

# Using Trypan Blue to Assess Yeast Cell Viability in the Undergraduate Biology Laboratory

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We describe a trypan blue dye assay for use in the assessment of yeast cell viability after exposure to UV radiation. This exercise can be implemented easily in the undergraduate biology teaching laboratory. Furthermore, this protocol can be expanded for students to use in carrying out their own inquiry-based laboratory exercises – investigating the relationship between relevant combinations of yeast strains and experimental conditions of interest. Students can be directed to complete this exercise in a single laboratory period, or allowed to independently conduct a more comprehensive experiment over the course of several weeks.

**Keywords:** trypan blue, cell viability, yeast cells, inquiry-based learning, inquiry-based laboratory

## Introduction

Inquiry-based laboratory experiences in science courses can help students understand science as a process while keeping them engaged and interested (AAAS 2011 and 2015). Here we describe a simple laboratory module that can ultimately be used by students in designing their own experiment, focused on exposing the budding yeast *Saccharomyces cerevisiae* to a particular insult and determining how it affects cell viability. By coupling the selection of different experimental treatments to appropriate strains of yeast, students can potentially answer a variety of biological questions. This laboratory module is adapted from a similar exercise designed to introduce students to mammalian cell culture and cell viability techniques using HeLa cells (Bowey-Dellinger et al. 2017).

## Yeast Strain Selection

The potential relationship between the strains of yeast selected for study and the insult(s) of choice is of particular importance. This protocol, adapted in part from *DNA Damage: Studying the Impact of UV Light* (Carolina

Biological Supply Company), utilizes two readily available and affordable yeast strains: a wild-type (WT) strain (Carolina, 173620) and a UV-sensitive (UVS) mutant (Carolina, 173624). Here we describe a laboratory module in which students expose both WT and UVS strains of yeast to ultraviolet radiation and examine its effect on cell viability using trypan blue. Specifically, trypan blue is a vital dye, absorbed by dead cells but excluded by living cells. Any strain of yeast, however, can be used by students in the context of an inquiry-based exercise. Ultimately, the strains of yeast available should guide students in selecting relevant treatment conditions for their own experiment.

## Instructional Time and Organization.

This laboratory module can easily be implemented in a single 2-hour general education biology course or a 3-hour course for biology majors. Depending on the strain(s) of yeast and insult(s) selected, students may require multiple laboratory periods to complete their experiment.

## Student Outline

### Objectives

- Use trypan blue to stain yeast cells
- Use disposable hemocytometers to count yeast cells and assess cell viability after UV exposure
- Generate appropriate graphs that depict cell concentrations and cell viability results

### Introduction

Today, you will be working with two different strains of yeast: a wild-type (WT) strain and a strain that is sensitive to UV light (UV-sensitive or UVS). You will expose both yeast strains to UV light and examine whether or not the viability of the cells is compromised. Trypan blue, a vital dye that is absorbed by dead cells and excluded by living cells, will be used to stain the yeast cells. Then, you will use hemocytometers with calibrated grids to count all of the living and dead cells present. This is a very efficient way to calculate cell concentrations and assess cell viability after a particular treatment (in this case, exposure to UV light).

### Methods and Data Collection

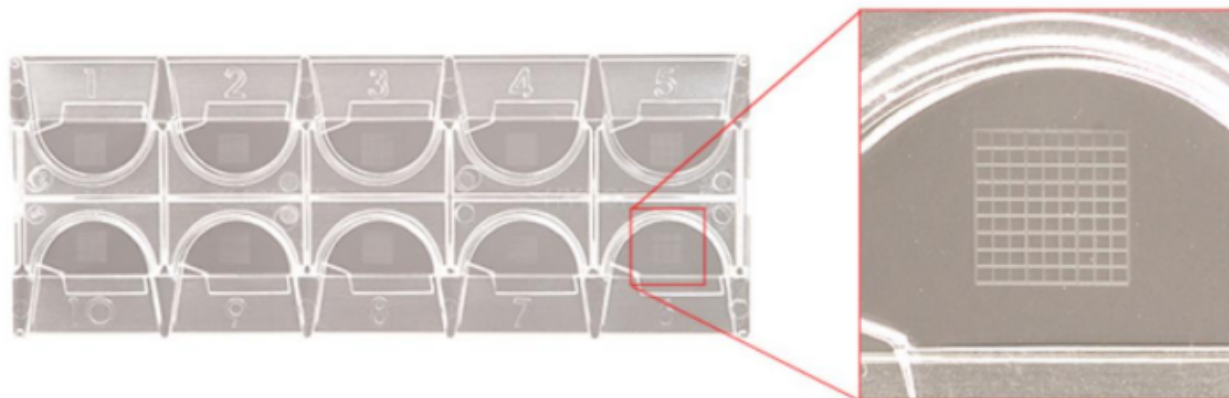
#### *Part A: Exposing Yeast Cells to UV Radiation*

