New method reveals parts of bacterium genome essential to life

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A team at the Stanford University School of Medicine has cataloged, down to the letter, exactly what parts of the genetic code are essential for survival in one bacterial species, *Caulobacter crescentus.*

They found that 12 percent of the bacteria's genetic material is essential for survival under laboratory conditions. The essential elements included not only protein-coding genes, but also regulatory DNA and, intriguingly, other small DNA segments of unknown function. The other 88 percent of the genome could be disrupted without harming the bacteria's ability to grow and reproduce.

The study, which was enabled by the team's development of an extremely efficient new method of genetic analysis, paves the way for better understanding of how bacterial life evolved and for improving identification of DNA elements that are essential for many bacterial processes, including the survival of pathogenic bacteria in an infected person. It will be published online Aug. 30 in *Molecular* Systems Biology .

"This work addresses a fundamental question in biology: What is essential for life?" said Beat Christen, PhD, one of the co-first authors of the new paper and a postdoctoral scholar in developmental biology. "We came up with a method to identify all the parts of the genome required for life."

The bacteria studied is a non-pathogenic freshwater species that has long

been used in molecular biology research. Its complete genome was sequenced in 2001, but knowing the letters in its genetic code did not tell the researchers which bits of DNA were important to the bacteria.

"There were many surprises in the analysis of the essential regions of Caulobacter's genome," said Lucy Shapiro, PhD, the paper's senior author. "For instance, we found 91 essential DNA segments where we have no idea what they do. These may provide clues to lead us to new and completely unknown bacterial functions." Shapiro is a professor of developmental biology and the director of the Beckman Center for Molecular and Genetic Medicine at Stanford.

Caulobacter's DNA, like that of most bacteria, is a single, ring-shaped chromosome. To perform their experiment, the researchers mutated many Caulobacter cells so that each cell incorporated one piece of artificial DNA at a random location in its chromosome. The artificial DNA, which was labeled so the scientists could find it later, disrupted the function of the region of bacterial DNA where it landed. Over two days, the researchers grew these mutants until they had about 1 million bacterial cells, and then sequenced their DNA. After intensive computer analysis, they created a detailed map of the entire bacterial genome to show exactly where the artificial DNA segments had been inserted in the chromosome of the surviving cells.

This mutation map contained many gaps — the regions of the DNA where no living bacteria had survived with an artificial DNA insertion. These regions, the researchers reasoned, must be essential for bacterial life since disrupting them prevented bacterial survival.

"We were looking for the dog that didn't bark," Shapiro said.

Scientists have used a similar mapping strategy to find essential genetic elements before, but the Stanford team added several innovations that

greatly improved the speed and resolution of the method.

"Our method is very streamlined," Christen said. "We can do an analysis that would have taken years in a few weeks. We can immediately go to the answer."

The new method collapses into a single experiment work that used to take dozens of experimental steps, and shifts the majority of the time needed for the research from laboratory work to data analysis.

In total, the essential Caulobacter genome was 492,941 base pairs long and included 480 protein-coding genes that were clustered in two regions of the chromosome. The researchers also identified 402 essential promoter regions that increase or decrease the activity of those genes, and 130 segments of DNA that do not code for proteins but have other roles in modifying bacterial metabolism or reproduction. Of the individual DNA regions identified as essential, 91 were non-coding regions of unknown function and 49 were genes coding proteins whose function is unknown. Learning the functions of these mysterious regions will expand our knowledge of bacterial metabolism, the team said.

The research team anticipates that the new technique will have several interesting uses in both basic and applied research. For instance, the technique provides a rapid and economical method to learn which genetic elements are essential in any microbial species.

"This would give fundamental information so we could determine which essential genetic elements are conserved through evolution," said co-author Harley McAdams, PhD, professor of developmental biology.

The scientists also pointed out that the method could be used to examine which DNA segments are essential for bacterial survival in specific circumstances, such as when pathogenic bacteria invade a host animal or

plant. Developing a comprehensive list of genetic elements that make a bacterial species infectious could lead to the identification of new anti-infective agents including new antibiotics.

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