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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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AFIT-ENP-MS-18-M-071

DEPARTMENT OF THE AIR FORCE AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

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AFIT-ENP-MS-18-M-100

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

THESIS

Presented to the Faculty

Department of Engineering Physics

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Nuclear Engineering

Todd A. Bryant, MS

Major, USA

March 2018

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

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AFIT-ENP-MS-18-M-100

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 singlestrand 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.



iv

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.

Todd A. Bryant



Table of Contents

Abstract	iv
Table of Contents	vi
List of Figures	ix
List of Tables	xii
I. Introduction	1
General Issue	1
Problem Statement	2
Research Objectives	2
Hypothesis	3
II. Literature Review	3
Chapter Overview	3
A Brief Description of Deinococcus radiodurans	3
DNA	5
DNA Damage from Direct and Indirect Actions	6
General DNA Repair Mechanisms	8
Deinococcus radiodurans DNA Damage and Repair	10
Role of <i>recF</i> in DNA Repair	12
Role of <i>uvrB</i> in DNA Repair	13
High LET and Low LET	14
Heavy Charged Particle Interactions	15
Deinococcus radiodurans and Mutant Strains	16
III. Methodology	
Chapter Overview	



Deinococcus Radiodurans uvrB Knockout	
Deinococcus Radiodurans recF knockout	28
Sample Culture Preparation	29
Sample Plate Preparation	31
UV Protocol Test	35
Rehydration of Samples and Spotting Post UV Irradiation	36
Colony Counting Post UV Irradiation	36
Oxygen Ion Generation	38
Oxygen Ion Dose Calculations	39
Oxygen Irradiation of Samples	42
Rehydration of Samples and Spotting Post Oxygen Irradiation	44
Colony Counting Post Oxygen Irradiation	44
Statistical Methods of Comparison	44
IV. Analysis and Results	46
Chapter Overview	46
UV Experiment	46
Oxygen Experiment	50
UV Experiment Findings	62
Oxygen Experiment Findings	62
V. Conclusions and Recommendations	63
Conclusions of Research	63
Recommendations for Future Research	67
Appendix A: WT UV Exposure Comparison	69
Appendix B: Mutant 1.5 UV Exposure Comparison	70



Appendix C: Mutant 5 UV Exposure Comparison	71
Appendix D: Mutant 8 UV Exposure Comparison	72
Appendix E: Mutant 11 UV Exposure Comparison	73
Appendix F: Mutant 16 UV Exposure Comparison	74
Appendix G: Mutant 6A UV Exposure Comparison	75
Appendix H: Mutant 27 UV Exposure Comparison	76
Appendix I: 500 Gy Oxygen Exposure Comparison	77
Appendix J: 1000 Gy Oxygen Exposure Comparison	78
Appendix K: 10,000 Gy Oxygen Exposure Comparison	79
Appendix L: Wild Type Oxygen Exposure Comparison	80
Appendix M: Mutant 1.5 Oxygen Exposure Comparison	81
Appendix N: Mutant 5 Oxygen Exposure Comparison	82
Appendix O: Mutant 8 Oxygen Exposure Comparison	83
Appendix P: Mutant 11 Oxygen Exposure Comparison	84
Appendix Q: Mutant 16 Oxygen Exposure Comparison	85
Appendix R: Mutant 6A Oxygen Exposure Comparison	86
Appendix S: Mutant 27 Oxygen Exposure Comparison	87
Bibliography	88



viii

List of Figures

Page
Figure 1. Composition of <i>Deinococcus radiodurans</i> nucleus. SEM image taken at
USAFSAM in 2016. [4] 6
Figure 2. The ESDSA repair process. Reprinted under the Creative Commons Attribution
License. [8] 11
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
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
Figure 5. Dilution series layout
Figure 6. Dilution series for an untreated sample
Figure 7. Stratalinker 1800 used to impart 9999 J m ⁻² of UV energy onto the samples 35
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
Figure 10. QASPR-3 Tandem target chamber with mounted x,y stage
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
Figure 14. Colony Averages Pre- and Post-UV Exposure
Figure 15. Colony Averages Pre- and Post-UV Exposure with Standard Deviation 48
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 49
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 50
Figure 18. Colony Averages Pre- and Post-Oxygen Exposure
Figure 19. Colony Averages Pre- and Post-Oxygen Exposure with Standard Deviation. 51
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 Deviation54
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
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
Figure 28. Colony comparison against wild type for 500Gy oxygen exposure 59
Figure 29. Colony comparison against wild type for 500Gy oxygen exposure with
standard deviation 59
standard deviation
standard deviation
standard deviation
standard deviation59Figure 30. Colony comparison against wild type for 1000Gy exposure60Figure 31. Colony comparison against wild type for 1000Gy oxygen exposure with standard deviation60Figure 32. Colony comparison against wild type for 10,000Gy exposure61
standard deviation59Figure 30. Colony comparison against wild type for 1000Gy exposure60Figure 31. Colony comparison against wild type for 1000Gy oxygen exposure with standard deviation60Figure 32. Colony comparison against wild type for 10,000Gy exposure61Figure 33. Colony comparison against wild type for 10,000Gy oxygen exposure with



xi

List of Tables

	Page
Table 1. Deinococcus radiodurans R1 Stain List	17
Table 2. uvrB Primer Sequences	19
Table 3. Genomic Template and PCR Cocktail	
Table 4. Plasmid Template and PCR Cocktail	
Table 5. PCR Primer Combinations	
Table 6. pUC19mPheS EcoRI Digest for NEBuilder Cloning	
Table 7. PCR Cocktail with Genomic Template for DR transformation	
Table 8. recF Primer Sequences	
Table 9. Oxygen Dose per Well	
Table 10. UV Exposure Colony Rollup – Raw Data	
Table 11. Oxygen Exposure Colony Rollup – Raw Data	52
Table 12. Statistically significant mutants compared to the untreated wild type	controls 56



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 upregulated 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 UvrA₂UvrB₂ 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 Uvr₂ 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.

#	Gene KO	Common Name	Proper Genotype
1	none	WT	
1.5	recF	recF	'merodiploid' WT
			and <i>∆recF::KAN</i>
5	DR_1279	Mn SOD	∆ <i>DR_1279</i> ::mlox
8	DR_1546	Cu/Zn SOD	ΔDR_1546::KAN
	DR_A0202	Cu/Zn SOD	∆ <i>DR_A0202::NAT</i>
11	bshA	Bacillithiol Biosynthesis	∆bshA::mlox
16	uvrB	uvrB	∆uvrB::KAN
6A	recF	recF	'merodiploid' WT
			and <i>∆recF::KAN</i>
27	uvrB	uvrB	∆uvrB::KAN

Table 1. Deinococcus radiodurans R1 Stain List



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 postirradiation 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 primersupplied 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.



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

Table 2. uvrB Primer Sequences

All six primer lyophilized powders were suspended to 100 μ M 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 10 μ M. 20 μ l of DR genomic stock at 529ng μ l⁻¹ was added to 180 μ l of water for a 1:10 dilution. The pUCIDT-Amp::KANkanp plasmid stock at an initial concentration of 144ng μ l⁻¹ was diluted 1:25 and then 1:100 to achieve 50pg μ l⁻¹ thus completing the template DNA dilutions. A PCR cocktail was created for a 50 μ l 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.

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

 Table 3. Genomic Template and PCR Cocktail

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

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



Molecular Biology Grade	21.5µl	64.5µl
Water		
10mM dNTPs	1µl	3μΙ
Kan Plasmid @ 50pg μl ⁻¹	2μΙ	6μΙ
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.

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

Table 5. PC	R Primer	Combinations
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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 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 1 minute, 66°C for 30 seconds, 72°C for 1 minute), then 72°C for 1 minute), then 72°C for 1 minute), then 72°C for 2 minutes then 30 cycles of (98°C for 1 minute, 66°C for 30 seconds, 72°C for 1 minute), then 72°C for 1 minute), then 72°C for 2 minutes and held at 4°C.

Following PCR amplification, 10μ l of 6x Sample Buffer was added to each 50μ l PCR reaction. The entire reaction was loaded in two lanes (30μ l per lane) of 0.8% agarose gel in 1xTBE with 5μ g 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 μ l⁻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 5 μ g 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	10μΙ
NEB EcoRl Enzyme U μl ⁻¹	4μΙ
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 Tag Master Mix in a 50µl reaction. The mixtures were then placed in a thermocycler programmed to take reaction through 98°C for 2 minutes, then 30 cycles of (98°C for 30 seconds, 68°C for 3 minutes), then 68°C for 5 minutes and hold at 4°C. 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 μ l⁻¹ and then 2 μ l 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.

PCR Cocktail with Genomic	1x reaction	15x reaction
Template		
Molecular Biology Grade Water	29µl	435µl
10mM dNTPs	1.5µl	22.5µl
Forward Primer @ 10µM	2μΙ	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	2μΙ	30µl
Total	48µl	720µl

 Table 7. PCR Cocktail with Genomic Template for DR transformation

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 CaCl₂ was added to a final concentration of 30mM CaCl₂ and the incubation was continued for 2 hours at 32°C and 220RPM. After the CaCl₂ 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.

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

Table 8. recF Primer Sequences

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 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 OD₆₀₀. If, after 4 hours, the OD₆₀₀ 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 10⁸ colony forming units (CFU) per ml. [4]



30

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, 60μ l 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 60μ l 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 200 μ l 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 20 μ l from the row into 180 μ l 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, 5μ l 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 singlechannel pipette and placing 60μ l 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.



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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.



38



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

Oxygen Ion Dose Calculations

Lenker established that a 60μ l 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 9.71 μ m 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.



43

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, 60μ l 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.



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 'm	eroploid'	WT and Δr	ecF::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 Δ	DR_1279:::	nlox (MnS	OD 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 ∆DR_	_1546::KAN	ΔDR_A02	02::NAT (Cu	ı/ZnSOD D	ouble 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	l) R1 ∆bshA	::mlox (Ba	acillithiol B	iosynthesi	s)		
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 Δυ	IVIB::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 'm	eroploid'	WT and ∆r	ecF::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 ΔυνrB::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			

Table 10. UV Exposure Colony Rollup – Raw Data





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 ($\Delta uvrB$) 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



Strain					R1 Wild T	vne		
CELLInput	21	15	9		NI WIG I	ype		
Untreated Unvacuumed	41	32						
Untreated Vacuumed	22	22	26	22				
500 Gy	26	22	20	/12				
1000 Gy	20	32	27	43				
1000 Gy	42	40	37	27				
10,000 Gy	28	23	32	28	D1 lm and a idl M/T a	nd Area Full AM		
Strain	0.7	0.7	6		RI meropioid Wi a	nd Drech::KAN		
CFO Input	2.7	2.7	0					
Untreated Unvacuumed	34	- 11						
Untreated Vacuumed	42	/	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			R	1 ∆DR_1	546::KAN ∆DR_A0202::N	IAT (Cu/ZnSOD Do	ouble 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				F	R1 ∆bshA::mlox (Bacilli	thiol 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	I				R1 ∆uvrB::	KAN		
CFU Input	15	25	21					
Untreated Unvacuumed	33	34						
Untreated Vacuumed	28	40	36	29				
500 Gv	43	41	29	37				
1000 Gv	39	42	45	43				
10.000 Gv	20	22	29	30				
Strain					R1 'meroploid' WT a	nd ArecE::KAN		
CELLInput	7	9	17					
Untreated Unvacuumed	21	37	1,					
Untreated Vacuumed	19	26	22	17				
500 Gy	24	20	10	21				
1000 Gy	24	22	21	26				
10.000 Gy	14	24	21	20				
Strain	14	22	27	22	D1 Aug - D-	KAN		
Sudifi CELLingut	45	04		1	кі <i>duvrB</i> ::	KA/V		
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				

Table 11. Oxygen Exposure Colony Rollup – Raw Data



The treated samples were then compared to the untreated wild type control population. The populations for Mutants #1.5 ($\Delta recF$), #8 (Cu/ZnSOD Double KO) and #16 ($\Delta uvrB$) showed a statistical difference at all exposure levels. Mutants #11 ($\Delta bshA$) and #27 ($\Delta uvrB$) 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 ($\Delta recF$). The only other population that fell within the rejection was Mutant #16 ($\Delta uvrB$) 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 ($\Delta recF$) and #6A ($\Delta recF$) 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 ($\Delta uvrB$) 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 ($\Delta recF$) 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 ($\Delta recF$) 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 ($\Delta uvrB$) 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 ($\Delta recF$) and #27 ($\Delta uvrB$) 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 ($\Delta recF$), #8 (Cu/ZnSOD Double KO) and #16 ($\Delta uvrB$) showed a statistical difference at all exposure levels. Mutants #11 ($\Delta bshA$) and #27 ($\Delta uvrB$) showed a statistical difference at 500Gy and 10,000Gy. These mutants all confirmed the hypothesis. Although



Mutant 6A ($\Delta recF$) 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 ($\Delta recF$) showed a difference at all exposure levels. The only other population to fall within the rejection region was Mutant #16 ($\Delta uvrB$) 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 ($\Delta recF$) and #6A ($\Delta recF$) 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 ($\Delta uvrB$) 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 μ m of the estimated 21.7 μ m 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.



65

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



66

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 Expos	ure Analysis												
Data													
Strain			R1	Wild	T I	vpe							
CEU Input	20	14				100							
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-CEU input}	2	N _{WT-Untreated}	8		n _{wt-uv}	8	Percent Kill	79				
	x-barwaceu input	17.0	x-barwater	12.5		x-barwr aw	2.6						
	SWT-CFU input	4.2	S _{WT-Untreated}	3.6		S _{WT-UV}	1.0						
Population Comparison	ns												
Comparison Set 12 - Wild Type													
H _o :	μ _{WT-CFU} input - μ _{WT-Untreated} = 0	Null	Hypothesis = The	ere is	no	difference bet	ween	the CFU input pop	oulatio	on and th	e Untrea	ated Pop	oulation
H _a :	$\mu_{\text{WT-CFU input}} - \mu_{\text{WT-Untreated}} > 0$	Alter	nate Hypothesis	= The	ere	is a difference	betw	een the CFU input	popu	lation an	d the Un	ntreated	Populati
Sp ²	13.7500												
t, test statistic	1.5350												
rejection region	$t \ge t_{\alpha}$												
α	0.05												
df	8												
ta	1.860												
p-value Since 1.5350 < 1.860. I do not reject	0.519325892 the null hypothesis, there is	no diff	erence between t	he CEL	J In	put population a	nd the	Untreated Populati	ons				
,,,,,,,													
H _o :	$\mu_{\text{WT-Untrested}} - \mu_{\text{WT-UV}} = 0$	Null	Hypothesis = The	ere is	no	difference bet	ween	the Untreated po	pulati	on and th	ne Treate	ed popu	lation
H _e :	$\mu_{WT-Untreated} - \mu_{WT-UV} > 0$	Alter	nate Hypothesis	= The	ere	is a difference	betw	een the Untreated	l popu	lation ar	id the U	V Treate	d Popula
Sp ²	7.0971												
t, test statistic	7.4042												
rejection region	$t \ge t_{\alpha}$												
α	0.05												
df	14												
ta	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 Exp	osure Analysis													
Data			\vdash											_
Strain			(1	5) P1 'meron	loid'	w/	Tand ArecE.:KAN							
CEU Input	3	4	- 1-		loid		I and Breet liver							
Untreated	15	24	2	2.6	27	2	22	15						
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														
	N13-CFU input	2		N1.5 -Untreated	8		n _{1.5-UV}	8	Percent Kill	67	1			
	x-bar - scall insut	3.5		x-bar, subtraster	2.1		x-bar, saw	0.7						
	Second	07		5. •	05		5	0.3						
	-13070 input			-1.3-Untreated			-1300							
Population Comparis	ons													
Comparison Set 13 - (1.5) R1 'm	eroploid' WT and ∆recF::KAN													
H _o :	μ _{1.3-CFU} input - μ _{1.3-Untreated} = 0	Null	Нур	othesis = The	re is	no	difference bet	ween th	ne CFU input pop	ulati	on ar	nd the Un	treated Po	pulation
H _a :	μ _{1.5-CFU} input - μ _{1.5-Untreated} > 0	Alter	rnat	e Hypothesis	= The	ere	is a difference	betwee	en the CFU input	popu	Iatio	n and the	Untreate	d Populati
s. ²	0.2700									-				
-p t test statistic	3 5298													
, corototione	0.5250													
rejection region	t > t _a													
a	0.05													
df	8													
ta	1.860													
p-value	0.519325892													
Since 3.5298 > 1.860, I do reject th	ne null hypothesis, there is a di	feren	ice b	etween the CF	U Inpi	ut p	opulation and th	e Untrea	ated Populations					
H _o :	$\mu_{1.3-\text{Untreated}} - \mu_{1.3-\text{UV}} = 0$	Null	Нур	othesis = The	re is	no	difference bet	ween th	ne Untreated pop	oulat	ion a	nd the Tre	eated pop	ulation
Н. :	$\mu_{1.5-Untreated} - \mu_{1.5-UV} > 0$	Alter	rnat	e Hypothesis	= The	ere	is a difference	betwee	en the Untreated	and	the U	V Treated	d Populati	on
e 2	0 1660		\vdash											
- test statistic	6 7337													
	0.7337													
rejection region	t > t _a													
α	0.05				-									
df	14													
ta	1.761													
p-value	0.519585629													
Since 6.7337 > 1.761, I do reject th	ne null hypothesis, there is a di	feren	ice b	etween the Un	treat	ed a	and the UV Treat	ed Popu	lations					



