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Characterization of the Effects of Heavy Charged Particle Exposure on the Radiation Resistant Bacterium Deinococcus radiodurans

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CHARACTERIZATION OF THE EFFECTS OF HEAVY CHARGED PARTICLE EXPOSURE ON THE RADIATION RESISTANT BACTERIUM *DEINOCOCCUS RADIODURANS*

THESIS

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Abstract

Although not an extremophile, *Deinococcus radiodurans* has proven to have exceptional resistance to ionizing radiation, specifically via gamma and X-ray photons. To date, no known experiments have bombarded the bacterium with charged particles larger than hydrogen. This study explored the effects on the organism's ability to survive high linear energy transfer heavy-charged particle exposures of oxygen ions. It also investigated the effects of low linear energy transfer ultraviolet radiation on various mutants.

Two *uvrB* mutants were created to ascertain the importance of the gene in single-strand break repair following gamma irradiation and two *recF* mutants were created to explore the role of the gene in double-strand break repair. The samples were desiccated to decrease the probability of indirect DNA damage. Following exposure the samples were rehydrated and counted. Comparisons were made against control samples and statistical differences were evaluated through a two population t-statistic test. The *uvrB* mutants displayed greater lethality than the wild type control and other mutants to gamma exposure and the *recF* mutants clearly experienced growth latency and greater lethality following oxygen ion exposure.

Acknowledgments

I would like to express my appreciation to my advisor, the faculty of ENP, the scientists and researchers of USAFSAM and the Sandia Ion Beam Laboratory, for their support, expertise, and good judgment. I would also like to thank my wife for her infinite patience while I worked to complete this project and her unwavering support throughout my career.

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CHARACTERIZATION OF THE EFFECTS OF HEAVY CHARGED PARTICLE EXPOSURE ON THE RADIATION RESISTANT BACTERIUM *DEINOCOCCUS RADIODURANS*

I. Introduction

General Issue

In the last 70 years the prospect of encountering high levels of ionizing radiation as a result of a nuclear event, radioactive particle release, space exploration, or nuclear-related medical treatments has garnered great attention across a wide array of professional communities. The United States Department of Defense (DoD) continues to investigate novel methods, techniques, and materials that may increase survivability of personnel. The Defense Threat Reduction Agency (DTRA) is currently funding studies for Basic Research for Combating Weapons of Mass Destruction (HDTRA-11-12-BRCWMD-BAA). DTRA specifically identifies "biological systems, including intact structures, metabolic products, or discrete components and pathways, as applied to protection of U.S. Forces during operations in areas actually or potentially contaminated by radiation. [1]" The expressed aim of this research is to investigate biological resistance mechanisms to radiation damage in the hope to one day contribute to personnel survivability in radiation environments. This fundamental research can concurrently benefit the health community by contributing to improved treatment options or even preventative care. In an attempt to better understand the mechanisms of radiation resistance, this project will examine various mutants of *Deinococcus radiodurans* (*D. radiodurans*) following exposure to heavy-charged particles to determine their survivability.

Although not an extremophile, *D. radiodurans* has long been acknowledged to have exceptional resistance to ionizing radiation, specifically gamma and X-ray bombardment. *D. radiodurans*' resistance is 200 times and 20 times that observed in *Escherichia coli* (*E. coli*), respectively [2]. The goal of this research is to identify DNA repair genes that enable *D. radiodurans* to survive large amounts of DNA damage through radiation (direct damage), oxidation (indirect damage) and desiccation [3]. Investigations will be made into cell survivability through exposure to a high flux of high linear energy transfer (LET) heavy charged particles (HCP). If primary DNA repair genes are identified, the applications within the DoD and health communities may be a profound improvement in the ability to protect humans from biological radiation damage.

Problem Statement

D. radiodurans has proven to be exceptionally resistant to gamma induced low-LET ionizing radiation and high-LET proton and neutron radiation [4] but there is no data on *D. radiodurans* resistance to high-LET heavy charged particle radiation.

Research Objectives

This research will attempt to accomplish three objectives. The first is to characterize wild-type *D. radiodurans* growth following a high-LET HCP exposure. The second is to characterize *D. radiodurans* mutant growth following the same exposure. The third objective is to identify additional genes that may contribute to wild-type *D. radiodurans* radiation resistance.

Hypothesis

The first hypothesis is that *D. radiodurans* exposed to high-LET HCP will exhibit a statistical difference from the untreated wild type control group. The second hypothesis is that mutants exposed to high-LET HCP will exhibit a statistical difference from their own untreated control group. The third hypothesis is that exposed DNA repair mutants will exhibit a statistically significant difference when compared to the exposed wild type. The null hypothesis is as follows: The populations of the experimental group (HCP radiated) and control group (no radiation) will show no statistical difference.

II. Literature Review

Chapter Overview

This chapter provides a brief description of *D. radiodurans*. It provides an overview of the structure of DNA, DNA repair, and *D. radiodurans'* unique DNA make-up. It discusses the major differences between high and low LET as well as the importance of choosing the right energy for this research. It also provides a brief discussion on heavy charged particle interactions with matter. This chapter concludes by identifying the theory and methods surrounding gene knockouts and mutant strain development

A Brief Description of *Deinococcus radiodurans*

D. radiodurans, a common bacteria found worldwide, was first noted as being highly radiation-resistant in the mid-1950s when it was found to remain as a contaminant after radioactive sterilization of tin cans during industrial canning. While the organism is benign to humans, its presence in the cans caused food to spoil. The industry used 4000 Gy

(Joules/kg) of gamma radiation assuming this dose was more than sufficient to kill any living organisms and completely sterilize the surfaces of the tin cans. There was little research interest in *D. radiodurans* from the 1950s until the early 2000s, when the scientific community began efforts to understand the processes contributing to *D. radiodurans'* extraordinary hardiness. These efforts were largely possible due to the rapidly increasing computing power enabling full sequencing of *D. radiodurans'* genomic construct. The body of work surrounding *D. radiodurans* investigations has grown considerably within the past decade. It has been moderately well-characterized while in a hydrated state, but up until 2016 [4] there had been no reports of it exposed to radiation while desiccated, nor had it been exposed to moderate doses of neutrons. To-date, no experimental investigations of desiccated *D. radiodurans* exposed to high energy charged particles heavier than hydrogen nuclei have been identified in the literature.

Scientists have proposed a number of possible mechanisms that contribute to the cell's ability to survive irradiation as well as desiccation. One theory suggests that a condensed genomic copy of its DNA contributes to its ability to reconstitute whole, complete, and error-free chromosomes following damage. [6] Another infers the bacterium has a unique ability to evade protein oxidation. [7] Cox et al. suggest that although these hypotheses may be correct to some degree, the primary mechanism for survival is *D. radiodurans'* exceptional ability to sustain and subsequently repair massive amounts of DNA damage. Consequently, this is likely the primary mechanism for its ability to also survive extreme desiccation. [8]

DNA

Deoxyribonucleic acid, typically referred to as DNA, contains all of an organism's genetic information. It is composed of a nitrogen-containing base, a pentose sugar, and a phosphate group. There are four primary nitrogen-containing base groups consisting of the purines, adenine and guanine, and the pyrimidines, cytosine and thymine. Each purine is hydrogen bonded to a pyrimidine into a base pair. [9] The sequence of these base pairs governs the production of all proteins within the cell. These proteins can then go on to perform their necessary functions.

Base pairs are formed in a tightly bound helix so the entire structure takes on the appearance of a spiral staircase. The outside of the "staircase" is composed of alternating covalently-bonded sugar and phosphate groups while the "rungs" between them are hydrogen-bonded purine-pyrimidine base pairs. The bonding of the purines and pyrimidines always follows a specific pattern; adenine bonds with thymine while cytosine bonds with guanine.

D. radiodurans' DNA consists of two large chromosomal segments and two smaller plasmid segments. [10] The cell, when viewed under a microscope, appears as a tetrad. In practical terms, this means that there are at least two copies, and possibly more depending on the growth state of the bacteria, of the full genomic sequence contained within every cell. This almost certainly contributes to its remarkable ability to repair DNA damage.

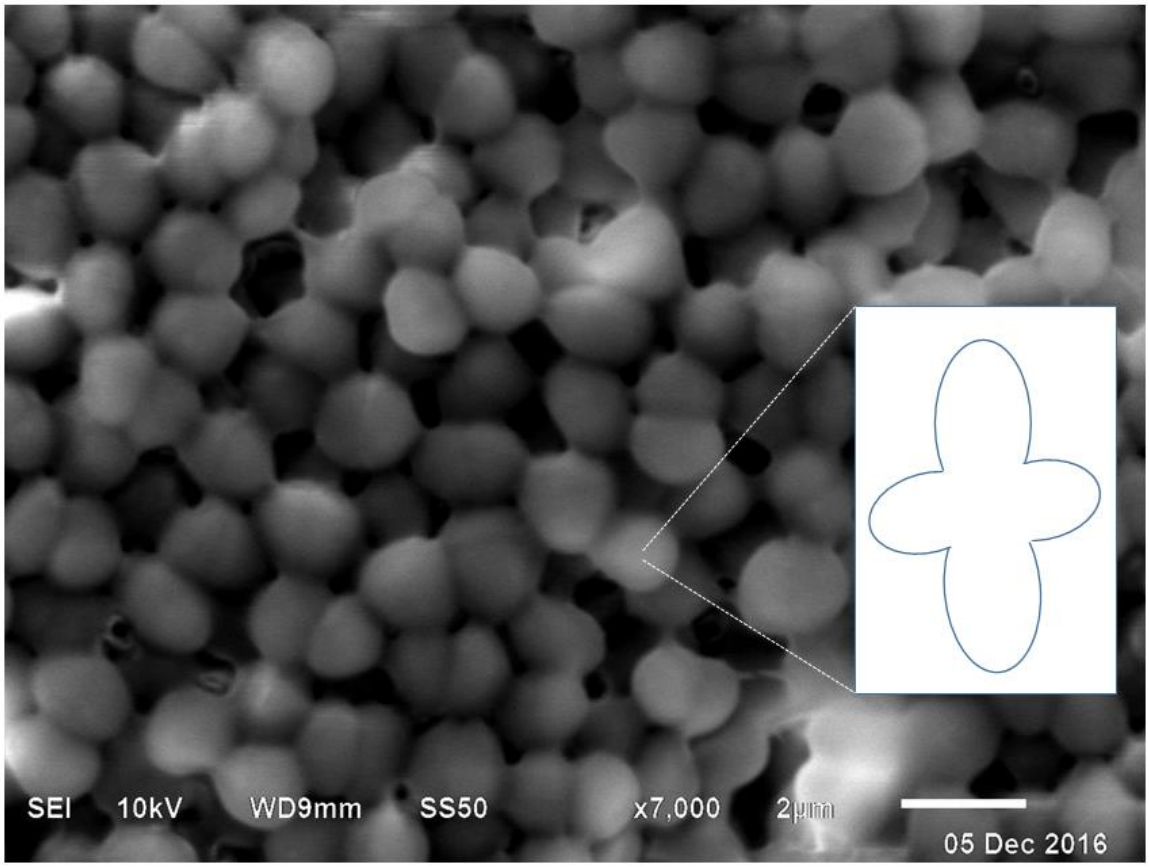

Figure 1. Composition of *Deinococcus radiodurans* nucleus. SEM image taken at USAFSAM in 2016. [4]

DNA Damage from Direct and Indirect Actions

When biological organisms or tissues are exposed to even moderate doses of radiation they inevitably sustain damage. Due to the relative volume of the organism in comparison to the volume in which the DNA is contained, the majority of the damage is incurred by the cell membranes, proteins, and other cell structures. However, with a high flux of ionizing particles one can expect that a portion of the damage will accrue in the DNA of the cells and can be statistically or experimentally determined. The damage is

categorized as either direct or indirect damage. While their mechanisms are different they both contribute to the total DNA damage experienced by a cell in a hydrated state.

As previously noted, an incident charged particle imparts most of its energy to the target medium at the end of its path length. If that happens to be in very close proximity to DNA, the Coulombic interactions of the now-stationary charged particle can tear apart multiple hydrogen and covalent bonds in its vicinity. The amount of ionizations created by this particle are log-linear in proportion to their molecular weight, meaning that larger particles create more ionizations at the end of their track than smaller particles. [12] This is more likely to result in double-stranded DNA breaks (DSB), which in many cases are lethal to a cell. Large sums of double-stranded breaks are almost universally lethal except in a very few cases, one of which is *D. radiodurans*.

When ionizing particles interact with the water contained within the cells reactive oxygen radicals are formed. According to Alpen, "the *primary products* are the excited water molecule, H_2O^*, and its immediate dissociation products, H· and OH·, and from the direct ionization of H_2O, H_2O^+ and e^- are produced." [12] If these reactive oxygen species (ROS) are formed in proximity to DNA, they can break bonds within the DNA backbone causing a single-strand break (SSB). This is referred to as indirect damage since the incident particle did not itself interact with the DNA but rather a secondary ionized product. Cells can typically repair a single-strand break without errors since the complement in the base pair is still present. A cell can be overwhelmed by single-strand breaks if they occur on opposite sides of the helix within about a 10 base pair region. The tension on the helix can break the hydrogen bonds resulting in an induced double-strand break. If the cell

experiences too many single strand and induced double-strand breaks it will certainly be more error prone and ultimately may not be capable of recovering.

General DNA Repair Mechanisms

DNA damage is inevitable throughout the lifespan of a cell. Indirect damage can occur through natural means such as from the interactions of reactive oxygen species that are produced by the cells as a result of normal metabolic processes. Direct and indirect damage can also occur through the interaction of foreign objects such as atomic particles or photons with atoms comprising the various components of the cell. There are five general repair pathways that are employed by the cell to maintain the integrity of the genomic information. Those pathways consist of base excision repair, mismatch repair, double strand break repair, nucleotide excision repair, and photoreactivation (or damage reversal.) [17]

Base excision repair is implemented when a single base experiences damage and either results in a base mismatch or a distortion (dimer) of the backbone. [18] As stated, indirect damage in the lifecycle is inevitable and can be a result of "simple chemical processes such as oxidation, hydrolysis, and methylation." [17] The damage, usually in the form of deamination, oxidation, or alkylation, is first recognized by an enzyme called glycosylase. Another enzyme called endonuclease cleaves the DNA on either side of the site and removes the damaged portion. Polymerase synthesizes replacement bases using the single-stranded portion on the opposing side of the helix as a template. The process is completed when the nicks are sealed by ligase. [18]

Mismatch repair is essentially a DNA audit for incorrect insertions, deletions, or mis-incorporation of bases during the replication process. The proof-reading process is strand-specific and is likely signaled to begin comparisons of the parent and daughter strands by unsealed ends of the backbone after replication. Once an error has been detected, the method for repair is similar to either base excision or nucleotide excision. [17]

When the hydrogen bond between the bases is broken, as may occur after interaction with a photoproduct, two adjacent bases can then bond with each other creating a bulge in the backbone. The nucleotide excision repair mechanism involves unwinding a portion in both the 3' and 5' directions. The section is then cleaved and removed by endonuclease. DNA polymerase uses the complementary bases as a template to repair the excised portion and the ends are sealed by ligase, much in the same manner as base excision repair. [17] [18]

Double strand breaks are especially serious and can often be lethal. The preferred method of repair is recombination since it is likely to result in exact copying of the original segment. The process includes formation of a Holliday junction (or four-way DNA junction). This is accomplished by accessing the homologous portion of another gene. One strand of the undamaged DNA crosses over and the displaced strand of the damaged DNA proceeds with a second cross-over. DNA polymerase then uses the undamaged portions as a template to create the complementary bases, endonuclease cleaves and removes the damaged portions, and ligase seals both ends of both genes. [18] The other method of DSB repair is nonhomologous end-joining which simply reattaches the two ends of damaged DNA, trims any excess, and seals the backbone. This is a quick method of repair, but also extremely error-prone and likely to results in lost genetic information.

Photoreactivation, or damage reversal is activated when DNA has sustained photo damage, typically from UV. This damage includes "two major lesions, cyclopyrimidine dimers and 6,4-photoproducts, which cross-link adjacent pyrimidines, resulting in a distortion of the DNA duplex." [17] This damage can be directly reversed by photolyase which reorders the incorrect covalent bonds between the adjacent bases. [18]

Deinococcus radiodurans DNA Damage and Repair

All organisms employ various methods of repairing DNA damage and each of these methods is controlled via their own sequences also held within the DNA. Some of the repair mechanisms are designed to repair SSB while others are for DSB. Zaradhka et al. noted six known repair mechanisms employed by prokaryotic and eukaryotic cells "either alone or in some combination to rejoin hundreds of partially overlapping chromosomal fragments [including] non-homologous end joining, homologous recombination at the fragment ends, intra- and interchromosomal single-strand annealing (SSA), synthesis-dependent-strand annealing (SDSA), break-induced replication, and copy choice." The authors then go on to state that they have excluded each of these repair mechanisms as being a primary mechanism attributable to DRs exceptional repair ability. They then identify a previously unknown mechanism that they term extended synthesis-dependent strand annealing (ESDSA) that is coupled with crossover to reassemble the fragmented DNA that results from either irradiation or desiccation. [13]

The main requirements for ESDSA to be implemented are two complete chromosomal copies where at least one experiences DSB. The process begins when two fragments with overlapping homologous base pairs "are used both as primers and as

templates for massive synthesis of complementary single strands." [13] ESDSA then assembles the smaller fragments into larger intermediates and then crossovers complete the recombination with the chromosomes arranged in a circular plasmid. [14] Figure 3. depicts the ESDSA mechanisms for repairing DNA damage.

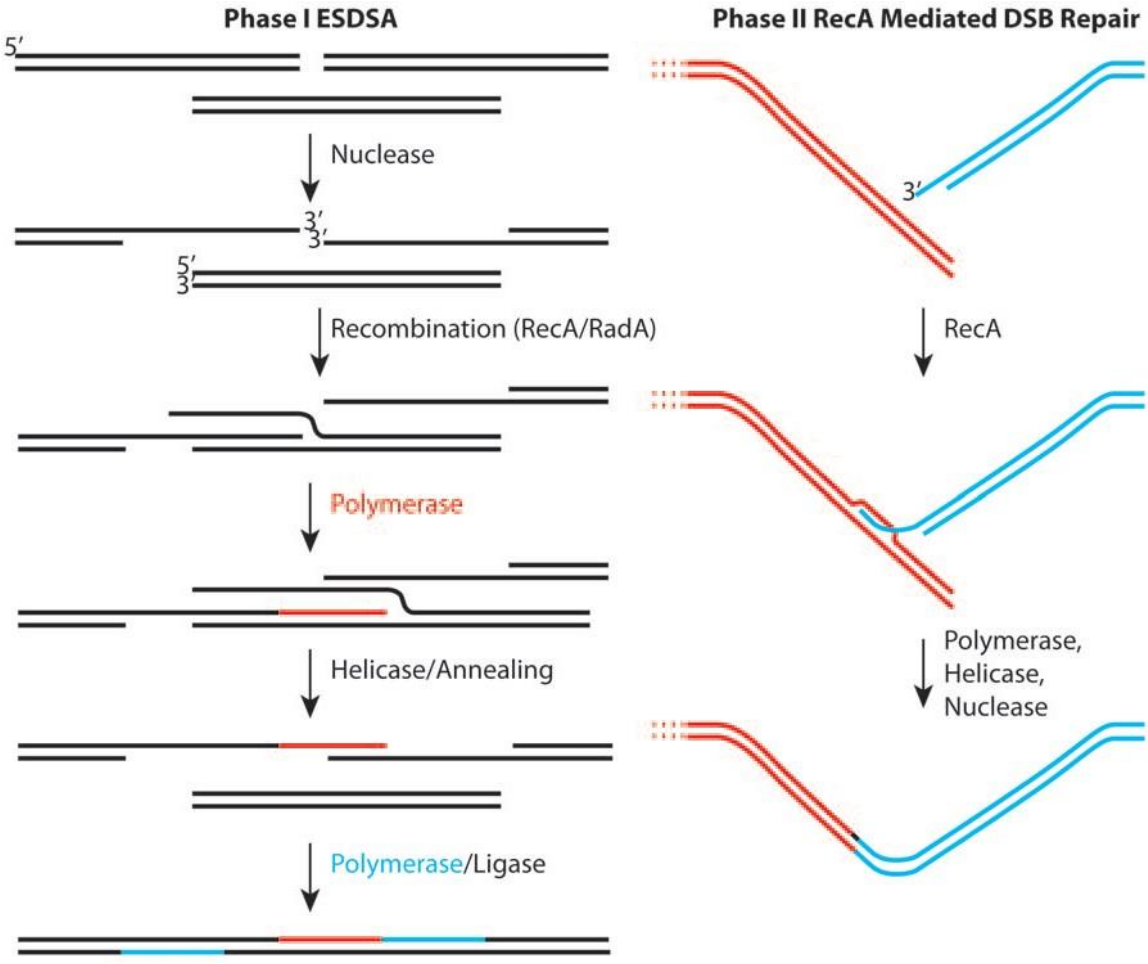

Figure 2. The ESDSA repair process. Reprinted under the Creative Commons Attribution License.

[8]

As far as can be ascertained through an exhaustive literature search, *D. radiodurans* is the only organism known to employ this mechanism for DNA repair. Using this method each bacterium can theoretically sustain hundreds of DSB with full recovery and minimal latency. While the bacterium is unique in employing this method, it should be noted that this is not unlike mechanisms employed by other organisms. *D. radiodurans* just seems to be more efficient in the employment of common repair pathways and takes a slightly different approach. The organism will only be incapable of repair if the fragments are too small to provide sufficient homologous overlaps of DNA with which to perform ESDSA.

