CUSTOM-MADE CELLS

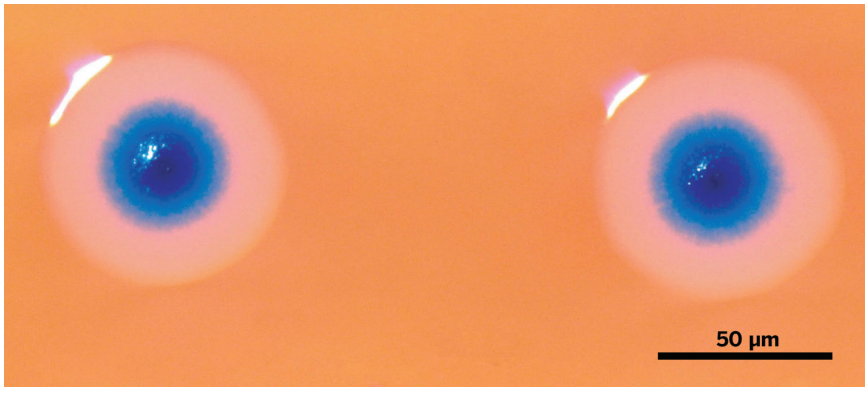
Synthetic biologists use **OPPOSING METHODS** to make microbes with tailored functions

DAVID PITTMAN, C&EN WASHINGTON

BEFORE DAWN one Monday morning this spring, Dan Gibson peered through an electron microscope in a Rockville, Md., lab. What the genetic researcher saw was a milestone in his field. Two blue blobs of cell colonies that resembled a pair of eyeballs stared back at him. A cell, the first controlled by a synthetic genome, had reproduced, proving it was functional.

Gibson's finding called for a cautiously worded 6 AM text message to his boss, genomics pioneer J. Craig Venter, to share the observation.

SCIENCE



PROOF OF FUNCTION A cell controlled by a synthetic genome was able to reproduce; colonies formed by it and its sibling resemble a pair of blue eyes.

"By the end of the day, we opened a bottle of champagne, and we all celebrated," says Gibson, a 33-year-old Buffalo, N.Y., native who also performed most of the group's experiments.

To produce those eyeball-like colonies, Venter's team of scientists synthesized the genome of the bacterium *Mycoplasma mycoides* and then transplanted it into a host cell, in this case *M. capricolum* (C&EN, May 24, page 10). It had taken almost six years, nearly $40 million in funding, and numerous setbacks, but Venter's vision had finally come to fruition.

Synthetic biologists had realized the possibility of building an entire genome—albeit one of the simplest known—that they could install in another cell.

The future is tantalizing. If researchers can build this basic genome, then they should someday be able to synthesize more complex ones that boast functions customized to match human needs.

"Maybe five, 10, or 15 years from now, we'll know what every gene does," says Gibson, a tall, lanky scientist who joined Venter's team in 2004. "We'll know exactly where we can design synthetic cells to do amazing things."

In the works are algae that produce hydrocarbons through photosynthesis. Building a year's stockpile of flu vaccine in a few days is also possible, Gibson notes. Having proven that they could re-create a bacterium such as *M. mycoides* with 1 million DNA base pairs, Venter and his team believe a larger organism with more than 30 million base pairs, such as algae, is within reach.

"When I talk to students, I tell them we are primarily limited now by our imaginations," says Venter, who helped map the human genome in 2000 and has twice been named one of the most influential people in the world by *Time* magazine. "I think it is a very exciting time that could influence almost every aspect of human life, and we want to drive that forward."

But the field of synthetic biology is split regarding how to accomplish its goal of building microbes that generate pharmaceuticals, fuels, or other hydrocarbons.

Venter and his team at J. Craig Venter Institute in both Rockville and San Diego are working to build entire genomes of small microbes from scratch. But nearly every other researcher in the field is taking a different course: designing a handful of customized genes to insert into existing organisms' genomes.

ALTHOUGH VENTER has yet to create a microbe capable of producing a viable product, others in the fast-growing field have already used synthetic biology to make limited forms of fuel, industrial chemicals, and pharmaceuticals (see page 25).

DuPont Tate & Lyle Bioproducts, a joint venture formed in 2005 between chemical giant DuPont and renewable ingredients manufacturer Tate & Lyle, uses synthetic biology to make 1,3-propanediol. Workers harness a genetically modified strain of *Escherichia coli* to turn corn syrup into the propanediol product, which DuPont then uses to make carpets, plastics, and other polymers.