Appendix C: Mutant 5 UV Exposure Comparison

Mutant 5 UV Expos	ure Analysis														
Data			-												+
Strain				(5) R1 ADR 1	779	mlo	ox (MpSOD KO)								+
CELLIpput	10	16		(5) KI BOK_1	273										+
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 ^{-3 dilution}		-			-	-		-							t
All exposures at 9999 Joules			-												+
Statistics															t
	N _{3-CEU input}	2		n _{a -Untreated}	8		n _{5-uv}	8	Percent Kill	58					
	x-bar scalling	13.0		x-bar-	8.9		x-bar	3.7							t
	Second and a	4.2		C	3.0		6	2.2						-	+
	-S-CFU input	7.2	•		0.0		-35-00	2.2						-	+
			-												+
Population Comparise	ons														
Comparison Set 14 - (5) R1 ADR	_1279::mlox (MnSOD KO)		-												+
H ₀ :	μ _{3-CFU input} - μ _{3-Untreated} = 0	Nul	I Hy	pothesis = The	re is	no	difference betw	veen t	the CFU input pop	oulatio	n and t	the Untr	eated Po	pulation	
H _a :	μ _{5-CFU input} - μ _{5-Untreated} > 0	Alte	erna	ate Hypothesis	= The	ere	is a difference	betwe	en the CFU input	popul	ation a	and the I	Untreater	d Populat	ci (
															t
5.2	15.3594														T
t test statistic	1 3314	1	-											-	+
															+
rejection region	t>t,		-												T
α	0.05		-												+
df	8														t
t _a	1.860														
p-value	0.519325892		-												+
Since 1.3314 < 1.860, I do not reje	ct the null hypothesis, there is	no di	ffer	ence between th	e CF	U Ir	nput population a	nd the	Untreated Populati	ons					+
H ₀ :	$\mu_{3-\text{Untreated}} - \mu_{3-\text{UV}} = 0$	Nul	I Hy	pothesis = The	re is	no	difference betw	veen t	the Untreated po	pulati	on and	the Trea	ated pop	ulation	
H _a :	$\mu_{\text{3-Untreated}} - \mu_{\text{3-UV}} > 0$	Alte	erna	ate Hypothesis	= The	ere	is a difference	betwe	en the Untreated	l and t	he UV T	[reated	Populatio	on	-
Sp ²	9.8131														+
t, test statistic	3.2960														
rejection region	$t \ge t_{\alpha}$														
α	0.05														
df	14														
ta	1.761														
p-value	0.519585629														
Since 3.2960 > 1.761, I do reject th	e null hypothesis, there is a di	ffere	nce	between the Un	treat	ed a	and the UV Treate	ed Pop	ulations						



Appendix D: Mutant 8 UV Exposure Comparison

Mutant 8 UV Expos	ure Analysis														
Data			\vdash			-				-	-				+
Strain	(8)	D1 A1	PP	1546-KAN ADP	A020	12	NAT (Cu/ZpSOD	Doubl	e KO)			1			+
CELLInout	(8)	19		_1340KAN 40K_	_H020	2	INAT (CU/21130D	Joubi	e koj						+
Untreated		8		5 2	5		5	3		-					+
Treated	0.8	2	1	4	15	2	17	12			-				+
All colony counts at 10 ^{-5 dilution}		-				-				-					t
All exposures at 9999 Joules			-												+
Statistics															Ť
	N _{8-CFU input}	2		N _{8-Untrested}	8		n _{s-uv}	8	Percent Kill	67					
	x-bar _{a-CEU input}	18.0		x-bar _{s-untreated}	5.3		x-bar _{s-uv}	1.7			-				
	SB-CFU input	1.4		S _{8-Untrested}	2.1		S _{8-UV}	1.0							1
															+
Population Comparise	ons														_
Companying Cat 15 (8) D1 ADD	154C	C	- 0			-				_					+
Comparison Set 15 - (6) KI ADK	1340KAN ADK_A0202IVAT	cu/2	na.	OD DOUBLE KOJ		-				_					+
н.		Nut	L H	vpothesis - The	re ic		difference bet	veen	the CELL input por	oulati	00.2	nd the lints	ested Po	nulation	+
	MacFu input Ma-Untreated = 0	Alte		to Hupothesis	- The		is a difference	hotur	on the CELL input		datio	on and the l	Introptor		
1'a -	MB-CFU input ~ MB-Untreated ~ U	Ante		ate hypothesis	- 1116		is a unreferice	Derwe	en tre cro mpu	, popu	liaut	un anu une v	Jinteatet	ropula	4
e 2	/ 1975		-			-					-				+
Sp t toot statistic	4.10/3		-			-				_	-				+
	/.0012		-			-				_	-				+
rejection region	tSt		-			-									+
a	0.05		-			-									+
df	0.05		-			-									+
t	1.860	1	1			-									+
	0 510325802		-			-				_	-				+
Since 7.8812 $>$ 1.860 L do reject th	e null hynothesis, there is a di) fferei	nce	hetween the CE	l Inn	ut i	onulation and the	e Untre	eated Populations						+
			1			Γ									t
H _o :	$\mu_{8 + \text{lotrested}} - \mu_{8 + \text{lot}} = 0$	Nul	I H	vpothesis = The	re is	nc	difference bet	veen	the Untreated po	pulat	ion a	and the Trea	ated popu	lation	T
Н, :	$\mu_{\text{B-Untreated}} - \mu_{\text{B-UV}} > 0$	Alte	erna	ate Hypothesis	= The	ere	is a difference	betwe	en the Untreate	d and	the l	UV Treated	Populatio	on	
5n ²	2.7711		-			-									+
t, test statistic	4.2351	1	1			-					-				t
		1	1			T					-				Ť
rejection region	t > t _e														
α	- 0.05		t		1	t					-				t
df	14		t								-				t
ta	1.761	1	Ĺ												Ť
p-value	0.519585629		t			t					-				t
Since 4.2351 > 1.761. I do reject th	e null hypothesis, there is a di	ffere	nce	between the Un	treat	ed	and the UV Treat	ed Pop	ulations						Ť



Appendix E: Mutant 11 UV Exposure Comparison

Mutant 11 UV Expo	sure Analysis													
Data										-				
Strain			11) (P1 AbshArmio	(Ba)	cill	ithial Biosynthe	cic)						
CELLInput	25	18		AT BOSHALINIO	(00)		fanor brosynaie	,3137						
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 ^{-3 dilution}														
All exposures at 9999 Joules														
Statistics														
	Dec. et al insta	2	11	Dec	8		Dec	8	Percent Kill	73				
	v-har	21.5		v-har	00		v-har	27						
	A DOI 11-CFU input	4.0		- Doi 11-Untreated	0.0		x 00111-0V	1.0			-			
	S11-CFU input	4.9		S11-Untreated	4.2		S11-UV	1.0						
Population Compariso	ons													
											-			
Comparison Set 16 - (11) R1 Abs	hA::mlox (Bacillithiol Biosy	nthes	sis)											
H _o :	μ _{11-CFU input} - μ _{11-Untrested} = 0	Null	Ну	pothesis = The	re is	no	difference betw	veen t	the CFU input pop	oulati	on a	and the Untr	eated Po	pulation
H _a :	μ _{11-CFU} input - μ _{11-Untrested} > 0	Alte	rnat	te Hypothesis	= The	ere	is a difference	betwe	en the CFU input	рори	lati	on and the	Untreated	l Populati
5n ²	18.6719													
t, test statistic	3.4030						<u> </u>							
rejection region	t > t _a													
α	0.05													
df	8													
ta	1.860													
p-value	0.519325892													
Since 3.4030 > 1.860, I do reject th	e null hypothesis, there is a di	fferer	ice I	between the CFI	J Inp	ut p	opulation and the	e Untre	eated Populations					
He:	Headersteen - Headers = 0	Null	Hv	oothesis = The	re is	no	difference bety	veen t	the Untreated po	pulat	ion :	and the Trea	ated popu	lation
Ha:	μ _{11-Untrested} - μ _{11-UV} > 0	Alte	rnat	te Hypothesis	= The	ere	is a difference	betwe	en the Untreated	and	the	UV Treated	Populatio	n
sp ²	10.1525													
t, test statistic	4.5350									-				
rejection region	t > t _a													
α	0.05													
df	14													
ta	1.761													
p-value	0.519585629	1												
Since 4.5350 > 1.761. I do reject th	e null hypothesis, there is a di	fferer	ice I	between the Un	treat	ed a	and the UV Treate	ed Pop	ulations					



Appendix F: Mutant 16 UV Exposure Comparison

Mutant 16 UV Expo	sure Analysis														
Data			-			-				-	-				+
Strain				(16)	R1 Ai	IVE	R-KAN								+
CELLInput	34	25		(10)											+
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															
	N16-CFU input	2		N _{16-Untrested}	7		n _{16-uv}	8	Percent Kill	74	-				
	x-bar _{16-CFU input}	29.5		x-bar _{16-Untreated}	15.1		x-bar _{16-uv}	3.9							
	S16-CFU input	6.4		S _{16-Untrested}	5.4		S _{16-UV}	2.6							_
															+
Population Comparise	ons														_
0	D. KAN		-			-					-				+
Comparison Set 17 - (16) RI Zuv	TDKAIN		-			-									+
u .		Nut		nothesis - The	ro ic		difference bet	voon	the CELL input no		00.7	nd the Lists	ested Re	nulation	+
10.	µ16-CFU input * µ16-Untreated = 0	Ales		/potriesis – rrie	TE 15		is a difference bett	veen		Julati	Un a	and the Unit	cateu ro	Denvio	
n _a .	µ16-CFU input ~ µ16-Untreated ~ U	Aite	ine	ate hypothesis	= 110	ere	is a difference	Detwe	en me cro input	popu	Jiatio	on and the t	Jnireated	1 Popula	10
_ 2	21.0510		-			-									+
Sp	31.0510		-			-				_	-				+
t, test statistic	3.2135		-			-				-	-				+
rejection region	t > t _a		-			-									+
α	0.05		-							_	-				+
df	7													-	t
t.	1.895	1													
p-value	0 519240411					-								-	+
Since 3.2135 > 1.895, I do reject th	e null hypothesis, there is a di	ı ffere	nce	between the CF	U Inp	utp	opulation and the	e Untre	eated Populations						+
-															
H _o :	$\mu_{16-Untreated} - \mu_{16-UV} = 0$	Nul	I Hy	pothesis = The	re is	no	difference bet	veent	the Untreated po	pulat	ion a	and the Trea	ated popu	ulation	
H _e :	$\mu_{16-Untreated} - \mu_{16-UV} > 0$	Alte	erna	ate Hypothesis	= The	ere	is a difference	betwe	en the Untreated	d and	the	UV Treated	Populatio	on	
Sp ²	17.2274		+			-									+
t, test statistic	5.2163														
rejection region	$t \ge t_{\alpha}$														
α	0.05														
df	13														
ta	1.771														
p-value	0.519558773														
Since 5.2163 > 1.771. I do reject th	e null hypothesis, there is a di	ffere	nce	between the Un	treat	ed a	and the UV Treat	ed Pop	ulations						



Appendix G: Mutant 6A UV Exposure Comparison

Mutant 6A UV Expo	sure Analysis												
Data													
Strain				(6A) R1 'meropl	oid'	w	and ΔrecF::KAN				_		
CFU Input	13	15		(orly the interop		_					_		
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 ^{-3 dilution}													
All exposures at 9999 Joules													
Statistics													
	n _{6A -CFU input}	2		N _{6A -Untreated}	8		n _{6A -UV}	8	Percent Kill	82			
	x-bareacturizent	14.0		x-bar souther and	6.1		x-bares and	1.1					
	Sector of the se	14		Same	3.4		5	0.4					
	->6A-CFU input	1.4		->6A-Untrested	0.4		S6A-UV	0.4					
Population Compariso	ons												
Comparison Set 18 - (6A) R1 'me	roploid' WT and ΔrecF::KAN												
H ₀ :	$\mu_{6A-CFU input} - \mu_{6A-Untrested} = 0$	Null	Ну	/pothesis = The	re is	no	difference bet	veen t	he CFU input po	oulatio	n and the Unt	treated Pop	pulation
H _a :	μ _{6A-CFU} input - μ _{6A-Untreated} > 0	Alte	rna	te Hypothesis	= The	ere	is a difference	betwe	en the CFU input	popula	ation and the	Untreated	l Populati
sp ²	10.6094												
t, test statistic	3.0582												
rejection region	t > t _a												
α	0.05												
df	8												
t _a	1.860												
p-value	0.519325892												
Since 3.0582 > 1.860, I do reject th	e null hypothesis, there is a di	fferer	nce	between the CFU	J Inp	ut p	population and th	e Untre	ated Populations				
H ₀ :	$\mu_{6A-Untreated} - \mu_{6A-UV} = 0$	Null	Ну	pothesis = The	re is	no	difference bet	veen t	he Untreated po	pulatio	n and the Tre	ated popu	lation
H _e :	μ _{6A-Untreated} - μ _{6A-UV} > 0	Alte	rna	te Hypothesis	= The	ere	is a difference	betwe	en the Untreater	d and th	ie UV Treated	J Populatio	'n
Sp ²	6.0131												
t, test statistic	4.0882												
rejection region	t > t _α												
α	0.05												
df	14												
ta	1.761												
p-value	0.519585629												
Since 4.0882 > 1.761, I do reject th	e null hypothesis, there is a di	fferer	nce	between the Un	treat	ed a	and the UV Treat	ed Pop	ulations				