Role of *recF* in DNA Repair

Following an event in which the organism experiences DNA damage there are a number of repair mechanisms that are moderated and regulated by various proteins. The formation of these proteins is encoded in the genome and the method by which they function is known as the pathway. The *recF* pathway has been noted as a critical component in the ESDSA process, particularly for DSB repair. The *recF* protein acts in concert with the *recO* and *recR* to "promote loading of *RecA* onto single stranded DNA" which are "dependent strand invasion [proteins] to prime DNA synthesis." [14] Bentchikou *et al*. note that cells devoid of the *recF* pathway showed impaired growth suggesting it is also involved in cell division.

While *recF* has been proven to play a central role in DNA repair, it should also be highlighted that no single recombination pathway works in isolation. Rather, they each are dependent on other pathways to completely and correctly reconstitute the genome following DSB. It should also be noted that different pathways play either critical or minor

roles in the repair process depending on the type and severity of the damage incurred (ie. a few SSB vs. many DSB.) It should also be noted that DSB repair mechanisms are up-regulated immediately prior to cell division since the gametes are broken during the division and crossovers are required to reconstruct the broken portions in both the parent and the daughter cells.

Role of *uvrB* in DNA Repair

The *uvrB* pathway also plays a central role in DNA repair, although it is believed to serve primarily as a SSB repair mechanism. As previously stated, no pathway works in isolation and this is especially true for *uvrB*. The pathway is actually a complex of proteins including both *uvrA* and *uvrB*. The repair process begins "when the $UvrA_2UvrB_2$ complex encounters a region of DNA which is distorted by the presence of a DNA lesion unconnected with transcription [in a] process known as global genome repair (GGR)." [16]

In GGR, once the Uvr_2 dimer is recruited to the site of DNA damage, it then "passes the damaged region of DNA to UvrB, which uses a beta-hairpin to verify the damaged nucleotide on one of the two DNA strands." [16] Once UvrB has locally unwound that portion of DNA and verified that there is a lesion, it then recruits UvrC to the site in order to excise the damaged region. At this point, the UvrB releases from the site and its role is complete. Surprisingly, the mean lifetime of a UvrB protein bound to a lesion is ~30s. [16] It is clear that UvrB plays a central role in DNA repair involving SSB, and it is also clear that the process proceeds rapidly allowing a cell to perform many repairs throughout a single division cycle. Since uvrB does not play a role in ESDSA it was chosen as the target gene for deletion in order to isolate the two repair pathways.

High LET and Low LET

A common misconception is that particles with higher energy will cause greater damage to a cell than particles with lower energy. While this may hold true at the macroscopic level or for high density materials, it can be quite the opposite for cellular interactions at the atomic level. Linear energy transfer describes the amount of energy an ionizing particle imparts to a material over a traversed distance.

$$LET = \frac{dE_L}{dx}$$

High LET particles deposit large amounts of energy into the target medium while low LET particles do not impart much energy in the medium through which it passes. The linear energy transfer is independent of the particle's kinetic energy. In the case of atomic particles, those with low LET are likely to pass completely through thin materials with low densities without depositing much energy while high LET particles are more likely to be stopped and deposit all their energy. For reference, an alpha particle incident to tissue with an initial energy of 5.3 MeV will end its track at about 35 μm. This means that nearly all of that energy will be deposited at a depth of 35 μm for *all* particles. [5]

The significance of the energy transfer is the likelihood that the particle will induce a break in DNA, particularly double-stranded, within a cell. In this research the samples will be desiccated to drastically reduce the possibility of HCP-water interactions (and the accompanying formation of ROS) during exposure so the preponderance of DNA damage will be direct. The energy will be selected such that most of the incident particles will deposit their energy at half the total depth of the samples and the energy will be sufficiently high to induce double-stranded DNA breaks.

Heavy Charged Particle Interactions

Photons such as gamma or UV primarily interact with matter via Compton scatter or the photoelectric effect. Compton scattering occurs when a photon is incident to a target atom. The atom recoils, carrying away some energy, and the photon is scattered at an angle with a longer wavelength (and lower energy.) The photoelectric effect occurs when a photon of sufficient threshold energy is incident to an electron resulting in the ejection (or emission) of the electron.

Uncharged neutrons interact with matter through elastic and inelastic scatter, neutron capture, or spallation. Elastic scatter involves an incident particle striking a target particle. Both recoil with a conservation of energy in the system. Inelastic scattering occurs when the incident particle has comparatively low kinetic energy. When it collides with a target particle all the energy is imparted and it comes to rest. Neutron capture involves a neutron merging with the nucleus of another atom to form a heavier isotope. This does not occur with heavy charged particles. Spallation refers to the emission of neutrons from a target nucleus after a high-energy particle has impacted it. In these cases, the incident photons or uncharged particles must be inside the range of the strong force (about 10^{-15} m.)

In contrast, heavy charged particles experience Coulombic interactions with the electrons and nuclei in the medium over much greater distances (about 10^{-12} m.) The majority of the energy is transferred at the end of the path length of the particle over a very small distance. One would expect that a given flux of HCPs would deposit more energy into a target than an equivalent flux of neutrons primarily due to the mass of the particle and the velocity it is travelling. The impact of indirect DNA damage through reactive oxygen species will be discussed but generally ignored because all target samples will be

desiccated. In this research it is expect that nearly every incident particle will interact with the sample in some manner and many of these interactions will result in direct damage in the form of a double-strand break.

Deinococcus radiodurans and Mutant Strains

Previous research conducted by the Air Force Institute of Technology (AFIT) in collaboration with the United States Air Force School of Aerospace Medicine (USAFSAM) utilized the R1 strain of *D. radiodurans*. Three mutant strains were subsequently developed at USAFSAM in order to investigate their survivability in UV, low LET neutron and high LET proton experiments. [4] Mutant #5 consisted of a manganese superoxide dismutase (SOD) knockout. Mutant #8 was a double copper/zinc SOD knockout. These genes were chosen for deletion because the production of SOD allows the cell to neutralize superoxide radicals into less harmful species. And finally, Mutant #11 was a *bshA* knockout. This gene produces an enzyme that is involved in the biosynthesis of a small molecule antioxidant, bacillithiol, which participates "in the destruction of reactive oxygen species and harmful xenobiotic agents." [11] Since all three of these mutant strains deal with reactive oxygen species that form when the cells are hydrated, they are expected to behave in a similar manner to wild type when exposed to both ultraviolet radiation and HCP while dehydrated.

In addition to the three mutants previously created, this project required the development of two additional mutant strains. Mutants #1.5 and #6A are *recF* knockouts while Mutants #16 and #27 are *uvrB* knockouts. As noted above, *recF* is a recombinant gene that aids in the repair of DNA damage, particularly from double-stranded breaks. The

uvrB gene aids in the repair of DNA damage, particularly from single-stranded breaks as are usually experienced from ultraviolet exposure. The *uvrB* and *recF* mutants are expected to experience greater kills rates following both UV and HCP exposure than wild type since these repair genes are necessary to repair direct DNA damage. The complete list of the mutants used in this research can be found in Table 1.

Table 1. *Deinococcus radiodurans* R1 Stain List

#	Gene KO	Common Name	Proper Genotype
1	none	WT	
1.5	*recF*	recF	'merodiploid' WT and *ΔrecF::KAN*
5	*DR_1279*	Mn SOD	Δ*DR_1279*::*mlox*
8	*DR_1546* *DR_A0202*	Cu/Zn SOD Cu/Zn SOD	Δ*DR_1546*::*KAN* Δ*DR_A0202*::*NAT*
11	*bshA*	Bacillithiol Biosynthesis	Δ*bshA*::*mlox*
16	*uvrB*	uvrB	*ΔuvrB::KAN*
6A	*recF*	recF	'merodiploid' WT and *ΔrecF::KAN*
27	*uvrB*	uvrB	*ΔuvrB::KAN*

III. Methodology

Chapter Overview

The purpose of this chapter is to describe the methods for achieving gene knockouts for the *uvrB* and *recF* genes. It provides a thorough examination of the process of sample preparation prior to treatment. In addition, it covers the selection of an appropriate bombarding particle as well as the calculation of the necessary energy and fluence to achieve proper exposures. It will conclude with a detailed explanation of the post-irradiation processing of the samples.

Deinococcus Radiodurans uvrB Knockout

Cloning of Knockout Plasmid

The first step in the homologous recombination knockout process involved ordering six primer sets from Integrated DNA Technologies (IDT Inc). These primer sets were used to PCR amplify 3 fragments of DNA. One fragment provided homology upstream of the target gene, a second fragment provide a selectable Kanamycin resistance gene that replaced the deleted gene sequence and the third fragment provided homology downstream of the deletion. The PCR primers were designed to have 10 to 20 base pair overhangs that are homologous to the fragments of DNA that will be linked adjacent. These primer-supplied short regions of homology provided the needed sequences for the NEBuilder cloning system to link multiple DNA fragments in a single reaction. Table 2 shows a complete list of the primer sequences.

Table 2. uvrB Primer Sequences

Primer Name	Sequence			
Puc_UvrBUp_Fwd	ttgtaaaacgacggccagtgTGC GCAAGGTACCGCAGATGC	Puc	Uvr_Upstream	Fwd
Kan_UvrBUp_Rev	acgaacggtaCCTGCGCGCC ACGACCAC	Kan	Uvr_Upstream	Rev
UvrBUp_Kan_Fwd	ggcgcgcaggTACCGTTCGTA TAGCATAC	Uvr_Upstream	Kan	Fwd
UvrBdown_Kan_Rev	tgccttctgcTACCGTTCGTAT AATGTATG	Uvr_downstream	Kan	Rev
Kan_UvrBdown_Fwd	acgaacggtaGCAGAAGGCA CGGCGGAA	Kan	Uvr_downstream	Fwd
Puc_UvrBdown_Rev	atccccgggtaccgagctcgGTA CCAGAAGCTGCTCAACAA ATGG	Puc	Uvr_downstream	Rev

All six primer lyophilized powders were suspended to 100µM in 0.1xTE Buffer at a pH of 7.5 while kept on ice to prevent degradation. Each primer was then diluted 1:10 in molecular biology grade water to 10µM. 20µl of DR genomic stock at 529ng μl^{-1} was added to 180µl of water for a 1:10 dilution. The pUCIDT-Amp::KANkanp plasmid stock at an initial concentration of 144ng μl^{-1} was diluted 1:25 and then 1:100 to achieve 50pg μl^{-1} thus completing the template DNA dilutions. A PCR cocktail was created for a 50µl polymerase chain reaction (PCR). The PCR is designed to amplify the DNA fragments in preparation

for the gene knockout construct cloning. Table 3 shows the complete PCR template cocktail.

Table 3. Genomic Template and PCR Cocktail

PCR Cocktail with Genomic Template:	1x reaction	8x reaction
Molecular Biology Grade Water	21.5µl	172µl
10mM dNTPs	1µl	8µl
DR Genomic @ 50ng μl^{-1}	2µl	16µl
5x Q5 Reaction Buffer	10µl	80µl
5x Q5 High GC Enhancer	10µl	80µl
Q5 DNA Polymerase	0.5µl	4µl
Total	45µl	360µl

The Kan primers require a plasmid template cocktail in order to properly amplify. Table 4 shows the complete PCR plasmid cocktail.

Table 4. Plasmid Template and PCR Cocktail

PCR Cocktail with Kan Plasmid Template:	1x reaction	3x reaction

Molecular Biology Grade Water	21.5µl	64.5µl
10mM dNTPs	1µl	3µl
Kan Plasmid @ 50pg μl^{-1}	2µl	6µl
5x Q5 Reaction Buffer	10µl	30µl
5x high GC Enhancer	10µl	30µl
Q5 DNA Polymerase	0.5µl	1.5µl
Total	45µl	135µl

Once the cocktail had been mixed, 2.5µl of each 10mM primer stock was added to 45µl of the genomic cocktail for a total of 50µl for PCR reactions 1 and 3. 2.5µl of each 10mM primer stock was added to 45µl of the plasmid cocktail for reaction 2. Table 5 shows the combinations for each reaction.

Table 5. PCR Primer Combinations

Reaction Number	Reaction Name	Forward Primer	Reverse Primer
1	uvrB up	Puc_uvrBUp_Fwd	Kan_UvrBUp_Rev
2	uvrB Kan	UvrBUp_Kan_Fwd	UvrBdown_Kan_Rev
3	uvrB down	Kan_UvrBdown_Fwd	Puc_UvrBdown_Rev

The reactions were moved to a thermocycler for amplification. The thermocycler was programmed to take reaction 2 through 98°C for 3 minutes, then 30 cycles of (98°C for 1 minute, 56°C for 30 seconds, 72°C for 1 minute), then 72°C for 2 minutes and hold at 4°C. The thermocycler was programmed to take reaction 1 through 98°C for 3 minutes then 30 cycles of (98°C for 1 minute, 72°C for 30 seconds, 72°C for 1 minute), then 72°C for 2 minutes and hold at 4°C . And finally, it was programmed to take reaction 3 through 98°C for 3 minutes then 30 cycles of (98°C for 1 minute, 66°C for 30 seconds, 72°C for 1 minute), then 72°C for 2 minutes and held at 4°C .

Following PCR amplification, 10µl of 6x Sample Buffer was added to each 50µl PCR reaction. The entire reaction was loaded in two lanes (30µl per lane) of 0.8% agarose gel in 1xTBE with 5µg ml^{-1} ethidium bromide and run for 30 minutes at 150 volts. The entire gel was placed over an ultraviolet light box and the bands visualized against a ladder standard. Using a razor blade, the ~1000 base pair bands were cut from the gel and distributed in 1.5ml microfuge tubes with no more than 300mg of gel slice per tube. The DNA fragments were isolated using a Qiagen gel extraction kit. Figure 4 depicts a sample placed over the UV light box.

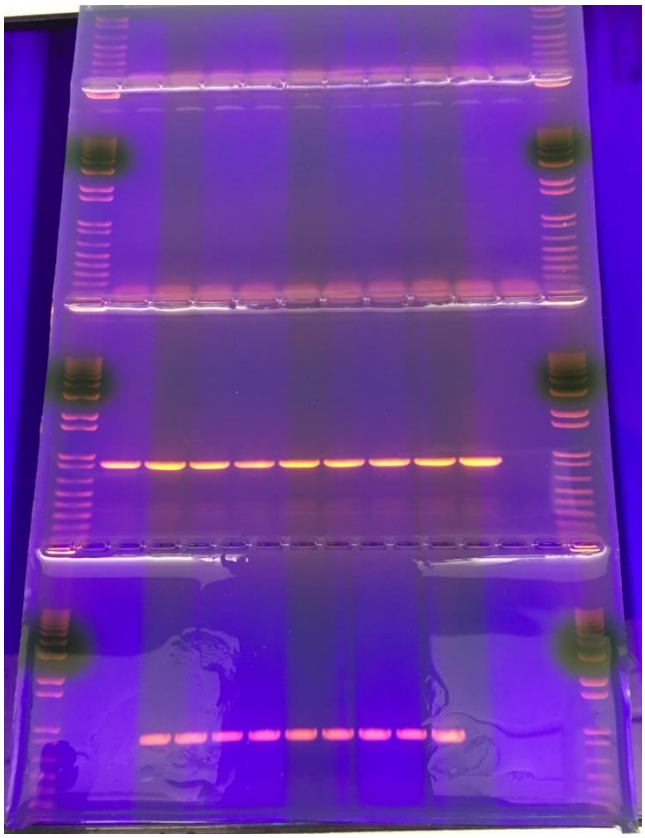

Figure 3. UV visualization of DNA fragments. The bright band consists of ~1000 base pair fragments as compared to the standard ladder at either end.

Escherichia coli Transformation

Following PCR amplification of the DNA insert fragments, an additional plasmid vector for cloning was prepared. *Escherichia coli* (*E. coli*) NEB5 alpha bacteria with the pUC19mPheS plasmid were streaked for isolation on an LB agar plate with 50µg ml^{-1} of Carbenicillin. The plates were then incubated overnight at 37°C in an unsealed plastic bag to prevent the agar from drying. The following day a single colony from the plate was inoculated into 30ml of LB broth with 50µg ml^{-1} of Carbenicillin in a 125ml flask. The flask was incubated overnight at 37°C and 220 RPM. The following day the media was

transferred to a 50ml conical tube and pelleted by centrifugation at 3500 RPM for 20 minutes. The pelleted cells were then re-suspended in 750µl of Qiagen P1 buffer and then another 750µl of Qiagen P2 buffer was added to the mixture in order to lyse the cells. The mixture was incubated for 5 minutes and then 1050µl of Qiagen N3 buffer was added to neutralize the alkaline solution as well as precipitate cell debris. The DNA was then isolated using Qiagen Mini spin columns and the concentration measured on a Nanodrop spectrophotometer.

Once the plasmid DNA was isolated and measured at 124.9ng μl^{-1}, a digest was set up to linearize the plasmid for use with the NEBuilder Cloning kit. The full reaction mixture is listed in Table 6. The mixture was incubated at 37°C overnight. The following morning sample buffer was added to the reaction and the entire mixture was loaded across three lanes of 0.8% agarose gel in 1xTBE with 5µg ml^{-1} of ethidium bromide and electrophoresed at 150 volts for 30 minutes. The gel was visualized on a UV light box and the plasmid DNA band at ~3895 base pairs was cut and distributed into 1.5ml microfuge tubes. The plasmid DNA was isolated using a Qiagen gel extraction kit and the concentration measured on a Nanodrop spectrophotometer.

Table 6. pUC19mPheS EcoRI Digest for NEBuilder Cloning

Digest Mix:	
Molecular Biology Grade Water	54µl
pUC19mPheS Plasmid DNA	32µl

10x NEB EcoRI Buffer	10µl
NEB EcoRI Enzyme U μl^{-1}	4µl
Total	100µl

Once the linearized plasmid DNA was isolated and the concentration was determined, the NEBuilder cloning kit was used to assemble the three PCR fragments and link them into the linearized plasmid backbone. To isolate intact gene knockout plasmids, 2µl of the NEBuilder reaction was used to transform *E. coli dam*$^-$ *dcm*$^-$ competent cells by the NEB Inc. protocol. The *E. coli dam*$^-$ *dcm*$^-$ were used to yield unmethylated plasmid DNA which transforms *D. radiodurans* at a much higher frequency than methylated DNA. After plating on LB agar containing 32ug/ml Kanamycin and incubation at 37°C overnight, four isolated colonies were picked, suspended in 20ul of LB broth and 2ul of this suspended bacteria were screened for correct clones by colony PCR using 2x Quickload Taq Master Mix in a 50µl reaction. The mixtures were then placed in a thermocycler programmed to take reaction through 98^0C for 2 minutes, then 30 cycles of (98^0C for 30 seconds, 68^0C for 3 minutes), then 68^0C for 5 minutes and hold at 4^0C. To observe if the bacteria had clones of the proper insert size of ~3000bp, 25ul of each reaction was electrophoresed in a similar manner as described above. Positive clones were chosen and the remaining 18µl of suspended colony was used to inoculate 30ml of LB broth with 32µg ml^{-1} Kanamycin in a 125ml flask. After an overnight incubation the cells were pelleted, lysed, and the plasmid DNA isolated. As an additional check for having the correct knockout plasmid clones, plasmids were diluted to 0.5ng μl^{-1} and then 2µl were placed into PCR tubes. These

templates were then mixed with a PCR cocktail and moved to a thermocycler programmed to take the reaction through 94°C for 2 minutes then 30 cycles of (94°C for 30 seconds, 60°C for 30 seconds, 65°C for 3 minutes), then 65°C for 10 minutes and held at 4°C . The full reaction cocktail can be found in Table 7.

Table 7. PCR Cocktail with Genomic Template for DR transformation

PCR Cocktail with Genomic Template	1x reaction	15x reaction
Molecular Biology Grade Water	29µl	435µl
10mM dNTPs	1.5µl	22.5µl
Forward Primer @ 10µM	2µl	30µl
Reverse Primer @ 10µM	2µl	30µl
5x LongAmp Taq Reaction Buffer	10µl	150µl
DMSO (Final Conc. 3%)	1.5µl	22.5µl
LongAmp Taq DNA Polymerase	2µl	30µl
Total	48µl	720µl

Following PCR, 20µl of the PCR reaction was mixed with 6µl of Orange G sample buffer and the mixture was loaded onto a 0.8% agarose gel with 0.5µg ml^{-1} of ethidium bromide and electrophoresed at 150 volts for 30 minutes. This PCR check confirmed the previous colony PCR result of having correct knockout clones. This process completed

"stitching" the DNA primer fragments into a final unmethylated plasmid ready for DR transformation.

Deinococcus radiodurans Transformation

D. radiodurans transformations to introduce the knockout plasmids into the cells allowing for the plasmid integration into the host chromosome by homologous recombination were carried out as follows. *D. radiodurans* R1 wild type bacteria were struck for isolation from a frozen glycerol stock on 1xTGY agar and incubated at 32°C for 2 days in an unsealed ziplock bag to prevent drying. A single colony was inoculated into 5mls of 1xTGY broth in a 14ml round bottom snap cap tube and incubated overnight at 32°C and 220 RPM. The overnight culture was diluted to an Abs. @ 600nM of 0.2-0.3 in 20mls of 1xTGY in a 125ml flask and incubated 2 hours at 32°C and 220RPM. After the 2 hours, 2.2mls of 300mM $CaCl_2$ was added to a final concentration of 30mM $CaCl_2$ and the incubation was continued for 2 hours at 32°C and 220RPM. After the $CaCl_2$ two hour incubation, 100µl aliquots of the culture were transferred to sterile 1.5ml microfuge tubes on ice. One µg of knockout plasmid DNA was added to the cells, mixed gently and incubated on ice for 1 hour. The DNA/cell mix was then transferred to 14ml round bottom snap-cap tubes containing 1ml of 1xTGY broth and were incubated overnight at 32°C and 220RPM for an extended grow out. The overnight grow out cultures were then diluted 1:10 7x in series (20µl in 180µl 1xTGY broth) using a multichannel pipet. Five microliters of each dilution of the dilution series was spotted on 1xTGY agar containing 16ug/ml kanamycin. The spots were allowed to dry and the plates were inverted and incubated at 32°C for 2-3 days. Any resulting Kanamycin resistant *D. radiodurans* colonies presumably had the plasmid integrated into the chromosome by single crossover homologous

recombination but the second homologous crossover event may or may not have occurred resulting in the complete final gene deletion.

