# The Effect of UV Radiation on the Survival of Yeast and its Implication to a Real-Life Situation

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## Biography

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# Introduction

Living organisms are used in many ways to test and monitor our environment. For example, canaries were used in mines to detect methane gas, and observing number and type of organisms can test water quality in streams. In this laboratory, we use *Saccharomyces cerevisiae* or budding yeast (Baker's yeast) to monitor the amount of ultraviolet radiation (UV) exposure.

UV radiation occurs naturally in sunlight and is responsible for the tanning and burning effects of the sun. UV radiation damages the DNA of skin cells ultimately leading to skin cancer, now considered the most common form of cancer in the USA (ACS, 2008). There are two types of UV radiation that reach the earth, UV-A and UV-B. Type UV-B exposure is the most intense, but UV-A can penetrate the skin much deeper (EPA, 2008). The amount of UV a person is exposed to, depends on the time of exposure and whether the skin is protected.

In this 4-week laboratory module, students expose yeast cells from "wild-type" and "unknown" strains to different amounts of UV radiation, and then measure the percent survival of these cells as an estimate of the amount of UV. The "unknown" is a *rad1* yeast mutant strain, which is hypersensitive to UV radiation due to a mutation in a DNA repair gene (Friedberg, 1998, Friedberg, 2001, Tomkinson, 1993). Next, working in groups and using information from the previous experiment, students design an experiment using survival of yeast to test the effect of a protective factor such as sunglasses, sunscreen, etc., against UV.

To help students see a connection between their experiments and a real-life situation, students read a scene from a case study at the start of each laboratory. It is story about a child with Xeroderma Pigmentosa (XP), a disease caused by mutations in a number of genes involved in DNA repair, some of which have homologs in yeast (Kraemer, 2008, Sijbers, 1996).

**Timeline**

| | |
|---|---|
| Week 1 | Read scene 1 of case study<br>Dilutions, plate yeast and expose to UV |
| Week 2 | Scene 1 presentations, read scene 2 of case study<br>Count surviving colonies, plot survival curve |
| Week 3 | Scene 2 presentations, read scene 3 of case study<br>Design and perform group experiment |
| Week 4 | Scene 3 presentations of case study<br>Analyze results of group experiment |

# Student Outline

**Learning goals:** After completing this laboratory module, you should be able to:

1. Demonstrate an understanding of the scientific method
2. Make dilutions, use sterile technique to plate a culture, and graph and interpret a survival curve
3. Design an experiment using the Scientific method with the appropriate controls and variables

**Week 1: Dilutions, plate yeast and expose to UV**

*Objective*: To study the effect of Ultraviolet (UV) radiation on the survival of yeast

*Pre-lab Discussion*

Determine the hypothesis, prediction, "independent" variable, "dependent" variable, "controlled" variables, and controls for the experiment.

Appropriate dilutions of a yeast culture will be plated on YPD plates to determine the number of viable cells. Plates will be exposed to UV radiation for different amounts of time after plating. The following week, count the number of colonies that survived on the YPD plates, and thus determine the extent of UV-induced killing.

