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CHARACTERIZATION OF NEUTRON AND PROTON EXPOSURE ON THE RADIATION RESISTANT BACTERIUM, *DEINOCOCCUS RADIODURANS*

THESIS

Ronald C. Lenker, Major, USA

AFIT-ENP-MS-17-M-100

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

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CHARACTERIZATION OF NEUTRON AND PROTON EXPOSURE ON THE RADIATION RESISTANT BACTERIUM, *DEINOCOCCUS RADIODURANS*

THESIS

Presented to the Faculty

Department of Engineering Physics

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Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Nuclear Engineering

Ronald C. Lenker, MS

Major, USA

March 2017

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CHARACTERIZATION OF NEUTRON AND PROTON EXPOSURE ON THE RADIATION RESISTANT BACTERIUM, *DEINOCOCCUS RADIODURANS*

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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.

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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.

Ronald C. Lenker

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CHARACTERIZATION OF NEUTRON AND PROTON EXPOSURE ON THE RADIATION RESISTANT BACTERIUM, *DEINOCOCCUS RADIODURANS*

I. Introduction

General Issue

Successfully surviving and navigating an irradiated battlefield, searching for survivors at the location of a nuclear reactor meltdown, or continuing to explore our solar system all involve exposure to ionizing radiation. As such, there continues to be a need within the United States Department of Defense and other governmental organizations to develop medical capabilities to either prevent or neutralize the biological damage caused by ionizing radiation. The Defense Threat Reduction Agency has a multiyear BAA for Basic Research for Combating Weapons of Mass Destruction (HDTRA-11-12-BRCWMD-BAA) to include "advancing knowledge to protect life."[1] The National Institute of Health also has research goals aligned to this endeavor, with "Determining mechanisms for radiation protection, mitigation and treatment."[1]

By investigating the mechanisms behind *Deinococcus radiodurans*'s (Dr) remarkable ability to resist ionizing radiation, we may further the understanding of how to protect human cells from the dangers of ionizing radiation. Specifically, investigations will be made into Dr's survivability in a neutron and proton environment, experiencing high linear energy transfer (LET) radiation.

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Problem Statement

The purpose of this research is to develop an understanding of Dr's ability to deal with varying levels of heavy charged particle (HCP) and neutron radiation measured in Grays (Gy). In SI units, the Gy is a Joule per kilogram (J/kg). Specifically, the type of HCP radiation to be researched is proton radiation. The overarching goal of this research is to test Dr's survivability in both neutron and proton environments. Populations exposed to varying levels of both neutron and proton radiation will be compared with non-irradiated control groups.

Hypothesis

The objective of these series of experiments is to test Dr's resistance to both neutron and proton radiation, at varying doses. The hypothesis: Dr demonstrates resistance to gamma induced ionizing radiation (low LET), but will not show similar resistance to neutron nor proton radiation (high LET). The null hypothesis: The populations of the experimental group (neutron or proton irradiated) and control group (no radiation) will not be statistically different.

Research Objectives

The research objectives are as follows:

1. Compare untreated samples of wild type Dr to samples with varying irradiation treatments of neutrons and protons.

2. Compare untreated samples of Dr mutants to samples with varying irradiation treatments of neutrons and protons.

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Assumptions/Limitations

There is no specifically known Relative Biological Effectiveness (RBE) for Dr, however the International Commission on Radiological Protection (ICRP) created a standard RBE based on the type of radiation and in some cases, such as neutrons, the particles' energy. Another way to measure radiation in addition to the Gy is the Sievert (Sv), which is also J/kg. However, Sieverts include a RBE. This RBE contains different weights depending on the type of radiation. For photons and electrons, a weighting factor of 1 is used. This means for low LET radiation, there is no difference between Gy and Sv.

However, there is a weight factor for both HCP and neutrons. In the case of HCP, such as the protons used in this experiment, the weighting factor is 20. This means that unlike radiations involving electrons and photons, where Gy and Sv are the same, the equivalent dose of proton radiation in Sv will be twenty times that of the absorbed dose in Gy. The weighting factor is slightly different for neutrons because it is based on their energy. For this experiment, a weighting factor of 10 corresponds to the neutrons of energy 2.45 MeV.[2]

For the experiments conducted on Dr, the intent is to look at how Dr reacts to high linear energy transfer (LET) as a result of the bombardment of protons and neutrons. In order to minimize the effects of low LET and radicals created in water, the samples are desiccated. In previous experiments it has been shown Dr is fairly impervious to desiccation and can be revived with few losses even after several weeks. All samples are expected to be desiccated for around two weeks or less. Further, they will be shipped in

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sterile containers to prevent contamination. However, they will be subjected to slight jarring and temperature fluctuations associated with shipping.

During the proton experiment, samples will need to be exposed to the environment of the ion beam laboratory while shifting their holder plate onto the stage of the ion beam. There is some risk of contamination during these periods, but will be mediated by as short as possible exposures and the samples will be covered following the end of proton irradiation.

Finally, there are only a limited number of samples that will be able to be radiated due time constraints of neutron generator / particle beam use. This will affect the depth of statistical data that can be gleaned from the experiments.

II. Literature Review

Chapter Overview

The purpose of this chapter is to enlighten the reader on the basic biology of Dr and its ability to repair itself following radiation treatment. The discussion will also delve into radiation itself by describing the differences of high and low LET. Finally, it will explain some of the Dr mutants used in the experiments.

A Brief Description of *Deinococcus radiodurans*

Deinococcus radiodurans is a robust bacterium that is known for its extraordinary resistance to ionizing radiation in the form of gamma radiation. In fact, this biological adaptation led to its discovery as a contaminant in radiation-sterilized corned beef cans in the mid-20th Century. This organism has the capacity to withstand massive DNA

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damage inflicted by ionizing radiation. For example, Bruch, et al. tested a Mn(II) speciation of Dr with doses up to 10 kGy of gamma rays with only a two log kill lethality.[3] "Well-aerated, exponential-phase cultures...will survive 5000 Gy of gamma radiation without loss of viability, and survivors are routinely recovered from cultures exposed to as much as 20 kGy".[4] The mechanisms for this biological adaptation are still being investigated, though they are suspected to be related to its DNA, its protective proteins, or as a by-product of its ability to overcome severe desiccation.[5]

Some of the features of this particular bacteria include two large chromosomes, and two smaller plasmids.[5] This genetic material is toroid in form. Dr is gram-positive, pigmented, and non-motile. Additionally, it is a non-spore forming, spherical bacterium whose size ranges of 1.5 to 3.5 microns in diameter, and exists in tetrads. It is capable of growing with a doubling time of about 80 minutes in a rich nutrient environment. [6]

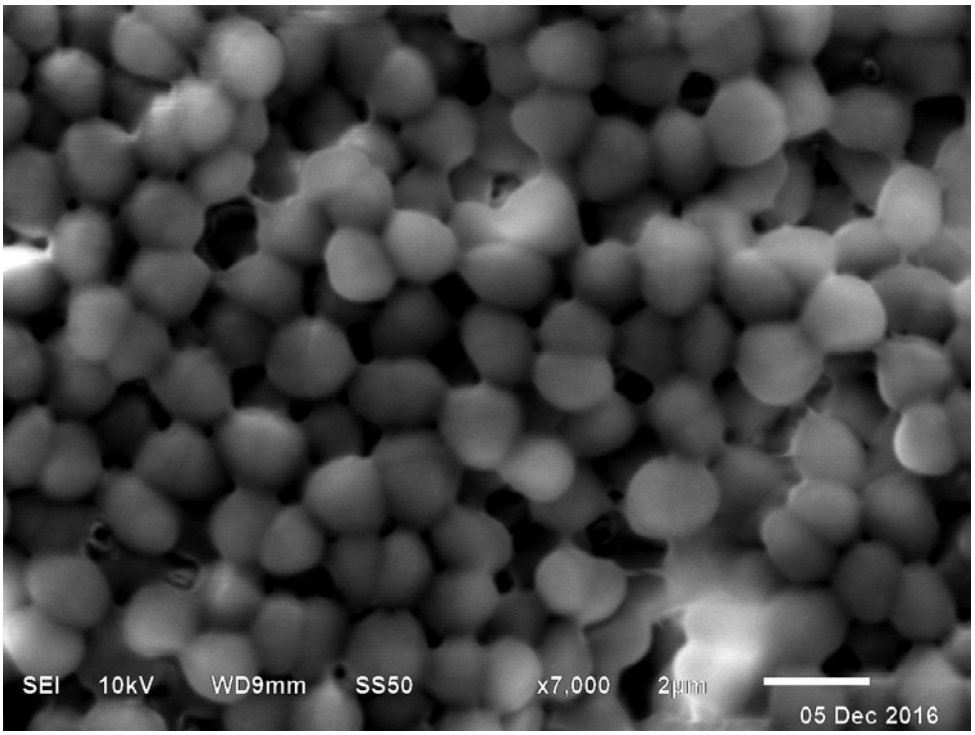

Figure 1. *Deinococcus radiodurans* taken by SEM at USAFSAM.

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High LET and Low LET

Linear energy transfer can be described as the "average energy locally imparted to the medium by a charged particle of specific energy traversing a distance."[7] In low LET, "the average spacing between energy transfer events along the track of the charged particle will be on the order of hundreds of nanometers." This means for low LET, you may only see an order of magnitude of 10 energy transfer events per μm. Examples of low LET radiation are gamma and electrons.

However, for high LET, "the formation of regions of ionization will be close together and will, in the limit, form a continuous chain, or column, of ionization damage."[7] Therefore, for high LET, one might see an order of magnitude of as high as 1000s of energy transfer events per μm. Examples of high LET radiation include alpha particles, protons, and neutrons.

Neutrons are not charged particles. However, neutrons will cause elastic, inelastic, non-elastic, neutron capture, and spallation events involving charged particles.[7] A charged particle has the intrinsic property of an electric charge and can be either positive or negative. An atom for example is made of protons which have a positive charge, electrons which have a negative charge, and neutrons which do not carry a charge. Atoms themselves are neutral as well, but may become ionized. This process happens when an electron is stripped off the atom and the resulting ion will have an overall positive charge.

Since we will be dealing with mono-energetic neutrons of 2.45 MeV, the events we will be concerned with include elastic, inelastic, and non-elastic scatter. A neutron elastic scatter is "the kinetic interaction of an energetic neutron with a nucleus of the

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absorbing medium in which classical kinematics describes the energy transfer. The elastic scattering process is important for neutrons with energies up to 14 MeV or so."[7] For neutrons that undergo inelastic scatter, the process is slightly different. In this case, an initial neutron will be absorbed within a target nucleus, creating a short-lived compound nucleus which then re-emits a neutron. This reaction will only occur if the initial neutron's energy "is greater than the threshold energy necessary for conservation of energy and momentum."[7] Finally, a non-elastic scatter is similar to an inelastic scatter, but after the neutron is captured, the re-emitted particle is not another neutron.[7] At this time, there has been very little experimentation involving high LET radiation and Dr.

Direct and Indirect Action

Both high LET and low LET can result in either direct or indirect action. In the case of indirect damage, the ionization and excitation of water by beta (electrons), gamma (photons), and HCP radiation result in the creation of radical species. For example, energetic photons may cause water to enter an excited state, then dissociate in H· and OH· radicals. Likewise, ionization of water results in H_20^+ and e^-. These products will go on to interact with other water molecules and hydrogen to form other radicals such as H_20^-, H·, and e_{aq}^-.[7] These radicals then attack cellular components including DNA.

In regards to direct damage, Alpen states, "Of greater importance with high LET radiations is the high likelihood that an ionizing event will occur directly in the important

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target bioactive molecule."[7] In this study, the bioactive molecule of consideration is deoxyribonucleic acid (DNA).

DNA

DNA is the genetic code found in all living organisms. The complex molecule's shape is that of a double-helix whose spiral is made up of two strands of monomer nucleotides. These nucleotides consist of a deoxyribose sugar molecule that is covalently bonded to a phosphate molecule, forming a sort of phosphate-sugar backbone. Like the rungs on a twisted ladder, this backbone also has base pair steps. Each base pair is a combination of a purine and a pyrimidine bound through hydrogen bonding. The purine Adenine bonds with the pyrimidine Thymine. Likewise, the purine Guanine bonds with the pyrimidine Cytosine. The order of the bases pairs forms the genetic code which tells a cell how to form the proteins necessary for cellular functions.[8]

The bases and sugar molecules of the DNA present targets, which both can undergo chemical reactions from the radicals mentioned in the previous section. The more damaging attack however, is when these radicals break the covalent bond between the sugar and phosphate molecules on the backbone. If this type of damage occurs to the DNA, the result may be either a single strand break (SSB) or a double strand break (DSB). In the case of a SSB, one of the two strands of DNA are severed. For DSBs, both DNA strands are severed in proximity of each other, usually within 10 base pairs or less. If a cell is unable to repair either a SSB or a DSB, the genetic code may be unusable by the cell. Without this information, mutations may occur or the cell may be unable to produce proteins needed for survival, resulting in cell death. Specifically, "for simpler

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organisms, such as bacteriophages and viruses...measurement of DSBs in organisms with double-stranded DNA precisely correlate with biological inactivation."[7]

DNA Damage from Direct and Indirect Actions

DNA damage may result from either direct or indirect damage. In general, a cell's DNA exposed to high LET often receives numerous DSBs, which completely sever the DNA. This is due to the more numerous events per distance as mentioned earlier. DSBs are "far more serious in the consequences for a cell…and repair of DSBs is an error-prone process that will frequently lead to mutation in the genome and/or loss of reproductive capacity."[7]

Indirect damage to DNA is the result of radicals created during indirect events. Low LET is usually the cause of the "indirect action of the products of radiolysis" which can result in SSBs.[7] SSBs are more readily repaired, though multiple SSBs in proximity can result in DSBs. Alpen further states, "it has been suggested that the high LET radiation…produces its damaging effect by production of double-strand breaks as single events, whereas low LET radiation is thought to produce a preponderance of damage through interaction of two sublethal events."[7]

Numerous studies involving low LET radiation (such as gamma and electrons) have led to further questions about Dr's radio-resistance. Is Dr able to survive due to having several copies of DNA available, the production of unique proteins which provide more protection to the DNA from radicals, a higher amount of scavengers which remove the radicals before they can attack its DNA, a higher functionality of repair enzymes

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capable of high fidelity DSB repair, presence of Manganese which seems to provide resistance, or some combination of the above?

Deinococcus radiodurans DNA Damage and Repair

Both high LET and low LET radiation affect a cell's DNA, causing either SSBs or DSBs. In order to repair SSBs, Dr uses a method of repair called excision repair. In this method, "the nucleotide excision repair removes pyrimidine dimers and oxidatively damaged DNA."[9] This is accomplished when the UvrA-UvrB protein complex, found in bacteria, locates and verifies the damage. The damaged area is removed and is filled by polymerase I. The repair is completed when DNA ligase I "seals the nick."[10] Polymerase I and ligase are enzymes involved in DNA repair.

Dr exhibits a two phase reconstruction of its DNA following DSBs. The first phase involves "a process dubbed extended synthesis-dependent single-strand DNA annealing (ESDSA)."[11]. In this process, shown in Figure 2, "chromosomal fragments with overlapping homologies are used both as primers and as templates for massive synthesis of complementary strands" and "depends on DNA polymerase I and incorporates more nucleotides than does normal replication in intact cells." [12] These newly created strands, which are complementary, become high-precision extensions which are able "join together contiguous DNA fragments into long, linear, double stranded intermediates."

This then leads into the second phase, which "involves RecA protein-mediated double strand break repair."[11] At this point, "these intermediates require RecA-dependent crossovers to mature into circular chromosomes that comprise double-stranded

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patchworks of numerous DNA blocks synthesized before radiation, connected by DNA blocks synthesized after radiation."[12]

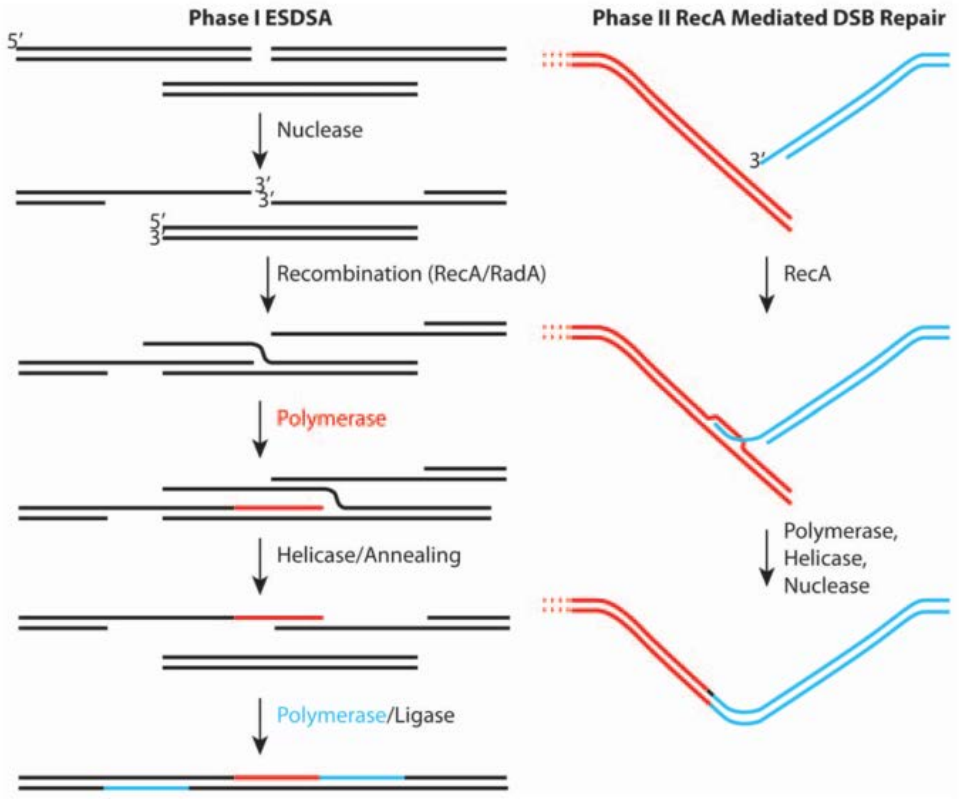

Figure 2. Two stages of genome reconstitution in *Deinococcus radiodurans*.[11]

Deinococcus radiodurans and Mutant Strains

The *Deinococcus radiodurans* 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). In addition to this wild-type (WT) strain, the laboratory staff, at USASAM, created 11 mutant strains. Three of these strains were selected for testing during both neutron and proton exposure and are listed in the Table 1.

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Table 1. *Deinococcus radiodurans* R1 Stain List

#	Gene KO	Common Name	Proper Genotype
1	none	WT	
5	DR_1279	Mn SOD	Δ*DR_1279*::*mlox*
8	DR_1546 DR_A0202	Cu/Zn SOD Cu/Zn SOD	Δ*DR_1546*::*KAN* Δ*DR_A0202*::*NAT*
11	BshA	Bacillithiol Biosynthesis	Δ*bshA*::*mlox*

Each of the mutants in the study has one or two genes removed that are suspected to have a role in the radio-resistance of Dr. This resistance involves the radicals created from the interaction of ionizing, low LET radiation and water as previously mentioned. In the case of Mutants #5 and #8, a superoxide dismutase (SOD) was removed or "knocked out" (KO). A SOD is an antioxidant enzyme which can break down a superoxide radical to a chemical less damaging to a cell. For Mutants #5 and #8, the metal cofactors are manganese (Mn) and copper (Cu) / zinc (Zn).

For Mutant #11, the gene KO is not a SOD. Instead it is bacillithiol A (BshA), which is "responsible for the first committed step in bacillithiol biosynthesis."[13] This compound is found in many Gram-positive bacteria, such as Dr. "It is involved in maintaining cellular redox balance as well as the destruction of reactive oxygen species."[13]

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Additionally, a laboratory strain of Escherichia coli (EC), common name DH5A, acquired from Protein Express, Inc. was used during the 3rd neutron irradiation experiment.

III. Methodology

Chapter Overview

The purpose of this chapter is to describe the methods used to conduct experimental procedures on Dr to test the hypothesis listed in the first chapter. This section begins with how Dr was prepared prior to irradiation. Next, a brief description of both neutron and proton generation is given. The next subsection looks at irradiation and rehydration of samples. Finally, an explanation on the methods of statistical analysis is given.

Deinococcus Radiodurans Sample Preparation

Initial Sample Growth

The bacteria preparation consisted of several steps, ultimately yielding a Dr sample that was 2-5 x 10^8 CFU/ml. These steps were conducted at USAFSAM. Initially, WT and the selected mutants were grown in a tryptone-glucose-yeast extract (TGY, with antibiotic selection of Nourseothricin (NAT) and Kanamycin (KAN) for mutant #8 only) culture medium (0.5 % tryptone, 0.3% yeast extract, 0.1% glucose). Colonies were streaked for isolation and incubated for 48 hours at 32 °C in unsealed plastic bags in order to prevent drying. After the 48 hours, a single colony per strain was inoculated into 5 ml of TGY culture medium using 14 ml round bottom tubes, again with antibiotics for

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mutant #8. The inoculated colonies were incubated overnight at 32 °C and 220 RPM for aeration. The following day, the cultures were diluted 1:100 (200 µl of overnight cell culture) into 20 ml of fresh TGY culture medium within a 150 ml flask with appropriate selection of antibiotics for mutant #8. The flasks were incubated overnight at 32 °C and 220 RPM.

After approximately 24 hours, the cultures were diluted to an optical density (OD_{600}) of 0.25 in 40 ml of TGY culture medium into 250 ml flasks. This was achieved using the Thermo Scientific NanoDrop 2000c Spectrophotometer and accompanying software. A 1:10 dilution sample of each Dr strain (100 µl of culture, 900 µl TGY) was added to a cuvette. The NanoDrop 2000c then took readings based on a 10mm pathlength of light. Below is a sample calculation showing how much culture needed to be added to achieve the OD_{600} of 0.25. The initial OD_{600} was multiplied by 10 to account for a 1:10 dilution. Tables of these measurements for each experiment appear in Appendix A.

$$40\ ml * \frac{.25}{4.97} = 2.0\ ml$$

The flasks were then incubated four hours at 32 °C and 220 RPM to achieve early log phase.

After the incubation period was completed, the cultures were concentrated 10x by centrifugation, with 30 ml of the cultures transferred into 50 ml conical tubes, set to 3500 RPM for 20 minutes in a table top centrifuge. During the spin, OD_{600} readings were taken to determine the CFU/ml post four hour incubation. A calculation was done to

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determine the amount of media to achieve an OD_{600} of 5. Tables of these calculations are found in Appendix A.

$$30\ ml * \frac{.624}{5} = 3.7\ ml$$

Next, the supernatant was poured off completely and the remaining pellets were re-suspended into fresh TGY culture media to achieve an OD_{600} of 5, which corresponds to 2-5 x 10^8 CFU/ml.

Sample Plate Preparation

In a biosafety cabinet, the samples were transferred to the wells of a 96 well plate column in order to easily deposit the samples onto the 96 well, flat bottom plate lids. The procedure was utilized for the first and second neutron experiments.

Using a multi-channel pipet, 60 µl of cells were transferred to the lid "wells" of three 96 well, flat bottom plate lids as shown in Figure 3. One plate lid was used as an untreated control, while the other two plate lids were irradiated. The lid wells were used instead of the actual wells because of the follow on experiments. Specifically, at Sandia National Lab using the QASPR-3 (Qualification Alternative to the Sandia Pulse Reactor 3) tandem ion beam, only a 96 well plate lid, not the plate, was initially thought to fit the sample stage in the QASPR-3's irradiation chamber, so all experimentation was completed using the lid wells.

