Molecular assays using genetic reporters are favored by researchers for their wide range of information including signaling molecule detection, but assay specificity can take years to develop [1]. Phage-assisted continuous evolution (PACE) enables accelerated protein engineering and more complex functions for assay development by increasing the scale of directed evolution by orders of magnitude. The Bartelle lab sought to develop a biosensor to detect Nitric Oxide (NO) as a pilot project to use PACE for biosensor development.

**Motivation**

Three different plasmids are required for PACE: a recombinant phage (RP), selection plasmid (SP), and a mutational plasmid (MP). Host cells are infected by the selection phage which induce positive or negative selection, while the mutagenesis plasmid increases mutations in the lagoon.

![Diagram](Fig 1)

**Research Methods**

Switching between on and off states for biosensor engineering is essential and requires both a positive and negative selection strategy. Thorough research was performed to choose these selectors.

- Positive selector: amplifies replication
- Negative selector: causes cell death

Recombinant $\phi$X174 plasmids will be constructed using the chosen selection mechanisms.

![Diagram](Fig 2)

**Progress**

An extensive literature review was performed to determine positive and negative selectors for $\phi$X174. Gene H, the minor spike protein, was chosen to act as the positive selector. Phage viability depends on relative expression levels [2]. A negative selector of MbcT/MbcA was chosen, which counters NAD+ phosphorylation, an essential cofactor for metabolism [3]. Constructs were planned using computer-aided molecular design and DNA was synthesized.

**Obstacles Overcome**

Multiple negative selection genes were considered for the molecular design of the constructs, including genes in the $\phi$X174 plasmid as well as toxin-antitoxin systems (RelE/RelB [4], MqsR/other antitoxins [5], and MbcT/MbcA). MbcT/MbcA was chosen for this system’s diverse opportunities for this project and potential future projects.

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