Identifying Mechanisms of Phage Defense in *Vibrio cholerae* Using High-Throughput Barcode Sequencing

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Introduction

- The aquatic pathogen *Vibrio cholerae* is the causative agent for the fatal enteric disease cholera[1] which actively afflicts populations in parts of Africa, Asia, and Haiti[2].
- Three major lytic phage, ICP-1, ICP-2, and ICP-3, have been identified from the stool samples of cholera patients, indicating their importance as evolutionary drivers in *V. cholerae*[3].
- Prior experiments have revealed that infection of *V. cholerae* with lytic phage is density-dependent, whereby phage predation was completely inhibited at high cell densities (Figure 2).
- This study seeks to elucidate the genes responsible for this deviation in phage defense using high-throughput transposon mutagenesis containing random genetic barcodes (Bar-Seq)[4].
- Particularly, we will be looking at the genes responsible for phage defense at varying cell densities to better understand how *V. cholerae* competes with environmental phage.

Methods

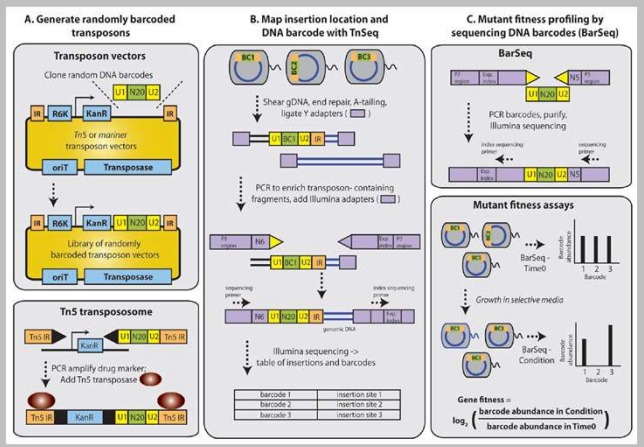

Figure 1. Diagram outlining the steps of developing a Bar-Seq library and conducting fitness assays.[4]

A. Create the transposon library: Mate *V. cholerae* with *mariner* transposon-containing *E. coli* cells. The transposon includes the inverted repeats, Kanamycin resistance, and an inserted 20-nucleotide random barcode sequence flanked by PCR priming sites.

B. Link barcodes to the transposon insertion sites: The gDNA is amplified using PCR priming sites, which allow for the barcodes to be mapped with a specific DNA segment. A database can then be generated allowing for simple tracking of gene fitness in the library by measuring the abundance of the hundreds of thousands of barcodes linked to specific genes.

C. Test mutant fitness in experimental conditions: Finally, the library can be challenged with a variety of different conditions to characterize gene fitness.

Results

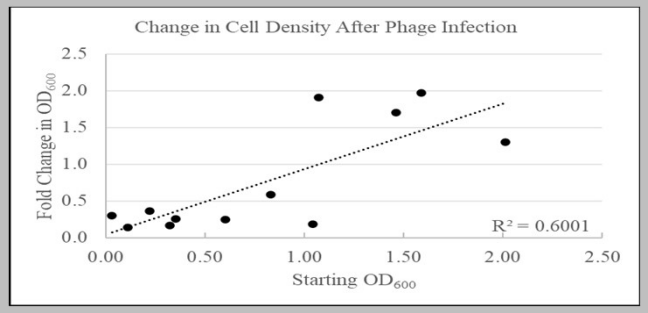

Figure 2. Density-Dependent Phage Infection Assay. Cultures of *V. cholerae* were infected with 10 µL of ICP-3 phage at the indicated starting densities. Cell density was remeasured 1 hour later to determine whether the cells could withstand the phage predation. Cultures with a low starting density (OD_{600} < 1.0) typically saw a 50% reduction in OD_{600} while those with a high starting density (OD_{600} > 1.0) continued to grow.

