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Characterization of Silicon Ion Exposure on Deinococcus radiodurans

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CHARACTERIZATION OF SILICON ION EXPOSURE ON *DEINOCOCCUS RADIODURANS*

THESIS

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Abstract

Deinococcus radiodurans (Dr) is a very robust bacterium known for its ability to survive in extreme environments. It "can survive drought conditions, lack of nutrients, and, most important, a thousand times more radiation than a person can". [1] Known for its ability to resist gamma radiation, Dr exhibits a unique capability to endure significant DNA damage. The exact reasons why are not yet understood but evidence suggests it is possibly related to its DNA, proteins, or possibly its resistance to desiccation. [2]

In this experiment desiccated wild type Dr and seven mutant strains were irradiated with heavy silicon ion beams. The strains were rehydrated after irradiation and colonies were counted to see if a significant kill rate was achieved. The results indicated a kill rate less significant than expected.

Acknowledgments

I would like to express my sincere appreciation to my faculty advisor, the faculty of ENP, and the scientists and researchers of USAFSAM and the Sandia Ion Beam Laboratory.

Richard F. Daughtry

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CHARACTERIZATION OF SILICON EXPOSURE ON *DEINOCOCCUS RADIODURANS*

I. Introduction

General Issue

One of the dangerous aspects of manned, deep space exploration is the body's exposure to ionizing radiation. The protective blanket provided by the Earth's atmosphere means our susceptibility of ionizing radiation is not as great. In the far reaches of outer space that protective environment is nonexistent, therefore the dangers posed by ionizing radiation are increased. Terrestrial concerns with exposure are found with workers responding to nuclear power plant accidents such as Fukushima, and Soldiers responding to nuclear detonations through the National Technical Nuclear Forensics (NTNF). Accordingly, various agencies of the United States Government seek to develop means in which to minimize or prevent the harmful effects posed by ionizing radiation.

In the 1950s, A.W. Anderson recognized the radiation resistance of *Deinococcus Radiodurans'* (Dr) when he noted the bacterium remained on food cans despite being subjected to gamma radiation. Further research conducted by D. Duggan and Anderson focused on Dr's resistance by varying pH levels [3]. Early research conducted into Dr has been in an attempt to determine the processes that undergird Dr's exceptional ability to resist ionizing radiation, and in the event of high-level exposure, its ability to repair its damaged DNA. By achieving further understanding of this phenomenon we hope to advance methods to protect human cells to the ill effects of elevated levels of ionizing

radiation. The investigation will specifically focus on Dr survivability in a high linear energy transfer (LET) silicon radiation environment.

Problem Statement

The literature review will show that Dr is extremely resistant to ionizing radiation. However, almost every reported exposure used gamma or electron beam radiation. To date there is a dearth of information relating to Dr's ability to withstand high LET radiation.

The purpose of this research is to understand Dr's ability to deal with varying doses of heavy charged particle (HCP) silicon radiation measured in Grays (Gy), (defined in SI units as a Joule per kilogram (J/kg)). The doses of silicon radiation Dr will be subjected to are 500, 1,000, and 10,000 Gy. Exposed Dr targets will be compared to non-irradiated controls. The significance of this experiment is to gain a better understanding of Dr's resistance by looking at direct damage caused by high LET silicon particles.

Hypothesis

The hypothesis is: exposed wild type and mutant strains of *D. radiodurans* will show a statistically significant reduction in colony numbers than non-exposed wild type and mutants. The null hypothesis is that there will not be a statistically significant reduction between irradiated and non-irradiated strains. Test statistics utilizing the one-tailed test will be used for colony comparisons with a 95% confidence rate.

II. Literature Review

Chapter Overview

This chapter will explain the biology of Dr and its repair mechanisms after being subjected to radiation. The differences between high and low linear energy transfer (LET) and its effect on Dr will also be discussed. DNA and its structure, types of damage, and repair mechanisms will also be addressed, followed by a description of the Dr mutants used during the course of research.

A Brief Description of *Deinococcus radiodurans*

Dr is a Gram-positive, red-pigmented, non-sporulating, nonpathogenic bacterium occurring in dyads and tetrads with an average cell diameter of 1 μm (range, 0.5 to 3.5 μm). [2] Other features include; two large chromosomes, "(2,648,615 and 412,340 base pairs), a megaplasmid (177,466 base pairs), and a small plasmid (45,702 base pairs) yielding a total genome of 3,284,123 base pairs, and two smaller plasmids". [2] The doubling time of Dr is approximately 80 minutes in a rich nutrient environment. [8]

Deinococcus radiodurans is best known for its resistance to ionizing gamma and ultraviolet (UV) radiation and desiccation as noticed by Anderson and Duggan in the 1950s. Furthermore, Slade, et al. noted that Dr survive[d], "7 kGy of ionizing radiation with marginal lethality (10%)" shattering its "3.28 Mb genome into 20–30 kb fragments by introducing 100–150 double-strand breaks and, presumably, at least 10 times as many single-strand breaks". Within 2.5 hours following exposure the Dr genome has reassembled itself. [4] The reasons for this remarkable ability to repair are still being studied. Blasius, et al. claimed Dr's resistance to radiation "cannot be related to

prevention of DNA damage, because DNA double-strand breaks are formed at the same rate in *Escherichia coli* and *D. radiodurans* when cells are irradiated under identical conditions. [5] Daly, et al. attribute Dr radioresistance to a "relationship between intracellular Mn/Fe concentration ratios" whereby the surviving cells contained "about 300 times more Mn and about three times less Fe than the most-sensitive cells". [6] Bertlet and Levine suggest proteins are what repair DNA damage in Dr. [7]

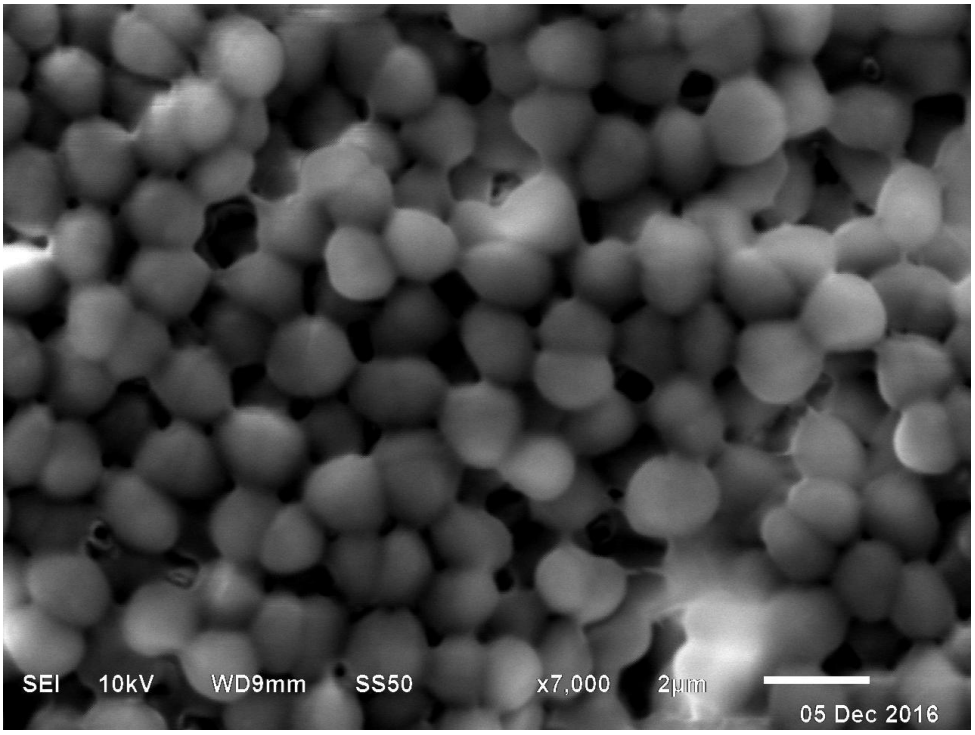


Figure 1. *Deinococcus radiodurans* taken by SEM at USAFSAM. [9]

High LET and Low LET

All matter is comprised of atoms. The three main particles that create the atom are the positively charged and relatively "heavy" proton, a nearly equally heavy but

neutrally charged neutron, and a smaller and lighter negatively charged electron. A stable atom is considered neutral; the composition of it does not tend to be positive (excess protons), or negative (excess electrons). Atomic stability is desired and atoms that are unstable will radiate energy until they achieve a stable ground state.

Radiation interacts with matter in two different ways depending upon how the energy is deposited. Linear energy transfer is a measurement of "the energy deposited per unit distance over the path of the radiation". [10] High LET is associated with heavy ions and alpha particles which deposit their energy quickly in matter and therefore have short ranges. [10] This loss of great energy over a short distance (approximately 0.1-1.0 mm) accounts for the high LET. Conversely, electrons and photons (gamma rays and x-rays) have low LET because their respective small size and neutral charge, and therefore do not lose their energy as rapidly and travel farther in matter (centimeters or longer) than the heavier charged particles. [10]

Most of the damage associated with low LET interaction is indirect. Ionization occurs when radiation has sufficient energy to remove electrons from a surrounding atom. Indirect damage is a result of ionization that happens when radiation ionizes or breaks apart a non "target" molecule, particularly water molecules. The primary cause for cell harm when a low LET radiation interaction occurs is the creation of a reactive oxygen species (ROS), in this case a hydroxyl radical (HO•), which is the primary radical created during the hydrolysis of water. [6] Radiation enters the cell and deposits energy that has the potential to ionize the water molecule within as such:

$$\text{radiation} \rightarrow H_2O \rightarrow H^+ + OH^-$$

The highly reactive OH^- radical subsequently attacks DNA as it looks for a donor electron to stabilize itself. Single stranded DNA breaks are often associated with indirect damage.

High LET, high energy loss within a short time and distance and in matter, inflicts a majority of its damage through direct damage cell as opposed to indirect damage through the creation of ROS. Heavy charged particles (HCP) enter matter and proceed in a generally straight path, stopping when their energy is depleted and imparting its energy directly into the molecule it stopped in, therefore high LET damage is less reliant upon ROS intermediaries and predominantly a result of the particle/target molecule interaction. In the case of DNA high LET damage usually manifests itself as double-stranded breaks (DSB). The rate at which this energy loss occurs is a "function of its residual energy". [11]

DNA

Deoxyribonucleic acid (DNA) is the molecule responsible for conveying genetic information found in living organisms. Located mostly inside the cell's nucleus, DNA is comprised of four bases; adenine (A), cytosine (C), guanine (G), and thymine (T) that bind A-T and C-G to form base pairs. The arrangements of base pairs determine what the organism may do regarding function, maintenance, and repair. These base pairs are attached to a phosphate and sugar molecule to form a nucleotide. Two nucleotides strands form a spiraled double helix that resembles a ladder. The backbone of the helix is created through covalent bonds between the sugar and phosphate groups. The two sides of the DNA "ladder: are held together by hydrogen bonds between the base pairs. [12]

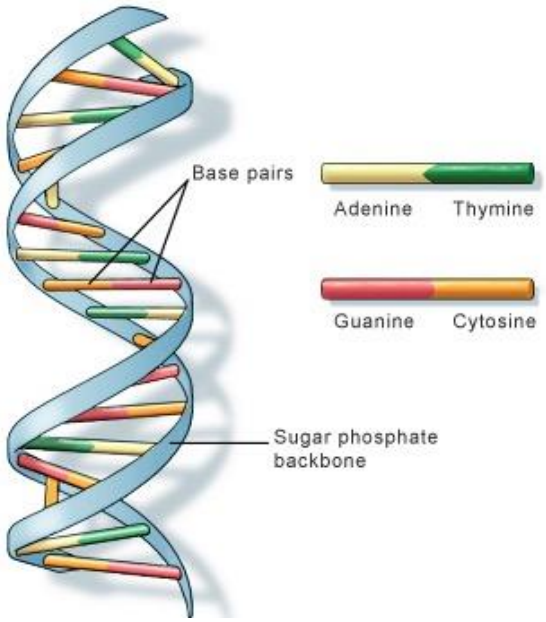


Figure 2. DNA Double Helix. [12]

DNA exposed to high LET generally results in numerous double strand breaks (DSBs) and DNA exposed to low LET typically experiences single-strand breaks (SSBs). A DSB is an alteration or break that occurs on both strands and results in a break of the covalently bonded sugar phosphate backbone. A SSB is an alteration or break that occurs on only one strand and results in a break of the covalently bonded sugar phosphate backbone of the broken strand. SSBs are considered less lethal and easier to repair as the overall integrity of the DNA molecule is retained, whereas a DSB will completely sever both backbones and result in loss of structural integrity. While SSBs are generally easier to repair, numerous SSBs on a strand of DNA within 6-10 base pairs can lead to DSBs. [11] DSBs occur when there is, "damage to the deoxyribose-phosphate backbone in two or more nearby locations. [11] DSBs are, "far more serious in the consequences

for a cell" and repair, "frequently lead[s] to mutation in the genome … and/or loss of reproductive capacity." [11]

Deinococcus radiodurans DNA Damage and Repair

As Slade notes, "[i]onizing radiation disintegrates the *D. radiodurans* genome by double-strand breakage into multiple fragments but also introduces at least 10-times more SSBs and many more sites of base damage". [2] Cellular desiccation also influences the amount of SSBs and DSBs damage received. A hydrated target will receive 2-3 orders of magnitude more SSBs and DSBs from gamma radiation than a desiccated target that can be attributed in part to the creation of ROS. [14]

The main repair mechanism for SSBs in DNA is excision. [11] As Edward Alpen explains in *Radiation Biophysics*, excision, "assumes the existence of a complementary strand as a template, so it serves only to repair single-strand breaks. [11] By their nature SSBs are less harmful to DNA than a complete severing of a strand caused by a DSB, therefore SSBs are easier to accurately repair. There are five components to excision repair: damage recognition, assemble the subunit, excise the damaged portion by making incisions on each side, resynthesize the excised gap, and ligate to regenerate the molecule. [15]

There are two methods of repair in the instance of DSBs; homologous recombination (HR) or nonhomologous end joining (NHEJ). [13] HR is essential to repair errors that occur during DNA replication. During HR an exchange of genetic information takes place between homologous sequences. [16] Usually, these are located on two copies of the same chromosome. [16] Despite its complexity homologous repair is

the preferred method as it is more accurate and uses a sister chromatid to ensure proper sequencing. [13] NHEJ, while indeed a faster repair mechanism, is more error prone than homologous recombination. NHEJ entails the rejoining of the two broken ends of the double helix through DNA ligation. [16] This is viewed as an "emergency solution" for the repair of DSB. [16] Figure 3 illustrates NHEJ and homologous end joining with the HR process.

