

Biologists are crafting libraries of interchangeable DNA parts and assembling them inside microbes to create programmable, living machines

SYNTHETIC LIFE

By W. Wayt Gibbs

Evolution is a wellspring of creativity; 3.6 billion years of mutation and competition have endowed living things with an impressive range of useful skills. But there is still plenty of room for improvement. Certain microbes can digest the explosive and carcinogenic chemical TNT, for example—but wouldn't it be handy if they glowed as they did so, highlighting the location of buried land mines or contaminated soil? Wormwood shrubs generate a potent medicine against malaria but only in trace quantities that are expensive to extract. How many millions of lives could be saved if the compound, artemisinin, could instead be synthesized cheaply by vats of bacteria? And although many cancer researchers would trade their eyeteeth for a cell with a built-in, easy-to-read counter that ticks over reliably each time it divides, nature apparently has not deemed such a thing fit enough to survive in the wild.

It may seem a simple matter of genetic engineering to rewire cells to glow in the presence of a particular toxin, to manufacture an intricate drug, or to keep track of the cells' age. But creating such biological devices is far from easy. Biologists have been transplanting genes from

one species to another for 30 years, yet genetic engineering is still more of a craft than a mature engineering discipline.

“Say I want to modify a plant so that it changes color in the presence of TNT,” posits Drew Endy, a biologist at the Massachusetts Institute of Technology. “I can start tweaking genetic pathways in the plant to do that, and if I am lucky, then after a year or two I may get a ‘device’—one system. But doing that once doesn't help me build a cell that swims around and eats plaque from artery walls. It doesn't help me grow a little microlens. Basically the current practice produces pieces of art.”

Endy is one of a small but rapidly growing number of scientists who have set out in recent years to buttress the foundation of genetic engineering with what they call synthetic biology. They are designing and building living systems that behave in predictable ways, that use interchangeable parts, and in some cases that operate with an expanded genetic code, which allows them to do things that no natural organism can.

This nascent field has three major goals: One, learn about life by building it, rather than by tearing it apart. Two, make genetic engineering worthy of its

REDESIGNED VIRUSES will help biologists learn how to build reliable genetic machines. A group at the Massachusetts Institute of Technology has reorganized the genome of the T7 bacteriophage drawn here.

BRYAN CHRISTIE DESIGN



DREW ENDY (*pictured*) and others at M.I.T. have designed and built more than 140 “BioBricks” (*in vials*). Each is a piece of DNA that performs a well-characterized function and interacts well with other genetic parts.

name—a discipline that continuously improves by standardizing its previous creations and recombining them to make new and more sophisticated systems. And three, stretch the boundaries of life and of machines until the two overlap to yield truly programmable organisms. Already TNT-detecting and artemisinin-producing microbes seem within reach. The current prototypes are relatively primitive, but the vision is undeniably grand: think of it as Life, version 2.0.

A Light Blinks On

THE ROOTS OF SYNTHETIC BIOLOGY extend back 15 years to pioneering work by Steven A. Benner and Peter G. Schultz. In 1989 Benner led a team at ETH Zurich that created DNA containing two artificial genetic “letters” in addition to the four that appear in life as we know it. He and others have since invented several varieties of artificially enhanced DNA. So far no one has made genes from altered DNA that are functional—transcribed to RNA and then translated to protein form—within living cells. Just within the past year, however, Schultz’s group at the Scripps Research Institute developed cells (containing normal DNA) that generate unnatural amino acids and string them together to make novel proteins [*see box on page 80*].

Overview/*Synthetic Biology*

- Molecular biology has been largely a reductive science that deduces the operation of living systems by breaking them apart.
- A growing number of synthetic biologists are taking a different approach: building machines from interchangeable DNA parts. The devices work inside living cells, from which they derive energy, raw materials, and the ability to move and reproduce.
- Synthetic biology has already produced microbes with a variety of unnatural talents. Some produce complex chemical ingredients for drugs; others make artificial amino acids, remove heavy metals from wastewater or perform simple binary logic.