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

Figure 3. This array depicts the location of each strain of Dr. Each strain (represented by number, i.e. 1 is WT, 5 is Mutant #5, etc.) was separated from the others by a row. This setup allowed for twelve samples per strain.

After reviewing several sample sizes, 60 µl drops were chosen as they provided the most level, even surface compared to other drop sizes. The plate lids were left within the BSL cabinet's laminar flow hood in order to dry overnight. After 24 hours of drying, the plate lids were placed on their respective plates and sealed with parafilm. They then sat desiccated for a day awaiting treatment. This was done in order to simulate shipping to Sandia National Laboratory for the proton experiment.

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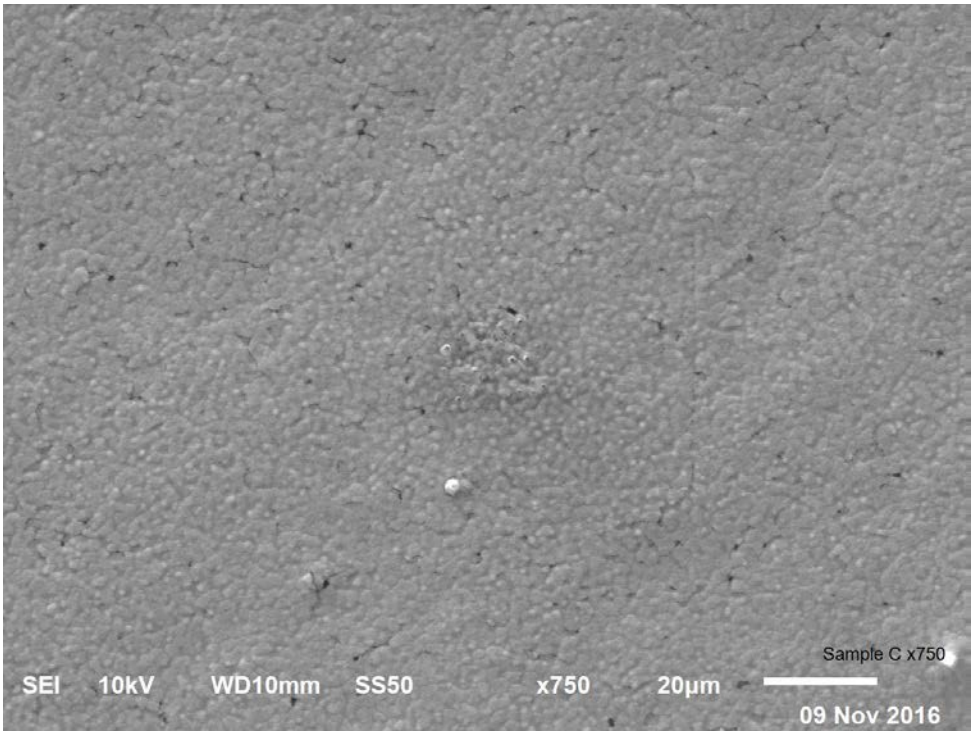

Figure 4. 60µl drop of Deinococcus radiodurans at 2-5 x 10^8 CFUs / ml count taken by SEM at USAFSAM.

A remaining 96 well, flat bottom plate with 40 µl of TGY in row A and 180 µl of TGY culture media in rows B-H was next used as a control to determine an initial CFU baseline. This baseline, referred to as a CFU input, provides a control for un-desiccated, non-irradiated bacteria. 60 µl drops of culture were added to row A, with the strains as follows: 1 1 1|5 5 5|8 8 8|11 11 11. The cells were then diluted 10 fold, seven times in series down the plate column by transferring 20 µl into the 180 µl of TGY media in rows B through H. Finally, 5 µl spots were transferred to TGY agar trays, which were then incubated for 48 hours at 32 °C in unsealed plastic bags in order to prevent drying.

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For the 3rd neutron experiment, EC was added. The cell culture media used for EC was LB broth (1.0 % tryptone, 0.5% yeast extract, and 0.5% sodium chloride) and EC was incubated in 37 ºC. The procedures above were followed with the additional of EC.

A modification from the procedure occurred when placing the samples onto the plate. Instead of using a multi-channel pipette, a single channel pipette was used in order to gain more precision when placing the drops in the center of their wells. Figure 5 shows how the samples were arrayed for the 3rd neutron experiment. Four plates were created for irradiation, with a fifth plate as an un-irradiated control.

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

Figure 5. For this experiment, fewer samples were used, but EC was included. Four samples per strain of bacteria were placed on each plate.

The plate setup for the proton experiment was modified as well. Two sets of plates (A & B) were created in the event any plate was damaged during shipping. Each

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set consisted of WT, and mutants 5, 8, and 11, with an untreated control plate. This time, each row of the samples were designated to receive varying amounts of proton irradiation. Another non-irradiated control was on the plate designated for irradiation that would also experience the same environmental condition inside the QASPR-3, minus irradiation. The untreated control plates of sets A and B had eight samples per strain. These setups are depicted in Figures 6 and 7, using WT as an example.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**100 Gy**			**1**
B												
C	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**500 Gy**			**1**
D												
E	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1000 Gy**			**1**
F												
G	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2500 Gy**			**1**
H												

Figure 6. The samples in columns 1-8, rows A, C, E, and G were set to receive various amounts of irradiation. These rows set to receive 100, 500, 1000, 2500 Gy respectively. All samples in column 12 did not receive any radiation. The 1 in each box represents wild type, but plates with the other mutants were also constructed.

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

Figure 7. Rows A, C, E, and G held WT, mutant 5, 8, and 11 respectively.

Neutron Generation

The Adelphi Technology, Inc. DD109.1 Neutron Generator was the source of neutrons for the irradiation of Dr. This neutron generator produces the neutrons via a Deuteron-Deuteron (D-D) reaction. It is capable of a neutron output of up to $1x10^9$ neutrons per second and can operate in a continuous or pulsed manner. The fast neutrons are produced mono-energetically at 2.45 MeV and the source size is approximately 16mm in diameter. This neutron generator operates with an ion beam supplied by a microwave plasma source. Microwave power is supplied by a magnetron. The ion source uses the electron cyclotron resonance effect to produce a high plasma density for the high current and high D+ content.[14]

The reaction of interest for neutron generation is the following:

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$$2D + 2D \rightarrow 3He\ (0.87\ MeV) + n\ (2.45\ MeV)$$

The generator is able to do this by using a titanium hydride target, which is impregnated with deuterium atoms. Deuterium gas is injected into the plasma chamber, which is ionized by the microwave source. A sufficient voltage, which overcomes the Coulomb barrier, is applied between the ion chamber and target. This accelerates the deuterium ions to the target, enabling them to fuse with the deuterons in the titanium. The products of this fusion are the 2.45 MeV neutrons and He. However, this reaction only occurs 50% of the time. The other 50% of the time the following reaction occurs [15]:

$$2D + 2D \rightarrow T + H$$

Neutron Dose Calculations

In order to calculate the dose of radiation via neutron exposures, the method as outlined by Cember in *Introduction to Health Physics* was followed. [16] Using N, the number of atoms/kg, f, the mean fractional energy transferred from neutron to scattered atom during collision with the neutron, and σ, the scattering cross section of the element for neutrons of energy E (2.45 MeV), the following value was found, as shown in Table 2.

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Table 2. *Deinococcus radiodurans* Cell Composition

Element	% Mass	N, atoms/kg	f	σ, cm^2	Nσf	
Oxygen	0.13	2.69E+25	0.111	8.45410E-25	2.524E+00	
Carbon	0.31	6.41E+24	0.142	1.58290E-24	1.441E+00	
Hydrogen	0.49	5.98E+25	0.5	2.59131E-24	7.748E+01	
Nitrogen	0.07	1.49E+24	0.124	1.30501E-24	2.411E-01	
				Σ Nσf	**8.169E+01**	**cm^2/kg**

The following references apply to the values on this table: % Mass[17], N [16], f[16], and σ[18]

Because the generator is able to produce a $1x10^9$ neutrons per second and geometry of the neutron generator results in a solid angle (Ω/4π) of 0.16, the result is a geometric attenuated source, S, of $1.60x10^8$ neutrons per second. The next consideration was the area, A, of a flat bottom, 96 well plate lid, whose total area is 109.269 cm^2. The following is calculated:

$$\dot{D}(E) = \mathrm{S} * \frac{1}{A} * \mathrm{E} * \Sigma\, \mathrm{N}\sigma\mathrm{f}$$

$$\dot{D}(2.45\ MeV) = \left(1.60x10^8 \frac{n}{s}\right) * \left(\frac{1}{109.269\ cm^2}\right) * (2.45\ MeV) * \left(81.69\ \frac{cm^2}{kg}\right)$$

$$* 1.6x10^{-13} \frac{J}{Mev} = 4.689x10^{-5}\ \frac{Gy}{s}$$

However, the dose rate is per the entire plate lid and the samples are per well of the plate lid. Each well represents 1.35% of the surface area of the sample plate.

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Therefore, the dose rate per well is reduced to 6.344×10^{-7} Gy/s. The following table depicts the dose per well based on the how the bacteria was irradiated.

Table 3. Neutron Dose per Well

	Hours	Dose (Gy)	Dose (Sv)
	5	1.1E-02	1.1E-01
Dose Per Well (sample)	10	2.3E-02	2.3E-01
	15	3.4E-02	3.4E-01
	20	4.6E-02	4.6E-01

Neutron Irradiation of Samples

For the 1st neutron experiment, three plates were taken to the neutron generator, located in Building 470 on Area B of Wright Patterson Air Force Base. The untreated plate was left outside of the neutron generator room, which is in the basement level of Building 470. The two treated plates were subjected to 5 hour and 10 hour neutron irradiation treatments, respectively. These plates were placed on the large cylinder of the neutron generator as close as possible to the source. The generator was run for five hours and the 5 hour treatment plate was removed and placed beside the untreated plate. The 10 hour treated plate received an additional 5 hours of neutron irradiation for a total of 10 hours. The same procedure was followed during the 2nd neutron experiment, only this time the first plate was removed at 15 hours and the second plate received a total of 20

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hours of irradiation. After both iterations, all three plates (untreated plus the two treated plates) were taken back to USAFSAM.

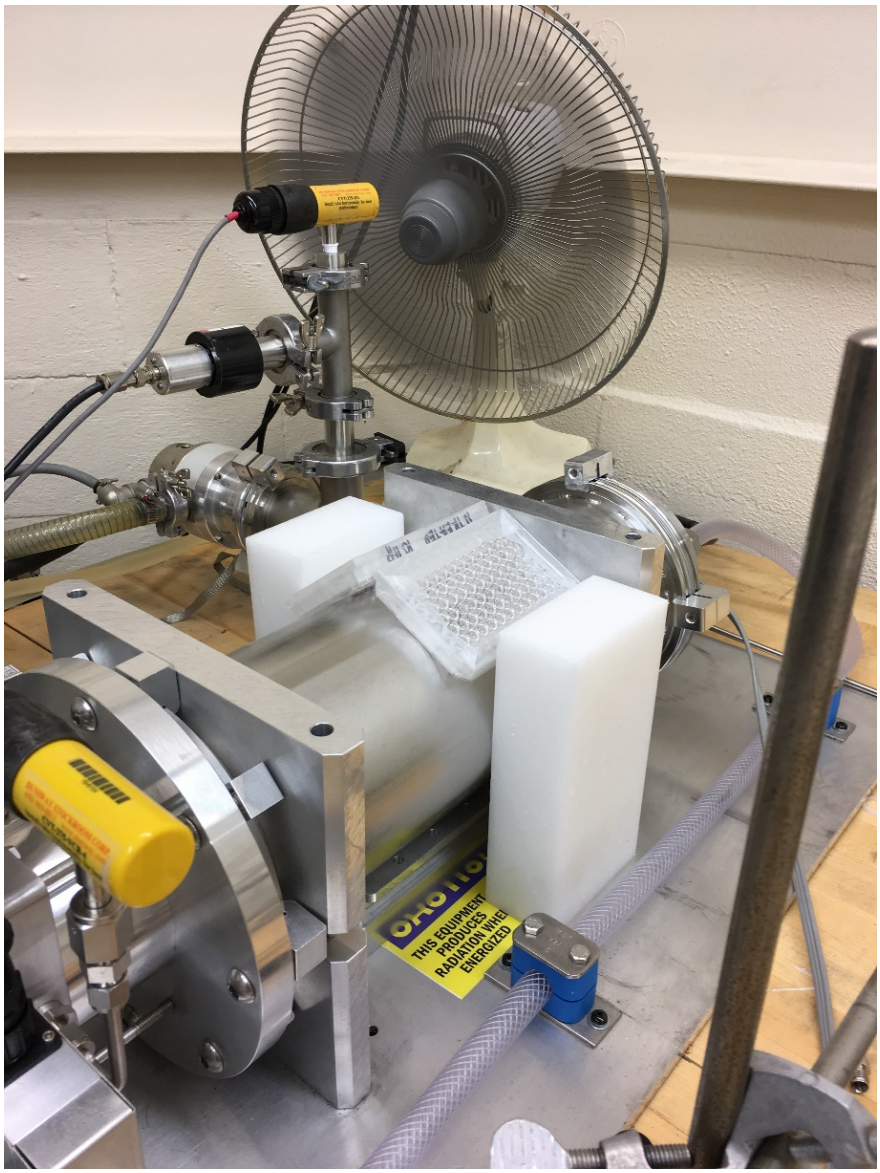

Figure 8. Two samples plates on the neutron generator.

For the 3rd neutron experiment, unlike the previous two neutron experiments, all four plates were irradiated during the same session. A specified plate was removed and

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placed outside the neutron generator room when the proper time of irradiation was achieved.

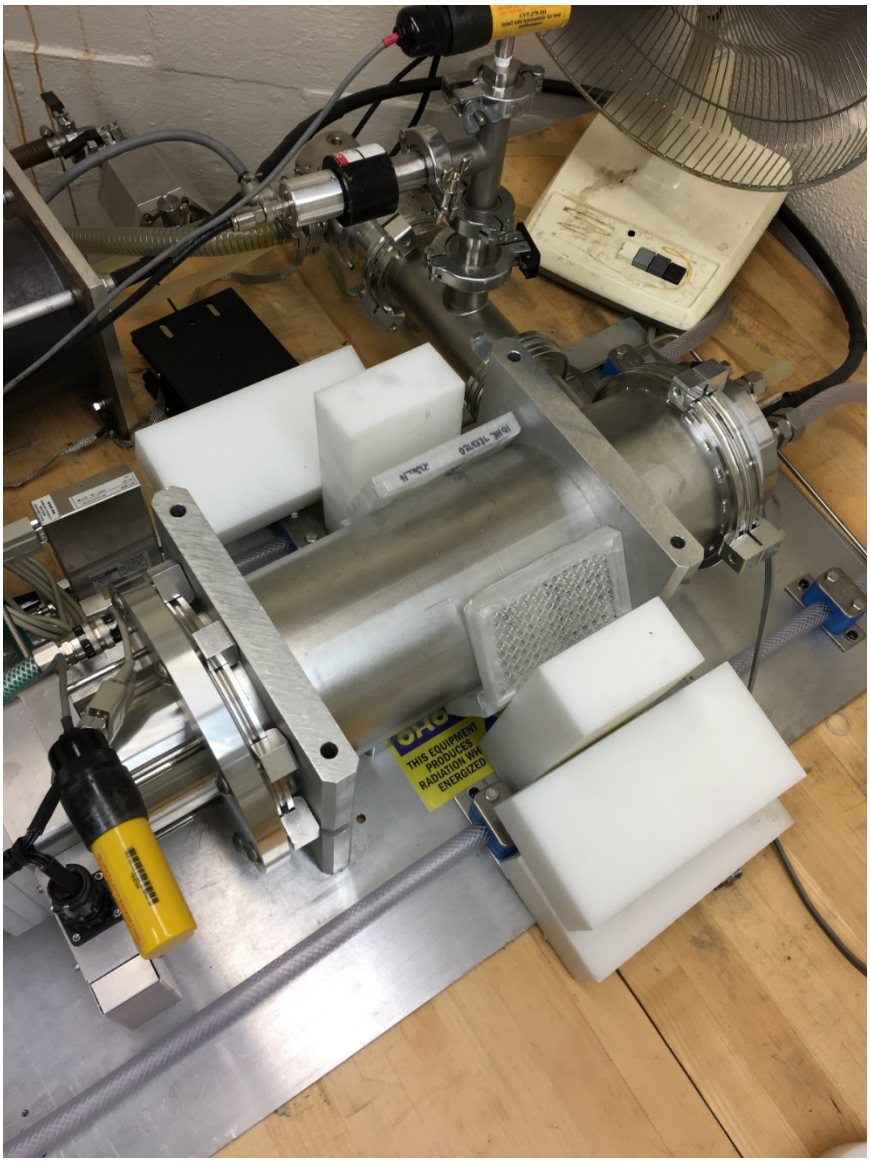

Figure 9. 4 treatment plates for irradiation by the neutron generator.

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Rehydration of Samples and Spotting Post Neutron Irradiation

After an approximate 24 hour waiting period to again to simulate shipping conditions, all three sample plates for the first and second neutron experiments were rehydrated with 60 µl of fresh TGY medium. The medium was pipetted up and down 20 times to re-suspend the cells. Next, the re-hydrated cells were pipetted up and down an additional 20 times to further re-suspend then transferred to a new 96 well, flat bottom plate. Another 40 µl of fresh TGY culture medium was added for a total of 100 µl of cell culture. The bacteria were then diluted 10 fold, seven times in series by transferring 20 µl in the 180 µl of TGY media. Finally, 5 µl spots were transferred to TGY agar trays, which were then incubated for 48 hours at 32 °C in unsealed plastic bags in order to prevent drying. The resulting colonies were then counted. This was the same serial dilution procedure as previously mentioned for the CFU input control.

In the case of the 3rd neutron experiment, a modification involved the re-hydration of the cells. The cells were diluted 10 fold, seven times in series down the plate column as previously mentioned. However, the additional 40 µl of TGY was not added to the 60 µl rehydrated spots in row A of the column well plate this time, resulting in all counts conducted at the 10^{-5}, not 10^{-4} dilution. Next, EC was spotted in 5 µl spots on LB agar, incubated for 24 hours, then the resulting colonies were counted. In addition to the 5 µl spots, 100 µl of Dr was spread on round TGY plates. This was done in order to decrease the variability of the experiment if possible. These trays were incubated for 48 hours.

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Colony Counting Post Neutron Irradiation

After the 48 hour incubation period, cell colonies were counted at the 4th dilution of each sample tray for the first and second neutron experiment. The cells were counted via visual inspection. The number of colonies per each sample was then recorded. Following the 3rd neutron experiment, the 100 µl spread plates were counted and recorded.

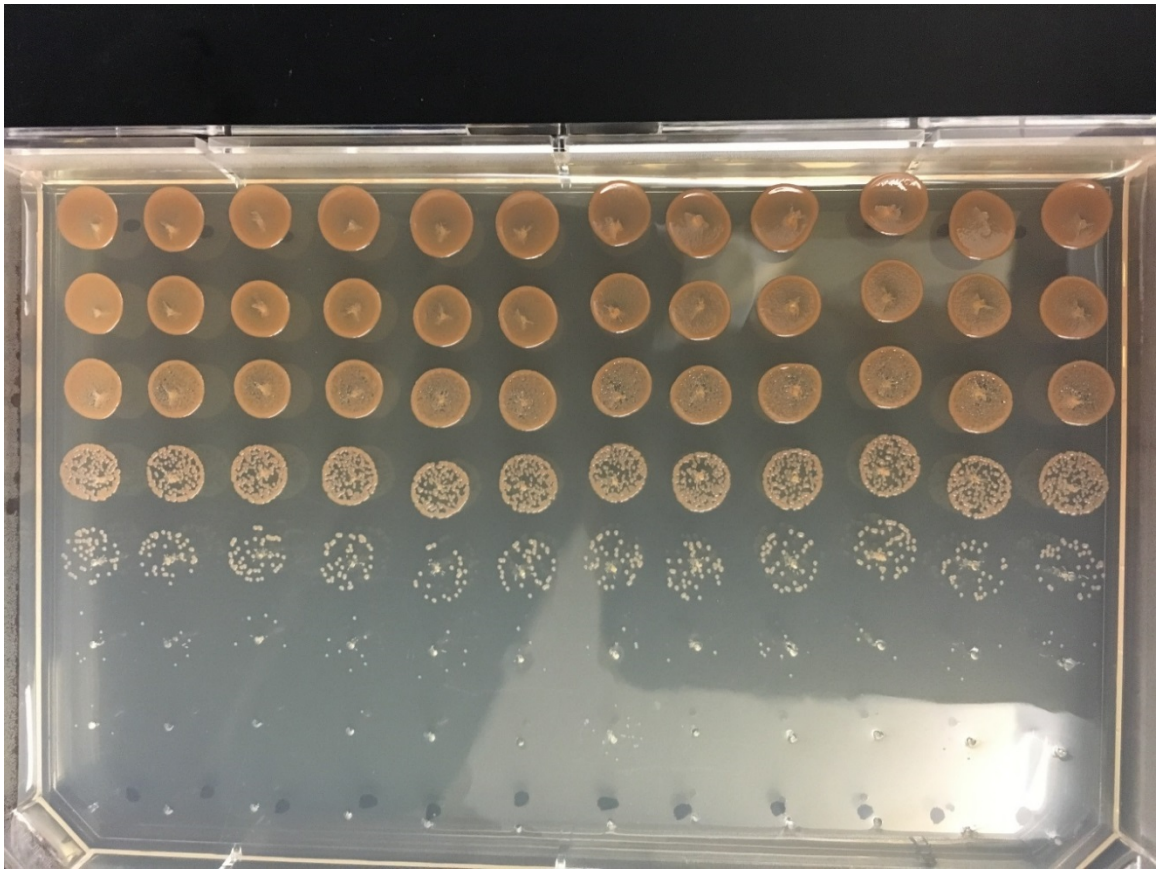

Figure 10. Wild Type *Deinococcus radiodurans* following a five hour neutron treatment in the 1st Neutron experiment.

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Proton Generation

The protons used for irradiation of Dr samples were generated by one of the Sandia National Laboratory's ion beams, QASPR-3. This device is a located at the Sandia National Laboratory's Ion Beam Lab located on Kirtland Air Force Base, New Mexico. This lab was opened in 2010 and is a "state-of-the-art facility using ion and electron accelerators to study and modify materials."[19] The QASPR-3 is a HVE 6 MV Tandem ion accelerator which "can accelerate most elements from hydrogen to gold. It is used for in-situ electrical testing, optical testing, and mechanical testing to determine the response of materials to radiation damage at various temperatures from -230 ºC to 1200 ºC. There is also a microbeam with a spot size of approximately 1 μm." [19] In the case of this experiment, the ion beam was used as proton radiation source.

