DNA of uncultured organisms sequenced using novel single-cell approach

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Scientists from the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) and the Bigelow Laboratory for Ocean Sciences have assembled high quality, contamination-free draft genomes of uncultured biodegrading microorganisms using a novel single cell genome sequencing approach. This proof of principle study, published in the April 23 edition of the journal *PLoS One*, offers researchers a new method to access and decipher the information embedded in genomes of interest with only minute quantities of DNA.

"Most of the microbial genomes sequenced to date are derived from organisms cultured in the laboratory," said DOE JGI Director Eddy Rubin. "We estimate that roughly 99.9 percent of the microbes that exist on this planet currently elude standard culturing methods, denying us access to their genetic material, so we have to explore other methods to characterize them. The power of single cell genomics is that it offers us the ability to sort out one cell from a complex environmental sample, liberate the DNA from that cell, and enzymatically produce millions of copies of that genome so that we have enough DNA to sequence it and characterize its metabolic potential.

"In its capacity as a national user facility, DOE JGI is dedicated to helping our users expand the utility of genomic information to advance DOE mission-relevant science—and in this particular case, building on our understanding of how the carbon balance is maintained in the ocean. The single cell approach will be of great interest to many of our users that have problems with accessing their particular target genomes."

Tanja Woyke and her colleagues at the DOE JGI sequenced genomes of two uncultured flavobacteria, marine microorganisms known for their biopolymer degradation capacity. The environmental sample for this work - surface ocean water - was collected in Maine's Boothbay Harbor. The two flavobacteria were chosen by Bigelow Laboratory collaborators Ramunas Stepanauskas and Michael Sieracki, who are particularly interested in genes encoding proteorhodopsins.

"Proteorhodopsins enable some microbial cells to harness the energy from sunlight in a process that is very different from photosynthesis," said senior author Stepanauskas. "Recent metagenomic studies revealed that proteorhodopsins are very abundant and diverse in the ocean. Using our single cell sequencing technology, we are starting to identify the specific group of microorganisms that carry proteorhodopsin genes, and to analyze the genomic context that may shed light on the role of proteorhodopsins in the ocean and their potential in biotechnology."

A technique called fluorescence activated cell sorting was used by the Bigelow scientists to pick out individual bacterial cells directly from the environmental sample. The single cells were then lysed (blown open) and a process called multiple displacement amplification was applied to make millions of copies of the bacterial genomes for sequencing. The resulting flavobacterial genome sequences are approximately 80 to 90 percent complete, a level sufficient, Woyke said, to prove the utility of the technique. Woyke credited DOE JGI's Cliff Han and his team at Los Alamos National Laboratory (LANL), which worked on closing gaps in the assembly.

Even though the flavobacteria sequenced are marine organisms, Stepanauskas pointed out that the single cell sequencing approach can be applied to organisms from a number of environments, including those microbial communities inhabiting extreme environments, such as hot pools, contaminated soil, and those constituting the human microbiome.

The technique bypasses the need for culturing before sequencing, he said, because only one cell is needed to decode a genome.

"As long as you can isolate a single cell, pick it from the environment, lyse it, you can generate millions of copies of that genome and gain access to the information inside that organism," Woyke confirmed. "One of the key issues that still needs refining is the lysis step, since many microbes will not lyse with alkaline solutions, the most common agent for the job. But we are actively working on that."

The capacity to sequence DNA from a single, uncultured cell was first documented in 2005 at Roger Lasken's team while he was at the New Haven-based company Molecular Staging, but the technique has yet to yield a completed genome. "If one copy of the genome stays intact, you should theoretically be able to finish a genome from a single cell," Woyke said. She also noted that other groups are working on pooling identical cells to have a better chance of achieving that goal.

"However, each microbial cell may turn out to be different, that's just one of the unanswered, basic questions in biology that may be finally addressed by single cell genomics," added Stepanauskas. "Even without completed genome assemblies, single cell sequencing offers radically new opportunities for the basic research and biotechnology applications of the microbial "uncultured majority"."

Woyke said they are currently working with several DOE JGI collaborators to apply the single cell approach to other organisms of interest. One of the projects involves examining the microbial communities within cow rumen to identify enzymes that break down cellulose from plant material that can be used for next-generation biofuels production.

Other authors on the study include DOE JGI's Gary Xie, Cliff Han,

Hajnalka Kiss, Jimmy Saw, Pavel Senin, and Chi Yang, Alex Copeland and Jan-Fang Cheng. Other collaborating institutions are the University of La Laguna (Spain), the University of Hawaii at Manoa and the National Yang-Ming University (Taiwan).

Source: DOE/Joint Genome Institute (news : web)