Benner and other “old school” synthetic biologists see artificial genetics as a way to explore basic questions, such as how life got started on earth and what forms it may take elsewhere in the universe. Interesting as that is, the recent buzz growing around synthetic biology arises from its technological promise as a way to design and build machines that work inside cells. Two such devices, reported simultaneously in 2000, inspired much of the work that has happened since.

Both devices were constructed by inserting selected DNA sequences into *Escherichia coli*, a normally innocuous bacterium in the human gut. The two performed very different functions, however. Michael Elowitz and Stanislaus Leibler, then at Princeton University, assembled three interacting genes in a way that made the *E. coli* blink predictably, like microscopic Christmas tree lights [*see box on opposite page*]. Meanwhile James J. Collins, Charles R. Cantor and Timothy S. Gardner of Boston University made a genetic toggle switch. A negative feedback loop—two genes that interfere with each other—allows the toggle circuit to flip between two stable states. It effectively endows each modified bacterium with a rudimentary digital memory.

To engineering-minded biologists, these experiments were energizing but also frustrating. It had taken nearly a year to create the toggle switch and about twice that time to build the flashing microbes. And no one could see a way to connect the two devices to make, for example, blinking bacteria that could be switched on and off.

“We would like to be able to routinely assemble systems from pieces that are well described and well behaved,” Endy remarks. “That way, if in the future someone asks me to make an organism that, say, counts to 3,000 and then turns left, I can grab the parts I need off the shelf, hook them together and predict how they will perform.” Four years ago parts such as these were just a dream. Today they fill a box on Endy’s desk.

Building with BioBricks

“THESE ARE GENETIC PARTS,” Endy says as he holds out a container filled with more than 50 vials of clear, syrupy fluid. “Each of these vials contains copies of a distinct section of DNA that either performs some function on its own or can be

HOW A GENETIC PART WORKS

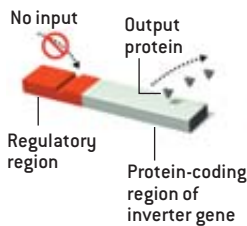
Assemblies of genes and regulatory DNA can act as the biochemical equivalent of electronic components, performing Boolean logic.

A COMPONENT

A biochemical inverter performs the Boolean NOT operation in response to an input signal, in the form of a protein encoded by another gene.

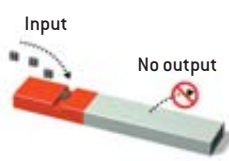
ON

When no input protein is present (input = 0), the inverter gene is “on”—it gives rise to its encoded protein (output = 1).



OFF

When input protein is abundant (input = 1), the inverter gene turns off (output = 0).



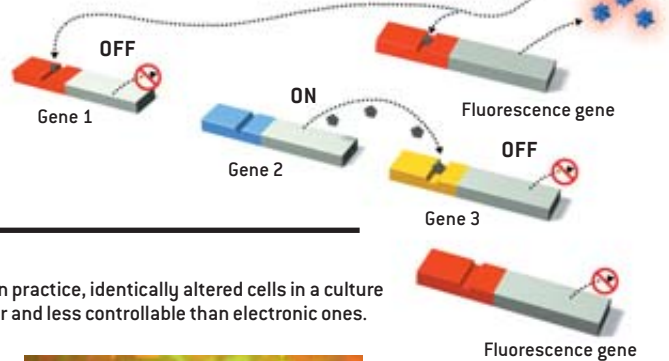
A CIRCUIT

One simple genetic circuit connects three inverters, each of which contains a different gene (gene 1, 2 or 3). The genes oscillate between on and off states as the signal propagates through the circuit. The behavior is monitored through a gene (far right) that intercepts some of the output protein generated by one of the inverter genes (gene 3) and gives rise to fluorescence in response.