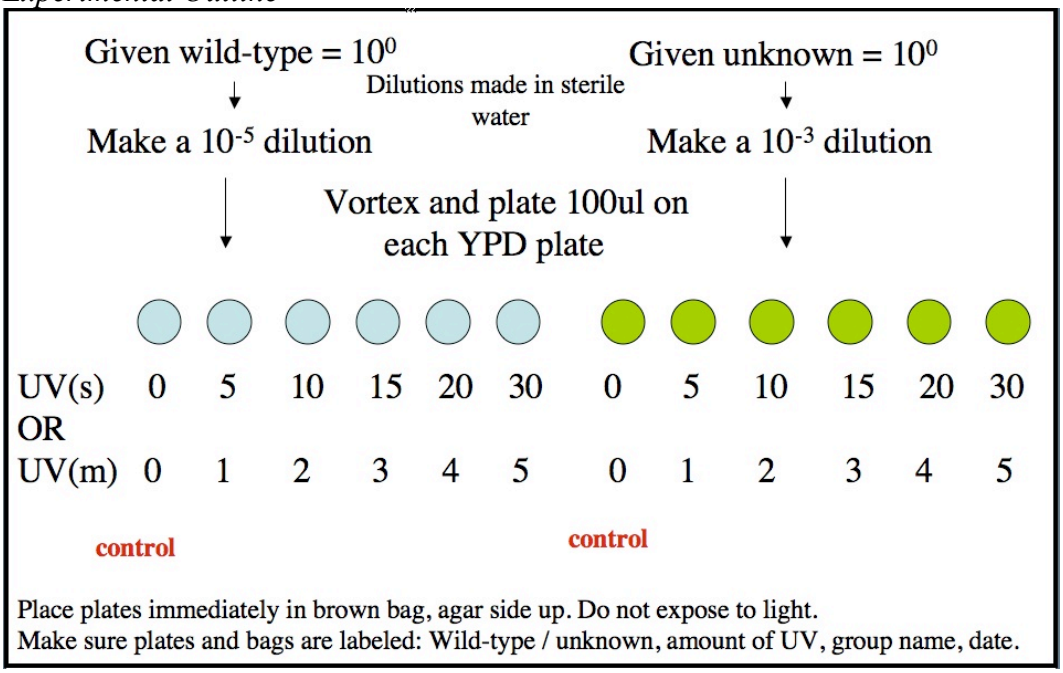
*Materials required for each group of four students*

| | |
|---|---|
| 12 YPD plate | Bunsen burner |
| Tube of sterile water 15ml | Brown paper bags |
| Sterilized microtubes | 300 ul of a $10^0$ culture of "wild-type" in a microtube |
| Micropipettes p10, p100, p1000 and tips | 150 ul of a $10^0$ culture of "unknown" in a microtube |
| Spreader to plate yeast | Sharpie |
| Ethanol to sterilize spreader | Vortexer |

*Common area*

UV-B transilluminator box shielded with a large plexiglass OR Stratalinker
Timer that counts seconds and minutes

*Experimental Outline*




*Experimental Procedure*

1) You are given two microtubes containing yeast cultures, one containing "wild-type $10^0$" and the other "unknown $10^0$". $10^0$ refers to undiluted culture, which was grown at 30°C for 24 hours.

