Open Access Directed Evolution Through Phage-Assisted Continuous Evolution (PACE)

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Motivation

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.

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 ϕX174 plasmids will be constructed using the chosen selection mechanisms.

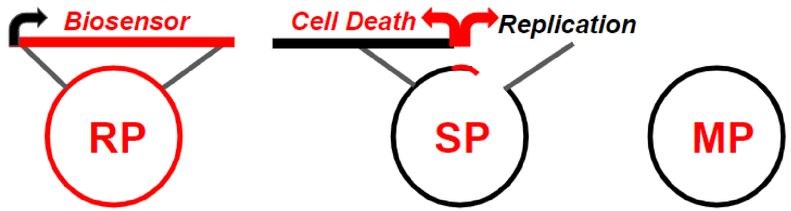

Fig 1. Three different plasmids are required for PACE: a recombinant phage (RP), selection plasmid (SP), and a mutational plasmid (MP). Host cells are infected by the selection phage which induce positive or negative selection, while the mutagenesis plasmid increases mutations in the lagoon.

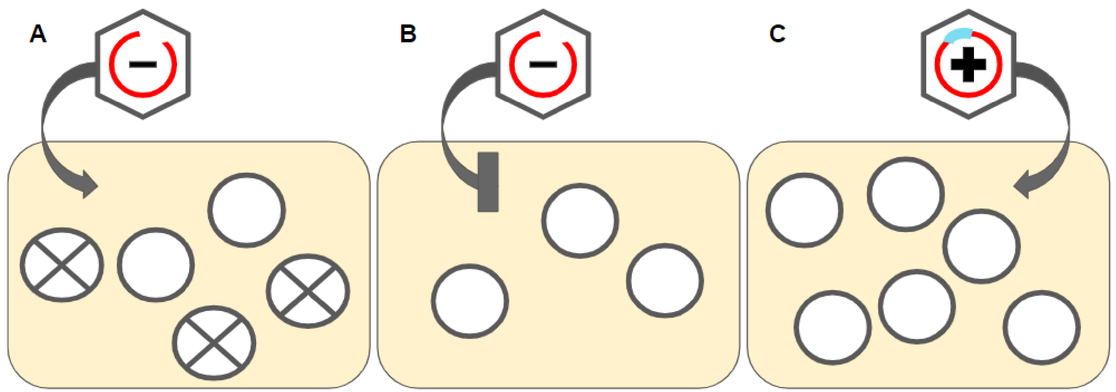

Fig 2. Selection strategies to facilitate on/off states. A toxin-antitoxin system was chosen as the negative selection gene. Gene H from the ϕX174 genome was chosen as the positive selection gene. **(A)** Toxin MbcT causes reduced viability while slowing phage replication, ultimately causing cell death. **(B)** The presence of antitoxin MbcA suppresses the expression of MbcT, resulting in cell protection during positive selection. **(C)** During the on state, a positive selector is expressed, and cells are able to replicate at an increased rate.

Progress

An extensive literature review was performed to determine positive and negative selectors for ϕX174. 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/McbA 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 ϕX174 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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References

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