Proton Dose Calculations

As mentioned earlier, a 60 μl drop, desiccated, is the target layer for the beam. Since the cells are spherical, ranging from 1.5 to 3.5 μm in diameter, an average diameter of 2.5 μm and an average radius is 1.25 μm was used for calculations. The 60 μl drop is taken from concentration of 2-5x10^8 CFU/ml. Again, taking the average, the concentration is 3.5x10^8 CFU/ml.

$$60\ \mu l * \frac{1\ ml}{1000\ \mu l} * \ 3.5\ x\ 10^8\ \frac{CFU}{ml} = 2.1\ x\ 10^7 CFU$$

So, in a 60 μl drop, it is expected to have 2.1 x 10^7 CFUs. Based on the average size and shape of Dr, the volume Dr in the drop is determined by the following:

$$Volume\ of\ Dr = \frac{4}{3} * \pi * (1.25\ x\ 10^{-6} m)^3 * 4 * 2.1x10^7 = 6.87\ x\ 10^{-10}\ m^3$$

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Assuming, at most, the layer will take up the entire lid plate well, whose area is 3.165 x 10^{-5} m^2, the layer depth is demonstrated via the follow equation:

$$Layer\ Depth\ of\ Dr = \frac{Volume\ of\ Dr}{3.165\ x\ 10^{-5}\ m^2} = 0.0000217\ m$$

This means that the 60 µl drop as a layer depth of 21.7 µm. The polystyrene plate lid has a thickness of 1.27 mm. The density of Dr is 0.9392 g/cm^3. [17]

Inputting the above layer measurements into SRIM and TRIM [20], it was determined that 4.5 MeV protons would deposit .85 eV/Angstrom into the Dr layer.

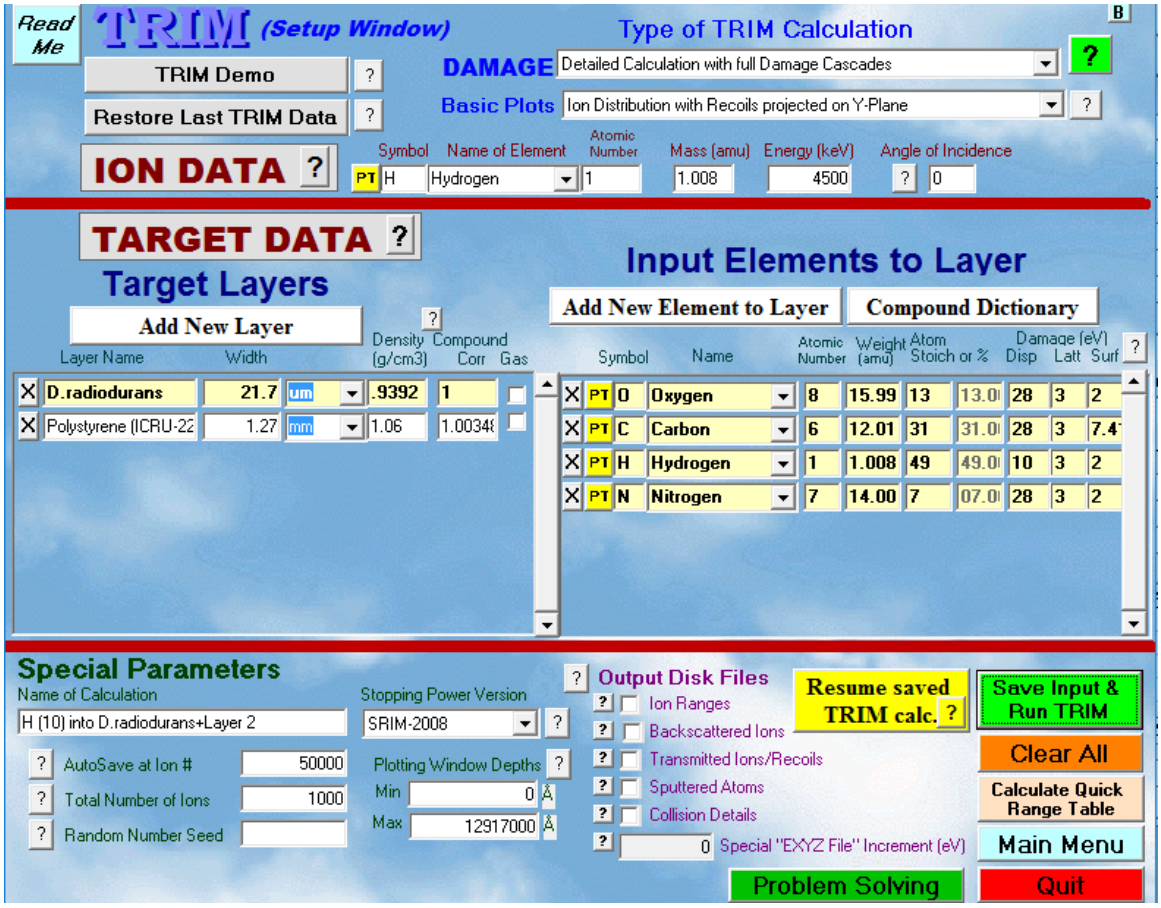

Figure 11. Input screen for TRIM, with the first layer of Dr and the second layer the plate lid.

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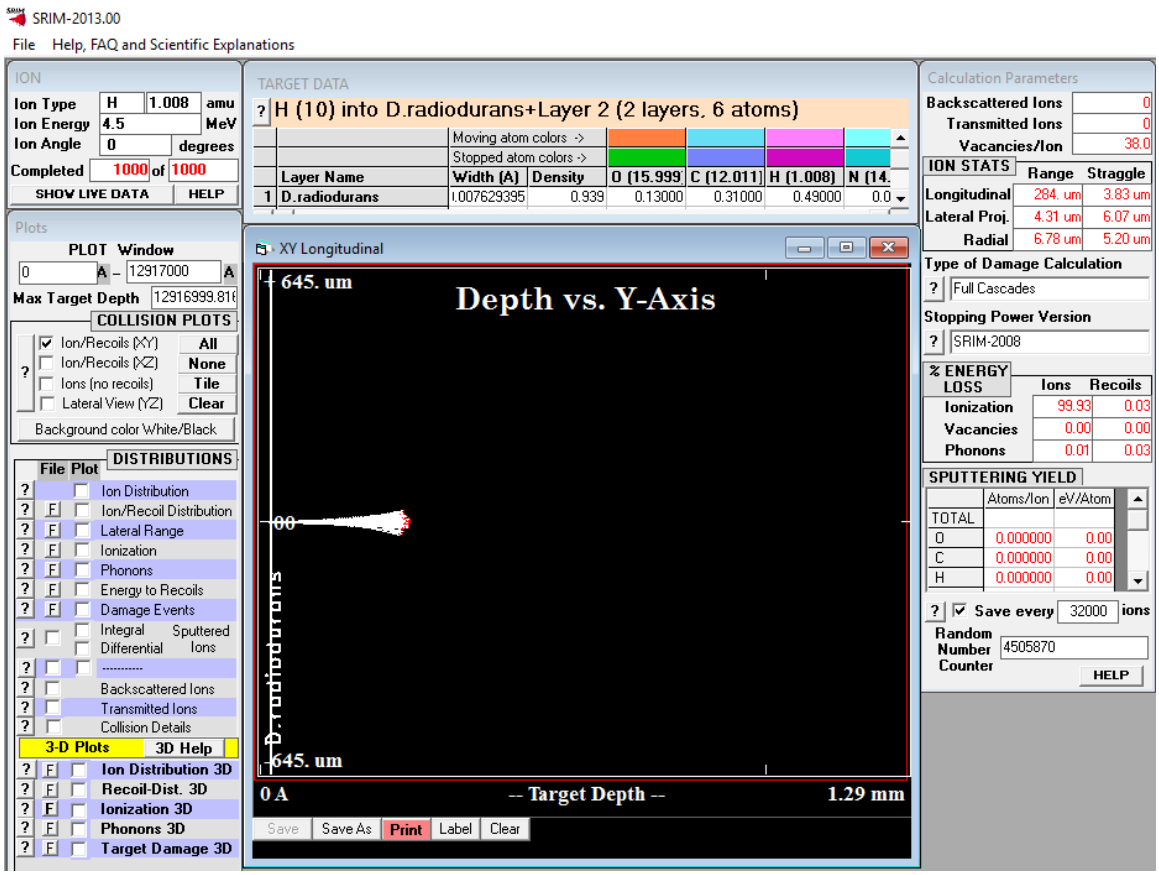

Figure 12. Based on the inputs in the previous figure, TRIM simulation of 4.5 MeV proton ions irradiating the Dr sample.

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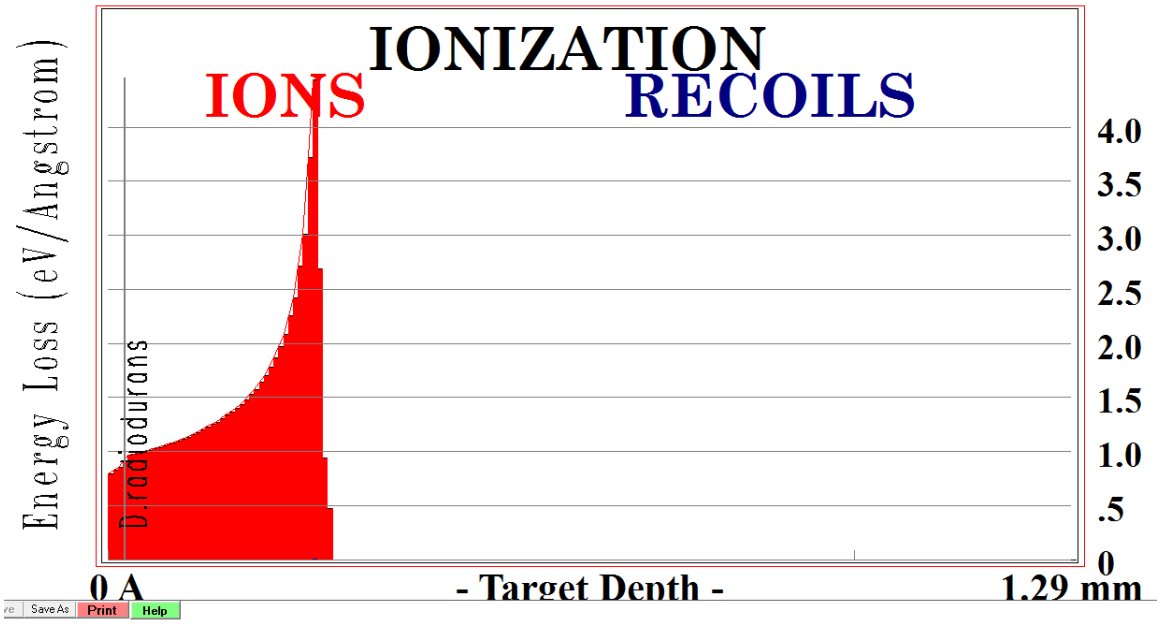

Figure 13. Chart created by TRIM showing the ionization in both Dr and the polystyrene lid. This shows the ionization in the Dr layer to be around 0.85 eV / Angstrom.

Knowing this ionization allows one to determine the fluence needed to achieve a certain dose of irradiation.

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

$$0.85 \frac{eV}{Angstrom-Ion} * \frac{1.6022\ x\ 10^{-19}\ J}{1\ eV} * \frac{1\ x\ 10^{8}\ Angstrom}{1\ cm} * \frac{cm^3}{0.9392\ g} * \frac{7.2\ x\ 10^{9}\ Ions}{1\ cm^2} * \frac{1000\ g}{1\ kg} =$$

$$104\ Gy \sim 100\ Gy$$

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Table 4. Proton Dose per Well

	Fluence (Ion/cm^2)	Dose (Gy)	Dose (Sv)
	7.20E+08	1.0E+01	2.1E+02
Dose Per Well (sample)	7.20E+09	1.0E+02	2.1E+03
	3.60E+10	5.2E+02	1.0E+04
	7.20E+10	1.0E+03	2.1E+04
	1.80E+11	2.6E+03	5.2E+04
	7.20E+11	1.0E+04	2.1E+05

Proton Irradiation of Samples

The dehydrated samples were shipped to the Ion Beam Lab which took two days. The radiation experiment lasted three days, which took place five days after the samples arrived at the Ion Beam Lab. Wild type Dr and mutants #5 and #8 were both irradiated with protons as shown in Figure 10 below, however mutant #11 was not due to time constraints. On a second Wild Type plate, one row was irradiated for a dose of 10 Gy and another row was irradiated for a dose 10,000 Gy.

The three controls mentioned earlier were devised for this experiment because Dr would experience longer times in a dehydrated state than experienced for the previous experiments.

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Each sample plate was adhered to the stage on the QASPR-3, which had limited mobility to move in the x and y directions, rotate, and move along the radius. Because of this, the ion beam's vacuum had to be evacuated and the plate repositioned for each row of irradiation.

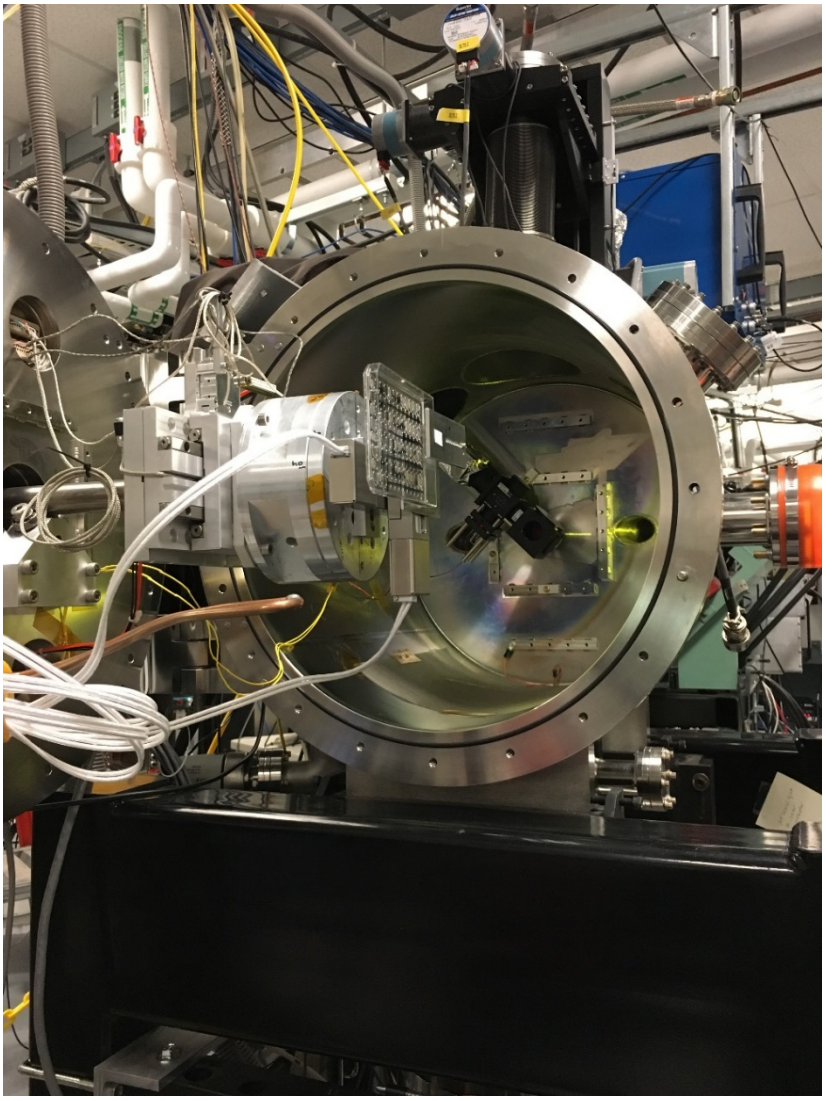

Figure 13. Dr sample plate attached to the stage of the QASPR-3.

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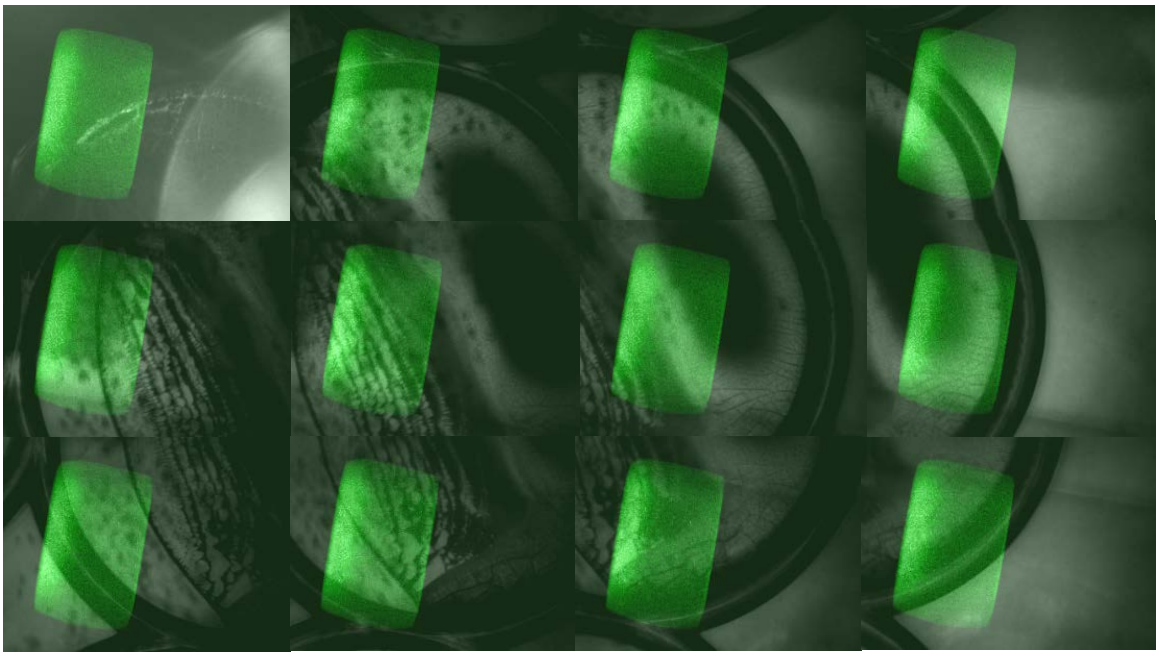

Figure 14. The QASPR-3 proton beam was able to hit the total area each well by firing shots in a grid pattern based on the area of the beam. Top Row: Shots 1-4; Center Row: Shots 5-8; Bottom Row: Shots 9-12.

At the beginning of each day of experimentation the beams conditions such as the beam current and area were validated. The beam itself was calibrated using a phosphorus target situated on the stage above the sample lid as shown in Figure 13. This enabled the operator of the beam to both validate the fluence in ions/cm^2 and the beam's width, which would determine the grid pattern of shots, such in Figure 14. The ion beam's fluence was always within ten percent of the requested fluence. The QASPR-3 was able to accelerate the protons in a directed beam so that the entire well was evenly covered with no overlap, with an example of a well in Figure 14. The samples were shipped the next day following the end of the experiment and arrived back at USAFSAM two days later.

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Rehydration of Samples and Spotting Post Proton Irradiation

After arriving back at USAFAM, the samples were rehydrated five days later. The process was similar to the rehydration of samples following the neutron experiments. All irradiated sample and control plates were rehydrated with 60 µl of fresh TGY medium. The medium was pipetted up and down 20 times to re-suspend the cells. Next, the re-hydrated cells were pipetted up and down an additional 20 times to further re-suspend then transferred to a new 96 well, flat bottom plate. The bacteria were then diluted 10 fold, seven times in series by transferring 20 µl in the 180 µl of TGY media. Finally, 5 µl spots were transferred to TGY agar trays, which were then incubated for 48 hours at 32 °C in unsealed plastic bags in order to prevent drying. The resulting colonies were then counted.

Colony Counting Post Proton Irradiation

After a 72 hour incubation period, cell colonies were counted at the 5^{th} dilution of each sample tray for the proton experiment. The cells were counted via visual inspection. The number of colonies per each sample was then recorded.

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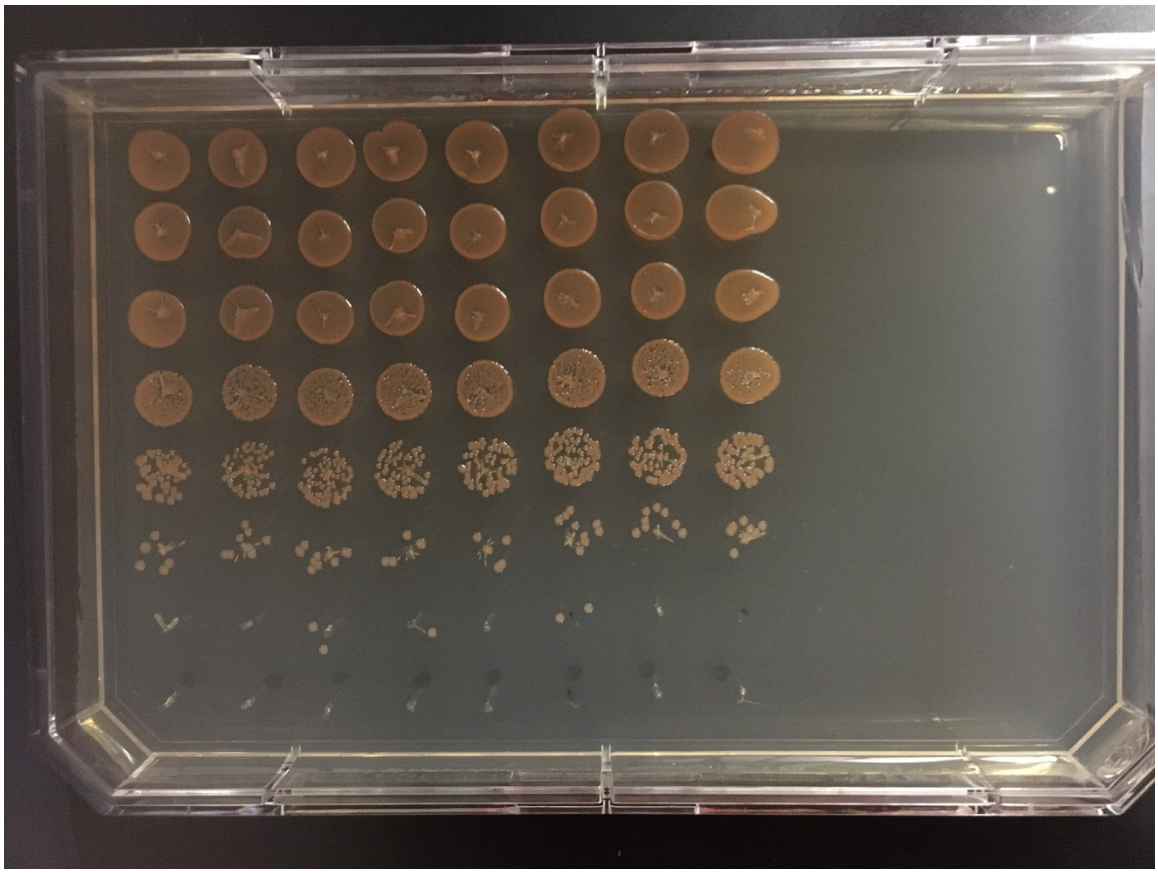

Figure 15. Wild Type Dr re-growth after 500 Gy irradiation. Colonies were counted at the 10^{-5} dilution.

Statistical Methods of Comparison

A statistical analysis was conducted between the following samples - CFU input control to non-irradiated control, and non-irradiated control to the irradiated sample populations. The statistical analysis consisted of a small, independent sample test of hypothesis for a population, μ_1, to another population, μ_2, using the Student's t-Statistic.[21] This method was chosen because of the small sample size (< 30 samples), with the following assumptions: 1 – the two samples are randomly selected in an independent manner from the two target populations, 2 – both samples' populations have

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distributions that are approximately normal, and 3 – the population variances are equal. Due to this, a pooled sample estimator, s_p^2, was used. This was calculated the following way:

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

where n is the number of samples per strain irradiation treatment and s_2 is the sample variance.