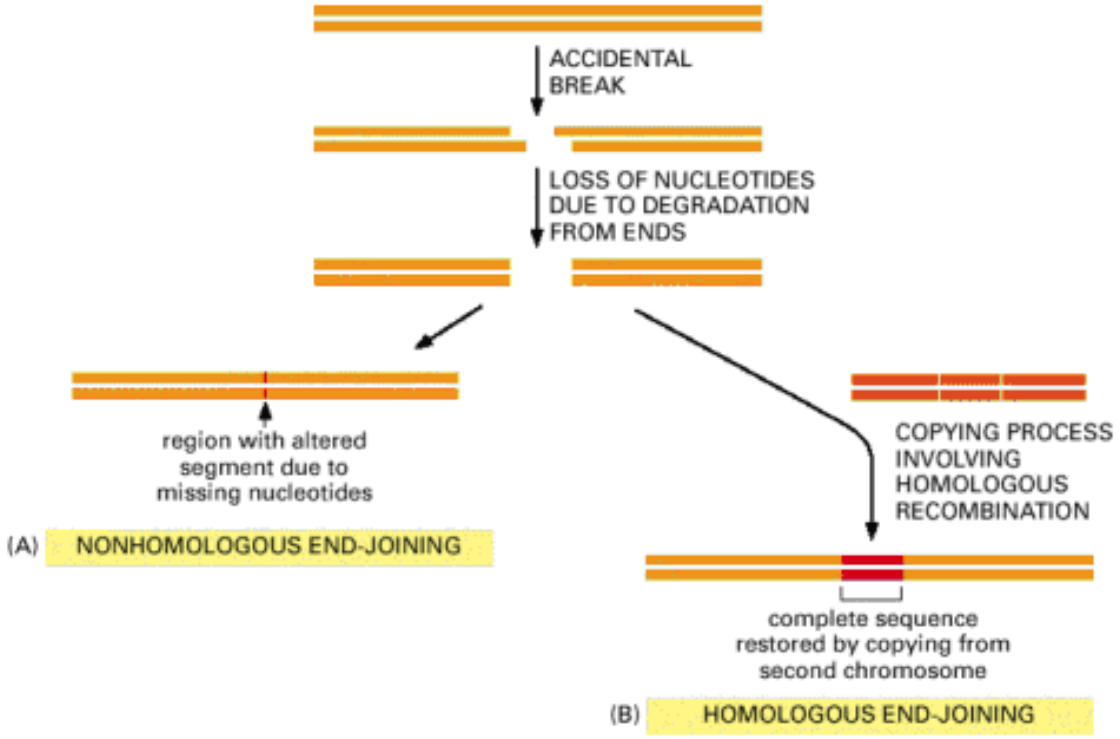


Figure 3. Two different types of end-joining for repairing double-stranded breaks. [16]

Deinococcus radiodurans and Mutant Strains

As with the previous experiment conducted by Lenker in 2017, the Dr. wild-type R1 strain selected for this experiment was acquired from the American Type Culture Collection (ATCC) for use by United States Air Force School of Aerospace Medicine

(USAFSAM). An additional seven mutant strains created by the laboratory staff at USAFSAM were used during the experiment. All eight strains were subjected to silicon ion irradiation at the Sandia Ion Beam Laboratory in Albuquerque, NM. Two control samples plates remained at USAFSAM and were not shipped to Sandia. The strains used for this experiment are listed in Table 1.

Table 1. *Deinococcus radiodurans* R1 strain list

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

As shown in Table 1, with the exception of the WT strain the mutants have had either one or two genes removed. It is believed these particular genes may affect the radiation resistance of Dr, as mentioned during the description of low LET interaction with the water within cells. Mutant 5 had one, and mutant 8 had two superoxide dismutase (SOD) gene(s) knocked out (KO) or removed. A SOD is a detoxifying enzyme and one of the three major cellular defense systems which protects against ionizing radiation by creating a less damaging chemical when reacting with a superoxide.

[16] The cofactor for Mutant 5 is manganese (Mn). The cofactors for Mutant 8 are copper (Cu) and zinc (Zn).

The KO gene for Mutants 16, and 27 is uvrB. uvrB functions during nucleotide excision repair, serving as an enzyme to assist in DNA repair. For Mutants 1.5 and 6A the KO gene is recF. recF serves a role during homologous recombination which is essential to repair errors that occur during DNA replication. Mutant 11 KO is BshA, or bacillithiol biosynthesis, a gene of uncertain function but believed to defend against peroxides and other reactive oxygen species. [17]

III. Methodology

Chapter Overview

The purpose of this chapter is to describe the methodology utilized to create the Dr used during the experiment. The first section will cover mutant creation, DNA amplification, plasmid creation and amplification, and Dr transfer to the knockout mutants. The following section will discuss the kill curve, the various stages of Dr growth, desiccation, and rehydration, and a description of the nature of the silicon ion beam. Finally, an explanation of counting methods is given.

Plasmid Prep of pUC19mPheS and PCR for *D. radiodurans* Gene Knock-outs

To prepare the plasmid, the E. coli NEB 5 alpha bacteria carrying pUC19mPheS was isolated from a frozen glycerol stock and streaked and incubated overnight at 37°C in an unsealed plastic bag to prevent drying. The following day a single colony from the plate was inoculated overnight at 37°C in 30ml of LB broth with 50µl/ml Carbenicillin in

a 125ml flask. The next day the overnight culture was pelleted in a 50ml conical tube at 3500 RPM for 20 minutes. Supernatant was poured off and the pellet was suspended in 750µl of Qiagen P1 buffer then added to 750µl of Qiagen P2 buffer, mixed, and incubated for 5 minutes to alkaline lyse the cells. 1050µl of Qiagen N3 buffer was mixed to neutralize the reaction. Cell debris was precipitated by centrifuge at 3500 RPM for 5 minutes. After centrifugation the aqueous portion was distributed equally between two 1.5ml microcentrifuge tubes and spun at maximum speed for 15 minutes to pellet any remaining precipitate. The remaining supernatant was loaded into Qiagen spin miniprep columns and spun for 1 minute. After discarding the flow through, 500µl of Qiagen PB buffer was added then spun for 1 minute. After discarding the flow through, 750µl of Qiagen PE buffer was added then spun for 1 minute before discarding the flow through again and spun once more for 2 minutes. The columns were then placed in sterile 1.5ml microfuge tubes. 100µl of Qiagen Elution buffer was added to each column and incubated at room temperature for 1 minute before spun at max speed for 1 minute to elute the plasmid DNA from the column. DNA tubes were placed on ice and the quality and quantity of the plasmid DNA was measured at 124.9ng/µl using the NanoDrop.

pUC19mPheS EcoRI Digest for NEBuilder Cloning

Following the NanoDrop measurement a digest mixture was created and incubated overnight at 37°C. Table 2 details the mixture.

Table 2. Digest mix

Molecular Biology Grade Water	54µl
pUC19mPheS Plasmid DNA at 124.9ng/µl	32µl
10X NEB EcoRI Buffer	10µl
NEB EcoRI Enzyme	4µl
Total	100µl

After incubation, 20µl (to 40µl) of 6x Sample Buffer was added and to and mixed with the plasmid digest. The mixture was loaded over 3 lanes of a 0.8% agarose gel in 1xTBE with 0.5µg/ml ethidium bromide and ran at 10 volts per centimeter distance. Using a razor blade and an UV light box, the ethidium bromide plasmid DNA bands (3895bp) were cut from the gel and distributed in 1.5ml microfuge tubes with no more than 300mg of gel slice per tube. The linearized plasmid DNA was isolated from the agarose with Qiagen gel extraction mini spin columns. Quantity and quality of the linearized plasmid DNA was measured with the NanoDrop.

PCR Amplification of Fragments for Gene Knock Out Construct Cloning

Twelve primers from IDT Inc. were suspended to 100µM in 0.1xTE buffer with pH of 7.5. Table 3 details the dilutions.

Table 3. Primer dilution mixtures

Primer Name	nmoles	µl of 0.1 TE for 100µM
Puc_RecFup_Fwd	26.6	266
Kan_RecFup_Rev	24.2	242
RecFup_Kan_Fwd	30.1	301
RecFdown_Kan_Rev	31.0	310
Kan_RecFdown_Fwd	19.3	193
Puc_RecFdown_Rev	27.7	277
Puc_UvrBUp_Fwd	30.5	305
Kan_UvrBUp_Rev	26.8	268
UvrBUp_Kan_Fwd	36.9	369
UvrBdown_Kan_Rev	26.2	262
Kan_UvrBdown_Fwd	24.7	247
Puc_UvrBdown_Rev	22.7	227

Each primer was diluted to 1:10 in molecular grade biology water to 10µM (10µl in 90µl of water). PCR templates were diluted to 50ng/µl in molecular grade biology water with 20µl of 529ng/µl *D. radiodurans* genomic DNA stock added to 180µl of water.
5µl of 144ng of pUCIDT-Amp::KANkanp plasmid was diluted to 50pg/µl in 120µl of molecular biology grade water. Twelve PCR reactions (6 duplicates) were set up for genomic and Kan plasmid templates. DNA fragments were then amplified in the thermocycler.

NEBuilder Construction of Knock-Out Plasmid

Several knockout plasmids were created based with NEBuilder protocols. Briefly the amplified fragments were combined with the plasmid backbone to create recF or uvR homologous regions (for gene knockout) along with a kanamycin selectable marker. Fragments 1 (recFup), 3 (recFdown), 4 (uvrBup), and 6 (uvrBdown) were diluted with water and DNA to 33ng/µl (~0.05 pmol). The NEBuilder HiFi DNA Assembly Master Mix improves the efficiency and accuracy of DNA assembly. It allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility by utilizing an exonuclease to create single-stranded 3′ overhangs that facilitate the annealing of the overlaps. The polymerase then fills in gaps within each annealed fragment while the DNA ligase seals nicks in the assembled DNA resulting in a double-stranded fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation of E coli. [18]

Table 4. NEBuilder reaction mixes

Additive	A		B	
	recF	uvrB	recF	uvrB
Water	4.5µl	5.5 µl	4 µl	5 µl
5’ Homology Fragment	1 µl	1 µl	1 µl	1 µl
Kan Fragment	2 µl	1 µl	2 µl	1 µl
3’ Homology Fragment	1 µl	1 µl	1 µl	1 µl
Plasmid Vector	1.5 µl	1.5 µl	2 µl	2 µl
2x NEBuilder Mix	10 µl	10 µl	10 µl	10 µl

Mixture was incubated at 50°C for 60 minutes then stored at -20°C.

Transformation of *E. coli* one-shot chemically competent cells

The two cell types (NEB 5-alpha and NEB dam$^-$ dcm$^-$) were thawed on ice. 2µl of the NEBuilder reaction was added and incubated on ice for 30 minutes. It was then heat shocked at 42°C for 30 seconds and incubated on ice for 5 minutes. 950µl of Super Optimal broth with Catabolite repression (SOC) media was added to the cell/DNA mix and incubated at 37°C for 60 minutes with 220 RPM shaking for aeration. The mixture was then pelleted in a microfuge at max speed for 2 minutes. 850µl of supernatant was removed and the cell was then pelleted in the remaining media. The suspension was spread plated on LB agar with 32µg/ml Kanamycin and incubated overnight at 37°C in an unsealed plastic bag to prevent drying.

Clone Construction Verification of *D. radiodurans* DNA Repair KO Clones Using Plasmid Template

Clone construction was verified by isolating plasmid DNA using Qiagen Spin Minipreps and analyzed with the NanoDrop. All plasmids were reduced to 0.5ng/µl in water with remaining undiluted plasmid stored at -20°C for use in transformation. 2µl of diluted plasmid templates were added to 16 PCR tubes with a 10x mix for uvrB and recF. The primer sets for recF were Puc_RecFup_Fwd and Puc_RecFdown_Rev. The primer sets for uvrB were Puc_UvrBUp_Fwd and Puc_UvrBdown_Rev. PCR cocktail mix is shown in Table 5.

Table 5. PCR cocktail with genomic template mix

Molecular Biology Grade Water	290µl
10mM dNTPs	15µl
Forward Primer @ 10uM	20µl
Reverse Primer @ 10uM	20µl
5x LongAmp Taq Reaction Buffer	100µl
DMSO (Final Conc. 3%)	15µl
LongAmp Taq DNA Pol	20µl
Total	480µl

2µl of each templates was added to 48µl of cocktail in PCR tubes and placed in the thermocycler under the following conditions: 94°C for 2 minutes, 30 cycles of (2 step PCR), of 94°C for 30 seconds, 65°C for 3 minutes, 65°C for 10 minutes, then held at 4°C. Once complete, 20µl of each reaction was mixed with 6µl of Orange G loading buffer and run on a 0.8% agarose gel in 1xTBE with 0.5µg/ml ethidium bromide at 10 volts per centimeter distance.