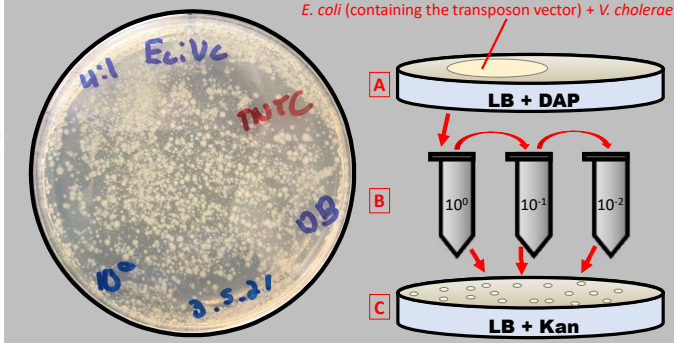

Figure 3. Creating Mutant Colonies. A. In order to transfer the plasmid from *E. coli* to *V. cholerae*, a colony mating was performed on LB-DAP. The *E. coli* is DAP auxotrophic and cannot grow in a DAP deficient medium. **B.** Afterward, the conjugate mixture is resuspended, and serial dilutions are then performed. **C.** Finally, the dilutions are plated on separate LB-Kan plates, which only allows *V. cholerae* cells that took up the plasmid to grow.

Table 1. Quantification of Successful Mutant Colonies

Dilutions	*4:1 Ratio*	*9:1 Ratio*	*19:1 Ratio*	*24:1 Ratio*
10^0	TNTC	TNTC	TNTC	TNTC
10^{-1}	22	TNTC	328	147
10^{-2}	7	6	9	2

Table 1. Quantification of Successful Mutant Colonies. Different conjugation ratios of *E. coli* to *V. cholerae* were analyzed to determine which would produce the greatest number of mutants. The 9:1 ratio (180 µL : 20 µL) produced the highest cell count on the 10^{-1} dilution, indicating that this is the optimal ratio to use. TNTC = Too Numerous To Count

Results

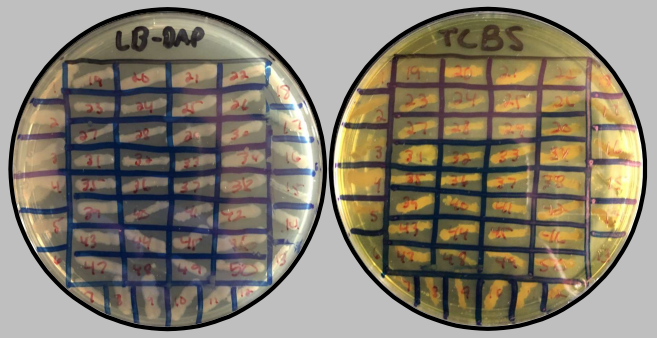

Figure 4. Confirmation of Mutant Colony Identity. Fifty random colonies were selected from a post-conjugation selective plate (LB-Kan) and transferred to both a TCBS plate and an LB-DAP positive control plate. TCBS is fermented by *Vibrio* species which turn the medium yellow. Colonies #6 and #13 did not turn the TCBS yellow; however, colony #6 did not grow on the LB-DAP positive control, meaning the colony was likely not viable. This confirms that *V. cholerae* is being properly selected for.

Conclusions

- The transfer of the transposon plasmid from *E. coli* to *V. cholerae* via conjugation on LB-DAP produced a high quantity of mutant cells containing barcode sequences that can be used to generate a Bar-Seq library.

Future Goals

- Once a genetically robust library is generated, the next step in our research is to sequence the library and begin the process of linking the 20-nucleotide barcodes to their respective genes.
- We will begin searching to identify the genes that contribute to phage defense in low, medium, and high cell densities to determine whether the same phage defense system is implemented in all three conditions.
- More broadly, the *V. cholerae* Bar-Seq library will be an invaluable tool for characterizing large segments of the genome that have previously been neglected and can be used in a wide range of applications.

References

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4. Wetmore, Kelly, et al. "Rapid Quantification of Mutant Fitness in Diverse Bacteria by Sequencing Randomly Bar-Coded Transposons." *mBio*, vol. 6, no. 3, Jun. 2015.

Acknowledgements

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