Introduction
Existing sunscreen compounds are associated with a range of health and environmental problems – such as accelerated skin damage under UV light, and coral bleaching. Cyanobacteria and several other organisms have evolved microbial sunscreens to survive in environments with high UV-A and UV-B radiation. These protective compounds, specifically mycosporines and mycosporine-like amino acids (MAAs), also defend against oxidative stress by quenching free radicals and preventing cellular damage.

A mycosporine-synthesizing gene cluster in the cyanobacteria *Anabaena variabilis* was characterized by Balskus and Walsh (2010). Our team seeks to optimize these genes for high-volume expression in *E. coli* and study their effects on bacterial survival rates under high-UVA environments.

### Directed Evolution

Evolve more UV resistant strains by successive rounds of selection and mutation

<table>
<thead>
<tr>
<th>A (NRPS-like enzyme)</th>
<th>B (ATP-grp)</th>
<th>O-MT</th>
<th>DHQS-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ava_3855</td>
<td>Ava_3856</td>
<td>Ava_3858</td>
<td>Ava_3850</td>
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**Directed Evolution**

#### UV Exposure

We mimic solar UV by combining reptile UVB lamps, with a UVA nail curing lamp:

**UV kill curve experiment**

For each time point, make a 1:10 dilution series, and spot on plates to count colony forming units (CFU)

**Results**

UV kill curves were generate for HB101, to establish baseline exposure levels for Directed Evolution. We also tested HB101 with the pGLO plasmid, to check if GFP has a protective effect. We see a 4-log decrease in viable cells after 75min exposure for HB101, and only 30 minutes for pGLO:

- **HB101**
- **HB101 + Ava_3858**
- **HB101 + Ava_3858-3855**
- **HB101 no-plasmid controls**

**Environmental, Ethical, Legal, Societal Issues**

Sunscreen has many health and environmental impacts we have only recently discovered. A portion of our project is public engagement and awareness of the impact through social media, our crowd funding campaign, and an online survey. We helped to educate that:

- **PABA**, a common ingredient in sunscreens, has been banned in the EU due to allergic dermatitis and photo sensitivity, and may even be carcinogenic

### Conclusions

We hope the standardization of these genetic components and their admission to the iGEM registry will help foster collaborative research into the potential applications of MAAs as alternatives to toxic synthetic sunscreens, protection for terraforming bacteria in harsh UV environments, and as antibiotic-free selectable markers for genetic engineering.

### Future Directions

It is our intention to continue the project gathering data and running additional generations of RCA Directed Evolution. We hope to be able to establish a successful strain of genes which express mycosporine under UV induced stress.

### Acknowledgments

- Built UV exposure rig to mimic solar UV
- Determined exposure level needed for >4 log decrease in Colony Forming Units (CFU)
- Demonstrated GFP is NOT an effective UV protectant for *E.coli*
- Synthesized and transformed *A. variabilis* shinoirin biosynthesis genes
- Submitted one gene (Ava_3856) to iGEM
- Measured UV absorption spectrum of whole methanol extract

**Corporate Sponsors**: IDT, The ODIN, NEB
**Community Support**: Indiegogo Donors
**DIY Bio Community**: Counter Culture Labs, Berkeley Bio Labs, BioCurious

**Citations**


**DIY Bio Community**: Counter Culture Labs, Berkeley Bio Labs, BioCurious

**Societal Issues**

- Building a DIYBio culture promotes interdisciplinary knowledge sharing, Loui Beniguel, Cirolo Contreras, Dante Giannini, Elisabetta Gionta, Paula Alcoch, Lucetta Gries, and Antonio Fantozzi.