D. radiodurans Transformation

D. radiodurans wildtype from a frozen glycerol stock was streaked for isolation on 1xTGY agar and incubated at 32°C for 2 days in an unsealed ziplock bag to prevent drying. A single colony from the plates was inoculated into 5mls of 1xTGY broth in a 14ml round bottom snap cap tube along with a blank 5ml control and incubated overnight at 32°C and 220 RPM. The overnight culture was diluted to an absorbance at 600nm between 0.2-0.3 which is approximately a 1:10 to 1:20 dilution. 2mls of the culture was

mixed into 20mls of 1xTGY in a 125ml flask and incubated 2 hours at 32°C and 220 RPM. After 2 hours, 2.2mls of 300mM $CaCl_2$ (~30mM final) were added and incubated for 2 hours at 32°C and 220 RPM. After the $CaCl_2$ 2 hour incubation, 100 µl of the culture were transferred to five sterile 1.5ml microfuge tubes and placed on ice. 1µg of plasmid DNA was added to each transformation and gently mixed and incubated on ice for 1 hour (as shown in Table 6).

Table 6. Plasmid DNA transformation mixture

#	Plasmid	Concentration (ng/µl)	µl volume to add
1	pUC19mPheS::KOuvrB::Kan	27	37
2	pUC19mPheS::KOuvrB::Kan	28.8	35
3	pUC19mPheS::KOuvrB::Kan	31.2	32
4	pUC19mPheS::KOrecF::Kan	30.8	33
5	pUC19mPheS::KOrecF::Kan	28.2	36
6	pUC19mPheS::KOrecF::Kan	22.8	44
7	Negative Control (No DNA)	N/A	0
8	Blank (No DNA/No Cells)	N/A	0

After 1 hour the DNA/cell mix was transferred to a 14ml round bottom snap-cap tube containing 1ml of 1xTGY and incubated overnight at 32°C and 220RPM to grow out. The following day the grown out cultures were diluted 1:10 7x in series (20ul in 180µl 1xTGY) using a multichannel pipet. 5µl of each dilution of the dilution series was spotted on 1xTGY agar containing 16ug/ml kanamycin and allowed to dry before the

plates were inverted and incubated for 48 hours at room temperature in an unsealed ziplock bag.

4-CP Selection and Screening of *D. radiodurans* Knockouts

5mM 4-CP 1xTGY agar plates with 16µg/ml Kanamycin were made mixing 500mls MQ water, 7.5g of agar, 2.5g of Tryptone, 1.5g of Yeast Extract, 0.5g of Dextrose, and 0.5g of 4-CP (4-chlorophenylalanine). The solution was placed in the autoclave and cooled to 55°C before 250µl of 32mg/ml Kanamycin was added and mixed. Plates were poured and left to solidify at room temperature for 48 hours.

Kan^R Colony Pick for Grow out and Patch for Direct 4-CP Selection

For each transformation, 5-10 colonies were selected and mixed into 5mls of 1xTGY broth with 16µg/ml kanamycin and incubated for grow out at 32°C and 220RPM for 48 hours. The grown out cultures were then diluted 1:10 7x in series (20ul in 180ul 1xTGY). 5µl of each dilution of the dilution series was spotted on 1xTGY agar and on 5mM 4-CP 1xTGY agar with both agar types containing 16µg/ml kanamycin. Once the spots dried, the plates were inverted and incubated at room temperature for 48 hours in an unsealed ziplock bag. After 48 hours, 5-10 colonies for each transformation were selected and patched to the same area on a 5mM 4-CP 1xTGY agar plate with 16µg/ml kanamycin. A loop was used to mix the patched bacteria and then streaked for isolation. Plates were then incubated at 32°C for 48 hours in an unsealed ziplock bag.

Screening of 4-CP Resistant and Kanamycin Resistant Colonies

To screen the colonies, 1 colony from each transformation was suspended in 5mls of 1xTGY broth with 16µg/ml kanamycin and incubated at 32°C 220 RPM for 48 hours.

After incubation, 500µl of culture was mixed with 500µl of 50% Glycerol/50% 1xTGY broth in a 1ml cryovial and stored at -80°C for glycerol stock. For genomic DNA isolation, 2mls of the culture was transferred to a 2ml microfuge tube and pelleted by centrifugation at max speed for 5 minutes. Supernatant was removed by pipet and the genomic DNA was isolated using the QIAamp DNA Mini kit with the gram positive bacteria protocol. The genomic DNA concentration was measured by NanoDrop and diluted to 50ng/µl in water. Remaining undiluted genomic DNA was stored at -20°C. 2ul of diluted genomic DNA templates were added to separate PCR tubes (16 total, 2 each from RAD2, RAD3, RBD3, UAD2, UAD4, UBD1, and a WT and No DNA control) for each primer mix. PCR Mix was 10x for uvrB and 10x for recF. Primer sets for recF were Puc_RecFup_Fwd and Puc_RecFdown_Rev. Primer sets for uvrB were Puc_UvrBUp_Fwd and Puc_UvrBdown_Rev. Table 7 details the PCR cocktail and genomic template mixture.

Table 7. PCR cocktail and genomic template mixture

Molecular Biology Grade Water	290µl
10mM dNTPs	15µl
Forward Primer @ 10uM	20µl
Reverse Primer @ 10uM	20µl
5x LongAmp Taq Reaction Buffer	100µl
DMSO (Final Conc. 3%)	15µl
LongAmp Taq DNA Pol	20µl
Total	480µl

48µl of cocktail was added and mixed to each 2µl template then put into the thermocycler under the following conditions: 94°C for 3 minutes, 30 cycles of (2 step PCR), 94°C for 30 seconds, 65°C for 3 minutes, 65°C for 10 minutes, and held at 4°C. 15µl of Orange G loading buffer was mixed with each 50ul PCR reaction and then the entire volume (65µl) ran on a 0.8% agarose gel in 1xTBE with 0.5µg/ml ethidium bromide at 10 volts per centimeter distance.

New Kan Selection PCR Check of *D. radiodurans* DNA Repair Knock-Out Clones Using Plasmid Template

Plasmid DNA was isolated using Qiagen Spin Minipreps by the manufacturer's protocol (16 total) and 4mls of culture. NanoDrop measured DNA concentration and 260/280 ratio. All plasmids were diluted to 0.5ng/µl in water with no dilution step larger than 1:100. Remaining undiluted plasmid was stored at -20°C for later transformations. 2µl of diluted plasmid templates was added to separate PCR tubes (48 total). Primer sets were diluted to 10µM (1:10 dilution of 100µM stock from IDT Inc.). Primer sets were as follow: recF whole: Puc_RecFup_Fwd and Puc_RecFdown_Rev, uvrB whole: Puc_UvrBUp_Fwd and Puc_UvrBdown_Rev, recF Kan: RecFup_Kan_Fwd and RecFdown_Kan_Rev, uvrB Kan: UvrBUp_Kan_Fwd and UvrBdown_Kan_Rev. The PCR mix for the primers is shown below in Table 8.

Table 8. PCR mix (15x for uvrB whole, recF whole, uvrB Kan, and recF Kan)

Molecular Biology Grade Water	435µl
10mM dNTPs	22.5µl
Forward Primer @ 10uM	30µl

Reverse Primer @ 10uM	30µl
5x LongAmp Taq Reaction Buffer	150µl
DMSO (Final Conc. 3%)	22.5µl
LongAmp Taq DNA Pol	30µl
Total	720µl

48ul of cocktail was added and mixed with 2ul of appropriate template then placed in the thermocycler. PCR conditions for full-length 3kb plus PCR were: 94°C for 2 minutes, 30 cycles of (2 step PCR) at 94°C for 30 seconds, 60°C for 30 seconds, 65°C for 3 minutes, then 65°C one cycle for 10 minutes and held at 4°C. PCR conditions for Kan 1kb plus PCR were: 94°C for 2 minutes, 30 cycles of (2 step PCR) at 94°C for 30 seconds, 60°C for 30 seconds, 65°C for 1 minute, then 65°C one cycle for 10 minutes and held at 4°C. 20µl of each reaction was mixed with 6µl of Orange G loading buffer then ran on a 0.8% agarose gel in 1xTBE with 0.5µg/ml ethidium bromide. The gel ran at 10 volts per centimeter distance.

PCR Check of *D. radiodurans* DNA Repair Knock out Clones Using Genomic Template

Genomic DNA was isolated using Qiagen QiaAmp Spin Mini-Columns by the manufacturer's protocol (30 total). DNA concentration and 260/280 ratio was then measured using the NanoDrop. Genomic DNA samples were diluted to 25ng/µl in water with the remaining undiluted sample stored at -20°C. 4µl of diluted plasmid templates were added to separate PCR tubes (34 total). Primer sets (Appendix A) were arranged as:

recF full length: Puc_RecFup_Fwd, Puc_RecFdown_Rev, uvrB full length: Puc_UvrBUp_Fwd, Puc_UvrBdown_Rev, recF intra-deletion: recFIntDelFwd, recFIntDelRev, uvrB intra-deletion: UvrBIntDelFwd, UvrBIntDelRev. PCR mix is shown in Table 9.

Table 9. PCR mix (20x for uvrB full length, uvrB internal deletion, recF full length, and recF internal deletion)

Molecular Biology Grade Water	540µl
10mM dNTPs	30µl
Forward Primer @ 10uM	40µl
Reverse Primer @ 10uM	40µl
5x LongAmp Taq Reaction Buffer	200µl
DMSO (Final Conc. 3%)	30µl
LongAmp Taq DNA Pol	40µl
Total	920µl

46µl of cocktail was added to each 4µl template and then placed in the thermocycler. Full length PCR conditions were set as: 94°C for 2 minutes, 30 cycles (3 step PCR) of 94°C for 30 seconds, 60°C for 30 seconds, 65°C for 4 minutes, one cycle at 65°C for 10 minutes, and held at 4°C. Intra-del PCR conditions were set as: 94°C for 2 minutes, 30 cycles (3 step PCR) of 94°C for 30 seconds, 56°C for 30 seconds, 65°C for 1 minute, one cycle at 65°C for 10 minutes, and held at 4°C. 15ul of Blue Juice loading buffer was added to each 50µl PCR reaction and ran on a 20µl 0.8% agarose gel in 1xTBE with 0.5µg/ml ethidium bromide. The gel ran 10 volts per centimeter distance until the loading buffer reached the bottom of the gel. At the conclusion of the gel run it was

noted the full length PCR did not work well due to the inability to get a clean recF mutant. This necessitated a re-transformation that would last for two weeks. Re-transformation yielded satisfactory results for uvrB

It was assumed a pure recF mutant was not obtained based upon electrophoresis gel results. These results demonstrated that recF mutants likely retained some degree of the WT genome in at least one copy of the chromosome.

D. radiodurans **Mutant Sample Prep for Dried Spot UVC Treatment Including** ***E. coli***

E. coli and five strains of *D. radiodurans* were streaked on agar plates then incubated for 48 hours at 32°C (37°C for *E. coli*). Strain list is shown in Table 10.

Table 10. Strain list for UVC treatment

Number	Strain	Plate
N/A	*E. coli* DH5α	LB agar
1	(1) *D. radiodurans* R1 WT	1xTGY agar
5	(5) *D. radiodurans* R1 DR_1279::mlox	1xTGY agar
8	(8) *D. radiodurans* R1 DR_1546::KAN DR_A0202::NAT	1xTGY agar KAN 16µg/ml NAT 50µg/ml
11	(11) *D. radiodurans* R1 DR_1279::mlox	1xTGY agar
16	(16) *D. radiodurans* R1 uvrB::KAN	1xTGY agar KAN 16µg/ml
27	(27) *D. radiodurans* R1 uvrB::KAN	1xTGY agar KAN 16µg/ml

After incubation, an individual colony from each strain was inoculated into 5ml of 1xTGY broth (LB broth for *E. coli*) and with the appropriate antibiotics added for each strain, then placed in 14ml round bottom tubes, and incubated at 32°C (37°C for *E. coli*) and 220 RPM overnight. The following day the cultures were diluted 1:100 (20µl of culture in 20ml broth) into the appropriate media type in 125ml vented cap flasks and incubated overnight. After incubation a measurement of the 600nm absorbance was taken from a 1:10 dilution of each culture (100µl of culture and 900µl of broth). The dilution was placed in a spectrophotometer cuvette and measured against a blank, broth only, cuvette. The NanoDrop readings of each cuvette determined the volume of culture needed to add to 40mls of broth to achieve an Abs. 600 of 0.25.

$$\frac{40mls * 0.25}{10 * Abs.600nm} = vol.of\ culture\ in\ mls\ needed$$

The calculated volume of each culture was added to 40mls of broth in a 250ml vented cap flask and incubated at 32°C (37°C for E. coli) 220 RPM for 4 hours (when *D. radiodurans* cultures reach early log phase). After incubation, 30ml of each culture was transferred to a 50ml conical tube. The bacteria were pelleted by centrifugation at 3500 RPM for 20 minutes. During the centrifugation, the 600nm Abs. values of each undiluted culture was read and the value was used to calculate the re-suspension volume for each culture to reach a 600nm Abs. value of 5 (2-5x10^8 CFU/ml for *D. radiodurans*).

$$\frac{30mls * Abs.600nm}{5} = vol.of\ broth\ in\ mls\ needed$$

After centrifugation, the supernatant was poured and pipetted off and the bacterial pellet was suspended in the calculated volume of broth for a 600nm Abs. value of 5.

In a laminar flow hood, a single channel pipet was used to deliver a 60µl drop to 2 plate lids in duplicate (see Table 11 below). One plate was for UVC treatment and the other was an untreated control. The plates remained in the hood overnight to dry.

