Supplemental Materials
for
Student-Designed High-Throughput Assays to Assess Effects of Growth Insults in Budding Yeast
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Received: 29 November 2017, Accepted: 23 April 2018, Published: 29 June 2018.

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Appendix 1: Teacher Materials and Preparation Notes for HTS Yeast-Growth Experiments.

Below are the requirements for one student group of 3-4 persons. Larger groups will require instructors to scale up preparation and materials needed.

Materials and Ordering Information:

<table>
<thead>
<tr>
<th>Materials</th>
<th>Catalog Number</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mL standardized 0.5 OD&lt;sub&gt;600 nm&lt;/sub&gt;/mL S. cerevisiae yeast strain cell suspension grown in 5 mL culture tubes containing YEPD medium*</td>
<td></td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>5 ml culture tubes</td>
<td>215088</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>BP29631</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>96-well microtiter plate</td>
<td>703469</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Microcentrifuge tubes (4)</td>
<td>215236</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Microcentrifuge tube holder (microwave safe)</td>
<td>215578</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Yeast extract-peptone-dextrose growth medium (YEPD)</td>
<td>BP2469100</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Sterile micropipette tips</td>
<td>215052</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>15 mL conical tubes</td>
<td>215095</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Foil</td>
<td></td>
<td>Grocery Store</td>
</tr>
<tr>
<td>Plastic wrap</td>
<td></td>
<td>Grocery Store</td>
</tr>
<tr>
<td>Petri Dishes</td>
<td>741249</td>
<td>Carolina Biological Supply</td>
</tr>
<tr>
<td>Agar</td>
<td></td>
<td>Carolina Biological Supply</td>
</tr>
</tbody>
</table>

*Directions for yeast growth are found under “yeast strains and growth.”

Equipment:
- Microwave oven (1100W)
- 30°C Shaking mini incubator
- Microplate reader or spectrophotometer
- Computers equipped with Microsoft Excel
- Sterile micropipettes

Yeast Strains and Growth Conditions

All materials and yeast strains can be purchased through Carolina Biological Supply (Burlington, NC) or Fisher Scientific (Raleigh, NC). Standard methods and media were employed for manipulations and growth of yeast (1). Briefly, S. cerevisiae strains were grown in 5 mL of yeast extract peptone dextrose (YEPD or YPD) medium at 30°C and 120 rpm for 24 h. Prior to lab experiments yeast cultures were
standardized to an OD$_{600 \text{ nm}}$ of 0.5/mL. Unless otherwise indicated, all growth assays were performed in YEPD medium.

*S. cerevisiae* strains used in this study are listed in Table 1.

**Table 1.** *S. cerevisiae* strains used in this study.

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Strain Genotype</th>
<th>Catalog Number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype (WT)</td>
<td>HAO</td>
<td>173620</td>
<td>Carolina Biological Supply <a href="http://www.carolina.com/yeast-genetics/saccharomyces-cerevisiae-ha0-strain-a-wild-type/173620.pr?question=">http://www.carolina.com/yeast-genetics/saccharomyces-cerevisiae-ha0-strain-a-wild-type/173620.pr?question=</a></td>
</tr>
<tr>
<td>UV-Sensitive (UVS)</td>
<td>G948-1C/ reunited rad1 rad18 phr1 ura3</td>
<td>173634</td>
<td>Carolina Biological Supply <a href="http://www.carolina.com/yeast-genetics/saccharomyces-cerevisiae-uv-sensitive-strain-g948-1cu-alpha-rad1-rad18-phr1-ura3-mutant-in-excision-repair/173634.pr?question=">http://www.carolina.com/yeast-genetics/saccharomyces-cerevisiae-uv-sensitive-strain-g948-1cu-alpha-rad1-rad18-phr1-ura3-mutant-in-excision-repair/173634.pr?question=</a></td>
</tr>
</tbody>
</table>

**Chemical/radiation insults**

More information about yeast cell manipulations can be found in the figure legends accompanying the relevant data or in the student protocols provided in the Appendices. In general, yeast cells were subjected to chemical insults by supplementing rich growth medium (YEPD) with the appropriate amount of compound for the specified amount of time with shaking at 30°C. Yeast cells were subjected to radiation insults by exposure to the radiation of interest for the specified amount of time prior to growth assays.

