BioBuilderClub

Ionizing Radiation Bio-sensor and Bio-reporter

Team members: Antonio Cen Wu, Maik Ho Cen Wu, and Mame Diop

Team Leader: Alfred Aung Lwin ; Mentor: Racquel Kim Sherwood

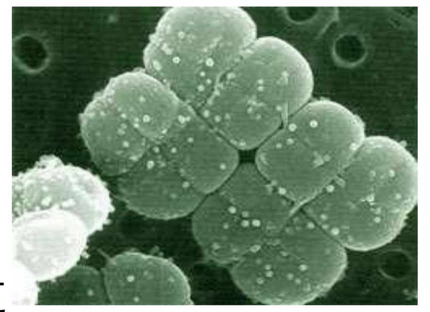
Manhattan Comprehensive Night & Day High School
New York

Abstract

Ionizing radiation is a form of radiation emitted from radioactive materials and nuclear reactions and from stars. Ionizing radiation comes into environment from natural sources such as soil and water and cosmic radiation, and from human activities, such as nuclear power plants, medical use of radiation and nuclear bomb tests, and discharges of nuclear wastes. There are 99 nuclear power plants currently operating in the 30 states in the US. They may pose great danger to public health during nuclear accidents. Exposure to high level of ionizing radiation can cause serious health risks, and long term exposure of low level ionizing radiation can cause cancers. In the event of nuclear accidents, commercial electronic radiation dosimeters are too expensive to be widely used for the detection of radiation levels in the environment. Moreover, some of those devices may not work efficiently because they are sensitive to temperature changes in the environment.

According to our literature review, *Deinococcus radioduran* bacteria is resistant to ionizing radiation, and It can survive even after exposure to 12,000 Gray doses of ionizing radiation. In human exposure, more than 75 rads, which is equivalent to (.75 Gray) can cause acute radiation syndrome, such as nausea, vomiting within hours and can be fatal in a few days or weeks.

Our research objective is to engineer a bio-sensor and bio-reporter to detect ionizing radiation from the environment and indicate the levels of radiation through generation of pigments.



Our desired biosensor consisting of two components:
- ❖ Component to detect ionizing radiation from environment
- ❖ Component for an efficient DNA repair mechanism for cell survival

Genes of interest from *Dinococcus radiodurans*:
- ❖ **(BBa-K1499200, BBa-K602016 and BBa-K602020 from iGEM Parts**

Our reporter :
- ❖ Beta-carotene as bio-reporter to show color for radiation doses.

Our desired chassis:
- ❖ E.coli strain (**MG1655-CB1000) Mutant strains**

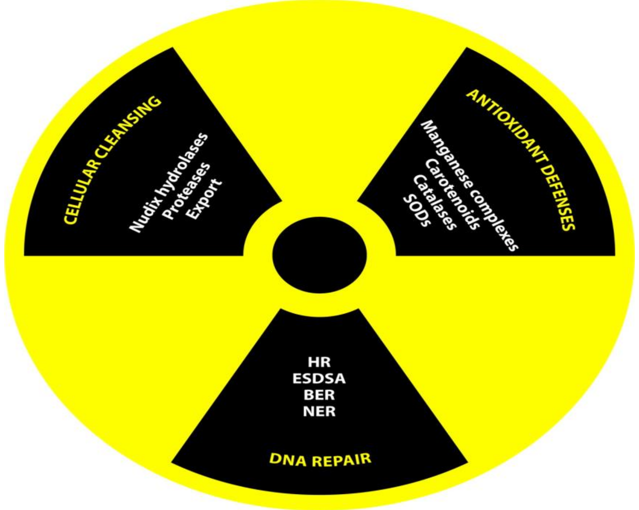
Overall goal: To engineer a biosensor and bioreporter capable of detecting and producing a signal in response to ionizing radiation.

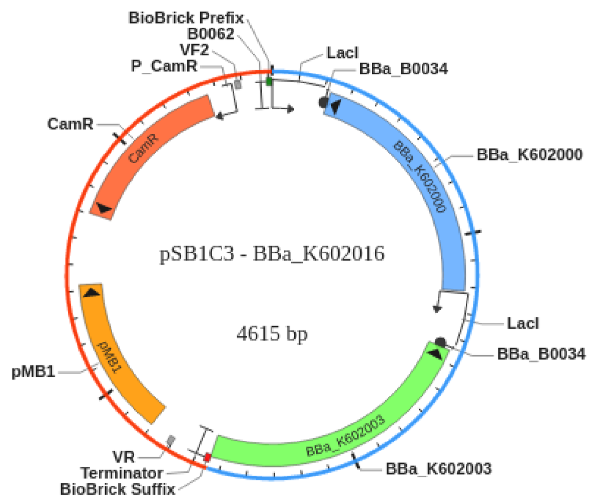
Phase I objective (Current Project): Use a gene from the world toughest bacteria, *Deinococus radiodurans* to engineer a chassis capable of withstanding physiologically relevant ionizing radiation.