Table 11. Plate Map for 60ul Drops

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	1		5		8		11	16	27		*E.coli*	
D												
E												
F	1		5		8		11	16	27		*E.coli*	
G												
H												

The samples used for spotting were also diluted 1:10 seven times in series (200µl of cells in plate well row A, then 20µl into 180µl of broth) and spotted 5µl onto 1xTGY agar in duplicate (LB agar for *E. coli*). This was to determine the input CFU and CFU loss due to drying and vacuuming. Once the spots were dry, the plates were incubated over the weekend in unsealed bags at 32°C (37°C for *E. coli* overnight). After drying in the laminar flow hood, the lids with dried spots were placed on the 96 well plate bottoms, wrapped with parafilm, and stored at room temperature in the dark. *E. coli* input CFU colonies were counted and recorded.

$$\frac{\text{Colony Count} * \text{Dilution Factor}}{5} * 60\mu l = CFU\ per\ 60\mu l\ spot$$

Vacuum and UVC Treatment

The plates were removed from dark storage, parafilm was removed and then the plates were placed in the ultracentrifuge (no rotor in the centrifuge) with the lids down. The ultracentrifuge was turned on and set to pull a vacuum. After 15 minutes the plates were removed. The UVC crosslinker was prepared and ran at 9999 J/m^2. Immediately after, the first plate lid was placed in the crosslinker and treated at 850 J/m^2. After treatment, the lids with the dry spots were placed on the 96 well plate bottoms, re-wrapped with parafilm, and stored at room temperature in the dark.

Re-suspension of Bacteria in Dried Spots; Dilutions and Plating

Dried spots were suspended with 60µl of broth and pipetted up and down 20 times. After all spots were suspended, each one was pipetted up and down 20 times before the volume was transferred to a well in row A of a 96 well plate. The samples used for spotting were also diluted 1:10 seven times in series (200µl of cells in plate well row A, then 20µl into 180µl of broth) and spotted 5µl onto 1xTGY agar in duplicate (LB agar for *E. coli*). This is to determine the untreated CFU and CFU loss due to UVC treatment. Once dry, the spots were incubated at 32°C (37°C for E. coli overnight) in unsealed bags for 2 days. Colonies were counted and recorded in the dilution so the CFU per 60µl spot could be calculated.

Table 12. *D. radiodurans* CFU counts normalized to 10-5

	Untreated (Drying and Vacuum only)				Drying, Vacuum, and UVC Treated			
Strain	A	B	C	D	A	B	C	D
1	9-5	5-5	19-5	13-5	9-5	11-5	7-5	19-5
5	9-5	9-5	24-5	10-5	15-5	19-5	11-5	11-5
8	7-5	2.1-5	19-5	7-5	9-5	9-5	20-4	2.9-5
11	13-5	8-5	20-5	13-5	50-5	17-5	18-5	18-5
16	9-5	21-5	26-5	15-5	18-5	20-5	21-5	100-5
27	11-5	9-5	24-5	17-5	19-5	24-5	10-5	23-5

Noticeable CFU loss between treated and untreated was seen in strain 8, C and D. An unexplained rise between treated and untreated was seen in strain 11 A and 16 D.

All four *E. coli* demonstrated CFU loss between treated versus untreated.

Table 13. *E. coli* treated and untreated CFU counts

E.coli Untreated (Drying and Vacuum only)	*E.coli* Drying, Vacuum, and UVC Treated
6-3	15-2
14-3	8-2
30-3	8-2
22-3	2-1

Silicon Dose Calculations

SRIM and TRIM was utilized to determine the proper silicon ion dose calculation. The layers were selected based upon materials the silicon ion beams would interact. Sandia Tandem Accelerator information is located in Appendix C.

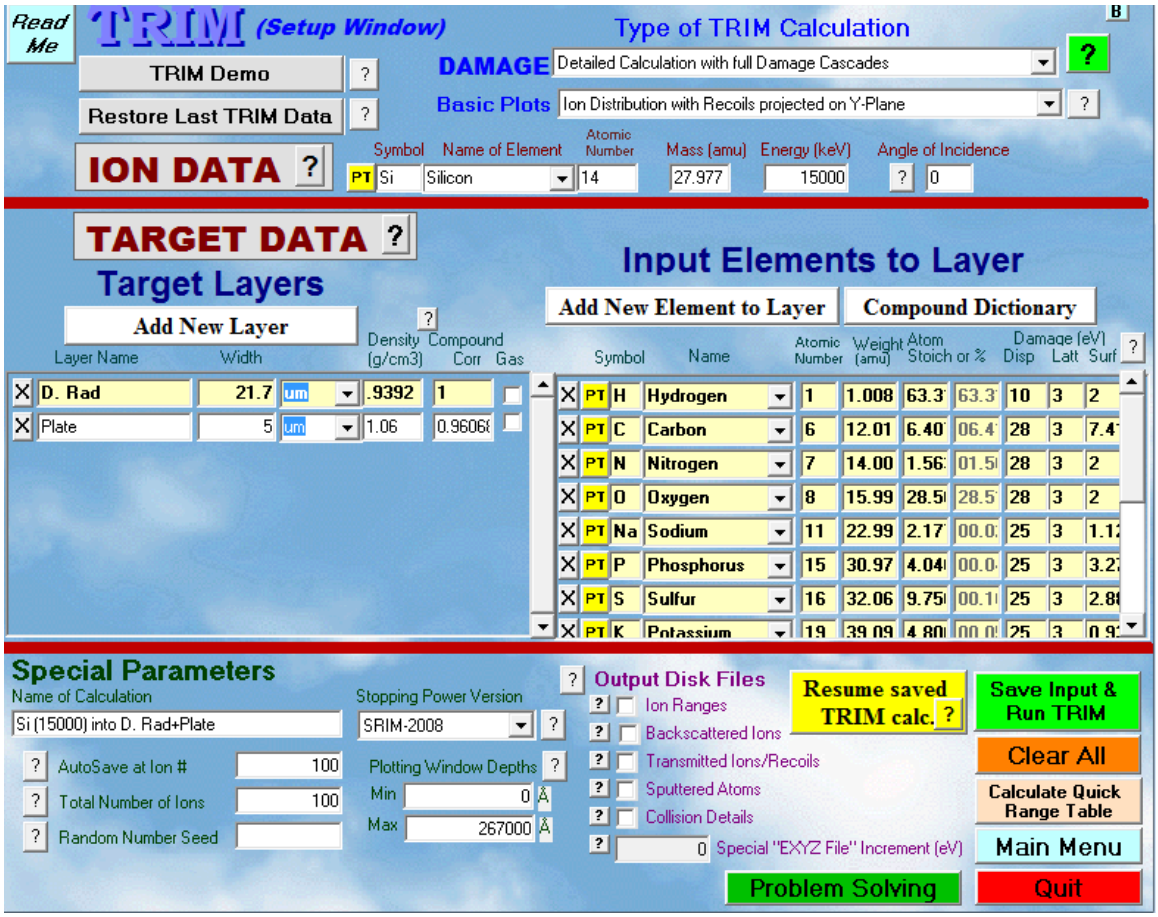


Figure 4. SRIM input screen with variables selected

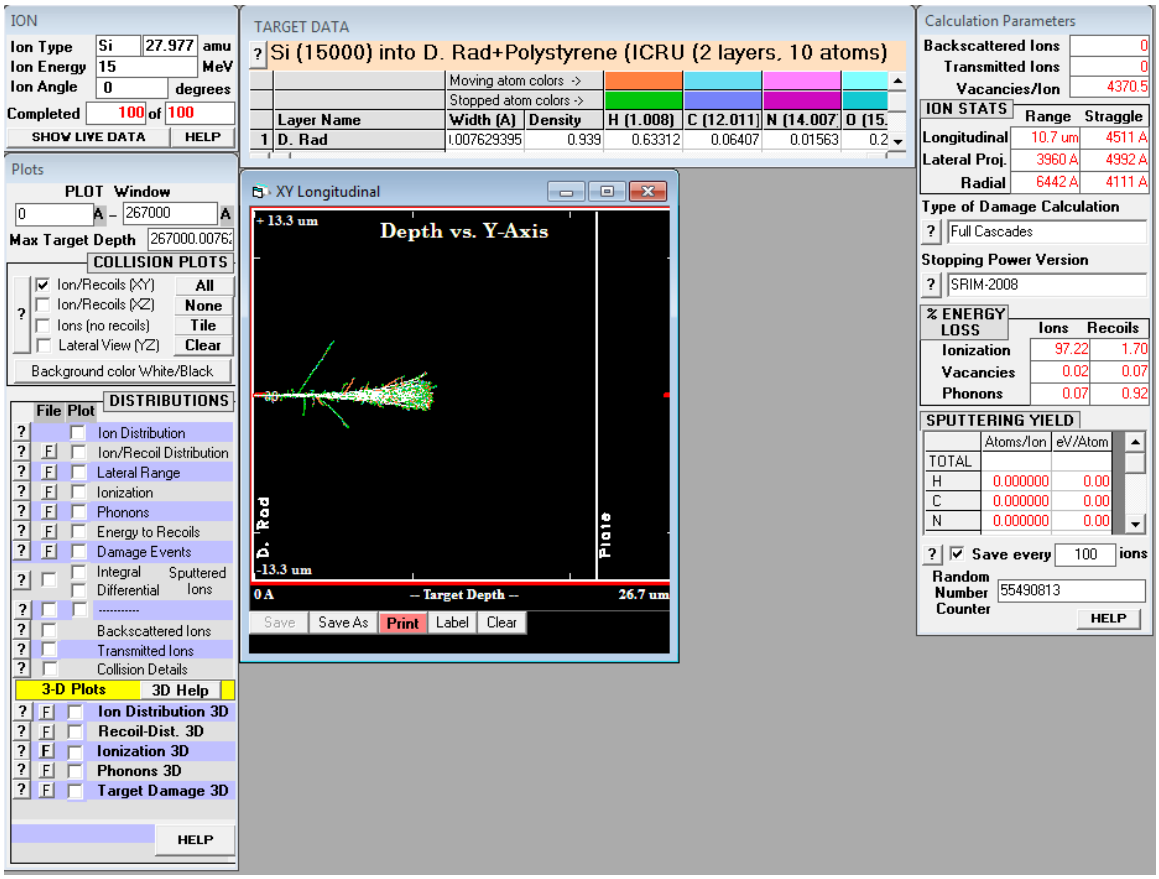


Figure 5. Based upon input from Figure 4. Simulation of 15 MeV silicon ions irradiating Dr.

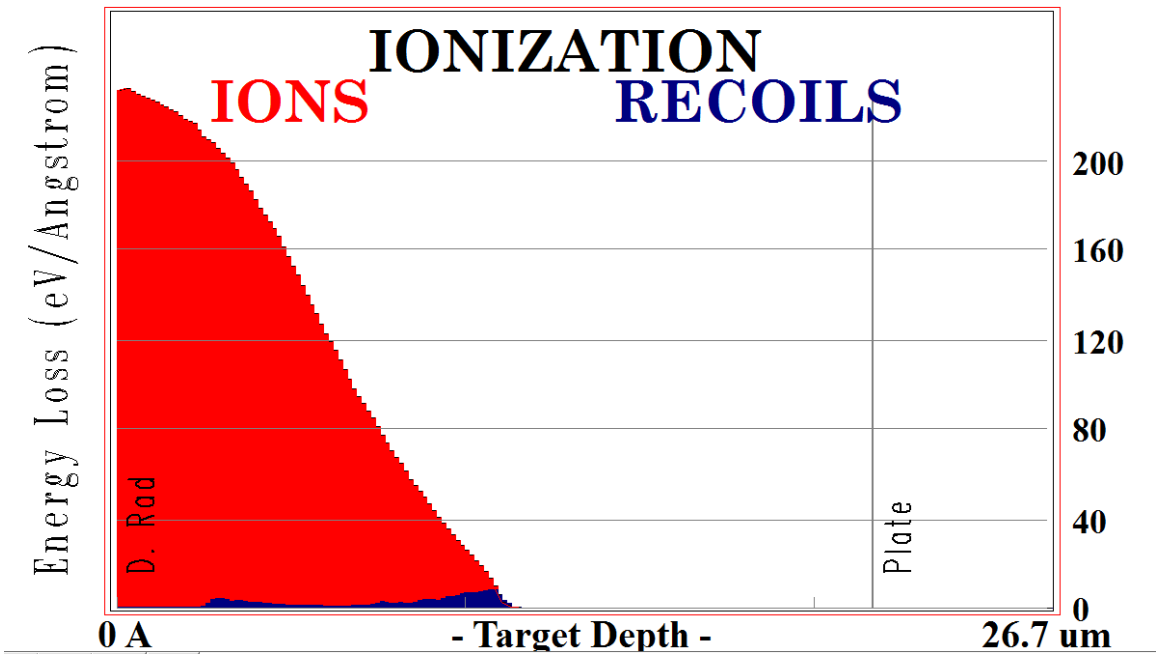


Figure 6. Chart created showing the ionization in Dr and the polystyrene lid measured around 250 eV/Angstrom. The red region measures energy loss to the target electrons. The blue recoil region measures energy loss due to recoil atom interaction.