1. You will receive two YEPD (rich media) agar plates that contain lawn cultures of yeast. One plate will have the wild-type (WT) strain of yeast, and the other will have the UV-sensitive (UVS) mutant strain of yeast.
2. On the bottom of the plates, use a permanent marker to draw a line that divides the agar into two equal parts. Label one side “FOIL” and the other side “UV”.
3. Remove the lid of the plate (but do not throw it away) and place the plate inside a clear plastic sandwich bag (or cover the plate surface with plastic wrap). Make sure the plastic is pulled taut over the plate so that it is smooth and not touching the surface of the agar.
4. Using aluminum foil, cover half of the plate labeled “FOIL” along the line you previously drew in order to protect those yeast cells from the UV rays.
5. Repeat steps three and four for the second plate.
6. Expose the yeast plates to UV radiation by using a benchtop UV light box for 5 minutes.
7. Remove the foil and plastic from the plates and place the lids back on.
8. Incubate the plates at room temperature for 10 minutes.
9. During this incubation period, prepare the materials for Part B by completing steps 10 and 11 in the protocol.

#### *Part B: Staining and Counting Cells*

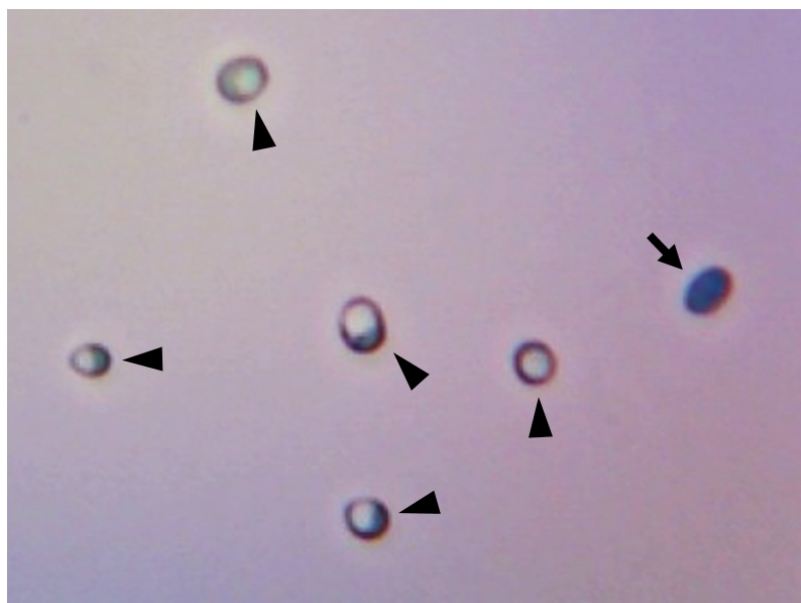
10. Label two sterile plastic culture tubes, one with “WT-FOIL” and the other “WT-UV.” Label two additional tubes, one with “UVS-FOIL” and the other with “UVS-UV.” Label four microcentrifuge tubes the same way.
11. Transfer 5 mL of YEPD liquid medium into each tube using a sterile pipette.
12. Using a sterile wood applicator, collect a sample of yeast from the half of the WT lawn culture that was protected from the UV (“FOIL”).
13. Add the sample into the culture tube labeled “WT FOIL” and pipette up and down to resuspend the cells.
14. Repeat steps 12 and 13 for each of the yeast samples. You should have four tubes total, 2 for WT and 2 for UVS.
15. Measure the optical density (OD) of each of the yeast suspensions and make adjustments as necessary to obtain an OD<sub>600</sub> value of 0.5 for each sample. (In general, 1 OD<sub>600</sub> = ~ 3x10<sup>7</sup> cells/mL)
16. Mix one of the yeast sample tubes by gently shaking (make sure the tube is capped). Transfer 20 μL of the cell suspension to a clean microcentrifuge tube labeled appropriately (in step 10) to match the correct sample.
17. Add 20 μL of trypan blue to the microcentrifuge tube.

18. Load 10  $\mu\text{L}$  of the cell/dye mixture into a single chamber on the counting slide. (see **Figure 1** below)



**Figure 1.** A disposable hemocytometer/counting slide and one of its counting grids/chambers. Each of the counting slides we will be using today contains a total of 10 counting chambers (see insert for counting grid). You load the cell suspension of interest into the desired chamber by micropipetting it through the slit found to the left of the chamber. The brand and model of the disposable hemocytometer slides is KOVA Glasstic and is further described in the Materials section.

19. Place the counting slide under a microscope, locate the grid on the slide, and count cells. Use the data table below for your convenience. See **Figure 2** below to have an idea of what dead/living cells look like under the microscope.



**Figure 2.** A micrograph showing viable and dead budding yeast cells. Live cells possess intact cell membranes that exclude trypan blue and are indicated by arrowheads. Non-viable cells, on the other hand, are stained dark blue (indicated by a complete arrow) as their membranes are permeable and take up the dye. Magnification 200x.