Emeryville, Calif.-based Amyris Biotechnologies is already using genetically modified yeast to create diesel fuel and hopes to churn out jet fuel in the future. Last month, it announced a commercial partnership with Sanofi-Aventis to mass-produce the antimalarial drug artemisinin through engineered yeast.

Neither Venter's top-down approach nor the rest of the field's bottom-up method is without scientific hurdles. Brilliant scientists across the globe continue to struggle with how to manipulate DNA for humanity's benefit.

Venter is frank when he talks about the time it took to complete the synthetic genome project. "I actually thought it was going to be a whole lot faster," the balding 63-year-old Utah native told members of

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Congress in a hastily convened hearing a week after his *Mycoplasma* announcement. "We feel bad it has taken us so long."

His lab had previously developed and published the individual steps needed for the project, but the team struggled with combining the steps into one process for this latest effort.

To build its genome, Venter's team of researchers had to synthesize a piece of DNA 18 times as long as any other previously produced in a lab. Aside from developing techniques to build and move such a massive genome, Venter's team was set back three months because of a single mutation—out of more than 1 million base pairs—in a gene involved in DNA replication.

Workers eventually found the pesky problem and corrected it. "The larger a piece of DNA is, the more likely you are to have a mutation," Gibson says. He adds that these mutations can creep in during genome sequencing or when yeast connects smaller DNA fragments into larger segments, as was the case with *M. mycoides*.

Venter's team also ran into trouble inserting their synthetic genome into a host cell.

For any synthetic genome project, geneticists need to find pairs of organisms that are sufficiently similar so that when a lab-prepared genome is inserted into a host cell, the genome is recognized and then survives and replicates.

"Right now, we have only one pair of organisms that anyone has ever demonstrated genome transplantation with," Gibson says. "One of the goals of the institute is to explore other pairs of organisms that can do this."

To protect the synthetic genome during transplantation, researchers can either delete genes in the host cell's DNA-restriction system or coat the incoming genome with an armor of methyl groups.

MANY IN synthetic biology praise Venter's work, saying the steps his lab developed to synthesize large DNA segments are useful for the entire field. Still, they caution that any tangible product achieved through his methods many be years away.

"In the future, this could be a really interesting way to produce chemicals, drugs, and fuels," says Jay D. Keasling, a chemical engineering professor at the University of California, Berkeley. "But you don't need to start by resynthesizing an entire chromosome. If we had done that, we still wouldn't be done with the malaria drug project," he adds, referring to work that helped launch Amyris.

Unlike Venter's team, Keasling's research group has already developed products with modified microbes. His team examined the DNA of the Chinese plant *Artemisia annua* to determine which part of its genome instructs it to make the precursor of the drug artemisinin. The researchers then took that snippet of DNA, re-created it, and transplanted it into yeast. Instead of fermenting a starch to produce ethanol, the specially engineered yeast transforms glucose into artemisinic acid, which the lab then turns into artemisinin (C&EN, Oct. 24, 2005, page 69).

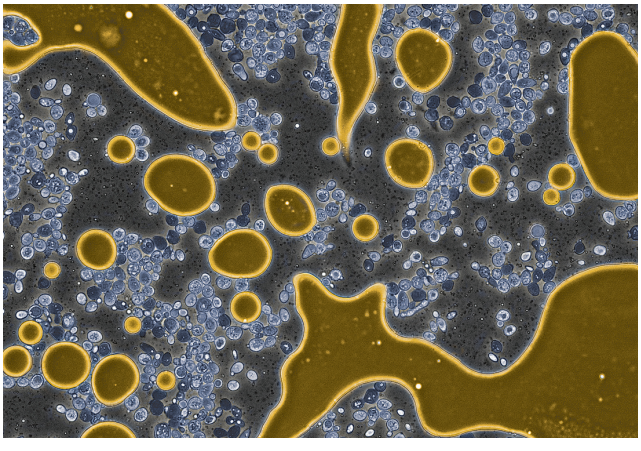
"So now you can make this huge beer fermenter that makes antimalarial drugs super cheap," says Jack D. Newman, cofounder of Amyris and its senior vice presi-

dent of research. The company employs the same method to make biofuels.

"It's kind of like once you know how to do computer programming, you can make a program that is an Excel spreadsheet or a Word file," says Newman, a former postdoc in Keasling's lab. "It's really quite amazing, all the different products synthetic biology can make."

Under Keasling's leadership, the Joint Bioenergy Institute, a research program funded by the Department of Energy, is trying to engineer yeast to feed off sorghum, dextrose, sucrose, and even corn syrup to produce hydrocarbons. Amyris already uses engineered yeast to produce diesel from sugarcane in Brazil. The company received a $24 million grant in December 2009 from DOE to look at domestic feedstock for fuel production through yeast.

