1. Introduction

It is still sharp in our memory that, on March 11, 2011, the Great East Japan Earthquake struck off the coast of Eastern Japan’s island and triggered a series of events that led to the nationwide nuclear crisis. Moved by that accident in iGEM 2011, we have built a synthetic biological dosimeter to detect the radiation for the need for low-cost, portable and easy-to-use dosimeter. In this year we further improved that “Bio-dosimeter”. As shown below, our “Bio-dosimeter” consists of two points: damage tolerance and radiation detection. To confer tolerance to E.coli, we introduced radiation resistance genes from Deinococcus radiodurans. For the detection of the radiation, we connected the native DNA damage response system of E.coli to production of pigment lycopene as an optical reporter.

3. Damage Tolerance

The bacterium D. radiodurans shows remarkable resistance to DNA damage caused by ionizing radiation, desiccation, UV radiation, oxidizing agents, and electrophilic mutagens. It has a complex DNA repair system comprising multiple unique proteins including the ones detailed below:

- **PprI**: can induce the gene expression of recA and pprA and enhance the enzyme activities of catalases. Experimental data indicates that PprI regulates multiple DNA repair protection pathways in response to radiation stress.
- **PprA**: plays a critical role in the radiation-induced non-homologous end-joining repair mechanism of D. radiodurans by preferentially binding to double-stranded DNA carrying strand breaks and catalyzing DNA end-joining reaction.
- **PprM**: is a modulator of the PprI-dependent radiation response mechanism of D. radiodurans and regulates the induction of PprA.
- **RecA**: plays a role in recombination repair, but has some differences from the E. coli RecA.

To induce DNA damage, we used: Mitomycin C to introduce interstrand cross-links into duplex DNA and break DNA chains, Hydrogen peroxide, a highly reactive compound, breaks not only DNA molecules but also various substances in the cell, such as organelles.

4. Detection of DNA Damage

**How to detect**

As the detector of the radiation, we chose the native DNA damage response system of E.coli, known as the SOS response. In the SOS response, DNA damage leads to RecA activation which then causes LexA auto-cleavage. LexA is a repressor of SOS genes; hence, the drop in LexA levels enables SOS genes to be expressed. Promoters of SOS genes contain the SOS box which is a conserved sequence that acts as the LexA binding site. We hoped that by using a SOS promoter it is possible to make downstream genes responsive to DNA damage, so that radiation.

**How to report**

We chose lycopene biosynthesis as a reporter as it neither requires addition of substrate or excitation at a specific wavelength. Lycopene biosynthesis is a stepwise process starting from farnesyl pyrophosphate (FPP) which is natively produced in E. coli. However, the conversion of colorless FPP to orange-red lycopene is catalyzed by a series of enzymes (CrtE, CrtB and CrtI) which are missing in E. coli.

**Parts & Characterization**

To measure the DNA damage detection, we use the DNA damaging agents as a source of DNA damage. To evaluate the promoter activity quantitatively and accurately, we employed the Dual Luciferase Reporter Assay System. We were trying to construct a promoter evaluation device utilizing freely and renilla luciferase, but not yet finished.

5. Summary & Future Work

- **Last year**, we cloned radioresistance genes from D. radiodurans genomic DNA and BioBricked them. And then we assayed these parts for their effects on DNA damage caused by UV radiation.

**This year**, we employed E.coli Rosetta strains for protein expression to make our parts functional. Then, we tried to assess its tolerance to various types of DNA damage (caused by mitomycin C and hydrogen peroxide) and to evaluate DNA damage detection more clearly.

6. Reference