AT 150 MINUTES

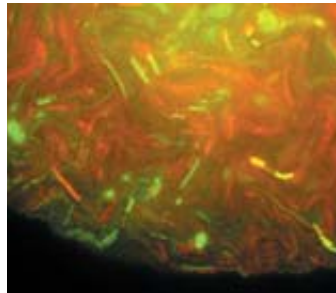
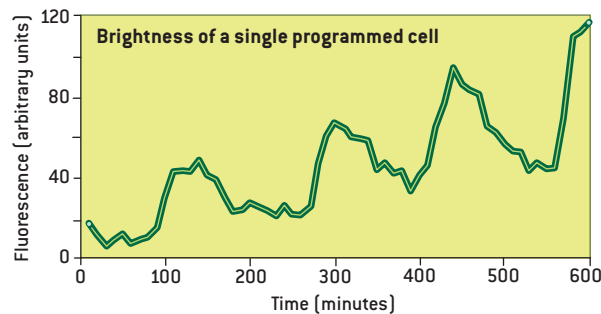


AT 200 MINUTES



A CIRCUIT IN ACTION

Cells containing such a circuit blink on and off repeatedly (graph). But in practice, identically altered cells in a culture (photograph) blink at varying rates, because genetic circuits are noisier and less controllable than electronic ones.



Fluorescence gene

used by a cell to make a protein that does something useful. What is important here is that each genetic part has been carefully designed to interact well with other parts, on two levels.” At a mechanical level, individual BioBricks (as the M.I.T. group calls the parts) can be fabricated and stored separately, then later stitched together to form larger bits of DNA. And on a functional level, each part sends and receives standard biochemical signals. So a scientist can change the behavior of an assembly just by substituting a different part at a given spot.

“Interchangeable components are something we take for granted in other kinds of engineering,” Endy notes, but genetic engineering is only beginning to draw on the power of the concept. One advantage it offers is abstraction. Just as electrical engineers need not know what is inside a capacitor before they use it in a circuit, biological engineers would like to be able to use a genetic toggle switch while remaining blissfully ignorant of the binding coefficients and biochemical makeup of the promoters, repressors, activators, inducers and other genetic el-

ements that make the switch work. One of the vials in Endy’s box, for example, contains an inverter BioBrick (also called a NOT operator). When its input signal is high, its output signal is low, and vice versa. Another BioBrick performs a Boolean AND function, emitting an output signal only when it receives high levels of both its inputs. Because the two parts work with compatible signals, connecting them creates a NAND (NOT AND) operator. Virtually any binary computation can be performed with enough NAND operators.

Beyond abstraction, standardized parts offer another powerful advantage: the ability to design a functional genetic system without knowing exactly how to make it. Early last year a class of 16 students was able in one month to specify four genetic programs to make groups of *E. coli* cells flash in unison, as fireflies sometimes do. The students did not know how to create DNA sequences, but they had no need to. Endy hired a DNA-synthesis company to manufacture the 58 parts called for in their designs. These new BioBricks were then added to



Living machines reproduce, but as they do, they mutate.

M.I.T.'s Registry of Standard Biological Parts. That online database today lists more than 140 parts, with the number growing by the month.

Hijacking Cells

AS USEFUL AS IT HAS BEEN to apply the lessons of other fields of engineering to genetics, beyond a certain point the analogy breaks down. Electrical and mechanical machines are generally self-contained. That is true for a select few genetic devices: earlier this year, for example, Milan Stojanovic of Columbia University contrived test tubes of DNA-like biomolecules that play a chemical version of tic-tac-toe. But synthetic biologists are mainly interested in building genetic devices within living cells, so that the systems can move, reproduce and interact with the real world. From a cell's point of view, the synthetic device inside it is a parasite. The cell provides it with energy, raw materials and the biochemical infrastructure that decodes DNA to messenger RNA and then to protein.