The lessons learned in reprogramming yeast and *E. coli* to make diesel can be applied to make other chemicals and hydrocarbons, Keasling points out. Yeast could churn out plastics, polymers, or lubricants. "There's no reason you can't produce every other molecule we get from petroleum," he says.



AMYRIS BIOTECHNOLOGIES

FUEL UP Genetically engineered yeast cells (gray-blue circles), which are typically 3–4 µm in diameter, brew renewable diesel (yellow and brown) by fermenting sugars.

Indeed, South San Francisco-based LS9, a privately held biotech firm that uses synthetic biology to create fuels, recently reengineered *E. coli* to produce chemicals such as fatty acids, aldehydes, and wax esters from glucose (C&EN, Feb. 1, page 11). Researchers boosted the bacteria's fatty acid output three- to fourfold by expressing and suppressing particular enzymes involved in the acid formation.

Despite the recent success by Keasling and others, the bottom-up method is not without limitations. For example, researchers still struggle with how to organize tens of thousands of DNA pairs to achieve specialized gene sequences, or circuits, that work. "We know of the words, but we don't know how to make sentences very well," Keasling says. "We can put all of the components together, but they often don't work."

AS A RESULT, it can take years to design, let alone build, a single genetic circuit such as the one Christopher A. Voigt's lab at the University of California, San Francisco, recently completed. Voigt's group designed the circuit to demonstrate the possibility of customizing an organism to sense environmental cues such as light and then alter its chemistry accordingly. The bacteria carrying Voigt's genetic circuit are so sensitive that they can grow only when a particular amino acid is present (*Nature,* DOI: 10.1038/nature04405).

The hoopla around Venter's recent work—which was published in *Science* magazine—notwithstanding, it has drawn criticism, Voigt says, because the team didn't synthesize a specialized genome, as most in the field are trying to do. "They just kind of rebuilt this natural genome," he explains. "They implied genome design in their paper, but it was a recapitulation of a natural genome. There was no design in it."

Other synthetic biologists have lauded Venter's recent work, however, for its ability to synthesize pieces of DNA several times larger than those previously generated.

Voigt calls the Venter team's recently developed technique of combining DNA pieces into a larger one the "Gibson Method," after the group's hard-working member. Voigt likens the process to throwing a handful of Legos in a bag and having them come together in just the right way. Gibson found a way to use what are essentially precisely placed molecular magnets that enable shorter pieces of DNA to snap together (*Nat. Meth.,* DOI: 10.1038/nmeth.1318).

"The construction techniques just got mature," Voigt says. "Now, it's going to be this massive application of them to different problems."

However, "Venter's approach is not yet practical because of its cost and time-consuming nature," says James J. Collins, director of the Center for BioDynamics at Boston University. But "I think the cost will eventually come down, and the techniques will become more efficient, leading to an enabling technology platform."

The Venter Institute, meanwhile, has its eyes set on more near-term prizes. The group is working with Swiss pharmaceutical company Novartis to develop a more rapid method for making flu vaccines than is currently possible. The premise is that synthetic biologists could build every permutation of the flu virus in the lab after sequencing its DNA. After that, growing millions of doses of any flu strain for a vaccine could take a matter of days rather than weeks via traditional methods.

"As we are in an exploratory phase evaluating this new technology, it is premature to share more details on research collaborations and discuss potential commercial applications at this time," Novartis said in a statement addressing progress.

Synthetic Genomics, the for-profit firm Venter cofounded with Hamilton O. Smith in 2005, hopes to leverage the technology to build algal fuel factories. Last summer, oil and gas giant ExxonMobil announced a $600 million partnership with the La Jolla, Calif.-based company to develop algae-derived biofuels. And last week, they opened a new greenhouse facility to test algae strains capable of producing hydrocarbons that can be processed into fuel for cars (see page 6). ExxonMobil plans to fund $300 million in research over five to six years in La Jolla while spending $300 million in its own labs.

With synthetic biology moving forward rapidly, Amyris' Newman called Venter's recent work inspirational for the whole field. He compared the *Science* paper with the first trans-Atlantic flight in 1919. It was 20 years after that historic aerial feat before Pan American Airways established the first regular, commercial trans-Atlantic passenger service. In the meantime, a vibrant commercial airline industry flew people locally.

Whether synthetic biologists use Venter's top-down approach to build whole genomes or nearly everyone else's bottom-up method of transplanting small customized genetic pathways into existing genomes, maybe one day they'll meet in the middle. ■