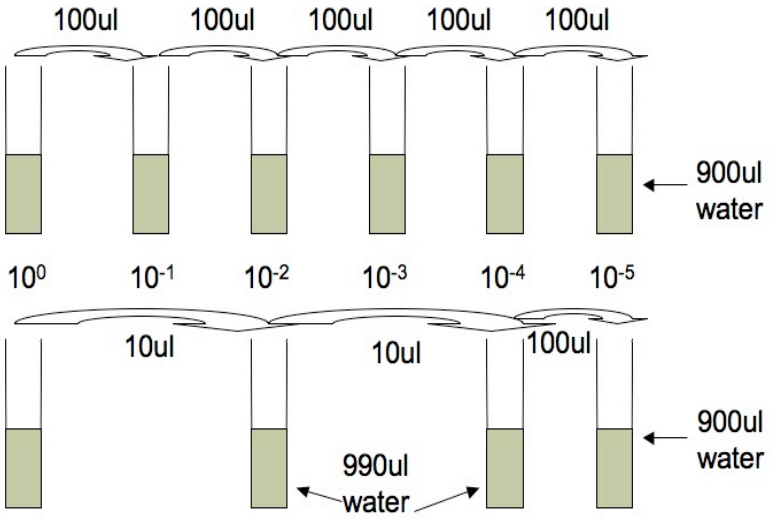



**Figure 1.** How to make dilutions

2) To make 1ml (1000ul) of a $10^{-5}$ dilution of the "wild-type" strain (see Figure 1) With a sharpie, label two sterile microtubes "W$10^{-2}$" and "W$10^{-4}$" respectively and pipette 990ul sterile water into them. Vortex the tube containing the $10^0$ "wild-type" culture and pipette 10ul of the culture into your $10^{-2}$ microtube. This is your $10^{-2}$ dilution. Vortex these cells well and then using a clean tip for your micropipette, pipette 10ul of the $10^{-2}$ dilution into the tube labeled $10^{-4}$. This is your $10^{-4}$ dilution. Next label a microtube "W$10^{-5}$", and pipette 900ul of sterile water into it. Vortex your $10^{-4}$ dilution well and then pipette (with a clean tip) 100ul of $10^{-4}$ dilution into the $10^{-5}$ tube. This is your $10^{-5}$ dilution.

3) Using sterile technique, make 1ml (1,000ul) of a $10^{-3}$ dilution of the " unknown" strain. Follow the figure and make the dilution using sterile water.

4) You will need six YPD plates to plate the $10^{-5}$ "wild-type" and the $10^{-3}$ "unknown" dilutions respectively (a total of 12 YPD plates). Label the bottom (agarose half, along the periphery) of each plate, as wild-type or unknown, time exposed to UV, and your group name.

5) Using sterile technique, plate 100ul of the "wild-type" $10^{-5}$ dilution on each of six appropriately labeled YPD plates. Then, plate 100ul of the "unknown" $10^{-3}$ dilution on six YPD plates. Before plating make sure you mix the culture well by vortexing or inversion.

6) Use only ONE source UV radiation for your entire experiment. **For the UV box (transilluminator):** Expose plates to UV= 0, 5, 10, 15, 20, 30 seconds, label your plates "wild-type UV=0s", "wild-type UV= 5s"……...**For the Stratalinker:** Expose your plates to UV= 0, 1, 2, 3, 4 , 5 minutes, label your plates "wild-type UV=0m", "wild-type UV= 1m"……...
Expose the plates to the designated amount of UV radiation. **Note that the lids of plates must be removed before exposure to UV.** The plates marked UV= 0 do not get UV treatment. Immediately after exposure place the plates in a brown paper bag to prevent

exposure to light. This is because yeast has a photolyase that uses the energy of visible light to "reverse" the UV- induced DNA damage; bagging the plates prevents the photoreversal reaction. Make sure you bag the unexposed plate along with the UV exposed plates for each dilution.

7) Label the outside of the brown bag with your course number and group name. Plates will be incubated at 30°C for 2 days.

**Week 2: Count surviving colonies, plot survival curve**

*Materials required for each group of four students*

4 Sharpies, 4 sheets of graph paper

1) Use the provided Sharpies to count the number of colonies on each plate and enter your observations in the tables below.

**Table 1.** Wild-type $10^{-5}$ dilution plated on YPD

| Time exposed to UV (seconds) | No. of colonies | % survival |
|---|---|---|
| 0 | | 100 |
| 5 | | |
| 10 | | |
| 15 | | |
| 20 | | |
| 30 | | |

**Table 2.** Unknown $10^{-3}$ dilution plated on YPD

| Time exposed to UV (seconds) | No. of colonies | % survival |
|---|---|---|
| 0 | | 100 |
| 5 | | |
| 10 | | |
| 15 | | |
| 20 | | |
| 30 | | |

2) Plot a single "survival curve" for both the wild-type and unknown. Plot % survival on the Y-axis and time exposed to UV in seconds on the X-axis. Which is more sensitive to UV, the wild-type or unknown?

*Homework Research*

Determine what cellular process could be affected in the unknown yeast strain to make it more sensitive to UV. Your answer should be maximum one page; double spaced, and should contain at least one original scientific research paper as a reference.

**Week 3: Design an experiment**

Groups (of 4 students) design their own experiment using survival of yeast to show the

protective effect of sunscreen, sunglasses, different fabrics, or anything they wish to bring to test. Experimental design must adhere to the scientific method, and must have appropriate controls.

