

Technologies for High-Throughput characterization of environmental isolates

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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.

Ongoing large scale high-throughput cultivation and targeted lower throughput approaches aim to recover the broad diversity of microbes from the Oak Ridge site that are exemplars of relevant metabolism occurring in this specific environment. With the overarching goal to understand the interactions of this large set of isolated microbes with their environment, the ENIGMA team has developed high-throughput technologies that allow large-scale phenotypic investigation and in-depth characterization.

Some of the molecular genetic tools we have developed, such as RB-TnSeq, led to a considerable amount of gene function inferences from gene-phenotype measurements. We are working on extending the application of this approach to previously genetically challenging microbes. We developed an automated platform for DNA Affinity Purification (DAP) – seq, a biochemical assay used to characterize protein-DNA interaction sites by in vitro affinity-based protein purification, DNA binding, and next generation sequencing. When applied in parallel, DAP-seq has the power to elucidate the DNA binding sites of a bacterium's entire repertoire of transcription factors.

Another approach, exometabolomics, is based on the comparison of inoculated vs. uninoculated media to identify secreted products and depleted metabolites. This provides direct biochemical observations on consumed and secreted metabolites which can be used to predict resource competition and cross-feeding in microbial consortia and communities. This approach has enabled the rapid profiling of substrate use via LC-MS/MS by FRC isolates. These efforts will result in a better understanding of the coupling between growth substrates, and microbial activity.

We combined RB-TnSeq and metabolomics to explore the impact of stresses on a biological system and showed that the use of multi-omics techniques provides a way to probe complex interactions.

Finally, we developed computational tools capable of incorporating our large-scale experimentally generated data (Fitness browser – <https://fit.genomics.lbl.gov/>) and providing reliable analyses for genome annotation (Gap Mind - <http://papers.genomics.lbl.gov/gaps>) facilitating data communication and availability to the scientific community at large.

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