Appendix H: Mutant 27 UV Exposure Comparison

Data (27) R1 Δων/8:XAN Strain (27) R1 Δων/8:XAN CFU Input 19 36 Untreated 12 16 13 7 17 8 8 All colony counts at 10 ^{4 existen} 2 1.7 1 2.5 1.5 1 All colony counts at 10 ^{4 existen} 2 1.7 1 2.5 1.5 1 2 1.6 1 1 1 2.5 1.5 1 1 2.5 1.5 1 1 2.5 1.5 1 </th <th>Mutant 27 UV Expo</th> <th>osure Analysis</th> <th></th>	Mutant 27 UV Expo	osure Analysis														
Strain (27) R1 Juv/E:XAN CFU input 19 36 Of Unput 18 12 16 17 8 8 Treated 2 1.7 1 2.5 1.1 2 2.5 1.5 All colony counts at 10 ^{4 sluton} All exposures at 999 Joules All exposures at 990 Joules All exposures at 990 Joules All exposures at 900 Journes	Data															
Control Carl / Carl Journal (Cr) / Carl Journal (Cr) / Carl Journal (Cr) Control (Cr) Contro (Cr) Control (Cr) <	Strain			-	(27)	D1 A.	wrF	R-KAN								
District	CEU Input	19	36		(27)		VIL									
Treated 2 1.7 1 2.5 1.1 2 2.5 1.5 All colony counts at 10 ^{6 shudes} All exposures at 999 Joules Percent Kill 85 Statistics Improvements 1.2 Improvements 8 Percent Kill 85 Kbargroup input 2.2 Improvements 1.2 Kbargroup input 8 Percent Kill 85 Population Comparisons Improvements 1.4 Improvements 1.4 Improvements 1.4 Improvements 1.4 Improvements 1.8 Improvements 1	Untreated	18	12	16	13	7	17	8	8							
All colony counts at 10 ^{4 dickin} All exposures at 9999 Joules Statistics	Treated	2	1.7	1	2.5	1.1	2	2.5	1.5							
All exposures at 9999 Joules Image: Construction of the second secon	All colony counts at 10 ^{-5 dilution}															
Statistics Important set in the set of the set o	All exposures at 9999 Joules															
n ₂₂ συ/ input 22 n ₂₂ συ/ input 27.5 k -bar (synthesize) 8 Percent KIII 85 A Strong input 12.0 Sgrow input 12.4 k -bar (synthesize) 1.8	Statistics															
k+barg-coluppet 27.5 k+barg-coluppet 12.0 k+barg-coluppet 12.0 k+barg-coluppet 12.0 k+barg-coluppet 12.0		n _{27-CEU input}	2		N _{27-Untreated}	8		n _{27-UV}	8	Percent Kill	85					
S22CRU leper 12.0 S22CRU leper 4.4 S22CRU 0.6 POpulation Comparisons Image: Comparison Set 19 - (27) R1 Δuv/B::KAN H ₀ : H _{27CPU leper} - H _{27U/UPDEFERS} = 0 Null Hypothesis = There is no difference between the CFU input population and the Untreated Pop H ₁ : H _{27CPU leper} - H _{27U/UPDEFERS} = 0 Null Hypothesis = There is a difference between the CFU input population and the Untreated Pop H ₁ : H _{27CPU leper} - H _{27U/UPDEFERS} = 0 Null Hypothesis = There is a difference between the CFU input population and the Untreated Pop H ₂ : H _{27CPU leper} - H _{27U/UPDEFERS} = 0 Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Pop H ₂ : H _{27CPU} leper S2433 Image: Comparison Set 19 - (27) R1 Δuv/B::KAN Image: Comparison Set 19 - (27) R1 Δuv/B::KAN H ₂ : H _{27CPU} leper Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population Set 19 - (27) R1 Δuv/B::KAN Image: Comparison Set 19 - (27) R1 Δuv/B::KAN Since 3.2433 > 1.860, 1 do reject the null hypothesis a difference between the CFU input population and the Untreated population		x-barzz-cell input	27.5		x-barzz-listrested	12.4		x-bar _{27-UV}	1.8							
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$		Samera	12.0		Samuel	44		5	0.6							
Population Comparisons Image: Comparison Set 19 - (27) R1 ΔuvB::KAN Image: Comparison Set 19 - (27) R1 ΔuvB::KAN Image: Comparison Set 19 - (27) R1 ΔuvB::KAN Ho : H22xCRU Input * H22XUITENEES = 0 NUII Hypothesis = There is no difference between the CFU input population and the Untreated Population and the Untreated Population and the Untreated Population region s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 34.7969 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN s_p^2 0.05 Image: Comparison Set 19 - (27) R1 ΔuvB::KAN Image: Comparison Set 19 - (27		-27-CFU Input	12.0		-2/-Untreated			-27-00	0.0							
Comparison Set 19 - (27) R1 Δ uvrB::KAN Null Hypothesis = There is no difference between the CFU input population and the Untreated Population	Population Comparis	ons														
Ho<: $\mu_{22-CPU input} + \mu_{22-Untreated} = 0$ Null Hypothesis = There is no difference between the CFU input population and the Untreated Population Ho<: $\mu_{22-CPU input} + \mu_{22-Untreated} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population s_p^2 34.7969 Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population s_p^2 34.7969 Alternate Hypothesis Alternate Hypothesis Alternate Hypothesis rejection region t > t_a a a a a a a aq 0.05 a <td>Comparison Set 19 - (27) R1 Δu</td> <td>vrB::KAN</td> <td></td>	Comparison Set 19 - (27) R1 Δu	vrB::KAN														
Hait $\mu_{22xCPU input } + \mu_{22xUntrested} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated of the statistic s_p^2 34.7969 1	H ₀ :	μ _{27-CFU} input - μ _{27-Untreated} = 0	Null	Ну	pothesis = The	re is	no	difference bet	ween t	he CFU input po	oulatio	on ar	nd the Untre	eated Por	pulation	
$\frac{s_p^2}{t, test statistic} = \frac{34.7969}{3.2433}$ $\frac{s_p^2}{t, test statistic} = \frac{3.2433}{3.2433}$ $\frac{s_p^2}{t, test statistic} = \frac{1}{10000000000000000000000000000000000$	H _a :	μ _{27-CFU} input - μ _{27-Untreated} > 0	Alte	rna	te Hypothesis	= The	ere	is a difference	betwe	en the CFU input	popu	latio	on and the l	Intreated	d Populati	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $																
t test statistic 3.2433 rejection region t > t_a α 0.05 df 8 t_a 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_o: $\mu_{27vuntreated} - \mu_{27vuv} = 0$ Null Hypothesis = There is no difference between the Untreated population and the UV Treated Population Sp ² 9.7531 t_test statistic 6.7643	s _n ²	34.7969														
rejection region t > t_a a 0.05 df 8 t_a 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 : $\mu_{27Vultreated} - \mu_{22VUV} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population H _a : $\mu_{27Vultreated} - \mu_{22VUV} > 0$ Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population s_p^2 9.7531 t_test statistic 6.7643	t, test statistic	3.2433														
rejection region t > t_a Image: Constraint of the statistic t > t_a t > t_a t > t_a <th <="" constraint="" of="" statistic<="" td="" the=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></th>	<td></td>															
α 0.05 0 </td <td>rejection region</td> <td>t > ta</td> <td></td>	rejection region	t > ta														
df 8 8 9	α	0.05														
t_a 1.860 Image: Constraint of the statistic 1.860	df	8														
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 : µ _{27VUNTREETED} - µ _{22VUV} = 0 H ₁ : µ _{27VUNTREETED} - µ _{22VUV} > 0 Alternate Hypothesis = There is a difference between the Untreated population and the UV Treated Population s _p ² 9.7531 t, test statistic 6.7643	t _α	1.860														
Since 3.2433 > 1.860, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Populations Ho .	p-value	0.519325892														
Ho μ27-Untreated - μ27-UV Null Hypothesis = There is no difference between the Untreated population and the Treated population Ho μ27-Untreated - μ27-UV Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population Fp ² 9.7531 P P t, test statistic 6.7643 P P P	Since 3.2433 > 1.860, I do reject th	he null hypothesis, there is a di	ffere	nce	between the CF	U Inp	ut p	opulation and th	e Untre	ated Populations						
Ho<: μ27-UNTreased - μ27-UV 0 Null Hypothesis = There is no difference between the Untreated population and the Treated population Ho<: μ27-UNTreased - μ27-UV 0 Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population Sp ² 9.7531 Image: Comparison of the treated for the treated fo																
Hai μ27-VUNTVENENCE - μ27-VUV > 0 Alternate Hypothesis = There is a difference between the Untreated and the UV Treated Population sp ² 9.7531 Image: Sp ² Imag	H _o :	$\mu_{27\text{-Untreated}} - \mu_{27\text{-UV}} = 0$	Null	I Hy	pothesis = The	re is	no	difference bet	ween t	he Untreated po	pulati	on a	nd the Trea	ited popu	lation	
sp ² 9.7531 t, test statistic 6.7643	H _a :	$\mu_{27\text{-Untrested}} - \mu_{27\text{-UV}} > 0$	Alte	rna	te Hypothesis	= The	ere	is a difference	betwe	en the Untreater	d and t	the L	JV Treated F	opulatio	n	
t, test statistic 6.7643	sp ²	9.7531														
	t, test statistic	6.7643														
relation region tot	rejection region	+ > +														
	a	0.05														
4 000 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	df	0.05		-												
	+	1 7 5 1														
4 1.701 1.70	- un lun	0.510505630														
produc 0.32505023	Since 6 7643 > 1 761 L do reject ti	e null hynothesis there is a di	ffere	nce	hetween the Un	treat	ed :	and the LIV Treat	ed Pop	ulations						



Appendix I: 500 Gy Oxygen Exposure Comparison

500 Gy Exposure Comparison																	
Data																	
Strain				500 G	Exposure												
(1) Wild Type (1.5) R1 'meroploid' WT and ArecF::KAN	26	6 32 2 3.5	36	43 3.9						_							
(5) R1 ΔDR_1279::mlox (MnSOD KO)	90	0 42	31	28													
(8) R1 ΔDR_1546::KAN ΔDR_A0202::NAT (Cu/ZnSOD Double KO) (11) R1 ΔbshA::mlox (Bacillithiol Biosynthesis)	27	7 28	33	32													
(16) R1 ΔυντΒ::KAN	43	3 41	29	37													
(5A) K1 meropioid wit and brechtskan (27) R1 duvr8::KAN	39	a 22 9 19	31	26													
All colony counts at 10 ^{-5 diution}																	
Statistics																	
	n _{wT-500}	4	n _{1.5 -50}	o 4	n _{s -500}	4	i n _{a-1}	900	4	n ₁₁₋₅₀₀	4	n _{us-500}	4	n ₆₄₋₅₀₀	4	n ₂₇₋₅₀₀	4
	X~wT-500	34.3	x-1.5-5	6.0	x-bar ₅	-500 47.8	x-b	ar ₈₋₅₀₀	27.0	x-bar ₁₁₋₅₀₀	28.3	x-bar ₁₆₋₅₀₀	37.5	x-bar ₆₄₋₅₀₀	24.0	x-bar ₂₇₋₅₀₀	28.8
	\$wT-500	7.1	\$1.5-50	4.0	\$5-500	28.8	S S2.5	00	4.5	\$11-500	11.5	Sus-500	0.2	S6A-500	5.1	\$27-500	8.4
			Perce	nt Ki 82.40876		-39.4161		1	21.16788		17.51825		-9.48905		29.92701		16.05839
Population Comparisons																	
Comparison Set 9 - 500 Gy Exposure																	
H ₀ : H_:	$\mu_{WT-500} - \mu_{1.5-500} = 0$	Alternate	othesis = There is Hypothesis = The	no difference b re is a differenc	etween the Wild te between the V	Type popula Wild Type por	tion and the M pulation and th	utant 1.5 e Mutan	Population t 1.5 Population								
	PRINK PLINK -																
Sp ²	33.5163	3															
t, test statistic	0.0340	D															
rejection region	t>t _a																
a df	0.05	6															
t _a	1.943	3															
p-value Since 6.8948 > 1.943, I do reject the null hypothesis, there is a c	0.519127341 difference between the V	1 Wild Type a	ind Mutant 1.5 Po	pulations													
H ₀ :	$\mu_{WT-500} - \mu_{S-500} = 0$	Null Hypo	othesis = There is Humotheric = The	no difference b	etween the Wild	Type popula	tion and the M	utant 5 P	opulation								
	F = 1 - 900 P3 - 500 C V			_ is a strike en	- seconden alle i		- The sector of the UT	enefi									
5p ²	440.2500	0															
t, test statistic	-0.9099	5															
rejection region	$t > t_x$																
α df	0.05	5															
t,	1.943	3															
p-value Since - 9999 < 1.943. Ldo not reject the null hypothesis, there is	0.519127341	1 the Wild T	we and Mutant S	Populations													
since 1985 (1996) rab not reject the num ripothesis, there is			pe and matant s	ropulations													
H ₀ :	μ _{wT-soo} - μ _{s-soo} = 0	Null Hypo	othesis = There is	no difference b	etween the Wild	Type popula	tion and the M	utant 8 P	opulation								
n.:	Pwt-soo * Ps-soo * 0	Alternate	Hypothesis = The	re is a differen	be between the v	wild type pop	pulation and th	e Mutan	t a Population								
5 ₈ ⁻¹	35.7917	7															
t, test statistic	1.7138	B															
rejection region	t > t _a																
α df	0.05	5															
t.	1.943	3															
p-value Since 1.7138 < 1.943. Like not reject the null hypothesis, there is	0.519127341 is no difference between	1 the Wild T	wee and Mutant 8	Populations													
H ₀ :	μ _{wT-500} - μ ₁₁₋₅₀₀ = 0	Null Hypo	othesis = There is	no difference b	etween the Wild	Type popula	tion and the M	utant 11	Population								
n ₂ :	Pwt-500 * P11-500 * 0	Atternate	Hypothesis = The	re is a differen	te between the t	who type pop	polation and th	emulari	t 11 Population								
5p ²	91.9167	7															
t, test statistic	0.8851	1															
rejection region	t > t _x																
α df	0.05	6															
t _e	1.943	3															
p-value Since 0.8851 < 1.943, I do not reject the null hypothesis, there i	0.519127341 is no difference between	1 the Wild T	vpe and Mutant 1	1 Populations													
H ₀ :	μ _{wT-500} · μ ₁₅₋₅₀₀ = 0	Alternate	othesis = There is Nonothesis = The	no difference b	etween the Wild	Type popula Wild Type por	tion and the M sulation and th	utant 16	Population t 16 Population								
	PRIVE PRIVE																
5 _p ²	44.6250	D															
to their distribution	-0.688	-															
rejection region	t>t _a																
α df	0.05	5															
t _e	1.943	3															
p-value Since -0.6880 < 1.943, I do not reject the null hypothesis, there	0.519127341 is no difference between	1 n the Wild	Type and Mutant	16 Populations													
H ₀ :	μ _{WT-500} · μ _{6A-500} = 0	Alternate	Hypothesis = There is Hypothesis = The	no difference b	etween the Wild	Type popula Wild Type por	tion and the M nulation and th	e Mutan	Population t 68 Population								
Sp ⁴	38.4583	3															
ty was automatic	2.3375	-															
rejection region	t>t _a																
α df	0.05	6															
t _a	1.943	3															
p-value Since 2.3375 > 1.943, I do reject the null hypothesis, there is a c	0.519127341 difference between the V	1j Wild Type a	ind Mutant 6A Po	pulations													
H ₀ :	$\mu_{WT-500} - \mu_{27-500} = 0$	Alternato	otnesis = There is Hypothesis = The	no difference b	etween the Wild	Type popula Wild Type cor	tion and the M	e Mutarr	Population								
	Pmi - 300 PM27 - 300 P - 3	. mernate				the part		- medi	openedon								
5,2 A Anna statistic	60.9167	7															
t, test statistic	0.9966	5															
rejection region	t>t _a																
α df	0.05	6															
t _e	1.943	3															



