

## Mobile Genetic elements in the ORFRC: Native plasmids and Viral Genomes

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**Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.**

Mobile genetic elements such as plasmids and viruses can shape the function and evolution of natural microbial communities. Both viruses and plasmids have, for example, been shown to harbor and transfer genes to microbial hosts that improve microbial fitness in particular environments or conditions. Here, we present a set of studies to identify and functionally characterize mobile genetic elements in ground water microbiomes at the Oak Ridge Reservation study site (ORFRC), which is contaminated with heavy metals and nitrate. First<sup>1</sup>, we optimized a cultivation-free method targeting plasmid DNA across a range of sizes and abundance to examine the plasmidome of groundwater samples. Plasmidome sequencing data from just two ground-water wells helped identify > 600 new assembled circular plasmids, many encoding metal and antibiotic resistance genes. In a follow-up study<sup>2</sup>, we examined in more detail the surprising presence of genes encoding mercury tolerance, despite mercury not being one of the major contaminants at this site. Many homologs of *mer* genes (e.g., *merA*) were found in several assembled plasmid scaffolds, including a plasmid found to be abundant (based on sequence coverage) in both groundwater samples. Since this abundant plasmid, p5343, was not isolated but rather inferred from assembly of plasmidome sequence, we synthesized this 8kb plasmid to test it experimentally. We developed a mercury tolerance assay in *E. coli* and established that the *mer* genes on p5343 were functional in conferring mercury tolerance. More importantly we found that p5343 was able to naturally transform into several isolates including two Gram-positive bacteria that have not been transformed before. In a third recent study<sup>3</sup> we re-analyzed this large sequence dataset for the presence of phage genomes. In collaboration with the DOE Joint Genome Institute (JGI) we discovered 200 phage genomes, infecting some of the dominant

microbes in the ecosystem. Several of these phage genomes encode predicted metal and antibiotic resistance genes, presumably providing a selective advantage to infected hosts. Taken together, these plasmidome and phageome studies provide a first overview of the diversity of mobile genetic elements in ORFRC groundwater, along with valuable future directions. The current database of > 600 plasmids and 200 viral genomes can be mined for parts such as origins for developing genetic tools for manipulating many model and ORFRC bacterial isolates. Functional studies of genes encoded on these plasmids and viral genomes will reveal key insight into metal and antibiotic resistance in this environment, highlighting functions encoded by mobile genetic elements. Study of additional wells, both ground water and sediment will provide rich information of mobile genes and their potential role in the microbial ecology of this site.

### **References**

1. Kothari et al mBio 2019 <https://doi.org/10.1128/mBio.02899-18>
2. Kothari et al mSystems 2019 <https://doi.org/10.1128/mSystems.00588-19>
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