**Table 2.** Chemical/radiation insults used in study.

<table>
<thead>
<tr>
<th>Insult</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave Radiation</td>
<td>0 s, 15 s, 30 s, 60 s</td>
</tr>
<tr>
<td>Ultraviolet Radiation* (sunlight)</td>
<td>15 minutes (with or without foil)</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>0, 0.05, 0.1, 0.2 µg/mL</td>
</tr>
</tbody>
</table>
*UV light box or transilluminator may be used instead of sunlight

**High Throughput Growth Curves and Data Analyses**

A microplate reader (BioTek) was programmed to collect kinetic optical density measurements at OD$_{600}$ nm every 30 minutes for 40 hours from each well of a 96-well microtiter plate as a measure of yeast growth. Experimental plates were maintained in the microplate reader for the duration of the 40 h experiment at 30°C with gentle agitation performed prior to OD$_{600}$ nm recordings. If a microplate reader is not available, a spectrophotometer may be used as an alternative to collect OD$_{600}$ nm measurements. Raw optical density measurements were then exported to Microsoft Excel and growth curves and standard deviation from wells of interest were generated by plotting the OD$_{600}$ nm as a function of time.

**Module Use at the University Level**

**College Student Pre-laboratory Preparation**

Before implementation of this module, university students are given *S. cerevisiae* as a model organism and are taught HTS methodologies to assess growth. Students are then divided into groups of 3-4 individuals. Each group is required to perform a 0.5-1 h literature search (using Google Scholar, PubMed or SciFinder) on the experimental variable of their choosing (i.e., microwave radiation, ultraviolet radiation, cell cycle inhibitor) that may influence yeast growth. After their literature search, student groups come together and discuss their findings with the class. Student groups are then required to formulate a hypothesis and design a simple way to test their hypothesis using the materials and equipment available including the appropriate controls (Appendix 1). Students then decide how to analyze their data and communicate their findings (Appendices 2-4). After approval from the instructor, students may begin their experiments.

**Instructor Notes.** Students found it challenging to narrow down variables and appropriate yeast dilutions that may affect yeast growth. Instructors therefore offered guidance by providing a scaffold of serial dilutions within a predetermined time frame (e.g. 40-48 h) and growth conditions to begin their studies. Students were prompted to research insults from the environment, chemical or otherwise that may affect growth in a negative way. Since this activity was used in a Cancer Biology course, students were
directed towards topics previously discussed in class such as cell cycle regulators, DNA-damage induced mutagenesis and others. Furthermore, students had difficulty in the ways in which to communicate their scientific process. We therefore found it helpful to gear their communications as a protocol to the lay public (e.g. high school students) and provided a layout of sections to include (as shown in Appendices 2-4), but omitting any primary data. However, instructors can allow students the free choice of how to communicate their work, such as a research presentation to the class, as series of ongoing “tweets” using Twitter with appropriate hashtags, a laboratory notebook entry or a formal laboratory report.

**Module Use at the High School Level**

The workflow designed and tested by university students was then communicated by the students as scientific protocols listed in the below appendices (2-4) with instructor modifications for clarity. These protocols served as structured inquiry activities at the high school level. However, high school teachers may decide to offer less structure thus emphasizing different learning outcomes by allowing students to choose their own insult, alter insult exposure times, utilize different yeast strains, and/or have them design their own procedures based on resources locally available.

*Instructor Notes.* It is important that high school students have some familiarity using graphing programs such as Microsoft Excel ahead of time. If instructors have students research additional background information, previous knowledge of PubMed, Google Scholar and/or SciFinder is also appropriate.

**What works**

*S. cerevisiae* growth conditions, serial dilutions, temperature, and OD$_{600}$ readings were predetermined by the instructor as optimal standard growth conditions. Any insult(s) and/or experimental variables students chose to test were therefore compared to this baseline. However, there are numerous areas for student-driven exploration within this broad context and are discussed below.
**Areas of student-driven exploration**

1. Hypothesis generation: University students apply their own unique hypothesis to agents that may affect yeast cell growth after a search of the scientific literature. This may include growth factors, such as additives in the growth medium or alterations in baseline temperature as examples.