*List of supplies available to each group to design their experiment*

| | |
|---|---|
| 6 YPD plate | Vortexer |
| Tube of sterile water 15ml | Brown paper bags |
| Bunsen burner | Sharpie |
| Spreader and ethanol to plate yeast | Saran wrap |
| 200 ul of a $10^0$ culture of "wild-type" in a microtube | Kinds of fabric (chiffon, lycra, cotton, polyester), Sunscreens (of various SPF's 15, 30, 60), Sunglasses |
| Micropipettes, sterilized microtubes and tips | Cardboard cutouts |

*Common area*
UV-B transilluminator box shielded with a large plexiglass or Stratalinker
Timer that counts seconds and minutes


**Figure 2**. Examples of student group experiments. A) Testing sunscreens of various SPF's. Sunscreen applied to saran wrap is attached to a cardboard cutout. B) Testing fabrics such as net (pink), lycra (yellow), cotton (white), and a leaf.

**Case Study**
**Introduction**
The case study is a story about a baby with a rare autosomal recessive disorder, Xeroderma pigmentosa (XP). The cells of these patients have defective Nucleotide excision repair (NER) machinery; hence the body is unable to repair DNA damage caused by UV exposure (Friedberg, 2001, Kraemer, 2008). As a result, patients develop lesions in their skin after even after the slightest exposure to sunlight. Mutations in at least 8 genes, all involved in NER, have been found to play a role in XP. One of the genes, XPF, is a homolog of *RAD 1* (Kraemer, 2008 and Sijbers, 1996).

**Learning goals**
1) Recognize that several human genes have homologs in other organisms
2) Understand how studying the phenotype of mutant organisms can be used to determine the function of the gene that is mutated.
3) Understand the use of model organisms to study the function of the genes and processes in humans.

**Student handouts**

*She danced in the light of the moon- Written by Allison D'Costa*

**Scene 1**

"Don't cry Ashley, this is your first hike, and you're going to love it", said Jack
"I just changed her diaper, I wonder why she is so uncomfortable", murmured Carol.
"This is a short trail, just another 45 minutes"
Ashley's crying got louder; she seemed to be in a lot of pain.
"Jack, her face is swollen and is beginning to blister. We must turn back. I don't understand what is going on", cried Carol.
Ashley howled all the way back. Nothing her parents did seemed to calm her down.
"Go straight to the emergency room," said Carol as she urged Jack to drive faster.

Dr. Beasley, a man in his sixties, listened carefully as Carol, who was now crying inconsolably, told him about Ashley.
"She has been screaming for the past hour. She gets a rash whenever we go out, its worse on her face, hands and legs. Her pediatrician, Dr. Smith, says it's some kind of allergy and has asked us to apply cortisone cream. Nothing is helping, I don't understand what is wrong with my child".
"Looks like we have a case of severe sunburn. The child looks to be in terrible pain", said Dr. Beasley. "How old is she?"
"She will be a year old next month"
'Let me take a closer look', said Dr. Beasley as he undressed Ashley, who was now screaming louder under the bright light of the examination table.
"She does have a lot of freckles. Her body is covered with blisters".
"Let me examine her eyes", said Dr. Beasley as he continued the examination.
"Dr. Beasley, she does not seem to like too much light. She always keeps her eyes shut, especially when we go outside. What do you think is wrong with Ashley?" asked Jack helplessly.
Dr. Beasley had a hunch, but he wanted to be absolutely sure before he made a diagnosis.
"Let me do a few tests. In the meantime, keep Ashley at home, away from any light".

