Ongoing Projects

NSF Award No. DBI-2400327

Project Number	Project Title	Principal Investigator	Home Institution	Project Description
UCR-003-I-F	Generation of defined transposon libraries in Vibro cholerae	Dr. Ansel Hsiao	UC Riverside	The overall goal of the project is to generate protocols for the automated assembly, manipulation, and duplication of an arrayed transposon mutant library for V. cholerae. We will use a Tn5 family transposon introduced into V. cholerae via conjugation from an Escherichia coli donor strain, pick colonies using the ExFAB colony picker, and then use ExFAB liquid handling robotic systems to automate PCR for identification of transposon insertion sites, and to generate multi-well libraries of mutant cultures and glycerol stocks of these libraries. At the end of this project, we will generate a defined mutant library of V. cholerae. We will use these mutant libraries to identify critical genetic determinants of pathogen fitness in the presence of different environmental stresses, including in animal systems with defined commensal microbial communities. The library can also be a resource for many laboratories, providing insertional mutants for proof-of-concept experiments without having to individually construct knockouts for individual genes. The protocols developed under this project can also be adapted for similar studies in other bacterial systems.
UCSB-008-I-D	Discovery and Functional Characterization of Novel Fusion Enzymes Driving Biosilification in Diatoms	Dr. Michelle O'Malley	UC Santa Barbara	The objective of this project is To understand and characterize novel proteins from diatoms responsible for synthesizing chemicals such as long-chain polyamines (LCPAs), a key contributor to diatom biosilification. We will leverage Ex-Fab's highthroughput capabilities to express these proteins in P. pastoris, which allows us to design, test, and build multiple protein variants by selectively removing different regions of the enzymes, dissecting the function of protein domains. Additionally, it enables extensive clone screening and optimization of expression conditions to improve yield and activity.
UCSB-009-I-D	Modern Analogues for Ancient Life: A Comparative Catalogue of Giant Bacteria Morphology	Dr. Jean-Marie Volland	UC Santa Barbara	The objective of this project is to create a comparative catalogue to bridge the gap between microbiologists studying living giant bacteria and micropaleontologists analyzing fossilized microbes. This catalogue will serve dual purposes: providing modern biologists with a visual survey of giant bacterial diversity—a resource that does not yet exist—and offering micropaleontologists updated diagnostic criteria for distinguishing giant acteria from other large microbes in the fossil record. The central feature of the catalogue will be a series of high-resolution 3D models that reveal both external and internal morphological features across a diverse set of extremophilic giant bacteria isolated from highly sulfidic marine environments—a research direction that directly supports Ex-FAB's mission to study life in extreme conditions.
UCR-010-I-D	High-throughput determination of growth rates for Neurospora crassa knockout mutants cultured under different conditions, followed by hierarchical clustering analysis to identify genes in shared pathways	Dr. Katherine Borkovich	UC Riverside	This project will use the BM3-BC automated colony handling robot, the SpImager, and ExFAB's PhenoTypic software to determine growth rates of Neurospora crassa knockout mutants cultured under different conditions. These data will inform efforts to identify N. crassa genes in shared pathways.

UCR-012-I-E	Phenotyping Rhodotorula spp. Tolerance to Heavy Metals for Bioremediation Applications	Dr. Jason Stajich	UC Riverside	In this study we will identify variation in tolerance to various heavy metals within a large collection of Rhodotorula isolates in order to evaluate their potential in bioremediation in contaminated environments and to understand the genetic basis for heavy metal tolerance. The project will utilize the S&P pinner and imager to create high resolution images that can be used with Phenotypic analysis software to understand the variation in tolerance and its genetic background.
UCSB-014-I-D	Imaging Kleptoplastidic Organelle Uptake Modifications in Ciliates via Enhanced Subcellular Structure Visualization	Dr. Michelle O'Malley, Dr. Holly Moeller	UC Santa Barbara	Alongside gaining insight into into the phenomenon of kleptoplasty in Mesodinium amoebae, the proposed work seeks to introduce expansion microscopy as a method to visualize subcellular structure of understudied microorganisms with novel biomolecular processes and design a streamlined pipeline for ExFAB users to easily adapt their organisms for this technique. Through characterizing structural modifications of stolen algal organelles by Mesodinium via expansion microscopy on the Cytation C10, we aim to inform future research on directed endosymbiosis and acquired metabolism to open novel avenues of bioproduction in biotechnologically relevant species.