- a. Note that:
- Dark blue cells = Dead cells
  - Light blue cells = Living cells
  - Write down how many squares were counted (see formula below)
  - Write down how many cells that were counted were dead (dark blue)
  - Write down how many cells that were counted were alive (light blue)

vi. Hint: total number of cells counted will be equal to dead (iv) + alive (v) cells

20. Repeat steps 16 through 19 for each of the yeast sample tubes. Record your data in the table below:

		Square 1		Square 2		Square 3		Square 4		Square 5		Total live cells	Total cells
	OD <sub>600</sub>	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead		
WT-F													
WT-UV													
UVS-F													
UVS-UV													

*Part C: Data Analysis—Calculating Cell Viability*

21. Using the data you collected, you can calculate cell concentration and cell viability. The calculations needed to do so are described below.

Cell Concentration

$$\left( \frac{\# \text{ cells}}{\# \text{ small squares}} \right) \times 7500 \times \text{Trypan Blue Dilution} = \# \text{ cells} / \text{mL}$$

Example: If you diluted your cells with an equal volume of trypan blue (2-fold dilution) and counted 90 cells in 18 squares, then the equation would be:

$$\left( \frac{90}{18} \right) \times 7500 \times 2 = 75,000 \text{ cells} / \text{mL} = 7.5 \times 10^4 \text{ cells} / \text{mL}$$

Cell Viability

Calculate the proportion of live cells using the following equation:

$$\left( \frac{\text{Total \# Live Cells}}{\text{Total \# of Cells (alive and dead)}} \right) \times 100 = \text{Cell Viability (\%)}$$

22. Calculate both the cell concentration and cell viability for each of your samples. Compare and contrast the values obtained for the WT and the UV sensitive strains, both in the “no UV” and the “UV” conditions. Record your data in the table below:

	Cell Concentration	Cell Viability
WT-F		
WT-UV		
UVS-F		
UVS-UV		

23. Use Microsoft Excel software to create a graph that displays your data appropriately.

## Materials

### Yeast Strains

The WT and UVS strains of yeast selected for this experiment are readily available from Carolina Biological Supply Company. These strains can be purchased individually (Carolina, 173620 and 173634) or as part of the laboratory activity kit *DNA Damage: Studying the Impact of UV Light* (Carolina, 173608). This protocol can easily be adapted for a variety of other strains of yeast with the help of databases, such as the *Saccharomyces* Genome Database (SGD; <https://www.yeastgenome.org>), to identify phenotypes or sensitivities of interest. The insults used to challenge WT and mutant yeast strains can be selected based on the reported phenotypes of different mutants of interest. The individual mutant yeast selected can be acquired from comprehensive yeast strain collections, such as the Yeast Knock Out Collection, available through Dharmacon or GE Lifesciences (<http://dharmacon.gelifesciences.com/cdnas-and-orfs/non-mammalian-cdnas-and-orfs/yeast/yeast-knockout-collection/>).

### Yeast Strain Growth

Standard methods and conditions were used to grow yeast in the context of this protocol. Yeast cells were grown at 30°C on a standard formulation of rich medium containing 1% yeast extract, 2% peptone, and 2% dextrose. Additional information about yeast growth methods, rich media recipes, and storage conditions can be found in *A Classroom Guide to Yeast Experiments* (Genetics Education Network 1996). Specifically, Part G of the aforementioned guide contains useful information on lab techniques for the culture and maintenance of yeast strains. Physical copies of this text are available through Carolina Biological Supply Company (173670); however, this publication is also available online free of charge (<https://www.phys.ksu.edu/gene/chapters.html>). Also, please go to our Instructor's Guide (Appendix A) for more tips and information on the handling of yeast.

### Generating the Yeast Strain Lawns

To generate the yeast lawn cultures, apply 500 µL of saturated liquid yeast culture (grown overnight) to an agar plate made of the desired media. Once plated, use a sterile cell spreader or glass beads to spread the suspension out evenly over the surface of the plate. Incubate the inoculated plates at 30°C for two days before use.

### Adapting the Yeast Growth Protocol

Certain adaptations of this laboratory exercise might not require lawn cultures of yeast cells at all, but warrant active liquid cultures instead. *A Classroom Guide*

*to Yeast Experiments* and other comparable laboratory manuals can be referenced to generate appropriate experimental protocols for answering a variety of biological questions. For advanced undergraduate courses, consider directing students to independently research these sources and design their own inquiry-based experiments.

### Exposure of Yeast Cells to Insult(s) of Choice

This protocol utilizes two different strains of yeast: a wild-type strain and a mutant strain with a known deficiency. Exposing cultures of both strains to a relevant insult, such as UV radiation, highlights the deficiency of the mutant strain and the ability of the wild-type strain to recover. Adaptations of this protocol could instead utilize a single, wild-type strain and examine how manipulating the intensity of a given insult compromises cell viability.

### Cell Staining with Trypan Blue

Samples of yeast cells suspended in rich media were stained using an equal volume of 0.4% trypan blue dye (Fisher Scientific, 15250061). While the dye is excluded from living cells, it is absorbed by dead cells, giving them a contrasting dark blue appearance.

