Leveraging A Global-Scale Atlas of Phage Host Range to Discover Novel Precision Antimicrobials

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Background: A growing global antimicrobial resistance (AMR) crisis fueled by the overuse of traditional antibiotics has created an urgent need not only for new antimicrobials, but entirely new approaches for sustained therapeutic development against existing and emerging pathogens. At the same time, the world faces the looming threat of catastrophic climate change, and methane emissions from agriculture are a key driver in overall greenhouse gas emissions.

Methods: We have adapted a unique genomic technology, proximity ligation sequencing, to harness the vast genetic diversity of microbial viruses (phages) to discover large-numbers of protein-based interventions against microbes. Proximity-guided metagenomics allows us to pinpoint the microbial targets of phages in situ, recovering the genomes of both microbe and phage without culturing or isolation of either party. Inherently, each lytic phage genome encodes one or more proteins (endolysins) which can lyse their host cell. Each of these proteins represents a powerful tool in the targeted destruction of a selected organism, and because we know which species of microbe each phage infects, we can identify candidate lysins from proximity-guided metagenomic data alone.

Results and **Discussion**: As part of our global-scale metagenomics project, we used our ProxiMeta platform to recover the target-resolved genomes of hundreds of thousands of phages across the tree of life. We will share published and unpublished work describing our discovery and evaluation of these lysins, and their applications in selectively removing methanogenic archaea in ruminants, and microbes involved in human disease.

Ultra-high throughput single-microbe sequencing enabled by semi-permeable capsules

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Background
Whole-genome and targeted sequencing open a window to understanding the diversity and function of unculturable microorganisms. On the one hand, metagenomic sequencing is attractive for its straightforward library preparation from bulk samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of cells in parallel. Here, we demonstrate how Semi-Permeable Capsule (SPC) technology enables a virtually unlimited number of processing steps on genetic material from thousands of individual microbes in parallel and allows for ultra-high-throughput single-cell sequencing.

Methods
This study aimed to demonstrate the use of SPCs for barcoding >10,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality. For proof-of-concept evaluation, we encapsulated well-characterized E. coli and B. subtilis bacteria into SPCs, lysed cells at alkaline conditions (pH 13), amplified their genomes, and employed a split-pool approach to add unique cellular barcodes.

Results
Upon sequencing of an aliquot of ~3,000 cells, important technical metrics, such as cross-contamination and genome recovery, were measured to assess the performance of the workflow. The results showed excellent genome retention within SPCs, with <1% of cross-contaminated genomes. Genome recovery analysis yielded a median coverage of 90% at a median sequencing depth of 8X for B. subtilis cells (1690 cells sequenced), and a median coverage of 63% at a median sequencing depth of 3X for E. coli cells (2023 cells sequenced) with clear indication for higher recovery at saturating sequencing.

Discussion
We conclude that the compartmentalization of microbial cells into SPCs allows the generation of high-quality whole-genome data at scale, and further apply the method on environmental samples.

Microbiome species profiling at scale with the Kinnex kit for full-length 16S rRNA sequencing

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Background: Targeted 16S sequencing is a cost-effective approach for assessing the bacterial composition of metagenomic communities and is particularly useful for low bacterial biomass samples. However, due to high similarity in 16S rRNA genes among related bacteria, sequencing the entire 16S gene (~1.5kb) with high accuracy is essential for precise species and strain identification. Comparative studies have shown that PacBio full-length (FL) 16S sequencing outperforms other methods for taxonomic resolution and accuracy. The Kinnex 16S rRNA kit, based on the multiplexed array sequencing (MAS-Seq) method, processes amplified, barcoded 16S amplicons and outputs a sequencing-ready library that results in up to a 12-fold throughput increase compared to standard FL 16S libraries for significantly higher throughput (up to 1,536 amplicons per SMRT Cell).

Methods: We tested the Kinnex 16S rRNA kit on a diverse range of samples including standards, feces, saliva, plant, wastewater, and swabs (skin, oral, vaginal, and veterinary wound). We analyzed the data using the HiFi-16S-workflow, that provides a FASTQ-to-report analysis for FL 16S HiFi reads.

Results: The results show that Kinnex 16S sequencing can yield >30k average reads per sample at 1,536-plex on a single Revio SMRT Cell or at a 768-plex on a Sequel IIe SMRT Cell. Comparing Kinnex 16S to standard FL 16S datasets, we found a high correlation and no bias in community compositions and were able to assign up to ~99% of denoised reads to species. In addition, because of the higher number of reads per sample, Kinnex 16S allows for more recovery of lower abundance species.

Discussion: With the Kinnex 16S rRNA kit, researchers may now multiplex more samples to dramatically reduce cost per sample or to profile each sample deeper with more reads/sample. The additional reads/sample along with better taxonomic resolution is advantageous for numerous environmental sample types which are often highly diverse.

Utilizing microbial genomic DNA libraries to discover modified nucleoside eraser enzymes

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Background: The number of known RNA modifications is ever increasing with over 170 modifications identified to date. The enzymes that install these modifications are called writer enzymes, whereas the enzymes that remove the modifications are called eraser enzymes. Many writer enzymes are known and well characterized. However, few eraser enzymes have been identified and studied.