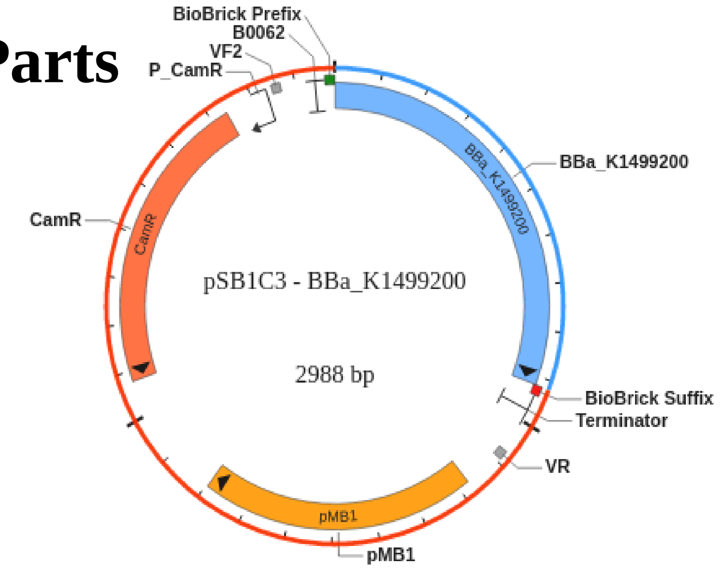
Limitation of our experiment:
- Time constraint and unavailability of the required lab materials

Nuclear Power Plant Accident

RAE System Gamma RAE II R Gamma Radiation Detector and Dosimeter
Base price: $999,999







Deinococus radiodurans

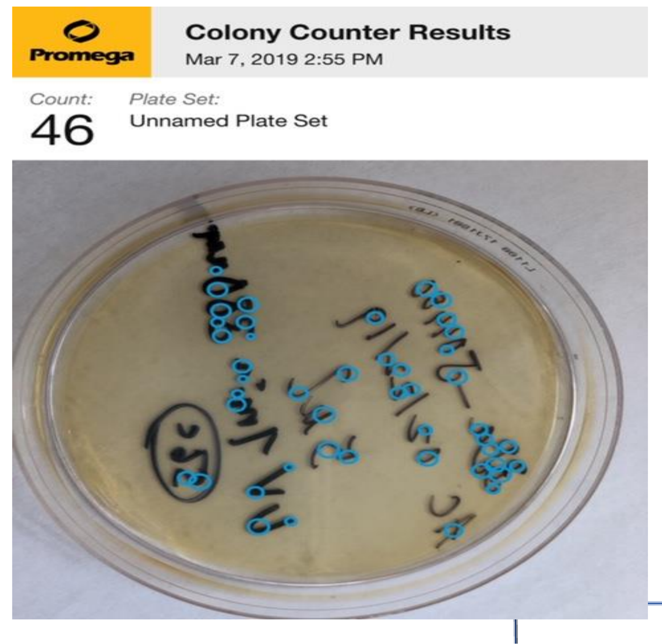
The world Toughest bacterium
Resistant to ionizing radiation dose of >12,000 Gy

Phases of Radiation Injury

Dose (Gy)	Prodromal Phase	Manifest Phase	Prognosis without Supportive Care
0.5–1.0	Mild	Modest decline in blood counts	Survival
1.0–2.0	Mild–moderate	Some bone marrow damage	Survival >90%
2.0–3.5	Moderate	Moderate–severe bone marrow damage	Probable survival
3.5–5.5	Severe	Severe bone marrow damage; modest GI damage	Death within 3.5–6 wk (50% of victims)
5.5–7.5	Severe	Pancytopenia and moderate GI damage	Death probable within 2–3 wk
7.5–10.0	Severe	Severe GI and bone marrow damage	Death probable within 2 wk
10	Severe	Severe GI damage, radiation-induced lung injury, altered mental status; at higher doses (>20.0 Gy), cardiovascular collapse, fever, shock	Death within 2 wk

Ann Intern Med. 2004;140:1037–1051.