### Cell Counting with Disposable Hemocytometers

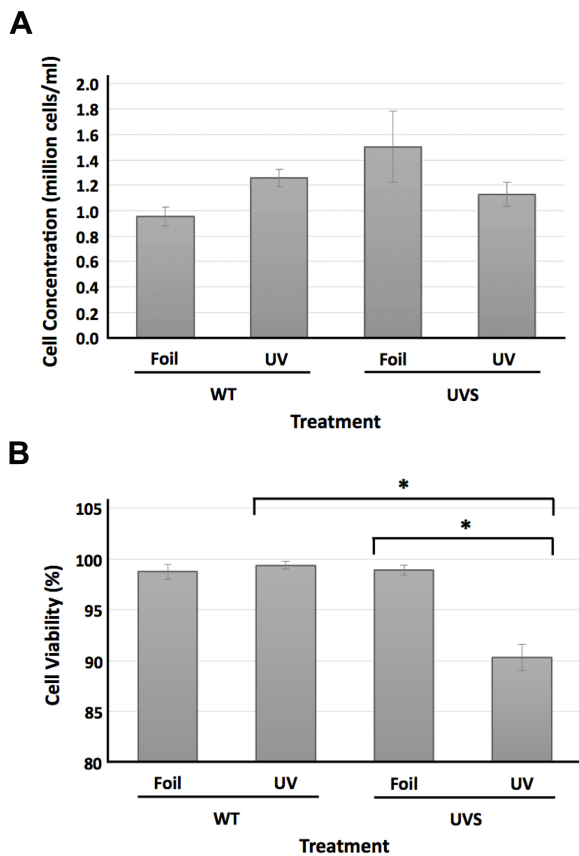
Disposable KOVA Glasstic slides (Fisher Scientific, 22270141) were used to count all of the living and dead cells in a specified number of grids in the counting chamber. These counts were used to calculate cell concentrations and to assess the cell viability in both yeast strains after exposure to UV radiation.

### Sample Student Deliverables/Analyses

As stated in the *Student Handout and Protocol* above, the students will collect their data from counting the cells, and use Microsoft Excel to create appropriate graphs for their findings. The graphs below show examples of data collected and graphed using this protocol.

Figure 3 visually displays the data obtained in the experiment. The first graph shows the total cell concentration in cells per milliliter for each of the yeast samples that were assayed for cell viability. The second graph demonstrates the cell viability that was calculated for each strain. This graph allowed students to visualize that the UV-sensitive yeast were harmed by exposure to the ultraviolet radiation; however, the WT yeast and the UV-sensitive yeast covered by foil were not affected to the same extent. There was significantly less death in the WT strains, and the foil was able to provide effective protection for the UV-sensitive strain. The horizontal axis represents the different yeast strains and exposures they received: WT-F for wild-type foil, WT-UV for wild-type UV radiation, and the same for the UV-sensitive strain.





**Figure 3.** Mean cell concentration (A) and cell viability (B) for each yeast strain with or without the exposure to UV radiation. Data were collected and averaged from three independent experiments. Error bars represent standard error of the mean. Asterisks indicate significant differences between the groups,  $*P < 0.01$ .

### Notes for the Instructor

See **Appendix A** for the *Instructor's Preparation Guide* that accompanies this laboratory module.

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**Candace Loftis** is a lab manager in the Department of Biology at HPU. She plans to return to school to further her career and transition into a medical field.

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**V. Neil Coffield** has been a faculty member at HPU since 2011. He teaches foundation courses for Biology majors and upper-level Virology and Immunology courses. His lab studies bone development in zebrafish embryos.

**Yewseok K. Suh** has been a faculty member at HPU since 2016, where he teaches foundation courses for Biology majors. His lab develops mechanism-based dietary agents for the prevention and treatment of cancer.

Appendix A

***Using Trypan Blue to Assess  
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**Instructor's Preparation Guide**

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## Summary

In this exercise, students will compare the viability of different strains of yeast cells growing in culture after exposure to UV radiation. Students will prepare suspensions of yeast cells to the specified concentration and then stain samples of their suspensions using the vital dye trypan blue. Next, students will load these samples of stained cells onto disposable hemocytometers and count both living and dead cells in the grid of the counting chamber. Students will be asked 1) to calculate the concentration and assess the viability of cells from the samples of each yeast strain and 2) to present their post-exposure yeast strain viability data in an appropriate graph. This exercise was originally designed for a 2-hour general education biology class of 18 students working in pairs, but could easily be expanded for a 3-hour laboratory class for biology majors (especially for those in which students can independently conduct a more comprehensive inquiry-based experiment over the course of several weeks).