The host cell, however, also adds a great deal of complexity. Biologists have invested years of work in computer models of *E. coli* and other single-celled organisms [see "Cybernetic Cells," *SCIENTIFIC AMERICAN*, August 2001]. And yet, acknowledges Ron Weiss of Princeton, "if you give me the DNA sequence of your genetic system, I can't tell you what the bacteria will do with it." Indeed, Endy recalls, "about half of the 60 parts we designed in 2003 initially couldn't be synthesized because they killed the cells that were copying them. We had to figure out a way to lower the burden that carrying and replicating the engineered DNA imposed on the cells." (Eventually 58 of the 60 parts were produced successfully.)

One way to deal with the complexity added by the cells' native genome is to dodge it: the genetic device can be sequestered on its own loop of DNA, separate from the chromosome of the organism. Physical separation is only half the solution, however, because there are no wires in cells. Life runs on "wet-ware," with many protein signals simply floating randomly from one part to another. "So if I have one inverter over here made out of proteins and DNA," Endy explains, "a protein signal meant for that part will also act on any other instance of that inverter anywhere else in the cell," whether it lies on the artificial loop or on the natural chromosome.

One way to prevent crossed signals is to avoid using the same part twice. Weiss has taken this approach in constructing a "Goldilocks" genetic circuit, one that lights up when a target chemical is present but only when the concentration is not

too high and not too low [see illustration on opposite page]. Tucked inside its various parts are four inverters, each of which responds to a different protein signal. But this strategy makes it much more difficult to design parts that are truly interchangeable and can be rearranged.

Endy is testing a solution that may be better for some systems. "Our inverter uses the same components [as one of Weiss's], just arranged differently," Endy says. "Now the input is not a protein but rather a rate, specifically the rate at which a gene is transcribed. The inverter responds to how many messenger RNAs are produced per second. It makes a protein, and that protein determines the rate of transcription going out [by switching on a second gene]. So I send in TIPS—transcription events per second—and as output, I get TIPS. That is the common currency, like a current in an electrical circuit." In principle, the inverter could be removed and replaced with any other BioBrick that processes TIPS. And TIPS signals are location-specific, so the same part can be used at several places in a circuit without interference.

The TIPS technique will be tested by a new set of genetic systems designed by students who took a winter course at M.I.T. this past January. The aim this year was to reprogram cells to work cooperatively to form patterns, such as polka dots, in a petri dish. To do this the cells must communicate with one another by secreting and sensing chemical nutrients.

"This year's systems were about twice the size of the 2003 projects," Endy says. It took 13 months to get the blinking *E. coli* designs built and into cells. But in the intervening year the inventory of BioBricks has grown, the speed of DNA synthesis has shot up, and the engineers have gained experience assembling genetic circuits. So Endy expects to have the 2004 designs ready for testing in just five months, in time to show off at the first synthetic biology conference, scheduled for this June.

Rewriting the Book of Life

THE SCIENTISTS WHO ATTEND that conference will no doubt commiserate about the inherent difficulty of engineering a relatively puny stretch of DNA to work reliably within a cell that is constantly changing. Living machines reproduce, but as they do they mutate.

"Replication is far from perfect. We've built circuits and seen them mutate in half the cells within five hours," Weiss reports. "The larger the circuit is, the faster it tends to mutate." Weiss and Frances H. Arnold of the California Institute of Technology have evolved circuits with improved performance