UCSB-016-I-E	Initial Exploratory Project_Identifying Fungal EV Markers within Anaerobic Gut Fungi Extracellular Vesicle-Like Particles throughTargeted Proteomics	Dr. Michelle O'Malley	UC Santa Barbara	In this work, we will characterize putative extracellular vesicles isolated from anaerobic gut fungi via mass spectrometry, Determining if the isolated particles, which exhibit hallmark extracellular vesicle characteristics through nanoparticle tracking and transmission electron microscopy, contain protein markers identified in other fungal species would provide additional evidence that these particles are indeed extracellular vesicles. This approach will also establish a targeted metabolomics workflow to identify proteins beyond the known markers and lay the groundwork for untargeted proteomic and metabolomic research, potentially uncovering novel molecules within extracellular vesicles, especially in the context of anaerobic gut fungi.
UCSB-017-I-D	Tracing 13C-Labelled Lignin Metabolites in Anaerobic Bacterial Communities via LC-MS	Dr. Michelle O'Malley	UC Santa Barbara	The objective of this work is to determine the mechanisms via which an anaerobic bacterial community enriched from an anaerobic digestor degrades lignin. We have preliminary compositional analysis data, and 2D NMR evidence suggesting that this community breaks down lignin bonds. However, we would like to confirm these results using targeted LC-MS detection of 13C labelled lignin breakdown products and a higher sensitivity 2D NMR experiment (NMR performed outside of UCSB).
UCSB-018-I-D	Spheroplasts Formation for Lignin-Digesting Anaerobic Gut Fungi	Dr. Michelle O'Malley	UC Santa Barbara	The general motive of this project is to develop genetic engineering tools for Anaerobic Gut Fungi (AGF) by pioneering and assessing high throughput fungal transformation methods. Specifically, this proposal covers the first step in the project, which is generation of a fungal protoplasts through an enzymatic digestion pipeline. We will perform automated confocal fluorescence microscopy to characterize the effect of enzymatic treatments on AGF cell wall degradation, varying enzyme composition, concentration, treatment time, and reaction temperature to find optimal conditions for protoplasting.
UCSB-019-I-E	Initial Exploratory Project_Probing Metabolomic and Proteomic Biosignatures in Sulfolobus-Integrated Europa Ocean Vents Systems to Model Europa Clipper	Dr. Jamie Snyder	Cal Poly Pomona	The objective of this work is to use ExFAB's MassSpec (MS) Suite to study the metabolomic and proteomic dynamics occurring within our Sulfolobus-integrated Europa ocean vent models. Using ExFAB's MALDI-TOF and Orbitrap, the Snyder Lab aims to compare the metabolites present in standard DSM 639 Sulfolobus acidocaldarius (Saci) archaeal strains versus Snyder Lab Saci strains that have adapted overtime to biofilm formation in Europa ocean vent conditions.
UCSB-020-I-D	Development of Microfluidic Platforms for Investigating Extremophiles	Dr. Todd Squires, Dr. Michelle O'Malley	UC Santa Barbara	In this project, we will Develop and validate novel microfluidic platforms for sorting and enriching extremophiles based on (i) droplet-based isolation and culture (Specific Aim 1), (ii) dynamic responses to environmental gradients such as chemical, thermal, or osmotic stresses (Specific Aim 2), and (iii) integration with ExFAB automation and analytical pipelines for scalable discovery workflows (Specific Aim 3). Devices will be designed to interface with ExFAB's Cytation C10 confocal imager for high-resolution droplet imaging and analysis.
UCSB-021-I-D	Build a method to overexpress oxygen-sensitive iron-sulfur proteins [FeFe]-hydrogenase and NADH dehydrogenase of anaerobic gut fungus Caecomyces churrovis	Dr. Michelle O'Malley	UC Santa Barbara	The goal of this project is to build a method to co-overexpress and purify two iron-sulfur enzymes, HydA ([FeFe]-hydrogenase) and NuoEF (NADH dehydrogenase), of the anaerobic gut fungus Caecomyces churrovis in E. coli BL21(DE3)ΔiscR. This is necessary for testing if these enzymes are key enzymes for H2 production by C. churrovis. Understanding the key enzymes for H2 production in the anaerobic gut fungi can inform engineering strategies for higher H2 production strains. In addition, a method for iron-sulfur proteins production can benefit future work on characterization of other iron-sulfur proteins.

