Genome Engineering of Bacteriophage MooMoo

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To successfully infect host cells, viruses must precisely control the expression of their genes. In temperate bacteriophage, the repressor plays a central role in determining whether the virus will grow lytically or integrate into the host genome and remain dormant. The goal of this research was to create a mutant phage that can only grow lytically. To accomplish this, we used the Bacteriophage Recombineering of Electroporated DNA (BRED) technique to delete the repressor gene in bacteriophage MooMoo. This technique requires electrocompetent Mycobacterium smegmatis cells that contain plasmid pJV53, which expresses proteins that promote recombination. MooMoo genomic DNA and a recombination substrate were simultaneously electroporated into the transformed bacterial strain to allow for recombination to occur. After incubation, plaques were picked and screened for the desired deletion. Plaques that tested positive for the deletion were purified. Surprisingly, MooMoo deletion mutants produced plaque phenotypes similar to wild-type phage. Because the integrase gene also regulates temperate phage life cycles, a deletion of both the repressor and integrase genes is currently being attempted. The BRED genome engineering technique can be applied to other temperate phages to produce therapeutic phages that can treat a wide range of bacterial diseases and target antibiotic-resistant microbes.

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