2. Experimental design: Students design their experimental procedure including type of insult, length of insult exposure, or dose of insult. Students may also opt to alter the interval of $\text{OD}_{600\ \text{nm}}$ growth readings as well as duration of shaking, but these would require baseline optimization by the students. We recommend this only if multiple lab sessions are devoted to troubleshooting.

3. Data analysis: Students empirically determine data to be analyzed with assistance from the Instructor where needed. Typically, students examine growth trends in their data by looking at the raw $\text{OD}_{600\ \text{nm}}$ values and then determine which dilutions would best represent their results. In our course, students utilized the microplate reader software (BioTek, Gen5) to create summary plots of multiple sets of growth data, and then chose a particular dilution series that showed the best reproducibility and statistical significance.

4. Conclusions and Communication: Students analyze their primary data and draw conclusions. They may, however, choose to communicate their findings in a number of ways such as an oral or poster presentation, laboratory notebook entry, laboratory report, infographic, and/or social media.
Appendix 2: Student Handout/Protocol—Assessing the Effects of Microwave Radiation on Yeast Growth.

I. Background

The causes of cancer can be divided into two main categories: harmful mutations passed on through genetic material (DNA) or lifestyle and other environmental factors leading to cellular transformation. Falling into the class of environmental factors, occupational exposure to microwave radiation has been shown to elevate cancer morbidity rates compared to non-exposed groups (2). Microwave radiation has also been shown to significantly accelerate the development of skin cancer and shortened the lifespan in mouse model systems (3). Microwave radiation falls at a wavelength of approximately 1e-2 meters on the electromagnetic spectrum, directly next to radio waves at 1e-3 meters. Both radio- and microwaves are types of ionizing radiation. Ionizing radiation carries enough energy to denature atoms and molecules in tissues, therefore altering essential biochemical reactions in the body. Individuals working in close proximity and frequency to sources of microwave radiation are at the greatest risk of its damaging effects. Specifically, DNA damage occurs when ionizing radiation causes double stranded breaks in the DNA. If the break cannot be repaired efficiently, it may result in a mutation or loss of protein product ultimately affecting its function. Changes in specific proteins such as tumor suppressors (proteins that suppress cell growth) and/or proto-oncogenes (proteins that increase cell growth) can result in cellular transformation.

To explore the effects of DNA damage, you will design an experiment testing various doses and exposure times of microwave radiation on cell viability in the eukaryotic model organism *S. cerevisiae*.

Student Learning Objectives

*By the end of this experiment students will:*

- Perform a scientific literature search
- Design a testable hypothesis
- Understand the purpose of using yeast as a model system
- Understand basic cellular processes affected by cancer
- Learn the basics of yeast cell culture
- Understand techniques employed in high-throughput data collection
- Learn how to use Microsoft Excel
- Analyze experimental data and construct a scientific figure

II. Experimental Procedure

1. Determine microwave treatment conditions by filling out Table 1 below. Refer to Table 2 as a guide.

Table 1. Experimental conditions for wild-type *S. cerevisiae* exposure to microwave radiation.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>*Microwave power setting</th>
<th>Microwave time setting (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>none</td>
<td>0 sec</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Check with your microwave’s user manual for help with achieving the power setting.
Table 2. Example experimental conditions for *S. cerevisiae* exposure to microwave radiation.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>*Microwave power setting</th>
<th>Microwave time setting (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>High power</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>High power</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>High power</td>
<td>60</td>
</tr>
</tbody>
</table>

2. Label 4 micro centrifuge tubes so that one represents each of your experimental conditions in Table 1.

3. Starting with the standardized 0.5 OD₆₀₀nm/mL *S. cerevisiae* wild-type strain provided, mix to homogeneity and pipette 200 microliters of the yeast cell suspension into each of the 4 labeled micro centrifuge tubes. *(Note: Microwaving larger volumes of cell suspension may result in spills upon microwave treatment)*

4. Microwave each of the tubes individually for the desired amount of time on high power. **UNCAP** the micro centrifuge tubes before microwaving *(caution: capped tubes will explode under pressure)* and use a microwave-safe tube rack.

Figure 1. Example set up for microwave radiation exposure of yeast using a 96-well microtiter plate.