Methods: To identify new eraser enzymes, we have designed a genetic selection system using auxotrophic strains of Escherichia coli deficient in either purine or pyrimidine biosynthesis. Using these strains, we can screen for the ability of E. coli to grow on minimal media supplemented with a modified nucleoside as the sole source of purines or pyrimidines. If E. coli is unable to grow on the provided modified nucleoside, then genomic DNA plasmid libraries can be used to identify enzymes from other species that can remove these modifications.

Results: We assessed the ability of the E. coli purine and pyrimidine auxotroph strains to grow on 19 different modified nucleosides. The auxotrophic E. coli strains were unable to grow on 16 of the compounds. We performed a genetic selection on these 16 compounds using 7 diffident genomic DNA libraries. From these selections, we identified 8 genes that allowed E. coli to grow on 2-thiouridine or 4-thiouridine. One of the enzymes from Thermococcus kodakarensis, named TudS_T for 4-thiouridine desulfurase, is the first example of a 4-thiouridine eraser enzyme from archaea shown to have activity in vivo.

Discussion: Combining genomic DNA libraries with auxotrophy-based genetic selection enables the discovery of novel enzymes. TudS_T does not show significant sequence or structural homology to known 4-thioruridine desulfurases and could not be identified using standard bioinformatic searches. This approach has allowed us to identify evolutionarily divergent enzymes and uncover new aspects of archaeal biology.

Lemur and Magnet: leveraging long reads for improved microbial community profiling

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Background
The emergence of long-read sequencing of microbiomes necessitates the development of new microbial community profilers tailored to long-read shotgun metagenomic datasets. Existing taxonomic read classification tools have established themselves as a *de facto* standard approach for taxonomic read classification and taxonomic profiling with short-read data.

Methods
We introduce Lemur and Magnet, a pair of tools optimized for lightweight and accurate taxonomic profiling from long-read shotgun metagenomic datasets. Lemur is a marker-gene based method that leverages an EM algorithm to reduce false positive calls while preserving true positives; Magnet makes detailed presence/absence calls for bacterial genomes based on whole-genome read mapping. By operating only on marker genes, Lemur is a comparatively lightweight software.

Results
Our results are threefold: 1) Lemur and Magnet provide accurate profiles of microbial communities, 2) The tandem is computationally efficient; it can run in minutes to hours on a laptop with 32 GB of RAM, even for large inputs; 3) Lemur has a small footprint; the marker gene database used by Lemur is only 4 GB and contains information from over 300,000 RefSeq genomes.

Lemur and Magnet are available at: https://github.com/treangenlab/lemur.

Discussion

Lemur and Magnet are tailored for long-read microbial community profiling. Lemur exhibits competitive performance by most standard metrics; when it is paired with Magnet, particularly in the presence of low-abundance or low-coverage data, it can improve precision by detecting and filtering out a large number of false positive calls.

Microbial Community Shifts Across Gradients of Urbanization

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Urbanization causes significant changes to soil ecosystems, particularly microbial communities within the affected soils. Microbes serve essential functions in wild ecosystems as they recycle nutrients, aid in plant growth, act as pathogens, and support the biodiversity within affected soils, and shifts in community composition can be indicative of functional changes that affect ecosystem fitness. Here, we explored microbial community shifts across gradients of urbanization, specifically 6 different soil types across different urban areas around California State University San Marcos in San Marcos, CA and Poway, CA: 1) native chaparral, 2) old fields (previously developed land that is no longer tilled or amended), 3) hedges dominated by Acacia longifolia, a nonnative shrub species, 4) turfgrass lawns, 5) soil beneath concrete, and 6) soil beneath asphalt. Total DNA was extracted from the sampled soils and underwent shotgun metagenomic sequencing, followed by analyses of relative composition, alpha diversity, beta diversity, and canonical-correlation analysis on the sequenced metagenomes. Results show lawns having the least diverse microbial communities (driven by soil moisture and pH), with soil beneath impervious surfaces having the strongest community profile shifts (driven by pH and bulk density).

Methane related cycle microbes in Croatian lakes

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Lakes play a crucial role in the global carbon cycle by being active sites for carbon transport, transformation and sequestration. The methane cycle in lakes involves a complex interplay of various microorganisms that mediate the production, consumption and flux of methane. These microorganisms include methanogens, methanotrophs and associated bacterial communities whose interactions profoundly influence the impact of methane on lacustrine environments. As a part of our study we investigated two different lake systems in Croatia: one oligotrophic - Plitvice Lakes and one mesotrophic with seasonal anoxia and salinization in the bottom of the lake - Baćina Lakes. The objectives were to investigate spatial and temporal variability of methane cycling microorganisms in the water column of four lakes in Croatia and their relation to environmental conditions. Using 16S rRNA gene sequencing and quantitative PCR we aimed to (1) investigate environmental parameters that shape the seasonality of methane-cycling microbial communities; (2) understand how methanotroph and methanogen communities vary along the oxycline. In the studied lakes, methanogens belonging to six orders within the phylum Euryarchaeota were identified: Methanobacteriales, Methanocellales, Methanofastidiosales, Methanomassiliicoccales and Methanomicrobiales and Methanosarciniales. Methanogens accounted for 0.004% to 2.2% of the total community in samples where they were detected. Five orders of methanotrophs were identified in the collected samples, including Methylacidiphilales, Methylococcales (represented by eight genera), Methylomirabilales, Rhizobiales, and Rokubacteriales. The relative abundance of methanotrophs, based on amplicon sequencing, ranged from 0.007% to 10% in the PA fraction and from 0.003% to 3.3% in the FL fraction. All lakes displayed distinct methanotrophic community compositions with seasonal variations and minor differences between the FL and PA fractions The qPCR results revealed variations in the abundance of pmoA and mrcA genes between the particle associated (PA) and free living (FL) fraction across the water column of all investigated lakes. Notably, the pmoA gene consistently exhibited higher abundance compared to the mcrA gene in all lakes, with a minimum tenfold difference. Metagenomes data analysis are in progress