## Laboratory Objectives

This exercise is designed to introduce students to the concepts of cell staining and viability and to reinforce the topics of experimental design and microscopy. In this exercise, students will:

- Expose different strains of yeast cells to UV radiation
- Calculate the concentration of yeast cells in a suspension
- Use trypan blue and hemocytometers to assess cell viability
- Generate an appropriate graph that represents their data
- Describe the relationship between staining and cell viability

## Anticipated Timelines

### Preparation

Prepare YEPD agar and broth media: 4 – 5 days in advance of the exercise

Streak primary yeast cell cultures: 3 – 4 days in advance of the exercise

Streak secondary yeast cell cultures: 2 days in advance of the exercise

Set out all needed equipment and supplies: the day of the exercise

### Procedure for General Education Biology (non-Majors) Course – 2 hours

Instructor's introduction to laboratory exercise ..... 15 minutes

Prepare two yeast cultures on agar plates for UV exposure ..... 5 minutes

Expose two yeast cultures on agar plates to UV radiation ..... 5 minutes

Incubate two yeast cultures on agar plates at room temperature ..... 10 minutes

Prepare culture tubes for the yeast cell suspensions .....	Concurrent
Create suspensions of the yeast cells and adjust optical density .....	15 minutes
Stain samples of each yeast cell suspension with trypan blue .....	5 minutes
Count living and dead yeast cells in each sample using hemocytometer .....	45 minutes
Calculate concentration and viability of yeast cells in each sample .....	10 minutes
Prepare an appropriate graph and description of results .....	10 minutes

### Yeast Strains, Reagents, Supplies and Equipment

The following tables outline the yeast strains, reagents, supplies and equipment recommended to conduct this laboratory exercise with a class of 18 – 24 students working in pairs. If ordering for multiple laboratory sections, check whether bulk purchasing options are available for each item, as this may further reduce the unit price. While the tables denote preferred vendors for each item, the prices listed do not reflect contract pricing extended to different institutions; suitable alternate items / vendors may be used. The prices listed also do not reflect potential shipping, handling and freight charges for each item.

#### Yeast Strains

Item	Quantity Recommended	Preferred Vendor	Vendor Product Number	List Price / Quantity
<i>Saccharomyces cerevisiae</i> , Wild-Type Strain	1	Carolina Biological	173620	\$10.50
<i>Saccharomyces cerevisiae</i> , UV-Sensitive Strain	1	Carolina Biological	173634	\$13.00
DNA Damage: Studying the Impact of UV Light Kit	Alternatively	Carolina Biological	173609	\$106.95

#### Reagents

Item	Quantity Recommended	Preferred Vendor	Vendor Product Number	List Price / Quantity
Yeast Extract	25 g	Fisher Scientific	BP1422-100	\$87.90 / 100 g

Peptone	50 g	Fisher Scientific	BP1420-100	\$74.12 / 100 g
Dextrose (Glucose)	50 g	Carolina Biological	857451	\$9.15 / 500 g
Agar	25 g	VWR	A10752-22	\$24.64 / 100 g
Phosphate Buffered Saline, pH 7.4, 1X	Alternative to YEPD broth	Fisher Scientific	10-010-023	\$19.97 / 500 mL
Trypan Blue Solution, 0.4%	500 $\mu$ L	Fisher Scientific	15-250-061	\$16.30 / 100 mL
Glycerol	Varies	VWR	AAAA16205-AP	\$28.45 / 500 mL
Ethanol, Laboratory Grade, 70%	Varies	Carolina Biological	861263	\$24.00 / 3.8 L
Bleach, 10%	Varies	Generic	N/A	N/A

## Supplies

Item	Quantity Recommended	Preferred Vendor	Vendor Product Number	List Price / Quantity
Wash Bottle, Ethanol, 500 mL	1	Fisher Scientific	S317323	\$7.32 / Each
General-Purpose Laboratory Labeling Tape	1	VWR	89097-986	\$44.41 / 6
Flask, Glass, 1000 mL	3	Fisher Scientific	S63274	\$66.25 / 6
Graduated Cylinder, 500 mL	1	Fisher Scientific	08-572-5F	\$77.29 / Each
Weigh Dishes	Varies	Fisher Scientific	S67090A	\$58.00 / 500
Spatula, Scoopula	6	Fisher Scientific	14-357Q	\$26.00 / 6
Stir Bar Kit, Octagonal	1	Fisher Scientific	14-513-82	\$128.00/ Each
Autoclave Gloves	1	VWR	32885-805	\$50.48 / pair

Petri Dishes, Stackable, 100 x 15 mm (sterile)	40	VWR	25384-342	\$300.72 / 500
Disposable Serological Pipettes, 25 mL	Varies	VWR	89130-900	\$257.30 / 200
Centrifuge Tubes, 15 mL (sterile)	Varies	VWR	490001-621	\$331.60 / 500
Black Permanent Markers	12	Generic	N/A	N/A
Wood Applicator Sticks (sterile)	25	Fisher Scientific	22-029-641	\$125.58 / 2000
Toothpick, Flat, Box	Alternative to Applicators	Carolina Biological	972126	\$2.00 / 750
Plastic Sandwich Bags	25	Generic	N/A	N/A
Aluminum Foil	Varies	Generic	N/A	N/A
Culture Tubes, Plastic, 17 x 100 mm (sterile)	25	VWR	60818-725	\$214.68 / 500
Autoclavable Test Tube Racks	12	Fisher Scientific	S43081	\$157.21 / 8
Graduated Transfer Pipettes (sterile)	25	Fisher Scientific	13-711-20	\$105.64 / 500
Disposable Cuvettes, 1.5 mL	Varies	Fisher Scientific	14-955-127	\$182.00 / 500
Racked Pipette Tips, 200 $\mu$ L	12	VWR	490000-380	\$80.56 / 10
Microcentrifuge Tubes, 1.7 mL	Varies	VWR	490004-436	\$82.26 / 500
Microtube Storage Racks	12	Fisher Scientific	05-541-1	\$53.98 / 5