The populations were then compared using a one-tailed test, with the subsequent equations showing the null hypothesis, H_0, the alternate hypothesis, H_a, the test statistic, t, each samples mean colony counts, $\bar{x}_1$ and $\bar{x}_2$, and the rejection region, t_a, which is based on $(n_1 + n_2 - 2)$ degrees of freedom. The variable, a, was 0.05 to reflect a 95 % confidence.[21]

$$H_0: (\mu_1 - \mu_1) = 0$$

$$H_a: (\mu_1 - \mu_1) > 0$$

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

$$Rejection\ Region: t > t_a$$

IV. Analysis and Results

Chapter Overview

The purpose of this chapter is to review the statistical analysis conducted between the irradiated sample colonies and their controls. All populations were compared with 95% certainty. The comparisons are broken down by experiment, with only the cases of

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statistical difference or close to statistical difference appearing the Tables 5 - 8. Close to statistical difference is defined as a difference of 0.1 or less between the t-statistics and the rejection region.

1st and 2nd Neutron Experiments

For the 1st and 2nd neutron experiments, the CFU input control and the non-irradiated sample control were compared. Then, the irradiated samples were compared to the non-irradiated controls for each strain. Figures 16 and 19 shows the total CFU count for each control and irradiated strain. Tables 5 and 6 depicts cases of statistical difference or cases that were close to statistical difference.

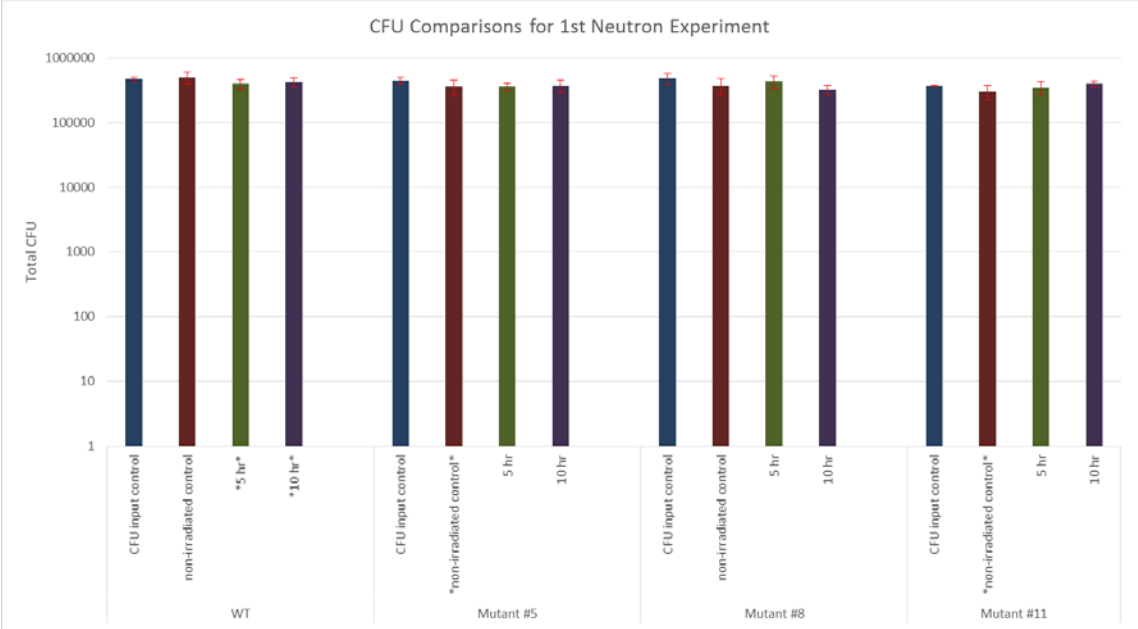

Figure 16. Total CFU comparison for the 1st Neutron Experiment.

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Table 5. 1st Neutron Experiment Statistically Significant Population Comparisons

Populations 1	Populations 2	Strain
Non-Irradiated Control	5 Hour Dose – 1.1 cGy	WT
Non-Irradiated Control	10 Hour Dose – 2.3 cGy	WT
CFU input Control	Non-Irradiated Control	5

The populations for the 5 and 10 hour irradiations of WT showed statistical differences from the non-irradiated controls. In each case, the test statistics were greater than the rejection region. In the listed comparison for Mutant #5, the test statistic was close to the border of the rejection region, but did not go into the rejection region.

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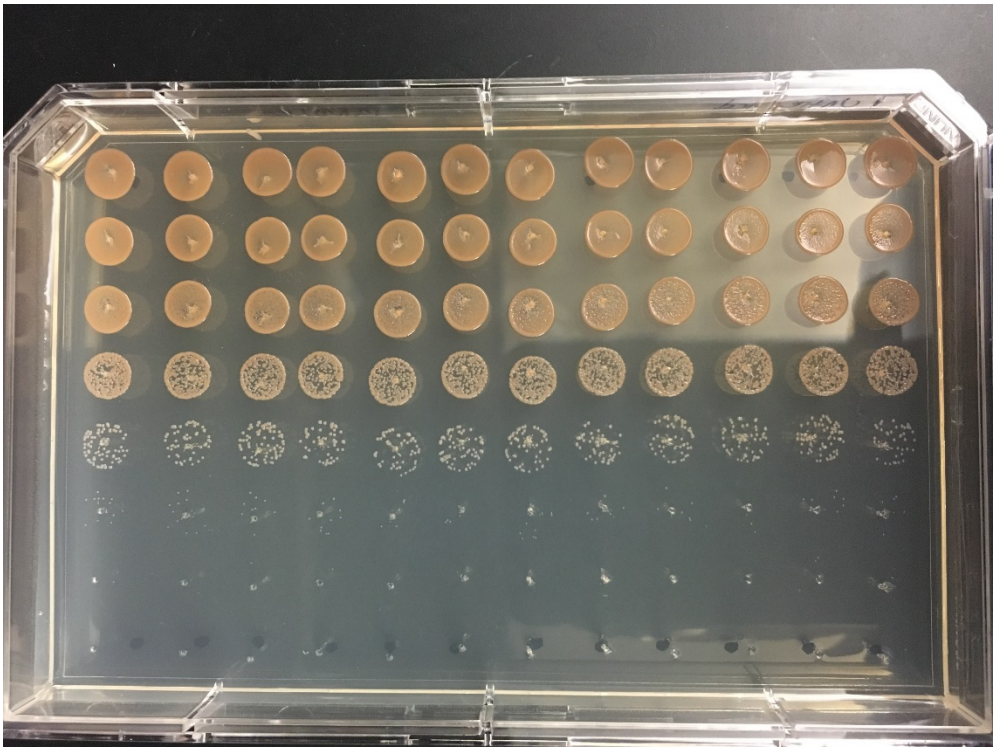

Figure 17. Dr Wild Type untreated with neutron radiation – 1st Neutron Experiment

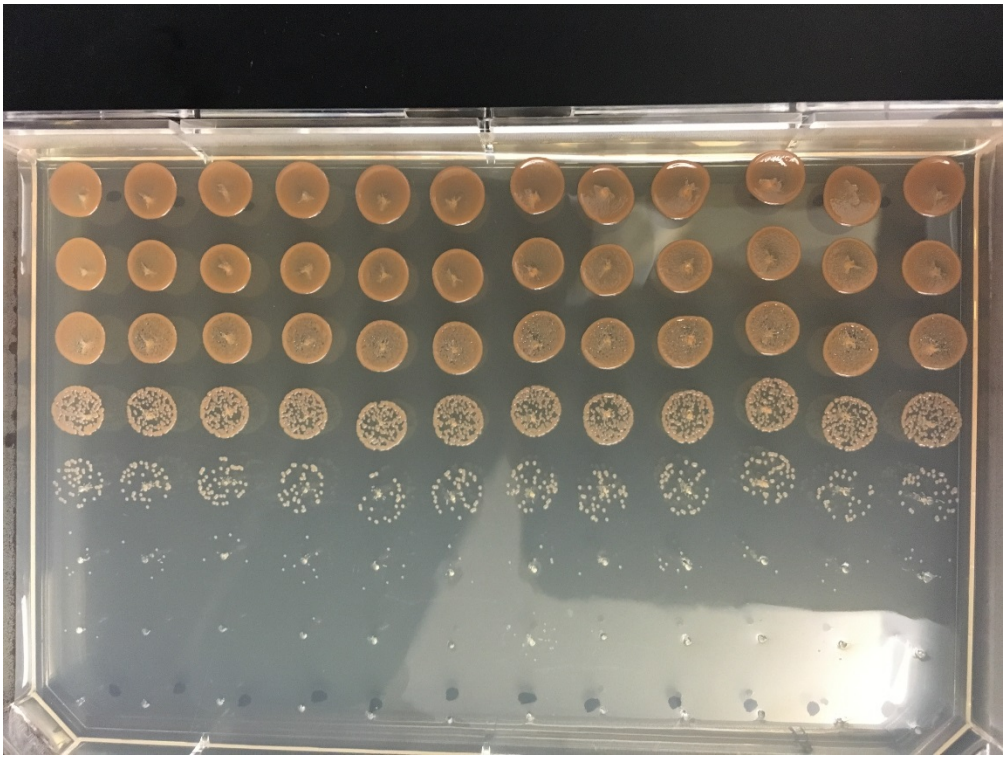

Figure 18. Dr Wild Type neutron irradiated for 5 hours – 1st Neutron Experiment

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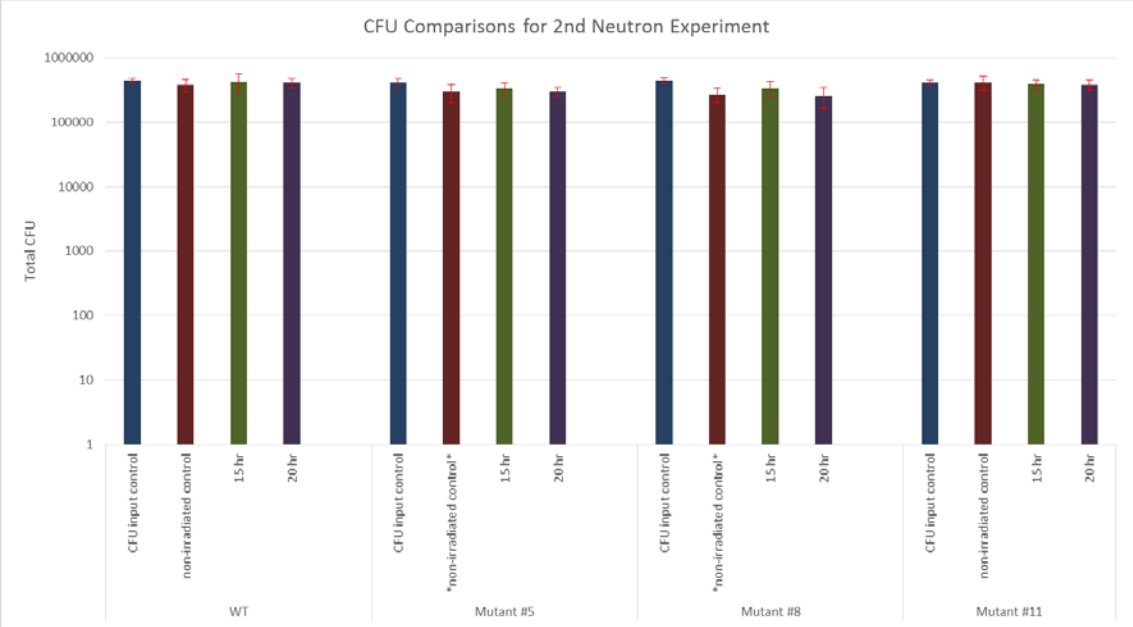

Figure 19. Total CFU comparison for the 2nd Neutron Experiment.

Table 6. 2nd Neutron Experiment Statistically Significant Population Comparisons

Populations 1	Populations 2	Strain
CFU input Control	Non-Irradiated Control	5
CFU input Control	Non-Irradiated Control	8

In regards to the control vs control comparison of Mutant #5, the test statistic was found to be in the rejection region. The control versus control comparison of Mutant #8 also demonstrated a difference in populations, where the test statistic was deep into the rejection region.

Upon reviewing the tables for the 1st and 2nd Neutron Experiments it can be seen that there does not seem to be any trends forming at these amounts of neutron radiation.

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Of all comparisons that showed a statistical difference or close to a statistical difference populations for these first two experiments, the latter two did not involve radiation, only dehydration.

3rd Neutron Experiment

The results from the third neutron radiation experiment are depicted next. For these comparisons, the CFU input control was not compared as it completed with 5 µl spots, not 100 µl spreads. This set of input controls was countable at the expected dilution.

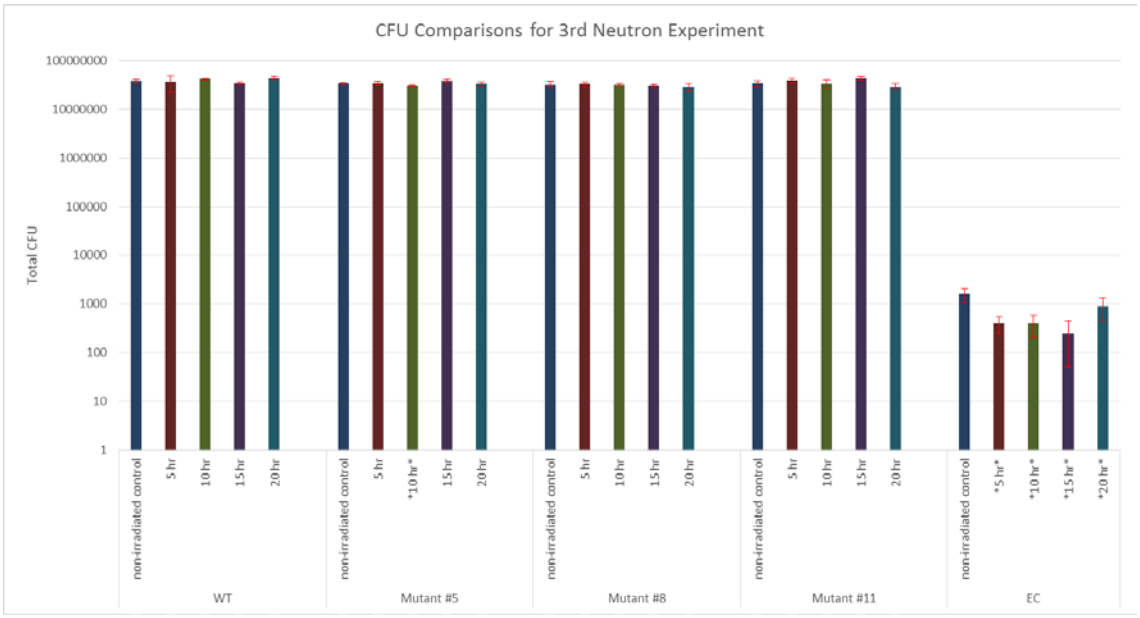

Figure 20. Total CFU comparison for the 3rd Neutron Experiment.

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Table 7. 3rd Neutron Experiment Statistically Significant Population Comparisons

Populations 1	Populations 2	Strain
Non-Irradiated Control	10 Hour Dose – 2.3 cGy	5
Non-Irradiated Control	5 Hour Dose – 1.1 cGy	EC
Non-Irradiated Control	10 Hour Dose – 2.3 cGy	EC
Non-Irradiated Control	15 Hour Dose – 3.4 cGy	EC
Non-Irradiated Control	20 Hour Dose – 4.6 cGy	EC

In regards to Mutant #5's entry, the test statistic was deeply within the rejection region. Like the previous experiments, no trends are readily apparent. This time, the only the difference between populations occurred between the non-irradiated control and 10 hour dose to Mutant #5's samples. However, *E. coli* did show a sensitivity to both desiccation and neutron treatment. EC's CFU input controls showed countable colonies starting at a 10^{-5} dilution, but the untreated control only had countable colonies at the 10^{-2} dilution. Additionally, the neutron radiation also had an effect on EC, unlike Dr.

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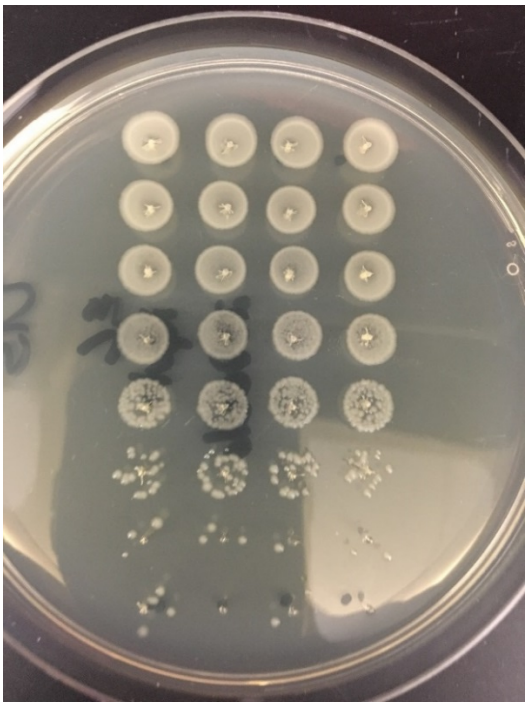

Figure 21. EC CFU input control, with countable colonies at the 10^{-5} dilution

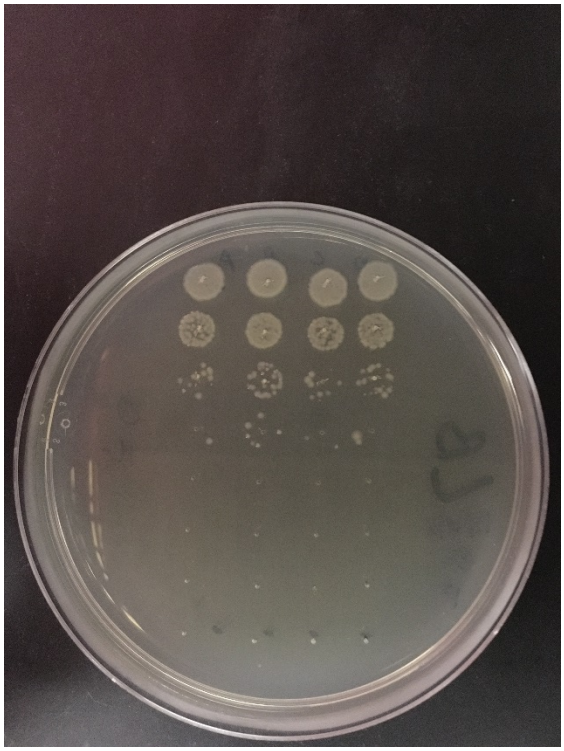

Figure 22. EC untreated control, with countable colonies at the 10^{-2} dilution.

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In every case of irradiation treatment, there was difference between that dose and the non-irradiated control. An interesting result in these comparisons is that while the test statistics for the 5, 10, and 15 hours irradiation treatments were extremely into the rejection region, the final dose, which was a higher irradiation, was not nearly as far in the rejection region as the others.

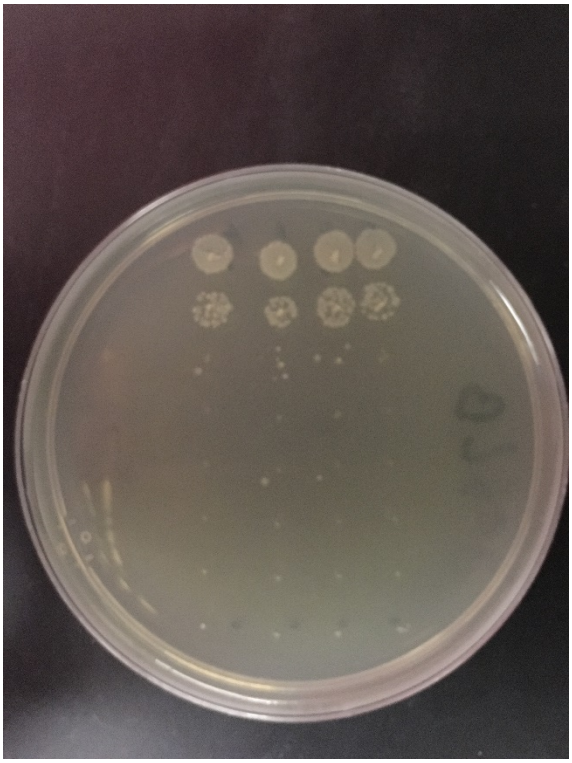

Figure 23. EC at 5 hours of neutron treatment.

1st, 2nd, 3rd Neutron Experiments Findings

For neutron radiation at this dose (cGy), it has been demonstrated that the hypothesis, which stated Dr would not resist neutron (high LET) radiation, was **not** upheld. Instead, in the vast majority of population comparisons, the null hypothesis,

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which stated the populations of the experimental groups (neutron radiated) and control groups (no radiation) would not be statistically different, could not be disproved.

Proton Experiment

The proton experiment had a total of three controls that were compared to each other, and the 3rd control was then compared to all the irradiated samples. These controls consisted of a CFU input control (Control 1), a Non-Irradiated Control – No Vaccum (NV, Control 2), and a Non-Irradiated Control – Vacuum (V, Control 3). This third control was on the plate with the treated samples, but was not treated itself. It did experience the same conditions inside the chamber of the QASPR-3, minus proton radiation.

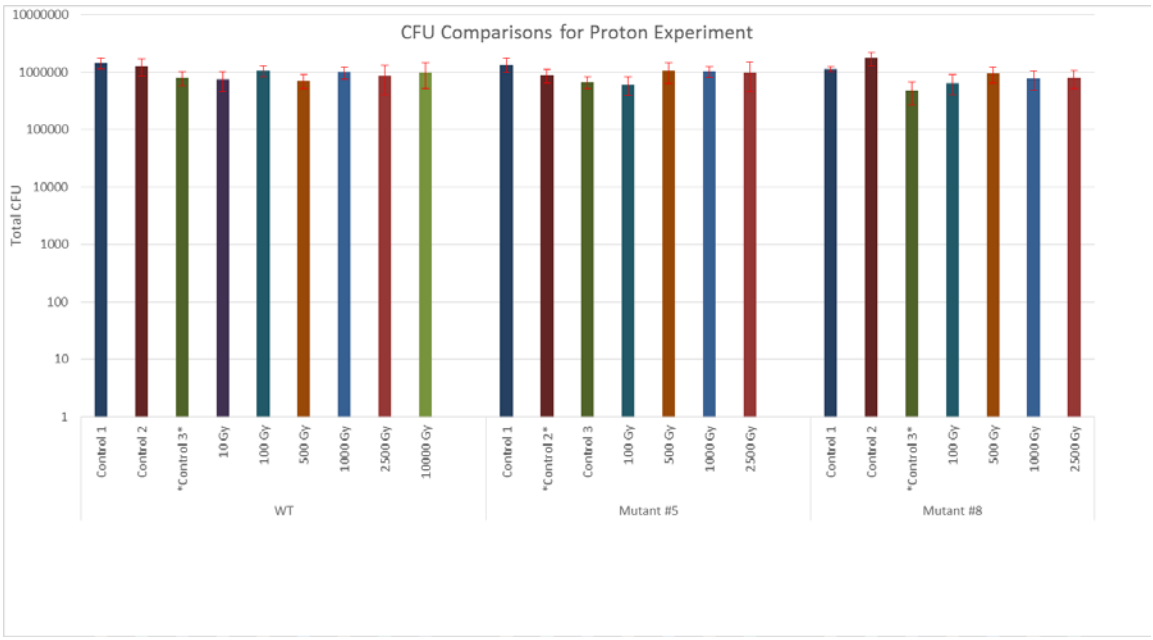

Figure 24. Total CFU comparison for the Proton Experiment.