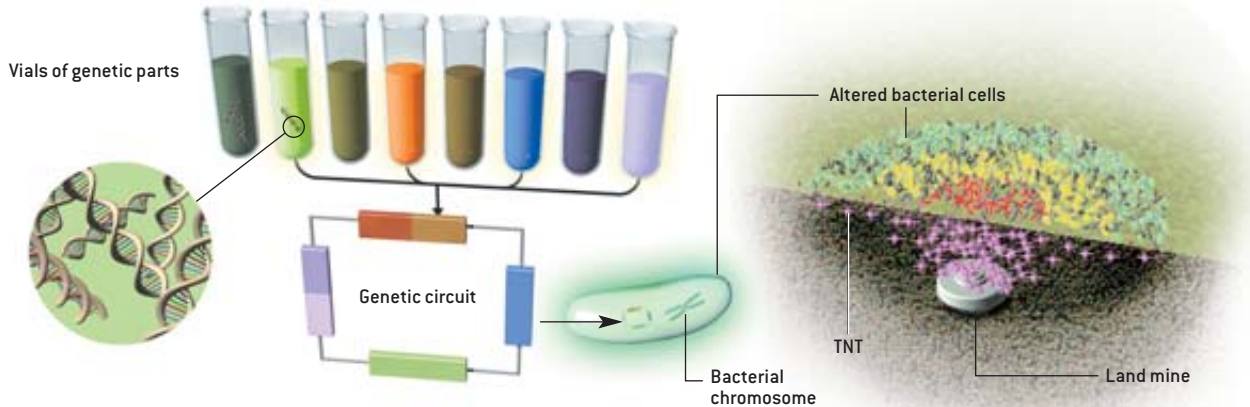
BUILDING A GENETIC MACHINE

A living TNT detector that reveals buried land mines could be made using genetic “Goldilocks” circuits that fluoresce only when the concentration of TNT is just right.

CONSTRUCTING A GENETIC TNT DETECTOR

Drawing from interchangeable DNA parts (*in test tubes*), engineers could assemble slightly different circuits. One would glow red, but only when the TNT concentration is high. A second might fluoresce yellow at medium levels of TNT, and a third could glow green at low concentrations.

Engineers would insert the circuits into three separate bacterial cultures. In the soil over a mine (*below*), TNT tapers off in a circular gradient. So a mixture of the altered cells would produce a fluorescent bull’s-eye centered on the mine.



A TNT DETECTOR IN ACTION

One design for a Goldilocks genetic circuit uses four interacting parts: a sensor, upper and lower thresholds, and an inverter. Each part has a distinctive behavior: the amount of protein output it produces varies as a function of the amount of input protein it receives. In the schematic

below for a red-glowing circuit, the graphs illustrate how each part adjusts its output over the full range of TNT concentrations. (The geometric shapes reflect output levels when the TNT concentration is in the “sweet spot” of, say, 4 percent.)

SENSOR

sends out two signals that are roughly proportional to the level of TNT within the cell. Importantly, the two signals are unequal: at a TNT level of 4 percent, one of the genes in the sensor (*dark purple*) churns out only half as much protein (*squares*) as does the other gene (*light purple*).