Appendix J: 1000 Gy Oxygen Exposure Comparison

1000 Gy Exposure Comparison																							
Data																							
Strain					1000	Gy Expos	ure																
(1) Wild Type (1.5) R1 'moraploid' WT and AmcEuKAN	42	2 40	37	37																			
(5) R1 ΔDR_1279::mlox (MnSOD KO)	29	36	31	34																			
(8) R1 &DR_1546::KAN &DR_A0202::NAT (Cu/ZnSOD Double KO (11) R1 &baba::mlox (Bacillithin) Biosynthesis)	25	25	24	27																			
(16) R1 &uvr8::KAN	43	42	45	43																			
(6A) R1 'meroploid' WT and ArecF::KAN	29	24	21	26																			
All colony counts at 10 ^{rd/uton}		37																					
Statistics																							
Statistics	0	4		0	4		0	4		0	4		0	4	0		4	0		4	0		4
	X-wT-1000	39.0		X-1.5-1000	6.8		x-bar _{5 -0000}	32.5		x-bar _{s-1000}	25.3		x-bar11-000	34.5	x-ba	18-1000	42.3	x-bar _{sa}	-1000	25.0	x-bar ₂₇	-1000	34.0
	S _{WT-1000}	2.4		\$1.5-0000	3.0		53-1000	3.1		5 ₈₋₁₀₀₀	1.3		S ₁₁₋₀₀₀₀	8.1	S16-0	00	2.5	S _{8A-0000}		3.4	\$ ₂₇₋₀₀₀₀		2.9
				Percent K	82.69231			16.66667			35,25641			11.53846		-	8.33333		35.8	9744		12.8	12051
Population Comparisons																							
Comparison Set 10 - 1000 Gy Exposure																							
н,:	$\mu_{WT-1000} - \mu_{1.5-1000} = 0$	Null Hypot	thesis = Th	ere is no d	ifference be	tween th	e Wild Typ	e population	n and the	Mutant 1.5	Population	1											
H _a :	μ _{wT-0000} - μ _{1.5-1000} > 0	Alternate F	Hypothesi	s = There i	s a differeno	e betwee	n the Wild	Type popula	ation and	the Mutant	t 1.5 Popula	ition											
5. ²	7.4583																						
t, test statistic	16.7003	1																					
rejection region	t>t.																						
a	0.05																						
df	6	5																					
p-value	0.519127341																						
Since 16.7003 ≥ 1.943, I do reject the null hypothesis, there is a	difference between t	the Wild Typ	pe and M	atant 1.5 P	opulations																		
He :	Harr. 1000 - Ha. 1000 = 0	Null Hypot	thesis = Th	ere is no d	lifference be	tween th	e Wild Typ	e population	n and the	Mutant 5 P	opulation												
Hat	μ _{ert-sooo} - μ _{s-sooo} > 0	Alternate H	Hypothesi	s = There i	s a differeno	e betwee	n the Wild	Type popula	ation and	the Mutant	5 Populati	on											
sp ⁻ t, test statistic	7.8333																						
rejection region	t>ta																						
a df	6.05																						
t _e	1.943	5																					
p-value Since 3.2844 > 1.943. I do reject the null hypothesis, there is a	0.519127341 difference between th	he Wild Type	e and Mu	tant 5 Pop	alations																		
H ₀ :	μ _{#IT-000} - μ ₈₋₀₀₀ = 0	Null Hypot	thesis = Th	iere is no d	lifference be	tween th	e Wild Typ	e population	n and the	Mutant 8 P	opulation												
H ₄ :	µ _{er⊤-0000} * µ ₈₋₀₀₀₀ ≥ 0	Alternate	Hypothesi	s = There i	s a differenc	e betwee	in the Wild	Type popula	ation and	the Mutant	t 8 Populati	on											
5 ₂ ²	3.7917																						
t, test statistic	9.9863	8																					
rejection region	t>t _a																						
α	0.05																						
t,	1.943																						
p-value	0.519127341	ī.																					
Since 9.9863 > 1.943, I do reject the null hypothesis, there is a	difference between th	he Wild Type	e and Mu	tant 8 Popi	alations																		
н.:	$\mu_{\rm WT-1000} \circ \mu_{\rm SI-1000} = 0$	Null Hypot	thesis = Th	iere is no d	lifference be	tween th	e Wild Typ	e population	n and the	Mutant 11	Population												
H _a :	μ _{жT-0000} - μ ₁₁₋₀₀₀₀ > 0	Alternate F	Hypothesi	s = There i	s a differeno	e betwee	n the Wild	Type popula	ation and	the Mutant	t 11 Populat	tion											
sj ²	35.8333																						
t, test statistic	1.0631	l																					
rejection region	t>t.																						
α	0.05																						
df	6	5																					
p-value	0.519127341	l I																					
Since 1.0631 < 1.943, I do not reject the null hypothesis, there	is no difference betwe	een the Wild	d Type an	d Mutant 1	1 Population	15																	
н.	μ _{wr-1000} - μ ₁₈₋₁₀₀₀ = 0	Null Hypot	thesis = Th	ere is no d	lifference be	tween th	e Wild Typ	e population	n and the	Mutant 16	Population												
H _a :	$\mu_{er\tau - 1000} - \mu_{16 - 1000} \ge 0$	Alternate F	Hypothesi	s = There i	s a differeno	e betwee	n the Wild	Type popula	ation and	the Mutant	t 16 Populat	tion											
.1	6.020																						
t, test statistic	-1.8571																						
a a a a a a a a a a a a a a a a a a a	0.05																						
df	6	5																					
t _s	0.519127241																						
Since -1.8571 < 1.943, I do not reject the null hypothesis, there	is no difference betw	een the Wil	ild Type ar	nd Mutant	16 Populatio	ins																	
		Null Hupot	thoric - Th	oro ir no r	efforcere bo	twoon th	o Wild Two	o population	and the	Mutort 64	Doculation												
нь.	µ _{WT-0000} = µ ₆₄₋₁₀₀₀ = 0 µ _{WT-0000} = µ ₆₄₋₁₀₀₀ ≥ 0	Alternate F	Hypothesi	s = There i	s a difference de	e betwee	in the Wild	Type popula	ation and	the Mutant	6A Popula	tion											
Sp ²	8.6667	<i>,</i>																					
t, test statistic	6.7234	•																					
rejection region	t>t _a																						
α df	0.05	5																					
t _s	1.943																						
p-value Since 6.7254 > 1.941.1 do reject the null humathatis, there is a	0.519127341 difference between th	he Wild Tyre	e and M-	tant 6A D-	pulations																		
there is a service of the main representation in the service of th		and type																					
H ₀ :	$\mu_{w\tau - 1000} \circ \mu_{27 - 1000} = 0$	Null Hypot	thesis = Th	ere is no d	lifference be	tween th	e Wild Typ	e population	n and the	Mutant 27	Population												
H _k :	μ _{wT-0000} - μ ₂₇₋₀₀₀₀ > 0	Alternate H	нypothesi	s = There i	s a differeno	e betwee	n the Wild	rype popula	ation and	the Mutant	27 Populat	tion											
5p ²	7.3333	8																					
t, test statistic	2.6112	1																					
rejection region	t>t _a																						
α	0.05																						
df t-	1 043																						
p-value	0.519127341																						
Since 2.0112 > 1.943, I do reject the null hypothesis, there is a	difference between th	he Wild Type	e and Mu	tant 27 Pop	outations																		



Appendix K: 10,000 Gy Oxygen Exposure Comparison

10000 Gy Exposure Comparison																				
Data																				
Strain					10000 Gy Ex	osure	_													
(1) Wild Type (1.5) R1 'meroploid' WT and &recF::KAN	20	3 23	32 9	2.3																
(5) R1 ΔDR_1279::mlox (MnSOD KO) (8) R1 ΔDR_1265::KAN ADR_40282::MAT (Cu/285OD Double KO	3	2 16	24	29																
(11) R1 AbshA::mlox (Bacillithiol Biosynthesis)	3:	1 25	33	26																
(16) R1 ΔuvrB::KAN (6A) R1 'meroploid' WT and ΔrecF::KAN	20	22 1 22	29	30																
(27) R1 ΔυνrB::KAN	3	5 14	22	33			_													
All colony counts at 10																				
Statistics																				
Statistics	Durg 19990	4	n.	5.0000	4	0	4		Pa	4	0	4	0.4.000	~	4	Des	4	217-0000	4	
	X*WT-10000	27.8	x	1.5-00000	5.1	x-bar ₅₋₃₀₀	25.3		x-bar ₈₋₀₀₀₀₀	21.0	x-bar11-1000	28.8	x-bar _{at}	r-00000 Z	5.3	x-bar64-00000	21.3	-bar ₂₇₋₁₀₀₀₀	26.0	
	S _{WT-00000}	3.7	51	3-10000	3.1	S ₅₋₁₀₀₀₀	7.0		5 ₈₋₁₀₀₀₀	1.4	\$11-00000	3.9	S18-3000	8	5.0	S ₅₄₋₀₀₀₀	5.4	27-00000	9.8	
			Pe	ercent Ki 8	1.71171		9.009009			24.32432		-3.6036		9.009	009		23.42342		6.306306	
Population Comparisons																				
Comparison Set 11 - 10000 Gy Exposure																				
н.:	μ _{w7-20000} - μ _{1.5-10000} = 0	Null Hypothe	esis = Ther	e is no diff	erence betwee	the Wild Ty	pe populat	tion and the	e Mutant 1.5	Population										
H _e :	μ _{wT-20000} - μ _{1.5-10000} > 0	Alternate Hy	pothesis =	There is a	difference bety	veen the Wik	d Type pop	ulation and	d the Mutan	t 1.5 Population										
5 ₀ ²	11.502	,																		
t, test statistic	9.454	9																		
rejection region	t>t _a																			
α df	0.0	5																		
t _s	1.94	3																		
p-value Since 9.4549 > 1.943, I do reject the null hypothesis, there is a	0.51912734 difference between the	e Wild Type an	nd Mutant	1.5 Popula	tions															
		No.71 Library Billion	asis - These	a la an diff		the part of T				and deliver										
n ₀ : H _s :	μ _{WT-00000} - μ _{S-00000} = 0 μ _{WT-00000} - μ _{S-00000} > 0	Alternate Hy	pothesis =	There is a	difference betwee	veen the Wild	d Type populat	cionand the pulation and	d the Mutan	t 5 Population										
1																				
s _p t, test statistic	0.632	5																		
relation ration	151																			
α	0.05	5																		
df t-	1.94	5																		
p-value	0.51912734	L																		
Since 0.6325 < 1.943, I do not reject the null hypothesis, there	is no difference betwe	en the Wild Ty	ype and Mi	utant 5 Pop	pulations															
Но:	$\mu_{w\tau-coooo} - \mu_{B-coooo} = 0$	Null Hypothe	esis = Ther	e is no diff	erence betwee	n the Wild Ty	pe populat	tion and the	e Mutant 8 P	opulation										
н,:	μ _{w7-0000} - μ _{e-0000} > 0	Alternate Hy	pothesis =	There is a	difference bety	reen the Wik	d Type pop	pulation and	d the Mutan	t 8 Population										
5g ²	7.791	7																		
t, test statistic	3.419	s																		
rejection region	t > t _a	5																		
df		5																		
t _e	0.51912734	8 L																		
Since 3.4198 > 1.943, I do reject the null hypothesis, there is a	difference between the	e Wild Type an	nd Mutant	8 Populati	ons															
Но:	μ _{wt-sooo} - μ _{ss-sooo} = 0	Null Hypothe	esis = Ther	e is no diff	erence betwee	n the Wild Ty	pe populat	tion and the	e Mutant 11	Population										
н.:	μ _{wT-00000} - μ ₁₁₋₀₀₀₀ > 0	Alternate Hy	pothesis =	There is a	difference bety	reen the Wik	d Type pop	oulation and	d the Mutan	t 11 Population										
5 ₉ ²	14.250	0																		
t, test statistic	-0.374	5																		
rejection region	t>t _a																			
α df	0.0	5																		
t _e	1.94	3																		
Since -0.3746 < 1.943, I do not reject the null hypothesis, there	e is no difference betwe	en the Wild T	Type and M	lutant 11 P	opulations															
He :	Har	Null Hypothe	esis = Ther	e is no diff	erence betwee	the Wild Ty	pe populat	tion and the	e Mutant 16	Population										
Hat	μ _{w7-0000} - μ ₁₈₋₁₀₀₀ > 0	Alternate Hy	pothesis =	There is a	difference bety	veen the Wik	d Type pop	pulation and	d the Mutan	t 16 Population										
c1	19.250																			
t, test statistic	0.805	3																		
rejection region	t>t _a																			
α df	0.0	5																		
t _a	1.94	8																		
p-value Since 0.8058 < 1.943, I do not reject the null hypothesis, there	0.51912734 is no difference betwee	t en the Wild Ty	ype and Mi	utant 16 Po	pulations															
H ₀ : H ₀ :	μ _{WT-20000} - μ _{EA-20000} = 0 μ _{WT-20000} - μ _{EA-20000} > 0	Alternate Hy	esis = Ther (pothesis =	e is no diff There is a	difference betwee	veen the Wild Ty	pe populat d Type pop	tion and the sulation and	e Mutant 6A d the Mutan	t 6A Population										
sp t, test statistic	21.250	i i																		
minition sealon	154																			
α	0.05	5																		
df t	1 94	3																		
p-value	0.51912734																			
Since 1.9941 > 1.943, I do reject the null hypothesis, there is a	difference between the	e Wild Type an	nd Mutant	6A Populat	tions															
Hot	$\mu_{WT-00000} - \mu_{27-L0000} = 0$	Null Hypothe	esis = Ther	e is no diff	erence betwee	the Wild Ty	pe populat	tion and the	e Mutant 27	Population										
n,:	μ _{WT-60000} - μ ₂₇₋₆₀₀₀₀ > 0	Atternate Hy	/potnesis =	nere is a	utterence betv	reen the Wild	u Type pop	Julation and	u (ne Mutan	∠/ Population										
5g ²	55.125																			
t, test statistic	0.333	5																		
rejection region	t>t _a																			
df	0.0	5																		
t _e p-value	0.51912734	B L																		
Since 0.3333 < 1.943, I do not reject the null hypothesis, there	is no difference betwee	en the Wild Ty	ype and Mi	utant 27 Po	pulations															



Appendix L: Wild Type Oxygen Exposure Comparison

Data Interface	Wild Type Analysis											
Nome Number of the second	Data											
Comparison Compari	Strain				1 14/12	100			-			
	Strain			K.		уре						
Unit and Engineering 13 3	CFU Input	21	15	9								
Unitedation for the set of the s	Untreated Unvacuumed	41	32									
300 by 3 <td>Untreated Vacuumed</td> <td>32</td> <td>33 2</td> <td>6 33</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	Untreated Vacuumed	32	33 2	6 33								
monor dia dia </td <td>500 Gv</td> <td>52</td> <td>22 2</td> <td>6 55</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	500 Gv	52	22 2	6 55								
Dist Org Dist Org Dist Dist <thdis< th=""> Dist Dist</thdis<>	ou ay	26	32 3	• 43					-			
Mathematic Mathema	1000 Gy	42	40 3	7 37								
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rejection region t b t a Image: Constraint of the sector												
α 0.05 0 <td>rejection region</td> <td>t > t_a</td> <td></td>	rejection region	t > t _a										
Owner Owner df 8 t_a 1.860 p-value 0.513225832 Since 1.7857 < 1.860, 1do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations	a	0.05										
ar 8 6 6 t_e 1.860		0.05										
ta 1.860 p-value 0.513225832 Since 1.7857 < 1.860, 1do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations	ar	8										
-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	ta	1.860										
pranue U.31323822 Since 1.7837 f 1.560, I do not reject the null hypothesis, there is no difference between the Untreated and the 10,000 Gy Treated Populations	- uslus	0.510000000										
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	0.100100	0 510225002										
	p-value	0.515525852										



80

Appendix M: Mutant 1.5 Oxygen Exposure Comparison

Mutant 1.5 Ana	lysis															
Data																
Strain				R1 'm	eroploid' V	VT and ∆re	CF::KAN									
CFU Input	2.7	2.7	6	i												
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 diutor}	•															
Ch. 11.11																
Statistics																
	n1.5-CFU input	3		n _{1.5-Untreated}	6					n _{1.5-500}	4	n _{1.5-1000}	4	n _{1.5-10000}	4	
	x-bar, continue	3.8		x-bar, cummer	24.3					x-bar, com	6.0	x-bar,	6.8	x-bar, r	5.1	
	c.	1.0		c.	16.7					c	4.0	c	2.0		2.1	
	P1.5-CFU input	1.9		P1.5-Untreated	10.7					⁹ 1.5-500	4.0	⁹ 1.5-1000	5.0	P1.5-10000	5.1	
Population Comp	arisons															
Comparison Set 2 - Mutant 1	.5															
н. :		Null Hype	thosis = Th	ore is no differ	ence hetw	oon the CE	ill input po	nulation a	nd the lint	treated Popu	lation					
	P1.5-CPU input P1.5-Untreated = 0	all second	the set less	There is no arrier		een ene er	o mpac po		in the one	incuted ropu	- detter					
n _a :	P1.5-CFU input - P1.5-Untreated > 0	Arternate	nypotnes	is = i nere is a di	merence b	erween th	е сно іпри	r populatio	m and the	ontreated P	opulation					
Sp ⁴	201.2276															
t, test statistic	-2.0471															
rejection region	t > t _a															
α																
df	0.03															
+	1 005	1														
ι <u>α</u>	1.033															
p-value	0.519240411															
Since -2.0471 < 1.895, I do no	ot reject the null hypothesis	, there is n	o differen	ce between the	CFU Input	populatio	n and the L	Intreated I	opulation	ns						
H ₀ :	$\mu_{1.5-\text{Untreated}} - \mu_{1.5-500} = 0$	Null Hypo	thesis = Th	nere is no differ	ence betw	een the Ur	ntreated po	pulation a	nd the Tre	eated popula	tion					
H _a :	μ _{1.5-Untreated} - μ _{1.5-500} >0	Alternate	Hypothesi	is = There is a di	fference b	etween th	e CFU inpu	t populatio	on and the	Untreated P	opulation					
c ²	191 2101															
3p	181.2101															
t, test statistic	2.1070															
rejection region	$t > t_{\alpha}$															
α	0.05															
df	8															
ta	1.860															
p-value	0.519325892	1														
Since 2.1070 > 1.860. I do reio	ect the null hypothesis, the	re is a diffe	rence bet	ween the Untre	ated and t	he 500 Gv 1	Freated Po	pulations								
	, p====, the															
н.:	0	Null Hurse	thesis – Th	ere is no diffor	ence hetw	een the Ur	treated or	nulation	nd the Tro	ated popula	tion					
101	m1.5-Untreated - M1.5-1000- U	. von nypo	uncard = 11		cince betw		eateu pu	-porocion a								
H _a :	$\mu_{1.5-\text{Untreated}} - \mu_{1.5-1000} > 0$	Alternate	Hypothes	ıs = There is a di	tterence b	etween th	e CFU inpu	t populatio	on and the	Untreated P	opulation					
sp ²	178.5104															
t. test statistic	2.0388	1														
	210500															
rejection region	+ 5 +															
rejection region	ις i _α															
u 	0.05															
at	8															
tα	1.860															
p-value	0.519325892															
Since 2.0388 > 1.860, I do rei	ect the null hypothesis. the	re is a diffe	rence bet	ween the Untre	ated and t	he 1000 Gv	Treated P	opulations								
	, , , , , , , , , , , , , , , , , , ,															
H. C		Null Hype	thosis = Tk	here is no diffor	once hetw	een the Ur	treated or	nulation a	nd the Tre	ated nonula	tion					
	P1.5-Untreated ~ P1.5-10000 = 0	. кол пуро	Greats = If	icre is no umen	crice betW	can die Of	eateu po	-puracion a	na cre tre							
H _a :	$\mu_{1.5-Untreated} - \mu_{1.5-10000} > 0$	Alternate	Hypothes	is = There is a di	tterence b	etween th	e CFU inpu	t populatio	on and the	Untreated P	opulation					
sp ²	178.7001															
t, test statistic	2,2318	1														
	2.2.310															
rejection region	151															
rejection region	$\iota \sim \iota_{\alpha}$															
α	0.05															
df	8															
tα	1.860															
p-value	0.519325892															
Since 2.2318 > 1.860. I do reio	ect the null hypothesis the	re is a diffe	rence bet	ween the Untre	ated and t	he 10.000 (Sy Treated	Population	15							
	, p , die					,										