Knockout Selection

To select for isolates where the second homologous crossover has occurred and thus have the complete gene deletion, 5-10 isolated Kanamycin resistant colonies from each transformation dilution spotting were picked and mixed on the agar surface in the same area of a 5mM 4-Chloro-Phenylalanine (4-CP) 1xTGY agar plate with 16ug/ml Kanamycin. From this mix area a loop was used to streak for isolation. These 4-CP patch plates were incubated at 32°C for 2 days in unsealed ziplock bags to prevent drying. The 4-CP is a counter-selection agent that prevents any bacteria with the mutated *pheS* gene (mPheS) from growing. Thus, only bacteria in which the second homologous recombination crossover event has occurred and have lost the knockout plasmid backbone containing the mPheS gene will grow. Isolated colonies that are resistant to Kanamycin and 4-CP were chosen, grown up, frozen down as glycerol stocks and had genomic DNA isolated. The genomic DNA was diluted and used as PCR template to confirm that the desired *uvrB* gene knockouts had been isolated. Two confirmed *uvrB* knockouts used in this study are Mutant #16 and Mutant #27.

***Deinococcus Radiodurans* recF knockout**

The *recF* knockout procedures were nearly identical to those described in the section above. The only difference was that the PCR amplification of the mutants did not indicate a complete loss of wild type *recF* despite the mutants being Kanamycin and 4-CP resistant. Attempts were made to segregate a fully deleted *recF* isolate but all efforts

resulted in a mix of wildtype and deleted *recF*. Therefore the *recF* mutants are merodiploids. Two samples were frozen as glycerol stocks and labeled as Mutant #1.5 and Mutant #6A. The recF primer sequences are listed in Table 8.

Table 8. recF Primer Sequences

Primer Name	Sequence			
Puc_RecFup_Fwd	ttgtaaaacgacggccagtgT GTGTTCGACCGCTTGCC	Puc	RecF_upstream	Fwd
Kan_RecFup_Rev	acgaacggtaTAGACAGG GCCGAGAGAC	Kan	RecF_upstream	Rev
RecFup_Kan_Fwd	gccctgtctaTACCGTTCGT ATAGCATAC	RecF_upstream	Kan	Fwd
RecFdown_Kan_Rev	catctcctcaTACCGTTCGT ATAATGTATG	RecF_downstream	Kan	Rev
Kan_RecFdown_Fwd	acgaacggtaTGAGGAGA TGCAAGCGGAGGG	Kan	RecF_downstream	Fwd
Puc_RecFdown_Rev	atccccgggtaccgagctcgTT CCGGCAGCGCGCGGTA	Puc	RecF_downstream	Rev

Sample Culture Preparation

All seven mutant strains as well as the wild type were streaked for isolation on Agar plates. Mutants #1.5, #16, #6A, and #27 were streaked on 1xTGY Kan 16µl ml^{-1}. Wild type #1 and Mutant #11 were streaked on 1xTGY, and Mutant #8 was streaked on 1xTGY

Kan 16µl ml^{-1} NAT 50µg ml^{-1}. The plates were incubated for 48 hours at 32°C in unsealed plastic bags. Single colonies were placed in 14ml round bottom snap cap tubes with 5ml of TGY broth and selective antibiotic(s) if necessary. The cultures were incubated overnight at 32°C and 220RPM for aeration. The cultures were then diluted 1:100 by placing 200µl of culture in 20ml of broth in a 125ml vented cap flask with appropriate selective antibiotics. The cultures were incubated overnight at 32°C and 220RPM for aeration. The cultures were diluted 1:10 by placing 100µl of culture in 900µl of broth and the 600nm absorbance was measured on the Nanodrop spectrophotometer against a broth blank. The amount of culture to be added to 40ml of TGY broth in order to achieve an Abs. at 600nm of 0.25 could then be calculated by the following equation.

$$40ml * \frac{0.25}{10 * Absorbance} = \#\ of\ ml\ of\ culture$$

The calculated culture volumes were added to 40ml of TGY broth in a 250ml vented cap flask and incubated for 4 hours at 32°C and 220 RPM for aeration. After the incubation period, 1ml of culture was placed on the Nanodrop spectrophotometer to measure the OD_{600}. If, after 4 hours, the OD_{600} readings were at 0.5 then the cultures were in early log phase. 30ml of each culture was placed in a 50ml tube and the cells were pelleted by centrifuge at 3500 RPM for 20 minutes. The supernatant was discarded. A calculation was performed to determine the appropriate amount of media to re-suspend the cells in to achieve an Abs. at 600nm of 5.

$$30ml * \frac{OD_{600}}{5} = \#\ ml\ of\ media$$

As shown by Lenker, an OD_{600} of 5 equates to 2-5 x 10^8 colony forming units (CFU) per ml. [4]

Sample Plate Preparation

After all eight strains of *D. radiodurans* were suspended to an Abs. at 600nm of 5 they were moved into a biosafety containment laminar flow hood in order to reduce the likelihood of contamination and aid in the drying process. Utilizing a single-channel pipette, 60µl of each strain was deposited into the corresponding "well" of a 96-well plate lid as shown in Figure 5. As is shown, the strain label was located in the well immediately above the 60µl spot. The tube was continually mixed by hand in order to ensure uniform density between the spots.

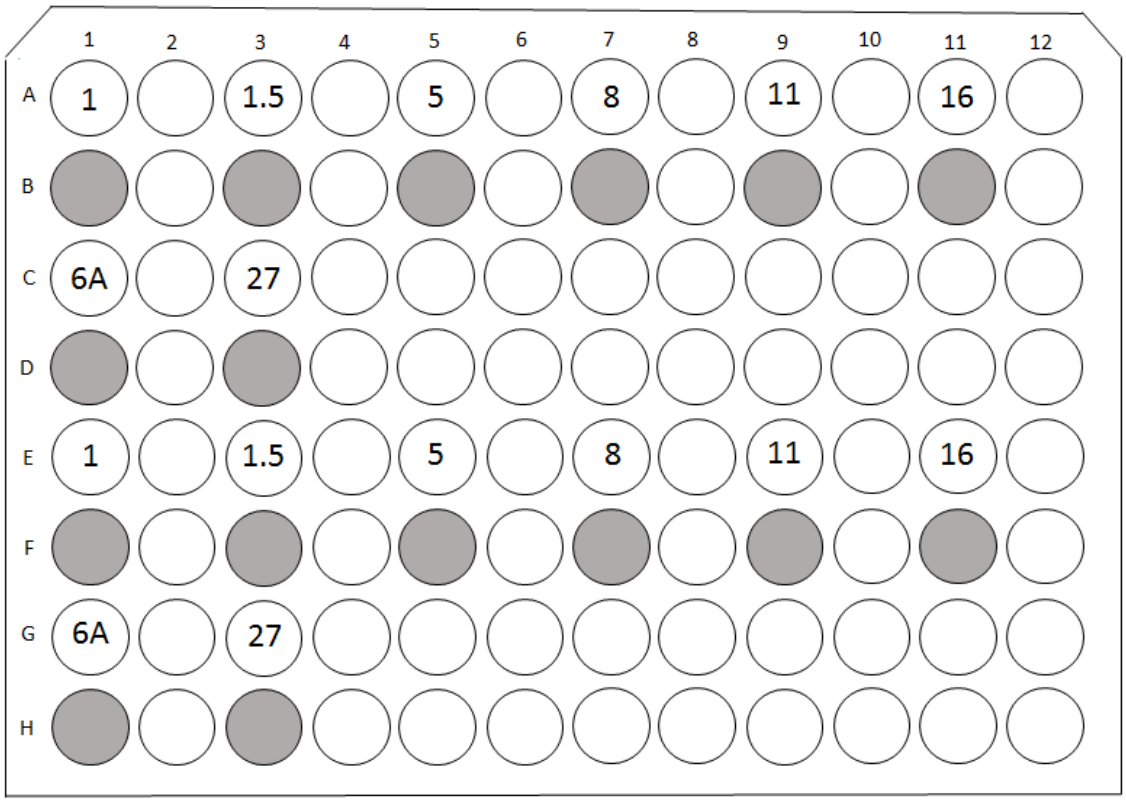

Figure 4. Sample plate configuration for every experiment. The shaded wells denote location of actual cell spots with strain labels located in the well immediately above the spot.

Once all the samples had been spotted to the plate lids, they were covered with the bottom portion of the 96-well plate slightly ajar. They were then allowed to dry overnight. Once all the samples had completely dried they were wrapped in parafilm and placed in a drawer either in preparation for shipment or to simulate shipping.

Three sets of serial dilutions were performed for each strain to determine the input CFU. The top well of the plate contained 200µl of each strain (ie. Cell A1 had #1, cell A2 had #1.5, cell A3 had #5, etc.) It was then diluted 1:10 through seven dilutions by pipetting 20µl from the row into 180µl of 1xTGY broth in the row below using a multichannel pipette. The dilution layout is depicted in Figure 6.

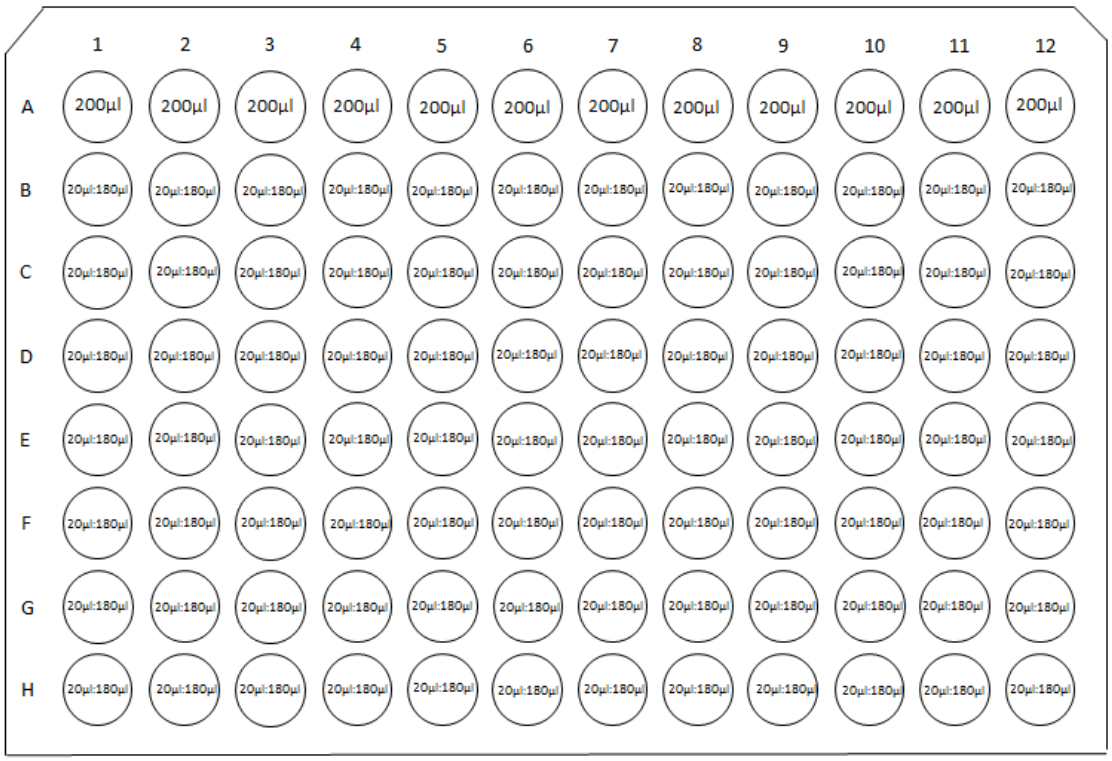

Figure 5. Dilution series layout.

Using a multichannel pipette, 5µl from each column were spotted to 1xTGY Agar plates. The spots were allowed to dry and then placed in unsealed plastic bags. The plates were then incubated at 32°C for 48 hours. Figure 7 depicts spotting from a dilution series.

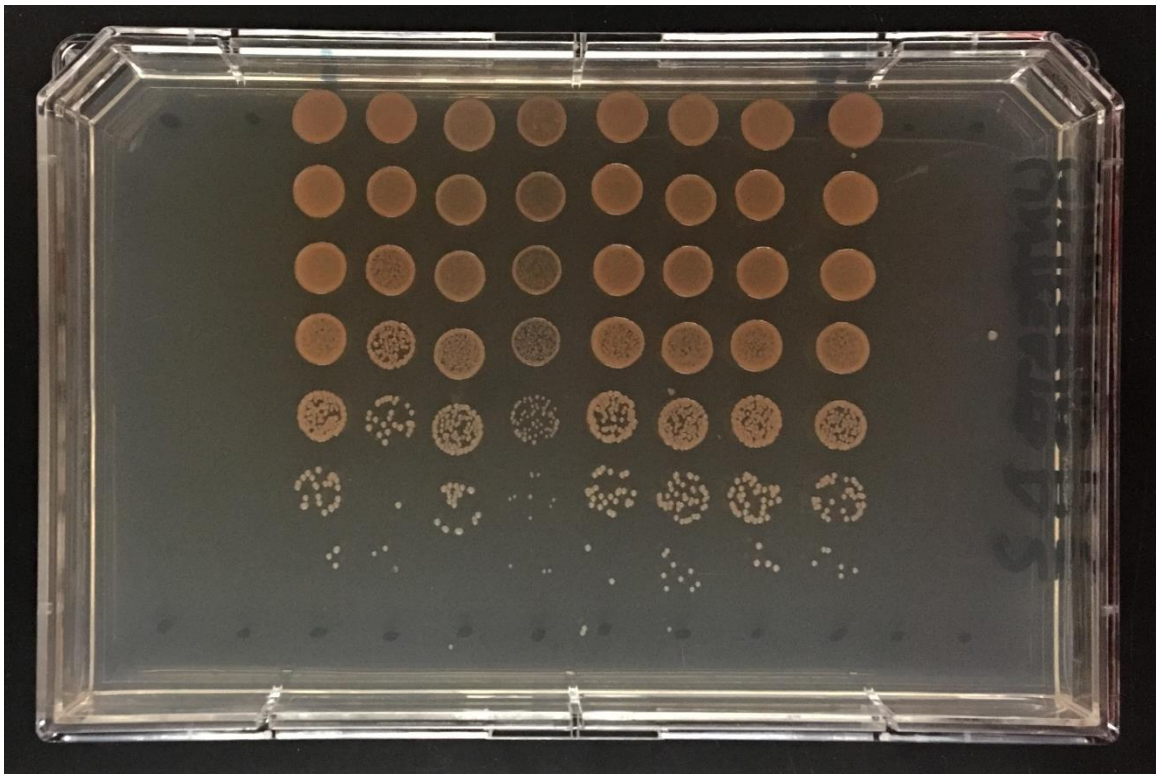

Figure 6. Dilution series for an untreated sample.

As noted by Lenker, the plate lids were used due to size constraints of the stage mounted in Sandia National Laboratory's (SNL) QASPR-3 (Qualification Alternative to the Sandia Pulse Reactor 3) tandem ion beam. [4] The same methods were employed in this experiment. The technicians at SNL had already worked with that geometry and it was thought to be in the best interest of time to replicate the setup. Ten total plates were shipped to SNL in a tightly packed and padded box. Two plates were destined to be untreated and unvacuumed experimental controls. Two plates were to be untreated but vacuumed, and then two plates were designated for each exposure level at 500, 1000, and 10,000 Gy. The samples were shipped two days after desiccation and arrived six days after desiccation. The first plate was mounted to the stage eight days following desiccation of the plates.

UV Protocol Test

The UV test consisted of eight total plates. After a day sitting in a drawer to simulate shipping the plates were vacuumed to 10^{-4} torr for 15 minutes. This was to simulate the vacuum process that the SNL samples would experience. Four were untreated and simply re-wrapped with parafilm and placed back in the drawer to simulate shipment from SNL. The remaining four were treated with 9999 J m^{-2} of UV in a UV Stratalinker 1800, shown in Figure 8.

Figure 7. Stratalinker 1800 used to impart 9999 J m^{-2} of UV energy onto the samples.

The samples were raised approximately 2cm in order to situate the plate lid as close to the sensor as possible. Following irradiation the plates were wrapped in parafilm and also placed in the drawer. This provided eight replicates of each strain treated, and eight replicates untreated.

Rehydration of Samples and Spotting Post UV Irradiation

The day following UV treatment, the samples were rehydrated by using a single-channel pipette and placing 60µl of fresh TGY broth. The samples were pipetted up and down approximately 20 times. The media remained on the plate lid while the next spot was rehydrated in the same manner. Once all eight strains were rehydrated they were again pipetted up and down approximately 20 times to ensure as many cells as possible were fully re-suspended. The ~60µl sample was placed in the top well of a 96-well plate and each strain was run through a full dilution series as depicted in Figure 4. Then 5µl from each well in the column was placed on a 1xTGY agar plate and the spots were allowed to dry. The plates were partially sealed in a plastic bag and incubated at 32°C for 48 hours.

Colony Counting Post UV Irradiation

Following the incubation period, individual colonies were visually counted. The untreated controls were all counted at the 10^{-5} dilution. Most of the treated colonies were counted at the 10^{-4} dilution. The colony counts are reported in Appendices A-H. Figure 9 shows an untreated control and Figure 10 shows a treated plate for comparison.

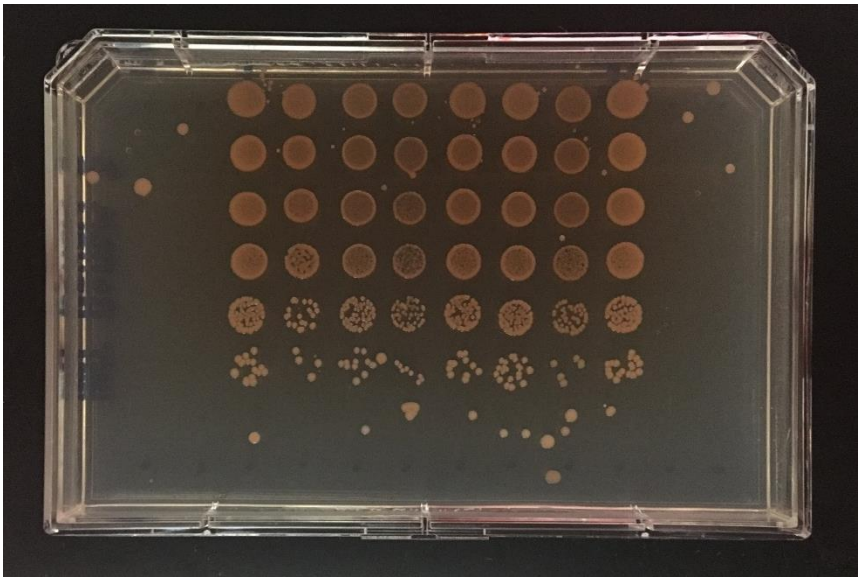

Figure 8. Untreated control plate from UV experiment. The strains, moving left to right, are wild type #1, Mutant #5, Mutant #8, Mutant #11, Mutant #16, Mutant #6A, and Mutant #27

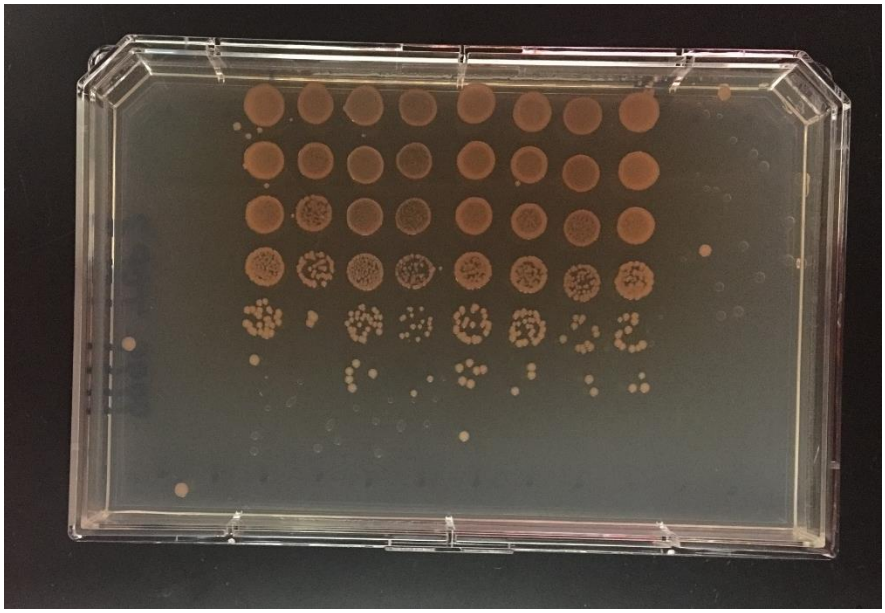

Figure 9. Treated plate from UV experiment. The strains, moving left to right, are wild type #1, Mutant #5, Mutant #8, Mutant #11, Mutant #16, Mutant #6A, and Mutant #27

Oxygen Ion Generation

SNL's QASPR-3 Tandem accelerator is capable of accelerating ions from hydrogen to gold in energies from 800keV to 10s of MeV. The ions are sputtered off of a negative ion source and then accelerated towards the positive terminal. The ions pass through a nitrogen stripper gas that removes the electrons. This produces a range of positive ions that are then accelerated away from the positive terminal. Magnets allow selection of a specific ion species and charge state on target. The landing energy is determined by multiplying the charge state plus one by the terminal voltage and is tuned by the terminal voltage.

