**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Genetic engineering of bacteriophages to understand their biology and utilise in biotech applications   |
| **Project Summary**  |
| There is an increasing awareness that multidrug resistance bacterial infections present a serious healthcare and economic crisis. The pandemic of antimicrobial resistant bacterial infections has already begun with an estimated 1.27 million deaths in 2019, attributed to antimicrobial resistant bacterial infections. A range of approaches are required to counter the increasing antimicrobial resistance problem. One such approach is the use of viruses that specifically kill bacteria- bacteriophages. The use of bacteriophages (phages) as therapeutics can be split into two approaches, the use of wild-phages and engineered phages. The use of “wild-phages” broadly involves the isolation of a large number of phage isolates and then the careful selection of phages that, on their own or in combination as a cocktail, provide the desired properties. While there is a long history of using “wild-phages” as therapeutics, there is relatively little research on the use of engineered phages for therapy.  The use of “wild-phages” is not without problems. Bacteria can evolve resistance to phages and phages have to be selected to have the desired host range and may contain genes encoding toxins. The use of engineered phages has many benefits, as it offers the potential to either remove detrimental traits from phages or add beneficial traits. E.g. to include anti-defences proteins that overcome the natural defences of bacteria to phage infection [[1]](https://paperpile.com/c/5eaDpw/eAYX). Thus, overcoming some of the limitations associated with the use of wild-phages. Using a synthetic biology approach to engineer phages offers the potential to rapidly add new properties that are desirable and provide traits that are useful for phage-therapy and other biotech applications. The potential of phage engineering is only just being realised, in part because of the lack of methods that allow rapid selection of phage mutants. We have previously used CRISPR based approaches for selection [[2]](https://paperpile.com/c/5eaDpw/RBtG), and have developed a simple colorimetric based method for the selection of phage mutants, which will be further developed (Figure 1).   Figure 1. Selection of phage mutants. Phages that have been engineered can be selected for by the “blue” plaque phenotype.  The overall aim of the project is to develop a minimal phage genome that can be utilised for biotech applications and to elucidate the function of phage genes. The objectives of the project are to:  * Further develop a colorimetric based selection technique for the creation of phage mutants.

This will involve optimising the system on a range of genetically different phages that infect *E. coli*, *Salmonella*.  * Engineer phages that target different bacterial hosts to display different fluorescent proteins on the capsids.

 This will allow rapid detection of specific bacteria within samples based, using a set of phages we have previously characterised for host range.  * Identify which genes are essential for phage SLUR96 infection.

This will allow the development of a phage chassis that can be used for future development of phage therapeutics. It is necessary to understand what phage genes are essential and under what conditions. To do this we will take a Random Barcode Transposon Sequencing (RB-TnSeq) approach using phage SLUR96.  * Create phage mutants that encode putative anti-phage defence systems

 Engineer phage SLUR96 to express putative anti-dense systems, then test these phages against strains of *E. coli* that express different anti-phage defence systems, to determine if these systems can be overcome. To be done in collaboration with researchers at Southampton University.  Techniques that will be undertaken during the projectMicrobiology – culturing of bacteria and phages  Molecular biology: PCR, Gibson Assembly, cloning, RB-TnSeq Bioinformatics: Genomic analysis of bacteriophage genomes   |
| **References** |
| 1. [Wu Y, Garushyants SK, van den Hurk A, et al (2024) Bacterial defense systems exhibit synergistic anti-phage activity. Cell Host Microbe.](http://paperpile.com/b/5eaDpw/eAYX)2. [Grigonyte AM, Harrison C, MacDonald PR, Montero-Blay A, Tridgett M, Duncan J, Sagona AP, Constantinidou C, Jaramillo A, Millard A (2020) Comparison of CRISPR and Marker-Based Methods for the Engineering of Phage T7. Viruses.](http://paperpile.com/b/5eaDpw/RBtG) |