**Scene 2**

There was silence as Carol and Jack waited anxiously for Dr. Beasley. It had taken a few weeks for Ashley's test results to come in, and they had a bad feeling that something was wrong with their baby.
Dr. Beasley entered the room with a lady. "This is Dr. Morgan. She is a genetic counselor". Carol and Jack sat up nervously. "Carol, Jack, I am afraid I have bad news. Ashley's tests show that she has a rare genetic disorder. We found her cell cultures to be extremely sensitive to UV. This explains why her skin develops lesions even after slight exposure to light. Dr. Morgan is going to help you understand Ashley's disorder. I am very sorry."
"Please, please tell us, how did Ashley get this disorder?" asked Jack, his voice shaking.

**Scene 3**

It was close to 5am. Carol had just read a story to Ashley and put her to bed. In an hour, Jack's alarm would go off. She turned on the radio to catch the news before she got some sleep.
NPR's Linda Wertheimer's voice came streaming in. Carol could not believe what she was hearing.
"Camp Sundown in New York's Hudson River Valley. It's for kids who have a rare skin disease. The

disease makes any exposure to sunlight or ultraviolet rays deadly. So these kids fish, ride horses, play games all night and sleep during the day. Dan and Caren Maher of Poughkeepsie started the camp three summers ago. Their six-year-old, Katie, has the disease. They say they wanted Katie to have a chance to go outside and play with other children for a few weeks of her life".

Carol was beginning to get very excited; she wanted to wake Jack up immediately but held back, she wanted to make sure this was really true. She jumped on the net to look for more information, and to her delight found that the next camp was scheduled the week of Ashley's birthday.

"Wake up Jack, you've got to see this. Camp Sundown, a camp for children like Ashley…"
"Where is it?" he asked.
"Somewhere in New York. We have to go".

Ashley blew out the five candles on her Barbie cake, as a crowd of 15 children and their families sang "Happy Birthday". Carol had tears in her eyes as Jack squeezed her hand.
"Coming to Camp Sundown is the best birthday present you could have given Ashley", he whispered. "She is beaming, I have never seen her so happy. We have only been here a day and she's behaving like she's known these children for years".
"We better catch up on some sleep; there is a seminar tomorrow at 10am for parents to learn about the latest research developments. It seems that scientists have found the genes that cause Ashley's disorder, and can you believe they have even found similar genes in yeast!"

**Implementation and making connections**

Each of the three scenes is handed out to students just before it is read in class; they only get to see one scene at a time. The scenes are read aloud, roles can be assigned. Students then discuss the contents with members of their group, and fill a table with columns "What do you know?" and "What do you need to know?" Questions and unknown terms become research for homework. The instructor facilitates discussion in each group without providing any answers. Each group must come up with one specific question that they would like to research and answer. The instructor makes sure there is little overlap between the questions proposed by groups. The following week, the groups come back with their answers and informally present their findings to the entire lab. Questions, discussion and feedback from other groups is encouraged. The next scene is read, and follows with discussion and presentation in the next lab (see Timeline).

Students initially wonder about the type of skin allergy the baby might have, and by scene two want to research genetic disorders that cause light-sensitivity. Following scene three, the symptoms, incidence, detection and genetics of Xeroderma pigmentosa (XP) and DNA repair mechanisms in cells are discussed. Students become very interested in the lifestyle of these patients.

Students must realize that the "unknown" in the experiment is somewhat similar to an XP patient, who is manifesting symptoms due to mutation in a DNA repair gene. From their homework research following week two, a few groups should have found yeast genes (*rad* genes) that play a role in DNA repair. They must understand that several human genes have homologs in other organisms, and therefore scientists use model organisms and mutants (like the "unknown") to study the function of genes. Since yeast has similar DNA repair genes as in humans, it is therefore being used as a model organism to understand how genes work to repair DNA in yeast, and therefore

similarly in humans.

**Use in an Elementary classroom**
**Sun Smart Lesson Plan:** Using Baker's Yeast as a Tool to Measure Sun Exposure

*Objectives:*
1) To help students gain a better understanding of the harmful effects of unprotected sun exposure
2) Provide students with sun safety habits to prevent harmful sun exposures
*Why:* Research scientists propose that sun exposure during childhood has a greater impact on skin cancer risk than sun exposure during adulthood.