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Table 8. Proton Experiment Statistically Significant Population Comparisons

Populations 1	Populations 2	Strain
Non-Irradiated Control (NV)	Non-Irradiated Control (V)	WT
CFU Input Control	Non-Irradiated Control (NV)	5
Non-Irradiated Control (NV)	Non-Irradiated Control (V)	8

For the Wild Type, the comparison showed a difference between the controls. Likewise, Mutant #5 also showed a difference in a control versus control comparison. This time, it was between the CFU input control and the Non-Irradiated Control – (NV). Finally, for Mutant #8, the t-statistics was well within the rejection region. Interestingly, there were no statistical differences between radiated and non-irradiation populations.

Much like the neutron experiments, it has been demonstrated that the hypothesis, which stated Dr would not resist proton (high LET) radiation, was **not** upheld. Instead, all of the population comparisons between the irradiated samples and the non-irradiated control in the vacuum supported the null hypothesis, which stated the populations of the experimental groups (proton radiated) and control groups (no radiation) would not be statistically different, could not be disproved.

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V. Conclusions and Recommendations

Conclusions of Research

These experiments have shown that not only is Dr resistant low LET radiation, but high LET radiation as well. For the neutron experiments, the low amount of radiation (no greater than cGy), seems to account for the lack of consistent effect of neutron irradiation. It was already demonstrated that Dr can receive a dose of 5 kGy of ionizing radiation of low LET with no lethality. [11] Likewise, previous experiments have shown a gamma dose of 10 kGy will still result in survival close to only 10^{-2} lethality.[3] It is reasonable to assume that the low amount of radiation is why the neutron irradiation resulted in no lethality.

However, at the surface, the proton experiment seems to be at odds with the findings of Paulino-Lima et al. In their study in regards to proton irradiation found in solar winds, the researchers used lower energy protons (200 keV protons, not 4.5 MeV protons) and had a greater LET (6.24 eV / Angstrom, compared to .86 eV / Angstrom). Taking this a step further, researchers found that dried plasmids exposed to 10 MeV protons, with 6.39 keV/µm LET resulted in 2.8 DSB/1000 Mbp-Gy.[22] The Mbp is the number of mega base pairs per plasmid. If you combine Dr's number of base pairs per DNA (3.06 Mbp) and plasmids (233 Kbp)[5], you get a total of 3.293 Mbp. Since both the energy and LET of the protons are on about the same order of magnitude (the LET for the proton experiment was .85 eV/Angstrom = 8.5 keV/ µm, and the energy of the protons was 4.5 MeV), an estimate of the number of DSB based on the number of Dr's

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Mbp and the irradiation dosage it received. This estimate is an upper level estimate, as the plasmids presented no other biological targets, unlike the cells of Dr.

$$\frac{2.8\ DSB}{1000\ Mbp - Gy} * 3.293\ Mbp * 10\ Gy = .09\ DSB$$

So, at 10 Gy, the Dr sample only incurred a faction of a DSB. Table 9 depicts the number of DSB estimated to have occurred based on the dose in Gy.

Table 9. Estimated Number of *Deinococcus radiodurans* DSBs at an LET of 8.5 keV/µm

Dose (Gy)	# of DSBs
10	.09
100	.90
500	4.6
1000	9.2
2500	23
10000	92

Minton and Daly have stated that "*D.radiodurans* exposed to 1.0 to 1.5 Mrad (1 rad = .01 Gy, so 1.0 to 1.5 Mrad = 10,000 to 15,000 Gy) gamma-irradiation sustains >120 DNA double strand per chromosome (In Minton and Daly's work, the term chromosome appears to equal the term genome); these double strand breaks are mended over a period of hours with 100% survival and virtually no mutagenesis.[23] At the maximum proton dose of 10000 Gy used for this experiment, only 92 DSBs are estimated to occur, so this

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may be why there were no differences between the non-irradiated controls and the proton irradiated samples.

In the solar wind experiment, a LET of 6.24 eV/Angstrom (62.4 keV), from 200 keV protons, was used.[17] This is an order of magnitude above what was done in the plasmid experiment. Assuming a linear relationship between LET and number of DSBs, it may be estimated that the Dr of that experiment experienced DSBs at an order of magnitude greater as well. Using the previous computational frame work:

$$\frac{28\ DSB}{1000\ Mbp - Gy} * 3.293\ Mbp * 10\ Gy = .90\ DSB$$

Table 10. Estimated Number of *Deinococcus radiodurans* DSBs at an LET of 62.4 keV/µm

Dose (Gy)	# of DSBs
10	.90
100	9.2
1000	92
10000	920

This may explain why data from this experiment showed a reduced survival rate at 1000 Gy (less than 2 log kill) and 10000 Gy (about 3 log kill). So one possible explanation for Dr's survival is that even though more energetic protons were used, an order of magnitude less of LET may have resulted in less damage overall to Dr's DNA.

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Interestingly, no single mutant stood out as being more sensitive to the proton irradiation. The mutant gene KOs were devised to disrupt pathways which protected against radicals resulting from indirect damage caused by low LET. This adds validity to the idea that indirect damage is more detrimental to Dr's ability to repair itself than direct damage.[22] Because of the ability to survive around a hundred DSBs, the protective mechanism at play seems to be Dr's capability to repair DNA DSBs.

Another major difference between the experiments was in the method used to create a sample. The researchers in *Survival of Deinococcus radiodurans Against Laboratory-Simulated Solar Wind Charged Particles* used a monolayer of cells. This was done to prevent irradiation shielding from dead cells. Because this experiment had more layers, there may have been some shielding. Likewise, some shielding may have occurred from the organic molecules of the TGY cell medium that did not evaporate while Dr was left to dehydrate under the biosafety cabinet.

Finally, the mechanisms normally associated with desiccation may have already been up-regulated during the de-hydration process. As such, this may have given Dr an advantage in repair during rehydration and re-growth.

Recommendations for Future Research

The results of these experiments certainly lead to more questions for future research. On such question is in regards to the neutron research. The neutron generator available at the Air Force Institute of Technology was somewhat limited in that it could only produce a 10^9 neutrons per second, without consideration of geometric attenuation. If possible, subjecting Dr to greater neutron fluxes may result in greater lethality than

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demonstrated in this experiment. Possible neutron sources include the Ohio State University Research Reactor, which is capable of neutron fluxes in the order of magnitude of 10^{13} n/cm^2/s, though these neutrons are thermal neutrons, not fast neutrons like those used in this experiment.[24] Another venue for greater neutron flux is the Spallation Neutron Source located at Oak Ridge National Laboratory.

Another interesting aspect of this research would be looking at another type of high LET radiation, such as alpha particles, which are essentially helium ions. The QASAR-3 is also able to produce this type of ion as well. If feasible, changing the sample preparation to a monolayer and washing of the cells to prevent shielding may also yield different results then were shown in the proton experiment during this research. Further researcher may also need to consider the LET, not the just the energy of the particles used for irradiation.

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Appendix A: Optical Density Measurements

Table 11. Initial Dr Optical Densities and Required Culture for an OD_{600} of 0.25 for 1st Neutron Experiment

Strain	Initial OD_{600}	Amount of Culture to Add to 40 ml TGY to achieve OD_{600} of 0.25
WT (1)	.566	1.8 ml
Mutant #5	.382	2.6 ml
Mutant #8	.497	2.0 ml
Mutant #11	.527	1.9 ml

Table 12. Initial Dr Optical Densities and Required Culture for an OD_{600} of 0.25 for 2nd Neutron Experiment

Strain	Initial OD_{600}	Amount of Culture to Add to 40 ml TGY to achieve OD_{600} of 0.25
WT (1)	.497	2.0 ml
Mutant #5	.390	2.6 ml
Mutant #8	.463	2.2 ml
Mutant #11	.508	2.0 ml

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Table 13. Initial Dr Optical Densities and Required Culture for an OD_{600} of 0.25 for 3rd Neutron Experiment

Strain	Initial OD_{600}	Amount of Culture to Add to 40 ml TGY / LB to achieve OD_{600} of .25
WT (1)	.542	1.9 ml
Mutant #5	.385	2.6 ml
Mutant #8	.342	2.9 ml
Mutant #11	.501	2.0 ml
EC	.424	2.4 ml

Table 14. Initial Dr Optical Densities and Required Culture for an OD_{600} of 0.25 for Proton Irradiation Experiment

Strain	Initial OD_{600}	Amount of Culture to Add to 40 ml TGY / LB to achieve OD_{600} of .25
WT (1)	.510	2.0 ml
Mutant #5	.326	3.1 ml
Mutant #8	.349	2.9 ml
Mutant #11	.491	2.0 ml

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Table 15. Post 4 Hour Incubation Optical Density and Amount of TGY required to achieve an OD_{600} of 5 for 1st Neutron Experiment

Strain	Post 4 Hour Incubation OD_{600}	Amount of TGY to Add to pellet to achieve OD_{600} of 5
WT (1)	.624	3.7 ml
Mutant #5	.712	4.3 ml
Mutant #8	.549	3.3 ml
Mutant #11	.761	4.6 ml

Table 16. Post 4 Hour Incubation Optical Density and Amount of TGY required to achieve an OD_{600} of 5 for 2nd Neutron Experiment

Strain	Post 4 Hour Incubation OD_{600}	Amount of TGY to Add to pellet to achieve OD_{600} of 5
WT (1)	.569	3.4 ml
Mutant #5	.574	3.4 ml
Mutant #8	.503	3.0 ml
Mutant #11	.681	4.1 ml

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Table 17. Post 4 Hour Incubation Optical Density and Amount of TGY required to achieve an OD_{600} of 5 for 3rd Neutron Experiment

Strain	Post 4 Hour Incubation OD_{600}	Amount of TGY / LB to Add to pellet to achieve OD_{600} of 5
WT (1)	.524	3.1 ml
Mutant #5	.620	3.7 ml
Mutant #8	.585	3.5 ml
Mutant #11	.787	4.7 ml
EC	2.133	12.8 ml

Table 18. Post 4 Hour Incubation Optical Density and Amount of TGY required to achieve an OD_{600} of 5 for Proton Irradiation Experiment

Strain	Post 4 Hour Incubation OD_{600}	Amount of TGY to Add to pellet to achieve OD_{600} of 5
WT (1)	.636	3.8 ml
Mutant #5	.773	4.6 ml
Mutant #8	.630	3.8 ml
Mutant #11	.765	4.6 ml

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Appendix B: Neutron Dose Calculations

2.45 MeV Neutrons										
Element	% Mass	N, atoms/kg	f	σ, cm^2	Nσf			σ, cm	ENDF/B-VII.1	http://www.nndc.bnl.gov/exfor/endf00.jsp
O-16	0.13	2.69E+25	0.111	8.45410E-25	2.524E+00			% Mass	Dr and Solar Wind article	
C-0	0.31	6.41E+24	0.142	1.58290E-24	1.441E+00					
H-1	0.49	5.98E+25	0.5	2.59131E-24	7.748E+01			other	Intro to Health Physics	
N-14	0.07	1.49E+24	0.124	1.30501E-24	2.411E-01				Cember	
				Σ Nσf	8.169E+01	cm^2/kg				
E	2.45	Mev								
Ω/4π	0.16									
S(from Generator)	1.00E+09	neutrons/s								
S(geometric attenuation)	1.60E+08	neutrons/s								
Plate Length	12.78	cm	https://fscimage.fishersci.com/images/D17414~.pdf							
Plate Width	8.55	cm								
Plate Area	109.269	cm^2								
Well Top Diameter	0.686	cm								
Well Top Area	1.478421	cm^2								
Surface Area Per Well	0.0135									
Dose Rate Per Plate	4.689E-05	Gy/s								
Dose Rate Per Well (sample)	6.344E-07	Gy/s								
	Hours	Dose (Gy)	Dose(Sv)							
Dose Per Well (sample)	5	1.1E-02	1.1E-01							
	10	2.3E-02	2.3E-01							
	15	3.4E-02	3.4E-01							
	20	4.6E-02	4.6E-01							

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Appendix C: Proton Dose Calculations

Ionization	0.85	eV/A-Ion						
ev to J	1.60E-19	J/eV				Fluence (Ions / cm^2)	Dose (Gy)	Dose (Sv)
Angstrom to cm	1.00E+08	Angstrom / cm				7.20E+08	1.0E+01	2.1E+02
Dr density	0.9392	g/cm^3				7.20E+09	1.0E+02	2.1E+03
g to kg	1000	g/kg				3.60E+10	5.2E+02	1.0E+04
						7.20E+10	1.0E+03	2.1E+04
						1.80E+11	2.6E+03	5.2E+04
						7.20E+11	1.0E+04	2.1E+05

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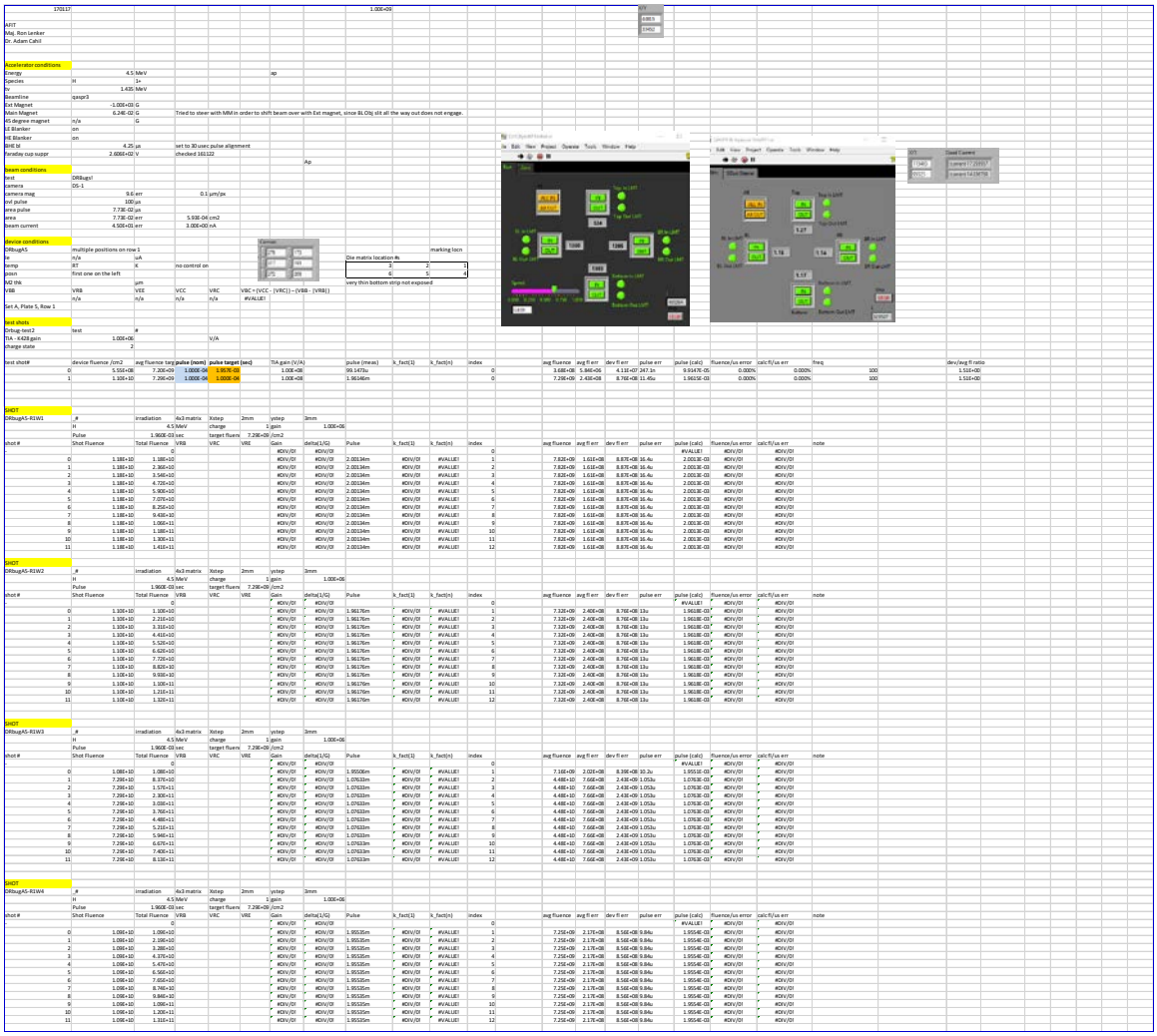
Appendix D: QASAR-3 Parameters



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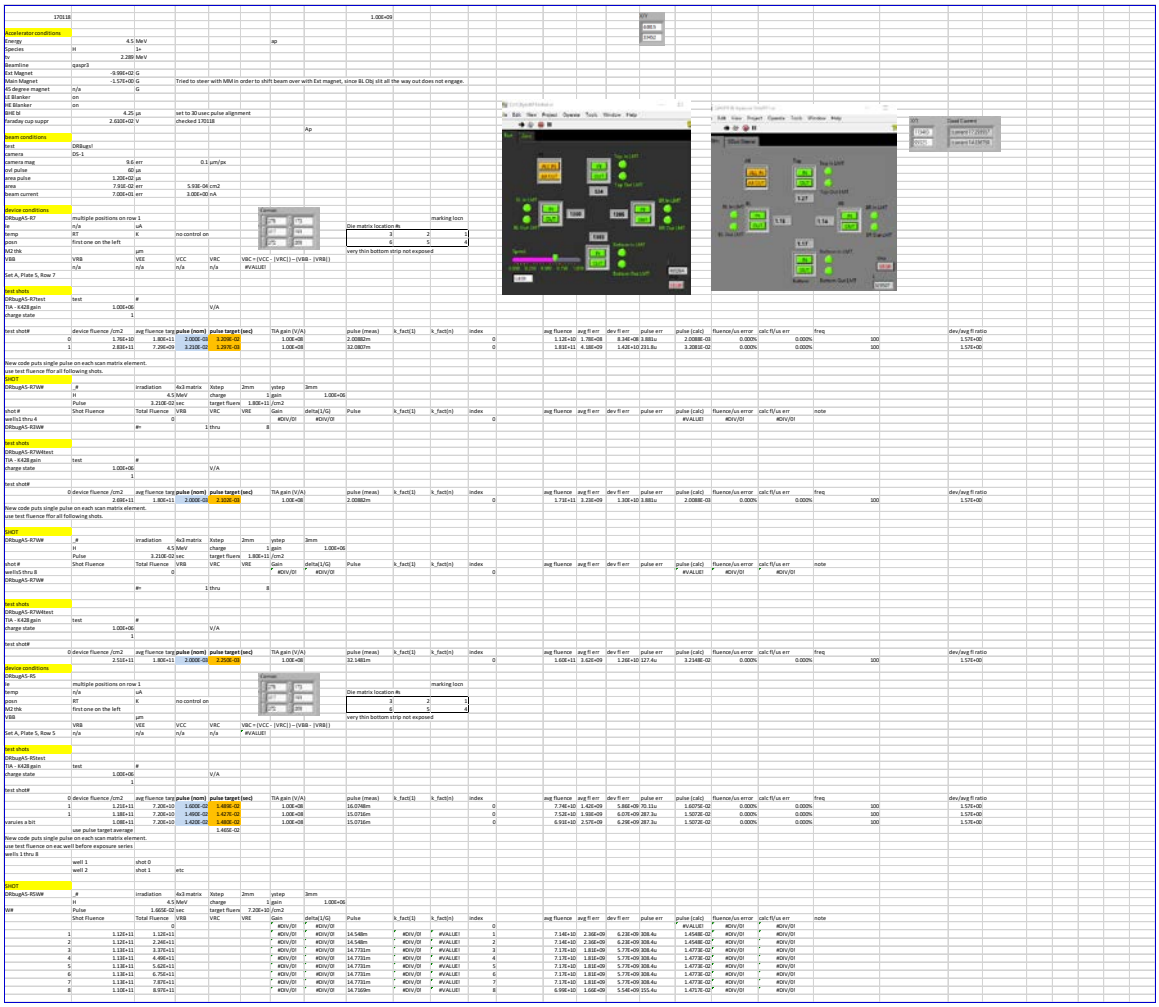

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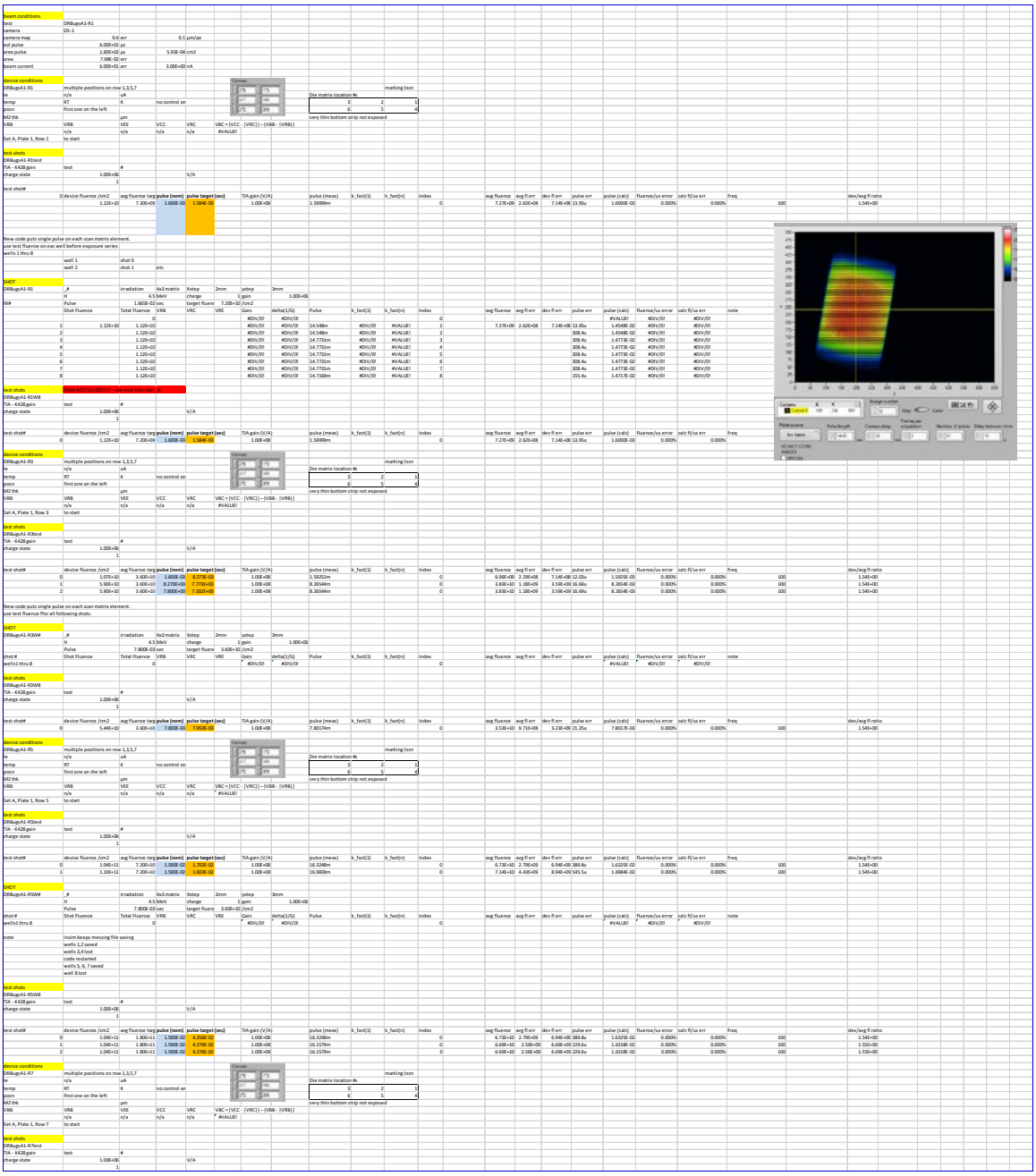