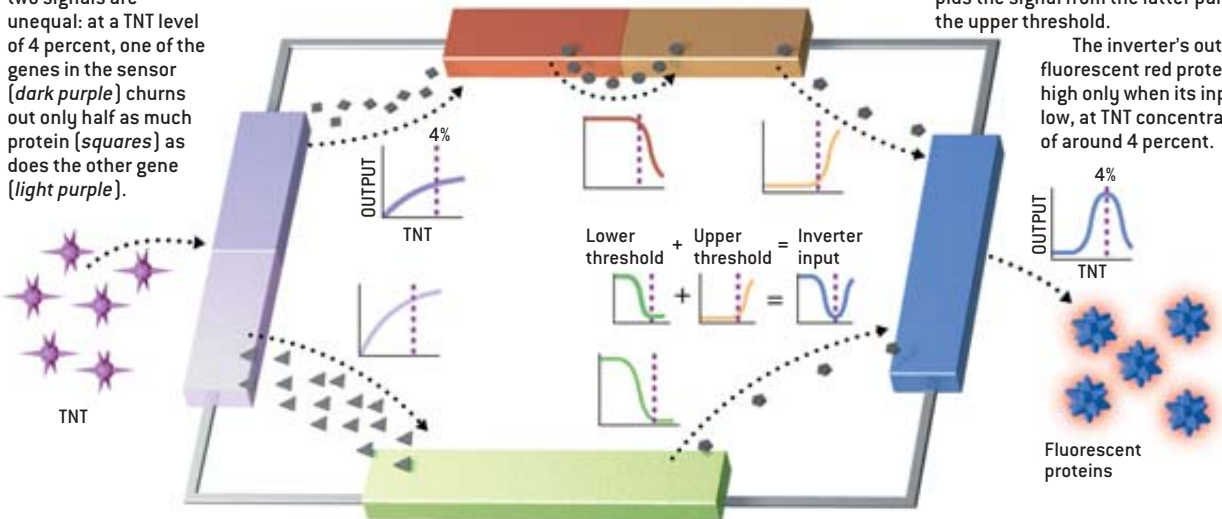
UPPER THRESHOLD

receives the weaker signal from the sensor. Output from the first gene in this part starts to fall dramatically but is still high when TNT levels are 4 percent. The next gene in the chain simply inverts whatever signal the first gene generates. So at 4 percent TNT concentration, the upper threshold sends very little protein to the next part (the inverter).

INVERTER

contains genes that express fluorescent proteins only when input signals from both thresholds are low. Its input (*pentagons*) is the sum of the protein signal produced by the lower threshold plus the signal from the latter part of the upper threshold.

The inverter’s output—a fluorescent red protein—is high only when its input is low, at TNT concentrations of around 4 percent.



LOWER THRESHOLD

emits the inverse of its input signal (*triangles*), which is the protein that the sensor produces most prolifically. This part’s output begins to fall steeply at TNT levels around 1 percent; by 4 percent TNT, the part produces almost no protein to send to the inverter.

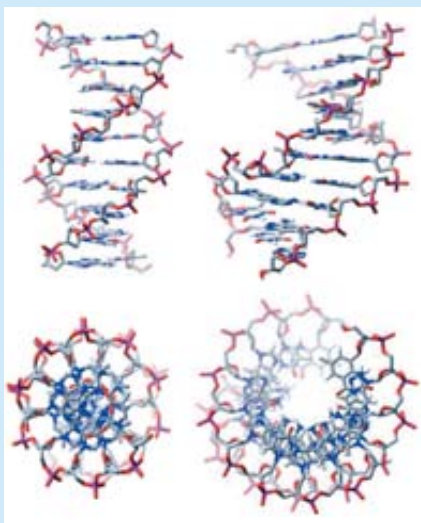
Life, but Not (Exactly) as We Know It

Life on earth has taken a tremendous range of forms, but all species arise from the same molecular ingredients: five nucleotides that form the building blocks for DNA and RNA, and 20 amino acids that serve as building blocks for proteins. (At least two additional natural amino acids are made by a few odd species.) These ingredients limit the chemical reactions that can happen inside cells and so constrain what life can do.

That constraint was eased in 2001, probably for the first time in more than three billion years. After years of trying, Lei Wang, Peter G. Schultz and their co-workers at the Scripps Research Institute in La Jolla, Calif., at last succeeded in adding to *Escherichia coli* bacteria all the genetic components the cells need to decode the three-nucleotide DNA sequence TAG into unnatural amino acids of various kinds.

It was a seminal step but an intermediate one, because amino acids by themselves have relatively few uses. The real goal is to modify cells so that they not only synthesize artificial amino acids but also string them together with natural amino acids to form proteins that no other form of life can make. Last year Schultz's group announced that it had done just that with *E. coli*, and in August the team reported its creation of a similarly talented form of yeast.