Phase I Method and materials:
- We transformed NEB 10-beta Competent E. coli with *D. radiodurans* UV DNA damage endonuclease, uvsE (BBa_K1499200 or RFP (BBa_J04450).
- We streaked the transformed cells into LB agar plates with chloramphenicol and inoculated 5 ul in 2ml of LB medium and incubated. Samples were incubated for 24 hrs.
- We conduced radiation tolerance assays using UV-A/UV-B irradiation
- We also conducted cell tolerance with Hydrogen peroxide, a DNA-damaging agent.
- Data was collected using Promega colony counter app.



Ionizing Radiation Bio-sensor and Bio-reporter

BioBuilderClub

Team members: Antonio Cen Wu, Maik Ho Cen Wu, and Mame Diop
Team Leader: Alfred Aung Lwin ; Mentor: Racquel Kim Sherwood

Manhattan Comprehensive Night & Day High School
New York

Result:

- UV radiation test and Hydrogen Peroxide test:
- 10-Beta E.coli containing a copy *D. radiodurans* uvsE had increased survival when compared to the control strain containing RFP

Radiation survival after exposure to UV A-380 nm or UVB-240 nm

Cells + pDNA	Type of UV exposure	Duration of exposure	Colony count before exposure	Colony count after exposure	Survival rate
10-beta + RFP	UVA-380nm	30 sec	122	59	48.3%
10-beta + uvsE	UVA-380nm	30 sec	87	66	75%
10-beta + RFP	UVA-380nm	1 minute	87	42	48%
10-beta + uvsE	UVB-380nm	1 minute	56	46	82%
10-beta + RFP	UVB-240nm	30 sec	155	69	44%
10-beta + uvsE	UVB-240nm	30 sec	146	65	44%
10-beta + RFP	UVB-240nm	1 minute	73	44	60%
10-beta + uvsE	UVB-240nm	1 minute	104	55	52%

Result:

