MOLECULAR ENGINEERING

Directed evolution made easy

Phage-assisted continuous evolution of proteins automates and accelerates selection, allowing hundreds of rounds of evolution to occur in a single week.

Researchers who want to engineer a protein to bind tightly to an antigen or to catalyze a given reaction often use a strategy called directed evolution. This approach randomly generates many variants of a gene of interest, then selects those versions encoding proteins that are best at performing some desired activity. In a typical round of evolution, genetic variants are produced, expressed and screened; those with the best performance are then selected and amplified. But directed evolution is labor-intensive. Each round typically requires days to extract and clone genes and to transform cells.

Generally, the more rounds of evolution, the better the engineered proteins, so researchers must make difficult choices between their time and the quality of their product. Now, researchers led by David Liu at Harvard University make the trade-off a little less difficult. Their system, called phage-assisted continuous evolution (PACE), allows dozens of rounds of evolution to occur in a single day. In essence, *Escherichia coli* and an associated bacterial virus, or phage, do the work of selecting which genes to pass on for subsequent rounds of evolution, without the need for any manual intervention.

Liu and graduate students Kevin Esvelt and Jacob Carlson engineered a system in which the only phage capable of replicating are those that also carry genes that produce desired activity. First, a crucial gene for phage replication is removed from the phage's genome and placed in an 'accessory plasmid' in *E. coli*. Expression of this gene can then be tied to a wide range of biological activities, says Liu: recombinase activity, polymerase activity, protein-protein interactions and even protein cleavage. Engineered bacteria and phage are then placed in vessels called lagoons, such that a continuous stream of *E. coli* move in and out of the vessel. The *E. coli* only stay in the lagoon for about 20 or 30 minutes, just long enough for phage infection and replication to occur. Only phage that activate the essential gene in the *E. coli* plasmid can reproduce and infect incoming bacteria. The better the phage is at inducing gene expression in the accessory plasmid, the more phage are produced. Rounds of selection continue as long as fresh *E. coli* are fed into the lagoon, but 'successful' phage can be isolated, sequenced and characterized at any point.

To see whether the process could evolve proteins with desired properties, the researchers set out three tests. Starting with the same T7 RNA polymerase, researchers selected for new versions that recognized a new promoter or began transcripts differently. In each case, PACE produced variants with high levels of the chosen activity in 1.5 to 8 days.

Setting up PACE should be simple in most biochemistry laboratories, says Liu. It requires standard equipment such as a peristaltic pump, flasks and tubing. "Very early on," Liu says, "we made a philosophical decision that we would not attempt to create a continuous system with microfluidics and robots and fancy machines, precisely because we wanted as many scientists as possible to be able to use PACE."

PACE will have broad applications, says Liu. It can create proteins with tailor-made properties that might be difficult to produce without hundreds or thousands of rounds of evolution. Another use is to address the basic science of molecular evolution. Even in this initial publication, the team showed that the same evolutionary outcome can be reached by disparate routes. In one case, two lagoons with identical starting conditions and selection pressure converged on the same set of mutations, but one lagoon produced the mutations within 24 hours whereas the other took 108 hours. It is easy to imagine experiments that examine the role of genetic drift and population size on evolutionary outcomes, says Liu. "We can replay very long evolutionary trajectories." Thus, PACE can be used to study problems that have so far been relegated to thought experiments. And doing so will not take millions of years or even multiple 'minipreps'.

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RESEARCH PAPERS

Esvelt, K.M. *et al.* A system for the continuous directed evolution of biomolecules. *Nature* **472**, 499–503 (2011).