*Introduction*

Prior to performing the experiment with elementary students, a sun safety lesson was used to teach the children about the harmful effects of UV exposure to their skin and eyes from both the sun and tanning beds, as well as an introduction to the use of Bakers yeast as a sensor for exposure to harmful substances. Information about good sun protection habits can be found at Center for Disease Control website (CDC, 2008). Bakers yeast are one-celled organisms that is added to bread to help it rise. These one-celled organisms are similar to the cells that make up our bodies, but because they are only one cell and we are made up of billions, yeast are affected much more by chemicals and radiation than we are. We can tell the harmful strength of chemicals and radiation by observing whether yeast live or die when exposed. Our bodies, on the other hand, have many protective abilities because we have so many cells, yet our cells can get sick and develop the illness cancer when exposed to a harmful substance such as UV radiation from the sun. We can use the survival of yeast cells to monitor how harmful the UV exposure is, and use that to compare to how our bodies may be affected.

After discussing the effects of sun exposure, have the children bring to the next class different things that they can use to protect themselves from sun exposure. These items may include hats, sunscreens of different SPF, sunglasses, lip balms, different weaves of clothing, different clothing colors, lotions etc. Using the scientific method, have students design a question and the appropriate hypothesis they will test. Make sure they identify variables and include controls such as no protective treatment and no sun exposure.

*Materials required for each group of four students*

| | |
|---|---|
| 4 YPD plates plated with $10^{-3}$ dilution of *rad 1* yeast strain | Sunscreens, various SPF, sunglasses, lip balm various types of cloth |
| Saran wrap, Sharpie | cell spreaders |

*Experimental Procedure*
1) Use two plates as controls, and two to test protective treatments.
2) Use a Sharpie to label the agar side (bottom) of the plates with student name, whether they are controls or have a protective treatment.
3) Apply protective treatments.
4) Set Petri dishes outside in the sun for 30 minutes covered with protecting material or just plastic

wrap. Place no sun exposure plates in the indoor incubation location.
5) Following sun exposure, incubate plates for 4 days at room temperature.

*Discussion*

Following the four-day incubation period, pass plates out to groups and allow the students to record their findings. Have each group share findings with the entire class. Review with a presentation and discussion on the importance of using sunscreen, sunglasses and covering the body prior to extensive sun exposure. Material for a presentation and question answer session with clickers can be obtained from the EPA sunwise website (EPA, 2008).

## Notes for Instructor

1) For 1 liter of YPD plates: 10g Bacto-yeast extract, 20g Bacto-peptone, 20g Dextrose, 40mg Adenine sulphate, 20g "technical agar" (Difco #281210) and water to make 1 liter. For YPD broth, omit the agar.
2) Fresh YPD plates of yeast "wild-type" and "unknown" must be streaked a week before lab. Colonies from these plates are used to setup the overnight culture. The "unknown" plate must be wrapped in foil (to prevent light exposure) before placing in the 30°C incubator
3) Setup overnight yeast culture $10^0$, a day (24 hours) before each lab. Take two large sterile test tubes and add 5ml YPD to each. Inoculate each with a large colony of wild-type and unknown, respectively. Grow for 24 hours in a shaker at 30°C and 220 rpm. This culture is called $10^0$. Vortex the culture before aliquoting.
4) Use either of two UV sources: Several plates can be placed at a time on the UV box or in the Stratalinker for exposure. UV Transilluminator (Fisher Scientific #FB-TI-816A, 312nm, 8X16 inches). Stratalinker (Stratagene UV Stratalinker 2400, UV-B bulbs)
5) Results: After each group of students has counted the number of colonies, ask them to write their data on the board for other groups to see and compare. Let them note that there was more killing in the "unknown" even though more cells were plated ($10^{-3}$ compared to $10^{-5}$). See sample results:

