Freedom and flexibility: Thinking outside the cell for functional genomics

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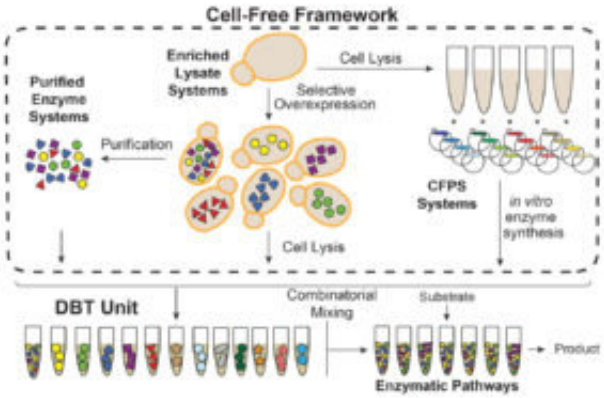

The cell-free systems approach outlined in the approved ETOP proposal starts by lysing pre-optimized cells from selected strains, and then working with the lysates from these strains to express genes and pathways of interest in cell-free platforms (CFPS) which can be mixed in cocktails of varying ratios for easily and rapidly characterizing novel and improved pathways, speeding up the “build” and “test” portion of the design-build-test (DBT) cycle. Credit: Mike Jewett

Over the past two decades, the U.S. Department of Energy (DOE) Joint Genome Institute (JGI), a DOE Office of Science User Facility at Lawrence Berkeley National Laboratory, has transitioned from a high-throughput genome sequencing center to a national user facility that provides researchers around the world with access to sequencing and computational analysis capabilities on projects relevant to the DOE missions of energy and environmental challenges. Along with advances

in sequencing technologies and capacities, JGI has developed capabilities such as single-cell genomics, synthetic biology, and metabolomics to move beyond generating a DNA sequence to understanding gene functions for a myriad of applications.

"JGI's strategic direction is to translate genomic information into functional understanding," noted Director Nigel Mouncey. "Today, the scale and cost of DNA sequencing have afforded the generation of an unprecedented level of gene and genome information for which relatively little is known regarding function. Thus, there is a critical need to rapidly, and at scale, assign validated function of genes, pathways and genomes."

Cell-based tests to determine metabolic function are challenging due to the need for cell growth, complex regulatory mechanisms, interference with or from other metabolic pathways and cellular processes. The latest proposal approved through the JGI's Emerging Technologies Opportunity Program (ETOP) is led by Hal Alper of the University of Texas at Austin and Michael Jewett of Northwestern University. Aided by nearly $500,000 in funding over two years from the JGI, the project aims to develop an optimized cell-free platform that will enable researchers to speed up the "build" and "test" portion of the design-build-test-analyze cycle in synthetic biology. Cell-free systems have been used successfully for individual protein expression over decades, but more recent applications have focused on enzyme screening, metabolic pathway design and prototyping and immune system characterization.

"What we're trying to develop is a generic platform that's pathway-agnostic, a seamless pipeline from DNA design to prototype," said Alper. "It's a tool for both discovery and understanding, but always a way to speed up cell engineering."

Launched in 2013, the ETOP aims to bring new technologies developed

at other institutions into the JGI, making them available to its users for energy and environment applications and adding value to the high throughput sequencing and analysis currently being done for JGI users.

The cell-free systems approach outlined in the proposal starts by lysing pre-optimized cells from selected strains, and then working with the lysates from these strains to express genes and pathways of interest which can be mixed in cocktails of varying ratios for easily and rapidly characterizing novel and improved pathways. Alper noted that this is a collaborative effort in which their labs will be handing off the technology development several times throughout the timeframe to enable progress.

"People have used cell-free frameworks to understand biochemistry for decades. What's new is idea of treating a pathway as something that can be built from enzyme cocktails," said Jewett. "In our design-build-test cycle, the unit isn't a plasmid or a construct, but a lysate enriched with pathway enzymes. You have freedom and flexibility; direct access to reaction conditions because you don't have cell walls, and it's useful for non-model organisms. This can accelerate design loops and can lead to the question, rather than taking 10,000 shots on goal in a month, can we do it in a week?"

"This ETOP will develop novel and scaleable cell-free expression platforms that are optimized for particular key nodes of metabolism and will be demonstrated for a range of biosynthetic genes and pathways," said Mouncey. "This technology is highly complementary to existing capabilities at JGI, and once in-house, will be combined in novel integrative workflows that allow for sophisticated genome mining to DNA synthesis to cell-free expression to high-throughput metabolomics to high-performance computing to characterize the function of 1000s of genes. Working with the leaders in this exciting field will lead to highly impactful and valuable technology for our Users."

Jewett added that the cell-free systems framework wasn't even possible two years ago. He cited the confluence of advances in DNA synthesis, improvements in cell-free biosynthesis capabilities, and genome engineering tools including novel strategies such as multiplexed CRISPR tools that have enabled this new platform.

"We hope to draw in researchers from the broader community that can leverage these new tools within the JGI," he added.

Provided by Lawrence Berkeley National Laboratory