Beam spot sizes vary between 0.001mm and 4mm and are, therefore, much too small to cover the entire cell spots in a single shot. SNL has developed an implantation technique where they characterize the beam and then move the sample on an x,y stage and "stitch" the shots together to achieve the desired total fluence. The error with this technique is typically 2-10%. The beam operates under a vacuum and is capable of 10^{-7} torr. The chamber and x,y stage are pictured in Figure 11.

Initial efforts were directed towards irradiating with helium ions. However, the beam was very unstable, likely due to the influence of local free fields. A heavier ion was desired in order to achieve a stable beam that could be easily and quickly characterized. For this reason, oxygen was chosen as the incident ion.

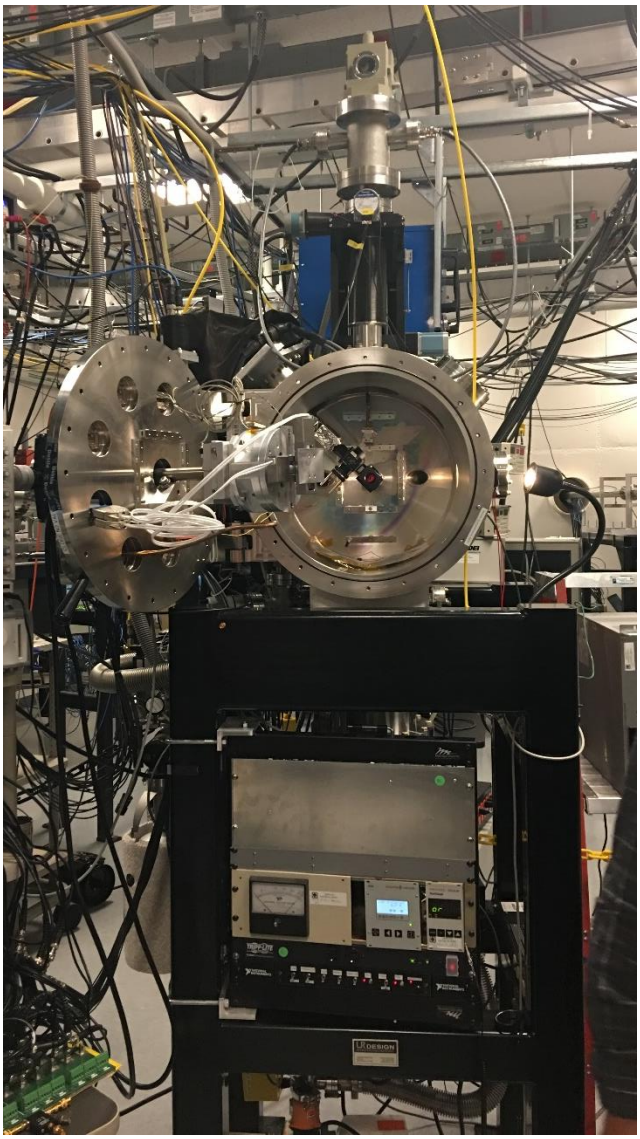

Figure 10. QASPR-3 Tandem target chamber with mounted x,y stage.

Oxygen Ion Dose Calculations

Lenker established that a 60µl drop desiccated on the plate lid creates an average cell layer depth of about 21.7 microns, which corresponds to 7 cell layers, with a cell density of 0.9392g cm^{-3} [4]. Since nearly all the energy of the bombarding particles is

imparted at the end of range, the experiment was designed to calculate the necessary energy that resulted in an ion range of approximately 9-11 microns. This was to ensure that the particles did not pass completely through the targets since it would impart very little energy and would be less likely to create DNA damage. The energy was calculated utilizing SRIM and TRIM [15] with the proscribed values. The input parameters, ion ranges, and ionization with energy loss plots are shown in Figures 12-14, respectively.

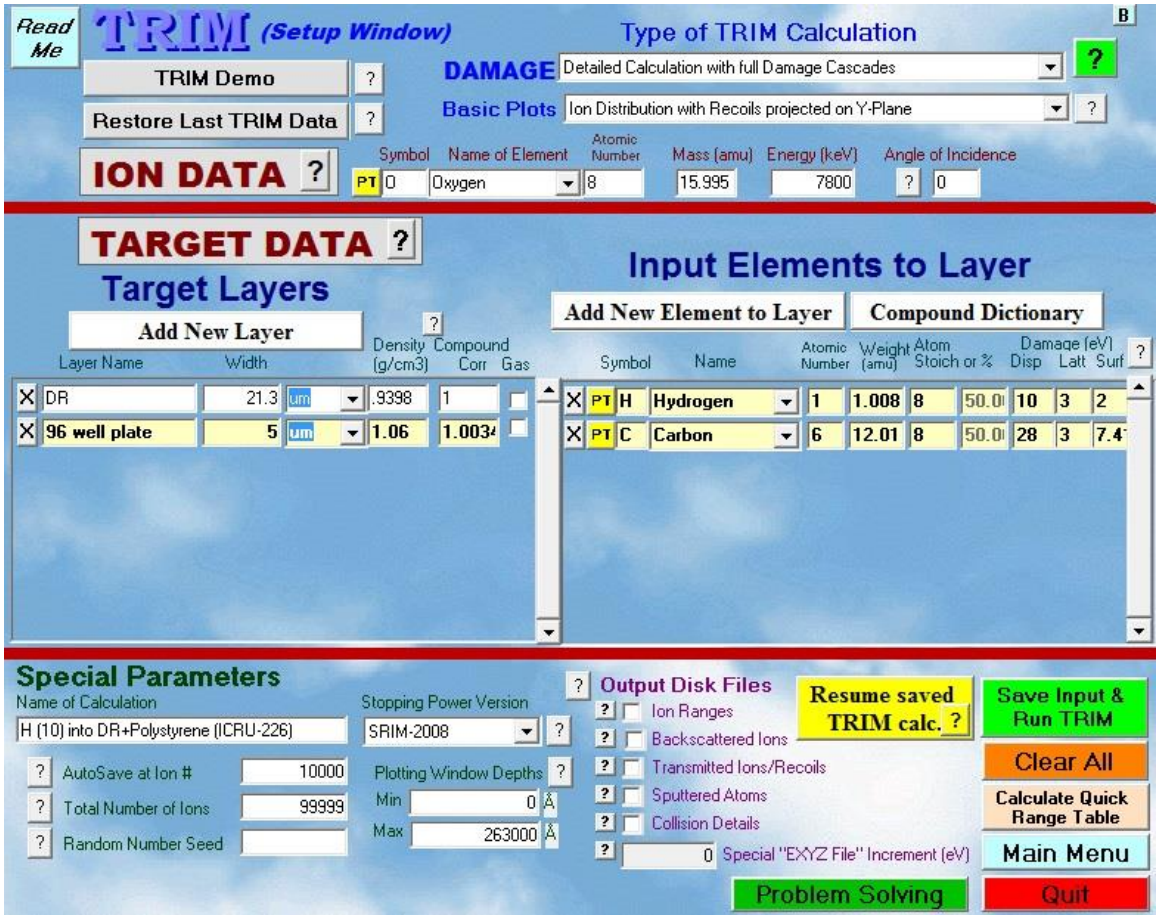

Figure 11. Input parameters for energy calculation of bombarding oxygen ions.

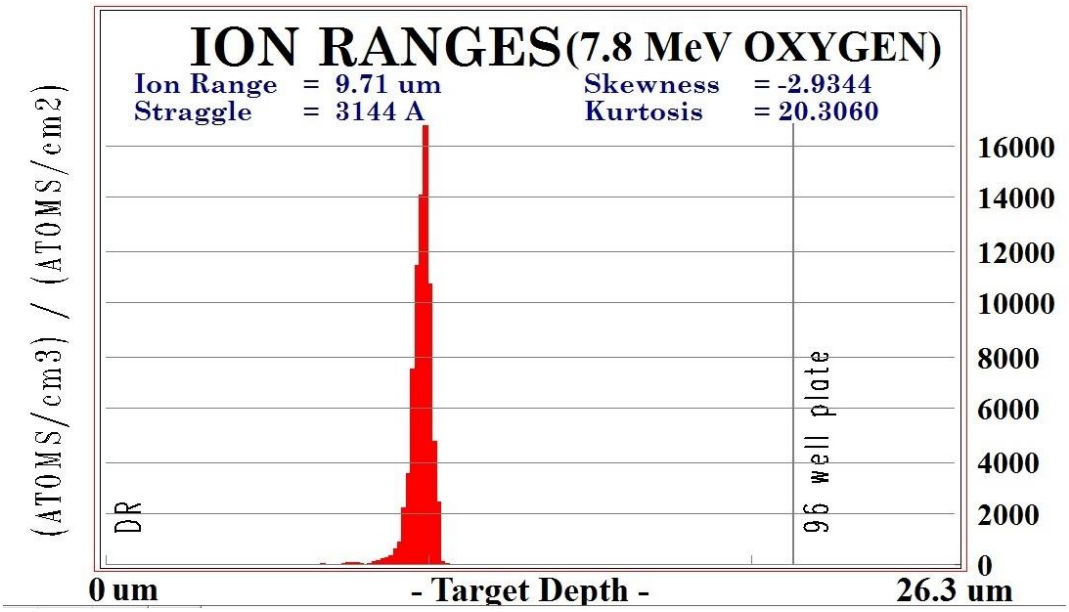

Figure 12. Ion ranges from 7.8MeV oxygen ions.

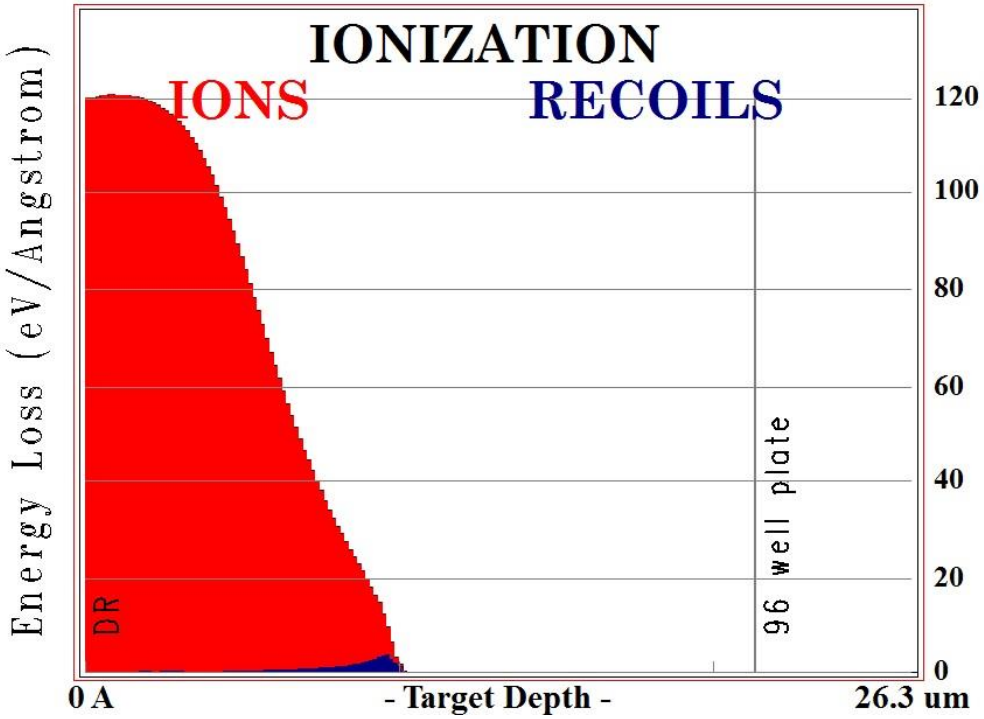

Figure 13. Energy loss of the bombarding particles due to ionizations in ev $Å^{-1}$. Recoils are the secondary ions created from knock-on collisions from the incident particles. The method of ionization is irrelevant to this research.

As can be seen in Figure 13, 7.8MeV oxygen ions have a range of 9.71μm which is just under half of the target cell depth. Theoretically, one could kill a maximum of three to four cell layers from the oxygen exposure, or about 50% of the total cells. The experiment entailed exposing samples to either 500, 1000, or 10,000 Gy of radiation. Using an energy loss value of 121 ev $Å^{-1}$ as shown in Figure 14 the necessary fluence can be calculated. The differences in exposure fluences merely impact the amount of time the gate is open on the beam line. Higher fluences equate to a greater number of particles incident to the target, but the energy (and resultant penetration) is the same for every exposure. The error in fluence values remained within two percent for the duration of the test. Target fluence values are shown in Table 9.

$$Dose = \frac{Ionization}{Density} * Fluence$$

Table 9. Oxygen Dose per Well

Target Exposure (Gy)	Fluence (ions cm^{-2}) (± 8%)
500	$2.42x10^{8}$
1000	$4.84x10^{8}$
10,000	$4.84x10^{9}$

Oxygen Irradiation of Samples

The samples were desiccated two days prior to shipment to SNL which took four days. Thirteen days after desiccation, the non-irradiated, vacuumed controls were placed

in the chamber under vacuum at 10^{-7} torr for 60 hours. The radiation experiment began 17 days after desiccation and concluded at 20 days. The samples were returned to USAFSAM at 25 days and rehydration began at 30 days. The non-irradiated, non-vacuumed control was included to determine cell survival after nearly a month spent dehydrated.

For the experiment, the plate lid was adhered to the x,y stage mounted within the QASPR-3 target chamber. The stage had limited mobility and only three wells could be irradiated at a time. Once complete, the chamber was re-pressurized and the plate lid was physically remounted in order to treat the next three rows. This resulted in each plate experiencing eight cycles of vacuum and re-pressurization.

Once the plate was mounted on the stage and the chamber was under vacuum, the beam spot size was characterized by placing it over a phosphorus target and measuring the intensity of the fluorescence via a camera. The size (area), shape, and intensity of the beam determined the number of shots required to cover the spots as well as the correction factor needed to "stitch" the shots together for a uniform exposure. Beam sizes varied from 1.5mm to 2mm so some spots required a 4x4 grid while others required a 4x5 grid to irradiate the entire sample. Once the beam was characterized, the software calculated the necessary time to keep the gate open on the beam line in order to achieve the desired fluence. Throughout the experiment, the beam never exceeded eight percent variation of the desired fluence and most of the spots were within five percent. On average, the technician was capable of completing one and a half plates per day and all exposures were completed within four days.

Rehydration of Samples and Spotting Post Oxygen Irradiation

The samples were rehydrated in an identical manner to those processed in the UV experiment. Using a single-channel pipette, 60µl of fresh TGY broth was placed on a spot. The samples were pipetted up and down approximately 20 times. The media remained on the plate lid while the next spot was rehydrated in the same manner. Once all eight strains were rehydrated they were again pipetted up and down approximately 20 times to ensure as many cells as possible were fully re-suspended. The ~60µl sample was placed in the top well of a 96-well plate and then each strain was run through a full dilution series. Then 5µl from each well in the column was placed on a 1xTGY agar plate and the spots were allowed to dry. The plates were then partially sealed in a plastic bag and incubated at 32°C for 48 hours.

Colony Counting Post Oxygen Irradiation

Following the incubation period, individual colonies were visually counted. The untreated controls were all counted at the 10^{-5} dilution. Most of the treated colonies were counted at the 10^{-4} and 10^{-5} dilutions. The colony counts were recorded and are reported in Appendices I-S.

Statistical Methods of Comparison

In accordance with previous *D. radiodurans* resistance research conducted at the Air Force Institute of Technology (AFIT) [4], a one-tailed Student's t-statistical analysis was performed to establish any statistical difference between the various populations. The method allows one to draw a conclusion with a given confidence the likelihood that two samples, μ_1 and μ_2, are the same. As noted by Lenker, this method is useful when working

with small sample sizes (< 30 samples) and is predicated on "the following assumptions: 1 – the two samples are randomly selected in an independent manner from the two target populations, 2 – both samples' populations distributions that are approximately normal, and 3 – the population variances are equal." [4]

This statistical method requires one to establish a null hypothesis, H_0, and an alternate hypothesis, H_a. In this experiment the null hypothesis generally proposed that the two samples were not statistically different where the alternate hypothesis proposed that they were statistically different. A test statistic, t, was calculated by the following equation:

$$t = \frac{(\overline{x_1} - \overline{x_2})}{\sqrt{s_p^2}(\frac{1}{n_1} + \frac{1}{n_2})}$$

where $\bar{x}$ is the mean colony count for each sample population, n is the number of samples in the population, and s_p^2 is the pooled sample estimator which was calculated via the following equation:

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

where s is the sample variance of each population. The degree of freedom is reflected in the denominator. For this experiment, α was set at 0.05 so all deductions are with 95% confidence. The rejection region of the hypothesis is if $t > t_a$ in which case one can conclude that the two populations are statistically different.

IV. Analysis and Results

Chapter Overview

The purpose of the chapter is to review the growth of the rehydrated wild type and mutant strains following UV and oxygen ion exposure compared with the CFU input and the untreated controls. It will also highlight the results that appear atypical, unexpected, or illogical. In these cases the causes that produced them will be diagnosed. It will cover the statistical comparisons as well as an evaluation of the biochemistry that resulted in those differences. The chapter will also cover some cases that did not show a statistical difference, although the majority of these instances will be ignored.

UV Experiment

For the UV experiment, the CFU input control was compared against the desiccated but untreated control group. Then the treated samples were compared to the untreated controls for each strain. Table 10 depicts the CFU counts pre- and post-UV exposure.

Table 10. UV Exposure Colony Rollup – Raw Data

Strain	R1 Wild Type							
CFU Input	20	14						
Untreated	15	11	14	13	17	15	6	9
UV Treated	3.1	2.2	2.1	1.9	2.3	2	2.5	5
Strain	(1.5) R1 'meroploid' WT and ΔrecF::KAN							
CFU Input	3	4						
Untreated	1.5	2.4	1.7	2.6	2.7	1.8	2.2	1.5
Treated	0.7	0.3	0.7	1.2	0.3	0.6	0.6	1
Strain	(5) R1 ΔDR_1279::mlox (MnSOD KO)							
CFU Input	10	16						
Untreated	11	5	13	10	12	12	4	4
Treated	1.5	2	2.6	3	5	5	2.6	8
Strain	(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)							
CFU Input	17	19						
Untreated	6	8	5	2	5	8	5	3
Treated	0.8	2	0.8	4	1.5	1.8	1.7	1.2
Strain	(11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)							
CFU Input	25	18						
Untreated	15	10	12	12	11	11	7	1
Treated	3	0.8	2	3	6	3	1.6	1.8
Strain	(16) R1 ΔuvrB::KAN							
CFU Input	34	25						
Untreated	19	15	15	19	12	21	5	
Treated	1	9	5	2.3	3	3	2.2	6
Strain	(6A) R1 'meroploid' WT and ΔrecF::KAN							
CFU Input	13	15						
Untreated	3	8	10	4	12	5	3	4
Treated	0.7	1.1	1.8	0.8	1.2	0.9	0.7	1.7
Strain	(27) R1 ΔuvrB::KAN							
CFU Input	19	36						
Untreated	18	12	16	13	7	17	8	8
Treated	2	1.7	0.9	2.5	1.1	2.3	2.5	1.5

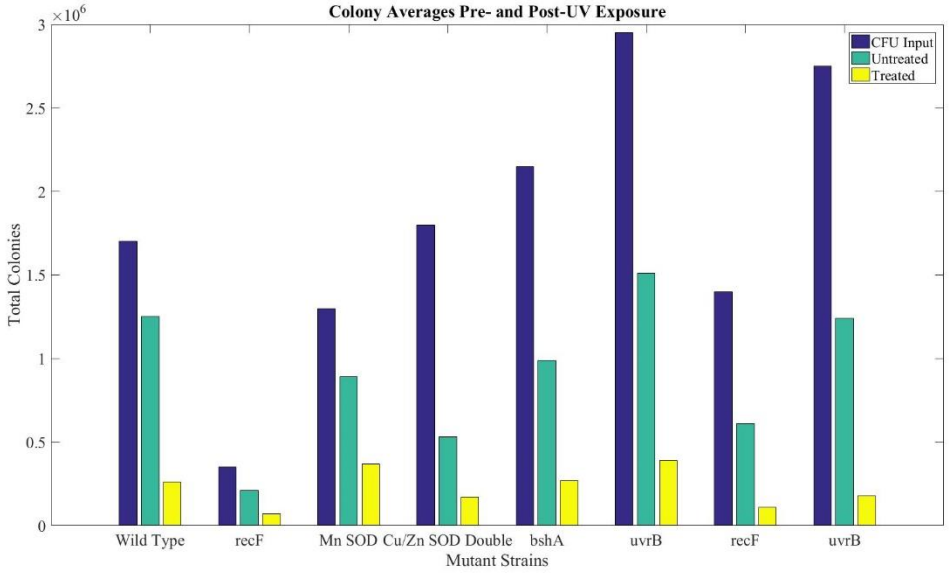

Figure 14. Colony Averages Pre- and Post-UV Exposure

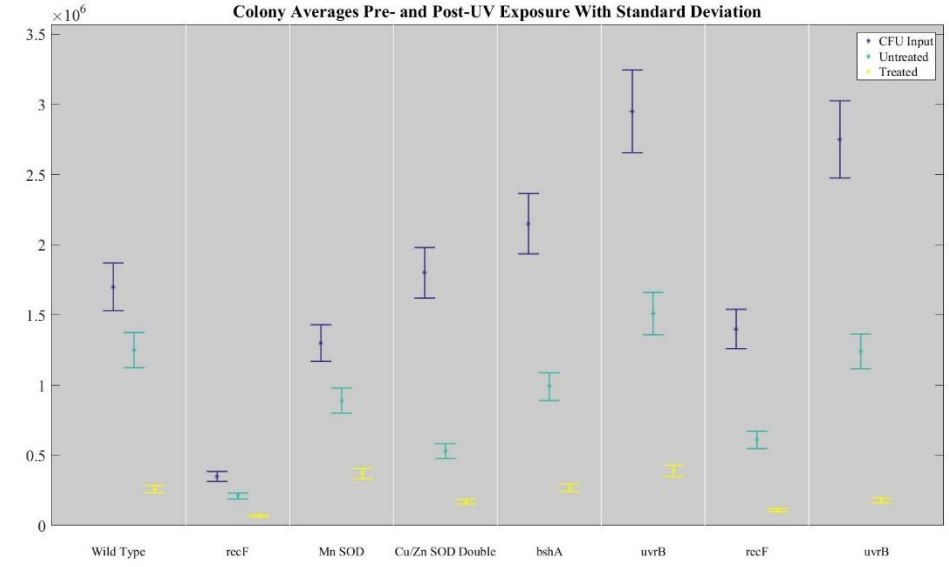

Figure 15. Colony Averages Pre- and Post-UV Exposure with Standard Deviation

The populations for every strain except #1 (wild type) and #5 (MnSOD KO) showed a statistical difference merely from the desiccation. Every strain tested in this experiment showed significant reductions in populations compared with the untreated controls, as shown above in Figure 15. The percentage of the population killed in the UV experiment ranged from #5 (MnSOD) at 58% to #27 (*ΔuvrB*) at 85%. It should be noted that the percentage kill is calculated against the untreated population rather than the CFU input. Figure 16 shows an untreated sample population for each mutant while Figure 16 shows a treated sample population for each mutant.