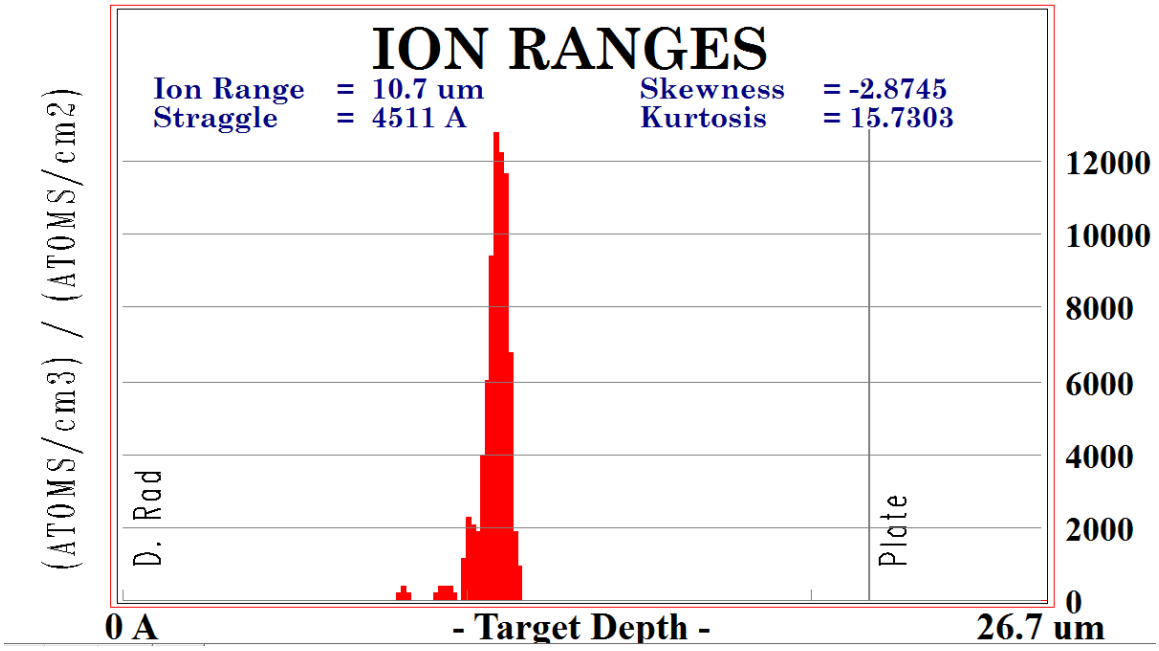


Figure 7. Chart showing the penetration depth of 15 MeV silicon ions into Dr. It is approximately 10.7um.

This ionization information is utilized to determine the fluence necessary to achieve a specific irradiation dose.

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

$$250\ \frac{eV}{Angstrom-Ion} * \frac{1.6022\ x\ 10^{-19}\ J}{1\ eV} * \frac{1\ x\ 10^{8}\ Angstrom}{1\ cm} * \frac{cm^3}{0.9392\ g} * \frac{1.17\ x\ 10^{8}\ Ions}{1\ cm^2} * \frac{1000\ g}{1\ kg} =$$

$$498.98\ Gy \sim 500\ Gy$$

IV. Analysis and Results

Chapter Overview

The purpose of this chapter is to show and describe the results of the experiments between irradiated samples and the control samples. The hypothesis stated there would be a statistically significant difference between irradiated samples and non-irradiated controls. The null hypothesis stated there would not be a statistically significant difference between the two groups. The results were compared using a one sided t-test with a confidence value of 95% certainty.

Colony Average Comparisons Pre- and Post Silicon Exposure

Silicon ions are heavy charged particles that will cause double strand breaks in the exposed cells. With the exception of WT, all mutants were grown with one or more knock-outs to inhibit post-irradiation cell repair. Single strand breaks created by reactive oxygen species should not be as much of a factor as double strand breaks created by interaction with silicon ions. The single strand breaks should be limited due to desiccation of one month and being subjected to the ion beam's vacuum of 10^{-7} Torr.

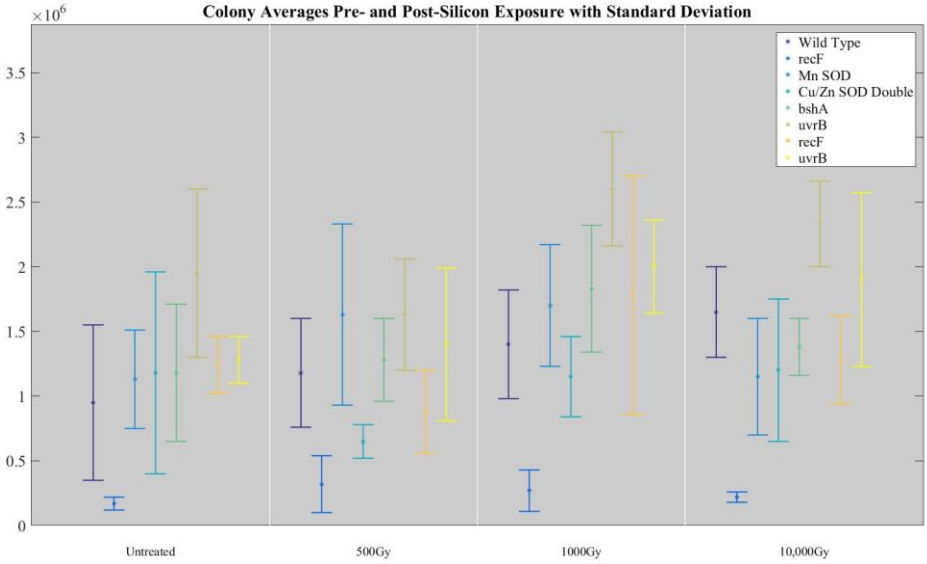


Figure 8. Comparison of strains by exposure.

Figure 8 shows a comparison of total colonies counts, normalized to 10-5 dilution, for each exposure condition. According to test statistics, colony counts for CFU inputs to desiccation in strains WT, 1.5, 5, 8, 11, and 27 accounted for all of the statistically significant results observed throughout the experiment. There were no statistically significant results observed in the any strains resulting from irradiation when compared their unexposed control. There was a statistically significant reduction when comparing WT treated at 1,000Gy to Mutant 1.5 treated at 1,000Gy and when comparing WT treated at 10,000Gy to Mutant 1.5 treated at 10,000Gy. Figure 5 shows the input CFU colonies after rehydration and spotting. The strains are WT, 1.5, 5, 8, 11, 16, 6A, and 27 in order from left to right.

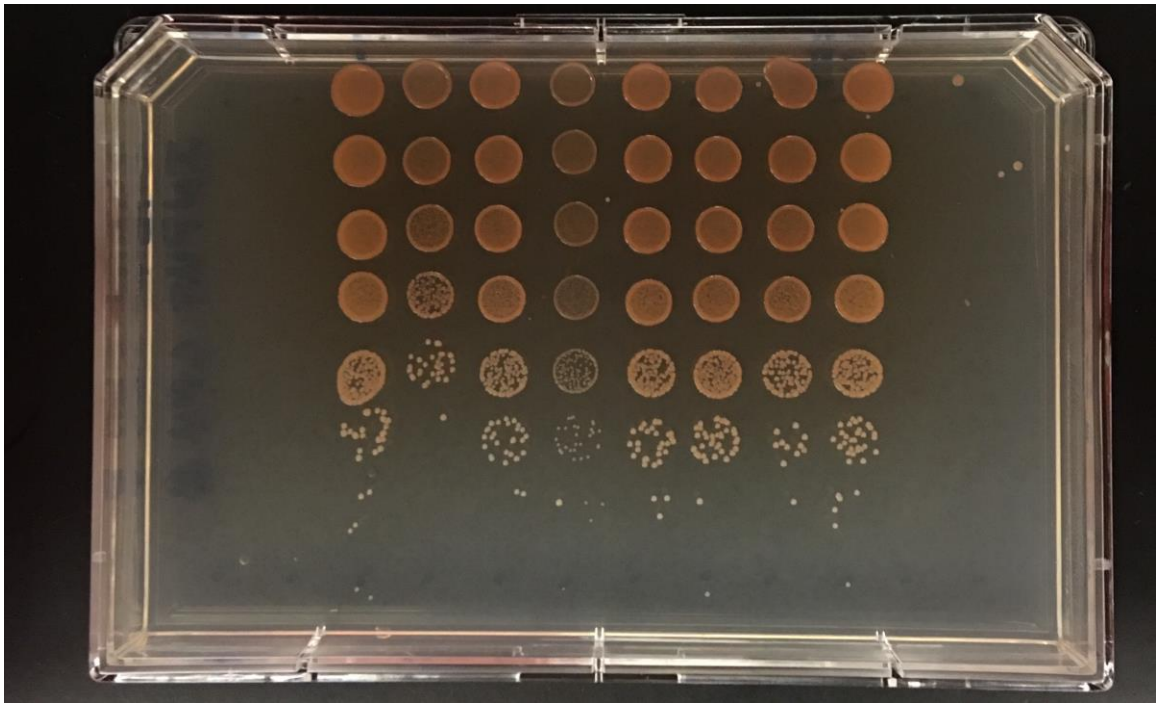


Figure 9. Rehydrated input CFU colonies.

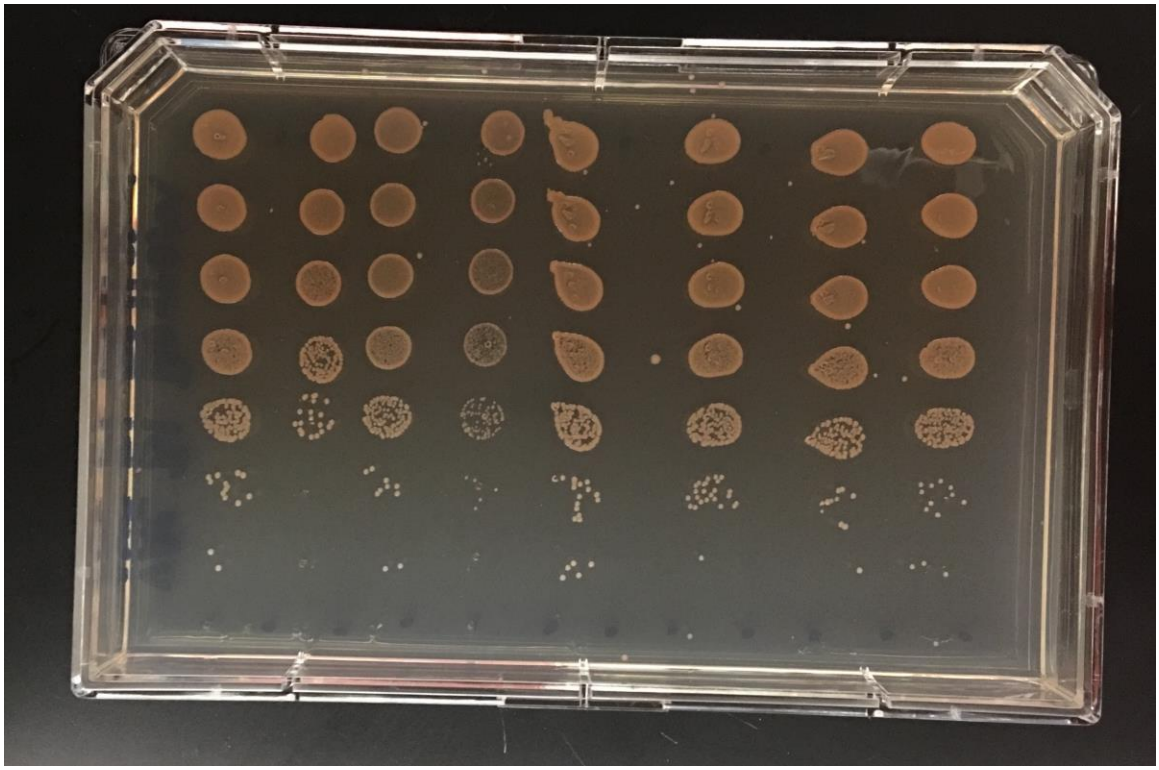


Figure 10. Desiccated and vacuumed CFU.

Figure 10 shows the rehydrated and spotted CFU for the desiccated and vacuumed strains. The strains are WT, 1.5, 5, 8, 11, 16, 6A, and 27 in order from left to right. The WT average colony input was 18.3. The WT control shows a 48% reduction from desiccation and vacuum with a gradual increase in colony count of 35.9% at 500Gy, 23.6% at 1,000,Gy and 10% at 10,000Gy exposure compared to the WT CFU input.

The 1.5 recF knock-out mutant was the weakest during the course of the experiment. The recF enzyme is necessary for repairs of double stranded DNA breaks. Accordingly, the mutant displayed the fewest number of CFU throughout all phases of the experiment. Although a statistically significant 45% CFU decrease was observed during desiccation, a 4% growth was observed during 500Gy irradiation, 14.5% growth and 28% growth was observed during 1,000Gy and 10,000Gy irradiation respectively.

Mutant 5 superoxide dismutase (SOD) knock-out is a manganese (Mn) transporter. Proteins necessary for DNA damage and repair are inhibited when *D. radiodurans* is grown in conditions limiting Mn^{2+}. [14] Statistically significant reduction in CFU input to desiccation and vacuuming was observed. However, no statistical reduction in growth was observed.

Mutant 8 is a double SOD knock-out of copper (Cu) and zinc (Zn) transport genes. These two cofactors, or enzymes, assist the cell in repair after desiccation and irradiation. A statistically significant reduction was observed from CFU input to desiccation and vacuuming.

Mutant 11 knock-out is BshA, or bacillithiol, a gene of uncertain function but believed to defend against peroxides and other reactive oxygen species. [17] This mutant

also displayed a statistically significant reduction from CFU input to desiccation. No statistically significant reduction was observed with the irradiated strains.

The knock-out gene for Mutant 16 was uvrB. uvrB functions during nucleotide excision repair, serving as an enzyme to assist in DNA repair.

Mutant 6A was a merodiploid recF knockout. recF is an essential enzyme necessary for error correction during DNA repair. Mutant 6A was the only strain in which growth (9.2%) was observed from CFU input to desiccation and vacuuming. Like mutant 16, no statistical significant reduction in colonies was observed. Like mutant 16, mutant 27 is a uvrB knockout. Statistically significant CFU reduction was observed from CFU input to desiccation and vacuuming. No other statistically significant reduction was observed.