Broad spectrum fatty acid assisted bacteriophage lysis of both gram negative and gram positive pathogens

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Background: Stenotrophomonas maltophilia (STM) is an environmental, gram-negative, multi-drug resistant bacteria that causes debilitating infections in immunocompromised people, such as cancer patients undergoing immunotherapy and persons with systemic airway infections. While phages have been successfully applied to treat infections caused by antibiotic resistant microbes, evolved resistance to phages and the highly personalized nature of phage therapy contribute to its lack of widespread use. Combinatorial approaches, such as those employing phages and antibiotics simultaneously, can increase the effectiveness of phage therapy. Here we explore naturally occuring fatty acids as therapeutic accompaniments to phages and antibiotics.

Methods: Checkerboard synergy assays were performed on STM strain K279 with phage ANB28 and saturated straight chain fatty acids with 1-9 carbons in both pH controlled and uncontrolled environments. OD measurements were taken at 24 hours and “interaction scores” were computed for each therapeutic combination. Phage-octanoic acid combinations were additionally tested against more than 100 strains of STM, Enterococcus, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli.

Results: Short chain fatty acids (C1-C5) reduce bacterial growth via pH, while medium chain fatty acids (C6 and above) reduce growth even when a neutral pH is maintained. Favorable interactions between phages and fatty acids increase as carbon chain length increases. Octanoic acid was shown to work cooperatively with bacteriophages to inhibit growth of over 90 species of E. faecalis and E. faecium, and ongoing experiments are exploring its effectiveness against the other four species mentioned above.

Discussion: Our results highlight the potential of fatty acids as antimicrobial agents. By pairing phages, fatty acids, and antibiotics, we hope to develop new treatments for infections caused by antimicrobial resistant bacteria.

High-throughput detection and evolutionary investigations of gene-clusters using the zol suite

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Background: Recently, several software have become available to visualize sequence-based relationships between homologous gene-clusters - co-located sets of genes. However, these software are largely limited in the information they provide and scale at which they can be applied.

Methods: To address these limitations, we developed fai and zol, bioinformatic tools that enable the rapid detection of homologous gene-clusters across thousands of genomes and subsequent evolutionary exploration of the genes within them using table-based reports. The suite further features auxiliary programs to: (i) assess the novelty and conservation of gene clusters within taxonomic contexts, (ii) visualize conservation and evolutionary statistics for individual genes across consensus depictions of gene clusters, and (iii) assess signatures of lateral transfer for gene clusters within different genomes.

Results: We demonstrate the utility of the zol suite through three distinct applications. First, we use the tools to perform a targeted search for a virus across a longitudinal metagenomic dataset profiling the microbiome of a lake. Second, we apply the tools to investigate evolutionary trends within biosynthetic gene clusters encoding for toxins and anti-insecticidal secondary metabolites in the fungal species of Aspergillus flavus. Third, to demonstrate the scale at which the programs can be applied, we assessed the conservation of a gene-cluster encoding for a cell-wall associated polysaccharide from Enterococcus faecalis across >5,000 genomes representing 92 distinct species from the diverse bacterial genus of Enterococcus. The polysaccharide encoding locus was found to be conserved in most but not all Enterococcus species and often featured a variety of distinct glycosyltransferases, including some unobserved in E. faecalis.

Discussion: In conclusion, the zol suite (https://github.com/Kalan-Lab/zol) aims to serve as a practical toolkit implementing a variety of functionalities for the investigation of gene clusters across large and taxonomically diverse microbial genomic and metagenomic datasets.

Standing Variation or De Novo Mutation: What Drives Drought Tolerance in Experimentally Evolved Rhizobium leguminosarum?

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Background: Drought, as a result of anthropogenic climate change, is known to have large ramifications for plant health and crop productivity, but plant microbiomes can assist plants under drought conditions. Among these are rhizobia that, while typically known for fixing nitrogen with legume, our work has shown can evolve to mediate plant drought tolerance in response to drought stress. Understanding the molecular mechanisms that confer this resistance is critical for interpreting the mechanisms altering symbiosis as well as leveraging microbiomes for plant productivity.

Methods: We generated reference-quality genomes for 155 experimentally evolved isolates of R. leguminosarum derived from 27 ancestral strains under four historical treatments (drought vs watered X nitrogen-fertilized vs unfertilized). We use a combination of bioinformatic tools to map derived strains back to this ancestral population and investigate the genetic basis for the extended phenotype of drought tolerance.

Results: We found evidence for selection on standing genetic variation depending on the watering environment, with populations evolved in wet conditions containing different makeup of ancestral strains compared to those from drought conditions. Experimentally evolved strains display little genetic difference at the symbiosis plasmid to their corresponding ancestors with; only 18 out of the 155 strains contain genetic differences in comparison to their ancestor. Furthermore, we find evidence for genetic differentiation at the regions housing both symbiosis and trehalose reuptake genes, when comparing drought-evolved to watered strains.