5. Label a sterile 96-well microtiter plate according to the desired treatment conditions. Be sure to allow three replicates for each treatment, including a control (no treatment). See Figure 1 above as an example.

6. Once microwave treatments are complete, pipette 50-100 μL from each treatment tube, including the control, into the appropriate A-row wells of a sterile 96-well microtiter plate—use the Figure 1 above as a guide.

7. Fill every well on the plate (B1 through H12) with 100 μL of YEPD. If available you may use a multichannel pipettor for this purpose.
8. Serially dilute the yeast suspension (1:10) from row A to row H within the same columns, by taking 10 μL of the preceding well and pipetting it onto the next well. For example, take 10 μL from well A1 and pipette into well B1, mix and repeat the dilution by taking 10 μL from B1 and placing into C1. Repeat until you have reached well H. You do not need to change pipette tips within rows. Mix each dilution thoroughly.

9. When you reach row H, discard 10 μL of medium to achieve a final volume in wells B1 through H12 of 100 μL.

10. Place the 96-well microtiter plate into the plate reader and monitor cellular growth at OD<sub>600nm</sub> and 30°C for the next 40-48 hours.

11. Generate a graph of yeast growth curves using Microsoft Excel. Plot OD vs. time for the average of each time point. Data points to be analyzed will be empirically determined based on your resulting data.

### III. Directions on Using Microsoft Excel to Plot Yeast Growth Curves and Standard Deviation

1. Obtain data file from instructor exported as a .xls file from the plate reader.
2. Open data file in Microsoft Excel (*Note: these directions are written for PC computers, and may vary slightly with different computer models or versions of Microsoft Excel*).
3. Take the averages of each radiation time group by creating 4 new columns, and labeling each Average 0 (control), Average 15 sec, Average 30 sec, and Average 60 sec, respectively.
4. In each separate Average column, in the formula line type “=AVG (highlight all cells from each time point to be averaged)”
5. Repeat for the remaining time groups.
6. Highlight the average column for each time group.
7. Click on the “Insert” tab on the top of the screen.
8. Under “Charts” section, click the “Scatter” tab.
10. Title the graph and label the x-axis “Time” and the y-axis “Optical Density” including appropriate units.

To determine and graph standard deviation, create an additional 4 separate columns labeled STD 0 (control), STD 15sec, STD 30sec, and STD 60sec.

12. In each STD column, in the formula line type “=STDEV(highlight all cells from each time point)”.
13. To add the STD to the graph, click on the line for the zero time point.
14. Click on “Add Chart Element”, “Error Bars”, “More Error Bar Options”.
15. Under “Error Amount”, click the circle entitled “Custom” and press the “Specify Value” button.
16. Under “positive error value”, highlight all the cells under the STD 0, and repeat for the “negative error value”.
17. Click “OK”.
18. Repeat for each time point.

### IV. Discussion/Analysis Questions

1. What is DNA damage? How does microwave radiation cause DNA damage? How do eukaryotic cells (like yeast) respond to this insult?
2. Examine your yeast growth curves. Do you notice any significant differences in growth between time of radiation exposure(s)?
3. Why do the yeast cells that were in the microwave for a longer period of time start growing later than the control group?
4. Why did some yeast cells grow even though they were exposed to microwave radiation?
5. How does your data compare to your original hypothesis?
6. Are there potential sources of error in your experiment? How would you design it differently the next time?
Appendix 3: Student Handout/Protocol—Assessing the Effects of UV Radiation on Yeast Growth.

This protocol has been adapted from Carolina’s DNA Damage Kit, [http://www.carolina.com/yeast-genetics/dna-damage-studying-the-impact-of-uv-light/FAM_173608.pr](http://www.carolina.com/yeast-genetics/dna-damage-studying-the-impact-of-uv-light/FAM_173608.pr)

I. Background
As a continuation of the effects of radiation-induced DNA damage on yeast growth (Appendix 2), students may choose ultraviolet radiation in addition or in lieu of microwave radiation for their selected insult. In this experiment, students have the opportunity to work with two different *S. cerevisiae* strains: 1) wild-type and 2) UV-sensitive (UVS) strain. Both strains will be exposed to UV light and growth effects analyzed.

