1. Introduction

On March 11, 2011, the Great East Japan Earthquake struck off the coast of Eastern Japan and triggered a nationwide nuclear crisis centered on the Fukushima 1 Nuclear Power Plant. The need for low-cost, portatile and easy-to-use dosimeters became apparent as measurements of radiation exposure could only be conducted at dedicated installations spaced far apart and the numbers reported only infrequently. Hence we have decided to tackle the building of a biological dosimeter. As shown below, there are two components to our "Bio-dosimeter": damage tolerance and detection. To confer tolerance to our E. coli chassis, we modularly introduced radiation resistance genes from the extremophilic bacterium Deinococcus radiodurans. For detection of DNA damage, we connected the native DNA damage response system of E. coli to color pigment production.

We also wanted to gauge how well a biological dosimeter would be received by the public. To that end, we conducted a survey in conjunction with the KIT Kyoto team, asking questions related to both synthetic biology and radioactivity. We discovered that most people are more concerned about the dangers of radiation than potential biohazards, and should support the development and use of our bio-dosimeters.

What is your stance towards buying genetically-modified (GM) food?

【Always buy non-GM】
【Try to buy non-GM】
【Don't really need GM】
【Buy whatever GM is available】
【Never noticed GM indication】

2. Damage Tolerance

Deinococcus radiodurans is a bacterium with remarkable resistance to DNA damage caused by ionizing radiation, desiccation, UV radiation, oxidizing agents, and electrophilic mutagens. It has a complex DNA repair system consisting of multiple unique proteins including the ones described below.

PprI, a protein unique to D. radiodurans, regulates multiple DNA repair and protection pathways, including PprM and RecA expression, as well as enhancing carotenoid activation.

Parts & Characterization

Single-gene parts: lacI, lacZ, IPTG, PprM, PprA, RecA

3. Detection of DNA Damage

If DNA is significantly damaged (e.g. by exposure to UV radiation or chemicals), synthesis of several DNA damage-related proteins occurs quickly. This reaction to DNA damage is the SOS response. Central to this response is RecA, which has multiple activities essential for the repair and maintenance of DNA. In the bacterial SOS response, it plays a co-protease role in the autolytic cleavage of the LexA repressor.

We decided to employ lycopene biosynthesis as a reporter as it neither requires addition of substrate (e.g. luciferase) or excitation at a specific wavelength (e.g. GFP). 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.

4. Summary & Future Work

In this project, we

- Biobricked four proteins (PprI, PprM, PprA, RecA) implicated in the DNA damage response of D. radiodurans and assayed these parts and their combinations for the effects on DNA damage tolerance
- put together a DNA damage detector device by combining a lycopene biosynthesis part (Cambridge, 2009) with the SOS promoter (Bangalore, 2006) and assayed the promoter’s response to varying levels of UV irradiation
- worked with the KIT-Kyoto team to conduct a widely-targeted survey of the public perception of radiation and synthetic biology in (West) Japan

We also propose the following future work for our project:

- testing the response of the SOS promoter to other sources of DNA damage (ionizing radiation, hydrogen peroxide, mitomycin C etc.)
- characterizing PprI, PprM, PprA, RecA and their combinations for their effects on tolerance towards other sources of DNA damage
- visually indicating the level of radiation exposure using multiple pigments/promoters
- incorporating DNA damage tolerance and detection into one device to build a complete Bio-dosimeter

5. References