Discussion: Our study indicates that the evolution symbiotically conferred drought resistance in rhizobia is likely a result of selection on standing variation in ancestral strains, and driven by selection acting at canonical symbiosis genes as well as those important for osmolytes that protect nodulating bacteroids from osmotic stress.

The diversity of Anaerostipes species in the gut

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Members of the class Clostridia, a polyphyletic group of gram-positive, spore-forming anaerobes in the Firmicutes phylum, inhabit a substantial portion of the human gastrointestinal tract. Despite a consistent, demonstrated value in maintaining Clostridial populations in the gut, the functional role of most commensal gut Clostridial species remains understudied. Here, we investigated genomic strain diversity of Anaerostipes, a prevalent butyrate-producer within the gut microbiota that has previously been associated with various disease states. To further understand the ecological and commensal role of various species from Anaerostipes, we conducted a genomic comparison across the Anaerostipes genera. We isolated Anaerostipes hadrus (n = 17) and Anaerostipes caccae (n = 4) from healthy humans (n = 5), initially identified using unique colony morphology and Sanger sequencing. We next conducted whole genome comparisons of our isolates and available genomes (n = 105) to investigate shared and unique metabolic functions between and within species. Based on a maximum likelihood tree constructed from single nucleotide polymorphisms (SNPs) present in the core genes across Anaerostipes genomes and average nucleotide identity (ANI), we observed 12 distinct species, with the majority of the strains classified to A. hadrus, A. hominis, or A. caccae. A.butyraticus, A. avistercoris, and A. excrementavium also appear to be closely related, as observed using ANI at a 95% cutoff. Pangenomic analyses suggests that Anaerostipes species have open pangenomes. Principal Coordinate Analysis (PCoA) of all genes identified using the Clusters of Orthologous Genes (COG) database demonstrated distinct clustering between A. hadrus and A. caccae, indicating additional significant metabolic differences. Nearly all Anaerostipes strains possessed propionate coA-transferase [E.C. 2.8.3.1], an enzyme that yields acetate. Collectively, these data indicate metabolic variance across Anaerostipes species that may influence their ability to coexist within the gut environment and variably influence human health.

Surveying Nitrogenase Diversity with Protein Structure Prediction Tools

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As global population increases, ammonia-derived fertilizers are essential to boost agricultural output. These fertilizers are produced by the energy-intensive Haber-Bosch process, which consumes 2% of the world's fossil fuel energy, necessitating more sustainable methods of nitrogen fixation. Diazotrophs catalyze ammonia production via the enzyme nitrogenase, which efficiently cleaves the nitrogen triple bond. Surveying nitrogenase structural diversity can streamline the selection of enzymes for electrochemical ammonia production and for agricultural soil amendments, circumventing the environmental burden of the Haber-Bosch process and nitrogen pollution. Because their activity underpins primary production, nitrogenases have been widely studied in model cultures. Yet, large gaps in understanding of nitrogenase diversity remain, especially in complex systems like native soil microbiomes, which are believed to harbor the most diverse set of nitrogenases. These gaps remain largely due to the biases imposed by culturing, amplicon sequencing, and the existence of pseudo-nif sequences (homologous nifH sequences not indicative of diazotrophy). Since nitrogenase diversity was last reviewed, scientists and engineers have massively expanded known microbial diversity, especially in the domain Archaea, and have acquired the ability to predict protein structures and interactions. We systematically applied these tools to update our understanding of nitrogenase structural diversity and to provide stable and compact scaffolds for the investigation of commercial nitrogenase use. NifDHK gene annotation from publicly available genomes and metagenome-assembled genomes (MAGs), in addition to MAGs from our previous soil characterizations, was followed by RoseTTAFold All-Atom protein-small molecule interaction predictions. Through this combined approach we will (1) identify the minimal motif necessary for triple bond cleavage, (2) elucidate the degree of variability in nitrogenase structure and cofactor interactions, and (3) propose stable and compact variants for investigation in industrial applications. As protein prediction tools are still in their infancy, our collaborator will validate the structural predictions using cryoEM imaging.

Bacterial RNA-seq from host deep tissue sites - Developing a new tool for studying bacterial phenotypic heterogeneity and antibiotic persistence

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Background: During an infection, individual bacterial cells are differentially exposed to a wide range of host-derived stressors. This can result in the emergence of phenotypically distinct subpopulations with reduced susceptibility to antibiotics – a phenomenon termed antibiotic persistence. When antibiotic treatment fails to clear these bacterial subpopulations, they can promote long-term and relapsing infections, posing a major therapeutic challenge.

Methods: We are developing a new bacterial RNA sequencing approach to study antibiotic persistence during infection in a mouse model of prolonged antibiotic treatment of a systemic infection. In our model, mice are inoculated intravenously with Yersinia pseudotuberculosis (Yptb). During systemic infection, Yptb colonizes deep tissue sites including the spleen, and forms microcolonies which serve as a powerful model for studying phenotypic heterogeneity and antibiotic persistence. Mice then receive 3 doses of doxycycline (Dox) intraperitoneally (at 48-, 96, and 144- hours post-infection). Mouse spleens are collected at different timepoints post-infection and post-treatment to quantify bacterial load (CFUs), visualize Yptb microcolony morphology, and prepare single cell suspensions for bacterial fluorescence-activated cell sorting (FACS) and RNA sequencing.