81

Mutant 5 Analysis Data R1 ADR_1279::mlox (MnSOD KC Strain CFU Input Untreated Unvacuumed Untreated Vacuumed 500 Gy 1000 Gy 10,000 Gy 23 12 34 31 42 10 37 21 90 29 21 28 34 28 31 31 36 16 24 29 All colony counts at 10^{-5 diution} Statistics n_{s-CFU input} s -Untreated n₅₋₁₀₀₀ x-bar₅₋₁₀₀₀ n₅₋₅₀₀ x-bar₅₋₅₀₀ 47.8 32.5 x-bars-cruinpy 15.0 x-bar_{5-Untreate} 28.7 x-bar₅₋₁₀₀₀₀ 25.3 7.0 6.7 28.8 3.1 7.0 S5-CFU input -1000 **Population Comparisons** Comparison Set 3 - Mutant 5 University of the second He: На : Hscruinput - Usuated > 0 Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Populatio 45,6190 sp* t, test statistic -2.8616 rejection region t>t_a 0.05 df 1.895 va 0.519240411 Since -2.8616 < 1.895, I do not reject the null hypothesis, there</td> the CFU Input population and the Untreated Populations н.: $\mu_{\text{5-Untreated}} - \mu_{\text{5-SOO}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population н.: Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population μ_{5-Untreated} - μ₅₋₅₀₀ > 0 338.760 t, test statistic -1.6063 rejection region $t \ge t_{\alpha}$ 0.05 α df 1.860 0.519 19325892 sis, there p-valu Since -1.6063 < 1.860, I do not reject the null hyp the Untreated and the 500 Gy Treated Population H_e: - μ_{s.tom}= 0 Null Hypothesis = There is no difference between the Untreated population and the Treated population He. H_a: H5-UNTREASEd - H5-1000 >0 Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population 31.2917 sp² t, test statistic -1.0616 rejection region $t > t_{\alpha}$ 0.05 α df 8 1.860 p-value 0.519325892 Since -1.0616 < 1.860, I do not reject the null hypothesis, there is no difference be tween the Untreated and the 1000 Gy Treated Populations Н₀ : Н₂ : μ_{5-Unterated} - μ₅₋₁₀₀₀₀ = 0 Null Hypothesis = There is no difference between the Untreated population and the Treated population Hs-unreased - Hs-10000 >0 Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population 46.010 sp* t, test statistic 0.7803 rejection region $t \ge t_{\alpha}$ 0.05 α df 8 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 N: Mutant 5 Oxygen Exposure Comparison



Appendix O: Mutant 8 Oxygen Exposure Comparison

Mutant 8 Analys	sis																
Data																	
Strain			R1	ΔDR 1546::KA	N ADR AND	02::NAT (0	Cu/ZnSOD	Double KO		1			1				
CFU Input	15	20	18	3									1				
Untreated Unvacuumed	21	35	i														
Untreated Vacuumed	23	18	24	1 23													
1000 Gy	27	20	21	1 27													
10,000 Gy	23	20	20	21													
All colony counts at 10 ^{-5 diution}																	
Statistics																	
	n _{8-CFU input}	3		n _{8-Untreated}	6					n ₈₋₅₀₀	4		n ₈₋₁₀₀₀	4	n ₈₋₁₀₀₀₀	4	
	x-bar _{8-CFU input}	17.7		x-bar _{8-Untreated}	24.0					x-bar ₈₋₅₀₀	27.0		x-bar ₈₋₁₀₀₀	25.3	x-bar ₈₋₁₀₀₀₀	21.0	
	S8-CFU input	2.5		S _{8-Untreated}	5.8					S ₈₋₅₀₀	4.5		S8-1000	1.3	S8-10000	1.4	
Donulation Compo	ricono																
Population Compa	Insons																
Comparison Set 4 - Mutant 8																	
H _o :	$\mu_{\text{S-CPU input}} - \mu_{\text{S-Untreated}} = 0$	Null Hypo	othesis = Th	here is no diffe	rence betv	veen the C	FU input p	opulation a	and the U	ntreated Pop	oulation						
H _a :	$\mu_{\text{B-CFU input}} - \mu_{\text{B-Untreated}} > 0$	Alternate	Hypothes	is = There is a o	difference	between t	he CFU inp	ut populati	on and th	e Untreated	Population	1					
Sp ²	25.8095																
t, test statistic	-1.7630																
rejection region	+ \ +																
rejection region	L 2 Lg																
df	0.05																
t.	1.895																
p-value	0.519240411																
Since -1.7630 < 1.895, I do not	reject the null hypothesis	, there is r	no differen	nce between th	e CFU Inpu	it populati	on and the	Untreated	Populati	ons							
H. f	U	Null Hype	thesis = Th	here is no diffe	rence hetv	veen the L	Intreated r	opulation	and the T	reated nonul	lation						
н	Ps-Untreated Ps-soo = 0	Alternate	Wynothes	is – There is a r	lifference l	hetween t	he CEll inn	ut nonulati	on and th	e Untreated	Population	,					
··a ·	M8-Untreated * M8-500 * V	Anternate	Typothes	is - mere is a c	interence	Jetweent	ne er o mp	ut populati	on and a	ie ontreated	Population						
s. ²	28,7500																
t, test statistic	-0.8668																
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,																	
rejection region	t>t _a																
α	0.05																
df	8																
ta	1.860																
p-value	0.519325892																
Since -0.8668 < 1.860, I do not	reject the null hypothesis	, there is r	no differen	nce between th	e Untreate	d and the	500 Gy Tre	ated Popul	ations								
u .		Mull they	thesis - T	horo is no diff-	ronce hat	voon the t	Intractor -	opulation	and the T	reated por	lation						
n ₀ .	μ _{8-Untreated} - μ ₈₋₁₀₀₀ = 0	мин нурс	unesis = If	nere is no diffe	nence betv	veen the U	ha critte	opulation	and the l	reated popul	nacion Descut :::						
на:	μ _{8-Untreated} - μ ₈₋₁₀₀₀ > 0	Alternate	Hypothes	as = There is a (anterence	between t	ne CFU inp	ut populati	on and th	e untreated	Population	1					
. 2	34 5000																
Sp ⁻	21.5938																
t, test statistic	-0.4167																
rejection region	tst																
a	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 r	no differen	nce between th	e Untreate	d and the	1000 Gy Tr	eated Popu	lations								
H _o :	$\mu_{\text{8-Untreated}} - \mu_{\text{8-10000}} = 0$	Null Hypo	othesis = Th	here is no diffe	rence betv	veen the U	Intreated p	opulation	and the T	reated popul	lation						
H _a :	$\mu_{\text{B-Untreated}} - \mu_{\text{B-10000}} > 0$	Alternate	Hypothes	is = There is a d	difference	between t	he CFU inp	ut populati	on and th	e Untreated	Population	1					
Sp ²	21.7500																
t, test statistic	0.9965																
rejection region	$t \ge t_{\alpha}$																
α	0.05																
dt	8																
t _α	1.860																
p-value	0.519325892	thors is	o diff	n hotura an 11	linterat	d and the f	0.000.000	reated Pr	ulation								
5000 0.9903 ~ 1.800, 1 00 NOT F	eject the null hypothesis,	arere is n	o amereno	between the	- oncreater	a anu trie 1	,000 GY I	reated POP	alacions								