Colony Comparison of Mutants to Untreated Control

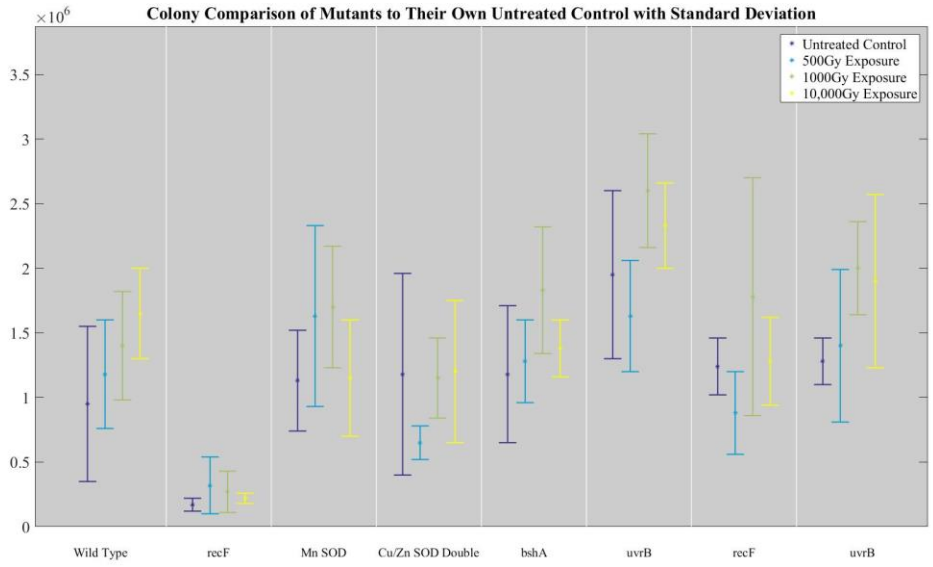


Figure 11. Colony comparison of mutants to their own untreated control.

Figure 11 shows colony counts for each mutant strain over all exposure conditions. No statistically significant difference is observed at any exposure dose when exposed bacteria are compared against their un-exposed control.

While not significant, reductions were observed in exposed bacteria. Reductions of 35.9%, 23.6%, and 10% were observed in the WT strain at 500, 1,000, and 10,000Gy respectively. It is suspected that an upregulation of repair enzymes is occurring after 500 and 1,000 Gy exposure, however this is not known for certain.

Reductions observed in mutant 1.5 were 14.5% and 28.2% at 1,000 and 10,000Gy respectively. 500Gy irradiation exhibited a 4% growth in CFU. The reason for this is not understood.

Mutant 5 exhibited reductions of 37.5%, 34.6%, and 55.7% at 500, 1,000, and 10,000Gy respectively.

Mutant 8 exhibited reductions of 71.3%, 49.2% and 47% at 500, 1,000, and 10,000Gy respectively.

Mutant 11 exhibited reductions of 44.5%, 20.6%, and 40.2% at 500, 1,000, and 10,000Gy respectively.

Mutant 16 exhibited reductions of 38.2%, 1.2%, and 11.7% at 500, 1,000, and 10,000Gy respectively.

Mutant 6A exhibited unique results with a 22.7% reduction at 500Gy but growth of -56.6% and -12.5% at 1,000 and 10,000Gy respectively.

Mutant 27 exhibited reductions of 49.3%, 27.7%, and 31.3% at 500, 1,000, and 10,000Gy respectively.

Unexpectedly, all irradiated samples fell within the rejection region when compared to their respective controls, therefore the hypothesis that there would be a statistically significant difference between desiccated, irradiated strains and desiccated, non-irradiated controls must be rejected. In this case the hypothesis must be rejected. Strains WT, 1.5, 5, 8, 11, and 27 were outside the rejection region with respect to CFU input and desiccation comparisons, and in this case, the hypothesis is not rejected.

V. Discussion/Conclusions

Prior to the experiment at Sandia it was expected that statistically significant decreases in exposed mutants and WT would be observed. It was hypothesized that the recF and uvrB mutants would lack critical repair enzymes which would increase the amount of damage observed from the high LET silicon ions. The Mn, Cu, and Zn SOD knockouts would have a decreased ability to prevent ROS damage, but with high LET silicon ions used to irradiate the Dr, ROS damage was not expected to be the dominant damage mechanism.

From the results it was observed that the only significant reduction was observed when non-desiccated bacteria were compared with desiccated or treated bacteria. Within exposed bacteria no significant reduction in growth was observed when untreated and treated bacteria of the same strain were compared. This is unexpected and an attempt will be made to interpret these results.

One interesting data point is the behavior of wild type bacteria exposed to high levels of radiation. The experimental data showed no significant difference between

unexposed wild type and wild type bacteria exposed to 500, 1,000 and 10,000 Gy. Based upon this experiment using silicon ions (atomic weight of 28.0855 amu) and the experiment conducted by Major Todd Bryant using oxygen ions (atomic weight 15.999 amu) [21], the data may suggest a relative biological effectiveness (RBE) for *D. radiodurans* of 1 for heavy charged particles. The molecular weight of silicon is 1.75 times oxygen yet both experiments returned similar post-exposure data despite using different ions. If true this could be a significant finding relative to Dr's radiation resistance. This would imply the Dr possesses a radioresistance to heavy ions that has not yet been seen in other organisms.

It was unexpected that statistical analysis of the irradiated strains indicated the hypothesis must be rejected. There were several reasons statistically significant results from irradiation were expected. First, it was expected that heavy charged particles (especially in a desiccated sample) would create predominantly more DSBs which are more damaging and difficult to repair than SSBs. Second, mutants were grown with specific genes knocked-out to inhibit DNA repair. Mutants 16 and 27 lacked uvrB, 1.5 and 6A lacked recF, and these knockouts should make DNA repair much more difficult to carry out, resulting in observable reductions in colony growth, which was not observed. Finally for many biological entities high LET particles have a high RBE with some estimates ranging from 10 to 60. [19]

Based upon the inconsistent data it is impossible to make a statement on the behavior of SOD/Bsh mutants. The genes targeted in these mutants help protect the bacteria against excess ROS, and hence are assumed to play a role in low LET resistance due to the high incidence of indirect damage. However, it was hypothesized that these

pathways would have less of a protective effect against high LET radiation, where direct damage is assumed to be the predominant mechanism. Therefore the lack of significant reduction in colony growth in these strains is not necessarily unexpected. However, based upon the lack of significant reduction in any mutant strain it is not possible to make a definitive statement about the behavior of these strains.

The most probable explanation to these unexpected results is the researcher's procedural laboratory inexperience. It is probable that laboratory experimental procedure during rehydration and dilution for spotting explains the absence of expected results. Once the plates returned from Sandia they were rehydrated, diluted and spotted. Plates waited for dilution in the order they were rehydrated. Many of the rehydrated plates sat idle for 30-60 minutes before dilution commenced. It is possible there was insufficient pipet mixing before spotting. This could result in highly concentrated inputs, particularly when extracted from the plate well bottom. Methodically processing post-irradiated plates from rehydration to spotting one at a time and strict attention to mixing would have likely prevented this outcome. The literature clearly indicates what the expected results should be when irradiating *D. radiodurans* strains grown with various knockouts. Those results were not observed in this experiment.

Recommendations for future experiments would be a repeat of this experiment using the same ion and improved reconstitution techniques in order to attain the expected results. If successful, the data could be compared with a similar experiment using a different heavy charged particle to determine if a RBE of 1 for *D. radiodurans* is a possibility.

Additionally, comet assays could be conducted to determine DNA damage. Treated and untreated cells are lysed and protease treated then immobilized in agarose on slides. The chromosomal DNA is electrophoresed from the lysed cells, stained with a DNA specific fluorescent dye and imaged using epifluorescence microscopy. [22] Comet assay is a method to determine actual chromosomal DNA damage and correlate that damage to kill curves. By observing the cells after exposure it may also be possible to calculate a repair curve for exposed *Dr.*

Appendix A: Primer Sets and Sequences

Table 14. recF Primer sequences

Primer Name	Sequence			
Puc_RecFup_Fwd	ttgtaaaacgacggccagtgTGTGTTCGACCGCTTGCC	Puc	RecF_upstream	Fwd
Kan_RecFup_Rev	acgaacggtaTAGACAGGGCCGAGAGAC	Kan	RecF_upstream	Rev
RecFup_Kan_Fwd	gccctgtctaTACCGTTCGTATAGCATAC	RecF_upstream	Kan	Fwd
RecFdown_Kan_Rev	catctcctcaTACCGTTCGTATAATGTATG	RecF_downstream	Kan	Rev
Kan_RecFdown_Fwd	acgaacggtaTGAGGAGATGCAAGCGGAGGG	Kan	RecF_downstream	Fwd
Puc_RecFdown_Rev	atccccgggtaccgagctcgTTCCGGCAGCGCGCGGTA	Puc	RecF_downstream	Rev

Primer Name	Sequence			
Puc_UvrBUp_Fwd	ttgtaaaacgacggccagtgTGCGCAAGGTACCGCAGATGC	Puc	Uvr_Upstream	Fwd
Kan_UvrBUp_Rev	acgaacggtaCCTGCGCGCCACGACCAC	Kan	Uvr_Upstream	Rev
UvrBUp_Kan_Fwd	ggcgcgcaggTACCGTTCGTATAGCATAC	Uvr_Upstream	Kan	Fwd
UvrBdown_Kan_Rev	tgccttctgcTACCGTTCGTATAATGTATG	Uvr_downstream	Kan	Rev
Kan_UvrBdown_Fwd	acgaacggtaGCAGAAGGCACGGCGGAA	Kan	Uvr_downstream	Fwd
Puc_UvrBdown_Rev	atccccgggtaccgagctcgTTCCGGCAGCGCGCGGTA	Puc	RecF_downstream	Rev

Appendix B: Dr Statistical Results

WT 1 Analysis

Data

Strain	1 (WT)			
CFU Input	19	15	21	n/a
Unused Controls in Albuquerque	9	7	18	4
500 Gy Treated Samples	10	10	9	18
1000 Gy Treated Samples	18	9	12	17
10000 Gy Treated Samples	19	14	20	13
Vacuumed only in Albuquerque	13	11	4	10

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	18.3	$\bar{x}_{1\text{-Unused}}$	9.5	$\bar{x}_{1\text{-500 Gy}}$	11.8	$\bar{x}_{1\text{-1000 Gy}}$	14.0	$\bar{x}_{1\text{-10000 Gy}}$	16.5	$\bar{x}_{1\text{-Vacuum}}$	9.5
	$s_{1\text{-CFU input}}$	3.1	$s_{1\text{-Unused}}$	6.0	$s_{1\text{-500 Gy}}$	4.2	$s_{1\text{-1000 Gy}}$	4.2	$s_{1\text{-10000 Gy}}$	3.5	$s_{1\text{-Vacuum}}$	3.9
	Percentage Kill (Compared to CFU Input)			48		35.9		23.6		10		48.18182

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	25.5333	
t, test statistic	2.2888	
rejection region	$t > t_\alpha$	
α	0.05	
df	5	
t_α	2.015	
p-value	0.51897085	

Since 2.2888 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	26.9583	

s_p^2	26.9583
t, test statistic	-0.6128
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.6128 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	27.1667
t, test statistic	-1.2210
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.12210 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	24.3333
t, test statistic	-2.0068
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -2.0068 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

1.5 Analysis

Data

Strain	1.5			
CFU Input	3.1	3	3.2	n/a
Unused Controls in Albuquerque	0.8	1.9	1.1	1.5
500 Gy Treated Samples	0.6	5	5	2.3
1000 Gy Treated Samples	5	1.4	1.9	2.3
10000 Gy Treated Samples	2.4	1.7	2.1	2.7
Vacuumed only in Albuquerque	2	1.9	2.3	2.1

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	3.1	$\bar{x}_{1\text{-Unused}}$	1.7	$\bar{x}_{1\text{-500 Gy}}$	3.2	$\bar{x}_{1\text{-1000 Gy}}$	2.7	$\bar{x}_{1\text{-10000 Gy}}$	2.2	$\bar{x}_{1\text{-Vacuum}}$	2.1
	$s_{1\text{-CFU input}}$	0.1	$s_{1\text{-Unused}}$	0.5	$s_{1\text{-500 Gy}}$	2.2	$s_{1\text{-1000 Gy}}$	1.6	$s_{1\text{-10000 Gy}}$	0.4	$s_{1\text{-Vacuum}}$	0.2
	Percentage Kill (Compared to CFU Input)			45		-4		14.5		28.22581		33.06452

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	0.1415
t, test statistic	4.8729
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 4,8729 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	2.4558

s_p^2	2.4558
t, test statistic	-1.3762
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.3762 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	1.4096
t, test statistic	-1.1316
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1,1316 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	0.2058
t, test statistic	-1.6365
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.6365 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

5 Analysis

Data

Strain	5 Dr R1ΔDr_1279::mlox (MnSOD KO)			
CFU Input	23	30	25	n/a
Unused Controls in Albuquerque	9	14	9	17
500 Gy Treated Samples	18	6	19	22
1000 Gy Treated Samples	22	13	13	20
10000 Gy Treated Samples	15	5	12	14
Vacuumed only in Albuquerque	15	8	9	9

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	26.0	$\bar{x}_{1\text{-Unused}}$	11.3	$\bar{x}_{1\text{-500 Gy}}$	16.3	$\bar{x}_{1\text{-1000 Gy}}$	17.0	$\bar{x}_{1\text{-10000 Gy}}$	11.5	$\bar{x}_{1\text{-Vacuum}}$	10.3
	$s_{1\text{-CFU input}}$	3.6	$s_{1\text{-Unused}}$	3.9	$s_{1\text{-500 Gy}}$	7.0	$s_{1\text{-1000 Gy}}$	4.7	$s_{1\text{-10000 Gy}}$	4.5	$s_{1\text{-Vacuum}}$	3.2
Percentage Kill (Compared to CFU Input)				57		37.5		34.6		55.76923		60.57692

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	14.5500
t, test statistic	5.0629
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 5.0626 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	32.5833

t, test statistic	-1.2388
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.2388 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	18.7917
t, test statistic	-1.8759
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.8759 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	17.9583
t, test statistic	-0.0834
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.0834 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