KOVA™ Glasstic™ Slide 10 with Grids	25	Fisher Scientific	22-270-141	\$180.56 / 100
4-Digit Counter	25	Carolina Biological	700680	\$13.25 / Each
Bench-Top Biohazard Bag Holder	12	VWR	11215-508	\$31.82 / Each
Biohazard Bags for Bag Holder	12	VWR	11215-857	\$24.29 / 100
Heavy-Duty Biohazard Disposal Bags	2	VWR	11215-866	\$614.81 / 200
Small Nitrile Gloves	Varies	VWR	490010-852	\$139.91 / 100
Medium Nitrile Gloves	Varies	VWR	490010-854	\$139.91 / 100
Large Nitrile Gloves	Varies	VWR	490010-856	\$139.91 / 100
X-Large Nitrile Gloves	Varies	VWR	490010-858	\$139.91 / 100
Safety Glasses	25	Fisher Scientific	S01821	\$44.00 / 12

## Equipment

Item	Quantity Recommended	Preferred Vendor	Vendor Product Number	List Price / Quantity
Refrigerator, 4°C	1	Generic	N/A	N/A
Portable Balance	1	VWR	10205-002	\$829.18
Deionizer	1	Fisher Scientific	09-033-10	Request a Quote
Hotplate / Magnetic Stirrer	1	VWR	490000-188	\$541.64
Steam Sterilizer	1	Sterilink, Inc.	N/A	Request a Quote
Pipette Controller	1	VWR	490017-208	\$401.93
Compact Microbiological Incubator	1	VWR	10200-150	\$693.84
Benchtop UV Transilluminators	Varies	Fisher Scientific	UV95045701	\$2,501.01

NanoDrop™ 2000c Spectrophotometer	1	Fisher Scientific	ND2000C	\$11,150.00
Compound Microscope	25	Associated Microscope Services	N/A	Request a Quote
Single-Channel Pipettor, 10–100 μL	12	VWR	490014-614	\$320.03
Biohazard Waste Container	1	Fisher Scientific	18-880A	\$132.00
Revco™ UxF - 86°C Upright UltraLow Temp Freezer	Optional	Fisher Scientific	13-990-608	\$22,714.00

## Yeast Culture Handling

### Care of yeast strains

Vendors often ship yeast strains on rich media in glass vials packed in ice. Once received, the vials should be stored at 4°C until the yeast is streaked onto YEPD agar plates in preparation for the laboratory exercise. The UVS yeast vial should be thoroughly wrapped in aluminum foil, to prevent any unintended exposure of the yeast to UV light. If long-term storage of the yeast strains is needed, yeast can be suspended in 15% glycerol and kept frozen at -80°C. The yeast can be revived (without thawing the frozen cultures) by scraping cells off of the top of the frozen culture and streaking them on YEPD agar plates.

In order to minimize potential contamination to the stock strains or any reagents, utilize aseptic techniques throughout the preparation of yeast cultures. These include, but are not limited to:

- a. Decontaminating the work surface and yeast culture vials with 70% ethanol
- b. Opening culture vials, flasks of media, petri dishes, culture tubes, reagent bottles and any other sterile materials only in close proximity to a Bunsen burner flame
  - o The flame assists by drawing air currents upwards
- c. Returning the cap or lid to every container whenever it is not being used
- d. Preventing applicators and pipettes from contacting anything but the yeast cultures inside vials, dishes or tubes once they have been removed from their sterile wrapper

### Disposal of contaminated materials

Discard all solid waste in an appropriate waste container after use: culture vials, petri dishes, wooden applicators, transfer pipettes, centrifuge tubes and any other contaminated materials should be collected in a biohazard waste container lined with a biohazardous waste bag.

Sterilize each sealed bag via autoclave at 121°C and 100 kPa above atmosphere pressure for 15 minutes. Waste can be discarded alongside other trash after decontamination.

Disinfect any liquid waste by adding enough bleach to the waste container to bring the final concentration of bleach to 10%. Expose the waste to bleach for at least one hour before flushing down the sink with plenty of water.

## Safety Guidelines

**This laboratory exercise should be conducted in accordance with your institution's chemical hygiene plan and / or biosafety plan.** *Saccharomyces cerevisiae* is classified as a biosafety level I organism, indicating that it poses little to no risk of infection to healthy adults. However, UV light boxes are sources of intense ultraviolet radiation, which can cause significant damage to a person's eyes and skin if left unprotected. While most benchtop models have built-in UV shields, users should still wear UV-rated eye protection, gloves and a long-sleeved lab coat.