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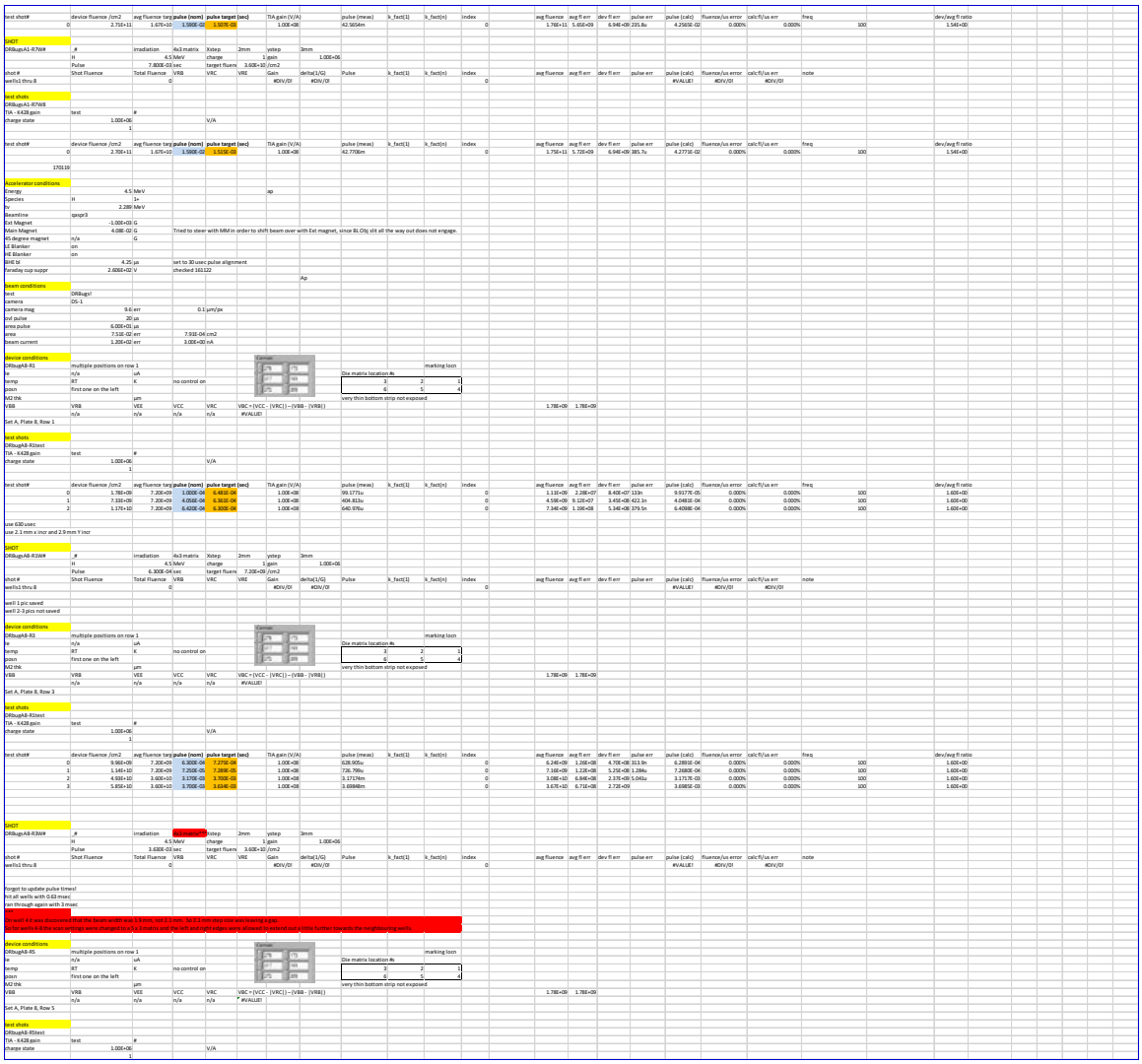

test shot#	device fluence /cm2	avg fluence targ	pulse (nom)	pulse target (sec)	TIA gain (V/A)		pulse (meas)	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	freq		dev/avg fl ratio
0	2.71E+11	1.67E+10	1.590E-02	1.507E-03	1.00E+08		42.5654m			0		1.76E+11	5.65E+09	6.94E+09	235.8u	4.256SE-02	0.000%	0.000%	100		1.54E+00
SHOT																					
DRbugsA1-R7W#	_#	irradiation	4x3 matrix	Xstep	2mm	ystep	3mm														
	H		4.5 MeV	charge		1 gain	1.00E+06														
	Pulse		7.800E-03 sec	target fluenc	1.60E+10	/cm2															
shot #	Shot Fluence	Total Fluence	VRB	VRC	VRE	Gain	delta(1/G)	Pulse	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	note	
wells1 thru 8		0				#DIV/0!	#DIV/0!				0						#VALUE!	#DIV/0!	#DIV/0!		
test shots																					
DRbugsA1-R7W8																					
TIA - 6428 gain	test	#																			
charge state	1.00E+06			V/A																	
	1																				
test shot#	device fluence /cm2	avg fluence targ	pulse (nom)	pulse target (sec)	TIA gain (V/A)		pulse (meas)	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	freq		dev/avg fl ratio
0	2.70E+11	1.67E+10	1.590E-02	1.515E-03	1.00E+08		42.7706m			0		1.75E+11	5.72E+09	6.94E+09	385.7u	4.277 1E-02	0.000%	0.000%	100		1.54E+00
170119																					
Accelerator conditions																					
Energy	4.5 MeV					ap															
Species	H	1+																			
fv	2.289 MeV																				
Beamline	qaspr3																				
Ext Magnet	-1.00E+03 G																				
Main Magnet	4.08E-02 G		Tried to steer with MM in order to shift beam over with Ext magnet, since BL Obj slit all the way out does not engage.																		
45 degree magnet	n/a	G																			
LE Blanker	on																				
ME Blanker	on																				
BME bl	4.25 µs		set to 30 usec pulse alignment																		
Faraday cup suppr	2.606E+02 V		checked 161122																		
						Ap															
Beam conditions																					
test	DRBugs!																				
camera	DS-1																				
camera mag	9.6 err		0.1 µm/px																		
bd pulse	20 µs																				
area pulse	6.00E+01 µs																				
area	7.51E-02 err		7.91E-04 cm2																		
beam current	1.20E+02 err		3.00E+00 nA																		
device conditions					Viewer																
DRbugsA8-R1	multiple positions on row 1									marking locn											
Ir	n/a	uA					Die matrix location #s														
temp	RT	K	no control on					2	2	1											
posn	first one on the left							6	5	4											
M2 thk		µm					very thin bottom strip not exposed														
VRB	VRB	VEE	VCC	VRC	VBC = (VCC - (VRC)) – (VRB - (VRB))							1.78E+09	1.78E+09								
	n/a	n/a	n/a	n/a	#VALUE!																
Set A, Plate 8, Row 1																					
test shots																					
DRbugsA8-R1test																					
TIA - 6428 gain	test	#																			
charge state	1.00E+06			V/A																	
	1																				
test shot#	device fluence /cm2	avg fluence targ	pulse (nom)	pulse target (sec)	TIA gain (V/A)		pulse (meas)	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	freq		dev/avg fl ratio
0	1.78E+09	7.20E+09	1.000E-04	6.481E-04	1.00E+08		99.1771u			0		1.13E+09	2.28E+07	8.40E+07	133n	9.917 7E-05	0.000%	0.000%	100		1.60E+00
1	7.33E+09	7.20E+09	4.050E-04	6.301E-04	1.00E+08		404.811u			0		4.59E+09	9.12E+07	3.45E+08	422.1n	4.0481E-04	0.000%	0.000%	100		1.60E+00
2	1.17E+10	7.20E+09	6.420E-04	6.309E-04	1.00E+08		640.976u			0		7.34E+09	1.19E+08	5.34E+08	379.5n	6.4098E-04	0.000%	0.000%	100		1.60E+00
use 630 usec																					
use 2.1 mm x incr and 2.9 mm Y incr																					
SHOT																					
DRbugsA8-R1W#	_#	irradiation	4x3 matrix	Xstep	2mm	ystep	3mm														
	H		4.5 MeV	charge		1 gain	1.00E+06														
	Pulse		6.300E-04 sec	target fluenc	7.20E+09	/cm2															
shot #	Shot Fluence	Total Fluence	VRB	VRC	VRE	Gain	delta(1/G)	Pulse	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	note	
wells1 thru 8		0				#DIV/0!	#DIV/0!				0						#VALUE!	#DIV/0!	#DIV/0!		
well 1 pic saved																					
well 2-3 pics not saved																					
device conditions					Viewer																
DRbugsA8-R3	multiple positions on row 1									marking locn											
Ir	n/a	uA					Die matrix location #s														
temp	RT	K	no control on					3	2	1											
posn	first one on the left							6	5	4											
M2 thk		µm					very thin bottom strip not exposed														
VRB	VRB	VEE	VCC	VRC	VBC = (VCC - (VRC)) – (VRB - (VRB))							1.78E+09	1.78E+09								
	n/a	n/a	n/a	n/a	#VALUE!																
Set A, Plate 8, Row 3																					
test shots																					
DRbugsA8-R1test																					
TIA - 6428 gain	test	#																			
charge state	1.00E+06			V/A																	
	1																				
test shot#	device fluence /cm2	avg fluence targ	pulse (nom)	pulse target (sec)	TIA gain (V/A)		pulse (meas)	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	freq		dev/avg fl ratio
0	3.66E+09	7.20E+09	8.300E-04	7.275E-04	1.00E+08		626.905u			0		6.24E+09	1.26E+08	4.70E+08	313.9n	6.2891E-04	0.000%	0.000%	100		1.60E+00
1	1.14E+10	7.20E+09	7.250E-05	7.289E-05	1.00E+08		726.799u			0		7.16E+09	1.23E+08	5.25E+08	1.284u	7.2683E-04	0.000%	0.000%	100		1.60E+00
2	4.99E+10	3.60E+10	3.170E-03	3.700E-03	1.00E+08		3.1717m			0		3.08E+10	6.84E+08	2.37E+09	5.041u	3.1717E-03	0.000%	0.000%	100		1.60E+00
3	5.85E+10	3.60E+10	3.700E-03	3.634E-03	1.00E+08		3.69848m			0		3.67E+10	6.71E+08	2.72E+09		3.6985E-03	0.000%	0.000%	100		1.60E+00
SHOT																					
DRbugsA8-R3W#	_#	irradiation	4x3 matrix	Xstep	2mm	ystep	3mm														
	H		4.5 MeV	charge		1 gain	1.00E+06														
	Pulse		3.630E-03 sec	target fluenc	3.60E+10	/cm2															
shot #	Shot Fluence	Total Fluence	VRB	VRC	VRE	Gain	delta(1/G)	Pulse	k_fact(1)	k_fact(n)	index		avg fluence	avg fl err	dev fl err	pulse err	pulse (calc)	fluence/us error	calc fl/us err	note	
wells1 thru 8		0				#DIV/0!	#DIV/0!				0						#VALUE!	#DIV/0!	#DIV/0!		
Forgot to update pulse times!																					
hit all wells with 0.63 msec																					
ran through again with 3 msec																					
As well 4 it was discovered that the beam width was 1.9 mm, not 2.1 mm. So 2.1 mm step size was leaving a gap.																					
In the wells 6-8 the spot settings were changed to 5 x 5 matrix and the left and right edges were allowed to extend out a little further towards the neighbouring wells.																					
device conditions					Viewer																
DRbugsA8-R5	multiple positions on row 1									marking locn											
Ir	n/a	uA					Die matrix location #s														
temp	RT	K	no control on					3	2	1											
posn	first one on the left							6	5	4											
M2 thk		µm					very thin bottom strip not exposed														
VRB	VRB	VEE	VCC	VRC	VBC = (VCC - (VRC)) – (VRB - (VRB))							1.78E+09	1.78E+09								
	n/a	n/a	n/a	n/a	#VALUE!																
Set A, Plate 8, Row 5																					
test shots																					
DRbugsA8-R5test																					
TIA - 6428 gain	test	#																			
charge state	1.00E+06			V/A																	
	1																				

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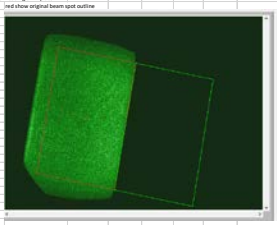

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Appendix E: *Deinococcus Radiodurans* Statistical Analysis

Protocol 4 Analysis

Data

Strain	1 (WT)											
CFU Input	44	49	48									
Untreated Samples	63	50	65	40	44	56	45	40	46	53	62	34
5 Hr Treated Samples	46	39	47	45	25	41	40	38	37	50	30	38
10 Hr Treated Samples	46	38	48	34	30	48	52	36	40	47	37	46
All colony counts at 10^{-4} dilution												

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Untreated}}$	12	$n_{1\text{-Treated 5 Hr}}$	12	$n_{1\text{-Treated 10 Hr}}$	12
	$\bar{x}_{1\text{-CFU input}}$	47.0	$\bar{x}_{1\text{-Untreated}}$	49.8	$\bar{x}_{1\text{-Treated 5 Hr}}$	39.7	$\bar{x}_{1\text{-Treated 10 Hr}}$	41.8
	$s_{1\text{-CFU input}}$	2.6	$s_{1\text{-Untreated}}$	10.1	$s_{1\text{-Treated 5 Hr}}$	7.1	$s_{1\text{-Treated 10 Hr}}$	6.9

Population Comparisons

Comparison Set 1 - Strain 1(WT)

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	86.8974	
t, test statistic	-0.4709	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since -.4709 < 1.771, 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	75.8333	
t, test statistic	2.8597	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since 2.8597 > 1.717, I do reject the null hypothesis, there is a difference between the Untreated and the 5 Hr 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	74.2424	
t, test statistic	2.2743	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since 2.2743 > 1.717, I do reject the null hypothesis, there is a difference between the Untreated and the 10 Hr Treated Populations

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Protocol 4 Analysis

Data

Strain	Mutant #5											
CFU Input	51	42	42									
Untreated Samples	57	34	40	31	39	34	31	23	24	39	42	34
5 Hr Treated Samples	32	39	41	30	35	42	42	29	32	40	34	32
10 Hr Treated Samples	46	30	37	40	37	18	41	41	47	33	34	43
All colony counts at 10^{-4} dilution												

Statistics

	$n_{S\text{-CFU input}}$	3	$n_{S\text{-Untreated}}$	12	$n_{S\text{-Treated 5 Hr}}$	12	$n_{S\text{-Treated 10 Hr}}$	12
	$\bar{x}_{S\text{-CFU input}}$	45.0	$\bar{x}_{S\text{-Untreated}}$	35.7	$\bar{x}_{S\text{-Treated 5 Hr}}$	35.7	$\bar{x}_{S\text{-Treated 10 Hr}}$	37.3
	$s_{S\text{-CFU input}}$	5.2	$s_{S\text{-Untreated}}$	9.0	$s_{S\text{-Treated 5 Hr}}$	4.8	$s_{S\text{-Treated 10 Hr}}$	7.9

Population Comparisons

Comparison Set 2 - Strain 5

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	72.2051	
t, test statistic	1.7016	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 1.7016 < 1.771, 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	51.9697	
t, test statistic	0.0000	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since 0.0000 > 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr 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	71.6780	
t, test statistic	-0.4581	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -0.4581 > 1.717, I do reject the null hypothesis, there is no difference between the Untreated and the 10 Hr Treated Populations

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Protocol 4 Analysis

Data

Strain	Mutant #8											
CFU Input	43	58	42									
Untreated Samples	58	43	42	38	25	28	26	26	41	33	45	44
5 Hr Treated Samples	48	46	39	37	39	37	45	54	41	67	35	34
10 Hr Treated Samples	26	26	31	30	43	28	37	33	31	26	39	36
All colony counts at 10^{-4} dilution												

Statistics

	$n_{8\text{-CFU input}}$	3	$n_{8\text{-Untreated}}$	12	$n_{8\text{-Treated 5 Hr}}$	12	$n_{8\text{-Treated 10 Hr}}$	12
	$\bar{x}_{8\text{-CFU input}}$	47.7	$\bar{x}_{8\text{-Untreated}}$	37.4	$\bar{x}_{8\text{-Treated 5 Hr}}$	43.5	$\bar{x}_{8\text{-Treated 10 Hr}}$	32.2
	$s_{8\text{-CFU input}}$	9.0	$s_{8\text{-Untreated}}$	10.1	$s_{8\text{-Treated 5 Hr}}$	9.5	$s_{8\text{-Treated 10 Hr}}$	5.6

Population Comparisons

Comparison Set 3 - Strain 8

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	97.9679	
t, test statistic	1.6043	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 1.6043 < 1.771, 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	95.3598	
t, test statistic	-1.5259	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -1.5259 > 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr 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	66.1174	
t, test statistic	1.5815	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since 1.5815 > 1.717, I do reject the null hypothesis, there is no difference between the Untreated and the 10 Hr Treated Populations

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Protocol 4 Analysis

Data

Strain	Mutant #11											
CFU Input	37	37	38									
Untreated Samples	43	30	32	42	21	32	30	25	21	32	20	33
5 Hr Treated Samples	31	31	26	28	42	44	38	37	29	47	40	21
10 Hr Treated Samples	44	36	34	38	42	44	39	46	38	42	40	33
All colony counts at 10^{-4} dilution												

Statistics

	$n_{11\text{-CFU input}}$	3	$n_{11\text{-Untreated}}$	12	$n_{11\text{-Treated 5 Hr}}$	12	$n_{11\text{-Treated 10 Hr}}$	12
	$\bar{x}_{11\text{-CFU input}}$	37.3	$\bar{x}_{11\text{-Untreated}}$	30.1	$\bar{x}_{11\text{-Treated 5 Hr}}$	34.5	$\bar{x}_{11\text{-Treated 10 Hr}}$	39.7
	$s_{11\text{-CFU input}}$	0.6	$s_{11\text{-Untreated}}$	7.5	$s_{11\text{-Treated 5 Hr}}$	8.0	$s_{11\text{-Treated 10 Hr}}$	4.1

Population Comparisons

Comparison Set 4 - Strain 11

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	47.8141	
t, test statistic	1.6243	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 1.6243 < 1.771, 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	60.1780	
t, test statistic	-1.3946	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -1.3946 > 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr 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	36.6174	
t, test statistic	-3.8793	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -3.8793 > 1.717, I do reject the null hypothesis, there is no difference between the Untreated and the 10 Hr Treated Populations

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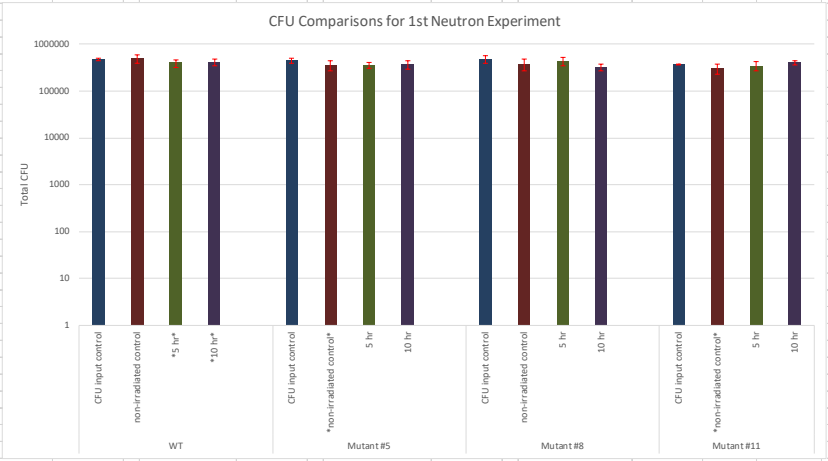
WT								**WT**	CFU input control	470000	26457.51311
n1 -CFU input	3	n1 -Untreated	12	n1 -Treated 5 Hr	12	n1 -Treated 10 Hr	12		non-irradiated control	498333.3	100709.6035
x-bar1-CFU input	47	x-bar1-Untreated	49.83333333	x-bar1-Treated 5 Hr	39.66666667	x-bar1-Treated 10 Hr	41.83333333		*5 hr*	396666.7	70881.89066
s1-CFU input	2.645751311	s1-Untreated	10.07096035	s1-Treated 5 Hr	7.088189066	s1-Treated 10 Hr	6.860073328		*10 hr*	418333.3	68600.73328
Mutant #5								**Mutant #5**	CFU input control	450000	51961.52423
n5 -CFU input	3	n5 -Untreated	12	n5 -Treated 5 Hr	12	n5 -Treated 10 Hr	12		*non-irradiated control*	356666.7	89679.56424
x-bar5-CFU input	45	x-bar5-Untreated	35.66666667	x-bar5-Treated 5 Hr	35.66666667	x-bar5-Treated 10 Hr	37.25		5 hr	356666.7	48492.42365
s5-CFU input	5.196152423	s5-Untreated	8.967956424	s5-Treated 5 Hr	4.849242365	s5-Treated 10 Hr	7.93295772		10 hr	372500	79329.5772
Mutant #8								**Mutant #8**	CFU input control	476666.7	89628.8644
n8 -CFU input	3	n8 -Untreated	12	n8 -Treated 5 Hr	12	n8 -Treated 10 Hr	12		non-irradiated control	374166.7	100585.4077
x-bar8-CFU input	47.66666667	x-bar8-Untreated	37.41666667	x-bar8-Treated 5 Hr	43.5	x-bar8-Treated 10 Hr	32.16666667		5 hr	435000	94628.46007
s8-CFU input	8.96288644	s8-Untreated	10.05854077	s8-Treated 5 Hr	9.462846007	s8-Treated 10 Hr	5.57320429		10 hr	321666.7	55732.0429
Mutant #11								**Mutant #11**	CFU input control	373333.3	5773.502692
n11 -CFU input	3	n11 -Untreated	12	n11 -Treated 5 Hr	12	n11 -Treated 10 Hr	12		*non-irradiated control*	300833.3	75131.19838
x-bar11-CFU input	37.33333333	x-bar11-Untreated	30.08333333	x-bar11-Treated 5 Hr	34.5	x-bar11-Treated 10 Hr	39.66666667		5 hr	345000	79943.16163
s11-CFU input	0.577350269	s11-Untreated	7.513119838	s11-Treated 5 Hr	7.994316163	s11-Treated 10 Hr	4.097301403		10 hr	396666.7	40973.01403



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Protocol 5 Analysis

Data

Strain	1 (WT)											
CFU Input	46	41	47									
Untreated Samples	36	37	36	25	33	28	40	40	36	38	47	59
15 Hr Treated Samples	23	34	48	18	46	49	42	50	61	36	55	48
20 Hr Treated Samples	35	46	42	41	31	27	42	46	46	39	54	40
All colony counts at 10^{-4} dilution												