"The translational machinery [that reads RNA to make proteins] in yeast is very similar to the translational machinery of



TWISTED LADDER OF DNA (above left, seen in side view and top view) may not be the only macromolecule capable of storing the blueprints for living organisms. Scientists are experimenting with semiartificial nucleic acids, such as xDNA (at right), that are more stable and thus less likely to suffer mutations.

humans," points out T. Ashton Cropp, a biologist in Schultz's lab. "So far we have produced six kinds of unnatural amino acids in yeast," and the scientists have begun adapting the systems to work in human kidney cells and in roundworms, Cropp says. "We're very close to having a system that can make two different unnatural amino acids and put them in the same protein," he

adds. "It is tricky because in order to do that, the cell has to decode a four-nucleotide DNA sequence," which, as far as anyone knows, no cell has ever done.

"This advance could foster developments with inestimable biomedical potential," suggests Brian L. Davis of the Research Foundation of Southern California in La Jolla. He envisions white blood cells that could make novel proteins to destroy pathogens or cancerous cells more quickly. Cropp says the technology is already producing new research tools, such as proteins that include fluorescent amino acids or that change behavior when they are exposed to light. "It allows us to attach polymers to therapeutic proteins, which makes them work better as drugs," Cropp notes.

Synthetic biologists also have been avidly tinkering with unnatural forms of DNA. Steven A. Benner and his associates at the University of Florida developed a six-letter genetic alphabet more than a decade ago; it was recently used to create a rapid test for the SARS virus. "We're playing around with a variant called TNA, where ribose is replaced with a slightly simpler sugar," says Jack W. Szostak of Massachusetts General Hospital. TNA and xDNA, created by Eric T. Kool of Stanford University, are more stable than DNA. That may make them better suited as a medium for reprogramming cells. First, however, scientists will have to get them working inside living organisms. —W.W.G.

using multiple rounds of mutation followed by selection of those cells most fit for the desired task. But left unsupervised, evolution will tend to break genetic machines.

"I would like to make a genetically encoded device that accepts an input signal and simply counts: 1, 2, 3, ... up to 256," Endy suggests. "That's not much more complex than what we're building now, and it would allow you to quickly and precisely detect certain types of cells that had lost control of their reproduction and gone cancerous. But how do I design a counter so that the design persists when the machine makes copies of itself that contain mistakes? I don't have a clue. Maybe we have to build in redundancy—or maybe we need to make the function of the counter somehow good for the cell."

Or perhaps the engineers will have to understand better how simple forms of life, such as viruses, have solved the problem of persistence. Synthetic biology may help here, too. Last November, Hamilton O. Smith and J. Craig Venter announced that their group at the Institute for Biological Energy Alternatives had re-created a bacteriophage (a virus that infects bacteria) called phiX174 from scratch, in just two weeks. The syn-

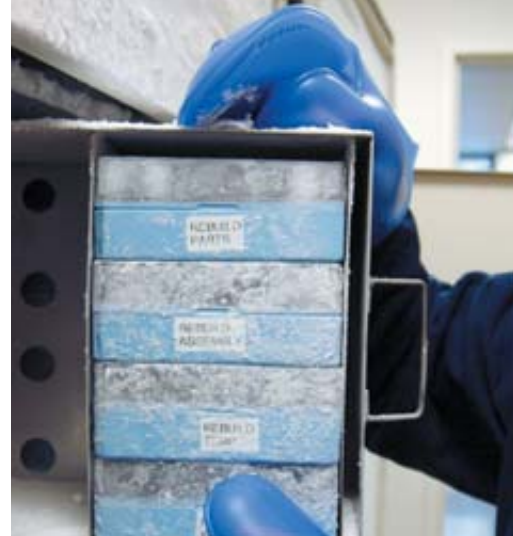
thetic virus, Venter said, has the same 5,386 base pairs of DNA as the natural form and is just as active.

"Synthesis of a large chromosome is now clearly in reach," said Venter, who for several years led a project to identify the minimal set of genes required for survival by the bacterium *Mycoplasma genitalium*. "What we don't know is whether we can insert that chromosome into a cell and transform the cell's operating system to work off the new chromosome. We will have to understand life at its most basic level, and we're a long way from doing that."

