'Magic pools' approach can hurry studies of novel bacteria

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To characterize the genes of newly identified bacteria, microbiologists often introduce mutations within the bacteria using mobile DNA segments called transposons to study the impact of these mutations.

However, the trial and error process to identify a functional transposon system for a new bacterium can be lengthy.

Now, scientists at the Lawrence Berkeley National Laboratory in California have developed a method to speed up the process.

In work published this week in *mSystems*, an open access journal from the American Society for Microbiology, the investigators described a method to study hundreds or thousands of different transposon systems in parallel against a target bacterium. Within what the investigators term "magic pools," the individual DNA vectors used to deliver the transposons contained different combinations of genetic material and antibiotic resistance markers. To enable the tracking of all of these different transposon vectors in parallel, the authors added a unique DNA barcode to each vector, such that they could be identified using DNA sequencing, a laboratory tool to determine the order of building blocks within DNA segments. After introducing mutations to a new bacterium with the magic pool, DNA sequencing of the barcode can be used to identify the vector design that is effective for making transposon mutants. Because all DNA "parts" are archived, it is straightforward to reconstruct the effective vector design.

The team used this magic pools method to construct large libraries of transposon mutants in five bacteria, including three from the phylum Bacteroidetes.

"Instead of testing one genetic method at a time and having it fail, we wanted to test hundreds or thousands of different genetic variants at the same time," said senior study author Adam M. Deutschbauer, Ph.D., a research scientist with the laboratory. "It's like if you had a lot of knobs you could turn to get something to work. We just turn all the knobs in multiple ways and ask, 'What was the best way of turning all of those knobs?' "

The methodology could be used to speed up understanding of novel microorganisms obtained from the human gut or the environment, like in soil, Deutschbauer said. The general strategy could also be used to accelerate the development of non-transposon-based genetic systems, such as CRISPR/Cas genome editing or plasmid-based overexpression, in microorganisms.

During their experiments, Deutschbauer and colleagues built four magic pools using the transposons Tn5 or mariner as well as markers for the antibiotics kanamycin or erythromycin. They tested two kanamycin magic pools against three environmental bacteria isolated from groundwater samples collected from a metal-contaminated site in Oak Ridge, Tenn., and two erythromycin magic pools against three bacteria from the Bacteroidetes family. For five of these bacteria, they generated genome-wide libraries of transposon mutants and measured the importance of nearly all genes for growth under laboratory conditions.

"There is so much about biology that we don't understand, that in my view it's advantageous just to try a lot of things in parallel and see what works, especially if the approach of testing things in parallel is very much streamlined," Deutschbauer said. "For the diversity of

microorganisms we're going to study in the future, methods of trial and error to develop new molecular genetic methodologies are going to be too slow and time-consuming. We need ways to accelerate the development of new genetic tools for nonmodel organisms."

More information: For data describing the magic pools, the full mutant libraries, and the mutant fitness assays, as well as source code, see genomics.lbl.gov/supplemental/magicpools

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