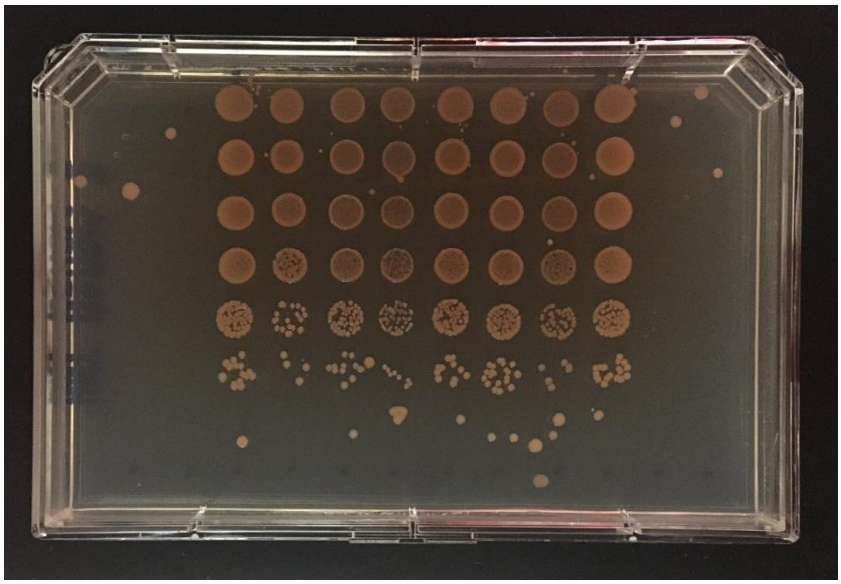

Figure 16. Untreated sample populations for UV experiment. The strains, moving left to right, are wild type #1, Mutant #5, Mutant #8, Mutant #11, Mutant #16, Mutant #6A, and Mutant #27

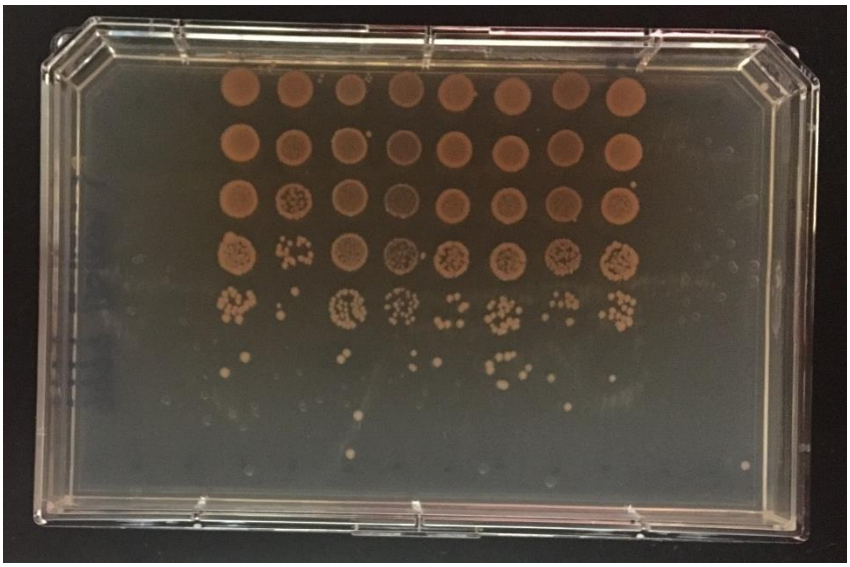

Figure 17. Treated sample population for the UV experiment. The strains, moving left to right, are wild type #1, Mutant #5, Mutant #8, Mutant #11, Mutant #16, Mutant #6A, and Mutant #27

Oxygen Experiment

For the oxygen ion experiment, the CFU input controls were first compared against the untreated, unvacuumed control group. In all cases, it was shown that there was no statistical difference between the CFU input and the controls. Next, the untreated and unvacuumed controls were compared against the untreated and vacuumed controls. In all cases, these were not shown to be within the rejection region. In order to increase the sample size and the statistical power, the unvacuumed and vacuumed controls were grouped and will hereafter just be referred to as untreated controls. Figure 18 shows the average colony counts for each mutant following exposure to oxygen ions. Table 11 shows the full colony counts of every sample included in the oxygen experiment.

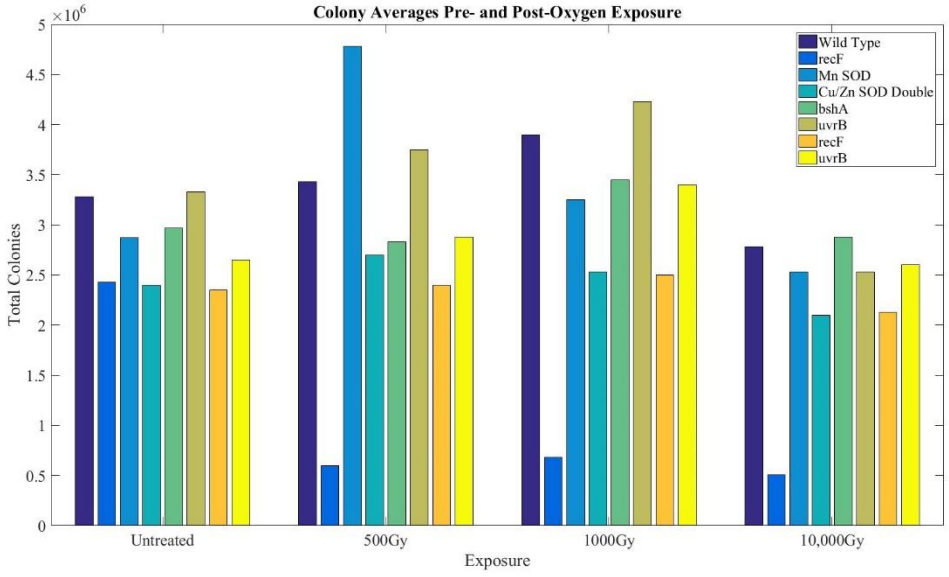

Figure 18. Colony Averages Pre- and Post-Oxygen Exposure

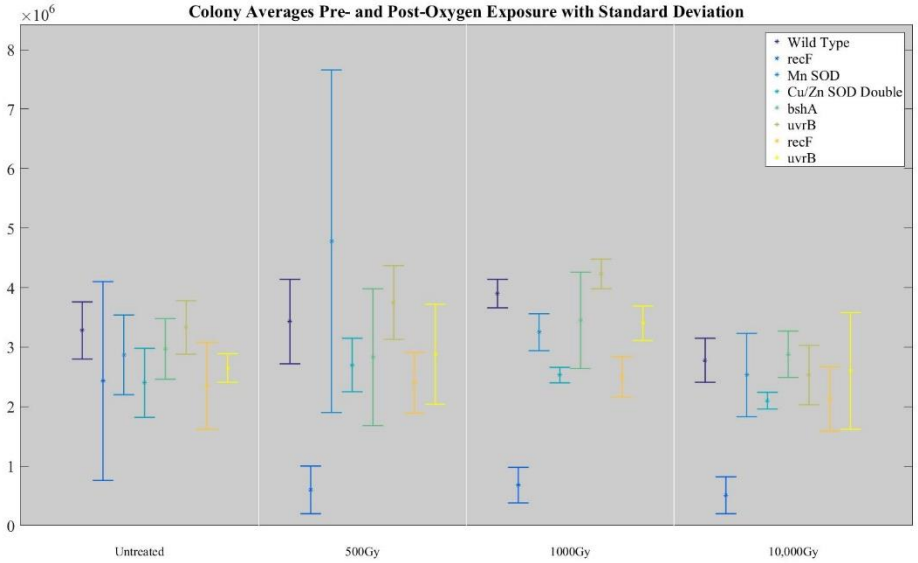

Figure 19. Colony Averages Pre- and Post-Oxygen Exposure with Standard Deviation

Table 11. Oxygen Exposure Colony Rollup – Raw Data

Strain	R1 Wild Type											
CFU Input	21	15	9									
Untreated Unvacuumed	41	32										
Untreated Vacuumed	32	33	26	33								
500 Gy	26	32	36	43								
1000 Gy	42	40	37	37								
10,000 Gy	28	23	32	28								
Strain	R1 'meroploid' WT and *ΔrecF::KAN*											
CFU Input	2.7	2.7	6									
Untreated Unvacuumed	34	11										
Untreated Vacuumed	42	7	10	42								
500 Gy	12	3.5	4.7	3.9								
1000 Gy	11	6	6	4								
10,000 Gy	3	6	9	2.3								
Strain	R1 *ΔDR_1279::mlox* (MnSOD KO)											
CFU Input	10	12	23									
Untreated Unvacuumed	37	34										
Untreated Vacuumed	21	31	28	21								
500 Gy	90	42	31	28								
1000 Gy	29	36	31	34								
10,000 Gy	32	16	24	29								
Strain	R1 *ΔDR_1546::KAN ΔDR_A0202::NAT* (Cu/ZnSOD Double KO)											
CFU Input	15	20	18									
Untreated Unvacuumed	21	35										
Untreated Vacuumed	23	18	24	23								
500 Gy	27	28	21	32								
1000 Gy	25	25	24	27								
10,000 Gy	23	20	20	21								
Strain	R1 *ΔbshA::mlox* (Bacillithiol Biosynthesis)											
CFU Input	10	14	20									
Untreated Unvacuumed	28	28										
Untreated Vacuumed	24	39	31	28								
500 Gy	34	11	33	35								
1000 Gy	43	24	33	38								
10,000 Gy	31	25	33	26								
Strain	R1 *ΔuvrB::KAN*											
CFU Input	15	25	21									
Untreated Unvacuumed	33	34										
Untreated Vacuumed	28	40	36	29								
500 Gy	43	41	29	37								
1000 Gy	39	42	45	43								
10,000 Gy	20	22	29	30								
Strain	R1 'meroploid' WT and *ΔrecF::KAN*											
CFU Input	7	9	17									
Untreated Unvacuumed	21	37										
Untreated Vacuumed	18	26	22	17								
500 Gy	24	22	19	31								
1000 Gy	29	24	21	26								
10,000 Gy	14	22	27	22								
Strain	R1 *ΔuvrB::KAN*											
CFU Input	15	21	21									
Untreated Unvacuumed	29	28										
Untreated Vacuumed	27	28	24	23								
500 Gy	39	19	31	26								
1000 Gy	34	37	35	30								
10,000 Gy	35	14	22	33								

The treated samples were then compared to the untreated wild type control population. The populations for Mutants #1.5 (*ΔrecF*), #8 (Cu/ZnSOD Double KO) and #16 (*ΔuvrB*) showed a statistical difference at all exposure levels. Mutants #11 (*ΔbshA*) and #27 (*ΔuvrB*) showed a statistical difference at 500Gy and 10,000Gy, but actually showed an increase in total colonies at 1000Gy when compared to the untreated wild type control. This anomaly has been assessed to be a procedural error resulting in poor mixing during the serial dilutions. Figures 19, 20 and 21 show the comparison plots for each mutant at 500Gy, 1000Gy and 10,000Gy, respectively. Table 12 shows the kill percentages for the three mutants that displayed a statistically significant difference at all exposure levels.

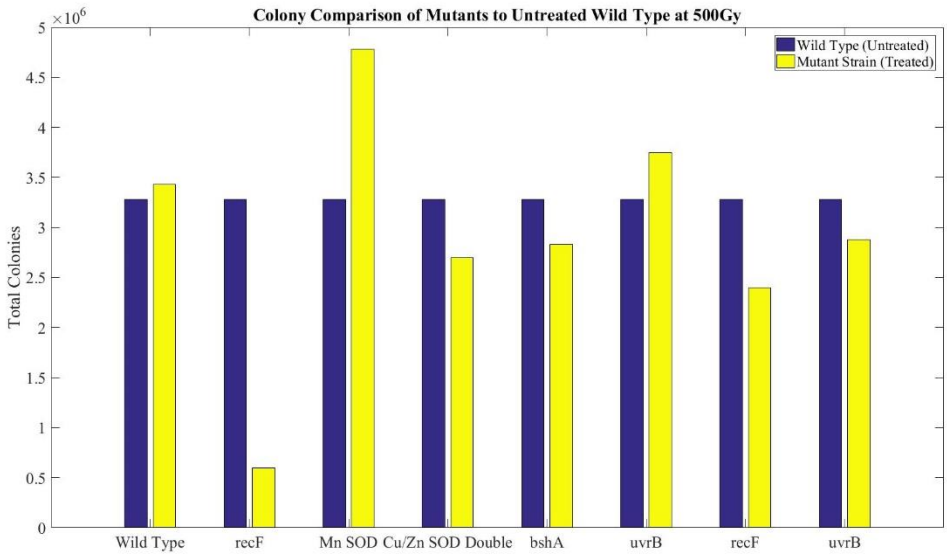

Figure 20. Colony Comparison of Mutants to Untreated Wild Type at 500Gy Oxygen Exposure

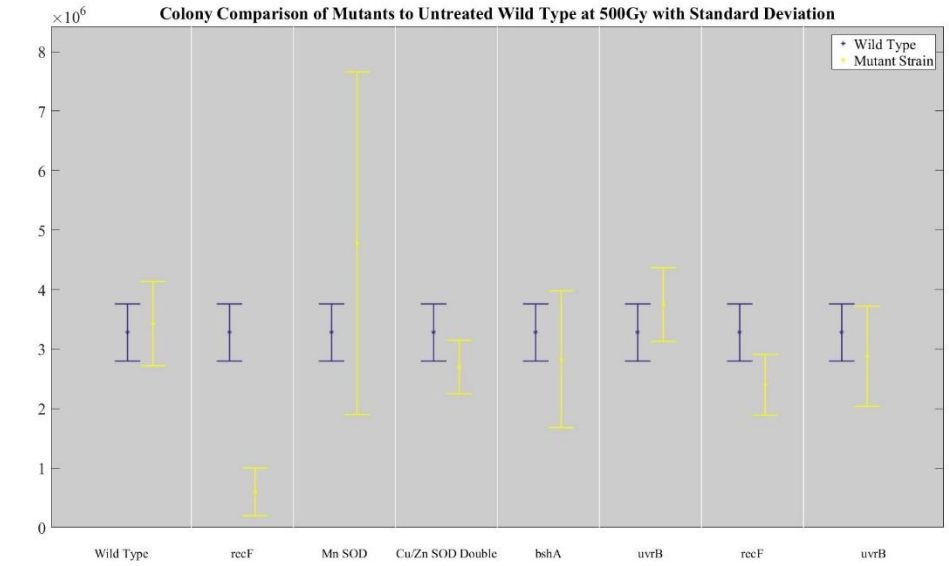

Figure 21. Colony Comparison of Mutants to Untreated Wild Type at 500Gy Oxygen Exposure with Standard Deviation

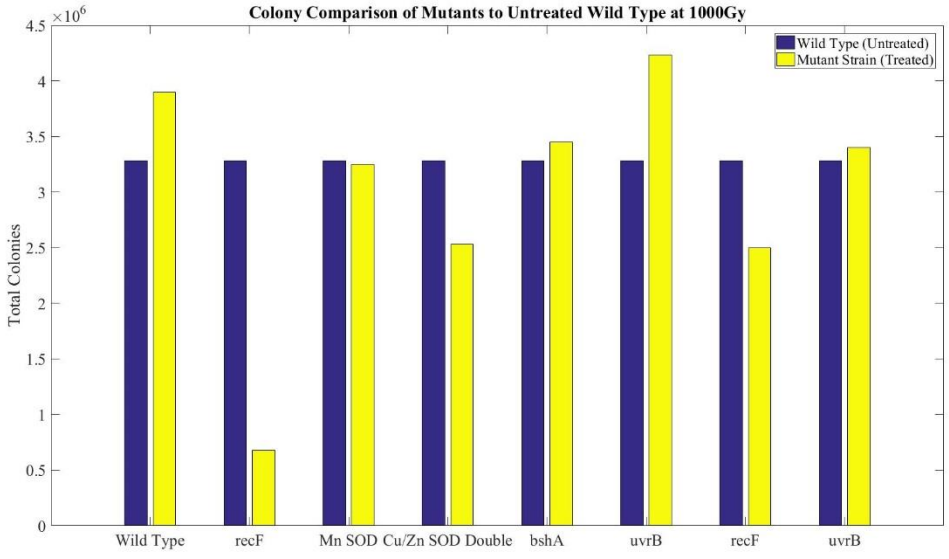

Figure 22. Colony Comparison of Mutants to Untreated Wild Type at 1000Gy Oxygen Exposure

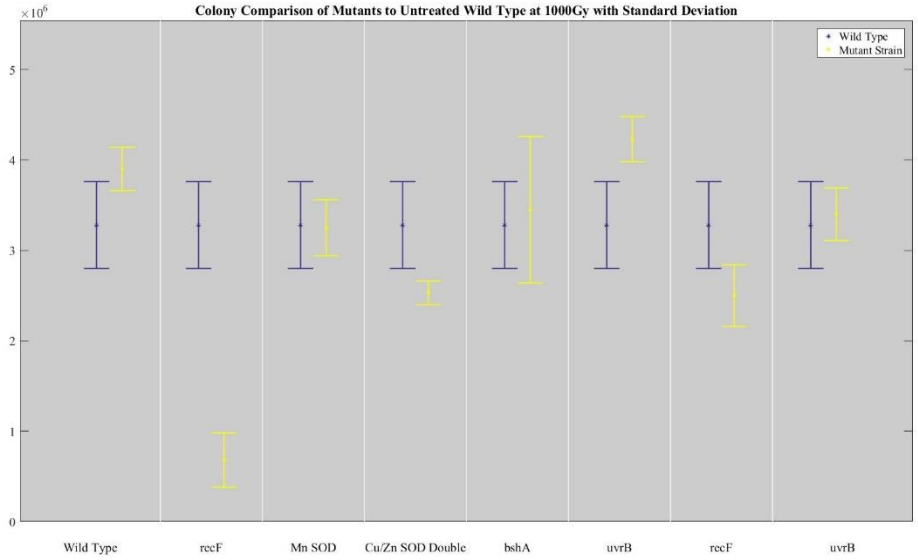

Figure 23. Colony Comparison of Mutants to Untreated Wild type at 1000Gy with Standard Deviation

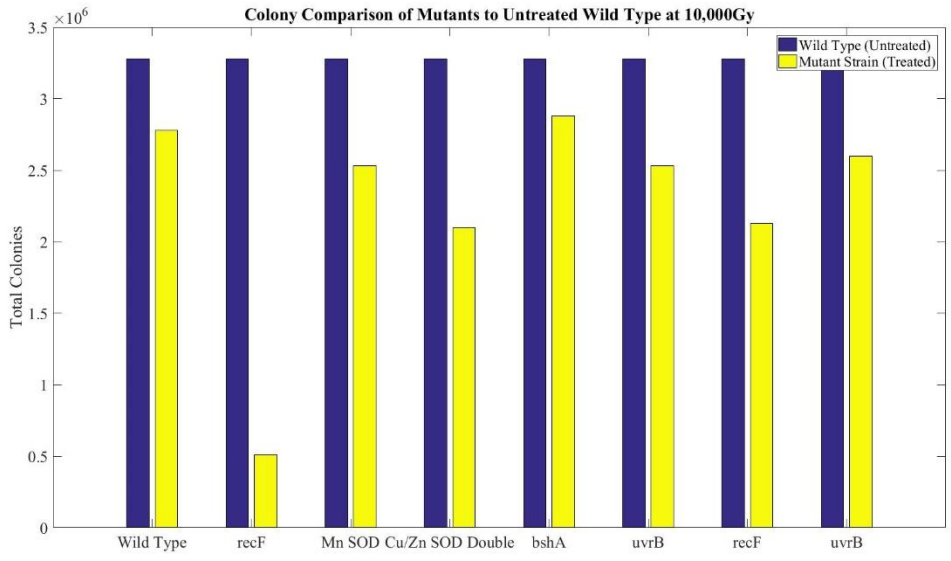

Figure 24. Colony Comparison of Mutants to Untreated Wild Type at 10,000Gy Oxygen Exposure

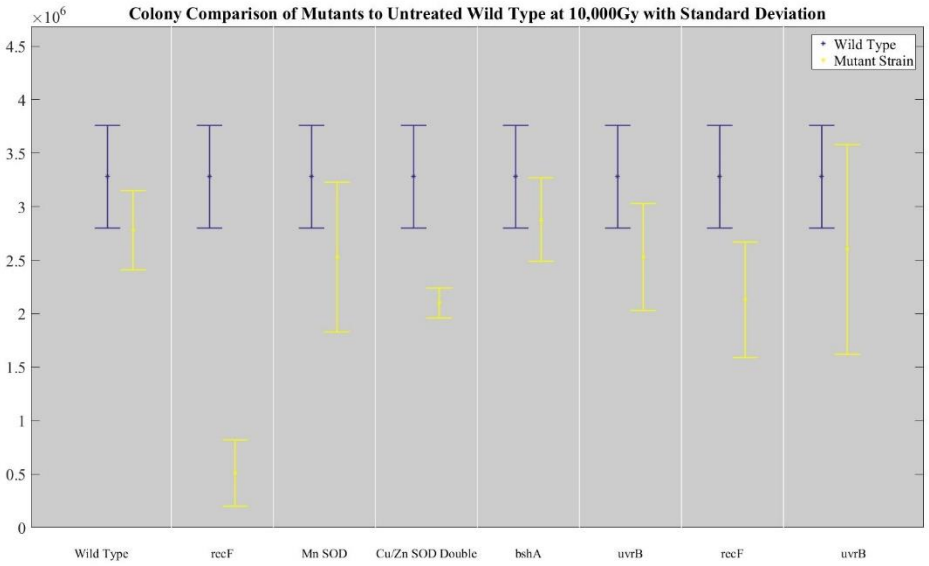

Figure 25. Colony omparison of mutants to untreated wild type at 10,000Gy with standard deviation

Table 12. Statistically significant mutants compared to the untreated wild type controls

Percentage Killed Compared to Untreated Wild Type			
	Exposure (Gy)		
Mutant	500	1000	10000
1.5	82	79	85
8	18	23	36
6A	27	24	35

The next step in the analysis was to compare each treated sample to its own untreated control group. This comparison yielded very few populations that fell within the rejection region. The only mutant to show a statistical difference at all exposure levels was

Mutant #1.5 (*ΔrecF*). The only other population that fell within the rejection was Mutant #16 (*ΔuvrB*) at 10,000Gy exposure although it did not do so with very strong correlation.