Student Learning Objectives
*By the end of this experiment students will:*
- Perform a scientific literature search
- Design a testable hypothesis
- Understand the purpose of using yeast as a model system
- Understand basic cellular processes affected by cancer
- Learn the basics of yeast cell culture
- Understand techniques employed in high-throughput data collection
- Learn how to use Microsoft Excel
- Analyze experimental data and construct a scientific figure

Safety
Prolonged exposure to Ultraviolet light (UV) produced by the sun or lamps, can have harmful effects to the eyes and skin. Therefore, students are instructed to wear the appropriate UV-blocking safety glasses and to avoid skin exposure while conducting UV-insults.

II. Experimental Procedure

1. Determine ultraviolet radiation treatment conditions for each yeast strain by filling out Table 1 below.

Table 1. Experimental conditions for *S. cerevisiae* strains exposure to ultraviolet radiation.

<table>
<thead>
<tr>
<th>Plate Description</th>
<th>Yeast Strain</th>
<th>Exposed to Light (plastic wrap) Ultraviolet Light Exposure Time</th>
<th>Protected from Light (foil) Ultraviolet Light Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. cerevisiae</em> wild-type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate Description</td>
<td>Yeast Strain</td>
<td>Exposed to Light (plastic wrap) Ultraviolet Light Exposure Time</td>
<td>Protected from Light (foil) Ultraviolet Light Exposure Time</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>---------------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td><em>S. cerevisiae</em> UVS mutant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. You will receive two petri dishes of rich medium containing lawns of the yeast strains of interest.
3. Draw a line on the bottom of each plate, dividing the agar into two equal parts. Label one half “foil”.
4. Remove the plate lid (save the lids for later, but do not place them on the plates during the sun treatment) and place each dish inside of a plastic bag (or cover the plate with plastic wrap). Pull the plastic covering taut. Make sure that the plastic covering is smooth and does not touch the agar surface.
5. Use the aluminum foil to cover the half of each of the plates labeled “foil” to shield these portions of the yeast lawn from the sun.
6. Expose the yeast to UV light or sunlight for the desired amount of time according to Table 1 above.
7. Replace the plastic and foil covering on plates with the lids.
8. Use a sterile pipette to transfer 5 mL of YEPD into each of two 15 mL conical tubes, label one “WT Foil” and the other “WT UV”.
9. One at a time, use a sterile micropipette tip to collect a clump of the WT yeast strain (both exposed to UV and protected from UV “foil”).
10. Smear the clump on the inside of an appropriately labeled 15 mL conical tube.
11. Resuspend cells into the YEPD by pipetting up and down. At the end of this step you should have two tubes for the WT strain, UV/sunlight and no UV (or “foil”).
12. Repeat steps 6-10 with the UVS strain. At the end of this step you should have a total of 4 conical tubes, 2 for WT and 2 for UVS.
13. Using a spectrophotometer, adjust the optical density of each of the yeast suspensions to 0.5 OD$_{600}$ nm.
14. Pipette at least 50 μL from each treatment tube, including the control, into the appropriate A-row wells—using Figure 1 as a guide.
15. Fill every well on the plate (B1 through H12) with 100 μL of YEPD media.
16. Serially dilute the yeast suspension (1:10) from row A to row H within the same column, by taking 10 μL of the preceding well and pipetting it onto the next well. For example, take 10 μL from well A1 and pipette into well B1, mix and repeat the dilution by taking 10 μL from B1 and placing into C1. Repeat until you have reached well H. You do not need to change pipette tips within columns. Mix each dilution thoroughly.
17. When you reach row H, discard 10 μL of YEPD medium to achieve a final volume in wells B1 through H12 of 100 μL.
18. Place the 96-well microtiter plate into the plate reader and monitor cellular growth at OD600 nm and 30°C for the next 40-48 hours.
19. Generate a graph of yeast growth curves using Microsoft Excel. Plot OD vs. time for each treatment. Data points to be analyzed will be empirically determined based on your resulting data.