Appendix P: Mutant 11 Oxygen Exposure Comparison

Data																
itrain				R1 ∆bshA	::mlox (Ba	illithiol Bi	osynthesis									
CFU Input	10	14	20													
Intreated Unvacuumed	28	28														
Intreated Vacuumed	24	39	31	28												
00 Gy	34	11	33	35												
000 Gy	43	24	33	38												
Ul colony counts at 10 ^{-5 div}	tion	23		20												
in colony counts at 10																
tatistics																
	n _{11-CFU input}	3		n ₁₁ -untreated	6					n ₁₁₋₅₀₀	4	n ₁₁₋₁₀₀₀		4	n ₁₁₋₁₀₀₀₀	4
	x-bar or cruised	14.7		x-bar.	29.7					x-bar	28.3	 x-bar	34.	5	x-bar.	28.8
	c .	5.0		c .	5.1					c	11.5	c	8	1	e	3.9
	P11-CFU input	5.0		"11 -Untreated	5.4					P11-500	****	-11-1000	0.	-	211-10000	
opulation Com	parisons															
maarican Sat 5 Mutant																
mparison set 5 - Mutant																
÷	$\mu_{\texttt{11-CFU input}} - \mu_{\texttt{11-Untreated}} = 0$	Null Hypo	thesis = Th	ere is no differ	ence betw	een the CF	U input pop	oulation an	d the Untr	eated Popu	lation					
4	μ _{11-CFU input} - μ _{11-Untreated} > 0	Alternate	Hypothesi	s = There is a di	fference b	etween th	e CFU input	populatio	n and the l	Untreated P	opulation					
1	25.7143															
test statistic	-4.1833															
jection region	t > t _a															
	0.05															
	7															
	1.895															
value	0.519240411															
nco 4 1922 < 1 995 I do i	of reject the null hypothesis	thoro is n	o difference	o hotwoon the	CELLInnut	nonulatio	n and the U	Introated B	onulation							
;:	μ ₁₁₋₀ meessed - μ ₁₁₋₅₀₀ = 0	Null Hypo	thesis = Th	ere is no differ	ence betw	een the Un	ntreated po	pulation ar	nd the Trea	ated populat	tion					
:	И11-0+rested - И11-500 = 0 И11-0+rested - И11-500 = 0	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw	een the Ur	ntreated po	pulation ar	nd the Trea	ated populat	tion					
;;	$\mu_{11\text{-Untreasted}} ^* \mu_{11\text{-500}} ^= 0 \\ \mu_{11\text{-Untreasted}} ^* \mu_{11\text{-500}} ^> 0$	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw fference b	een the Un	ntreated po e CFU input	pulation ar	nd the Trea	ated populat Untreated P	tion opulation					
); ;; 2	$\label{eq:massed_product} \begin{split} \mu_{11} & _{\text{OPERANG}} & ~ \mu_{11} & _{\text{ODE}} = 0 \\ \mu_{11} & _{\text{OPERANG}} & ~ \mu_{11} & _{\text{ODE}} > 0 \\ & & $	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw fference b	een the Un	ntreated po e CFU input	pulation ar	nd the Tree	ated populat	tion					
;; ;; 2 2	μ_{11} -onesand * μ_{11} -one = 0 μ_{11} -onesand * μ_{11} -one > 0 66.0104 0.2001	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betwo	een the Ur	ntreated po e CFU input	pulation ar	nd the Trea	ated populat Untreated Pi	tion					
;: ;: a test statistic	$\begin{array}{c} \mu_{11}, _{00704005} + \mu_{11}, _{000} = 0 \\ \mu_{11}, _{00704005} + \mu_{11}, _{000} > 0 \\ \hline & 66.0104 \\ 0.2701 \end{array}$	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw	een the Ur	ntreated po	pulation ar	nd the Trea	ated populat	ion opulation					
;; ;; test statistic	μ ₁₁ - υπιατική "μ ₁₁ - 300 = 0 μ ₁₁ - υπιατική "μ ₁₁ - 300 > 0 66-0104 0.2701	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betwo	een the Ur	treated po e CFU input	pulation ar	nd the Trea	ated populat Untreated Pi	cion opulation					
;: ;: test statistic jection region	μ _{11 - 00} - μ _{11 - 000} = 0 μ _{11 - 00} - μ _{11 - 000} = 0 66 - 0.04 0 - 0.2701 t>t _a	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betwo	een the Ur	ntreated po	pulation ar	nd the Trea	ated populat	ion					
; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;	$\begin{array}{c} \mu_{11}, \mu_{11}, \mu_{11}, \mu_{22} = 0 \\ \mu_{11}, \mu_{12}, \mu_{23}, \mu_{23} > 0 \\ \hline & 66, 0104 \\ 0.2701 \\ t > t_0 \\ t > t_0 \\ 0.0 \\ \end{array}$	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betwe	een the Ur atween the	treated po e CFU input	pulation ar	nd the Trea	ated populat	ion opulation					
; ; z test statistic jection region	μ ₁₁₋₀ mmands * μ ₁₁₋₀₀₀ = 0 μ ₁₁₋₀ mmands * μ ₁₁₋₀₀₀ > 0 666.0104 t>t ₀ 0.2701 t>t ₀ 8 0.055 8 9 pcm	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw fference b	een the Ur etween th	treated po	pulation ar	nd the Trea	ted populat Jntreated P	ion opulation					
o: ; test statistic ejection region	$\mu_{11-000000000}^{-} \mu_{11-0000}^{-} = 0$ $\mu_{11-00000000}^{-} \mu_{11-0000}^{-} > 0$ $\frac{66.0104}{0.2701}$ $t > t_g$ 0.005 8 1.8680 0.005000000000000000000000000000000000	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di	ence betw fference b	een the Ur	treated po e CFU input	pulation ar	nd the Tree	ated populat Untreated Pi	tion					
: : : : : : : : : : : : : : : : : : :	$\begin{array}{c} \mu_{11:00000005}^{} - \mu_{11:000}^{} = 0 \\ \mu_{11:00000000}^{} - \mu_{11:00000000000000000000000000000000000$	Null Hypo Alternate	thesis = Th Hypothesi	ere is no differ s = There is a di e between the	ence betw fference b	een the Ur atween the	ntreated po e CFU input	pulation ar populatio	nd the Trea n and the l	ated populat	tion opulation					
: : est statistic eetion region value nee 0.2701 < 1.860, I do n	$\begin{array}{c} \mu_{11} \mbox{-}$	Null Hypo Alternate there is no	thesis = Th Hypothesi D difference	ere is no differ s = There is a di e between the	ence betw fference b	een the Ur etween the	ntreated po e CFU input	pulation ar populatio	nd the Tree	ated populat	tion opulation					
: : test statistic jection region value cce 0.2701 < 1.860, I do n :	μ _{11 - OPTIMINE} - μ _{11 - 000} = 0 μ _{11 - OPTIMINE} - μ _{11 - 000} > 0 66.0104 0.2701 t > t _a 0.055 8 0.519325892 ot reject he null hypothesis, μ _{11 - OPTIMINE} - μ _{11 - 000} = 0	Null Hypo Alternate there is no Null Hypo	thesis = Th Hypothesi o difference thesis = Th	ere is no differ s = There is a di e between the ere is no differ	ence betw fference b Untreated	een the Un etween the and the 50 een the Un	ntreated po e CFU input	pulation ar populatio d Populati	nd the Tree n and the I ons nd the Tree	ited populat Untreated Pi	tion					
: : test statistic ection region value co.22701 < 1.860, I do n : :	$\begin{array}{c} \mu_{11}, \omega_{00} = 0 \\ \mu_{11}, \omega_{00} = 0 \\ 0.001$	Null Hypo Alternate there is no Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference b Untreated ence betw fference b	and the 50	treated po e CFU input	pulation ar populatio d Populati pulation ar	ons n and the Tree	ted populat Untreated P defense of the second	tion opulation tion opulation					
; ; test statistic jection region value nce 0.2701 < 1.860, I do n ; ;	μ1 - Unmanted * μ11 - 500 = 0 μ1 - Unmanted * μ11 - 500 > 0 66.0104 0.27001 t > t ₀ 0.055 8 0.5153225592 ot reject the null Hypothesis, μ1 - Unmanted * μ1. 2000 = 0 μ1 - Unmanted * μ1. 3000 > 0	Null Hypo Alternate there is no Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference b Untreated ence betw fference b	een the Ur atween the and the 50 een the Ur atween the	ntreated po e CFU input 0 Gy Treate ntreated po e CFU input	pulation ar populatio d Populati pulation ar populatio	nd the Tree n and the l ons n and the Tree	ated populat Untreated Pi ated populat Untreated Pi	ion opulation					
;: ; test statistic jection region value nce 0.2701 < 1.860, I do n ; ;	$\begin{array}{l} \mu_{11}, \omega_{11} = 0 \\ \mu_{11}, \omega_{12} = 0 \\ \mu_{11}, \omega_{12} = 0 \\ 0.6, 0104 \\ 0.7011 \\ t > t_{s_1} \\ 0.05 \\ 0.5, 1325 \\ 0.6, 13.660 \\ 0.5, 1325 \\ 0.6, 13.660 \\ 0.5, 1325 \\ 0.711 \\ 0.05 \\ 0.5, 1325 \\ 0.5, 1005 \\ 0.5, 10$	Null Hypo Alternate there is no Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference b Untreated ence betw fference b	een the Ur etween th and the 50 een the Ur etween th	ntreated po e CFU input 0 Gy Treate ntreated po e CFU input	pulation ar populatio d Populati pulation ar populatio	nd the Tree n and the I ons n and the I	ted populat Untreated Pi ted populat Untreated Pi	tion opulation tion opulation					
: est statistic jection region value value : : : : : : : : : : : : :	H1: -Зиталаст - H1: -300 = 0 H1: -Зиталаст - H1: -300 > 0 66:0104 0.2701 t > t _a 0.05 8 0.15325823 ot reject the null hypothesis, H1: -Jumenset = H1: -300 > 0 - H1: -Jumenset = H1: -300 > 0 - 40.7921 T -	Null Hypo Alternate there is nc Null Hypo	thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference br Untreated ence betw fference bet	and the 50	ntreated po e CFU input 0 Gy Treate treated po e CFU input	pulation an populatio d Population population an	nd the Trees and the test of test	ated populat Untreated Pi sted populat Untreated Pi	ion ion ion opulation					
: est statistic ection region value cce 0.2701 < 1.860, i do n : : est statistic	μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo > 0 66.0104 0.2701 t > t _q 0.055 8 0.519325892 ot reject the null hypothesis, μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 μ ₁₁ - one states = μ ₁₁ - soo = 0 0 - 1.1724	Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw Ifference be Untreated ence betw	een the Ur etween th and the 50 een the Ur etween th	treated po e CFU input	pulation an populatio d Population an population	nd the Tree on and the I	ted populat Untreated P Intreated Populat	ion opulation ion					
: est statistic ection region value co 2,2701 < 1.860, I do n : : est statistic ection region	μ1: -000000000 = 0 μ1: -000000000000000000000000000000000000	Null Hypo Alternate there is nc Null Hypo	thesis = Th Hypothesi difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference br Untreated ence betw fference br	een the Ur etween th and the 50 een the Ur etween th	treated po e CFU input 0 Gy Treated treated po e CFU input	pulation an populatio td Populatio pulation an	nd the Treat an and the I	ated populat Untreated P sted populat Untreated P	ion ion					
: est statistic ection region value ce 0.7701 < 1.860, I do n : : est statistic ection region	μ1 - Unmanter * μ1 - 500 = 0 μ1 - Unmanter * μ1 - 500 > 0 66.0104 0.2701 t > t _g 0.05 8 0.519325592 ott reject the null Hypothesis, μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ1 - Unmanter * μ1 - 100° = 0 μ2 - 100° = 0 μ3 - 10° = 0 μ4 - 10° = 0	Null Hypo Alternate	thesis = Th Hypothesi b difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference b Untreated ence betw	een the Ur and the 50	ntreated po e CFU input 0 Gy Treated e CFU input	pulation an populatio d Population an population an	nd the Tree ons n and the l	ted populat Untreated P ted populat Untreated P	ion opulation ion opulation					
: est statistic est statistic est statistic est of the statistic est statistic est statistic est statistic estion region	μ1: -unreases - μ1: 500 = 0 μ1: -unreases - μ1: 500 > 0 66:0104 0.2701 t>t_a 0.05 0.5513255623 trictertenul "Pi1: 500" 0 μ1: -unreases - "μ1: 500" 0 μ1:	Null Hypo Alternate	thesis = Th Hypothesi a difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence between betwe	zen the Ur tween th and the 50 zen the Ur etween th	treated po cFU input 0 Gy Treate treated po c CFU input	pulation an populatio d Population an population	nd the Tree an and the l ons nd the Tree n and the l	ted population	tion uion tion					
: est statistic ection region alue ce 0.2701 < 1.860, I do n : : est statistic ection region	$\begin{array}{c} \mu_{11} : \text{unmasses}^{-1} \mu_{11} : \text{scos} = 0 \\ \mu_{11} : \text{unmasses}^{-1} \mu_{11} : \text{scos} > 0 \\ \hline & 66.0104 \\ 0.02701 \\ t > t_{g} \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.05 \\ 0.00 \\ 0.07917 \\ 0.07917 \\ 0.07917 \\ 0.07917 \\ 0.07917 \\ 0.07917 \\ 0.05$	Null Hypo Alternate	thesis = Th Hypothesi b difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw.	een the Un etween th and the 50	treated po c FU input 0 Gy Treate treated po e C FU input	pulation an populatio d Population an population	nd the Tree n and the I ons nd the Tree	ted populat Intreated P ted populat	ion opulation ion					
est statistic ection region alue ece 0.2701 < 1.860, I do n est statistic est statistic ection region	$\begin{array}{c} \mu_{11}, \omega_{11}, u_{11}, u_{22} = 0 \\ \mu_{11}, \omega_{12}, u_{22}, u_{23} = 0 \\ (66,0104) \\ (66,0104) \\ (701)$	Null Hypo Alternate there is nc	thesis = Th Hypothesi D difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference b Untreated ence betw ence betw	and the 50	treated po c FU input	pulation an populatio d Populatio pulation an populatio	nd the Tree an and the l ons and the Tree	ted populat	ion opulation ion opulation					
: est statistic eetion region value cee 0.2701 < 1.860, I do n : : est statistic ection region value ce - 1.1724 < 1.860, I do J	µ1: -(личкие - №1: 500 = 0 µ1: -(личкие - №1: 500 = 0 66:0104 0.070 t>ta 0.055 8 0.055 8 0.19325682 µ1: -(личкие - №1: -000 = 0 1: -1.1724 t>ta 0.055 8 0.13225822 null hypothesis, 0.051325822 null hypothesis,	Null Hypo Alternate there is nc Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di	ence betw fference be Untreated ence betw fference be untreated	and the 10	treated po CFU input 0 Gy Treated po e CFU input	pulation an populatio d Populatio pulation an populatio	nd the Tree	ted population of the second sec	ion opulation ion opulation					
: cest statistic eetion region value ce 0.2701 < 1.860, i do n : : est statistic eetion region value real-11724 < 1.860, i do n	µ1: -зичания " µ1: 500 = 0 µ1: -зичания " µ1: 500 > 0 66:0104 0.2701 t > t ₀ 0.055 8 0.519325692 0t - eject the null hypothesis, µ1: -зичания" - µ1: 200" 0 µ1: -зичания" - µ1: 200" 0 40.7917 -1.1724 t > t ₀ 0.0519325892 0.05	Null Hypo Alternate there is nn Null Hypo Alternate	thesis = Th Hypothesi > difference Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the	unce between the set of the set o	and the 50 and the 50 and the 50 and the 1	treated po c FU input 0 Gy Treate treated po e c FU input	pulation an populatio d Populatio pulation an populatio ted Populatio	ons ons and the l	ted population of the second sec	ion Jon opulation					
:	$\begin{array}{c} \mu_{11:00=0000} = 0 \\ \mu_{11:00=00000} = 0 \\ \mu_{11:00=000000} = 0 \\ 0.0000000000000000000000000000000$	Null Hypo Alternate there is nc Null Hypo there is n Null Hypo	thesis = Th Hypothesi o difference thesis = Th Hypothesi o difference thesis = Th	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ	untreated	een the Ur and the 50 een the Ur stween th	treated po e CFU input 0 Gy Treated threated po e CFU input treated po 0000 Gy Treated treated po	pulation an populatio d Populatio pulation an populatio ted Populatio	nd the Tree of the time of the	ted population	ion opulation ion uon					
: : : : : : : : : : : : : : : : : : :	μ1 - University * μ11 - 500 = 0 μ1 - University * μ11 - 500 > 0 μ1 - University * μ11 - 500 > 0 0.2701 t > t _a 0.055 8 0.519325892 ot reject the null hypothesis, μ1 - University * μ11 - 2000 * 0 μ1 - University * μ11 - 2000 * 0 40.7917 -1.1724 t > t _a 0.0519325892 0.05 1.1724 1.2724 t > t _a 0.05 8 0.519325892 0.519325892 0.519325892 μ1 - University * μ1, 10000 * 0 μ1 - University * μ1, 10000 * 0	Null Hypo Alternate there is no. Null Hypo there is n Alternate	thesis = Th Hypothesi b difference thesis = Th Hypothesi thesis = Th	ere is no differ = There is a di e between the ere is no differ = There is a di ce between the ere is no differ = There is a di	untreated	and the 50 and the 50 and the 10 and the 11 and the 11	treated po c CFU input 0 Gy Treate treated po a CFU input treated po y Treat	pulation an populatio d Populatio pupulation an populatio ted Populatio	ons ons tions	ted populat Intreated P Intreated P Untreated P Intreated P	ion opulation opulation opulation					
:	$\begin{array}{c} \mu_{11}, \mu_{11}, \mu_{11}, \mu_{20} = 0 \\ \mu_{11}, \mu_{11}, \mu_{20} = 0 \\ (6.0104) \\ (0.701) \\ (0$	Null Hypoo Alternate there is no Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ee between the ere is no differ s = There is a di	ence between ference between betwe	een the Ur tween th and the 50 een the Ur and the 10 een the Ur	e CFU input 0 Gy Treated treated po c CFU input treated po c FU input	pulation an populatio d Populatio pulation an populatio pulation an populatio	nd the Tree of the	ted population	ion opulation ion ion					
<pre>;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;;</pre>	µ1	Null Hypo Alternate there is no. Null Hypo Alternate Null Hypo	thesis = Th Hypothesi b difference thesis = Th Hypothesi o difference thesis = Th	ere is no differ = There is a di e between the ere is no differ = There is a di ce between the ere is no differ = There is a di	Untreated Untreated Ence betw. Untreated ence betw.	and the 50 and the 50 and the 50 and the 10 seen the Ur and the 11	treated po c CFU input 0 Gy Treated po c CFU input treated po c CFU input	pulation an populatio d Populatio pulation an population an population an population an	ons ons d the Trees ons d the Trees and the I	ted populat Intreated P ted populat Intreated P ted populat	ion join opulation					
: is in the statistic is it is	μ1: -umments - μ1: 500 = 0 μ1: -umments - μ1: 500 > 0 66:0104 0.757 1> t _a 0.05 8 1.860 0.519325822 ot reject the null hypothesis, μ1: -ummants - μ1: 500 = 0 μ1: -ummants - μ1: 5000 = 0	Null Hypoo Alternate there is no Null Hypoo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ere is no differ s = There is a di	Untreated Untreated Untreated Untreated Untreated	een the Ur tween th and the 50 een the Ur etween th tween the Ur tween th	0 Gy Treated po CFU input 0 Gy Treated po CFU input 0000 Gy Treated po CFU input	pulation an populatio d Populatio pulation an population an population and populatio	ons ons of the Trees of the Trees of the Trees of the Trees	ted population of the second sec	ion opulation ion opulation					
: : : : : : : : : :	μ1: Jonesses - μ1: 300 = 0 μ1: Jonesses - μ1: 300 > 0 66.0104 0.2701 t > t _a 0.05 8 0.13322582 0.05 μ1: Jonesses - μ1: 3007 = 0 μ1: Jonesses - μ1: 4007 = 0 0.519325822 t > t _a 0.05 8 1.1724 t > t _a 0.519325822 tot reject the null hypothesis, 8 1.1860 0.519325822 tot reject the null hypothesis, 9 μ1: Jonesses - μ1: 10007 = 0	Null Hypo Alternate there is nc Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ = There is a di e between the ere is no differ = There is a di ce between the ere is no differ s = There is a di	Untreated	and the 50	treated po c FU input 0 Gy Treate treated po c FU input treated po c FU input	pulation an populatio d Populatio pulation an populatio populatio populatio	ons ons tions d the Trees and the t	ted population of the second sec	ion opulation ion opulation					
: isi isi statistic iest statistic value co.2701 < 1.800, 1 do n : : : iest statistic lection region ralue ci-1,1224 < 1.860, 1 do n : : : : : : : : : : : : : : : : : : :	$\begin{array}{c} \mu_{11} \mbox{-}$	Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi o difference thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di	Untreated Untreated Untreated Untreated Untreated	and the 50 and the 50 een the Ur etween the and the 1 etween the	0 Gy Treated 0 Gy Treated 0 CFU input treated po 0000 Gy Treated 0000 Gy Treat	pulation an populatio ted Population population an population an population	ons ons tions	tted population of the second se	ion cion cion opulation opulation					
:	$\begin{array}{c} \mu_{11} \mbox{-}$	Null Hypo Alternate there is nc Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi hesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di	Untreated	and the SO and the SO and the SO and the Ur tween the tween the Ur tween the Ur	treated po CFU input O Gy Treate d po e CFU input treated po e CFU input	pulation an populatio d Populatio pulation an populatio population	ons ons tions d the Trees	ted population of the populati	tion opulation ilon opulation					
: est statistic est statistic iection region relue est statistic ection region : : est statistic ection region : : est statistic est statistic	µ1 - ситение * №11 - 500 = 0 №11 - ситение * №11 - 500 > 0 №11 - ситение * №11 - 500 > 0 0.2701 t > t ₀ 0.055 8 0.5159225892 ot reject the null hypothesis, µ1 - ситение * №11 - 200° 0 µ1 - ситение * №11 - 200° 0 №11 - 200° 0 №11 - 200° 0 №11 - 200° 0 №11 - 200° 0	Null Hypo Alternate there is nn Null Hypo Alternate	b difference o difference thesis = Th Hypothesis	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di	Untreated Untreated Untreated Untreated Untreated	and the 50 and the 50 een the Ur etween th een the Ur even the Ur	0 Gy Treated po CFU input Ureated po e CFU input Ureated po Gy Treated po e CFU input	pulation an populatio d Populatio pupulation an population an population and population and population and population	ons ons tions	ted populat Intreated P Intreated P Intreated P Intreated P	ion jopulation opulation jopulation					
:	μ1: -0==0 μ1: -0==0 μ1: -0==== - 66:0104 - 0:05 8 0:05 8 0:05 8 0:05 9 μ1: -0==== 0.05 0:05 8 0:05 8 μ1: -0=== -1.1724 μ1: -0=== -1.1724 t>t_s 0.05 0:05 8 -1.1724 1.1724 t>t_s 0.05 0:0525822 1.1860 0:05325822 1.192 ut -== -1.1724 t>t_s -0.055 0:05225822 1.192 ut -== -1.1724 t>t_s -1.1724 t>t_s -0.055 0:0525825 -0.055 0:054 -0.055	Null Hypo Alternate there is nc Null Hypo Alternate	thesis = Th Hypothesi o difference thesis = Th Hypothesi thesis = Th Hypothesi	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di	Untreated Untreated ence betw Untreated fference betw fference betw	and the S0	treated po CFU input 0 Gy Treated po e CFU input treated po e CFU input	pulation an populatio d Populatio pulation an populatio populatio	ons ons d the Trees and the I	ted population of the populati	ion opulation					
;; ; <t< td=""><td>μ1: -unement = μ1: 500 = 0 μ1: -unement = μ1: 500 > 0 μ1: -unement = μ1: 500 > 0 1> ta 0.05 1> ta 0.05 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.160 0.51932582 0.3044 0.3044 0.3044 0.3044 0.3044 0.3044</td><td>Null Hypo Alternate there is nn Null Hypo Alternate</td><td>b difference o difference thesis = Th Hypothesis</td><td>ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di</td><td>Untreated Untreated Untreated Untreated Untreated</td><td>and the 50 and the 50 een the Ur etween th even the Ur tween th</td><td>0 Gy Treated po e CFU input Ureated po e CFU input treated po e CFU input</td><td>pulation an populatio d Populatio pupulation an population an population and population and population and population</td><td>ons ons tions</td><td>ted populat Intreated P Intreated P Intreated P Intreated P</td><td>ion jopulation opulation ion opulation</td><td></td><td></td><td></td><td></td><td></td></t<>	μ1: -unement = μ1: 500 = 0 μ1: -unement = μ1: 500 > 0 μ1: -unement = μ1: 500 > 0 1> ta 0.05 1> ta 0.05 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.51932582 0.160 0.51932582 0.3044 0.3044 0.3044 0.3044 0.3044 0.3044	Null Hypo Alternate there is nn Null Hypo Alternate	b difference o difference thesis = Th Hypothesis	ere is no differ s = There is a di e between the ere is no differ s = There is a di ce between the ere is no differ s = There is a di	Untreated Untreated Untreated Untreated Untreated	and the 50 and the 50 een the Ur etween th even the Ur tween th	0 Gy Treated po e CFU input Ureated po e CFU input treated po e CFU input	pulation an populatio d Populatio pupulation an population an population and population and population and population	ons ons tions	ted populat Intreated P Intreated P Intreated P Intreated P	ion jopulation opulation ion opulation					