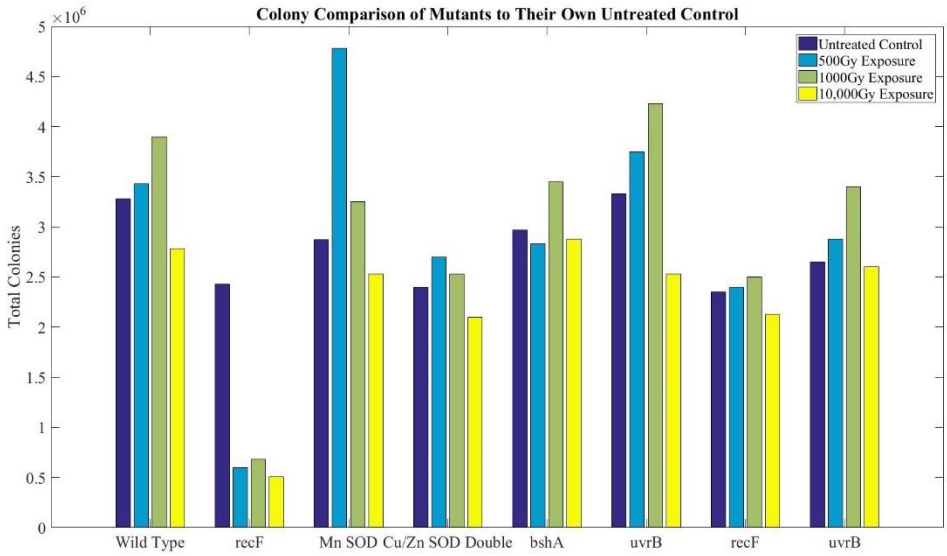

Figure 26. Colony comparison of mutants to their own untreated control

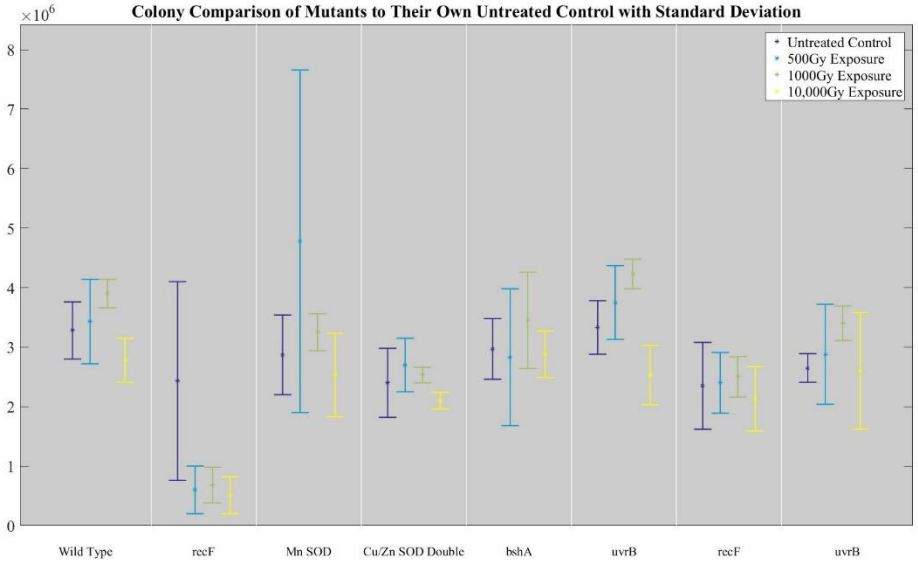

Figure 27. Colony comparison of mutants to their own untreated control with standard deviation

The final step in the analysis was to compare each mutant to the wild type at each exposure level. This comparison yielded the most results with Mutants #1.5 (*ΔrecF*) and #6A (*ΔrecF*) showing a statistically significant difference to the exposed wild type at all three exposure levels. Mutant #8 (Cu/ZnSOD Double KO) showed a difference at 1000Gy and 10,000Gy while Mutants #5 (MnSOD KO) and #27 (*ΔuvrB*) only showed a difference at 1000Gy.

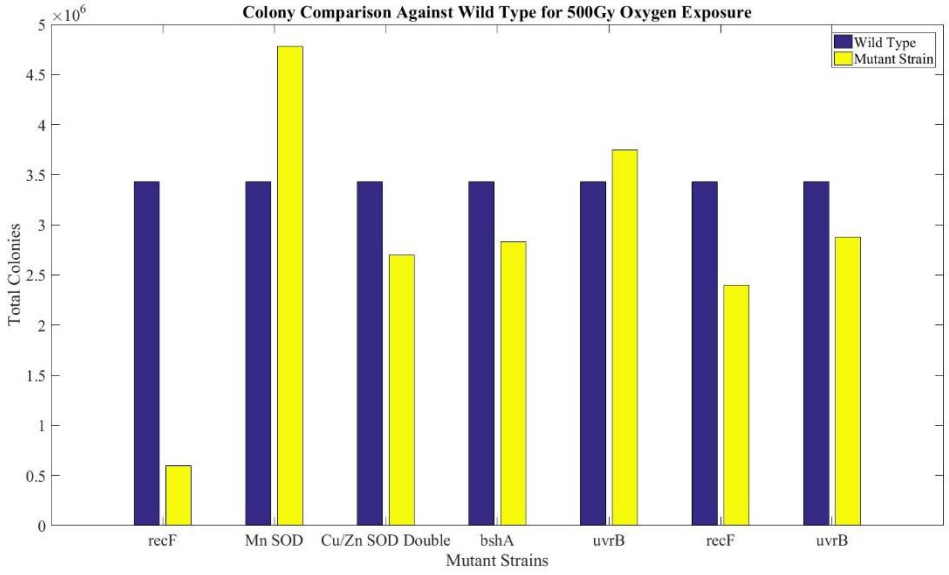

Figure 28. Colony comparison against wild type for 500Gy oxygen exposure

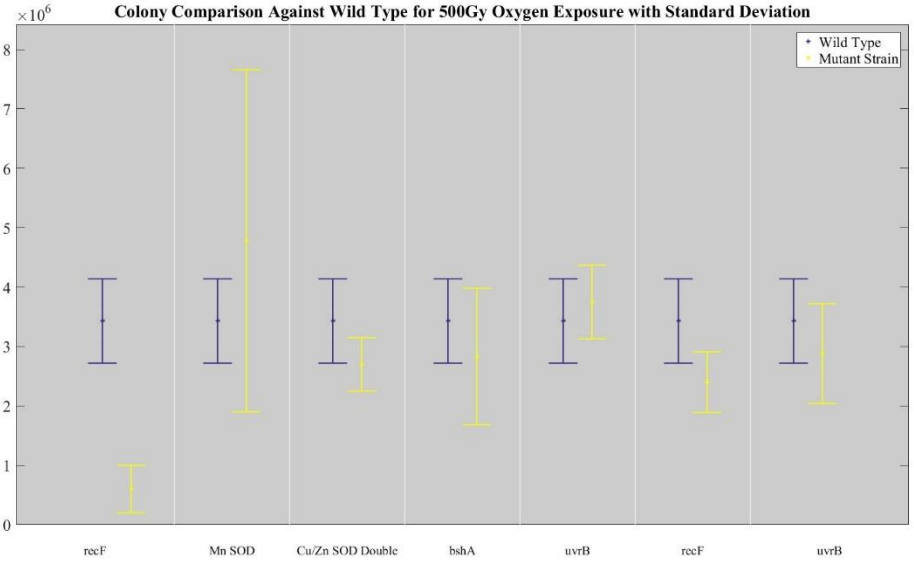

Figure 29. Colony comparison against wild type for 500Gy oxygen exposure with standard deviation

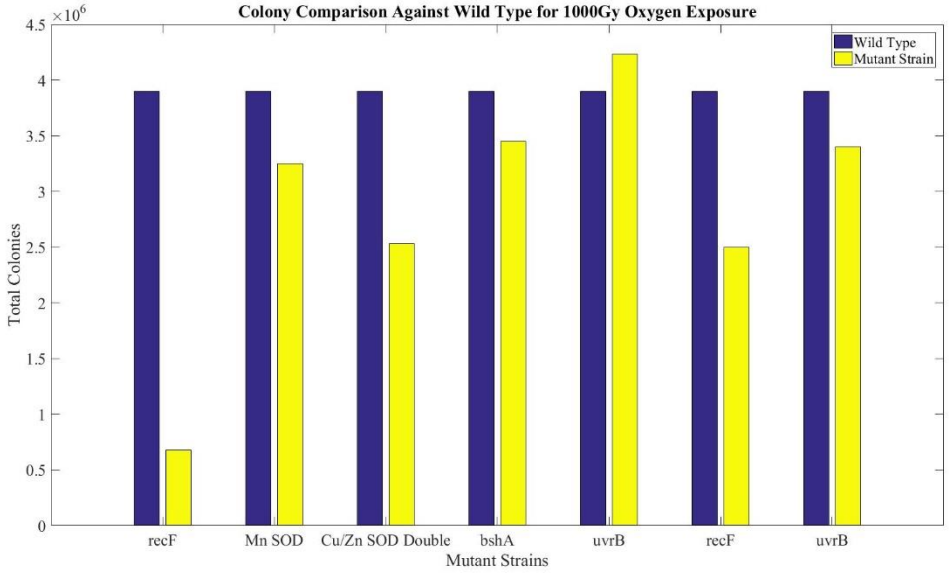

Figure 30. Colony comparison against wild type for 1000Gy exposure

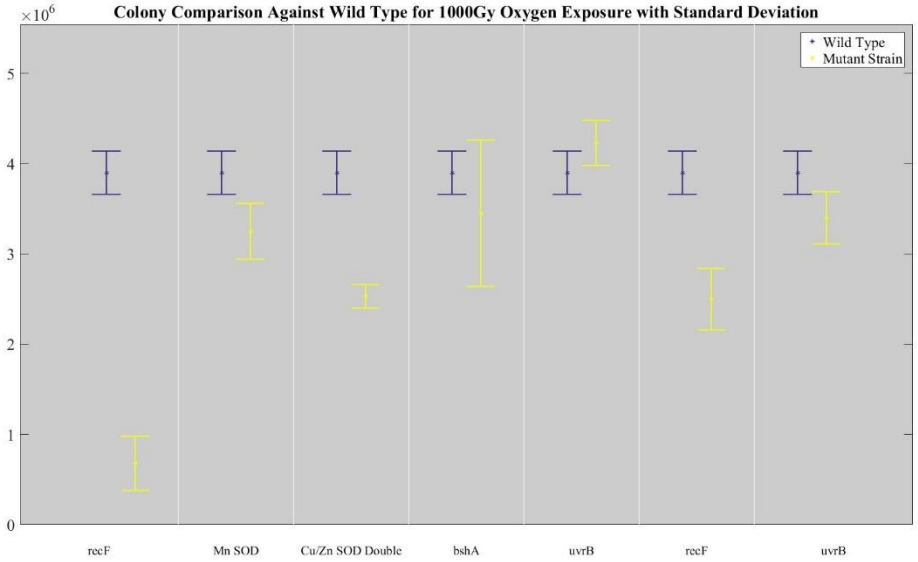

Figure 31. Colony comparison against wild type for 1000Gy oxygen exposure with standard deviation

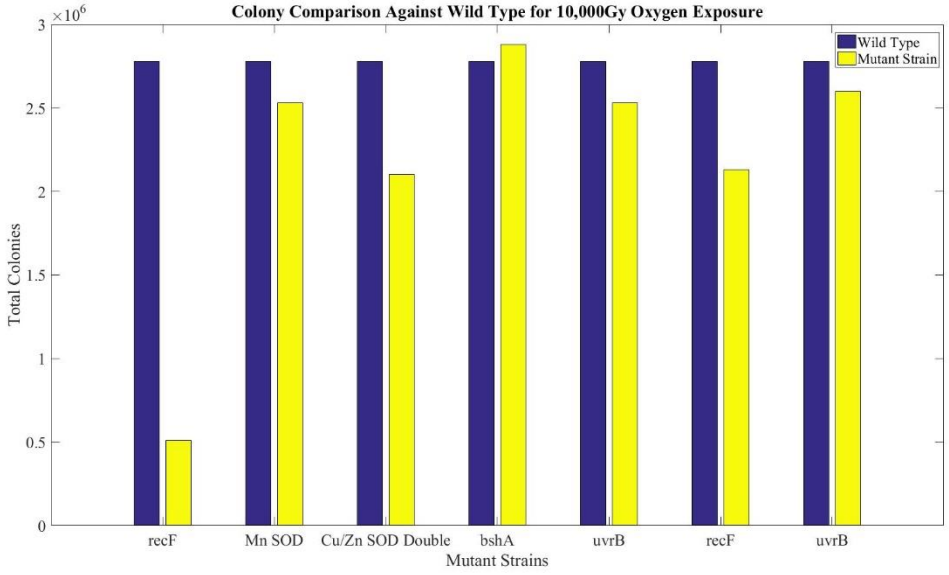

Figure 32. Colony comparison against wild type for 10,000Gy exposure

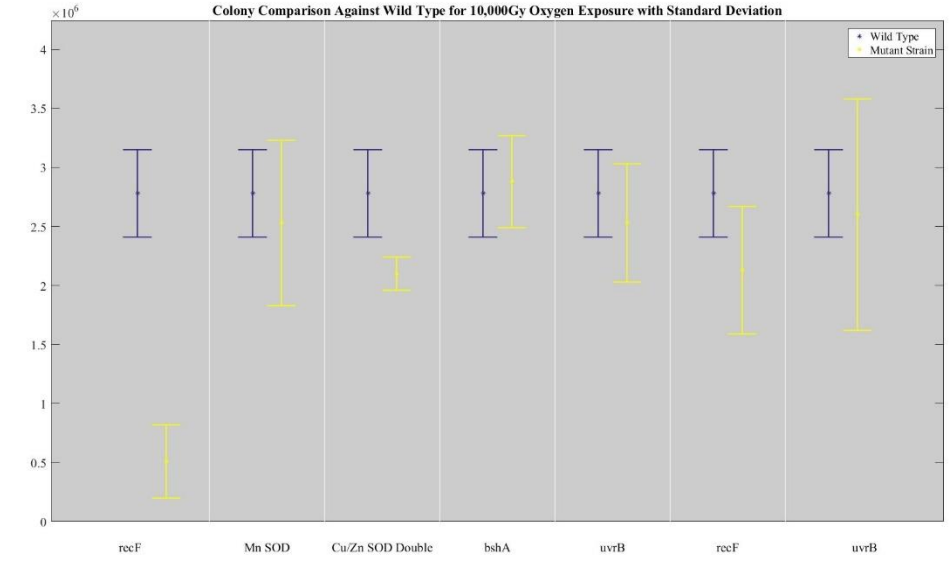

Figure 33. Colony comparison against wild type for 10,000Gy oxygen exposure with standard deviation

UV Experiment Findings

While the UV experiment was intended mostly to develop and become familiar with the procedures for desiccation, rehydration, serial dilutions, and spotting in preparation for the HCP experiment at SNL, some interesting trends resulted. For UV radiation at 9999J cm^{-2} it has been demonstrated that all but wild type #1 and Mutant #8 (Cu/ZnSOD Double KO) showed a statistically significant reduction in population densities merely from the desiccation and vacuum process. Of even more importance, every treated mutant as well as the wild type in this experiment fell very firmly within the rejection region when compared to their own untreated populations.

Nearly all DNA damage from UV radiation would be in the form of SSB. If enough SSB breaks accumulate, one could also expect induced DSBs to form. With this in mind it would be expected that Mutants #16 and #27 may show evidence of increased kill rates, or at least growth latency since the *uvrB* gene is instrumental is SSB repair. This was proven since the mutants experienced 74% and 85% kills, respectively. However, the evidence indicates that *every* strain experienced at least 50% kills with some approaching 80%. Mutants #1.5 and #6A (*ΔrecF*) showed growth latency throughout the experiments indicating that recF plays a fundamental role in DNA replication during cell division.

Oxygen Experiment Findings

The HCP experiment also yielded some very important, and in some ways expected, results that support all three hypotheses. For oxygen ions accelerated to 7.8MeV, it has been demonstrated that both Mutants #1.5 and #6A (*ΔrecF*) display statistical differences when compared to the untreated and treated wild type controls for all exposure

levels. However, only Mutant #1.5 shows any statistical difference when compared to its own untreated control.

Mutants #5 (MnSOD KO) and #27 (*ΔuvrB*) were in the rejection region at the 1000Gy exposure level, but were not at the 10,000Gy exposure. At first glance, this seems to suggest that there was a greater response at the lower exposure levels. However, this is misleading since, in all cases, the populations decreased at 10,000Gy. The statistical comparison is really comparing the rate at which the populations decrease, so Mutants #1.5 (*ΔrecF*) and #27 (*ΔuvrB*) decreased at a greater rate at 1000Gy but were at about the same rate for 10,000Gy. This does not suggest that the bacteria experienced recovery at the higher exposure level.

It should be noted that for every mutant, the desiccated controls all showed population increases from the CFU input. This defies logic if one assumes that all procedures were performed in the exact same manner. Since this was the case for all mutants it can only be deduced that there was some procedural error that contributed to the discrepancy. For this reason, the CFU inputs were ignored and the treated populations were only compared to the untreated populations.

V. Conclusions and Recommendations

Conclusions of Research

UV Experiment

The results of the UV experiment are generally consistent with past research suggesting that a dose of 10,000Gy of gamma radiation will result in about 10^{-2} lethality. [19] While all mutants displayed at least 50% kill rates, both of the *uvrB* mutants showed

85% lethality. This is well below two-log lethality, but that should be expected considering the total absorbed dose is only about 400Gy of low-LET radiation.

The primary damage from UV radiation results in the formation of thymine and pyrimidine dimers and SSB. The uvrB repair gene is necessary in the recruitment of both uvrA and uvrC. These genes help accomplish the excision of the identified lesion. Since the *uvrB* mutant was unable to utilize this pathway for repairing the damage, and it is the preferred pathway for SSB, the results of the experiment appear to be within the bounds of expectation.

The samples were only desiccated for a day prior to UV treatment, so they were relatively "wet" compared to the samples that went to SNL. It is probable that they were not yet in a stationary or "dormant" phase at the time of irradiation making them more susceptible to radiation-induced damage. It is also probable that there were more oxide radicals present in this experiment. Finally, repair mechanisms are up-regulated following a prolonged period of dehydration. In the case of the UV-treated cells, they likely never achieved a dry enough state to initiate this acceleration of repair pathways. In light of these factors, the UV results are not particularly surprising.