III. Directions on Using Microsoft Excel to Plot Yeast Growth Curves and Standard Deviation

1. Obtain data file from instructor exported as a .xls file from the plate reader.
2. Open data file in Microsoft Excel. (Note: these directions are written for PC computers, and may vary slightly with different computer models or versions of Microsoft Excel)
3. Take the averages of each yeast strain/treatment by creating 4 new columns, and labeling each Average Wildtype (WT) Foil, Wildtype (WT) Sunlight, UVS Foil, UVS Sunlight, respectively.
4. In each separate Average column, in the formula line type “=AVG (highlight all cells from each time point to be averaged)”.
5. Repeat for the remaining yeast strain/treatment groups.
6. Highlight the average column for each yeast strain/treatment group.
7. Click on the “Insert” tab on the top of the screen.
8. Under “Charts” section, click the “Scatter” tab.
10. Title the graph and label the x-axis “Time” and the y-axis “Optical Density” including appropriate units.
11. To determine and graph standard deviation (STD), create an additional 4 separate columns labeled STD Wildtype (WT) Foil, STD Wildtype (WT) Sunlight, STD UVS Foil, and STD UVS Sunlight.
12. In each STD column, in the formula line type “=STDEV(highlight all cells from each time point)”.
13. To add the STD to the graph, click on the line for the Wild Type Foil.
14. Click on “Add Chart Element”, “Error Bars”, “More Error Bar Options”.
15. Under “Error Amount”, click the circle entitled “Custom” and press the “Specify Value” button.
16. Under “positive error value”, highlight all the cells under the STD Wildtype (WT) Foil, and repeat for the “negative error value”.
17. Click “OK”.
18. Repeat for each yeast strain/treatment.

IV. Discussion/Analysis Questions

1. What is DNA damage? How does ultraviolet radiation cause DNA damage? How do eukaryotic cells (like yeast) respond to this insult?
2. Examine your yeast growth curves. Do you notice any significant differences in growth between time of radiation exposure(s)?
3. Why do the yeast cells that were in the ultraviolet for a longer period of time start growing later than the control group?
4. Why did some yeast cells grow even though they were exposed to ultraviolet radiation?
5. For students who completed both microwave and ultraviolet radiation experiments:
   a. Compare and contrast DNA damage caused by ultraviolet vs microwave radiation?
   b. Based on your results from the two experiments, which insult was the most toxic to cells AND why? Defend your answer using your growth curve data and information found from the scientific literature.
Appendix 4: Student Handout/Protocol-Assessing the Effects of Rapamycin on Yeast Growth.

I. Background

Nutrient depletion in *S. cerevisiae* causes a wide-array of cellular and biochemical changes that result in terminal cell cycle arrest called G0 or stationary phase (4, 5). Yeast cells in stationary phase have 1n DNA content, stop replication and look larger than usual under a microscope. Furthermore, *S. cerevisiae* in stationary phase, reduce the amount of protein synthesis and exhibit a pattern of RNA polymerase II (RNA pol II) transcripts, which is distinct from cells in the rest of the cell cycle. The signaling pathway responsible for the changes by RNA pol II in G0 cells is the TOR (for target of rapamycin) pathway. In the laboratory, we can chemically induce the G0 state by using the antifungal/antibiotic rapamycin. Rapamycin has been shown to inhibit cell proliferation by interfering with the TOR signaling pathway. Of relevance, current cancer therapies utilize mammalian TOR inhibitors to suppress the growth of cancer. In this experiment, you will design an experiment where you will treat wild-type *S. cerevisiae* with varying concentrations of rapamycin and examine its effects on yeast cell growth.

Learning Objectives
*By the end of this experiment students will:*
- Perform a scientific literature search
- Design a testable hypothesis
- Understand the purpose of using yeast as a model system
- Understand cell cycle
- Learn the basics of yeast cell culture
- Understand techniques employed in high-throughput data collection
- Learn how to use Microsoft Excel
- Analyze experimental data and construct a scientific figure

Safety

When handling rapamycin wear standard personal protective equipment which includes gloves, safety glasses and a lab coat.

II. Experimental Procedure

1. Determine rapamycin treatment conditions by filling out Table 1 below. Refer to Table 2 as a guide.

   **Table 1.** Experimental conditions for wild-type *S. cerevisiae* exposure to rapamycin.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Rapamycin µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Example experimental conditions for *S. cerevisiae* exposure to rapamycin.

