Researchers Clone and Engineer Bacterial Genomes in Yeast and Transplant Genomes Back into Bacterial Cells

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Researchers at the J. Craig Venter Institute (JCVI), a not-for-profit genomic research organization, published results today describing new methods in which the entire bacterial genome from *Mycoplasma mycoides* was cloned in a yeast cell by adding yeast centromeric plasmid sequence to the bacterial chromosome and modified it in yeast using yeast genetic systems.

This modified bacterial chromosome was then isolated from yeast and transplanted into a related species of bacteria, Mycoplasma capricolum, to create a new type of M. mycoides cell.

This is the first time that genomes have been transferred between branches of life—from a prokaryote to eukaryote and back to a prokaryote. The research was published by Carole Lartigue et al in Science Express.

Hamilton Smith, M.D., one of the leaders of the JCVI team said, “I believe this work has important implications in better understanding the fundamentals of biology to enable the final stages of our work in creating and booting up a synthetic genome. This is possibly one of the most important new findings in the field of synthetic genomics.”

The research published today was made possible by previous breakthroughs at JCVI. In 2007 the team published results from the

transplantation of the native M. mycoides genome into the M. capricolum cell which resulted in the M. capricolum cell being transformed into M. mycoides. This work established the notion that DNA is the software of life and that it is the DNA that dictates the cell phenotype.

In 2008 the same team reported on the construction of the first synthetic bacterial genome by assembling DNA fragments made from the four chemicals of life—ACGT. The final assembly of DNA fragments into the whole genome was performed in yeast by making use of the yeast genetic systems. However, when the team attempted to transplant the synthetic bacterial genome out of yeast into a recipient bacterial cell, all the experiments failed.

The researchers had previously established that no proteins were required for chromosome transplantations, however they reasoned that DNA methylation (a chemical modification of DNA that bacterial cells use to protect their genome from degradation by restriction enzymes, which are the proteins that cut DNA at specific sites) might be required for transplantation. When the chromosome was isolated direct from the bacterial cells it was likely already methylated and therefore transplantable due to it being protected from the cells restriction enzymes.

In this study, the team began by cloning the native M. mycoides genomeinto yeast by adding a yeast centromere to the bacterial genome. This is the first time a native bacterial genome has been grown successfully in yeast. Specific methylase enzymes were isolated from M. mycoides and used to methylate the M. mycoides genome isolated from yeast. When the DNA was methylated the chromosome was able to be successfully transplanted into a wild type species of M. capricolum. However, if the DNA was not first methylated the transplant experiments were not successful. To prove that the restriction enzymes

in the M. capricolum cell were responsible for the destruction of the transplanted genome the team removed the restriction enzyme genes from the M. capricolum genome. When genome transplantations were performed using the restriction enzyme minus recipient cells, all the genome transplantations worked regardless of if the DNA was methylated or not.

“The ability to modify bacterial genomes in yeast is an important advance that extends yeast genetic tools to bacteria. If this is extendable to other bacteria we believe that these methods may be used in general laboratory practice to modify organisms,” said Sanjay Vashee, Ph.D., JCVI researcher and corresponding author on the paper.

The team now has a complete cycle of cloning a bacterial genome in yeast, modifying the bacterial genome as though it were a yeast chromosome and transplanting the genome back into a recipient bacterial cell to create a new bacterial strain. These new methods and knowledge should allow the team to now transplant and boot up the synthetic bacterial genome successfully.

The research published today by JCVI researchers was funded by the company Synthetic Genomics Inc., a company co-founded by Drs. Smith and Venter.

Provided by J. Craig Venter Institute