All students should have received laboratory safety training prior to participating in this exercise and must adhere to safe laboratory practices throughout the duration of the exercise. These practices include, but are not limited to:

- Wearing appropriate clothing (closed-toed shoes) and protective equipment in the lab
- Decontaminating all work bench surfaces and washing hands before leaving the lab
- Never eating, drinking, chewing gum, smoking or applying makeup while in the lab

## Supplemental Information

Additional background information on *Saccharomyces cerevisiae*, its reproductive cycle, genetics, and response to radiation can be found in *A Classroom Guide to Yeast Experiments* (Genetics Education Network 1996). This publication also details basic genetics, UV, and mutation experiments, which your biology major students may find useful in developing their own comprehensive independent research projects. Physical copies of this resource are available for purchase from Carolina Biological Supply Company (173670), although it can also be accessed free of charge through The GENE Project's website here: (<https://www.phys.ksu.edu/gene/chapters.html>).

## Laboratory Preparation

Read through both the *Instructor's Preparation Guide* and the *Student Guide* in their entirety to become familiar with the reagents, supplies, equipment and protocols that will be used.

The preparer should wear nitrile gloves, a lab coat and safety glasses, and should demonstrate safe and responsible laboratory practices throughout the preparation of the media



and yeast cultures. Proper use of aseptic techniques will not only help minimize the potential for contamination to the cultures, but also protect the user from biohazardous materials.

### **Prepare YEPD agar and broth media: 4 – 5 days in advance of the exercise**

This exercise was originally designed for a single 2-hour general education biology class of 18 students working in pairs. If preparing media for larger classes or additional laboratory sections, adjust the amount of agar and broth media that needs to be prepared accordingly.

1. **Label the outside of the 1000 mL glass flasks using labeling tape and a marker**
  - a. Write the following information on the outside of two of the flasks
    - i. YEPD agar (the media type), your initials and the date
  - b. Write the following information on the outside of one of the flasks
    - i. YEPD broth (the media type), your initials and the date
2. **Dissolve yeast extract (1%), peptone (2%), dextrose (2%), and agar (2%) in 500 mL of dH<sub>2</sub>O**
  - a. Use a graduated cylinder to add 500 mL of dH<sub>2</sub>O to each of the three flasks
    - i. Add an octagonal magnetic stir bar to each flask of water
  - b. Add 5 g of yeast extract, 10 g of peptone and 10 g of dextrose to each flask
  - c. Add an additional 10 g of agar to the two flasks labeled “YEPD agar”
    - i. Be careful to not add any agar to the “YEPD broth” flask
  - d. Place one of the flasks on the magnetic stirplate and stir the media until the reagents have dissolved evenly. Repeat with the two remaining flasks
3. **Sterilize flasks of media via autoclave using an appropriate liquid cycle with slow exhaust**
  - a. In a liquid cycle, steam is exhausted slowly allowing liquids to cool; this helps minimize the likelihood that liquids “boil over” during the autoclave cycle
  - b. Cover the mouth of each flasks with aluminum foil (taping it down if necessary)
  - c. Wear appropriate personal protective equipment when loading materials into autoclave (lab coat, safety glasses, heat-resistant gloves and closed-toe shoes)
  - d. All of flasks should be placed into a secondary containers, made of heat-resistant polypropylene or stainless steel, to capture and liquids that might boil over
4. **Pour sterile YEPD agar in petri dishes; aliquot the sterile YEPD broth into conical tubes**
  - a. Wear appropriate personal protective equipment when removing materials from autoclave (lab coat, safety glasses, heat-resistant gloves and closed-toe shoes)
  - b. Use magnetic stir plates to stir the media and allow time for the flasks to cool
  - c. Pour enough agar into each petri dish so that the bottom of the plate is covered
    - i. 500 mL of media should yield anywhere from 15 – 20 plates
    - ii. Allow the agar to solidify before disturbing the petri dishes

- d. Use a 25 mL serological pipette to aliquot 10 mL of YEPD broth into 15 ml tubes
  - i. Used to create cell suspensions and to adjust optical density
5. **Store YEPD agar plates and YEPD broth tubes in refrigerator at 4°C until needed**
  - a. YEPD agar plates should be stored upside-down in plastic sleeves to prevent potential condensation on the lid from dripping down onto the agar surface

### **Streak primary yeast cell cultures: 4 days in advance of the exercise**

1. **Utilize aseptic techniques throughout the preparation of yeast cultures**
  - a. Decontaminate the work surface and stock vials with 70% ethanol
  - b. If possible, streak plates in close proximity to a Bunsen burner flame
2. **Label the bottom of two YEPD agar plates using a permanent marker**
  - a. Write WT, YEPD, your initials and the date on the first plate
  - b. Write UVS, YEPD, your initials and the date on the second plate
3. **Inoculate each plate with the appropriate strain by streaking in a zigzag pattern**
  - a. Use a sterile wood applicator to collect yeast from the wild-type stock vial and gently spread the yeast cells across a third of the WT-labeled YEPD agar surface
  - b. Use a second sterile wood applicator to continue spreading yeast cells across a different third of the agar surface, beginning from the streak in the first section
  - c. Use another sterile wood applicator to finish spreading yeast cells across the remaining third of the agar surface, beginning from the streak in the second section
  - d. Repeat this process to spread UV-sensitive yeast cells across the UVS-labeled plate
4. **Store the inoculated YEPD agar plates upside-down in an incubator at 30°C for 48 hours**
  - a. The UVS-labeled YEPD agar plate should be thoroughly wrapped in aluminum foil

### **Streak secondary yeast cell cultures: 2 days in advance of the exercise**

This exercise was originally designed for a single 2-hour general education biology class of 18 students working in pairs. If preparing secondary cultures for larger classes or additional laboratory sections, adjust the number of plates that need to be inoculated accordingly.