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Rapamycin µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

2. Label 4 microcentrifuge tubes so that one represents each of your experimental conditions in Table 1.
3. Your instructor will provide you with an overnight culture of wild-type *S. cerevisiae*.
4. Using a spectrophotometer, adjust the optical density of the yeast suspension to OD$_{600}$ of 0.5/mL. If the culture is too dense, you may need to dilute the culture in YEPD.
5. Transfer 1 mL of the OD$_{600}$ of 0.5/mL into four individual microcentrifuge tubes.
6. Label each tube with the rapamycin dose determined in Table 1.
7. Centrifuge each tube at 5000 rpm for 5 min to pellet the yeast cells.
8. Gently resuspend (by pipetting up and down) the yeast cell pellet in each tube by adding 1 mL YEPD containing the appropriate concentration of rapamycin (Rap).
9. Pipette 50 µL from each treatment, including the control, into the appropriate A-row wells—use Figure 1 as a guide.

**Figure 1.** Example set up for rapamycin treatment of yeast using a 96-well microtiter plate.

10. Fill every well on the plate (B1 through H12) with 100 µL of YEPD media.
11. Serially dilute the yeast suspension (1:10) from row A to row H within the same column, by taking 10 µL of the preceding well and pipetting it onto the next well. For example, take 10 µL from well A1 and pipette into well B1, mix and repeat the dilution by taking 10 µL from B1 and placing into C1. Repeat...
until you have reached well H. You do not need to change pipette tips within columns. Mix each dilution thoroughly.
12. When you reach row H, discard 10 μL of YEPD medium to achieve a final volume in wells B1 through H12 of 100 μL.
13. Place the 96-well microtiter plate into the plate reader and monitor cellular growth at OD\textsubscript{600} nm and 30°C for the next 40-48 hours.
14. Generate a graph of yeast growth curves using Microsoft Excel. Plot OD vs. time for each treatment, including the appropriate units. Data points to be analyzed will be empirically determined based on your resulting data.

III. Directions on Using Microsoft Excel to Plot Yeast Growth Curves and Standard Deviation
1. Obtain data file from instructor exported as a .xls file from the plate reader.
2. Open data file in Microsoft Excel (Note: these directions are written for PC computers, and may vary slightly with different computer models or versions of Microsoft Excel)
3. Take the averages of each yeast strain/treatment by creating 4 new columns, and labeling each Average 0.0 μg/mL Rap, 0.05 μg/mL Rap, 0.1 μg/mL Rap, and 0.2 μg/mL Rap, respectively.
4. In each separate Average column, in the formula line type “=AVG (highlight all cells from each time point to be averaged)”
5. Repeat for the remaining treatment groups.
6. Highlight the average column for each treatment group.
7. Click on the “Insert” tab on the top of the screen.
8. Under “Charts” section, click the “Scatter” tab.
10. Title the graph and label the x-axis “Time” and the y-axis “Optical Density” including appropriate units.
11. To determine and graph standard deviation (STD), create an additional 4 separate columns labeled STD 0.0 μg/mL Rap, STD 0.05 μg/mL Rap, STD 0.1 μg/mL Rap, and STD 0.2 μg/mL Rap.
12. In each STD column, in the formula line type “=STDEV(highlight all cells from each treatment)”. 
13. To add the STD to the graph, click on the line for the 0.0 μg/mL Rap.
14. Click on “Add Chart Element”, “Error Bars”, “More Error Bar Options”.
15. Under “Error Amount”, click the circle entitled “Custom” and press the “Specify Value” button.
16. Under “positive error value”, highlight all the cells under the STD 0.0 μg/mL Rap, and repeat for the “negative error value”.
17. Click “OK”.
18. Repeat for each drug treatment group.

IV. Discussion/Analysis Questions
1. How does nutrient depletion stop cells from dividing?
2. How can manipulating the cell cycle be useful as a cancer therapy?
3. Examine your yeast growth curves. Do you notice any significant differences in growth between rapamycin treatment(s)?
4. Why do the yeast cells treated with higher doses of rapamycin start growing later than the control group?
5. Why did some yeast cells grow even though they were exposed to rapamycin?
6. How does your data compare to your original hypothesis?
7. Are there potential sources of error in your experiment? How would you design it differently the next time?

References for Appendices 2-4