8 Analysis

Data

Strain	8 Dr R1ΔDr_1546::KANΔDr_A0202:NAT			
CFU Input	25	25	18	n/a
Unused Controls in Albuquerque	13	22	4	18
500 Gy Treated Samples	7	6	8	5
1000 Gy Treated Samples	10	8	15	13
10000 Gy Treated Samples	13	4	15	16
Vacuumed only in Albuquerque	17	6	5	9

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	22.7	$\bar{x}_{1\text{-Unused}}$	11.8	$\bar{x}_{1\text{-500 Gy}}$	6.5	$\bar{x}_{1\text{-1000 Gy}}$	11.5	$\bar{x}_{1\text{-10000 Gy}}$	12.0	$\bar{x}_{1\text{-Vacuum}}$	9.3
	$s_{1\text{-CFU input}}$	4.0	$s_{1\text{-Unused}}$	7.8	$s_{1\text{-500 Gy}}$	1.3	$s_{1\text{-1000 Gy}}$	3.1	$s_{1\text{-10000 Gy}}$	5.5	$s_{1\text{-Vacuum}}$	5.4
	Percentage Kill (Compared to CFU Input)			48		71.3		49.3		47.05882		59.19118

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	42.6833
t, test statistic	2.1878
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 2.1878 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	30.9583

t, test statistic	1.3344	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	
Since 1.3344 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations		
H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	34.9583	
t, test statistic	0.0598	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	
Since 0.0598 < 1.934, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations		
H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	45.1250	
t, test statistic	-0.0526	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	
Since -0.0526 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations		

11 Analysis

Data

Strain	11 Dr R1ΔbshA::mlox			
CFU Input	24	20	25	n/a
Unused Controls in Albuquerque	12	16	6	18
500 Gy Treated Samples	14	8	14	15
1000 Gy Treated Samples	20	22	20	11
10000 Gy Treated Samples	17	13	13	12
Vacuumed only in Albuquerque	5	15	12	10

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	23.0	$\bar{x}_{1\text{-Unused}}$	11.8	$\bar{x}_{1\text{-500 Gy}}$	12.8	$\bar{x}_{1\text{-1000 Gy}}$	18.3	$\bar{x}_{1\text{-10000 Gy}}$	13.8	$\bar{x}_{1\text{-Vacuum}}$	10.5
	$s_{1\text{-CFU input}}$	2.6	$s_{1\text{-Unused}}$	5.3	$s_{1\text{-500 Gy}}$	3.2	$s_{1\text{-1000 Gy}}$	4.9	$s_{1\text{-10000 Gy}}$	2.2	$s_{1\text{-Vacuum}}$	4.2
	Percentage Kill (Compared to CFU Input)			49		44.6		20.7		40.21739		54.34783

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	19.6000
t, test statistic	3.3271
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 3.3271 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	19.1250

t, test statistic	-0.3234

rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.3234 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	26.1250
t, test statistic	-1.7985

rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.7985 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	16.4583
t, test statistic	-0.6972

rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.6972 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

16 Analysis

Data

Strain	16 Dr R1ΔuvrB::KAN			
CFU Input	29	30	20	n/a
Unused Controls in Albuquerque	23	18	20	8
500 Gy Treated Samples	17	22	13	13
1000 Gy Treated Samples	31	23	24	n/a
10000 Gy Treated Samples	21	20	27	25
Vacuumed only in Albuquerque	35	23	15	14

All colony counts at 10^{-5} dilution

Statistics

$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	3	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
$\bar{x}_{1\text{-CFU input}}$	26.3	$\bar{x}_{1\text{-Unused}}$	19.5	$\bar{x}_{1\text{-500 Gy}}$	16.3	$\bar{x}_{1\text{-1000 Gy}}$	26.0	$\bar{x}_{1\text{-10000 Gy}}$	23.3	$\bar{x}_{1\text{-Vacuum}}$	21.8
$s_{1\text{-CFU input}}$	5.5	$s_{1\text{-Unused}}$	6.5	$s_{1\text{-500 Gy}}$	4.3	$s_{1\text{-1000 Gy}}$	4.4	$s_{1\text{-10000 Gy}}$	3.3	$s_{1\text{-Vacuum}}$	9.7
Percentage Kill (Compared to CFU Input)		26		38.3		1.27		11.70886		17.40506	

Population Comparisons

H_0 : $\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$ Null Hypothesis = There is no difference between the CFU input population and the Untreated Population

H_a : $\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	37.4833
t, test statistic	1.4614
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 1.4614 < 2.015, I do not reject the null hypothesis, there is no difference between the CFU Input population and the Untreated Population

H_0 : $\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the Treated population

H_a : $\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$ Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	30.2500

t, test statistic	0.8357
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.8357 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	32.9500
t, test statistic	-1.4826
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since -1.4826 < 2.015, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	26.5833
t, test statistic	-1.0286
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.0286 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

6A Analysis

Data

Strain	6A Dr R1 'meroploid' WT & ΔrecF::KAN			
CFU Input	10	10	14	n/a
Unused Controls in Albuquerque	13	17	12	13
500 Gy Treated Samples	11	6	12	6
1000 Gy Treated Samples	14	16	10	31
10000 Gy Treated Samples	16	8	13	14
Vacuumed only in Albuquerque	13	9	9	13
All colony counts at 10^{-5} dilution				

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	11.3	$\bar{x}_{1\text{-Unused}}$	12.4	$\bar{x}_{1\text{-500 Gy}}$	8.8	$\bar{x}_{1\text{-1000 Gy}}$	17.8	$\bar{x}_{1\text{-10000 Gy}}$	12.8	$\bar{x}_{1\text{-Vacuum}}$	11.0
	$s_{1\text{-CFU input}}$	2.3	$s_{1\text{-Unused}}$	2.2	$s_{1\text{-500 Gy}}$	3.2	$s_{1\text{-1000 Gy}}$	9.2	$s_{1\text{-10000 Gy}}$	3.4	$s_{1\text{-Vacuum}}$	2.3
	Percentage Kill (Compared to CFU Input)			-9.2		22.8		-57		-12.5		2.941176

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	5.0833	
t, test statistic	-0.6049	
rejection region	$t > t_\alpha$	
α	0.05	
df	5	
t_α	2.015	
p-value	0.51897085	

Since -0.6049 < 2.015, I do not reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population
s_p^2	7.5833	

t, test statistic	1.8616
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 1.8616 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	44.5833
t, test statistic	-1.1384
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.1384 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	8.2500
t, test statistic	-0.1846
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.1846 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

27 Analysis

Data

Strain	27 Dr R1ΔuvrB::KAN			
CFU Input	28	25	30	n/a
Unused Controls in Albuquerque	12	16	13	15
500 Gy Treated Samples	6	13	19	18
1000 Gy Treated Samples	15	20	23	22
10000 Gy Treated Samples	29	15	17	15
Vacuumed only in Albuquerque	14	10	13	9

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Unused}}$	4	$n_{1\text{-500 Gy}}$	4	$n_{1\text{-1000 Gy}}$	4	$n_{1\text{-10000 Gy}}$	4	$n_{1\text{-Vacuum}}$	4
	$\bar{x}_{1\text{-CFU input}}$	27.7	$\bar{x}_{1\text{-Unused}}$	12.8	$\bar{x}_{1\text{-500 Gy}}$	14.0	$\bar{x}_{1\text{-1000 Gy}}$	20.0	$\bar{x}_{1\text{-10000 Gy}}$	19.0	$\bar{x}_{1\text{-Vacuum}}$	11.5
	$s_{1\text{-CFU input}}$	2.5	$s_{1\text{-Unused}}$	1.8	$s_{1\text{-500 Gy}}$	5.9	$s_{1\text{-1000 Gy}}$	3.6	$s_{1\text{-10000 Gy}}$	6.7	$s_{1\text{-Vacuum}}$	2.4
	Percentage Kill (Compared to CFU Input)			54		49.4		27.7		31.3253		58.43373

Population Comparisons

H_0 :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} = 0$	Null Hypothesis = There is no difference between the CFU input population and the Untreated Population
H_a :	$\mu_{1\text{-CFU input}} - \mu_{1\text{-Untreated}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	4.5333
t, test statistic	9.1729
rejection region	$t > t_\alpha$
α	0.05
df	5
t_α	2.015
p-value	0.51897085

Since 9.1729 > 2.015, I do reject the null hypothesis, there is a difference between the CFU Input population and the Untreated Population

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	19.3333

t, test statistic	-0.4020
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -0.4020 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 500Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	8.0000
t, test statistic	-3.6250
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -3.6250 < 1.943, I donot reject the null hypothesis, there is no difference between the Untreated and the 1000Gy Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the CFU input population and the Untreated Population

s_p^2	24.3333
t, test statistic	-1.7918
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -1.7918 > 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10000Gy Treated Populations

Appendix C: Sandia's Tandem Accelerator

The Sandia Tandem Accelerator is a High Voltage Engineering 6 MV EN Tandem accelerator capable of accelerating a range of ions from hydrogen to gold over a range of energies from 800 keV to 10's of MeV on target. The ions are generated using negative ion sources, these ions are accelerated towards the positive terminal of the accelerator where the ions are run through a nitrogen gas channel which strips the electrons from the ions and produces a range of positively charged ions. These positive ions are then accelerated away from the positive terminal. The desired ion species and charge state is then selected using a mass analyzing magnet and directed to the end-station. The landing energy of the ions is then:

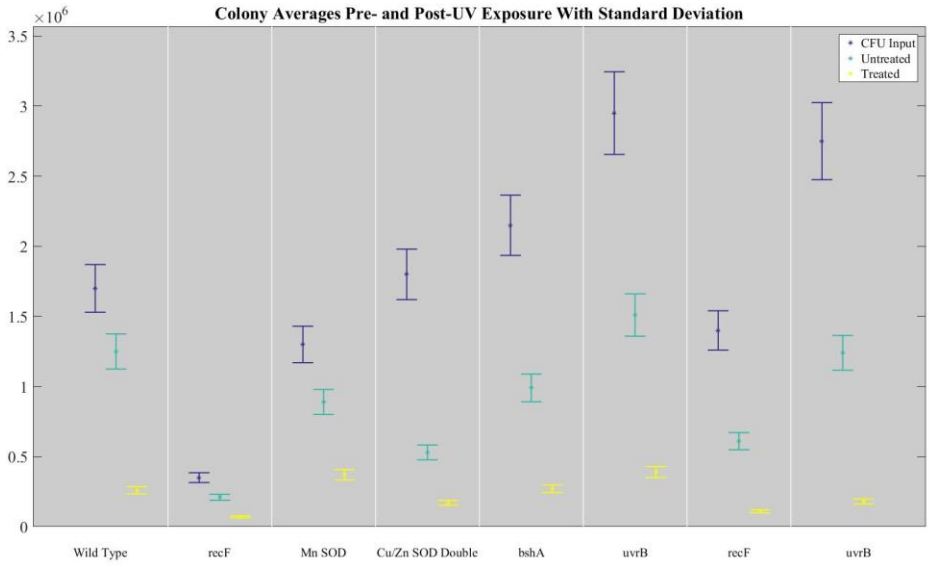
$$\text{Landing Energy} = (\text{charge state} + 1) * \text{terminal voltage} \quad (1)$$

In the selected end-station the ion fluence is determined using the beam area, beam current and the beam pulse length on target. As our beam spots sizes are typically between 0.001 to 4 mm we either measure the beam or irradiate the target. We have developed a reproducible methodology that allows us to determine the fluence and then irradiate the target using a range of pulse lengths from <20 ns to DC. The error in the ion fluence is typically between 2-10% of the total fluence.

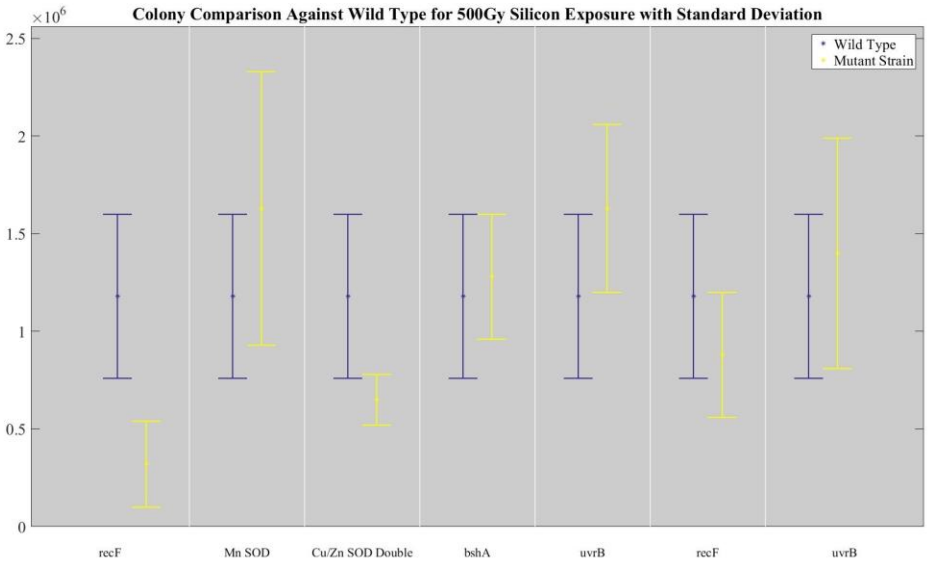
To accommodate large areas samples we have developed an implantation technique where we characterize the beam and then perform a series of irradiations where we move