84

Appendix Q: Mutant 16 Oxygen Exposure Comparison

Mutant 16 Anal	ysis															
Data																
Strain				1	R1 // //	rB::KAN										
CFU Input	15	25	i 21	L												
Untreated Unvacuumed	33	34														
Untreated Vacuumed	28	40	36	5 29												
500 Gy	43	41	. 25	37												
10,000 Gy	20	22	29	30												
All colony counts at 10 ^{-5 diutor}	1															
Statistics																
	n _{16-CFU input}	3		n _{16-Untreated}	6					n ₁₆₋₅₀₀	4	n ₁₆₋₁₀₀₀	4	n ₁₆₋₁	0000	4
	x-bar sectulated	20.3		x-bar subtrasted	33.3					x-bar	37.5	x-bar te-too	42.3	x-ba	F16-1000	25.3
	S16-CFU input	5.0		S _{16-Untreated}	4.5					S ₁₆₋₅₀₀	6.2	S ₁₆₋₁₀₀₀	2.5	S ₁₆₋₁₀	2000	5.0
Population Comp	arisons															
Population comp	ansons															
Comparison Set 8 - Mutant 1	6															
H _o :	$\mu_{16\text{-}CFU \text{ input}} - \mu_{16\text{-}Untreated} = 0$	Null Hypo	othesis = Ti	here is no differ	ence betw	veen the C	FU input po	pulation a	nd the Unt	treated Popul	ation					
H _a :	$\mu_{16\text{-}CFU \text{ input}} \circ \mu_{16\text{-}Untreated} > 0$	Alternate	Hypothes	is = There is a d	ifference b	oetween ti	ne CFU inpu	it populati	on and the	Untreated Po	opulation					
c 2	34 -000															
^a p t tost statistic	21.4286															
i, iest statistic	-3.9/16	1														
rejection region	t>t,															
α	0.05															
df	7															
tα	1.895															
p-value	0.519240411															
Since -3.9716 < 1.895, I do no	t reject the null hypothesis,	there is n	o differen	ce between the	CFU Input	populatio	n and the U	Intreated I	Population	IS						
H _o :	$\mu_{16-Untreated} - \mu_{16-500} = 0$	Null Hypo	othesis = Ti	here is no differ	ence betw	veen the U	ntreated p	opulation a	ind the Tre	eated populat	ion					
H _a :	$\mu_{16-Untreated} - \mu_{16-500} > 0$	Alternate	Hypothes	is = There is a d	ifference b	between ti	ne CFU inpu	it populati	on and the	Untreated Po	opulation					
sp ²	26.7917															
t, test statistic	-1.2471															
rejection region	$t > t_{\alpha}$															
α	0.05															
dt	8															
τα	1.860															
p-value	0.519325892	there in	o d!ff	o hotu*	Links t	l and the T	00.007	ad Domit	lone							
511CE -1.24/1 < 1.860, I do no	creject the null hypothesis,	unere is n	o ameren	Le petween the	untreated	and the 5	oo Gy Trea	ea Popula	uons							
и -		Null the	thesis - T	hara is no diff	ance both	ieen the !!	Intreated -	nulation :	nd the Tra	asted populat	ion					
	M16-Untreated * M16-1000 = U	ман нурс	voiesis = 11	nere is no differ	ence betw	wen me U	na eateu p	opulation i	nu de tre	area populat	1011					
H _a :	μ _{16-Untreated} - μ ₁₆₋₁₀₀₀ >0	Alternate	Hypothes	is = There is a d	itterence b	between t	ne CFU inpu	it populati	on and the	Untreated Po	opulation					
,																
5p ⁴	14.7604															
t, test statistic	-3.5955															
rejection region	t > t _a															
α	0.05															
dt	8															
t _α	1.860															
p-value	0.519325892	altern i	1166		11-14-1	landet i	000 6 7	ted fr - 1								
since -3.5955 < 1.860, I do no	creject the null hypothesis,	unere is n	o amereni	ce between the	Untreated	and the 1	000 Gy Tre	nea Popul	ations							
ц.,		Null the	thoris - T	horo is no diff-	onco het	ioon the !!	ntroated -	opulation	nd the T-	atod name	ion					
10.	P15-Untreated - P15-10000 = 0	мин нурс	vinesis = 11	nere is no differ	ence betw	reen the U	na eated p	opulation a	niu ule fre	aceu populat	ion 					
H _a :	μ _{16-Untreated} - μ ₁₆₋₁₀₀₀₀ >0	Alternate	Hypothes	is = There is a d	ITTerence b	between ti	ne CFU inpu	it populati	on and the	Untreated Po	opulation					
Sp ⁴	21.7604															
t, test statistic	2.6845															
rejection region	t>t _a															
α	0.05															
df	8															
t _α	1.860															
p-value	0.519325892															
Since 2.6845 > 1.860, I do reje	ect the null hypothesis, ther	e is a diffe	rence bet	ween the Untre	ated and t	he 10,000	Gy Treated	Population	15							



