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Systems Microbiology

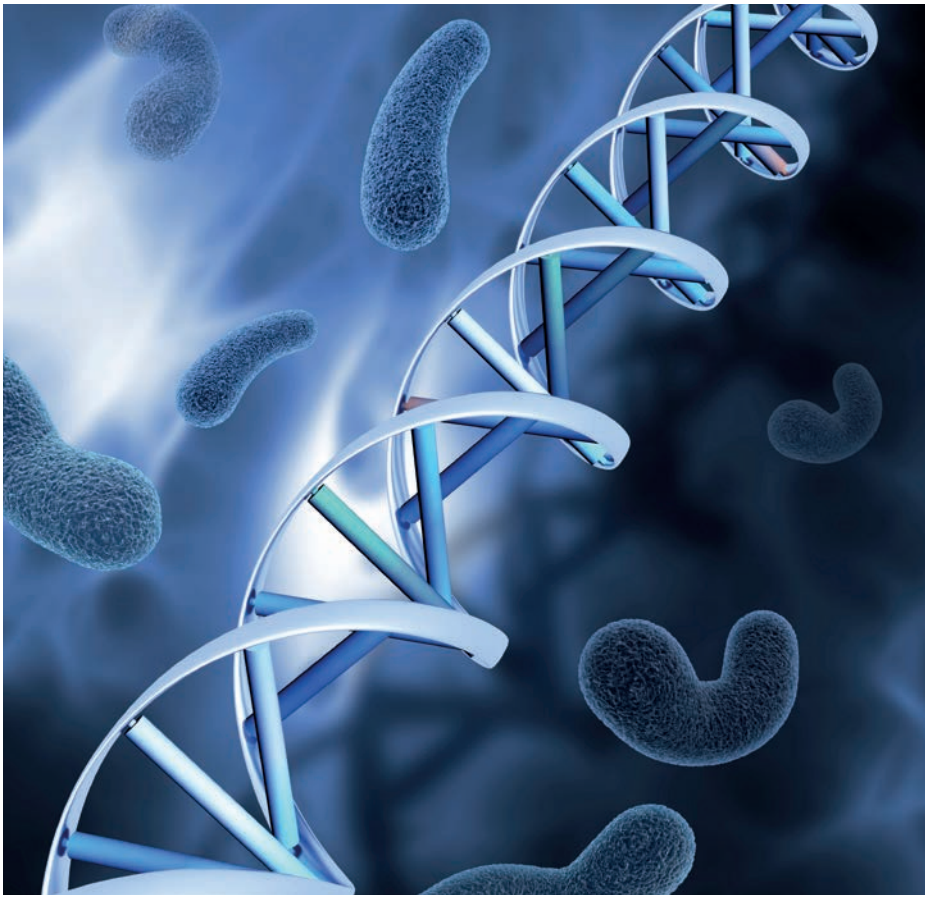
Gene function revealed in *E. coli* library

A new library of thousands of bacterial strains could help scientists understand gene function in cells

The systematic deletion of each gene in one strain of a bacterium widely used in recombinant DNA studies has allowed researchers at Japan's NAIST to create a library that could be crucial in discovering how cells work.

Although many components of the molecular machinery inside cells are now well understood, less is known about how these individual components work together as a system. The genome is the blueprint for this system. However, despite the entire genetic sequences of many organisms having been determined, it is still unclear what each gene does — even in simple organisms like bacteria. Ascertaining gene functions is crucial for gaining a complete understanding of how cells work.

To assist scientists study the functions of individual genes, a team of researchers led by Hirotada Mori at the NAIST Graduate School of Biological Sciences has produced a library of thousands of strains of the K-12 variant of the bacterium *Escherichia coli*; each strain in this library has a single gene deleted[1]. Mori's team used an efficient, state-of-the-art technique to realize this, enabling them to succeed where previous attempts had failed.



Systematic deletion of genes in K-12 bacteria created thousands of different strains that make up the Keio collection.

The team substituted one gene at a time with another gene that gave cells resistance to the antibiotic Kanamycin, enabling selective growth of the modified K-12 cells. The modified cells were then collected. The antibiotic-resistance gene can be cut out if it is desired to avoid polar effects — leaving the DNA as though the gene had never existed.

The researchers repeated the process 4,288 times — once for each K-12 gene — and successfully produced 3,985 unique K-12 strains. The failure to produce bacteria in which the approximately 330 remaining genes were deleted indicates that these genes are essential for the cell's survival.

The team collated the bacterial strains into a library, which they called the Keio collection, and made it available to all researchers. "Making these resources open to the public is so important for science," explains Mori. "Research can be performed in parallel across the world, and accumulation of the results will accelerate research to understand cells."

The research not only produced a complete library, but it allowed the team to perfect their technique and build on it. "Three years were required to complete the first library," says Mori, "but experience allowed us to construct a second, improved deletion library in six months. We have also developed a method to generate double-knockout strains of bacteria by combining two deletion strains."

Mori says that these strains with two genes deleted will enable researchers to study the interactions of genes, with the potential to provide even greater insight into how cells work.

Reference

1. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y. *et al.* Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology* **2,** 2006.0008 (2006).

More information about the group's research can be found at the Systems Microbiology Laboratory webpage:
http://bsw3.naist.jp/eng/courses/courses302.html