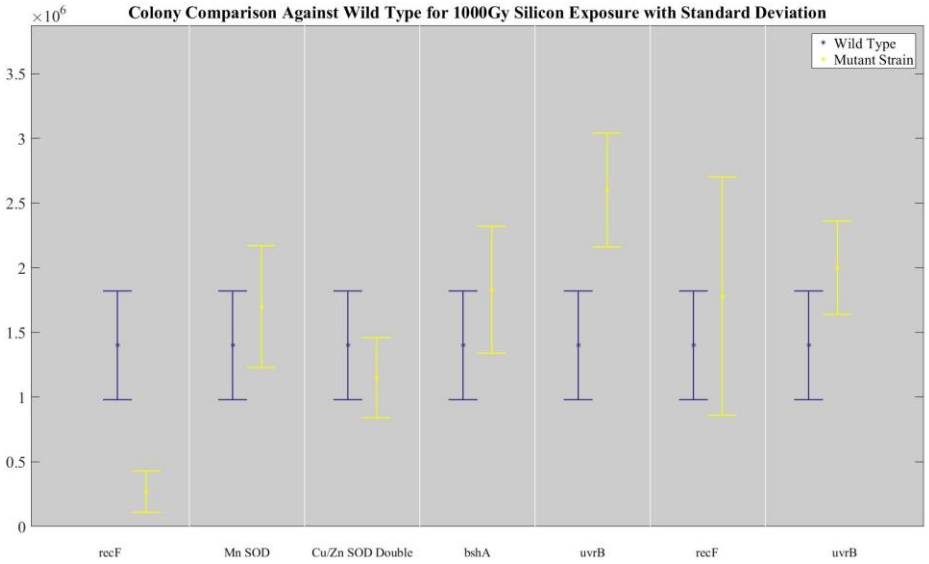
the sample under the beam using an x,y stage. This allows us to implant over a large (up to 1 inch) area using our small beams. [20]

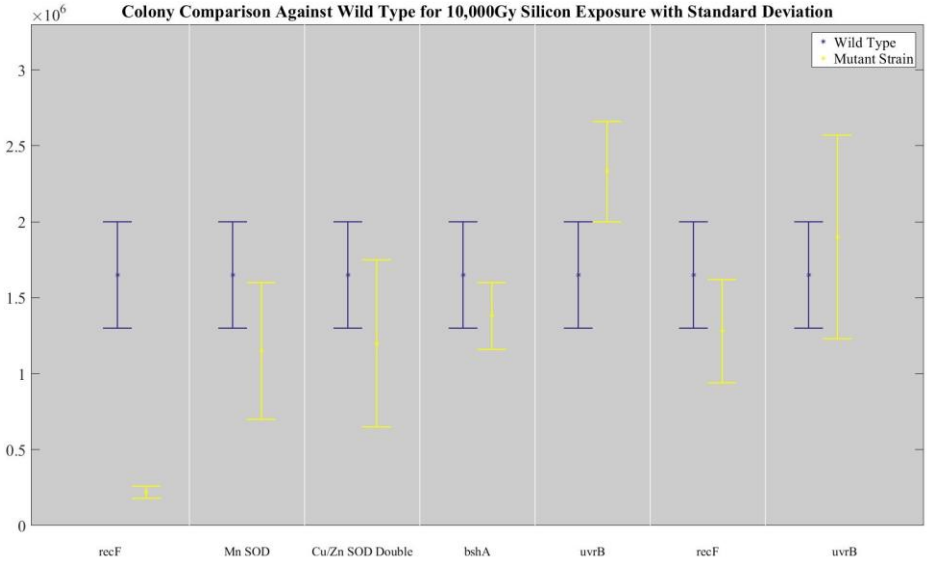
Appendix D: UVC Rollup of WT and Mutant Strains



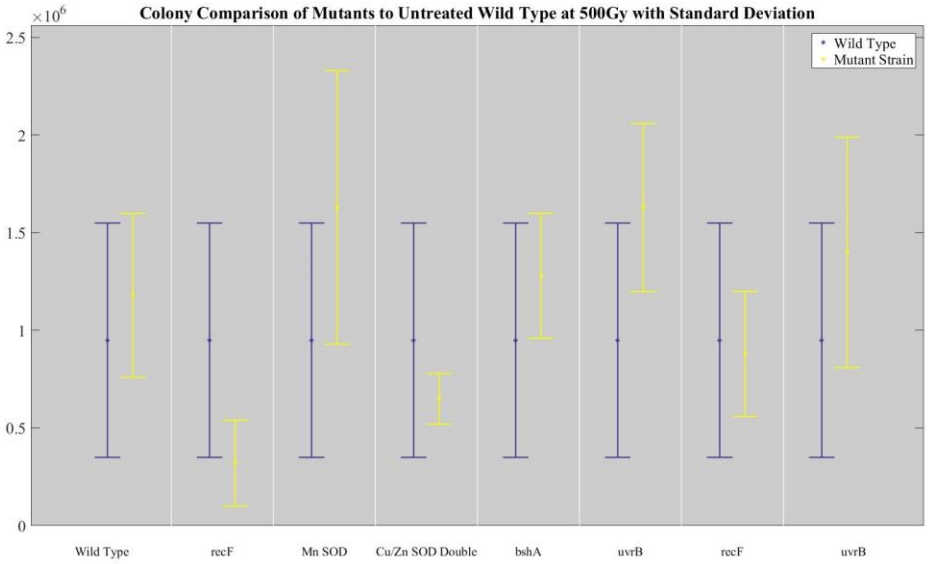
Appendix E: Colony Comparison Against Treated WT

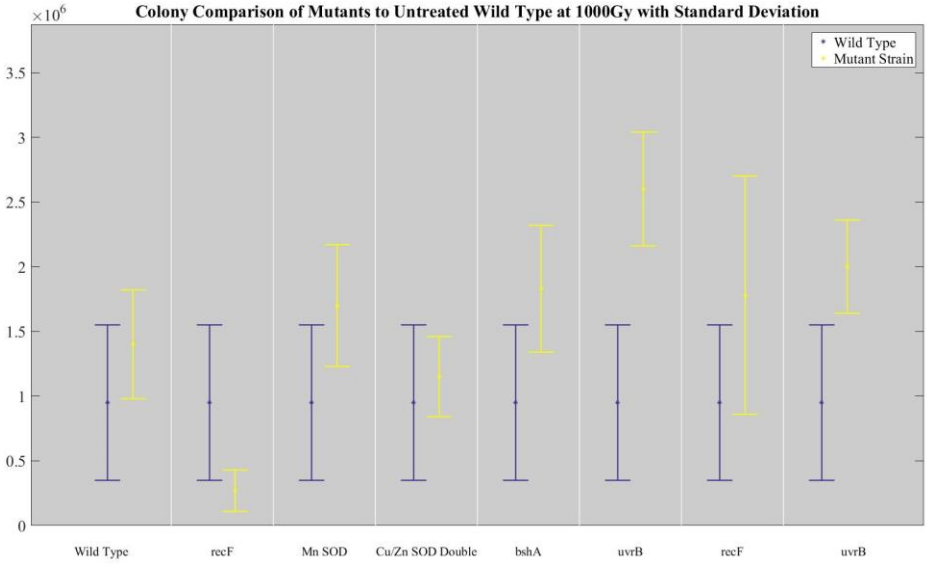


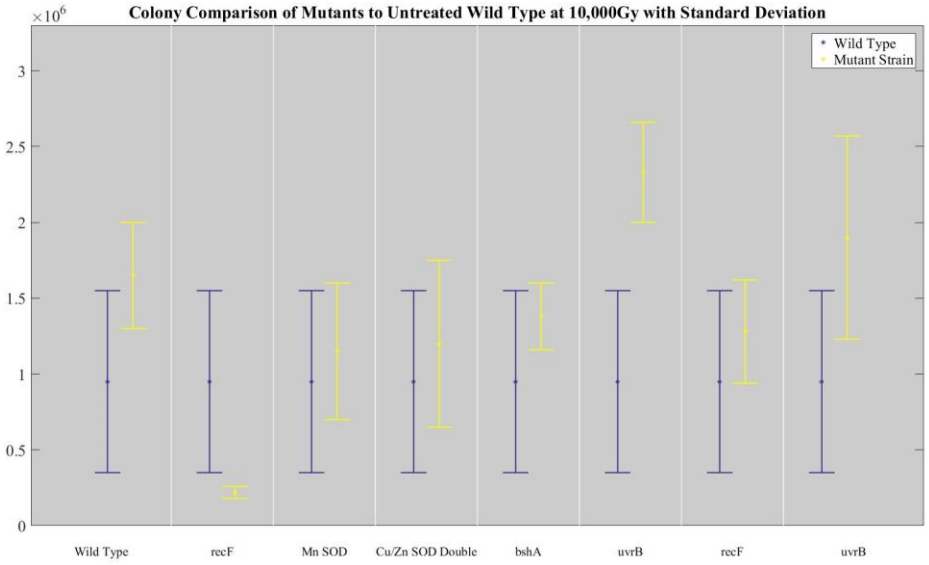




Appendix F: Colony Comparisons of Treated Mutant Against Untreated WT




Colony Comparison of Mutants to Untreated Wild Type at 1000Gy with Standard Deviation
×10^6
Wild Type
Mutant Strain
3.5
3
2.5
2
1.5
1
0.5
0
Wild Type
recF
Mn SOD
Cu/Zn SOD Double
bshA
uvrB
recF
uvrB


Colony Comparison of Mutants to Untreated Wild Type at 10,000Gy with Standard Deviation
×10^6
Wild Type
Mutant Strain
3
2.5
2
1.5
1
0.5
0
Wild Type
recF
Mn SOD
Cu/Zn SOD Double
bshA
uvrB
recF
uvrB

Bibliography

[1] S. E. DeWeerdt, "Genome News Network", http://www.genomenewsnetwork.org/articles/07_02/deinococcus.shtml, accessed Jul. 2017

[2] D. Slade and M. Radman, "Oxidative Stress Resistance in Deinococcus Radiodurans," Microbiology and Molecular Biology Reviews, p. 133, Mar. 2011

[3] D. Duggan, A. Anderson, and P. Elliker, "Inactivation of the Radiation-Resistant Spoilage Bacterium Micrococcus radiodurans," Appl Microbiol. 11(5): 398–403. Sep. 1963

[4] D. Slade, A. Lindner, G. Paul, and M Radman, "Recombination and Replication in DNA Repair of Heavily Irradiated Deinococcus radiodurans," Cell, DOI: 10.1016/j.cell.2009.01.018, April 2009

[5] M. Blasius, U. Hübscher, and S. Sommer, "Deinococcus radiodurans: What Belongs to the Survival Kit?," Critical reviews in Biochemistry and Molecular Biology, 43:3, 221-238

[6] M Daly, E. Gaidamakova, V Matrosova, A. Vasilenko, M Zhai, et al., "Protein oxidation implicated as the primary determinant of bacterial radioresistance," PLoS Biol 5(4): e92. doi:10.1371/journal.pbio.0050092, 2007

[7] B. Berlett and R. Levine, "Designing antioxidant peptides," Redox Rep. 2014 March; 19(2): 80–86. doi:10.1179/1351000213Y.0000000078

[8] O. White et. al, "Genome Sequence of the Radioresistant Bacterium Deinococcus radiodurans R1," Science. 1999 Nov 19; 286(5444): 1571–1577.

[9] R. Lenker, "Characterization of Neutron and Proton Exposure on the Radiation Resistant Bacterium, Deinococcus Radiodurans," AFIT Thesis, March 2017

[10] K. Krane, "Introductory Nuclear Physics," John Wiley and Sons, Rev. Edition, 1988

[11] E. Alpen, "Radiation Biophysics," Academic Press, 2nd Edition, 1998

[12] "Genetics Home Reference", U.S. National Library of Medicine, https://ghr.nlm.nih.gov/primer/basics/dna, accessed Dec. 2017

[13] D. Lewis, "Lesson 6 Lecture: Radiation Health Physics," NENG 630, Spring 2017.

[14] M. Daly, "Death by protein damage in irradiated cells," DNA Repair 11 (2012) 12–21, Oct. 2011

[15] J.T. Reardon, and A. Sancar, "Excision Repair," Encyclopedia of Genetics, pp. 673–675, 2001

[16] O. Desouky, N Ding, G Zhou, "Targeted and non-targeted effects of ionizing radiation," Journal of Radiation Research and Applied Sciences, 19 March 2015

[17] D. Klawuhn, "The Effects of Ionizing Radiation and Oxidizing Species on Strains of Deinococcus radiodurans Lacking Endogenous Oxidative Protection Methods," AFIT Thesis, March 2016

[18] "NEBuilder® HiFi DNA Assembly Master Mix/NEBuilder HiFi DNA Assembly Cloning Kit," Instruction Manual, Introduction, p. 2

[19] F. A. Cucinotta, M. Y. Kim and L. J. Chappell, "Space Radiation Cancer Risk Projections and Uncertainties – 2010," NASA, p. 60, 2010

[20] E. S. Bielejec, personal communication, Dec. 1, 2017

[21] T. Bryant, “Characterization of the Effects of Heavy Charged Particle Exposure on the Radiation Resistant Bacterium Deinococcus Radiodurans,” AFIT Thesis, Feb. 2018
[22] C. McPherson, *Deinococcus radiodurans* Comet Assay, protocol instructions, 2017

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14. ABSTRACT

Deinococcus radiodurans is a robust bacterium that is known for its extraordinary resistance to ionizing radiation. In general, many of the investigations of this bacterium's resistance have revolved around low linear energy transfer radiation, such as gamma and electron radiation. This study explored *Deinococcus radiodurans*'s ability to survive high linear energy transfer radiation, specifically proton and neutron radiation. *Deinococcus radiodurans* was dehydrated to reduce the effects of low linear energy transfer radiation. The bacteria were exposed to both neutron and proton radiation of varying amounts and rehydrated. The resulting colonies were counted and compared to colonies of non-irradiated control samples using a two population, t-statistic test. With few, non-trend forming exceptions, the results of these comparisons showed, with 95% certainty, that there was no statistical difference between the non-irradiated controls and the irradiated samples.

15. SUBJECT TERMS

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