SIP-OMICS: A Semi-Automated Pipeline for Isotopically-Targeted Community Analysis and Metagenomics

Erin Nuccio[1], **Steve Blazewicz[1]*** (blazewicz 1@llnl.gov), Ashley Campbell[1], Marissa Lafler[1], Rex Malmstrom[2], **Bruce Hungate[3], Egbert Schwartz[3]**, **Jennifer Pett-Ridge[1]**

[1]Lawrence Livermore National Laboratory, Livermore, CA; [2]Joint Genome Institute, Berkeley, CA; [3]Northern Arizona University, Flagstaff, AZ

Website: https://sc-programs.llnl.gov/soil-microbiome

Project Goals: Microorganisms play key roles in soil carbon turnover and stabilization of persistent organic matter via their metabolic activities, cellular biochemistry, and extracellular products. Microbial residues are the primary ingredients in soil organic matter (SOM), a pool critical to Earth's soil health and climate. We hypothesize that microbial cellular-chemistry, functional potential, and ecophysiology fundamentally shape soil carbon persistence, and we are characterizing this via stable isotope probing (SIP) of genome-resolved metagenomes and viromes. We focus on soil moisture as a 'master controller' of microbial activity and mortality, since altered precipitation regimes are predicted across the temperate U.S. *Our SFA's ultimate goal is to determine how microbial soil ecophysiology, population dynamics, and microbe-mineral-organic matter interactions regulate the persistence of microbial residues under changing moisture regimes.*

Abstract: Linking the identification of uncultivated microbes with their environmental function is a key ambition for microbial ecologists. While many techniques attempt to meet this goal, stable isotope probing—SIP—remains the most comprehensive for studying whole microbial communities *in situ*. In DNA-SIP, microbes who take up an isotopically heavy substrate end up with heavier DNA, which can be divided into multiple fractions containing DNA of different densities. Compounds labeled with ^{13}C or ^{15}N are frequently used to study the ecophysiology of organisms that consume a substrate of interest, while ^{18}O water is used as a universal tracer to measure the taxon-specific growth of all active taxa. However, SIP is not as broadly used as it could be because it requires specialized equipment, requires expensive reagents, is relatively low throughput and very time-consuming.

We designed a high throughput semi-automated DNA-SIP pipeline that can be combined with either amplicon or metagenomic sequencing. Our pipeline decreases operator time, reduces operator error, and improves reproducibility by targeting the most labor-intensive steps of traditional SIP—fraction collection, cleanup, and DNA processing. Fractionation is accomplished by connecting a SIP tube to an HPLC fraction collector (Agilent Infinity Fraction Collector), which aliquots four SIP tubes into a single plate 96-well plate. DNA precipitation and Picogreen quantification are then automated on a liquid handling robot (Hamilton STAR), which allows us to process density fractions from 16 SIP samples simultaneously. In addition, we have developed a method for pre-screening nucleic acids for isotopic enrichment, to ensure samples are adequately enriched prior to density gradient separation. Since establishing our pipeline, we have run over 1000 SIP samples, including well-replicated studies of annual grassland soil taxa active during key points in the water-year (fall wet up, spring growing season), plus analyses of

soil water limitation, redox, habitat and mycorrhizal effects. Overall, the pipeline reduces the per sample processing time from 9 hours to 1.7 hours, with ca. 30 minutes of manual work per sample. Using *in silico* analysis, we determined that 9 fractions are an ideal number to identify enriched organisms in samples with greater than 5 atom percent enrichment—this level of resolution balances of financial costs of extra fractions versus the benefits of error reduction [1].

We further improved our SIP practices by including internal standards during ultracentrifugation, which can calibrate per sample conversions of GC content to mean weighted density and determine the ^{18}O atom percent enrichment of a taxon's genome. The internal standards are two 9Kb PCR products of known isotopic enrichment. The more isotopically enriched standard typically appears in ultracentrifuge tubes approximately 0.05 g/ml heavier than the non-enriched standard, indicating about 3.3 extra neutrons per base pair are required for a DNA molecule to be isolated in a subsequent fraction. Using this methodology, the atom percent enrichment of a taxon's genome can be quantified in comparison to the internal calibration standard, which greatly improves the reproducibility of SIP runs because the calculation of sequence enrichment does not rely solely on refractometry. Refractometry measurements may vary between different operators and laboratories leading to incorrect measurements of isotopic enrichment of DNA contained in that fraction. Our semi-automated SIP approach and calibration of internal standards should make isotope-enabled techniques more high-throughput, more reproducible, accessible to the greater scientific community and allow better comparison of results among different experiments.

References

1. Sieradzki, E.T., et al., *Measurement Error and Resolution in Quantitative Stable Isotope Probing: Implications for Experimental Design.* mSystems, 2020. **5**(4): p. e00151-20.

This research is based upon work of the LLNL 'Microbes Persist' Soil Microbiome SFA, supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Number SCW1632 to the Lawrence Livermore National Laboratory, and a subcontract to Northern Arizona University. Work at Lawrence Livermore National Laboratory was performed under U.S. Department of Energy Contract DE-AC52-07NA27344.