UCSB-023-I-E	Optimizing methods to characterize small polar organic molecules	Dr. Morgan Raven, Dr. David Valentine	UC Santa Barbara	In this project we will develop improved methods to characterize small polar molecules that are found in low concentrations in the ocean and are highly redox sensitive. We will be reacting monosaccharides with differing types and concentrations of sulfur species and using the orbitrap mass spectrometer to identify and characterize the reaction products, and the triple quad MS to quantify the free sulfur left in solution.
UCSB-024-E-D	Elucidating the metabolic flux in Vibrio natriegens-based cell-free protein expression systems	Dr. Javin Oza	Cal Poly SLO	This project aims to leverage Vibrio natriegens' exceptional capacity for protein expression and metabolic flexibility to establish a cell-free system with unprecedented protein expression kinetics in vitro. V. natriegens has proven to be a viable chassis for cell-free protein expression, however, reaction kinetics and protein yields do not out-perform those of E. coli, an observation that is at odds with in vivo phenotypes. Our hypothesis is that V. natriegens cell-free systems are limited by metabolic constraints. The project will utilize targeted metabolomics to 1) diagnose metabolic bottlenecks and 2) guide reaction condition optimization.
UCSB-025-E-F	High-Throughput Elicitation of Natural Product Biosynthesis in Cellulolytic Clostridia	Dr. Ben Woolston	Northeastern	The objective of this project is to identify new secondary metabolites in one particularly gifted group of anaerobes – the cellulolytic Clostridia – using the capabilities of ExFAB to screen five microbes against a large library of potential inducers, identifying conditions that result in novel MS features for further study. Beyond the immediate scientific goal, this work will also establish and validate a high-throughput elicitation screening (HiTES) platform, which will be of broad interest to the ExFAB community.
UCSB-026-E-D	Identifying ferredoxin nicotinamide oxidoreductase (Fnor) enzymes for ethanol production by functional complementation in Thermoanaerobacterium saccharolyticum	Dr. Dan Olson	Dartmouth College	We recently characterized a set of 27 ferredoxin nicotinamide oxidoreductase (Fnor) enzymes. We found several with a desired combination of both high thermostability and "true" FNOR activity, that should enable them to function as part of a thermophilic ethanol production pathway. However, ethanol yield and titer were relatively low, and we suspect that one or more additional mutations may be needed to enable the desired pathway to function well. The overall goal of this project is to screen different combinations of fnor genes, chromosomal loci, and background strains to identify these mutations.
UCSB-027-E-F	High-Throughput Optimization of Anaerobic Growth Conditions for the Model Halophile Haloferax volcanii	Dr. Jamie Foster	University of Florida	We aim to Optimize anaerobic growth conditions for the haloarchaeon Haloferax volcanii. The optimization will be achieved by implementing high-throughput automation and liquid-handling to systematically phenotype H. volcanii under various anaerobic growth conditions. The outcomes of this effort will improve the understanding of anaerobic metabolism in halophilic archaea and may aid future bioremediation and biotechnology efforts.
UCSB-028-E-D	High-Throughput Screening of Environmental Microbiomes for Anaerobic Bacterial Lignin Degraders	Dr. Brady Cress	UC Berkeley	Identifying lignin-degrading metabolisms is of paramount importance for complete and efficient utilization of plant feedstocks for fuel and specialty chemical production. Lignin is an untapped source of carbon for fuel and chemical production. We aim to conduct high-throughput screening of microbiomes from lignin-rich environments on lignin waste streams to select for metabolisms evolved to digest lignin polymers, and identify novel pathways of interest for industrial bioprocessing. By utilizing an automated assembly and screening process, we can vastly increase the number of lignin streams, environmental samples, conditional elements (e.g. pH, temperature), and functional readouts (e.g. lignin depletion, metabolic output, cellular growth, metagenomics and metatranscriptomics) to precisely and quantitatively characterize microbial activity and extract high performing strains and consortia.