Results: For initial bacterial cell sorts, mice were inoculated with Yptb, and a subset of mice were treated with Dox at 48 hours post-infection. With FACS, we successfully collected different sized pools of bacterial cells (10 and 100 cells) from spleens collected 48 hours post-infection from Dox-treated and untreated mice (N=4). Additionally, our RT-qPCR results demonstrate that we can isolate RNA from small pools of cells.

Discussion: While mechanisms responsible for antibiotic persistence have been described in vitro, the development of new therapeutic approaches to specifically target bacterial subpopulations is currently hindered by our lack of understanding of antibiotic persistence development and dynamics during infection. Ultimately, we hope that identifying the mechanisms that promote antibiotic persistence during infection will yield new potential therapeutic targets and strategies.

Growth and whole genome sequencing of diatom consortia enriched from Canadian alkaline soda lakes

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Background: The alkaline soda lakes situated in the Cariboo Plateau are home to bacteria, archaea, eukaryotes, fungi, and viruses. These soda lakes are highly productive environments due to high concentrations of bicarbonate resulting in the development of dense, thick microbial mats. To this day, only one mitogenome and plastid genome sequences are available for a diatom species isolated from Turkish alkaline soda lakes. Here, we aim to understand the adaptations of diatoms enriched from Canadian alkaline soda lakes via whole genome sequencing.

Methods: To enrich diatoms, we used urea, 0.58M alkalinity, 0.03M silica, and grown at 4°C. DNA was extracted and size selected using SRE XS kit. Nanopore sequencing was conducted using Ligation kit. Nanopore reads were base-called in real time using Guppy as well as offline using Dorado. Metagenome assembly was carried out with Flye and Barrnap was used to predict ribosomal RNA. rRNA sequences were classified using arb-silva database. Contigs were annotated using MetaErg. BLASTp was used to match coding sequences to the NCBI database.

Result: A contig with sequencing depth of 604x and length of 65,042-bp, was identified as chloroplast. Among the coding sequences identified were RuBisCO small and large subunits, genes associated with Photosystem I and II, and genes associated with ATP synthase. Based on BLASTp, these genes were 99% match to plastid genome of Nitzschia anatoliensis. Assembly and annotation of the nuclear genome is still in progress.

Discussion: Here we aimed to perform whole genome sequencing of diatoms enriched from Canadian soda lakes. Diatoms were grown at a high sodium (bi)carbonate concentration and high pH. So far, we have identified the plastid genome of diatom, which was very similar to the plastid genome of Nitzschia anatoliensis, isolated from Turkish soda lakes. Our research showed that this is a prominent, globally distributed high-pH-adapted species of diatom.

Benchmarking tools for plasmid reconstruction from short-read assemblies of diverse bacterial species

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Background
Accurate identification and reconstruction of plasmids are crucial to understanding the evolution and spread of antibiotic resistance and bacterial virulence. Although long-read sequencing technologies have made plasmid reconstruction easier, the lower cost of short-read sequencing and the massive size of existing short-read databases make short-read-based plasmid investigations highly relevant. Previous benchmarking efforts of tools for short-read-based plasmid identification and reconstruction focused on a narrow range of species and did not include many recently published tools. To establish best practices for plasmid detection using short reads, we benchmarked the performance of the most recent and widely used plasmid identification and reconstruction (i.e., contig binning) tools using short-read assemblies from diverse genera.

Methods
We compared 12 plasmid identification and 4 plasmid reconstruction tools with Illumina short-read assemblies from a diverse collection of Enterobacterales and enterococci. The ground-truth set of plasmids was determined using complete or near-finished assemblies generated with Illumina and Oxford Nanopore data.

Results
Most plasmid identification tools achieved better results for Enterobacterales than for Enterococcus (F1-score of up to 93% vs. 85%, respectively), likely due to differences in reference database representation. The top four identification tools had similar performance and included both graph-based (PlasmidEC and gplas2) and non-graph-based approaches (PlaScope and Plasmer), though PlaScope performed slightly better for Enterobacterales and Plasmer slightly better for Enterococcus. As expected, assemblies having longer contigs led to better plasmid identification. Gplas2 outperformed all other plasmid reconstruction tools, achieving a similar performance for Enterobacterales and Enterococcus. Nevertheless, plasmid reconstruction proved challenging, with the best tool perfectly reconstructing only 47.3% of Enterobacterales and 49.2% of enterococcal plasmids.

Discussion
In addition to aiding the selection of the optimal tools for plasmid identification and reconstruction from short-read data, our work highlights the shortcomings of existing methods for plasmid identification and reconstruction, especially for species underrepresented in databases.

Deep sequencing identifies the role of within-host evolution in an Enterococcus faecium outbreak

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Linking evolutionary processes within hosts to population level patterns is crucial for understanding microbial populations. These processes shape the emergence and spread of antibiotic resistance, virulence, and inter-strain competition. Here, we develop a protocol to study the within-host variation of the opportunistic gut bacteria Enterococcus faecium. We then apply the method to study the evolution of E. faecium in a 7-patient hospital outbreak.
We first validate our protocol using synthetic communities consisting of a mixture of two isolates. Each isolate was grown overnight separately, mixed at 1:1, 1:3, and 1:9 volume ratios, and plated on two different media types. All colonies were removed from the plates, DNA was extracted, and sequencing was performed using the Illumina platform. Reads were mapped to an internal reference. We then applied a series of filters and characterized the sensitivity and specificity of the deep sequencing variant calling pipeline. Single nucleotide polymorphisms (SNPs) present in the synthetic community reflected their expected mixture ratios. Structural variants were less identifiable in the mixed population. Applying this method to outbreak samples, we find evidence for low levels of diversity. Minor alleles detected at one time point were often not observed at other time points. Cocolonization between different MLSTs was detected in one patient. Variation arising within-host was rarely found between-hosts, with one SNP found to be shared between multiple patients. Overall, our results suggest deep sequencing may be a useful tool for investigating the within-host diversity of E. faecium, especially in patients with cocolonizing strains. When applied to a specific hospital outbreak, we find low E. faecium diversity within hosts and minimal shared variation between hosts.