Statistics

	$n_{1\text{-CFU input}}$	3	$n_{1\text{-Untreated}}$	12	$n_{1\text{-Treated 15 Hr}}$	12	$n_{1\text{-Treated 20 Hr}}$	12
	$\bar{x}_{1\text{-CFU input}}$	44.7	$\bar{x}_{1\text{-Untreated}}$	37.9	$\bar{x}_{1\text{-Treated 15 Hr}}$	42.5	$\bar{x}_{1\text{-Treated 20 Hr}}$	40.8
	$s_{1\text{-CFU input}}$	3.2	$s_{1\text{-Untreated}}$	8.7	$s_{1\text{-Treated 15 Hr}}$	12.7	$s_{1\text{-Treated 20 Hr}}$	7.3

Population Comparisons

Comparison Set 1 - Strain 1(WT)

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	65.9679	
t, test statistic	1.2875	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 1.2875 < 1.771, 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	118.2689	
t, test statistic	-1.0323	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -1.0323 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 15 Hr 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	64.5076	
t, test statistic	-0.8641	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -0.8641 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 5 Analysis

Data

Strain	Mutant #5											
CFU Input	34	48	41									
Untreated Samples	33	44	30	17	21	43	17	30	36	33	31	20
15 Hr Treated Samples	27	28	40	42	38	30	41	32	33	39	20	26
20 Hr Treated Samples	32	33	39	30	30	28	32	22	24	27	33	26
All colony counts at 10^{-4} dilution												

Statistics

	$n_{5\text{-CFU input}}$	3	$n_{5\text{-Untreated}}$	12	$n_{5\text{-Treated 15 Hr}}$	12	$n_{5\text{-Treated 20 Hr}}$	12
	$\bar{x}_{5\text{-CFU input}}$	41.0	$\bar{x}_{5\text{-Untreated}}$	29.6	$\bar{x}_{5\text{-Treated 15 Hr}}$	33.0	$\bar{x}_{5\text{-Treated 20 Hr}}$	29.7
	$s_{5\text{-CFU input}}$	7.0	$s_{5\text{-Untreated}}$	9.2	$s_{5\text{-Treated 15 Hr}}$	7.0	$s_{5\text{-Treated 20 Hr}}$	4.6

Population Comparisons

Comparison Set 1 - Strain 5

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	79.6090	
t, test statistic	1.9823	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 1.9823 > 1.771, 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	67.3144	
t, test statistic	-1.0201	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -1.0201 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 15 Hr 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	53.2538	
t, test statistic	-0.0280	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -0.280 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 5 Analysis

Data

Strain	Mutant #8											
CFU Input	47	39	46									
Untreated Samples	22	20	31	20	20	29	30	21	27	31	40	34
15 Hr Treated Samples	37	43	23	31	40	54	36	31	27	32	24	20
20 Hr Treated Samples	28	36	29	33	26	35	18	18	5	18	30	31
All colony counts at 10^{-4} dilution												

Statistics

	$n_{8\text{-CFU input}}$	3	$n_{8\text{-Untreated}}$	12	$n_{8\text{-Treated 15 Hr}}$	12	$n_{8\text{-Treated 20 Hr}}$	12
	$\bar{x}_{8\text{-CFU input}}$	44.0	$\bar{x}_{8\text{-Untreated}}$	27.1	$\bar{x}_{8\text{-Treated 15 Hr}}$	33.2	$\bar{x}_{8\text{-Treated 20 Hr}}$	25.6
	$s_{8\text{-CFU input}}$	4.4	$s_{8\text{-Untreated}}$	6.5	$s_{8\text{-Treated 15 Hr}}$	9.6	$s_{8\text{-Treated 20 Hr}}$	9.1

Population Comparisons

Comparison Set 3 - Strain 8

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	39.1474	
t, test statistic	4.1886	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since 4.1886 > 1.771, 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	67.2992	
t, test statistic	-1.8164	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since -1.8164 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 15 Hr 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	62.9924	
t, test statistic	0.4629	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since .4629 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 10 Hr Treated Populations

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Protocol 5 Analysis

Data

Strain	Mutant #11											
CFU Input	38	46	38									
Untreated Samples	50	37	44	33	38	42	56	42	34	52	47	20
15 Hr Treated Samples	36	33	37	55	43	34	46	35	43	38	36	30
20 Hr Treated Samples	41	45	39	40	37	37	29	50	39	46	30	25
All colony counts at 10^{-4} dilution												

Statistics

	$n_{11\text{-CFU input}}$	3	$n_{11\text{-Untreated}}$	12	$n_{11\text{-Treated 15 Hr}}$	12	$n_{11\text{-Treated 20 Hr}}$	12
	$\bar{x}_{11\text{-CFU input}}$	40.7	$\bar{x}_{11\text{-Untreated}}$	41.3	$\bar{x}_{11\text{-Treated 15 Hr}}$	38.8	$\bar{x}_{11\text{-Treated 20 Hr}}$	38.2
	$s_{11\text{-CFU input}}$	4.6	$s_{11\text{-Untreated}}$	9.8	$s_{11\text{-Treated 15 Hr}}$	6.9	$s_{11\text{-Treated 20 Hr}}$	7.3

Population Comparisons

Comparison Set 4 - Strain 11

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	84.2244	
t, test statistic	-0.0985	
rejection region	$t > t_\alpha$	
α	0.05	
df	13	
t_α	1.771	
p-value	0.519558773	

Since -.0985 < 1.771, 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	71.3598	
t, test statistic	0.7008	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since .07008 < 1.717, I do not reject the null hypothesis, there is no difference between the Untreated and the 15 Hr 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	74.5417	
t, test statistic	0.8748	
rejection region	$t > t_\alpha$	
α	0.05	
df	22	
t_α	1.717	
p-value	0.519713215	

Since 0.8748 > 1.717, I do reject the null hypothesis, there is a difference between the Untreated and the 20 Hr Treated Populations

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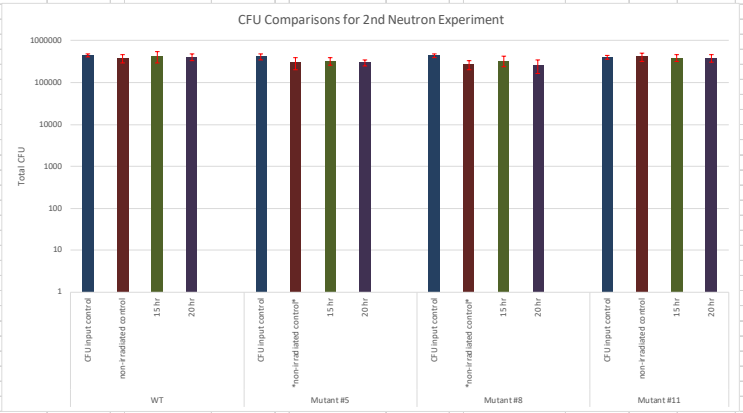
													CFU	Std. Dev.
	WT										**WT**	CFU input control	446666.7	32145.50254
All colony counts at 10^{-4} dilution	n1 -CFU input	3	0	n1 -Untreated	12	0	n1 -Treated 15 Hr	12	0	n1 -Treated 20 Hr	12		non-irradiated control	379166.7	87225.76072
10000	x-bar1-CFU input	44.66666667	0	x-bar1-Untreated	37.91666667	0	x-bar1-Treated 15 Hr	42.5	0	x-bar1-Treated 20 Hr	40.75		15 hr	425000	126670.6538
	s1-CFU input	3.214550254	0	s1-Untreated	8.722576072	0	s1-Treated 15 Hr	12.66706538	0	s1-Treated 20 Hr	7.275425636		20 hr	407500	72754.25636
	Mutant #5											**Mutant #5**	CFU input control	410000	70000
	n5 -CFU input	3	0	n5 -Untreated	12	0	n5 -Treated 15 Hr	12	0	n5 -Treated 20 Hr	12		*non-irradiated control	295833.3	92289.89242
	x-bar5-CFU input	41	0	x-bar5-Untreated	29.58333333	0	x-bar5-Treated 15 Hr	33	0	x-bar5-Treated 20 Hr	29.66666667		15 hr	330000	70323.92584
	s5-CFU input	7	0	s5-Untreated	9.228989242	0	s5-Treated 15 Hr	7.032392584	0	s5-Treated 20 Hr	4.618802154		20 hr	296666.7	46188.02154
	Mutant #8											**Mutant #8**	CFU input control	440000	43588.98944
	n8 -CFU input	3	0	n8 -Untreated	12	0	n8 -Treated 15 Hr	12	0	n8 -Treated 20 Hr	12		*non-irradiated control	270833.3	65429.81435
	x-bar8-CFU input	44	0	x-bar8-Untreated	27.08333333	0	x-bar8-Treated 15 Hr	33.16666667	0	x-bar8-Treated 20 Hr	25.58333333		15 hr	331666.7	95805.99083
	s8-CFU input	4.358898944	0	s8-Untreated	6.542981435	0	s8-Treated 15 Hr	9.580599083	0	s8-Treated 20 Hr	9.119991361		20 hr	255833.3	91199.91361
	Mutant #11											**Mutant #11**	CFU input control	406666.7	46188.02154
	n11 -CFU input	3	0	n11 -Untreated	12	0	n11 -Treated 15 Hr	12	0	n11 -Treated 20 Hr	12		non-irradiated control	412500	97805.46555
	x-bar11-CFU input	40.66666667	0	x-bar11-Untreated	41.25	0	x-bar11-Treated 15 Hr	38.83333333	0	x-bar11-Treated 20 Hr	38.16666667		15 hr	388333.3	68600.73328
	s11-CFU input	4.618802154	0	s11-Untreated	9.780546555	0	s11-Treated 15 Hr	6.860073328	0	s11-Treated 20 Hr	7.309188903		20 hr	381666.7	73091.88903



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Protocol 6 Analysis

Data

Strain	1 (WT)			
Untreated Samples	332	415	352	410
5 Hr Treated Samples	423	167	387	473
10 Hr Treated Samples	391	415	420	439
15 Hr Treated Samples	348	356	322	366
20 Hr Treated Samples	473	447	407	437

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{-Untreated}}$	4	$n_{1\text{-Treated 5 Hr}}$	4	$n_{1\text{-Treated 10 Hr}}$	4	$n_{1\text{-Treated 15 Hr}}$	4	$n_{1\text{-Treated 20}}$	4
	$\bar{x}_{1\text{-Untreated}}$	377.3	$\bar{x}_{1\text{-Treated 5 H}}$	362.5	$\bar{x}_{1\text{-Treated 10 Hr}}$	416.3	$\bar{x}_{1\text{-Treated 15 Hr}}$	348.0	$\bar{x}_{1\text{-Treat}}$	441.0
	$s_{1\text{-Untreated}}$	41.6	$s_{1\text{-Treated 5 Hr}}$	135.0	$s_{1\text{-Treated 10 Hr}}$	19.8	$s_{1\text{-Treated 15 Hr}}$	18.8	$s_{1\text{-Treated 20 H}}$	27.3

Population Comparisons

Comparison Set 1 - Strain 1(WT)

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 5 Hr Treated Population
H_a :	$\mu_{1\text{-Untreated}} - \mu_{1\text{-Treated 5 Hr}} > 0$	Alternate Hypothesis = There is a difference between the Untreated population and the 5 Hr Treated Population
s_p^2	9978.9583	
t, test statistic	0.2088	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since 0.2088 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr 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 10 Hr Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 10 Hr}} > 0$	Alternate Hypothesis = There is a difference between the Untreated population and the 10 Hr Treated Population
s_p^2	1058.9167	
t, test statistic	-1.6949	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since -1.6949 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 10 Hr Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 15 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the 15 Hr Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 15 Hr}} > 0$	Alternate Hypothesis = There is a difference between the Untreated population and the 15 Hr Treated Population
s_p^2	1041.1250	
t, test statistic	1.2820	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since 1.2820 < 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 15 Hr Treated Populations

H_0 :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 15 Hr}} = 0$	Null Hypothesis = There is no difference between the Untreated population and the 20 Hr Treated population
H_a :	$\mu_{1\text{-untreated}} - \mu_{1\text{-treated 15 Hr}} > 0$	Alternate Hypothesis = There is a difference between the Untreated population and the 20 Hr Treated Population
s_p^2	1235.7917	
t, test statistic	-2.5646	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since -2.5646 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 6 Analysis

Data

Strain	Mutant #5			
Untreated Samples	332	353	354	334
5 Hr Treated Samples	372	365	335	315
10 Hr Treated Samples	295	310	315	311
15 Hr Treated Samples	345	404	398	389
20 Hr Treated Samples	356	298	313	348

All colony counts at $10^{-5\ dilution}$

Statistics

	$n_{5\text{-Untreated}}$	4	$n_{5\text{-Treated 5 Hr}}$	4	$n_{5\text{-Treated 10 Hr}}$	4	$n_{5\text{-Treated 15 Hr}}$	4	$n_{5\text{-Treated 20 Hr}}$	4
	$\bar{x}_{5\text{-Untreated}}$	343.3	$\bar{x}_{5\text{-Treated 5 Hr}}$	346.8	$\bar{x}_{5\text{-Treated 10 Hr}}$	307.8	$\bar{x}_{5\text{-Treated 15 Hr}}$	384.0	$\bar{x}_{5\text{-Treated 20 Hr}}$	328.8
	$s_{5\text{-Untreated}}$	11.9	$s_{5\text{-Treated 5 Hr}}$	26.6	$s_{5\text{-Treated 10 Hr}}$	8.8	$s_{5\text{-Treated 15 Hr}}$	26.7	$s_{5\text{-Treated 20 Hr}}$	27.7

Population Comparisons

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

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

s_p^2	423.2500
t, test statistic	-0.2406
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.2406 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr Treated Populations

H_0 : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 10 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 10 Hr Treated population

H_a : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 10 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 10 Hr Treated Population

s_p^2	108.9167
t, test statistic	4.8106
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 4.8106 > 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 10 Hr Treated Populations

H_0 : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 15 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 15 Hr Treated population

H_a : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 15 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 15 Hr Treated Population

s_p^2	427.4583
t, test statistic	-2.7874
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -2.7874 < 1.943, I do not reject the null hypothesis, there is not difference between the Untreated and the 15 Hr Treated Populations

H_0 : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 15 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 20 Hr Treated population

H_a : $\mu_{5\text{-untreated}} - \mu_{5\text{-treated 15 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 20 Hr Treated Population

s_p^2	454.9167
t, test statistic	0.9614
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 0.9614 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 6 Analysis

Data

Strain	Mutant #8			
Untreated Samples	333	311	382	276
5 Hr Treated Samples	317	364	345	307
10 Hr Treated Samples	333	331	304	317
15 Hr Treated Samples	316	329	300	285
20 Hr Treated Samples	233	332	320	269

All colony counts at $10^{-5\ dilution}$

Statistics

	$n_{8\text{-Untreated}}$	4	$n_{8\text{-Treated 5 Hr}}$	4	$n_{8\text{-Treated 10 Hr}}$	4	$n_{8\text{-Treated 15 Hr}}$	4	$n_{8\text{-Treated 20 Hr}}$	4
	$\bar{x}_{8\text{-Untreated}}$	325.5	$\bar{x}_{8\text{-Treated 5 Hr}}$	333.3	$\bar{x}_{8\text{-Treated 10 Hr}}$	321.3	$\bar{x}_{8\text{-Treated 15 Hr}}$	307.5	$\bar{x}_{8\text{-Treated 20 Hr}}$	288.5
	$s_{8\text{-Untreated}}$	44.4	$s_{8\text{-Treated 5 Hr}}$	26.1	$s_{8\text{-Treated 10 Hr}}$	13.5	$s_{8\text{-Treated 15 Hr}}$	19.1	$s_{8\text{-Treated 20 Hr}}$	46.0

Population Comparisons

Comparison Set 1 - Strain 1(WT)

H_0 : $\mu_{8\text{-Untreated}} - \mu_{8\text{-Treated 5 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 5 Hr Treated Population

H_a : $\mu_{8\text{-Untreated}} - \mu_{8\text{-Treated 5 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 5 Hr Treated Population

s_p^2	1324.2917
t, test statistic	-0.3012
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since -.3012 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr Treated Populations

H_0 : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 10 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 10 Hr Treated population

H_a : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 10 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 10 Hr Treated Population

s_p^2	1076.2917
t, test statistic	0.1832
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since .1832 < 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 10 Hr Treated Populations

H_0 : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 15 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 15 Hr Treated population

H_a : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 15 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 15 Hr Treated Population

s_p^2	1167.6667
t, test statistic	0.7450
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since .7450 < 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 15 Hr Treated Populations

H_0 : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 15 Hr}} = 0$ Null Hypothesis = There is no difference between the Untreated population and the 20 Hr Treated population

H_a : $\mu_{8\text{-untreated}} - \mu_{8\text{-treated 15 Hr}} > 0$ Alternate Hypothesis = There is a difference between the Untreated population and the 20 Hr Treated Population

s_p^2	2042.3333
t, test statistic	1.1579
rejection region	$t > t_\alpha$
α	0.05
df	6
t_α	1.943
p-value	0.519127341

Since 1.1579 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 6 Analysis

Data

Strain	Mutant #11			
Untreated Samples	295	396	306	358
5 Hr Treated Samples	349	420	371	421
10 Hr Treated Samples	339	411	330	269
15 Hr Treated Samples	416	435	407	486
20 Hr Treated Samples	226	301	319	326

All colony counts at 10^{-5} dilution

Statistics

$n_{11\text{-Untreated}}$	4	$n_{11\text{-Treated 5 Hr}}$	4	$n_{11\text{-Treated 10 Hr}}$	4	$n_{11\text{-Treated 15 Hr}}$	4	$n_{11\text{-Treated 20 Hr}}$	4
$\bar{x}_{11\text{-Untreated}}$	338.8	$\bar{x}_{11\text{-Treated 5}}$	390.3	$\bar{x}_{11\text{-Treated 10 H}}$	337.3	$\bar{x}_{11\text{-Treated 15 Hr}}$	436.0	$\bar{x}_{11\text{-Treated 20 Hr}}$	293.0
$s_{11\text{-Untreated}}$	47.0	$s_{11\text{-Treated 5 Hr}}$	36.1	$s_{11\text{-Treated 10 Hr}}$	58.2	$s_{11\text{-Treated 15 Hr}}$	35.3	$s_{11\text{-Treated 20 Hr}}$	45.9

Population Comparisons

Comparison Set 1 - Strain 1(WT)

H_0 :	$\mu_{11\text{-Untreated}} - \mu_{11\text{- Treated 5 Hr}} =$	Null Hypothesis = There is no difference between the Untreated population and the 5 Hr Treated Population
H_a :	$\mu_{11\text{-Untreated}} - \mu_{11\text{-Treated 5 Hr}} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 5 Hr Treated Population
s_p^2	1756.2500	
t, test statistic	-1.7379	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since -1.7379 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 5 Hr Treated Populations

H_0 :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 10 Hr}} =$	Null Hypothesis = There is no difference between the Untreated population and the 10 Hr Treated population
H_a :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 10 Hr}} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 10 Hr Treated Population
s_p^2	2797.9167	
t, test statistic	0.0401	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since .0401 < 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 10 Hr Treated Populations

H_0 :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 15 Hr}} =$	Null Hypothesis = There is no difference between the Untreated population and the 15 Hr Treated population
H_a :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 15 Hr}} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 15 Hr Treated Population
s_p^2	1729.4583	
t, test statistic	-3.3071	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since -3.3071 < 1.943, I do reject the null hypothesis, there is a difference between the Untreated and the 15 Hr Treated Populations

H_0 :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 15 Hr}} =$	Null Hypothesis = There is no difference between the Untreated population and the 20 Hr Treated population
H_a :	$\mu_{11\text{-untreated}} - \mu_{11\text{-treated 15 Hr}} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 20 Hr Treated Population
s_p^2	2158.7917	
t, test statistic	1.3925	
rejection region	$t > t_\alpha$	
α	0.05	
df	6	
t_α	1.943	
p-value	0.519127341	

Since 1.3925 < 1.943, I do not reject the null hypothesis, there is no difference between the Untreated and the 20 Hr Treated Populations

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Protocol 6 Analysis

Data

Strain	EC										
Untreated Samples	15	23	11	16	24	11	13	13			colony counts at 10^{-2} dilution
5 Hr Treated Samples	2	4	3	3	5	7	4	4			colony counts at 10^{-2} dilution
10 Hr Treated Samples	2	5	3	4	8	4	2	4			colony counts at 10^{-2} dilution
15 Hr Treated Samples	1	1	7	3	2	1	3	2			colony counts at 10^{-2} dilution
20 Hr Treated Samples	8	5	10	12	7	3	17	9			colony counts at 10^{-2} dilution

Statistics

	$n_{EC\text{-}Untreated}$	8	$n_{EC\text{-}Treated\ 5\ Hr}$	8	$n_{EC\text{-}Treated\ 10\ Hr}$	8	$n_{EC\text{-}Treated\ 15\ Hr}$	8	$n_{EC\text{-}Treated\ 20\ Hr}$	8
	$\bar{x}_{EC\text{-}Untreated}$	15.8	$\bar{x}_{EC\text{-}Treated\ 5}$	4.0	$\bar{x}_{EC\text{-}Treated\ 10\ H}$	4.0	$\bar{x}_{EC\text{-}Treated\ 15\ Hr}$	2.5	$\bar{x}_{EC\text{-}Treated\ 20\ Hr}$	8.9
	$s_{EC\text{-}Untreated}$	5.1	$s_{EC\text{-}Treated\ 5\ Hr}$	1.5	$s_{EC\text{-}Treated\ 10\ Hr}$	1.9	$s_{EC\text{-}Treated\ 15\ Hr}$	2.0	$s_{EC\text{-}Treated\ 20\ Hr}$	4.3

Population Comparisons

Comparison Set 1 - Strain 1(WT)

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

s_p^2	14.1071
t, test statistic	6.2567
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 6.2567 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the 5 Hr Treated Populations

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

s_p^2	14.8214
t, test statistic	6.1041
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 6.1041 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the 10 Hr Treated Populations

H_0 :	$\mu_{EC\text{-}Untreated} - \mu_{EC\text{-}Treated\ 15\ Hr} =$	Null Hypothesis = There is no difference between the Untreated population and the 15 Hr Treated population
H_a :	$\mu_{EC\text{-}Untreated} - \mu_{EC\text{-}Treated\ 15\ Hr} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 15 Hr Treated Population

s_p^2	14.9643
t, test statistic	6.8504
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 6.8504 > 1.761, I do reject the null hypothesis, there is a difference between the Untreated and the 15 Hr Treated Populations

H_0 :	$\mu_{EC\text{-}Untreated} - \mu_{EC\text{-}Ctreated\ 15\ Hr} =$	Null Hypothesis = There is no difference between the Untreated population and the 20 Hr Treated population
H_a :	$\mu_{EC\text{-}Untreated} - \mu_{EC\text{-}treated\ 15\ Hr} >$	Alternate Hypothesis = There is a difference between the Untreated population and the 20 Hr Treated Population

s_p^2	22.3125
t, test statistic	2.9109
rejection region	$t > t_\alpha$
α	0.05
df	14
t_α	1.761
p-value	0.519585629

Since 2.9109 > 1.761 I do reject the null hypothesis, there is a difference between the Untreated and the 20 Hr Treated Populations

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WT

n1 -Untreated	4	n1 -Treated 5 Hr	4	n1 -Treated 10 Hr	4	n1 -Treated 15 Hr	4	n1 -Treated 20 Hr	4		
x-bar1-Untreated	377.25	x-bar1-Treated 5 Hr	362.5	x-bar1-Treated 10 Hr	416.25	x-bar1-Treated 15 Hr	348	x-bar1-Treated 20 Hr	441		
s1-Untreated	41.56420736	s1-Treated 5 Hr	135.0197516	s1-Treated 10 Hr	19.75474627	s1-Treated 15 Hr	18.83259586	s1-Treated 20 Hr	27.27636339		