Oxygen Experiment

The first hypothesis for this experiment stated that *D. radiodurans* exposed to high-LET HCP will exhibit a statistical difference from the untreated wild type control group. Mutants #1.5 (*ΔrecF*), #8 (Cu/ZnSOD Double KO) and #16 (*ΔuvrB*) showed a statistical difference at all exposure levels. Mutants #11 (*ΔbshA*) and #27 (*ΔuvrB*) showed a statistical difference at 500Gy and 10,000Gy. These mutants all confirmed the hypothesis. Although

Mutant 6A (*ΔrecF*) was created in the exact same manner as Mutant #1.5 it unexpectedly did not confirm the first hypothesis.

The second hypothesis for this experiment stated that mutants exposed to high-LET HCP will exhibit a statistical difference from their own untreated control group. Mutant #1.5 (*ΔrecF*) showed a difference at all exposure levels. The only other population to fall within the rejection region was Mutant #16 (*ΔuvrB*) at 10,000Gy exposure, although it did so with a weak correlation. These results seem to indicate that the threshold of damage to achieve lethality was not met, although it may merely be a function of the maximum depth of exposure and the probability of getting statistically significant kills.

The third hypothesis for this experiment stated that exposed DNA repair mutants will exhibit a statistically significant difference when compared to the exposed wild type. Mutants #1.5 (*ΔrecF*) and #6A (*ΔrecF*) showing a statistically significant difference to the corresponding exposed wild type at all three exposure levels which was more in line with the expected results. Mutant #8 (Cu/ZnSOD Double KO) showed a difference at 1000Gy and 10,000Gy suggesting that the exposure threshold lies somewhere between 500 and 1000Gy. Mutants #5 (MnSOD KO) and #27 (*ΔuvrB*) only showed a difference at 1000Gy.

The validity of a previous experiment was questioned because the energy was so high, most of it was deposited beyond the cell layers. This experiment was designed to place the Bragg peak in the middle of the full cell depth to ensure that all the energy was deposited within the media. 7.8MeV oxygen ions were calculated to penetrate about 10 μm of the estimated 21.7 μm depth. This suggests that even if one could kill every cell within the exposure depth, it would only equate to half of the total cells (discounting those that die due to desiccation.) This makes the analysis for the first two hypotheses difficult.

However, hypothesis three negates the need to take this into account because the comparison is to the treated wild type at the corresponding exposure. Any mutants that are accepted under the third hypothesis have most certainly experienced enhanced lethality due solely to the exposure.

Previous research indicated that *D. radiodurans* could experience 10,000Gy of gamma irradiation with two-log kills, which is astounding. However, this research showed that wild type *D. radiodurans* can sustain 10,000Gy of HCP exposure with less than two-log lethality. Relative biological effectiveness (RBE) is a weighting factor that expresses the ratio of the effectiveness of one type of radiation to another. In mammalian cells, HCP often have an RBE of 5-10 meaning that a dose is 5-10 times more damaging than an equivalent dose of gamma irradiation. The implication from this experiment is that for *D. radiodurans*, HCP have an RBE of 1. This is unlike any other cell that has ever been identified in the literature.

While this research did not achieve full gene knockout for the *recF* mutants, these experiments have clearly demonstrated that the gene plays a critical role in DSB repair. In contrast with previous studies that established *D. radiodurans* could withstand in excess of 10,000Gy of ionizing radiation, the *recF* mutants experienced over 80% lethality at a mere 500Gy of high-LET HCP bombardment. Surprisingly, the double copper/zinc SOD mutant experienced considerably greater kill rates from the oxygen exposure than either of the *uvrB* mutants even though it was designed to disrupt superoxide radical damage induced via low-LET radiation.

Throughout the duration of this study, the *recF* mutants displayed noticeable growth latency, even prior to irradiation. The gene is not only involved in DSB repair, but

it also plays a role in replication. There is clear evidence that *D. radiodurans* cannot survive without the gene since the knockout was not complete, so it follows that even a meroploid will struggle throughout its life cycle.

The general proposal that D. radiodurans' ability to efficiently and correctly repair its DNA following damage is the primary resistance mechanism to radiation-induced cell death is supported through this research. In particular, DSB repair appears to be what sets this organism apart from all others. It has shown that it is capable of sustaining tens to hundreds of DSB and reconstruct correct copies of its genome in order to continue its lifecycle. When one of the DSB repair pathways is compromised, it exhibits growth inhibition as well as poor resistance. Had the researchers been able to achieve a pure knockout, it is probable that the bacterium would have displayed a response to high-LET HCP bombardment that was on par with most other known organisms.

Recommendations for Future Research

There are a number of interesting results that beg further research. It is clear that the recF repair pathway is critical for DSB repair as was experienced through HCP bombardment. Bentchikou *et al.* identified uvrD as involved in the RecFOR pathway for DNA repair. [14] In this research, uvrB and recF were chosen specifically to isolate SSB and DSB repair mechanisms. However, uvrD plays a role in both repair mechanisms and may prove to be a vital link in the process. If the uvrD gene could be deleted, it would be beneficial to repeat the HCP bombardment in the same manner as this research.

While this project yielded interesting results, the error in the colony counts is such that it is unlikely to produce useable kill curves. The error almost certainly derived from a

procedural error in the rehydration and serial dilution, so there is no requirement to alter the irradiation parameters in order to achieve accurate curves. Also, more untreated control replicates would aid in the statistical comparisons.

Finally, there is some debate whether the method for calculating the required energy for the bombarding particles and the subsequent required fluence is accurate. It is possible that the particles penetrated much farther than expected, in which case the energy deposition would be much less in the target layers. There is also some doubt if the density of the cells changes significantly following desiccation. If the density decreases by even a third, the SRIM/TRIM model changes considerably. What this means in practical terms is that the chosen energy was too high and the majority of the energy was deposited beyond the cell layers. An accurate measurement of the density the dried spots would quickly answer this question.

Appendix A: WT UV Exposure Comparison

Wild Type UV Exposure Analysis

Data

Strain	R1 Wild Type								
CFU Input	20	14							
Untreated	15	11	14	13	17	15	6	9	
Treated	3.1	2.2	2	1.9	2.3	2	2.5	5	

All colony counts at 10^{-5} dilution

All exposures at 9999 Joules

Statistics

$n_{WT\text{-}CFU\ input}$	2	$n_{WT\text{-}Untreated}$	8	$n_{WT\text{-}UV}$	8	Percent Kill	79
$\bar{x}_{WT\text{-}CFU\ input}$	17.0	$\bar{x}_{WT\text{-}Untreated}$	12.5	$\bar{x}_{WT\text{-}UV}$	2.6		
$s_{WT\text{-}CFU\ input}$	4.2	$s_{WT\text{-}Untreated}$	3.6	$s_{WT\text{-}UV}$	1.0		

Population Comparisons

Comparison Set 12 - Wild Type

H_0 :	$\mu_{WT\text{-}CFU\ input} - \mu_{WT\text{-}Untreated} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{WT\text{-}CFU\ input} - \mu_{WT\text{-}Untreated} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	13.7500
t, test statistic	1.5350
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 1.5350 < 1.860, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}UV} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}UV} > 0$	Alternate Hypothesis = There is a difference between the Untreated population and the UV Treated Popula

s_p^2	7.0971
t, test statistic	7.4042
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 7.4042 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix B: Mutant 1.5 UV Exposure Comparison

Mutant 1.5 UV Exposure Analysis

Data

Strain	(1.5) R1 'meroploid' WT and ΔrecF::KAN								
CFU Input	3	4							
Untreated	1.5	2.4	2	2.6	2.7	2	2.2	1.5	
Treated	0.7	0.3	1	1.2	0.3	1	0.6	1	
All colony counts at 10^{-5} dilution									
All exposures at 9999 Joules									

Statistics

	$n_{1.5\text{-CFU input}}$	2	$n_{1.5\text{-Untreated}}$	8	$n_{1.5\text{-UV}}$	8	Percent Kill	67
	$\bar{x}_{1.5\text{-CFU input}}$	3.5	$\bar{x}_{1.5\text{-Untreated}}$	2.1	$\bar{x}_{1.5\text{-UV}}$	0.7		
	$s_{1.5\text{-CFU input}}$	0.7	$s_{1.5\text{-Untreated}}$	0.5	$s_{1.5\text{-UV}}$	0.3		

Population Comparisons

Comparison Set 13 - (1.5) R1 'meroploid' WT and ΔrecF::KAN

H_0 :	$\mu_{1.5\text{-CFU input}} - \mu_{1.5\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1.5\text{-CFU input}} - \mu_{1.5\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	0.2700
t, test statistic	3.5298
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 3.5298 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	0.1668
t, test statistic	6.7337
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 6.7337 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix C: Mutant 5 UV Exposure Comparison

Mutant 5 UV Exposure Analysis

Data

Strain	(5) R1 ΔDR_1279::mlox (MnSOD KO)								
CFU Input	10	16							
Untreated	11	5	13	10	12	12	4	4	
Treated	1.5	2	3	3	5	5	2.6	8	

All colony counts at 10^{-5} dilution

All exposures at 9999 Joules

Statistics

	$n_{5\text{-CFU input}}$	2	$n_{5\text{-Untreated}}$	8	$n_{5\text{-UV}}$	8	Percent Kill	58
	$\bar{x}_{5\text{-CFU input}}$	13.0	$\bar{x}_{5\text{-Untreated}}$	8.9	$\bar{x}_{5\text{-UV}}$	3.7		
	$s_{5\text{-CFU input}}$	4.2	$s_{5\text{-Untreated}}$	3.9	$s_{5\text{-UV}}$	2.2		

Population Comparisons

Comparison Set 14 - (5) R1 ΔDR_1279::mlox (MnSOD KO)

H_0 :	$\mu_{5\text{-CFU input}} - \mu_{5\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{5\text{-CFU input}} - \mu_{5\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	15.3594
t, test statistic	1.3314
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 1.3314 < 1.860, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	9.8131
t, test statistic	3.2960
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 3.2960 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix D: Mutant 8 UV Exposure Comparison

Mutant 8 UV Exposure Analysis

Data

Strain	(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)								
CFU Input	17	19							
Untreated	6	8	5	2	5	8	5	3	
Treated	0.8	2	1	4	1.5	2	1.7	1.2	
All colony counts at 10^{-5} dilution									
All exposures at 9999 Joules									

Statistics

	$n_{s\text{-CFU input}}$	2	$n_{s\text{-Untreated}}$	8	$n_{s\text{-UV}}$	8	Percent Kill	67
	$\bar{x}_{s\text{-CFU input}}$	18.0	$\bar{x}_{s\text{-Untreated}}$	5.3	$\bar{x}_{s\text{-UV}}$	1.7		
	$s_{s\text{-CFU input}}$	1.4	$s_{s\text{-Untreated}}$	2.1	$s_{s\text{-UV}}$	1.0		

Population Comparisons

Comparison Set 15 - (8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)

H_0 :	$\mu_{s\text{-CFU input}} - \mu_{s\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{s\text{-CFU input}} - \mu_{s\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	4.1875
t, test statistic	7.8812
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 7.8812 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{s\text{-Untreated}} - \mu_{s\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{s\text{-Untreated}} - \mu_{s\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	2.7711
t, test statistic	4.2351
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 4.2351 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix E: Mutant 11 UV Exposure Comparison

Mutant 11 UV Exposure Analysis

Data

Strain	(11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)								
CFU Input	25	18							
Untreated	15	10	12	12	11	11	7	1	
Treated	3	0.8	2	3	6	3	1.6	1.8	
All colony counts at 10^{-5} dilution									
All exposures at 9999 Joules									

Statistics

	$n_{11\text{-CFU input}}$	2	$n_{11\text{-Untreated}}$	8	$n_{11\text{-UV}}$	8	Percent Kill	73
	$\bar{x}_{11\text{-CFU input}}$	21.5	$\bar{x}_{11\text{-Untreated}}$	9.9	$\bar{x}_{11\text{-UV}}$	2.7		
	$s_{11\text{-CFU input}}$	4.9	$s_{11\text{-Untreated}}$	4.2	$s_{11\text{-UV}}$	1.6		

Population Comparisons

Comparison Set 16 - (11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)

H_0 :	$\mu_{11\text{-CFU input}} - \mu_{11\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{11\text{-CFU input}} - \mu_{11\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	18.6719
t, test statistic	3.4030
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 3.4030 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	10.1525
t, test statistic	4.5350
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 4.5350 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix F: Mutant 16 UV Exposure Comparison

Mutant 16 UV Exposure Analysis

Data

Strain	(16) R1 ΔuvrB::KAN								
CFU Input	34	25							
Untreated	19	15	15	19	12	21	5		
Treated	1	9	5	2.3	3	3	2.2	6	

All colony counts at 10^{-5} dilution

All exposures at 9999 Joules

Statistics

	$n_{16\text{-CFU input}}$	2	$n_{16\text{-Untreated}}$	7	$n_{16\text{-UV}}$	8	Percent Kill	74
	$\bar{x}_{16\text{-CFU input}}$	29.5	$\bar{x}_{16\text{-Untreated}}$	15.1	$\bar{x}_{16\text{-UV}}$	3.9		
	$s_{16\text{-CFU input}}$	6.4	$s_{16\text{-Untreated}}$	5.4	$s_{16\text{-UV}}$	2.6		

Population Comparisons

Comparison Set 17 - (16) R1 ΔuvrB::KAN

H_0 :	$\mu_{16\text{-CFU input}} - \mu_{16\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{16\text{-CFU input}} - \mu_{16\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	31.0510
t, test statistic	3.2135
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since 3.2135 > 1.895, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	17.2274
t, test statistic	5.2163
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since 5.2163 > 1.771, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix G: Mutant 6A UV Exposure Comparison

Mutant 6A UV Exposure Analysis

Data

Strain	(6A) R1 'meroploid' WT and ΔrecF::KAN								
CFU Input	13	15							
Untreated	3	8	10	4	12	5	3	4	
Treated	0.7	1.1	2	0.8	1.2	1	0.7	1.7	

All colony counts at 10^{-5} dilution

All exposures at 9999 Joules

Statistics

	$n_{6A\text{-}CFU\ input}$	2	$n_{6A\text{-}Untreated}$	8	$n_{6A\text{-}UV}$	8	Percent Kill	82
	$\bar{x}_{6A\text{-}CFU\ input}$	14.0	$\bar{x}_{6A\text{-}Untreated}$	6.1	$\bar{x}_{6A\text{-}UV}$	1.1		
	$s_{6A\text{-}CFU\ input}$	1.4	$s_{6A\text{-}Untreated}$	3.4	$s_{6A\text{-}UV}$	0.4		

Population Comparisons

Comparison Set 18 - (6A) R1 'meroploid' WT and ΔrecF::KAN

H_0 :	$\mu_{6A\text{-}CFU\ input} - \mu_{6A\text{-}Untreated} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{6A\text{-}CFU\ input} - \mu_{6A\text{-}Untreated} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	10.6094
t, test statistic	3.0582
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 3.0582 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}UV} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}UV} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	6.0131
t, test statistic	4.0882
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 4.0882 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix H: Mutant 27 UV Exposure Comparison

Mutant 27 UV Exposure Analysis

Data

Strain	(27) R1 ΔuvrB::KAN								
CFU Input	19	36							
Untreated	18	12	16	13	7	17	8	8	
Treated	2	1.7	1	2.5	1.1	2	2.5	1.5	

All colony counts at 10^{-5} dilution

All exposures at 9999 Joules

Statistics

	$n_{27\text{-CFU input}}$	2	$n_{27\text{-Untreated}}$	8	$n_{27\text{-UV}}$	8	Percent Kill	85
	$\bar{x}_{27\text{-CFU input}}$	27.5	$\bar{x}_{27\text{-Untreated}}$	12.4	$\bar{x}_{27\text{-UV}}$	1.8		
	$s_{27\text{-CFU input}}$	12.0	$s_{27\text{-Untreated}}$	4.4	$s_{27\text{-UV}}$	0.6		

Population Comparisons

Comparison Set 19 - (27) R1 ΔuvrB::KAN

H_0 :	$\mu_{27\text{-CFU input}} - \mu_{27\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{27\text{-CFU input}} - \mu_{27\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populati

s_p^2	34.7969
t, test statistic	3.2433
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 3.2433 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations

H_0 :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-UV}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-UV}} > 0$	Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population

s_p^2	9.7531
t, test statistic	6.7643
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 6.7643 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the UV Treated Populations

Appendix I: 500 Gy Oxygen Exposure Comparison

500 Gy Exposure Comparison

Data

Strain	500 Gy Exposure			
(1) Wild Type	26	32	36	43
(1.5) R1 'meroploid' WT and ΔrecF::KAN	12	3.5	4.7	3.9
(5) R1 ΔDR_1279::mlox (MnSOD KO)	90	42	31	28
(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)	27	28	21	32
(11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)	34	11	33	35
(16) R1 ΔuvrB::KAN	43	41	29	37
(6A) R1 'meroploid' WT and ΔrecF::KAN	24	22	19	31
(27) R1 ΔuvrB::KAN	39	19	31	26

All colony counts at 10^{-6} dilution

Statistics

n_{WT-500}	4	$n_{1.5-500}$	4	n_{5-500}	4	n_{8-500}	4	n_{11-500}	4	n_{16-500}	4	n_{6A-500}	4	n_{27-500}	4			
$\bar{x}_{WT-500}$	34.3	$\bar{x}_{1.5-500}$	6.0	$\bar{x}_{5-500}$	47.8	$\bar{x}_{8-500}$	27.0	$\bar{x}_{11-500}$	28.3	$\bar{x}_{16-500}$	37.5	$\bar{x}_{6A-500}$	24.0	$\bar{x}_{27-500}$	28.8			
s_{WT-500}	7.1	$s_{1.5-500}$	4.0	s_{5-500}	28.8	s_{8-500}	4.5	s_{11-500}	11.5	s_{16-500}	6.2	s_{6A-500}	5.1	s_{27-500}	8.4			
		Percent Ki	82.40876		-39.4161		21.16788		17.51825		-9.48905		29.92701		16.05839			

Population Comparisons

Comparison Set 9 - 500 Gy Exposure

H_0 : $\mu_{WT-500} - \mu_{1.5-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 1.5 Population

H_a : $\mu_{WT-500} - \mu_{1.5-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 1.5 Population

s_p^2	33.5163
t, test statistic	6.8948
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 6.8948 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 1.5 Populations

H_0 : $\mu_{WT-500} - \mu_{5-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 5 Population

H_a : $\mu_{WT-500} - \mu_{5-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 5 Population

s_p^2	440.2500
t, test statistic	-0.9099
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -.9099 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 5 Populations

H_0 : $\mu_{WT-500} - \mu_{8-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 8 Population

H_a : $\mu_{WT-500} - \mu_{8-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 8 Population

s_p^2	35.7917
t, test statistic	1.7138
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 1.7138 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 8 Populations

H_0 : $\mu_{WT-500} - \mu_{11-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 11 Population

H_a : $\mu_{WT-500} - \mu_{11-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 11 Population

s_p^2	91.9167
t, test statistic	0.8851
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.8851 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 11 Populations

H_0 : $\mu_{WT-500} - \mu_{16-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 16 Population

H_a : $\mu_{WT-500} - \mu_{16-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 16 Population

s_p^2	44.6250
t, test statistic	-0.6880
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.6880 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 16 Populations

H_0 : $\mu_{WT-500} - \mu_{6A-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 6A Population

H_a : $\mu_{WT-500} - \mu_{6A-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 6A Population

s_p^2	38.4583
t, test statistic	2.3375
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 2.3375 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 6A Populations

H_0 : $\mu_{WT-500} - \mu_{27-500} = 0$ — Null Hypothesis = There is no difference between the Wild Type population and the Mutant 27 Population

H_a : $\mu_{WT-500} - \mu_{27-500} > 0$ — Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 27 Population

s_p^2	60.9167
t, test statistic	0.9966
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943

Appendix J: 1000 Gy Oxygen Exposure Comparison

1000 Gy Exposure Comparison

Data

Strain	1000 Gy Exposure			
(1) Wild Type	42	40	37	37
(1.5) R1 'meroploid' WT and ΔrecF::KAN	11	6	6	4
(5) R1 ΔDR_1279::mlox (MnSOD KO)	29	36	31	34
(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)	25	25	24	27
(11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)	43	24	33	38
(16) R1 ΔuvrB::KAN	39	42	45	43
(6A) R1 'meroploid' WT and ΔrecF::KAN	29	24	21	26
(27) R1 ΔuvrB::KAN	34	37	35	30

All colony counts at $10^{-6 \text{ dilution}}$

Statistics

$n_{WT\text{-}1000}$	4	$n_{1.5\text{-}1000}$	4	$n_{5\text{-}1000}$	4	$n_{8\text{-}1000}$	4	$n_{11\text{-}1000}$	4	$n_{16\text{-}1000}$	4	$n_{6A\text{-}1000}$	4	$n_{27\text{-}1000}$	4		
$\bar{x}_{WT\text{-}1000}$	39.0	$\bar{x}_{1.5\text{-}1000}$	6.8	$\bar{x}\text{-bar}_{5\text{-}1000}$	32.5	$\bar{x}\text{-bar}_{8\text{-}1000}$	25.3	$\bar{x}\text{-bar}_{11\text{-}1000}$	34.5	$\bar{x}\text{-bar}_{16\text{-}1000}$	42.3	$\bar{x}\text{-bar}_{6A\text{-}1000}$	25.0	$\bar{x}\text{-bar}_{27\text{-}1000}$	34.0		
$s_{WT\text{-}1000}$	2.4	$s_{1.5\text{-}1000}$	3.0	$s_{5\text{-}1000}$	3.1	$s_{8\text{-}1000}$	1.3	$s_{11\text{-}1000}$	8.1	$s_{16\text{-}1000}$	2.5	$s_{6A\text{-}1000}$	3.4	$s_{27\text{-}1000}$	2.9		
		Percent K	82.69231		16.66667		35.25641		11.53846		-8.33333		35.89744		12.82051		