UCR-029-E-D	Extreme osmotolerance in bacteria isolated from concrete	Dr. Julia Maresca	SUNY College of Environmental Science and Forestry	Concrete is the most-used building material in the world, but has no measurable nutrients, no measurable water activity, high salt concentrations, and a pH close to 12.5. It is, therefore, a very common habitat that is inhospitable to most life. Despite the harsh conditions, concrete hosts a small but diverse community of microbes both on its surface and inside the matrix. Over the past 14 years, we have isolated a variety of bacteria from concrete, and here we propose to evaluate their tolerance of the combined stresses of high salt and high pH. These isolates grow in circumneutral, low-salt conditions in the laboratory, but tolerate higher-pH, higher-salt conditions. They include at least one novel species and may be sources for extremotolerant enzymes that can be used for bioproduction. Alternatively, the organisms themselves could be used for remediation or other applications in salty, arid environments, such as dump sites for fracking fluids, concrete-stabilized boreholes, or desert soils.

UCR-030-E-D	Decoding Bacterial Phenotypes Through Systematic Cultivation Design	Dr. Paul Carini	University of Arizona	We propose to leverage ExFAB's automated phenotyping capabilities to characterize 90 soil bacterial isolates from a geographically diverse California culture collection. These isolates were obtained using orthogonal array (OA) design—a systematic approach that efficiently samples multidimensional growth parameter space. By phenotyping all 90 isolates across 9 OA media conditions used for isolation (systematically varying temperature, pH, salinity, and carbon source), we will establish quantitative links between genotype, phenotype, geography, and growth environment. This project addresses ExFAB's mission to develop high-throughput workflows for extreme and exceptional microbes while introducing OA methodology as a generalizable framework for cultivation-based microbial ecology
UCR-031-E-D	Determining Morphological Differences Associated with Phenotypic Instability in Biomanufacturing	Dr. Mark Blenner	University of Delaware	Failure to scale in biomanufacturing remains a persistent threat to a biomanufacturing-enabled bioeconomy. The underlying design principles for making stable microbial strains that retain production characteristics as cultivation times extend with increasing scale are not known.This project aims to identify stable and unstable strains of engineered beta-carotene producing strains of Yarrowia lipolytica, determine what morphological features are associated with these phenotypes, and how these dynamics of morphological changes correlate stability phenotypes. We further will evaluate the effect of media and cultivation condition on stability and morphology.
UCR-032-E-E	High-throughput Plate-Based Imaging of ectomycorrhizal fungi Morphology	Dr. Rolando Perez	Stanford University	This exploratory project will establish and validate a compact, automated imaging workflow for capturing and quantifying Suillus pungens and Suillus subaureus colony morphology in 24-well agar plates. The goal is to confirm feasibility and reproducibility of ExFAB-compatible procedures for plate preparation, imaging, and morphometric analysis using a minimal number of samples. These results will serve as the technical foundation for a larger developmental project integrating morphological and metabolomic datasets to link phenotype and metabolic state.
UCR-033-E-D	Developing and piloting high-throughput, solid-state Ganoderma growth and phenotyping methods for predictive modeling of mycelium biomaterials	Dr. Rachel Linzer	Open Fung	This project aims to lay the foundations for model development by developing high-throughput methods for handling Ganoderma and assessing basic phenotypes in solid states, conducting a pilot study evaluating these phenotypes across process-relevant growth conditions, then sharing these methods and data to inform other fungal innovators.
UCR-034-E-E	Method Development to Enable High-Throughput Discovery of Small Molecule Lanthanide Chelators from Methylobacterium	Dr. Allegra Aron	University of Denver	This project aims to develop a method for small-scale (<4 mL) culturing of Methylobacterium for high-throughput mass spectrometry-based metallophore discovery and metabolomics analysis. If this optimization on small scale successfully recapitulates the supernatant metabolomics on a large scale (20-40 mL), subsequent experiments could enable us to discover the chemical diversity of lanthanide chelators produced by a group of 50-100 phyllosphere and rhizosphere isolates. The team of PIs recently discovered the first biological small molecule lanthanide chelator; however, how biology has evolved to solubilize lanthanides more broadly across lanthanide-utilizing environmental bacteria remains an open question that this proposal aims to tackle.
UCSB-035-E-D	Screening of Halophilic Bromoperoxidase Activity Using Automated Fluorescent Assay with LC-MS Validation	Dr. Sabine Rech	San Jose State University	The objective of this exploratory project is to test the feasibility of detecting bromoperoxidase enzyme activity from halophilic bacterial lysates using ExFab’s automated microplate and imaging systems. Specifically, we aim to evaluate whether a HOBr-selective fluorescent probe can reproducibly measure enzyme activity across a small set of high-salt and pH conditions, establishing a reliable, miniaturized workflow for future developmental-scale optimization.
