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"Plug and Play" Comes to Synthetic Biology

Howard Hughes Medical Institute researchers have developed a strategy for generating "plug-and-play" components for synthetic genetic networks that may someday nudge algae to create biofuels, help microbes make new materials, or even lend greater precision to beer brewing.

Those components could make life easier for synthetic biologists, who use engineering design principles to assemble genes and proteins into novel biological systems. HHMI investigator James J. Collins says his new tools should reduce the extensive tweaking required to make synthetic gene networks operate correctly. "This will fast-track the field of synthetic biology," Collins said. "It enables one to make predictable networks."

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— James J. Collins

Collins and his colleagues at Boston University described their research in an April 19, 2009, advance online publication in the journal *Nature Biotechnology*.

Collins applies the basic rules for how genes and proteins operate to the new field of synthetic biology, which brings engineers and biologists together to construct biological machines from parts, such as genes, proteins, and signaling pathways. But synthetic biology faces a significant challenge. Even when scientists know how a particular component acts in nature, its behavior isn't predictable when assembled into a network.

While it can take synthetic biologists a month or so to piece together a network, Collins says "it can take months if not a year or years to get the network to behave. You have to do an awful lot of tweaking to get your network to work the way you want." That tweaking translates into many hours in the lab introducing genetic mutations to fine-tune imperfect parts, substituting alternative components into a system, or adding new features to

counterbalance problems.

Collins realized that synthetic biologists needed off-the-shelf, plug-and-play biological parts that behave as expected when installed in networks. A large, well-characterized collection of parts would be extremely useful to researchers. Since small changes to one component can dramatically affect the behavior of the entire system, it's important to know how each one works, Collins says.

Collins and colleagues Tom Ellis and Xiao Wang, all members of the Boston University Center for BioDynamics, set out to create and test a supply of component parts in a fast, efficient manner. They focused their attention on one commonly used class of parts, gene promoters that tell genes when to turn on protein expression and when to shut it down. By linking promoters to particular genes, biologists can manipulate the regulation of those genes.

The team created 20 different promoters that shut down gene expression in the presence of the antibiotic tetracycline. Each of the promoters had a slightly different sensitivity to tetracycline and a different amount of influence over gene expression. In the experiment, the promoters controlled expression of a gene for green fluorescent protein.

The researchers developed their promoter library, carefully characterizing each promoter by measuring the amount of fluorescent protein it expressed. They followed the same procedure to create another library of 20 variations on a second type of promoter. Next, they created a trial network containing one promoter from each of the two libraries. The performance of this single network provided the data that Collins' group used to create a predictive model that could then be applied to the hundreds of potential pairings using the two promoter libraries.

The trial network pitted gene-against-gene in a sort of tug-of-war, with each promoter attempting to turn off the other. "Each gene wants to be on, and each gene wants to shut the other off," Collins explained. When the tetracycline-regulated promoter won, it expressed green fluorescent protein. Timing how long it took the tetracycline-regulated promoter to win gave Collins enough information to use the model to accurately predict the timing of gene expression in all future tugs-of-war between any combination of promoters in his two libraries.

"This is the key to the whole piece," Collins said. "If you construct one representative network out of a possible several hundred and characterize it, you can predict how those components will behave when put them together in several different networks.

"A similar idea would be, if you have a 7-year-old boy, you can analyze how the boy behaves on his own. And if you have four other 7-year-old boys, you can analyze how each of them behaves alone. But you can't really say how they will behave when they're put together. There are interactions you can't predict. But if you observe two boys together, you'll be better able to predict how each individual will behave with other boys," Collins said.

Finally Collins' group used their two-promoter network as a timer to control the flocculation of yeast, a step that occurs in brewing when yeast cells clump together and fall to the bottom of the fermentation tank. Ideally, flocculation should occur when fermentation is complete. The group used three different promoter combinations and tested the combinations against their model to predict when flocculation would occur for each combination. The model proved robust, showing the promise of creating a storehouse of characterized parts researchers may draw upon.

"I envision we'll be able to generate extensive libraries that will be appropriately characterized," Collins said. "These libraries could be broadly used in the synthetic biology community."

According to Collins, the time savings could be significant. It took Collins' group a couple of weeks to create the network on which they based their predictive model. But once that work was done, they used the model to predict the behavior of 440 networks, knowing which promoters he could pull from the shelf to tightly control flocculation. "You could easily build in parallel, in a matter of a couple of weeks, a number of networks and they'll behave the way you like," he said.

The practice could be extended to creating networks of numerous components, each with predictable, characterized behavior, he said. "Now the possibilities become huge. You're into thousands of possibilities."

"We envision this approach can be extended to other components, to proteins and RNAs, and to more complicated networks," Collins said. It's an important advance that will bring synthetic biologists closer to making microbes do science's bidding to produce new materials or fuels, he said.