Population Comparisons

Comparison Set 10 - 1000 Gy Exposure

H_0 : $\mu_{WT\text{-}1000} - \mu_{1.5\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 1.5 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{1.5\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 1.5 Population

s_p^2	7.4583
t, test statistic	16.7003
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 16.7003 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 1.5 Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{5\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 5 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{5\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 5 Population

s_p^2	7.8333
t, test statistic	3.2844
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 3.2844 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 5 Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{8\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 8 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{8\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 8 Population

s_p^2	3.7917
t, test statistic	9.9863
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 9.9863 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 8 Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{11\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 11 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{11\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 11 Population

s_p^2	35.8333
t, test statistic	1.0631
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 1.0631 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 11 Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{16\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 16 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{16\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 16 Population

s_p^2	6.1250
t, test statistic	-1.8571
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.8571 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 16 Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{6A\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 6A Population

H_a : $\mu_{WT\text{-}1000} - \mu_{6A\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 6A Population

s_p^2	8.6667
t, test statistic	6.7254
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 6.7254 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 6A Populations

H_0 : $\mu_{WT\text{-}1000} - \mu_{27\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 27 Population

H_a : $\mu_{WT\text{-}1000} - \mu_{27\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 27 Population

s_p^2	7.3333
t, test statistic	2.6112
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 2.6112 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 27 Populations

Appendix K: 10,000 Gy Oxygen Exposure Comparison

10000 Gy Exposure Comparison

Data

Strain	10000 Gy Exposure			
(1) Wild Type	28	23	32	28
(1.5) R1 'meroploid' WT and ΔrecF::KAN	3	6	9	2.3
(5) R1 ΔDR_1279::mlox (MnSOD KO)	32	16	24	29
(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO)	23	20	20	21
(11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)	31	25	33	26
(16) R1 ΔuvrB::KAN	20	22	29	30
(6A) R1 'meroploid' WT and ΔrecF::KAN	14	22	27	22
(27) R1 ΔuvrB::KAN	35	14	22	33

All colony counts at 10^{-6} dilution

Statistics

$n_{WT\text{-}10000}$	4	$n_{1.5\text{-}10000}$	4	$n_{5\text{-}10000}$	4	$n_{8\text{-}10000}$	4	$n_{11\text{-}10000}$	4	$n_{16\text{-}10000}$	4	$n_{6A\text{-}10000}$	4	$n_{27\text{-}10000}$	4
$\bar{x}_{WT\text{-}10000}$	27.8	$\bar{x}_{1.5\text{-}10000}$	5.1	$\bar{x}_{5\text{-}10000}$	25.3	$\bar{x}_{8\text{-}10000}$	21.0	$\bar{x}_{11\text{-}10000}$	28.8	$\bar{x}_{16\text{-}10000}$	25.3	$\bar{x}_{6A\text{-}10000}$	21.3	$\bar{x}_{27\text{-}10000}$	26.0
$s_{WT\text{-}10000}$	3.7	$s_{1.5\text{-}10000}$	3.1	$s_{5\text{-}10000}$	7.0	$s_{8\text{-}10000}$	1.4	$s_{11\text{-}10000}$	3.9	$s_{16\text{-}10000}$	5.0	$s_{6A\text{-}10000}$	5.4	$s_{27\text{-}10000}$	9.8
		Percent K	81.71171		9.009009		24.32432		-3.6036		9.009009		23.42342		6.306306

Population Comparisons

Comparison Set 11 - 10000 Gy Exposure

H_0 : $\mu_{WT\text{-}10000} - \mu_{1.5\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 1.5 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{1.5\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 1.5 Population

$s_{\bar{x}}^2$	11.5029
t, test statistic	9.4549
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 9.4549 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 1.5 Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{5\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 5 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{5\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 5 Population

$s_{\bar{x}}^2$	31.2500
t, test statistic	0.6325
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.6325 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 5 Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{8\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 8 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{8\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 8 Population

$s_{\bar{x}}^2$	7.7917
t, test statistic	3.4198
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 3.4198 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 8 Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{11\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 11 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{11\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 11 Population

$s_{\bar{x}}^2$	14.2500
t, test statistic	-0.3746
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.3746 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 11 Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{16\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 16 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{16\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 16 Population

$s_{\bar{x}}^2$	19.2500
t, test statistic	0.8058
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.8058 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 16 Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{6A\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 6A Population

H_a : $\mu_{WT\text{-}10000} - \mu_{6A\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 6A Population

$s_{\bar{x}}^2$	21.2500
t, test statistic	1.9941
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 1.9941 > 1.943, I do reject the null hypothesis, there is a difference between the Wild Type and Mutant 6A Populations

H_0 : $\mu_{WT\text{-}10000} - \mu_{27\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Wild Type population and the Mutant 27 Population

H_a : $\mu_{WT\text{-}10000} - \mu_{27\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the Wild Type population and the Mutant 27 Population

$s_{\bar{x}}^2$	55.1250
t, test statistic	0.3333
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.3333 < 1.943, I do not reject the null hypothesis, there is no difference between the Wild Type and Mutant 27 Populations

Appendix L: Wild Type Oxygen Exposure Comparison

Wild Type Analysis

Data

Strain	R1 Wild Type			
CFU Input	21	15	9	
Untreated Unvacuumed	41	32		
Untreated Vacuumed	32	33	26	33
500 Gy	26	32	36	43
1000 Gy	42	40	37	37
10,000 Gy	28	23	32	28

All colony counts at 10^{-5} dilution

Statistics

$n_{WT\text{-}CFU\ input}$	3	$n_{WT\text{-}Untreated}$	6	[redacted]		$n_{WT\text{-}500}$	4	$n_{WT\text{-}1000}$	4	$n_{WT\text{-}10000}$	4
$\bar{x}_{WT\text{-}CFU\ input}$	15.0	$\bar{x}_{WT\text{-}Untreated}$	32.8			$\bar{x}_{WT\text{-}500}$	34.3	$\bar{x}_{WT\text{-}1000}$	39.0	$\bar{x}_{WT\text{-}10000}$	27.8
$s_{WT\text{-}CFU\ input}$	6.0	$s_{WT\text{-}Untreated}$	4.8			$s_{WT\text{-}500}$	7.1	$s_{WT\text{-}1000}$	2.4	$s_{WT\text{-}10000}$	3.7

Population Comparisons

Comparison Set 1 - Wild Type

H_0 : $\mu_{WT\text{-}CFU\ input} - \mu_{WT\text{-}Untreated} = 0$ Null Hypothesis = There is no difference between the CFU Input population and the Untreated Population

H_a : $\mu_{WT\text{-}CFU\ input} - \mu_{WT\text{-}Untreated} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	26.6905
t, test statistic	-4.8817
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -4.8817 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}500} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}500} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	33.4479
t, test statistic	-0.3795
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.3795 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}1000} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}1000} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	16.6042
t, test statistic	-2.3445
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -2.3445 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}10000} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{WT\text{-}Untreated} - \mu_{WT\text{-}10000} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	19.4479
t, test statistic	1.7857
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 1.7857 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations

Appendix M: Mutant 1.5 Oxygen Exposure Comparison

Mutant 1.5 Analysis

Data

Strain	R1 'meroploid' WT and *ΔrecF::KAN*			
CFU Input	2.7	2.7	6	
Untreated Unvacuumed	34	11		
Untreated Vacuumed	42	7	10	42
500 Gy	12	3.5	4.7	3.9
1000 Gy	11	6	6	4
10,000 Gy	3	6	9	2.3

All colony counts at 10^{-5} dilution

Statistics

$n_{1.5\text{-CFU input}}$	3	$n_{1.5\text{-Untreated}}$	6	[redacted]		$n_{1.5\text{-500}}$	4	$n_{1.5\text{-1000}}$	4	$n_{1.5\text{-10000}}$	4
$\bar{x}_{1.5\text{-CFU input}}$	3.8	$\bar{x}_{1.5\text{-Untreated}}$	24.3			$\bar{x}_{1.5\text{-500}}$	6.0	$\bar{x}_{1.5\text{-1000}}$	6.8	$\bar{x}_{1.5\text{-10000}}$	5.1
$s_{1.5\text{-CFU input}}$	1.9	$s_{1.5\text{-Untreated}}$	16.7			$s_{1.5\text{-500}}$	4.0	$s_{1.5\text{-1000}}$	3.0	$s_{1.5\text{-10000}}$	3.1

Population Comparisons

Comparison Set 2 - Mutant 1.5

H_0 : $\mu_{1.5\text{-CFU input}} - \mu_{1.5\text{-Untreated}} = 0$ Null Hypothesis = There is no difference between the CFU input population and the Untreated Population

H_a : $\mu_{1.5\text{-CFU input}} - \mu_{1.5\text{-Untreated}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	201.2276
t, test statistic	-2.0471
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -2.0471 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-500}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-500}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	181.2101
t, test statistic	2.1070
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 2.1070 > 1.860, I do reject the null hypothesis, there is a difference between the Untreated and the 500 Gy Treated Populations

H_0 : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-1000}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-1000}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	178.5104
t, test statistic	2.0388
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 2.0388 > 1.860, I do reject the null hypothesis, there is a difference between the Untreated and the 1000 Gy Treated Populations

H_0 : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-10000}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{1.5\text{-Untreated}} - \mu_{1.5\text{-10000}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	178.7001
t, test statistic	2.2318
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 2.2318 > 1.860, I do reject the null hypothesis, there is a difference between the Untreated and the 10,000 Gy Treated Populations

Appendix N: Mutant 5 Oxygen Exposure Comparison

Mutant 5 Analysis

Data

Strain	R1 *ΔDR_1279::mlox* (MnSOD KO)			
CFU Input	10	12	23	
Untreated Unvacuumed	37	34		
Untreated Vacuumed	21	31	28	21
500 Gy	90	42	31	28
1000 Gy	29	36	31	34
10,000 Gy	32	16	24	29

All colony counts at 10^{-5} dilution

Statistics

$n_{5\text{-CFU input}}$	3	$n_{5\text{-Untreated}}$	6	[redacted]		$n_{5\text{-500}}$	4	$n_{5\text{-1000}}$	4	$n_{5\text{-10000}}$	4
$\bar{x}_{5\text{-CFU input}}$	15.0	$\bar{x}_{5\text{-Untreated}}$	28.7			$\bar{x}_{5\text{-500}}$	47.8	$\bar{x}_{5\text{-1000}}$	32.5	$\bar{x}_{5\text{-10000}}$	25.3
$s_{5\text{-CFU input}}$	7.0	$s_{5\text{-Untreated}}$	6.7			$s_{5\text{-500}}$	28.8	$s_{5\text{-1000}}$	3.1	$s_{5\text{-10000}}$	7.0

Population Comparisons

Comparison Set 3 - Mutant 5

H_0 :	$\mu_{5\text{-CFU input}} - \mu_{5\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{5\text{-CFU input}} - \mu_{5\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	45.6190
t, test statistic	-2.8616
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -2.8616 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-500}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-500}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	338.7604
t, test statistic	-1.6063
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -1.6063 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-1000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-1000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	31.2917
t, test statistic	-1.0616
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -1.0616 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-10000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{5\text{-Untreated}} - \mu_{5\text{-10000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	46.0104
t, test statistic	0.7803
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.7803 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations

Appendix O: Mutant 8 Oxygen Exposure Comparison

Mutant 8 Analysis

Data

Strain	R1 *ΔDR_1546::KAN ΔDR_A0202::NAT* (Cu/ZnSOD Double KO)			
CFU Input	15	20	18	
Untreated Unvacuumed	21	35		
Untreated Vacuumed	23	18	24	23
500 Gy	27	28	21	32
1000 Gy	25	25	24	27
10,000 Gy	23	20	20	21

All colony counts at 10^{-3} dilution

Statistics

	$n_{S\text{-CFU input}}$	3	$n_{S\text{-Untreated}}$	6	[redacted]	$n_{S\text{-500}}$	4	$n_{S\text{-1000}}$	4	$n_{S\text{-10000}}$	4
	$\bar{x}_{S\text{-CFU input}}$	17.7	$\bar{x}_{S\text{-Untreated}}$	24.0		$\bar{x}_{S\text{-500}}$	27.0	$\bar{x}_{S\text{-1000}}$	25.3	$\bar{x}_{S\text{-10000}}$	21.0
	$s_{S\text{-CFU input}}$	2.5	$s_{S\text{-Untreated}}$	5.8		$s_{S\text{-500}}$	4.5	$s_{S\text{-1000}}$	1.3	$s_{S\text{-10000}}$	1.4

Population Comparisons

Comparison Set 4 - Mutant 8

H_0 :	$\mu_{S\text{-CFU input}} - \mu_{S\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{S\text{-CFU input}} - \mu_{S\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	25.8095
t, test statistic	-1.7630
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -1.7630 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-500}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-500}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	28.7500
t, test statistic	-0.8668
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.8668 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-1000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-1000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	21.5938
t, test statistic	-0.4167
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.4167 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-10000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{S\text{-Untreated}} - \mu_{S\text{-10000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	21.7500
t, test statistic	0.9965
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.9965 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations

Appendix P: Mutant 11 Oxygen Exposure Comparison

Mutant 11 Analysis

Data

Strain	R1 *ΔbshA::mlox* (Bacillithiol Biosynthesis)			
CFU Input	10	14	20	
Untreated Unvacuumed	28	28		
Untreated Vacuumed	24	39	31	28
500 Gy	34	11	33	35
1000 Gy	43	24	33	38
10,000 Gy	31	25	33	26

All colony counts at 10^{-5} dilution

Statistics

	$n_{11\text{-CFU input}}$	3	$n_{11\text{-Untreated}}$	6	[redacted]	$n_{11\text{-500}}$	4	$n_{11\text{-1000}}$	4	$n_{11\text{-10000}}$	4
	$\bar{x}_{11\text{-CFU input}}$	14.7	$\bar{x}_{11\text{-Untreated}}$	29.7		$\bar{x}_{11\text{-500}}$	28.3	$\bar{x}_{11\text{-1000}}$	34.5	$\bar{x}_{11\text{-10000}}$	28.8
	$s_{11\text{-CFU input}}$	5.0	$s_{11\text{-Untreated}}$	5.1		$s_{11\text{-500}}$	11.5	$s_{11\text{-1000}}$	8.1	$s_{11\text{-10000}}$	3.9

Population Comparisons

Comparison Set 5 - Mutant 11

H_0 :	$\mu_{11\text{-CFU input}} - \mu_{11\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{11\text{-CFU input}} - \mu_{11\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	25.7143
t, test statistic	-4.1833
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -4.1833 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-500}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-500}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	66.0104
t, test statistic	0.2701
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.2701 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-1000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-1000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	40.7917
t, test statistic	-1.1724
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -1.1724 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-10000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-10000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	21.7604
t, test statistic	0.3044
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.3044 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated Unvacuumed and the 10,000 Gy Treated Populations

Appendix Q: Mutant 16 Oxygen Exposure Comparison

Mutant 16 Analysis

Data

Strain	R1 *ΔuvrB::KAN*			
CFU Input	15	25	21	
Untreated Unvacuumed	33	34		
Untreated Vacuumed	28	40	36	29
500 Gy	43	41	29	37
1000 Gy	39	42	45	43
10,000 Gy	20	22	29	30

All colony counts at 10^{-5} dilution

Statistics

	$n_{16\text{-CFU input}}$	3	$n_{16\text{-Untreated}}$	6	[redacted]	$n_{16\text{-500}}$	4	$n_{16\text{-1000}}$	4	$n_{16\text{-10000}}$	4
	$\text{x-bar}_{16\text{-CFU input}}$	20.3	$\text{x-bar}_{16\text{-Untreated}}$	33.3		$\text{x-bar}_{16\text{-500}}$	37.5	$\text{x-bar}_{16\text{-1000}}$	42.3	$\text{x-bar}_{16\text{-10000}}$	25.3
	$s_{16\text{-CFU input}}$	5.0	$s_{16\text{-Untreated}}$	4.5		$s_{16\text{-500}}$	6.2	$s_{16\text{-1000}}$	2.5	$s_{16\text{-10000}}$	5.0

Population Comparisons

Comparison Set 8 - Mutant 16

H_0 :	$\mu_{16\text{-CFU input}} - \mu_{16\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{16\text{-CFU input}} - \mu_{16\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	21.4286
t, test statistic	-3.9716
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -3.9716 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-500}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-500}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	26.7917
t, test statistic	-1.2471
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -1.2471 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-1000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-1000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	14.7604
t, test statistic	-3.5955
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -3.5955 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-10000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{16\text{-Untreated}} - \mu_{16\text{-10000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	21.7604
t, test statistic	2.6845
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 2.6845 > 1.860, I do reject the null hypothesis, there is a difference between the Untreated and the 10,000 Gy Treated Populations

Appendix R: Mutant 6A Oxygen Exposure Comparison

Mutant 6A Analysis

Data

Strain	R1 'meroploid' WT and *ΔrecF::KAN*			
CFU Input	7	9	17	
Untreated Unvacuumed	21	37		
Untreated Vacuumed	18	26	22	17
500 Gy	24	22	19	31
1000 Gy	29	24	21	26
10,000 Gy	14	22	27	22

All colony counts at 10^{-3} dilution

Statistics

	$n_{6A\text{-}CFU\ input}$	3	$n_{6A\text{-}Untreated}$	6	[redacted]	$n_{6A\text{-}500}$	4	$n_{6A\text{-}1000}$	4	$n_{6A\text{-}10000}$	4
	$\bar{x}_{6A\text{-}CFU\ input}$	11.0	$\bar{x}_{6A\text{-}Untreated}$	23.5		$\bar{x}_{6A\text{-}500}$	24.0	$\bar{x}_{6A\text{-}1000}$	25.0	$\bar{x}_{6A\text{-}10000}$	21.3
	$s_{6A\text{-}CFU\ input}$	5.3	$s_{6A\text{-}Untreated}$	7.3		$s_{6A\text{-}500}$	5.1	$s_{6A\text{-}1000}$	3.4	$s_{6A\text{-}10000}$	5.4

Population Comparisons

Comparison Set 7 - Mutant 6A

H_0 :	$\mu_{6A\text{-}CFU\ input} - \mu_{6A\text{-}Untreated} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{6A\text{-}CFU\ input} - \mu_{6A\text{-}Untreated} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	46.5000
t, test statistic	-2.5924
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -2.5924 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}500} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}500} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	43.4375
t, test statistic	-0.1175
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.1175 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}1000} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}1000} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	37.9375
t, test statistic	-0.3773
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.3773 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}10000} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{6A\text{-}Untreated} - \mu_{6A\text{-}10000} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	44.5313
t, test statistic	0.5223
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.5223 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations

Appendix S: Mutant 27 Oxygen Exposure Comparison

Mutant 27 Analysis

Data

Strain	R1 ΔuvrB::KAN			
CFU Input	15	21	21	
Untreated Unvacuumed	29	28		
Untreated Vacuumed	27	28	24	23
500 Gy	39	19	31	26
1000 Gy	34	37	35	30
10,000 Gy	35	14	22	33

All colony counts at 10^{-5} dilution

Statistics

$n_{27\text{-CFU input}}$	3	$n_{27\text{-Untreated}}$	6	[redacted]		$n_{27\text{-500}}$	4	$n_{27\text{-1000}}$	4	$n_{27\text{-10000}}$	4
$\bar{x}_{27\text{-CFU input}}$	19.0	$\bar{x}_{27\text{-Untreated}}$	26.5			$\bar{x}_{27\text{-500}}$	28.8	$\bar{x}_{27\text{-1000}}$	34.0	$\bar{x}_{27\text{-10000}}$	26.0
$s_{27\text{-CFU input}}$	3.5	$s_{27\text{-Untreated}}$	2.4			$s_{27\text{-500}}$	8.4	$s_{27\text{-1000}}$	2.9	$s_{27\text{-10000}}$	9.8

Population Comparisons

Comparison Set 8 - Mutant 27

H_0 :	$\mu_{27\text{-CFU input}} - \mu_{27\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{27\text{-CFU input}} - \mu_{27\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	7.6429
t, test statistic	-3.8366
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since -3.8366 < 1.895, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Populations

[redacted]

H_0 :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-500}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-500}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	30.2813
t, test statistic	-0.6334
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -0.6334 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 500 Gy Treated Populations

H_0 :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-1000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-1000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	6.9375
t, test statistic	-4.4113
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -4.4113 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000 Gy Treated Populations

H_0 :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-10000}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-10000}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	39.9375
t, test statistic	0.1226
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since 0.1226 < 1.860, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations

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Although not an extremophile, *Deinococcus radiodurans* has proven to have exceptional resistance to ionizing radiation, specifically via gamma and X-ray photons. To date, no known experiments have bombarded the bacterium with charged particles larger than hydrogen. This study explored the effects on the organism's ability to survive high linear energy transfer heavy-charged particle exposures of oxygen ions. It also investigated the effects of low linear energy transfer ultraviolet radiation on various mutants. Two *uvrB* mutants were created to ascertain the importance of the gene in single-strand break repair following gamma irradiation and two *recF* mutants were created to explore the role of the gene in double-strand break repair. The samples were desiccated to decrease the probability of indirect DNA damage. Following exposure the samples were rehydrated and counted. Comparisons were made against control samples and statistical differences were evaluated through a two population t-statistic test. The *uvrB* mutants displayed greater lethality than the wild type control and other mutants to gamma exposure and the *recF* mutants clearly experienced growth latency and greater lethality following oxygen ion exposure.

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