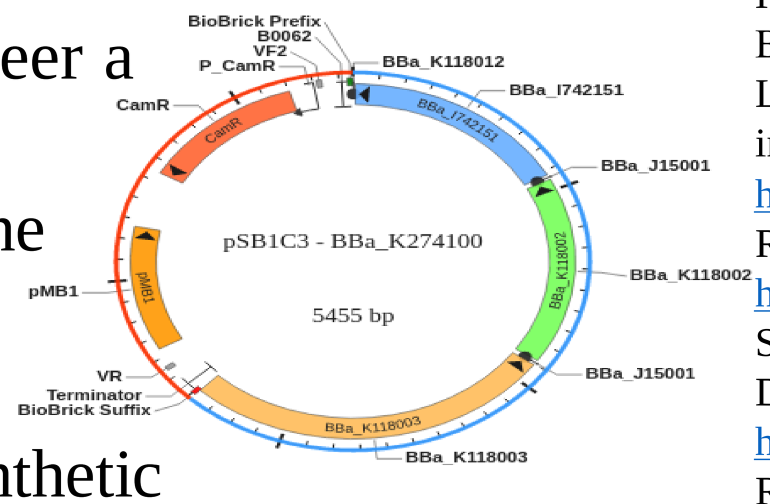
- **Hydrogen Peroxide test:**

Cell survival after exposure to 3% Hydrogen peroxide

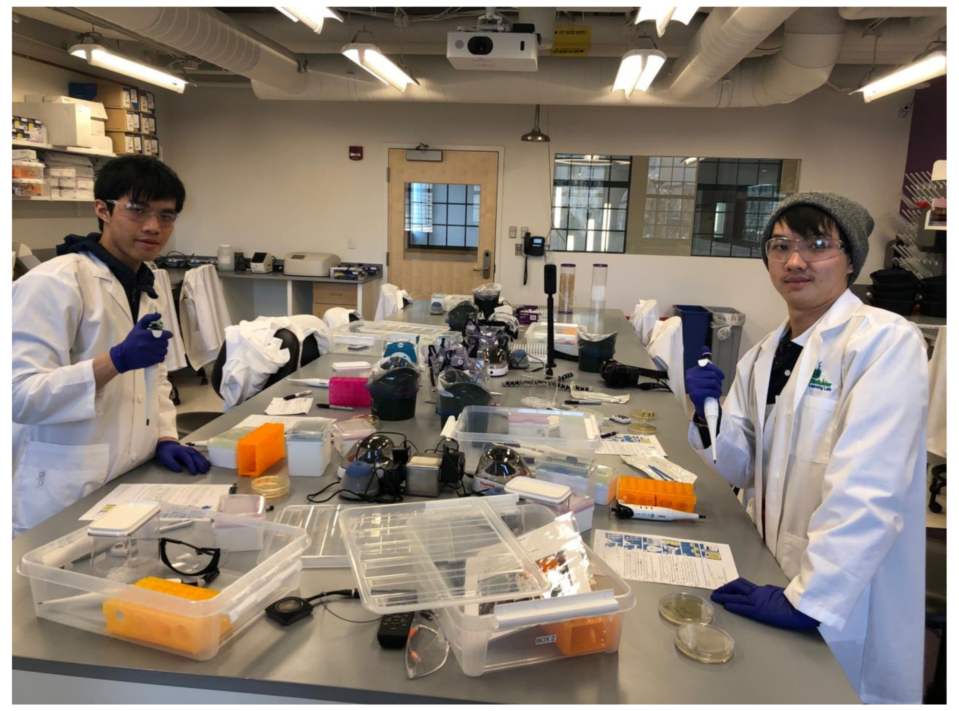
Cells + pDNA	Duration of exposure	Colony count before exposure	Colony count after exposure	Survival rate
10-beta + RFP	24 hrs	82	10	12%
10-beta + uvsE	24 hrs	127	11	8.6%
10-beta + RFP	24 hrs	86	13	15%
10-beta + uvsE	24 hrs	142	18	12%
10-beta + RFP	24 hrs	150	11	7.3%
10-beta + uvsE	24 hrs	73	6	8.26%
10-beta + RFP	24 hrs	104	10	9.6%
10-beta + uvsE	24 hrs	123	11	8.9%

Conclusion

- In the first phase , we had planned to use mutant E.coli strain **(MG1655-CB1000).** However we were unable to gain access to the strain. Instead we successfully engineered "NEB Competent Cells" to be resistant to radiation.
- **In the future we plan to use the chassis that we generated in these experiments to** engineer a biosensor in our chassis (10-beta + uvsE Engineered Phase I) and use Beta-Carotene BBa_K274100 as our bioreporter



- (Plasmid encodes the beta carotene biosynthetic pathway components which will expressed in response to radiation.



References:

.Battista, J. R., A. M. Earl, and M. J. Park. 1999. Why is Deinococcus radiodurans so resistant to ionizing radiation? Trends Microbiol. 7:362-365. [PubMed] https://www.ncbi.nlm.nih.gov/pubmed/10470044
Krisko, A., & Radman, M. (2013). Biology of extreme radiation resistance: the way of Deinococcus radiodurans. *Cold Spring Harbor perspectives in biology*, *5*(7), a012765. doi:10.1101/cshperspect.a012765
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3685888/
Kuldell Natalie, Bernstein Rachel, Ingram Karen, M. Hart Kathryn AC. Biobuilder: Synthetic Biology in the lab. Sebastopol, CA (USA): O' Reilly Media, Inc.; 2015.
Lockhart JS, DeVeaux LC (2013) The Essential Role of the *Deinococcus radiodurans ssb* Gene in Cell Survival and Radiation Tolerance. PLoS ONE 8(8): e71651.
https://doi.org/10.1371/journal.pone.007165
Ruder, Kate.2004. A Fight about the Toughest Microbe on Earth
http://www.genomenewsnetwork.org/articles/2004/10/15/radiodurans.php
Slade D, Radman M. 2011. Oxidative stress resistance in Deinococcus radiodurans. Microbiol Mol Biol Rev 75: 133–191.
https://www.ncbi.nlm.nih.gov/pubmed/21372322
Registry of standard Biological Parts [Internet], http://parts.igem.org/Main_Page
iGEM Osaka2011.
http://2011.igem.org/Team:Osaka/Team

WHO (World Health Organization), 2016 April. Ionizing radiation, health effects and protective measures https://www.who.int/news-room/fact-sheets/detail/ionizing-radiation-health-effects-and-protective-measures

Acknowledgements

Our MCNDHS BioBuilder Team would like to express our sincere gratitude to Dr. Natalie Kuldell, and Carla Boon for their advice and support given to us during the project. We are also would like to extend our heartfelt thanks to our mentor Dr. Racquel Kim Wood for her constant advice to us throughout the project. We also very much would like to thank our Principal Michael Toise, Asst. Principal (Science) Mark Testa and other faculty for their avid support. We are grateful to Ms. Margaret Aylward, and CDI staffs for their generous support.