GENOMICS

Bacterial genomes by the droplet

To produce enough raw material for high-quality whole-genome sequencing, researchers capture and grow single, culture-resistant bacteria in gel microdroplets.

The bacterial universe is vast and dimly understood. Knowing the complete genome of individual bacteria from the environment would go a long way to aid our understanding of how microbes function in complex communities, but producing quality whole-genome sequences from single cells is notoriously difficult. Cliff Han has found that the best way to study single genomes may be to multiply them.

Han and his team at the Los Alamos National Laboratory have spent the last 10 years 'finishing' bacterial genomes after completing work on the human genome. Finishing refers to filling gaps in a genome assembled from short sequence reads, and it requires a supply of genome template from cultured cells. As the quality of first draft genomes improved, the group began shifting their attention to sequencing the genomes of bacteria that could not be cultured in isolation.

Multiple displacement amplification can bulk up the tiny amount of DNA in a bacterium's lone chromosome for sequencing. The method generates heaps of DNA but is biased toward amplifying some parts of the genome over others, leading to uneven coverage and difficulties with assembly.

Single cells pose additional problems. "If you really have one copy of the genome, for sure it will be broken when the cell is being lysed," says Han. This causes many gaps due to poor amplification at the breaks, he explains. The small quantity of template also makes the process vulnerable to amplifying contamination disproportionately.

A frustrating year of work on single-cell genomics paralleled another project in Han's lab to develop culturing methods. One approach described more than 10 years ago uses porous gel microdroplets to culture bacteria in close communication with one another and with access to fresh nutrients. Han wondered, "Why not … combine these two things together and do a limited culture [of single cells] even though many of the cells don't grow or can't be isolated by themselves?"

Microdroplets are formed when a purified mixture of bacteria is added to an agarose gel emulsion that is then cooled to trap cells in the matrix. Using a low concentration of bacteria ensures that only 10%–20% of the microdroplets will contain cells, nearly all at single occupancy, says Han. Cells are grown to a limited extent, and members of each colony are essentially identical genetically. A cell sorter can then identify droplets containing microcolonies for independent amplification and sequencing.

The group began by sequencing both ends of the human gut. They screened amplified products from oral and stool-derived microcolonies using the 16S ribosomal DNA marker and chose a set of highly related genomes from each for whole-genome sequencing.

As compared to related genomes sequenced from single cells, microcolonies gave more even coverage, a lower proportion of contamination and less fragmented genome assemblies. Single-cell assemblies typically recover about 50% of the genome, whereas Han notes that as few as 30 cells in a microcolony push recovery to near 100%. The high-quality individual assemblies allowed the researchers to study sequence changes within species that would be averaged with bulk approaches such as shotgun sequencing of community DNA.

In the current protocol, samples are cultured overnight before gel is added, meaning there is a period of competitive growth that can skew membership from that of the original community. The use of artificial medium may also preclude the growth of many bacteria, even in limited culture. It may be difficult to capture and detect rare species, and differences within each colony make the approach unsuitable for studying gene or protein expression derived from single cells. However, the method did capture broad bacterial diversity including all common gut taxa.

Han's group is working on devices that will allow gel microdroplets containing bacteria to be incubated in the same environment from which they are isolated. Soon, whole single-cell genomes may be dredged from lakes and rivers, wetlands and hot pools, damp soils and sediments, pond scum and sewage effluent.

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RESEARCH PAPERS

Fitzsimons, M.S. *et al*. Nearly finished genomes produced using gel microdroplet culturing reveal substantial intraspecies genomic diversity within the human microbiome. *Genome Res*. doi:10.1101/gr.142208.112 (14 March 2013).