1. **Utilize aseptic techniques throughout the preparation of yeast cultures**
  - a. Decontaminate the work surface and stock vials with 70% ethanol
  - b. If possible, streak plates in close proximity to a Bunsen burner flame
2. **Label the bottom of 18 YEPD agar plates using a permanent marker**
  - a. Write WT, YEPD and the date on the first nine plates
  - b. Write UVS, YEPD and the date on the second nine plates

- c. Leave space for the students to write their own initials
3. **Inoculate each plate with the appropriate strain by streaking in a “lawn” pattern**
  - a. Use a sterile wood applicator to collect a single colony of yeast from the primary WT YEPD agar plate and gently spread the yeast cells across the entire surface of a new WT-labeled YEPD agar plate. Rotate the plate as you continue to spread the yeast
    - i. Repeat until all nine WT-labeled YEPD agar plates have been inoculated
  - b. Use a sterile wood applicator to collect a single colony of yeast from the primary UVS YEPD agar plate and gently spread the yeast cells across the entire surface of a new UVS-labeled YEPD agar plate. Rotate the plate as you continue to spread the yeast
    - i. Repeat until all nine UVS-labeled YEPD agar plates have been inoculated
4. **Store the inoculated YEPD agar plates upside-down in an incubator at 30°C for 48 hours**
  - a. The UVS-labeled YEPD agar plates should be thoroughly wrapped in aluminum foil

**Set out all needed equipment and supplies – the day of the exercise**

1. **Provide copies of the *Student Handout and Protocol* for each student**
2. **Take the YEPD agar plates with the WT and UVS yeast lawn cultures out of the incubator**
3. **Place the following equipment and supplies at every station for each student pair**

1 WT yeast lawn culture on YEPD plate	1 UVS yeast lawn culture on YEPD plate
2 compound microscopes	2 KOVA Glasstic® slide 10 with grids
1 P100 micropipette and 200 $\mu$ L tip box*	10 graduated plastic transfer pipettes*
4 plastic culture tubes* and tube rack	2 aliquots of YEPD broth in 15 mL tubes
10 1.7 mL microcentrifuge tubes*	1 microcentrifuge tube rack
4 wooden applicator sticks*	1 aliquot of 0.4% trypan blue dye
1 fine-tip black permanent marker	2 plastic sandwich bags and aluminum foil
1 tally register with 4-digit counter	1 biohazardous waste stand with bag

\*Ideally, all of the tips, transfer pipettes, tubes and applicators will be sterile

4. **Ensure that your students have access to nitrile gloves and UV-rated safety glasses**
5. **Set out the benchtop UV transilluminator and the spectrophotometer**
  - a. Providing access to multiple UV boxes and spectrophotometers will help limit bottlenecks during the exercise that might cause delays in the anticipated timeline
  - b. “Blank” the spectrophotometer using a cuvette of sterile YEPD broth before class
  - c. Ensure that there is an adequate supply of disposable cuvettes for students to use

## **Instructor's Procedure**

### **General Education Biology (non-majors)**

1. Provide a brief introduction to yeast, including information on some of the similarities and differences between the wild-type and UV-sensitive strains.
2. Discuss the anticipated timeline for the laboratory exercise, highlighting data collection, calculations, and the generation of an appropriate graph of the data.
3. Review relevant laboratory safety guidelines prior to beginning the exercise, including the correct use of personal protective equipment, safe operation of the UV box, as well as protocols for proper disposal of solid and liquid waste.
4. Assist students as they prepare their lawn cultures for exposure to UV light. Remind students to properly label the bottom of each half of their agar plates.
5. Load all of the agar plates onto the UV transilluminator for your students.
  - a. Be sure to wear UV-rated eye protection, gloves and a long-sleeved lab coat. This limits the need to supply lab coats to your entire class.
6. Assist students as they prepare their cell suspensions. Demonstrate how to correctly load the cuvettes into the spectrophotometer to measure the optical density ( $OD_{600}$ ) of their yeast suspension. A 0.5  $OD_{600}$  reading is ideal to facilitate accurate and timely cell counts of each of the different samples. Assist students as needed in adjusting the concentration of their suspensions.
7. Remind students to mix 20  $\mu\text{L}$  of trypan blue with a 20  $\mu\text{L}$  sample of each of their yeast suspensions, and not the entire 5 mL of suspension they prepared.
8. Review correct use of a compound microscope. Demonstrate how to use a KOVA Glasstic® slide 10 with grids and trypan blue to count living and dead cells. Assist students as they collect their data and calculate the concentration and viability of yeast cells in each of their different treatment groups.
9. Review expectations for the graph the students should generate of their data.

## Mission, Review Process & Disclaimer

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