**Table 1.** Wildtype $10^{-5}$

| UV (seconds) | No. of colonies | % survival |
|---|---|---|
| 0 | 190 | 100 |
| 5 | 149 | 78.4 |
| 10 | 120 | 63.2 |
| 15 | 53 | 27.9 |
| 20 | 35 | 18.4 |
| 30 | 2 | 1.05 |

**Table 2.** Unknown $10^{-3}$

| UV (seconds) | No. of colonies | % survival |
|---|---|---|
| 0 | ~4000 | 100 |
| 5 | 1 | 0.025 |
| 10 | 0 | 0 |
| 15 | 0 | 0 |
| 20 | 0 | 0 |
| 30 | 0 | 0 |

6) Group experiment guidelines: Only "wild-type" strain used.
For the UV box: UV emitted from bottom, for Stratalinker: UV emitted from top.
*a) To test the protective effect of sunglasses:* UV box: place the sunglasses on UV box, then inverted plate on top. Stratalinker/ sun exposure: place the sunglasses on

top of the plate (outside of sunglasses facing UV source). Use a Sharpie to mark the orientation of sunglasses on the plate.

*b) To test the protective effect of sunscreen:* Using clips, attach a piece of saran wrap onto the cardboard frame (see Figure 2) you are provided. Apply sunscreen on the saran wrap. UV box: Place the cardboard frame on top of the UV box, then the inverted plate on top of it. Stratalinker/ sun exposure: Place the cardboard frame on top of the plate.

c) *To test the protective effect of a type of fabric:* Squares of fabric (cotton, polyester, net, lycra, chiffon etc.) are provided. UV box: Place the square of cloth on top of the UV box, then the inverted plate on top of it. Stratalinker/ sun exposure: Place the fabric on top of the plate.

7) Elementary school guidelines: Best performed in spring or early fall, between 10 am – 2 pm. The "unknown" *rad1* strain is used because it shows a much more dramatic effect compared to wild-type when exposed to sunlight for 30minutes. The students do not know that they are using a mutant strain. Plating of cells must be done by the instructor in class using disposable cell spreaders. Instruct students on application of sunscreen, sunglasses etc., as in Group experiment guidelines.

## Literature cited

American Cancer Society. Cancer Facts and Figures 2008. Accessed September 30, 2008. Available at http://www.cancer.org/docroot/STT/content/STT_1x_Cancer_Facts_and_Figures_2008.asp?from=fast Center for Disease Control and Prevention. 2008. Available at http://www.cdc.gov/cancer/skin/pdf/sunsafety_v0908.pdf. Accessed September 20, 2008

Environmental Protection Agency. www.epa.gov/sunwise Accessed September 30, 2008

Friedberg, E. C., W. J. Feaver, W .Huang, M.S. Reagan, W. A. Ramos, K. Rodriguez, S. H. Reed, A.

E. Tomkinson, S. Wei, Z.Y. You. Nucleotide excision repair in yeast: recent progress and implications. 1998. Nucleic Acids Mol. Biol., 12:125-139.

Friedberg, E. C., 2001. How nucleotide excision repair protects against cancer. Nat. Rev. Cancer, 1: 22–33.

Kraemer, K. H. 2008. Xeroderma Pigmentosa. GeneReviews April 22, 2008 http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=xp

Sijbers, A. M., W. L. de Laat, R. R. Ariza, M. Biggerstaff, Y. F. Wei, J. G. Moggs, K. C. Carter, B. K. Shell, E. Evans, M. C. de Jong, S. Rademakers, J. de Rooij, N. G. Jaspers, J. H. Hoeijmakers, and R. D. Wood. 1996. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. Cell, 86(5): 811-22.

Tomkinson, A. E., A. J. Bardwell, L. Bardwell, N. J. Tappe, and E. C. Friedberg. 1993. Yeast DNA repair and recombination proteins Rad1 and Rad10 constitute a single-stranded-DNA endonuclease. Nature, 362(6423): 860-2.

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