Quantification of the replicability of differential gene expression analysis between two growth conditions of Variovorax paradoxus

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Background: Differences in gene expression drive differences in behavior and function across the whole tree of life. RNA sequencing (RNA-seq) is the primary method of assessing differential gene expression. However, RNA-seq experiments do not replicate perfectly. In this study, we analyze four Variovorax paradoxus datasets to quantify the replicability of differential gene expression results. Variovorax paradoxus is a beta proteobacterium that plays an important role in soil metabolism and in plant growth.

Method: Variovorax paradoxus was sequenced during log and stationary growth, at temperatures of 25 and 30 degrees Celcius. Each combination of growth phase and temperature was sequenced from multiple experiments, sometimes years apart. Tables, figures, and plots were finalized using Bowtie2, SAMtools, and DESeq2. Genes having a p-value less than 0.05 were deemed substantially differentially expressed in the DESeq2 differential expression analysis. Variant calling was performed by BCFtools so to identify SNPs and indels with QUAL score greater than 50.

Results: While there was significantly more agreement between the experiments than would be expected by chance, there were also significant discrepancies between experiments. The highest Pearson correlation of p values between experiments of equivalent design was 0.083 and the lowest one was 0.075, and the highest correlation of log fold changes was 0.28 and the lowest was 0.16. Despite these weak correlations, PCA analysis of PC1 (explains 45% of the variance) and PC2 (explains 15% of the variance revealed that log and biofilm samples clustered with their own category across all experiments. Variant calling on all datasets revealed differences in number of variants between experiments, potentially suggesting a biological reason for some gene expression change.

Discussion: Although differential gene expression analysis is a key method for identifying true biological differences between growth conditions, all results must be interpreted with the understanding that they might not be fully replicable.

Utilizing maternal vaccination to combat neonatal gut colonization by Klebsiella pneumoniae

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BACKGROUND
Klebsiella pnuemoniae (Kp) is a leading global cause of neonatal mortality from bloodstream infection (BSI). The preterm infant gut harbors identical strains of BSI-causing bacteria, indicating direct gut-to-bloodstream dissemination. Maternal vaccination could leverage passive transfer of maternal antibodies to minimize gut Kp colonization.

METHODS
Gnotobiotic mouse dams were colonized at birth with infant stool microbes, including a BSI-causing O1 Kp subtype. At mating, dams received three doses of an O1 bioconjugate vaccine or EPA control. Anti-O1 breastmilk IgA and IgG and anti-O1 serum IgG from dams and pups were quantified by ELISA. DOL 14 pups were orogastrically challenged with 10^7 CFUs of the O1 Kp strain. Stools were plated on Klebsiella ChromoSelect agar to measure Kp abundance, then shotgun metagenomically sequenced and analyzed taxonomically with MetaPhLan4.

RESULTS
Serum and breastmilk from O1-immunized dams had greater concentrations of anti-O1 antibodies than controls. Serum concentrations of anti-O1 IgG obtained pre- and post-O1 challenge were greater in offspring from immunized vs. non-immunized dams. Peak concentrations of anti-O1 IgG occurred at DOL 14 and decreased through DOL 21 in both pup groups, but offspring of O1-immunized dams demonstrated less loss of specific antibody over time than controls. No difference in Klebsiella stool abundance was observed among DOL 14 pups from control or O1-immunized dams. However, after Kp challenge, pups from O1-immunized dams had lower Klebsiella fecal content (median 2x10^4 vs. 6x10^5 CFUs/mg, $p<0.0001$) and relative abundances (median 11% vs. 27%, $p<0.0005$) than controls (two-tailed Mann-Whitney test).

DISCUSSION
O1-immunized dams and their offspring experienced greater anti-O1 antibody responses than their control counterparts. Pups from O1-immunized dams also had 30-fold fewer fecal Kp CFUs/mg after Kp challenge. Maternal vaccination does not impact initial Kp gut colonization but may augment protection during subsequent Kp exposure.

