metabolism." *Biochemistry and Molecular Biology Education* 35 (5): 359–363. doi:10.1002/bmb.85.

Mordacq, J., and R. Ellington. 2008. "The Yeast Two-Hybrid Assay." In *Tested Studies for Laboratory Teaching*, Vol. 29, edited by K. L. Clase, 167–187. Proceedings of the 29th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 433 pages.

Odom, D. P., and M. J. Grossel. 2002. "Using the Two-Hybrid Screen in the Classroom Laboratory." *Cell Biology Education* 1 (1): 43–62.

Oelkers, P. M. 2017. "Semester-Long Inquiry-Based Molecular Biology Laboratory: Transcriptional Regulation in Yeast." *Biochemistry and Molecular Biology Education* 45 (2): 145–151. doi:10.1002/bmb.21023.

Parra, K. J., M. P. Osgood, and D. L. Pappas. 2010. "A Research-Based Laboratory Course Designed to Strengthen the Research-Teaching Nexus." *Biochemistry and Molecular Biology Education* 38 (3): 172–179. doi:10.1002/bmb.20358.

Parra-Belky, K. 2002. "Identification of Yeast V-ATPase Mutants by Western Blots Analysis of Whole Cell Lysates." *Journal of Chemical Education* 79 (11): 1348–1350. doi:10.1021/ed079p1348.

Parra-Belky, K., K. McCulloch, N. Wick, R. Shircliff, N. Croft, K. Margalef, J. Brown, T. Crabill, R. Jankord, and E. Waldo. 2005. "Immunoprecipitation and Characterization of Membrane Protein Complexes from Yeast." *Biochemistry and Molecular Biology Education* 33 (4): 289–292. doi:10.1002/bmb.2005.49403304289.

Peters, E. 2005. "Reforming Cookbook Labs." *Science Scope.* 29 (3): 16–21.

Raghevendran, V., J. Nielsen, and L. Olsson. 2005. "Teaching Microbial Physiology Using Glucose Repression Phenomenon in Baker's Yeast as an Example." *Biochemistry and Molecular Biology Education* 33 (6): 404–410. doi:10.1002/bmb.2005.49403306404.

Ratcliff, W. C., A. Raney, S. Westreich, and S. Cotner. 2014. "A Novel Laboratory Activity for Teaching about the Evolution of Multicellularity." *The American Biology Teacher* 76 (2): 81–87. doi:10.1525/abt.2014.76.2.3.

Reinking, L. N., J. L. Reinking, and K. G. Miller. 2004. "Fermentation, Respiration & Enzyme Specificity: A Simple Device & Key Experiments with Yeast." *The American Biology Teacher* 56 (3): 164–168. doi:10.2307/4449781.

Safranek, W. W. 2014. "Yeast Identification by DNA Sequencing in an Undergraduate Mycology Laboratory." *Journal of Microbiology and Biology Education.* 15 (1): 26–27. doi:10.1128/jmbe.v15i1.598.

Sánchez, N. S., and M. Königsberg. 2006. "Using Yeast to Easily Determine Mitochondrial Functionality with 1-(4,5-Dimethylthiazol-2-Yl)-3,5-Diphenyltetrazolium Bromide (MTT) Assay." *Biochemistry and Molecular Biology Education* 34 (3): 209–212.

van Seters, J. R., J. P. J. Sijbers, M. Denis, and J. Tramper. 2011. "Build Your Own Second-Generation Bioethanol Plant in the Classroom!." *Journal of Chemical Education* 88 (2): 195–196. doi:10.1021/ed100791w.

Shortlidge, E. E., and S. E. Brownell. 2016. "How to Assess Your CURE: A Practical Guide for Instructors of Course-Based Undergraduate Research Experiences." *Journal of Microbiology and Biology Education* 17 (3): 399–408. doi:10.1128/jmbe.v17i3.1103.

Silverstein, T. P. 2016. "The Alcohol Dehydrogenase Kinetics Laboratory: Enhanced Data Analysis and Student-Designed Mini-Projects." *Journal of Chemical Education* 93 (5): 963–970. doi:10.1021/acs.jchemed.5b00610.

Sleister, H. M. 2007. "Isolation and Characterization of *Saccharomyces cerevisiae* Mutants Defective in Chromosome Transmission in an Undergraduate Genetics Research Course." *Genetics* 177 (2): 677–688. doi:10.1534/genetics.107.076455.

