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Genome Watch

A toolkit for microbial community editing

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This month's Genome Watch highlights recently reported methods that enable genome editing of microbes within phylogenetically diverse communities.

Ever since the surprising discovery that a wide range of environmental and host-associated habitats are colonized with diverse communities of largely uncultivated and uncharacterized microbes, microbial ecologists have struggled to figure out who they are and what they are doing. Sequence-based methods have had remarkable success in identifying these organisms, at least in terms of placing them on the phylogenetic tree. Multi-omics methods (metagenomics, metatranscriptomics, metaproteomics, metabolomics) have enabled significant progress in understanding what they are doing, yet remain frustratingly observational and inferential, due to our inability to cultivate and manipulate many of the key players. Experiments with synthetic communities, made up of cultivated isolates representative of a given environment, have enabled powerful reductionist experiments, but leave many dark corners of the microbial world unilluminated.

Enter ET-seq and VcDART, methods described in a recent paper by Rubin et al.[1]. While CRISPR-Cas genome editing methods have revolutionized genetic manipulation of animals, plants, fungi and bacteria, editing specific genes within specific genomes in microbial communities has posed many challenges. One is simply introducing DNA into the desired microbial cells, for which multiple methods exist but none are universally effective; the other is targeting the material to desired genomic locations, which can be done routinely for many isolates, yet most microbes are uncultivated. While various groups have had some success in introducing genetic material into microbial communities via plasmid transfer or phage infection[2,3], these methods are largely crude and unpredictable. And a prerequisite for accurate editing is a complete genome of the organism being manipulated, which for microbes would usually mean deep sequencing of DNA isolated from a pure culture to generate a high-quality genome.

Rubin et al. combined methods to tackle each of these obstacles in turn, starting with a synthetic community made up of isolated soil bacteria whose genomes had been sequenced. They introduced a randomly integrating mobile genetic element into the community, using multiple techniques to transform the DNA into cells, and assessed the success of each method through targeted sequencing of the regions flanking the element and mapping of these sequence reads back to the genomes. By this method, termed environmental transformation sequencing or ET-seq, they identified organisms that were receptive to transformation in the community context. In parallel, they developed a method to deliver a complete genome editing package based on an RNA-guided transposase on a single plasmid – which they termed DNA-editing all-in-one RNA-guided CRISPR-Cas transposase (DART; specifically, VcDART for the version using Vibrio cholerae enzymes). By targeting a gene within a tractable genome with VcDART, as well as a "safe site" expected to have no fitness effect, and assessing integration efficiency by the same targeted sequencing used for ET-seq, the authors could quantify the fitness effect of the gene disruption within the community context.

While these experiments used cultivated isolates combined into a synthetic community, they could technically be applied to any community whose member genomes had been characterized by deep metagenome sequencing and assembly into metagenome-assembled genomes or MAGs. Indeed, Rubin et al. applied similar methods to a community directly enriched from infant gut and were able to insert a selectable marker into a specific strain of interest.

So where could this lead? One thing the team hasn't done yet is edit a so-called "uncultivable" bacterium – one with no close cultivated representatives – but ET-seq provides an approach to quantitatively assess transformation efficiency across an entire community so that different methods can be tried. Even for the "cultivables," this suite of tools enables direct testing of gene function hypotheses in a community context without

laborious isolation and axenic cultivation. The resulting improvements in genome annotation could change the landscape for predictive microbiome modeling, enabling microbiome-based solutions to health and environmental challenges.

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Competing interests
The author declares no competing interests

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