Exploring diatom composition in eutrophic areas of the Great Lakes through rbcl DNA metabarcoding and shotgun metagenomics

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Cladophora is a green alga that has flourished in great abundance in the Great Lakes area due to an increase in eutrophication. Cladophora-associated diatoms are primary food sources for the freshwater organisms that reside in the Great Lakes and are often used as markers to monitor the ecological health of aquatic systems. These microscopic single-celled algae are especially important since they contribute greatly to carbon dioxide removal and are estimated to produce up to nearly half of the world's oxygen. To assess the diatom communities in the Great Lakes area, Cladophora samples collected by U.S. Geological Survey researchers from eutrophic sites around Lake Michigan were processed, and had their DNA sequenced. Two standard microbial diversity survey techniques were employed: ribulose-1,5-biphosphate carboxylase large subunit (rbcL) DNA metabarcoding and shotgun metagenomics. A total of 24 samples tagged with the rbcL primers were amplified and sequenced. DADA2 was used to filter, trim, generate error profiles, cluster, and extract sequencing variants. The shotgun metagenomics dataset was originally used to explore nitrogen-fixing (nifH) microbial communities associated with Cladophora. Shotgun metagenomics is technique that employs sequencing every single gene found in a sample, which allows for processing of entire microbial communities. FASTQ files of this dataset were obtained through NCBI's Sequencing Read Archive and reprocessed to assess the Great Lakes diatom composition. Using both of these methods, subsequent taxonomic composition and alpha/beta diversity statistical analysis were conducted. We will present a comparison between the two sequencing methods used for assessing diatom diversity, and we will discuss the taxonomic abundance and composition of diatoms in the Great Lakes. The results of this study will increase our understanding of the potential uses of metabarcoding and metagenomics to highlight shifts in microbial community compositions as a response to environmental and ecological changes.

Optimizing Saccharomyces boulardii Gene Expression for In Vivo Therapeutic Delivery

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Saccharomyces boulardii is a probiotic yeast known for its rapid growth at 37 °C, ease of genetic transformation, and ability to colonize the gut and produce therapeutics. To enhance its ability as a therapeutic carrier in vivo, precise control of its gene expression is desirable. Specifically, programming this yeast to sense when it has entered the gut would allow engineers to develop strains that can exhibit desirable properties during bioreactor cultivation (e.g. fast growth rate, nutrient storage) and switch their metabolism when they enter the gut (e.g. high product formation). For this purpose, promoters that alter their activity between nutrient media and the gut environments are necessary. Therefore, S. boulardii was administered via gavage to germ-free mice. Following euthanasia, RNA was extracted from the cecum and colon. Additionally, RNA from yeast cells growing in nutrient media under aerobic and anaerobic conditions at various time points was also collected. The RNA samples were sequenced, aligned to the S. boulardii genome, and analyzed for Differentially Expressed Genes (DEGs) using DESeq2. DEGs were identified with a log fold change threshold of 2 and a p-value of less than 0.01. A total of 10 genes were identified, five of which exhibit high activity under aerobic conditions and five of which are active under anaerobic conditions. 15 plasmids were constructed by ligating the promoter region of these genes to a mCherry fluorescent protein sequence. We are currently working on detecting these promoters' ability to activate a fluorescent protein in both in vivo and in vitro environments. These findings will enhance the potential of S. boulardii as a versatile and efficient therapeutic carrier, enabling it to alter its metabolism after administration.

primerForge: a Python program for identifying primer pairs capable of distinguishing groups of genomes from each other

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Background:
It is useful to be able to distinguish between specific strains of microorganisms in a given population. While whole-genome sequencing and phylogenetic analyses can be employed, these techniques are often slow and can be resource intensive. Polymerase chain reaction (PCR) can be used to amplify regions of genetic material that are specific to the strain(s) of interest. PCR is faster, less expensive, and more accessible than whole genome sequencing, so having a PCR-based approach can accelerate the detection of specific strain(s) of microbes and facilitate diagnoses and/or population studies.
Traditional primer design involves the selection of a target region of DNA to amplify, followed by primer pair selection and subsequent validation of the primer pair. Identifying a good pair of primers and a suitable target region often requires several iterations of the primer design process, which can be tedious and time consuming.

Methods:
primerForge identifies all suitable pairs of primers capable of producing PCR products of a specific size in a set of whole genome sequences. Optionally, it can also filter those primer pairs and limit the output to primer pairs that can be used to distinguish one set of genomes from another set of genomes via PCR amplification. primerForge relies on the khmer package to extract k-mers from genomic sequences and the primer3-py package to evaluate specific characteristics of primer pairs including melting temperature, hairpin potential, and dimer formation.

Results:
primerForge finds primer pairs that can distinguish between strains of microorganisms in the same species in under an hour with minimal resources. It performs better than swga.

Discussion:
primerForge can be used for surveillance of an outbreak clone of a particular pathogen. A laboratory could develop a set of PCR primers to track the population of this outbreak clone.

Phenotypic, Genomic, and Transcriptomic Analysis of Agrobacterium tumefaciens Strains

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Agrobacterium tumefaciens is a tool for genetic engineering based on its ability of transferring T-DNA on its tumor-inducing plasmid (pTi) to the host genome. Although A. tumefaciens strains are diverse in genome and virulence, the disarmed strains used in Agrobacterium-mediated transformation are mostly derived from strain C58. In our characterization of 30 wild-type A. tumefaciens strains, the strain with best transformation ability, 1D1108, is 3.8- fold stronger than C58. However, this phenotype does not correlate to gene content similarity. To characterize the genes that contribute high virulence, we conducted RNA sequencing to 1D1108 under acid or acetosyringone (AS) induction in vitro, and to 1D1108 isolated from infiltrated Nicotiana benthamiana. ~1,300 differential expression genes (DEGs) genes were identified in planta, which is around 10-fold larger than the DEGs between in vitro conditions. Beside the known acid or AS regulated genes, the role of other plant regulated genes in infection are mostly unclear. Further transcriptome analysis revealed the difference between the in vitro and in planta condition in gene expression pattern. In addition, differences were found when comparing 1D1108 dataset to published C58 transcriptome dataset. These results suggest that 1D1108 might have some different strategies during infection, and in planta transcriptome could provide us a comprehensive information of gene regulation during Agrobacterium infection. More in planta transcriptome dataset will be collected for comparison and further investigations of putative candidates are required to confirm their roles and mechanisms. The knowledge may be used for future synthetic biology work to improve Agrobacterium-mediated transformation.