Smith, G. P., M. Golomb, S. K. Billstein, and S. M. Smith. 2015. "The Luria-Delbru Ck Fluctuation Test as a Classroom Investigation in Darwinian Evolution." *The American Biology Teacher* 77 (8): 614–619. doi:10.1525/abt.2015.77.8.8.

Stambuk, B. U. 2002. "Transcriptional and Posttranslational Regulation of a Membrane Nutrient Transporter." *Biochemistry and Molecular Biology Education* 30 (6): 388–393. doi:10.1002/bmb.2002.49403006013.

Taipa, M. A., A. M. Azevedo, A. L. Grilo, P. T. Couto, F. A. G. Ferreira, A. R. M. Fortuna, I. F. Pinto, R. M. Santos, and S. B. Santos. 2015. "Student Collaboration in a Series of Integrated Experiments to Study Enzyme Reactor Modeling with Immobilized Cell-Based Invertase." *Journal of Chemical Education* 92 (7): 1238–1243. doi:10.1021/ed500842p.

Tatina, R., M. Mansor, and S. Maierin. 2001. "Phosphate Uptake in Carrot & Yeast." *The American Biology Teacher* 63 (7): 498–502. doi:10.2307/4451170.

Teixeira, M. C., P. M. Santos, C. Rodrigues, and I. Sá-Correia. 2009. "Teaching Expression Proteomics: From the Wet-Lab to the Laptop." *Biochemistry and Molecular Biology Education* 37 (5): 279–286. doi:10.1002/bmb.20315.

Timerman, A. P., A. M. Fenrick, and T. M. Zamis. 2009. "The Isolation of Invertase from Baker's Yeast: A Four-Part Exercise in Protein Purification and Characterization." *Journal of Chemical Education* 86 (3): 379–381. doi:10.1021/ed086p379.

Vallen, E. 2002. "Analysis of Protein Localization and Secretory Pathway Function Using the Yeast *Saccharomyces cerevisiae*." *Cell Biology Education* 1: 173–192. doi:10.1187/cbe.02-08-0027.

Walker, D. E., G. P. Lutz, and C. J. Alvarez. 2008. "Development of a Cross-Disciplinary Investigative Model for the Introduction of Microarray Techniques at Non-R1 Undergraduate Institutions." *CBE – Life Sciences Education* 7 (1): 118–131. doi:10.1187/cbe.07-01-0006.

Will, T. J., M. K. McWatters, and K. L. McQuade. 2006. "Exploring the Ubiquitin-Proteasome Protein Degradation Pathway in Yeast." *Biochemistry and Molecular Biology Education* 34 (6): 444–446. doi:10.1002/bmb.2006.494034062683.

Willhite, D. G., and S. E. Wright. 2009. "Detergent-Based Isolation of Yeast Membrane Rafts." *Biochemistry and Molecular Biology Education* 37 (6): 349–354. doi:10.1002/bmb.20339.

Williamson, B. 1999. "Red Mutant Hunt with *Saccharomyces cerevisiae*." In *Tested Studies for Laboratory Teaching*, Vol. 20, edited by S. J. Karcher, 123–135. Proceedings of the 20th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). 399 pages.

Witherow, D. S., and S. Carson. 2011. "A Laboratory-Intensive Course on the Experimental Study of Protein–Protein Interactions." *Biochemistry and Molecular Biology Education* 39 (4): 300–308. doi:10.1002/bmb.20506.

Wolyniak, M. J. 2013. "Improved Student Linkage of Mendelian and Molecular Genetic Concepts through a Yeast-Based Laboratory Module." *Biochemistry and Molecular Biology Education* 41 (3): 163–172. doi:10.1002/bmb.20679.

Zimmermann, F. K., R. Kern, and H. Rasenberger. 1975. "A Yeast Strain for Simultaneous Detection of Induced Mitotic Crossing over, Mitotic Gene Conversion and Reverse Mutation." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 28 (3): 381–388. doi:10.1016/0027-5107(75)90232-8.

Zimmermann, F. K. 1975. "Procedures Used in the Induction of Mitotic Recombination and Mutation in the Yeast *Saccharomyces cerevisiae*." *Mutation Research/Environmental Mutagenesis and Related Subjects* 31 (2): 71–86. doi:10.1016/0165-1161(75)90069-2.