

A systematic approach to scaling up synthetic biology

LEAN AND SMART DATA TOOLS are key to building a robust economy based on products created by engineered plants and microbes.

A series of streamlined computational research tools will help Japanese synthetic biologists produce rapid results for sustainable industries that use living organisms to produce base materials.

This 'bioeconomy' could include everything from fragrances that incorporate substances metabolized by engineered strains of bacteria to palm oil alternatives produced by yeasts, explains Sachiyo Aburatani, who helps steer the Computational Bio Big Data laboratory at Japan's National Institute of Advanced Industrial Science and Technology.

"Just by trying to substitute 10% of all palm oil to sustainable alternatives, there's already a demand for 60 million tonnes of oil per year," Aburatani points out. "Using substitutes from yeast could lead to as much as a reduction of 240 million tonnes of CO₂ annually."

Japanese synthetic biology has an advantage: the country's history with fermented foods, such as soy sauce and pickles, has seen research strengths in two vital fields — fermentation and microbes. With this foundation and a systematized research approach, scientists

expect to make rapid progress toward a bioeconomy over the next 10 years.

Using leaner data

Aburatani has been working on lean data tools, including gene network maps. "International teams are using machine learning to identify genes in an organism that can be modified to help it generate usable amounts of a product. But to do this, you need tens of thousands of data points for each study," she explains. "It's very expensive. Instead, in Japan, we're developing modelling techniques that can achieve similar levels of precision with realistic amounts of data, several hundred data points, at a minimum."

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For example, with statistical methods, Aburatani constructs her network maps to estimate the cause and effect between genes that contribute to the



production of a compound. "When a gene is in action, it switches other genes on and off, creating a very complicated and precise control network system within an organism," she explains. "If this network can be reconstructed, a useful control factor to adjust the target substance can often be discovered."

In an experiment using this computational technique, Aburatani found a gene, in which the a function was previously unknown, that contributes to doubling oil yield in yeast from the *Lipomyce* genus. Once systems like this are revealed, says Aburatani, the necessary data needed to make discoveries in similar areas should be reduced by tenths, or even hundreds.

Bio-production pathway tools

Tomokazu Shirai, who leads the Cell Factory Research Team at RIKEN, is designing artificial metabolic pathways. His team has established a computational tool called BioProV that pulls data from enzyme databases to find reactions that could comprise a new route.

He says cells are often modified based on computer reconstructions of their metabolism that predict how their product yield would change after editing a specific gene. But Shirai thinks this isn't enough. "To make an entire industry, we also need take the opposite approach, where we start from a desired product and find a way to make it," he says.

With BioProV Shirai's team can suggest the insertion of

candidate genes into a microbe or plant cell that codes for enzyme catalysts that correspond to desired chemical reactions. "This way, we can construct a pathway to synthesize non-natural compounds using enzymes an organism doesn't naturally produce," he explains.

Shirai's group also harness a tool called M-Path, developed by Michihiro Araki at Kyoto University, to analyse metabolic reactions and design new metabolic pathways. "These tools," he says, expand the repertoire of possible modifications. "We're no longer limited to optimizations on enzymes and reactions that already exist."

Accelerating production

Tomohisa Hasunuma, who leads

the Engineering Biology Research Center at Kobe University, is using tools like M-Path to enhance real-world outputs.

"Our group is working to make new metabolic designs a reality through automated processes, so that high-performing cells can be produced in a shorter time," he explains.

So far, his team has established the machinery to synthesize long chains of DNA, which are inserted in cells to bulk-edit genes. "At the moment it's the most precise tool of its kind. We reduced by half the amount of time it takes for synthesis, and the cost to a tenth."

His group is working on two compounds that can be used to manufacture painkillers,



A silkworm enzyme was inserted into *Escherichia coli*'s metabolism to increase the output of tetrahydropapaveroline and reticuline by more than a factor of seven each.



Bacteria are fed sugars (fermented) as they produce painkiller compounds.

tetrahydropapaveroline and reticuline. With insights from M-Path and an iterative breeding and research process called the design, build, test, and learn (DBTL) cycle, his team increased the microbial titres of tetrahydropapaveroline by up to a factor of eight and reticuline by more than a factor of seven.

This was achieved after M-Path calculations showed that an enzyme found in silkworms could be used as a more efficient alternative for two bacterial enzymes, a metabolic shortcut. The team then artificially fine-tuned the silkworm enzyme to fit more naturally within the *Escherichia coli* bacterium's metabolism.

"We've made tremendous progress in metabolic design and automated cell culture

techniques, and finally have all the research components to breed efficient bacteria using the DBTL cycle," says Hasunuma. "This breeding will be a crucial next step to scale up the production process and establish a competitive industry in which target compounds can be mass produced."

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■



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