Establishment of a Working System for Engineering Programmable Bacteriophages

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Enteropathogenic bacteria are a common source of newborn deaths, and Shiga toxin-producing Enterotoxigenic Escherichia coli is one of the most common bacterial pathogens found in infants. Bacteriophages naturally have narrow host specificity and lyse specific strains of bacteria. In order to develop a platform to engineer synthetic bacteriophages that have programmable host specificity with the ability to lyse multiple specific strains of bacteria, a working system for engineering the programmable bacteriophages is needed. Using plaque assays and time course experiments, the ideal incubation temperature, length of incubation, and phage dilution of T3 and T7 phages were determined. Various plaque assay methods were used to create a reliable method for determining the phage titer and for phage propagation. Using the established plaque assay method, the titers of the T3 and T7 stocks were determined and were found to be consistent and replicable. The T7 genomic DNA was isolated and a PCR was performed with various sizes of DNA fragments. The ideal size of a fragment that can be amplified was found to be 5,000 base pairs. Colonies of E. coli DH5a that are resistant to T7 were isolated using a plaque assay, and the number of resistant colonies found in various dilutions of T7 was counted. The number of resistant colonies of DH5a increases as the phage becomes more dilute. A working protocol for transforming phage DNA into yeast and rebooting the phages was established. In order to do so, a yeast transformation was performed for positive and negative controls and successfully transformed DNA into yeast. The wild-type T7 genome captured in a yeast artificial chromosome was successfully transformed into E. coli and rebooted to form viable phages.

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