Appendix R: Mutant 6A Oxygen Exposure Comparison

	iysis															
Data																
train				R1 'me	roploid' W	T and ∆rea	F::KAN					1				
FU Input	7	9	17	r												
ntreated Unvacuumed	21	37														
ntreated Vacuumed	18	26	22	17												
10 GY	24	22	19	31												
1.000 GV	14	24	21	20												
Il colony counts at 10 ^{-5 diutio}	n		21													
in colony counts at 10																
tatistics																
	N _{6A -CFU input}	3		n _{6A -Untreated}	6					n _{6A-500}	4	n ₆₄₋₁₀₀₀	4		n _{6A-10000}	4
	x-bar _{64-CFU input}	11.0		x-bar _{6A-Untreated}	23.5					x-bar _{6A-500}	24.0	x-bar _{6A-1000}	25.0		x-bar ₆₄₋₁₀₀₀₀	21.3
	S6A-CFU input	5.3		S _{6A} -Untreated	7.3					S ₆₄₋₅₀₀	5.1	S _{6A-1000}	3.4	L .	S _{6A-10000}	5.4
opulation Comp	arisons															
opulation comp																
mparison Set 7 - Mutant 6	A															
	$\mu_{6A-CFU \text{ input}} - \mu_{6A-Untreated} = 0$	Null Hypo	thesis = Th	nere is no differ	ence betw	een the Cl	FU input po	pulation a	nd the Untr	reated Popu	lation					
:	$\mu_{6A-CFU \text{ input}} - \mu_{6A-Untreated} \ge 0$	Alternate	Hypothes	is = There is a d	fference b	etween th	e CFU inpu	t populatio	n and the	Untreated P	opulation					
	46.5000															
est statistic	-2.5924															
ection region	$t \ge t_{\alpha}$															
	0.05															
	7	1														
	1.895															
alue	0.519240411															
nce -2.5924 < 1.895, I do no	t reject the null hypothesis, t	there is no	difference	e between the	CFU Input	opulation	and the Ur	ntreated Po	pulations							
	,,															
:	HeA-Untrested - HeA-500 = 0	Null Hypo	thesis = Th	nere is no differ	ence betw	een the U	ntreated po	opulation a	nd the Trea	ated popula	tion					
:	H64-Untreased ~ H64-500 = 0 H06-Untreased ~ H64-500 > 0	Null Hypo Alternate	thesis = Th Hypothesi	nere is no differ is = There is a d	ence betw	een the U etween th	ntreated po	pulation a	nd the Trea	ated popula Untreated P	tion opulation					
:	H64-Untreasted - H64-500 = 0 H64-Untreasted - H64-500 > 0	Null Hypo Alternate	thesis = Th Hypothesi	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated po	opulation a	nd the Trea	ated popula Untreated P	tion opulation					
: :	Ифа-Untreased - Ифа-500 = 0 Ифа-Untreased - Ифа-500 > 0 43,4375	Null Hypo Alternate	thesis = Tł Hypothesi	here is no differ is = There is a d	ence betw	een the U etween th	ntreated po ne CFU inpu	pulation a	nd the Tre:	ated popula Untreated P	tion opulation					
: : : est statistic	И ₄₄₋₀₀₇₁₁₀₈₆ - И ₄₆₋₀₀₀ = 0 И ₄₆₄₋₀₀₇₁₁₀₈₆ - И ₄₆₄₋₀₀₀ > 0 - 43.4275 - 0.1175	Null Hypo Alternate	thesis = Th Hypothes	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated pc	ppulation a	nd the Tree	ated popula Untreated P	tion					
: : est statistic	Ицалитична" Ицалоо = 0 Ицалитична" Ицалоо > 0 43.4375 -0.1175	Null Hypo Alternate	thesis = Th Hypothes	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated pc	ppulation a	nd the Trea	ated popula Untreated P	tion					
:	Наконтелле ° Накоо = 0 Наконтелле ° Накоо > 0 43.4375 -0.1175 t.> t.	Null Hypo Alternate	thesis = Th Hypothes	here is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated pc	ppulation a	nd the Trea	ated popula Untreated P	tion opulation					
: : z test statistic jection region	Ина. оптете ° Ина. 600 = 0 Нас. оптете ° Ина. 600 > 0 43.4375 -0.1175 t>t _a 0.05	Null Hypo Alternate	thesis = Tł Hypothes	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated pc	ppulation a	nd the Tree	ated popula Untreated P	tion opulation					
: : test statistic jection region	Иалонтината " Иалоо = 0 Иалонтината " Иалоо > 0 43.4375 -0.1175 t > t _a 0.055 о	Null Hypo Alternate	thesis = Tł Hypothes	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated po	pulation a	nd the Trea	ated popula Untreated P	tion					
: : est statistic ection region	Ифа.оптивне - Ифа.000 = 0 Ифа.оптивне - Ифа.000 > 0 43.4375 0.1175 t>t _a 0.055 8 9 - 0.055	Null Hypo Alternate	thesis = Tř Hypothes	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated po	ppulation a	nd the Trea	ated popula Untreated P	tion opulation					
: est statistic ection region	H44.0199805 * H44.000 = 0 H44.0199805 * H44.000 > 0 43.4375 -0.1175 t > t _a 0.005 8 1.18600 0 + 0.4347	Null Hypo Alternate	thesis = Th	ere is no diffe is = There is a d	ence betw fference b	een the U	ntreated po	pulation a t populatic	nd the Trea	ated popula Untreated P	tion opulation					
: : ection region /alue pre-0.1175 < 1.960 Ldm or	Haaumennes [−] Haasoo = 0 Haaumennes [−] Haasoo > 0 43.4375 -0.1175 t>t _a 0.05 8 0.05 8 1.860 0.051923582 trelet the null burowches [−]	Null Hypo Alternate	thesis = Th Hypothesi	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated po e CFU inpu	pulation a t populatic	nd the Treation and the	ated popula Untreated P	tion opulation					
est statistic ection region alue ce-0.1175 < 1.860, I do no	И _{44.00} тенне ⁺ И _{44.00} = 0 И ₄₄₋₀₀ тенне [−] И _{44.00} > 0 43.4375 -0.1175 t > t _a 0.05 8 1.860 0.513925892 t reject the null hypothesis, t	Null Hypo Alternate	thesis = Th Hypothes difference	nere is no differ is = There is a d	ence betw fference b	een the U etween th	ntreated po the CFU input	pulation a t populatic	nd the Tree	ated popula Untreated P	tion opulation					
est statistic ection region elue e-0.1175 < 1.860, I do nc	Hacommand - Hacoo = 0 Hacommand - Hacoo > 0 43.4375 -0.1175 t>t _a 0.05 8 1.860 0.51932582 t reject the null hypothesis, t	Null Hypo Alternate	thesis = Th Hypothesi difference	ere is no differ is = There is a d between the	ence betw fference b	een the U etween th and the 50	ntreated po e CFU inpu 0 Gy Treate	pulation a t populatio d Populatio	nd the Tree	ated popula Untreated P	tion					
: est statistic ection region ralue ce=0.1175 < 1.860, I do nc	μ _{4A-00} trunte ² μ _{4A-00} = 0 μ _{4A-00} trunte ² μ _{4A-00} > 0 43.4375 -0.1175 t>t _a 0.05 12.50 0.5139325892 t reject the null hypothesis, t μ _{4A-00} =0	Null Hypo Alternate	thesis = TF Hypothes difference thesis = TF	ere is no differ is = There is a d between the ere is no differ	ence betw fference b	een the U etween th and the 50 een the U	ntreated po e CFU inpu 0 Gy Treate	pulation a	nd the Tree	ated popula Untreated P	tion opulation					
: est statistic ection region value ce=0.1175 < 1.860, I do nc :	H44.0HHHHHH - H44.000 = 0 H44.0HHHHH - H44.000 > 0 43.4375 -0.1175 t > t _q 0.055 8 1.1660 0.513125 tt reject the null hypothesis, I H44.0HHHHH - H44.000 = 0 H44.0HHHHH - H44.000 = 0 H44.0HHHHH - H44.000 = 0	Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi	ere is no differ is = There is a d between the ere is no differ is = There is a d	ence betw fference b Jntreated ence betw fference b	een the U etween th and the 50 een the U etween th	o Gy Treated pre CFU inpute o Gy Treate ntreated po	ppulation a t population d Population at population a	nd the Tree in and the ons ind the Tree in and the	ated popula Untreated P ated popula Untreated P	tion opulation tion opulation					
: est statistic ection region value ce-0.1175 < 1.860, I do nc : :	Иза. оптенне - Иза. 600 = 0 Иза. оптенне - Иза. 600 = 0 43.4375 -0.1175 t > t _a 0.05 8 0.519325892 t reject the null hypothesis, 1 Иза. 005 9 8 0.519325892 Haa. ontenne - Иза. 600 = 0 Нао. оптенне - Иза. 600 = 0 Нао. оптенне - Иза. 600 = 0	Null Hypo Alternate	thesis = Tł Hypothes difference thesis = Tł Hypothes	ere is no differ is = There is a d e between the is = There is a d	ence betw fference b Jntreated ence betw fference b	een the U etween th and the 50 een the U etween th	o Gy Treated ntreated po o Gy Treate	pulation a t population d Population ppulation a t populatio	nd the Trea n and the ons nd the Trea	ated popula Untreated P ated popula Untreated P	tion opulation tion opulation					
: est statistic ection region value cce -0.1175 < 1.860, I do nc :	μ46.0012002 ° μ46.000 ° 0 μ43.4375 -0.1175 t > t _a 0.05 t > t _a 0.05 t = 0.05 t = 0.05 0.0137325892 treject the null hypothesis, t μ64.001200 ° 0 μ64.001200 ° 0 37.9375	Null Hypo Alternate	thesis = Tr Hypothesi difference thesis = Tr Hypothes	nere is no differ is = There is a d between the is nere is no differ is = There is a d	ence betw fference b Jntreated ence betw fference b	een the U etween th and the 50 een the U etween th	o Gy Treated pc CFU inpu o Gy Treated pc CFU inpu	pulation a t populatio d Populatio pulation a t populatio	nd the Tre- n and the ons nd the Tre-	ated popula Untreated P ated popula Untreated P	tion opulation tion opulation					
: est statistic ection region elue ce -0.1175 < 1.860, I do nc : : :	μ ₆₄₋₀₀₇₁₄₉₈₅ * μ _{64.900} = 0 μ ₆₄₋₀₀₇₁₄₉₈₅ * μ _{64.900} > 0 43.4375 -0.1175 t> t _g 0.05 8 0.519325892 treject the null hypothesis, t μ ₆₄₋₀₀₇₁₄₉₆ * μ _{64.000} = 0 μ ₆₄₋₀₀₇₁₄₉₆ * μ _{64.000} > 0 37.93975 -0.3773	Null Hypo Alternate	thesis = Th Hypothes difference thesis = Th Hypothes	nere is no differ is = There is a d between the t nere is no differ is = There is a d	ence betw fference b Jntreated ence betw fference b	een the U etween th and the 50 een the U	o Gy Treated Po Gy Treated ntreated po ee CFU inpu	pulation a t populatio d Populatio pulation a t populatio	nd the Trea in and the ons nd the Trea in and the	ated popula Untreated P ated popula	tion opulation tion opulation					
est statistic ection region alue ce=0.1175 < 1.860, I do no : : : :	Иак.онтенте [™] Иакоо = 0 Иак.онтенте [™] Иакоо > 0 43.4375 -0.1175 t > t _a 0.05 8 1.860 0.513925892 t reject the null hypothesis, t Иак.онтенте [™] Иак.1000 = 0 Иак.онтенте [™] Иак.1000 = 0 37.9375 -0.3773 -0.3773	Null Hypo Alternate	thesis = Th Hypothes difference thesis = Th Hypothes	ere is no differ is = There is a d between the rere is no differ is = There is a d	ence betw ffference b Jntreated ence betw fference b	een the U etween th and the 50 een the U	ntreated po e CFU inpu 0 Gy Treate htreated po e CFU inpu	pulation at population	nd the Tree.	ated popula Untreated P ated popula Untreated P	tion opulation					
est statistic ection region alue ce -0.1175 < 1.860, I do nc : : : : : : : : : : : : : : : : : : :	На4.0711112 ° На4.500 ° 0 На2.0711112 ° На4.500 ° 0 43.4375 43.4375 4 > t _q 0.055 8 1.680 0.5139325992 treject the null hypothesis, 1 На4.071112 ° На4.000 ° 0 На2.07112 ° На4.000 ° 0 37.9375 -0.3773 t > t _q	Null Hypo Alternate	thesis = Th Hypothes difference thesis = Th Hypothes	ere is no differ is = There is a d between the ere is no differ is = There is a d	ence betw fference b	een the U etween th and the 50 een the U etween th	o Gy Treated ntreated po Gy Treated ntreated po ee CFU inpu	d Population and Population	nd the Tree in and the ons ind the Tree	ated popula Untreated P sted popula Untreated P	tion opulation					
sst statistic sction region alue ee -0.1175 < 1.860, I do no set statistic sction region	μ _{4A.00} τυπτσ ² μ _{4A.600} = 0 μ _{4A.00} ×0 -0 43.4375 -0.1175 t>t _a 0.05 display -0.1375 t>t _a 0.05 1.800 0.519325892 treject the null hypothesis, I -0.3773 μ _{4A.007} =0 -0.3773 t>t _a 0.05	Null Hypo Alternate	thesis = Tr Hypothes difference thesis = Tr Hypothes	ere is no differ is = There is a d between the t here is no differ is = There is a d	ence betw fference b Jutreated ence betw	een the U etween th and the 50 een the U etween th	ntreated pc e CFU inpu 0 Cy Treated ntreated pc e cFU inpu	d Population	nd the Tree ons nd the Tree nd the Tree	ated popula Untreated P ated popula Untreated P	tion tion tion					
est statistic cction region alue e=-0.1175 < 1.860, I do no est statistic ection region	H44.0000000 - 90 H44.000000 - 90 H44.000000 - 0.1175 t > t _a 0.005 t > t _a 0.005 t > t _a 0.005 t > t _a 0.005 - 0.1175 -	Null Hypoo	thesis = 17 Hypothes difference Hypothes	ere is no differ is = There is a d between the ere is no differ is = There is a d	ence betw fference b Jntreated	een the U etween th and the 50 een the U etween th	ntreated pc CFU inpu O Gy Treate Intreated pc E CFU inpu	d Population and the second se	nd the Tree on and the ons	ated popula Untreated P sted popula Untreated P	tion copulation tion					
est statistic ection region alue e-0.1175 < 1.860, I do no est statistic est statistic	Has.corrente ² Has.coc = 0 Has.corrente ² Has.coc > 0 43.4375 -0.1175 -0.1175 t > t _a 0.05 8 0.0519325892 0 0.519325892 t reject the null hypothesis, t Has.corrente ² Has.corrente ² Has.corrente ² Has.corrente ² 0.075 t > t _a 0.0519325892 0 37.9375 -0.3773 -0.3773 t > t _a 0.055 8 0.051 8 1.860	Null Hypo Alternate	thesis = Th Hypothes difference thesis = Th Hypothes	e between the is = There is a d between the is = There is no differ is = There is a d	ence betw fference b Jntreated ence betw fference b	een the U etween th and the 50 een the U etween th	ntreated pp e CFU inpu e Gy Treate Intreated pp Intreated pp	pulation at population of popu	nd the Tree on and the	ated popula Untreated P ated popula	tion					
st statistic ction region ilue e = 0.1175 < 1.860, I do no st statistic ction region	μ46.00112002 ° μ46.000 ° 0 μ46.0011200 ° 0 43.4375 -0.1175 t > t _a 0.05 8 0.1850 0.053325892 treject the null hypothesis, I μ46.001120 ° 0 μ46.001120 ° 0 μ56.001120 ° 0 μ66.001120 ° 0 μ61.000 ° 0 8 0.053 8 0.13925892 0.513925892	Null Hypo Alternate	thesis = Th Hypothesi difference thesis = Th Hypothesi	ere is no differ is = There is a d between the rere is no differ is = There is a d	ence betw Ifference b	een the U etween th een the 50 een the U etween th	ntreated pc CFU inpu	pulation at population at popu	nd the Tree ons ons nd the Tree	ated popula Untreated P	tion tion tion					
est statistic ection region alue e - 0.1175 < 1.860, I do no est statistic ection region alue ec - 0.3773 < 1.860, I do no	Ча⊾онтине ⁻ Ча⊾оо ⁻ 0 Ча⊥онтине ⁻ Ча⊥оо ⁻ 0 43.4375 -0.1175 t > t _a 0.05 8 0.519325892 43.4375 1.860 0.519325892 1.860 0.519325892 1.860 0.519325892 1.860 0.519325892 t > t _a 8 8 8 8 8 8 8 8 8 8 8 8 8	Null Hypo Alternate	thesis = Th Hypothes difference Hypothes difference	ere is no differ is =There is a d between the t nere is no differ is = There is a d	Intreated	een the U	ntreated pp ee CFU inpu 0 Gy Treated ee CFU inpu ee CFU inpu	pulation a t populatio d Population t population t population	ons the Tree.	ated popula Untreated P ated popula	tion					
est statistic ection region alue ce-0.1175 < 1.860, I do no est statistic ection region alue ce-0.3773 < 1.860, I do no	Иа	Null Hypo Alternate	thesis = Th Hypothes difference Hypothes difference	ere is no differ is = There is a d between the is ere is no differ is = There is a d between the is	ence between the second s	een the U etween th and the 50 een the U etween th	ntreated pc e CFU inpu	d Population and Popu	ond the Tree of th	ated popula untreated P ated popula Untreated P	tion opulation tion opulation					
: est statistic ection region elue ce -0.1175 < 1.860, I do nc : est statistic ection region ralue ce -0.3773 < 1.860, I do nc	Ива. сотичине - Ива. 600 = 0 Ива. сотичине - Ива. 600 > 0 43.4375 -0.1175 1.0175 t>t_ 0.05 8 0.519325692 1.660 0.519325692 -0.3773 -0.3773 -0.3773 -0.3733 t>t_s 0.051932692 treject the null hypothesis, I 0.051932692 t.860 0.51932692 treject the null hypothesis, I 1.660 0.51932692 treject the null hypothesis, I 1.660 0.51932692	Null Hypo Alternate	thesis = Th Hypothes difference difference difference	ere is no differ is = There is a d between the is = There is a d for the is = There is a d between the is = there is no differ	ence betw Ifference b Jntreated Jntreated Jntreated	een the U etween th een the U een the U etween th und the 10	ntreated pc ec CFU inpu 0 Gy Treate ec CFU inpu 00 Gy Treated pc 00 Gy Treated pc	opulation at population at pop	nd the Tree ons ons nd the Tree in and the Tree lions	ated popula Untreated P ated popula Untreated P	Lion opulation Lion					
: : : est statistic : : : : : : : : : : : : :	Чалонтине [•] Чалоо ⁼ 0 Чалонтине [•] Чалоо ^{>} 0 43.4375 -0.1175 t > t _a 0.05 8 1.800 0.513325892 t reject the null hypothesis, t Чалонтине ⁻ Чалоо ^{>} 0 37.9375 t > t _a 0.05 8 0.513325892 0.3773 t > t _a 0.05 8 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.513225892 1.800 0.51325892 1.800 1.800 0.51325892 1.800 1	Null Hypo Alternate	thesis = Th Hypothes difference Hypothes difference	e between the l	Intreated	een the U etween th eand the 50 een the U etween th etween the U een the U	ntreated pc e CFU inpu 0 Gy Treate threated pc e CFU inpu 00 Gy Treate	pulation a d Populatio ed Population ed Population	nd the Tree.	ated popula ated popula ated popula Untreated P	tion copulation copulation					
: ection region elue ection region est statistic ection region est statistic ection region value cc=0.3773 < 1.860, I do nc : :	Ифа. сотичине * Ифа.600 = 0 Ирал. сотичине * Ифа.600 > 0 43.4375 -0.1175 t> t_ 0.05 8 0.519325892 reject the null hypothesis, I Ha.comeanse * Ифа.come > 0 1.560 0.519325892 1.600 0.519325892 1.600 0.519325892 1.560 0.0519325892 1.560 0.519325892 reject the null hypothesis, I Paa.comeanse * Haa.come > 0 0.519325892 Reject the null hypothesis, I Paa.comeanse * Haa.come > 0	Null Hypo Alternate	thesis = 17 Hypothes difference thesis = 17 Hypothes difference	e between the s = There is a d between the ere is no differ s = There is a d between the s = There is a d	ence betw Ifference b JIntreated ence betw Ifference b Ifference b	een the U etween th and the 50 een the U etween th the 10 een the U	ntreated pc ec CFU inpu 0 Cy Treated ntreated pc ec CFU inpu 00 Gy Treated pc 00 Gy Treated Dc 00 Gy Treated	opulation at population at pop	nd the Tree ons and the Tree in and the Tree in and the Tree	ated popula Untreated P ated popula ated popula	tion opulation tion tion opulation					
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Appendix S: Mutant 27 Oxygen Exposure Comparison

Mutant 27 Anal	ysis														
Data															
Strain					R1 Δμ	rB::KAN						1			
CEU Input	15	2	1 21												
Untreated Unvacuumed	29	2.	8												
Untreated Vacuumed	27	28	- 8 24	23											
500 Gy	39	19	9 31	26											
1000 Gv	34	3	7 35	30											
10 000 GV	35	14	1 22	33											
All colony counts at 10 ⁻⁵ diuton															
All colony counts at 10															
Statistics															
					6						4				4
	27 -CFU input		2	27 -Untreated	0					27-500		127-1000	-	127-10000	4
	x-bar _{27-CFU input}	19.0	0	x-bar _{27-Untreated}	26.5					x-bar ₂₇₋₅₀₀	28.8	x-bar ₂₇₋₁₀₀₀	34.0	x-bar ₂₇₋₁₀₀₀₀	26.0
	S27-CFU input	3.5	5	S27-Untreated	2.4					S ₂₇₋₅₀₀	8.4	S ₂₇₋₁₀₀₀	2.9	S27-10000	9.8
Denulation Comm															
Population Comp	ansons														
Comparison Set 8 - Mutant 27															
H _o :	μ _{27-CPU input} - μ _{27-Untreated} = 0	Null Hype	othesis = Th	here is no differ	ence betw	een the C	FU input po	pulation a	nd the Untr	reated Popu	lation				
H _a :	Hard - Ha	Alternate	e Hypothes	is = There is a di	fference b	etween th	ne CFU input	t populatio	on and the	Untreated P	opulation				
-	The second s														
e 2	7														
эр	7.6429														
t, test statistic	-3.8366	2													
rejection region	t > t _a														
α	0.05	5													
df	7	1													
t,	1 895	5													
n value	0 519240411														
Since 2 9266 < 1 905 Lide not	roject the pull hypothesis	thoro is n	o difforon	o hotwoon the	CELLInnut	nonulatio	n and the U	ntroated B	onulations						
Since - 5.8500 < 1.895, 1 uo not	reject the null hypothesis,	ulere is li	ounrerenc	e between the	CFO input	populatio	n and the O	ntreated P	opulations	•					
H ₀ :	$\mu_{27 - Untreated} - \mu_{27 - 500} = 0$	Null Hyp	othesis = Th	here is no differ	ence betw	een the U	ntreated po	pulation a	nd the Tre	ated popula	tion				
H _a :	μ _{27-Untreated} - μ ₂₇₋₅₀₀ >0	Alternate	e Hypothes	is = There is a di	fference b	etween th	ne CFU input	t populatio	on and the	Untreated P	opulation				
e ²	20 2912														
3p	30.2013														
t, test statistic	-0.6334	1													
rejection region	t>ta														
α	0.05	5													
df	8	1													
t _a	1.860														
p-value	0.519325892	2													
Since -0.6334 < 1.860. I do not	reject the null hypothesis	there is n	o differenc	e between the	Untreated	and the S	00 Gy Treate	ed Ponulat	ions						
	,						, mean	puidt							
и.		Nullia	thoric c Th	aro is no differ	onco hotu	oon the !!	ntroated	pulation -	nd the Tra	tod populat	tion				
n ₀ .	H27-Untreated - H27-1000= U	мин нур	ornesis = Th	iere is no ditter	ence betw	een (ne U	intreated po	pulation a	na me Tre	areo hobrila.	uon				
H _a :	$\mu_{27-\text{Untreated}} - \mu_{27-1000} > 0$	Alternate	e Hypothes	is = There is a di	fference b	etween th	ne CFU inpu	t populatio	on and the	Untreated P	opulation				
5 ₀ ²	6,9375	5													
t test statistic	A 4440	i i													
sy seat atotistic	-4.4113	-													
rejection region	t>t _a														
α	0.05	5													
df	8	3													
t,	1.860														
n-value	0 510225002														
Since -4 4113 < 1 960 Lide not	reject the null hypothesis	there is a	o difforence	e hetween the	Untreated	and the f	000 Gy Tree	ted Popula	tions						
SINCE -4.4115 < 1.800, 1 00 NO	reject the null hypothesis,	aiere is n	o unerenc	e verween the	onceated	anu me 1	oou oy irea	ceu ropula	GOIIS						
							1								
H _o :	$\mu_{27 \text{-Untreated}} - \mu_{27 \text{-10000}} = 0$	Null Hype	othesis = Th	nere is no differ	ence betw	een the U	ntreated po	pulation a	nd the Tre	ated popula	tion				
H _a :	μ _{27-Untreated} - μ ₂₇₋₁₀₀₀₀ >0	Alternate	e Hypothes	is = There is a di	fference b	etween th	ne CFU input	t populatio	on and the	Untreated P	opulation				
-					-										
- 2															
Sp	39.9375														
t, test statistic	0.1226	i i													
rejection region	t>t _n														
a.	u 0.00														
df.	0.05														
ui	8														
τα	1.860	1													
p-value	0.519325892	2													
Since 0.1226 < 1.860, I do not	reject the null hypothesis,	there is no	difference	e between the L	Intreated	and the 10	,000 Gy Trea	ated Popul	ations						



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