Mutant #5

n5 -Untreated	4	n5 -Treated 5 Hr	4	n5 -Treated 10 Hr	4	n5 -Treated 15 Hr	4	n5 -Treated 20 Hr	4		
x-bar5-Untreated	343.25	x-bar5-Treated 5 Hr	346.75	x-bar5-Treated 10 Hr	307.75	x-bar5-Treated 15 Hr	384	x-bar5-Treated 20 Hr	328.75		
s5-Untreated	11.8708326	s5-Treated 5 Hr	26.56281863	s5-Treated 10 Hr	8.770214745	s5-Treated 15 Hr	26.72077843	s5-Treated 20 Hr	27.72934667		

Mutant #8

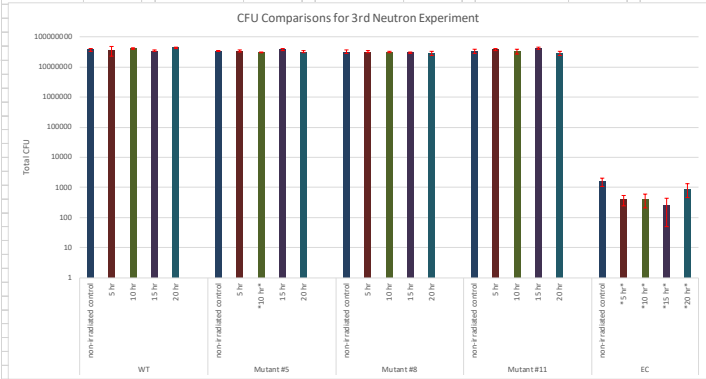
n8 -Untreated	4	n8 -Treated 5 Hr	4	n8 -Treated 10 Hr	4	n8 -Treated 15 Hr	4	n8 -Treated 20 Hr	4		
x-bar8-Untreated	325.5	x-bar8-Treated 5 Hr	333.25	x-bar8-Treated 10 Hr	321.25	x-bar8-Treated 15 Hr	307.5	x-bar8-Treated 20 Hr	288.5		
s8-Untreated	44.38092683	s8-Treated 5 Hr	26.05602937	s8-Treated 10 Hr	13.52466882	s8-Treated 15 Hr	19.12241268	s8-Treated 20 Hr	45.98912915		

Mutant #11

n11 -Untreated	4	n11 -Treated 5 Hr	4	n11 -Treated 10 Hr	4	n11-Treated 15 Hr	4	n11-Treated 20 Hr	4		
x-bar11-Untreated	338.75	x-bar11-Treated 5 Hr	390.25	x-bar11-Treated 10 Hr	337.25	x-bar11-Treated 15 Hr	436	x-bar11-Treated 20 Hr	293		
s11-Untreated	47.02747424	s11-Treated 5 Hr	36.06822239	s11-Treated 10 Hr	58.17430704	s11-Treated 15 Hr	35.31760656	s11-Treated 20 Hr	45.89117562		

EC

nEC -Untreated	8	nEC -Treated 5 Hr	8	nEC -Treated 10 Hr	8	nEC-Treated 15 Hr	8	nEC-Treated 20 Hr	8		
x-barEC-Untreated	15.75	x-barEC-Treated 5 Hr	4	x-barEC-Treated 10 Hr	4	x-barEC-Treated 15 Hr	2.5	x-barEC-Treated 20 Hr	8.875		
sEC-Untreated	5.092010549	sEC-Treated 5 Hr	1.511857892	sEC-Treated 10 Hr	1.927248223	sEC-Treated 15 Hr	2	sEC-Treated 20 Hr	4.323936698		



		CFU	Std. Dev.
WT	non-irradiated control	37725000	4156420.736
	5 hr	36250000	13501975.16
	10 hr	41625000	1975474.627
	15 hr	34800000	1883259.586
	20 hr	44100000	2727636.339
Mutant #5	non-irradiated control	34325000	1187083.26
	5 hr	34675000	2656281.863
	10 hr	30775000	877021.4745
	15 hr	38400000	2672077.843
	20 hr	32875000	2772934.667
Mutant #8	non-irradiated control	32550000	4438092.683
	5 hr	33325000	2605602.937
	10 hr	32125000	1352466.882
	15 hr	30750000	1912241.268
	20 hr	28850000	4598912.915
Mutant #11	non-irradiated control	33875000	4702747.424
	5 hr	39025000	3606822.239
	10 hr	33725000	5817430.704
	15 hr	43600000	3531760.656
	20 hr	29300000	4589117.562
EC	non-irradiated control	1575	509.2010549
	5 hr	400	151.1857892
	10 hr	400	192.7248223
	15 hr	250	200
	20 hr	887.5	432.3936698

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Protocol 7 Analysis

Data

Strain	1 (WT)								
Control 1 - CFU Input	16	16	11						
Control 2 - Untreated, No Vacuum	10	13	12	12	9	21			
Control 3 - Untreated. Vacuum	8	8	12	5	8	6	9		
10 Gy Treated Samples	10	4	5	6	10	6	11		
100 Gy Treated Samples	7	11	8	12	13	13	9	12	
500 Gy Treated Samples	6	8	9	4	5	9	9	7	
1000 Gy Treated Samples	8	11	14	12	8	8	11	8	
2500 Gy Treated Samples	8	8	15	7	8	8	1	15	
10000 Treated Samples	7	8	14	16	9	12	12	1	

All colony counts at 10^{-5} dilution

Statistics

	$n_{1\text{- control 1}}$	3	$n_{1\text{- control 2}}$	6	$n_{1\text{- control 3}}$	7	$n_{1\text{- 10 Gy}}$	7	$n_{1\text{- 100 Gy}}$	8	$n_{1\text{- 500 Gy}}$	8	$n_{1\text{- 1000 Gy}}$	8	$n_{1\text{- 2500 Gy}}$	8	$n_{1\text{- 10000 Gy}}$	8
	$\bar{x}_{1\text{-control 1}}$	14.3	$\bar{x}_{1\text{-control 2}}$	12.8	$\bar{x}_{1\text{-control 3}}$	8.0	$\bar{x}_{1\text{-10 Gy}}$	7.4	$\bar{x}_{1\text{-100 Gy}}$	10.6	$\bar{x}_{1\text{-500 Gy}}$	7.1	$\bar{x}_{1\text{-1000 Gy}}$	10.0	$\bar{x}_{1\text{-2500 Gy}}$	8.8	$\bar{x}_{1\text{-10000 Gy}}$	9.9
	$s_{1\text{-control 1}}$	2.9	$s_{1\text{-control 2}}$	4.3	$s_{1\text{-control 3}}$	2.2	$s_{1\text{-10 Gy}}$	2.8	$s_{1\text{-100 Gy}}$	2.3	$s_{1\text{-500 Gy}}$	2.0	$s_{1\text{-1000 Gy}}$	2.3	$s_{1\text{-2500 Gy}}$	4.7	$s_{1\text{-10000 Gy}}$	4.7

Population Comparisons

H_0 : $\mu_{1\text{-Control 1}} - \mu_{1\text{-Control 2}} = 0$ Null Hypothesis = There is no difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

H_a : $\mu_{1\text{-Control 1}} - \mu_{1\text{-Control 2}} > 0$ Null Hypothesis = There is a difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

s_p^2	15.3571
t_s test statistic	0.5413
rejection region	$t > t_\alpha$
α	0.05
df	7
t_α	1.895
p-value	0.519240411

Since 0.5413 < 1.895, I do not reject the null hypothesis, there is no difference between the Control 1 and Control 2

H_0 : $\mu_{1\text{-Control 2}} - \mu_{1\text{-Control 3}} = 0$ Null Hypothesis = There is no difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

H_a : $\mu_{1\text{-Control 2}} - \mu_{1\text{-Control 3}} > 0$ Null Hypothesis = There is a difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

s_p^2	10.9848
t_s test statistic	2.6212
rejection region	$t > t_\alpha$
α	0.05
df	11
t_α	1.796
p-value	0.519490029

Since 2.6212 > 1.943, I do reject the null hypothesis, there is s difference between the Control 2 and Control 3 Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-10 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 10 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-10 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 10 Gy treatment Population

s_p^2	6.4762
t_s test statistic	0.4201
rejection region	$t > t_\alpha$
α	0.05
df	12
t_α	1.782
p-value	0.519527501

Since 0.4201 < 1.782, I do not reject the null hypothesis, there is no difference between the Control 3 and the 10 Gy Treated Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-100 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 100 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-100 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 100 Gy treatment Population

s_p^2	5.2212
t_s test statistic	-2.2197
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since -2.2197 < 1.771, I do not reject the null hypothesis, there is no difference between the Control 3 and the 100 Gy Treated Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-500 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 500 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-500 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 500 Gy treatment Population

s_p^2	4.3750
t_s test statistic	0.8083
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since 0.8083 < 1.771, I do not reject the null hypothesis, there is no difference between the Control 3 and the 500 Gy Treated Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 1000 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 1000 Gy treatment Population

s_p^2	5.2308
t_s test statistic	-1.6896
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since -1.5896 < 1.771, I do not reject the null hypothesis, there is no difference between the Control 3 and the 1000 Gy Treated Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 2500 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 2500 Gy treatment Population

s_p^2	14.2212
t_s test statistic	-0.3843
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since -0.3843 < 1.771, I do not reject the null hypothesis, there is no difference between the Control 3 and the 2500 Gy Treated Populations

H_0 : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 10000 Gy treatment Population

H_a : $\mu_{1\text{-Control 3}} - \mu_{1\text{-1000 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 10000 Gy treatment Population

s_p^2	14.2212
t_s test statistic	-0.9607
rejection region	$t > t_\alpha$
α	0.05
df	13
t_α	1.771
p-value	0.519558773

Since -0.9607 < 1.771, I do not reject the null hypothesis, there is no difference between the Control 3 and the 10000 Gy Treated Populations

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Protocol 7 Analysis

Data

Strain	Mutant #5								
Control 1 - CFU Input	18	11	12						
Control 2 - Untreated, No Vacuum	4	7	11	11	10	10	8	10	
Control 3 - Untreated. Vacuum	7	8	5						
100 Gy Treated Samples	3	3	8	5	7	9	7	7	
500 Gy Treated Samples	8	10	8	16	11	16	5		
1000 Gy Treated Samples	9	14	10	13	8	8	10		
2500 Gy Treated Samples	4	10	7	12	8	10	21	6	

All colony counts at 10^{-5} dilution

Statistics

	$n_{S\text{-control 1}}$	3	$n_{S\text{-control 2}}$	8	$n_{S\text{-control 3}}$	3	$n_{S\text{-100 Gy}}$	8	$n_{S\text{-500 Gy}}$	7	$n_{S\text{-1000 Gy}}$	7	$n_{S\text{-2500 Gy}}$	8
	$\bar{x}_{S\text{-control 1}}$	13.7	$\bar{x}_{S\text{-control 2}}$	8.9	$\bar{x}_{S\text{-control 3}}$	6.7	$\bar{x}_{S\text{-100 Gy}}$	6.1	$\bar{x}_{S\text{-500 Gy}}$	10.6	$\bar{x}_{S\text{-1000 Gy}}$	10.3	$\bar{x}_{S\text{-2500 Gy}}$	9.8
	$s_{S\text{-control 1}}$	3.8	$s_{S\text{-control 2}}$	2.4	$s_{S\text{-control 3}}$	1.5	$s_{S\text{-100 Gy}}$	2.2	$s_{S\text{-500 Gy}}$	4.2	$s_{S\text{-1000 Gy}}$	2.4	$s_{S\text{-2500 Gy}}$	5.2

Population Comparisons

H_0 : $\mu_{S\text{-Control 1}} - \mu_{S\text{-Control 2}} = 0$ Null Hypothesis = There is no difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

H_a : $\mu_{S\text{-Control 1}} - \mu_{S\text{-Control 2}} > 0$ Null Hypothesis = There is a difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

s_p^2	7.7269
t, test statistic	2.5462
rejection region	$t > t_\alpha$
α	0.05
df	9
t_α	1.833
p-value	0.519392768

Since 2.5462 > 1.833, I do reject the null hypothesis, there is a difference between the Control 1 and Control 2

H_0 : $\mu_{S\text{-Control 2}} - \mu_{S\text{-Control 3}} = 0$ Null Hypothesis = There is no difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

H_a : $\mu_{S\text{-Control 2}} - \mu_{S\text{-Control 3}} > 0$ Null Hypothesis = There is a difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

s_p^2	5.0602
t, test statistic	1.4501
rejection region	$t > t_\alpha$
α	0.05
df	9
t_α	1.833
p-value	0.519392768

Since 1.4501 < 1.833, I do not reject the null hypothesis, there is no difference between the Control 2 and Control 3 Populations

H_0 : $\mu_{S\text{-Control 3}} - \mu_{S\text{-100 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 100 Gy treatment Population

H_a : $\mu_{S\text{-Control 3}} - \mu_{S\text{-100 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 100 Gy treatment Population

s_p^2	4.3935
t, test statistic	0.3817
rejection region	$t > t_\alpha$
α	0.05
df	9
t_α	1.833
p-value	0.519392768

Since 0.3817 < 1.833, I do not reject the null hypothesis, there is no difference between the Control 3 and the 100 Gy Treated Populations

H_0 : $\mu_{S\text{-Control 3}} - \mu_{S\text{-500 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 500 Gy treatment Population

H_a : $\mu_{S\text{-Control 3}} - \mu_{S\text{-500 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 500 Gy treatment Population

s_p^2	13.5476
t, test statistic	-1.5373
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -1.5373 < 1.860, I do not reject the null hypothesis, there is no difference between the Control 3 and the 500 Gy Treated Populations

H_0 : $\mu_{S\text{-Control 3}} - \mu_{S\text{-1000 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 1000 Gy treatment Population

H_a : $\mu_{S\text{-Control 3}} - \mu_{S\text{-1000 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 1000 Gy treatment Population

s_p^2	4.7619
t, test statistic	-2.4033
rejection region	$t > t_\alpha$
α	0.05
df	8
t_α	1.860
p-value	0.519325892

Since -2.4033 < 1.860, I do not reject the null hypothesis, there is no difference between the Control 3 and the 1000 Gy Treated Populations

H_0 : $\mu_{S\text{-Control 3}} - \mu_{S\text{-2500 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 2500 Gy treatment Population

H_a : $\mu_{S\text{-Control 3}} - \mu_{S\text{-2500 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 2500 Gy treatment Population

s_p^2	21.5741
t, test statistic	-0.9805
rejection region	$t > t_\alpha$
α	0.05
df	9
t_α	1.833
p-value	0.519392768

Since -0.9805 < 1.833, I do not reject the null hypothesis, there is no difference between the Control 3 and the 2500 Gy Treated Populations

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Protocol 7 Analysis

Data

Strain	Mutant #8								
Control 1 - CFU Input	10	12	12						
Control 2 - Untreated, No Vacuum	18	21	13	20	23	11	13	22	
Control 3 - Untreated. Vacuum	3	3	7	6					
100 Gy Treated Samples	4	6	6	11	7	4	9	5	
500 Gy Treated Samples	7	11	14	7	9	7	13	7	
1000 Gy Treated Samples	7	6	5	8	4	11	8	12	
2500 Gy Treated Samples	11	6	10	8	12	4	7	6	

All colony counts at 10^{-5} dilution

Statistics

	$n_{8\text{-control 1}}$	3	$n_{8\text{-control 2}}$	8	$n_{8\text{-control 3}}$	4	$n_{8\text{-100 Gy}}$	8	$n_{8\text{-500 Gy}}$	8	$n_{8\text{-1000 Gy}}$	8	$n_{8\text{-2500 Gy}}$	8
	$\bar{x}_{8\text{-control 1}}$	11.3	$\bar{x}_{8\text{-control 2}}$	17.6	$\bar{x}_{8\text{-control 3}}$	4.8	$\bar{x}_{8\text{-100 Gy}}$	6.5	$\bar{x}_{8\text{-500 Gy}}$	9.4	$\bar{x}_{8\text{-1000 Gy}}$	7.6	$\bar{x}_{8\text{-2500 Gy}}$	8.0
	$s_{8\text{-control 1}}$	1.2	$s_{8\text{-control 2}}$	4.7	$s_{8\text{-control 3}}$	2.1	$s_{8\text{-100 Gy}}$	2.4	$s_{8\text{-500 Gy}}$	2.9	$s_{8\text{-1000 Gy}}$	2.8	$s_{8\text{-2500 Gy}}$	2.8

Population Comparisons

H_0 : $\mu_{5\text{-Control 1}} - \mu_{5\text{-Control 2}} = 0$ Null Hypothesis = There is no difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

H_a : $\mu_{5\text{-Control 1}} - \mu_{5\text{-Control 2}} > 0$ Null Hypothesis = There is a difference between the Control 1 (CFU Input) population and the Control 2 (Untreated / Unopened Lid) Population

s_p^2	17.1713
t, test statistic	-2.2427
rejection region	$t > t_\alpha$
α	0.05
df	9
t_α	1.833
p-value	0.519392768

Since -2.2427 < 1.833, I do not reject the null hypothesis, there is not a difference between the Control 1 and Control 2

H_0 : $\mu_{5\text{-Control 2}} - \mu_{5\text{-Control 3}} = 0$ Null Hypothesis = There is no difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

H_a : $\mu_{5\text{-Control 2}} - \mu_{5\text{-Control 3}} > 0$ Null Hypothesis = There is a difference between the Control 2 (Untreated / Unopened Lid) population and the Control 3 (On Treatment Lid) Population

s_p^2	16.4625
t, test statistic	5.1818
rejection region	$t > t_\alpha$
α	0.05
df	10
t_α	1.812
p-value	0.519446506

Since 5.1818 > 1.812, I do reject the null hypothesis, there is a difference between the Control 2 and Control 3 Populations

H_0 : $\mu_{5\text{-Control 3}} - \mu_{5\text{-100 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 100 Gy treatment Population

H_a : $\mu_{5\text{-Control 3}} - \mu_{5\text{-100 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 100 Gy treatment Population

s_p^2	5.4750
t, test statistic	-1.2213
rejection region	$t > t_\alpha$
α	0.05
df	10
t_α	1.812
p-value	0.519446506

Since -1.2213 < 1.812, I do not reject the null hypothesis, there is no difference between the Control 3 and the 100 Gy Treated Populations

H_0 : $\mu_{5\text{-Control 3}} - \mu_{5\text{-500 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 500 Gy treatment Population

H_a : $\mu_{5\text{-Control 3}} - \mu_{5\text{-500 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 500 Gy treatment Population

s_p^2	7.2625
t, test statistic	-2.8025
rejection region	$t > t_\alpha$
α	0.05
df	10
t_α	1.812
p-value	0.519446506

Since -2.8025 < 1.812, I do not reject the null hypothesis, there is no difference between the Control 3 and the 500 Gy Treated Populations

H_0 : $\mu_{5\text{-Control 3}} - \mu_{5\text{-1000 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 1000 Gy treatment Population

H_a : $\mu_{5\text{-Control 3}} - \mu_{5\text{-1000 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 1000 Gy treatment Population

s_p^2	6.6625
t, test statistic	-1.8189
rejection region	$t > t_\alpha$
α	0.05
df	10
t_α	1.812
p-value	0.519446506

Since -1.8189 < 1.812, I do not reject the null hypothesis, there is no difference between the Control 3 and the 1000 Gy Treated Populations

H_0 : $\mu_{5\text{-Control 3}} - \mu_{5\text{-2500 Gy}} = 0$ Null Hypothesis = There is no difference between the Control 3 (Untreated / On Treatment Lid) population and the 2500 Gy treatment Population

H_a : $\mu_{5\text{-Control 3}} - \mu_{5\text{-2500 Gy}} > 0$ Null Hypothesis = There is a difference between the Control 3 (Untreated / On Treatment Lid Lid) population and the 2500 Gy treatment Population

s_p^2	6.6750
t, test statistic	-2.0542
rejection region	$t > t_\alpha$
α	0.05
df	10
t_α	1.812
p-value	0.519446506

Since -2.0542 < 1.812, I do not reject the null hypothesis, there is no difference between the Control 3 and the 2500 Gy Treated Populations

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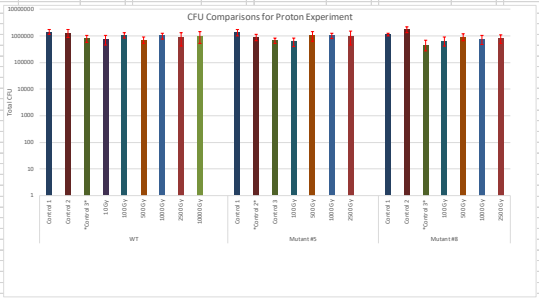
Mutant #5															
n5- control 1	3	n5 - control 2	8	n5 - control 3	3	n5 - 100 Gy	8	n5- 500 Gy	7	n5 - 1000 Gy	7		n5 - 2500 Gy	8	
x- bar5- control 1	13.66666667	x- bar5- control 2	8.875	x- bar5- control 3	6.666666667	x- bar5- 100 Gy	6.125	x- bar5- 500 Gy	10.57142857	x- bar5- 1000 Gy	10.28571429		x- bar5- 2500 Gy	9.75	
s5- control 1	3.785938897	s5- control 2	2.416461403	s5- control 3	1.527525232	s5- 100 Gy	2.232071427	s5- 500 Gy	4.157609203	s5- 1000 Gy	2.360387377		s5- 2500 Gy	5.2030211	
Mutant #8															
n8 - control 1	3	n8 - control 2	8	n8 - control 3	4	n8 - 100 Gy	8	n8- 500 Gy	8	n8 - 1000 Gy	8		n8 - 2500 Gy	8	
x- bar8- control 1	11.33333333	x- bar8- control 2	17.625	x- bar8- control 3	4.75	x- bar8- 100 Gy	6.5	x- bar8- 500 Gy	9.375	x- bar8- 1000 Gy	7.625		x- bar8- 2500 Gy	8	
s8- control 1	1.154700538	s8- control 2	4.657942526	s8- control 3	2.061552813	s8- 100 Gy	2.449489743	s8- 500 Gy	2.924648941	s8- 1000 Gy	2.774243784		s8- 2500 Gy	2.7774603	



		CFU	Std. Dev.
WT	Control 1	1433333.333	288675.1346
	Control 2	1283333.333	426223.7284
	Control 3	800000	223606.7977
	10 Gy	742857.1429	281999.6623
	100 Gy	1062500	232609.4213
	500 Gy	712500	195940.9532
	1000 Gy	1000000	232992.949
	2500 Gy	875000	470372.1931
	10000 Gy	987500	470372.1931
Mutant #5	Control 1	1366666.667	378593.8897
	Control 2	887500	241646.1403
	Control 3	666666.6667	152752.5232
	100 Gy	612500	223207.1427
	500 Gy	1057142.857	415760.9203
	1000 Gy	1028571.429	236038.7377
	2500 Gy	975000	520302.11
Mutant #8	Control 1	1133333.333	115470.0538
	Control 2	1762500	465794.2526
	Control 3	475000	206155.2813
	100 Gy	650000	244948.9743
	500 Gy	937500	292464.8941
	1000 Gy	762500	277424.3784
	2500 Gy	800000	277746.0299

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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

Deinococcus radiodurans, proton, neutron, linear energy transfer

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