UCR-039-I-F	The unresolved paradox of mutualism-breakdown in rhizobia	Dr. Joel Sachs	UC Riverside	Here we propose genomic analyses to study how and why mutualism breakdown events occur. Specifically, I propose to use ExFAB’s resources to isolate and sequence rhizobia at the nexus of soil and plant associated microenvironments to assess i) the evolutionary forces in the soil and the genomic changes they cause in rhizobia, and ii) how plant hosts select for symbiotic capacity and shape genome architecture of rhizobia.
UCR-040-I-F	Elucidating trade-offs during microbial symbiont adaptation	Dr. Joel Sachs	UC Riverside	The proposed experiments will characterize existing Bradyrhizobium variation in ecologically relevant traits and identify the phenotypes favored under these conditions providing valuable information for risk assessment and management of legume populations.

UCSB-041-I-E	Synthetic Control of Flavin Energetics for Artificial Electron Bifurcation	Dr. Brandon Greene	UC Santa Barbara	We aim to advance fundamental understanding of flavin-based electron bifurcation and establish mechanistic principles for programmable redox partitioning, bridging microbial bioenergetics with synthetic biology. By integrating protein engineering, synthetic cofactors, and advanced characterization within ExFAB, this work opens new avenues for energy-efficient biosynthesis, artificial electron bifurcation, and sustainable catalytic platforms, addressing critical challenges in bioenergy and carbon management.
UCSB-042-I-E	Filamentous large sulfur bacteria and their BGC potential for novel natural drug discovery	Dr. Jean-Marie Volland	UC Santa Barbara	Filamentous large sulfur bacteria have so far been deemed unculturable, so we rely on environmental samples for our experiments. These filaments are usually a heterogeneous mix of filaments with and without epibionts and of different diameters. To examine the difference in metabolites produced by the aposymbiotic and symbiotic filaments, we need a high-throughput screening process to separate them from each other. If successful, we will have a comprehensive list of all the metabolites, both primary and secondary, classified using GNPS2 for these filamentous large sulfur bacteria. We will also be able to identify the big secondary metabolite hits we find in the genome during BGC mining.
UCR-043-I-D	Genome-wide index transposon library for the multi-stress tolerant yeast Kluyveromyces marxianus	Dr. Ian Wheeldon	UC Riverside	Determining the genetic underpinning that enables K. marxianus to tolerate toxic saponins in agave juice still remains unclear — an indexed transposon library will enable the functional genomic screening experiments to address this question. ExFab equipment and workflows, such as the Opentrons liquid handler and S&P robotic pinning and imaging, will be used to generate and screen the transposon library
UCSB-017-I-F	Isolating Individual Bacterial Species from an Anaerobic Lignin Degrading Community to Probe Anaerobic Lignin Degradation Mechanisms	Dr. Michelle O'Malley	UC Santa Barbara	The objective of this study is to deconstruct a complex ~40-member anaerobic bacterial consortium to identify the specific drivers of anaerobic lignin modification. Isolating and identifying the species within the ~40-member bacterial community would give us a much clearer insight into bacterial community dynamics when faced with metabolizing a challenging substrate such as lignin, particularly under anaerobic conditions. Utilizing automated colony picking and liquid handling, we will generate a comprehensive library of isolates to assemble combinations of reduced complexity (<5 member) synthetic communities (SynComs) to identify which species are necessary to recapitulate the lignin degradation observed from the native community, unlocking unique anaerobic mechanisms used by native communities to break down unpretreated lignin.
UCSB-044-I-E	Understanding Deep-Sea Adaptation in Marine Nitrifiers Through Physiology and Genetic Tool Development	Dr. Michelle O'Malley, Dr. Alyson Santoro	UC Santa Barbara	The goal of this research is to use the high throughput capabilities of ExFAB to deeply explore the parameter-space of genetic engineering of non-model, marine organisms with the ultimate goal of a successful transformation system in ammonia-oxidizing archaea (AOA). This will include optimizing both DNA delivery methods, and selection/screening methods generally for marine, non-model microorganisms, in hopes to apply the methodology to AOA. To establish genetic tools for high salinity, chemolithoautotrophic, organisms that exist in a nitrifying community such as AOA, this work will first aim to assess and validate these tools in nitrite-oxidizing bacteria (NOB). Specifically, Nitrococcus mobilis (Nb-231) due to its well-understood genome.