Investigating the origin of S. aureus infections in people who inject drugs

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Background: Injection drug use (IDU) is an ongoing crisis that is complicated by IDU-associated Staphylococcus aureus bloodstream infections (IDU-BSI). The epidemiology, transmission, and biology of S. aureus IDU-BSI must be defined to develop harm reduction campaigns.

Methods: We performed whole genome long and short read sequencing of Staphylococcus aureus isolates from 45 IDU-BSI, 51 non-IDU-BSI (cBSI), 3 IDU-syringes, and 32 community syringes from a large US midwestern hospital and city. Hybrid assemblies of S. aureus were generated using Unicycler and clonality was assessed with Snippy using a core genome alignment cutoff of 15 SNPs. Cytokine response to S. aureus infection by IDU status was determined using a 40-plex chemokine panel on patient blood.

Results: We show that 30/32 S. aureus isolates from community syringes were separated by fewer than 4 SNPs with a minimum and average ANI% of 99.9806 and 99.9964. cBSI (14/51) and IDU-BSI (12/45) patients are equally as likely to share S. aureus isolates with less than 15 SNPs between skin and blood samples. We identified two IDU-BSI patients that share S. aureus bloodstream isolates with 10 SNPs that are also highly related to 30/32 of the community isolates by less than 3 SNPs. 2/3 of individuals had S. aureus isolates with 0 SNP differences between their syringe and blood samples. IDU-BSI patients had 10 significantly depressed cytokines associated with primary immune system recruitment and activation in response to S. aureus infections compared to cBSI patients ($p < 0.05$, Wilcoxon).

Conclusions: In a community with limited access to needle exchange programs, we identify clonal expansion of S. aureus isolated from syringes suggesting that drug paraphernalia may be acting as fomites of S. aureus transmission. Depressed patient cytokine responses suggest S. aureus may be able to cause BSI in IDU patients through direct bloodstream introduction bypassing primary immune responses.

Reconstructing the evolutionary history of halo-alkaliphilic bacteria

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Soda lakes are among the most alkaline aquatic environments on earth and among the most productive ecosystems known. High pH, high salinity, and extensive free radical activity lead to extreme conditions for survival of aquatic life. Due to their high phosphate concentration, they are also considered a promising candidate habitat for the origin of life. Here, we put that idea to the test by searching for an ancient genetic repertoire among genes necessary for survival in alkaline soda lakes. To identify those genes, we collected 498 microbial genomes from alkaline soda lakes worldwide. Additionally, we collected 1,985 genomes from marine environments and 1,317 genomes from other environments as outgroups. We then extracted 45 clades, each consisting of a subclade with at least three genomes from alkaline soda lakes and a sister-subclade with three genomes from oceans. We are now using Bayesian approaches, such as stochastic character mapping, to reconstruct the evolutionary history of the adaptation of modern bacteria in alkaline environments. This will enable us to identify genes enriched or adapted in genomes of halo-alkaliphilic bacteria. Through this study, we aim to uncover the key genes and possible mechanisms of bacterial evolution and adaptation in the halo-alkaliphilic environment, thereby possibly gaining an understanding of the evolution of life in early Earth environments.

Community Analysis of Grapevine Rhizosphere Bacteria during Peak Growth Season

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Background. California's wine industry is the fourth largest in the world, but this economic impact has a vast ecological cost as typical cultivation techniques rely heavily on pesticides and fertilizers, often leading to land degradation and pollution. Sustainable agriculture techniques are an important potential way to reduce these costs and make the industry more resilient against climate change induced stress. Many studies have shown that plants' interactions with microbial communities confer functional benefits, and that inoculation of plant growth-promoting bacteria may be an effective strategy for promoting crop productivity.

Methods. Rhizosphere and bulk soil samples were collected over 6 weeks from the Ted and Chris Robb Garden at the University of the Pacific in Stockton, CA. Rhizosphere samples were collected from three grape varieties: Vitis labrusca 'Eastern Concord', Vitis vinifera 'Golden Muscat', and Vitis vinifera 'Flame Seedless.' DNA was extracted from rhizosphere samples of each variety and bulk soil (250 mg). Rhizosphere and bulk samples from the earliest and latest timepoints and a midpoint collection were prepared using the Oxford Nanopore 16S Barcoding Kit 24v14. Microbiome and mixed DNA standards (Zymo) were used to validate the sequencing protocol, and no input samples were tested to ensure low kit contamination. Flongle sequencing was used to validate the preparation, and subsequent data collection was performed using the MinION Mk1b.

Results. High quality full length 16S sequencing samples were analyzed using the EPI2ME pipeline to evaluate community structure. Alpha and beta diversity measurements were used to evaluate each sample's sequencing depth and variance between samples.

Discussion. A time course of the bacterial community of the grape rhizosphere utilizing 16s rRNA community analysis was analyzed across the prime growing season. This work will provide baseline information that we can use to guide potential microbial interventions aimed at sustainable agriculture.