Re-creating a virus letter-for-letter does not reveal much about it, but what if the genome were dissected into its constituent genes and then methodically put back together in a way that makes sense to human engineers? That is what Endy and colleagues are doing with the T7 bacteriophage. "We've rebuilt T7—not just resynthesized it but reengineered the genome and synthesized that," Endy reports. The scientists are separating genes that overlap, editing out redundancies, and so on. The group has completed about 11.5 kilobases so far and expects to finish the remaining 30,000 base pairs by the end of 2004.

“The people in this class are happy and building nice, constructive things, as opposed to new species of virus or new kinds of bioweapons.”

—Drew Endy, M.I.T.



Beta-Testing Life 2.0

SYNTHETIC BIOLOGISTS have so far built living genetic systems as experiments and demonstrations. But a number of research laboratories are already working on applications. Martin Fussenegger and his colleagues at ETH Zurich have graduated from bacteria to mammals. Last year they infused hamster cells with networks of genes that have a kind of volume control: adding small amounts of various antibiotics turned the output of the synthetic genes to low, medium or high. Controlling gene expression in this way could prove quite handy for gene therapies and the manufacture of pharmaceutical proteins.

Living machines will probably find their first uses for jobs that require sophisticated chemistry, such as detecting toxins or synthesizing drugs. Last year Homme W. Hellinga of Duke University invented a way to redesign natural sensor proteins in *E. coli* so that they would latch onto TNT or any other compound of interest instead of their normal targets. Weiss says that he and Hellinga have discussed combining his Goldilocks circuit with Hellinga's sensor to make land-mine detectors.

Jay Keasling, who recently founded a synthetic biology department at Lawrence Berkeley National Laboratory (LBNL), reports that his group has engineered a large network of wormwood and yeast genes into *E. coli*. The circuit enables the bacterium to fabricate a chemical precursor to artemisinin, a next-generation antimalarial drug that is currently too expensive for the parts of the developing world that need it most.

Keasling says that three years of work have increased yields by a factor of one million. By boosting the yields another 25- to 50-fold, he adds, “we will be able to produce artemisinin-based dual cocktail drugs to the Third World for about one tenth the current price.” With relatively simple modifications, the bio-engineered bacteria could be altered to produce expensive chemicals used in perfumes, flavorings and the cancer drug Taxol.

Other scientists at LBNL are using *E. coli* to help dispose of nuclear waste as well as biological and chemical weapons. One team is modifying the bacteria's sense of “smell” so that the bugs will swim toward a nerve agent, such as VX, and digest it. “We have engineered *E. coli* and *Pseudomonas aeruginosa* to precipitate heavy metals, uranium and plutonium on their cell wall,” Keasling says. “Once the cells have accumulated the metals, they settle out of solution, leaving cleaned wastewater.”

Worthy goals, all. But if you become a touch uneasy at the

thought of undergraduates creating new kinds of germs, of private labs synthesizing viruses, and of scientists publishing papers on how to use bacteria to collect plutonium, you are not alone.

In 1975 leading biologists called for a moratorium on the use of recombinant-DNA technology and held a conference at the Asilomar Conference Grounds in California to discuss how to regulate its use. Self-policing seemed to work: there has yet to be a major accident with genetically engineered organisms. “But recently three things have changed the landscape,” Endy points out. “First, anyone can now download the DNA sequence for anthrax toxin genes or for any number of bad things. Second, anyone can order synthetic DNA from offshore companies. And third, we are now more worried about intentional misapplication.”

So how does society counter the risks of a new technology without also denying itself all the benefits? “The Internet stays up because there are more people who want to keep it running than there are people who want to bring it down,” Endy suggests. He pulls out a photograph of the class he taught last year. “Look. The people in this class are happy and building nice, constructive things, as opposed to new species of virus or new kinds of bioweapons. Ultimately we deal with the risks of biological technology by creating a society that can use the technology constructively.”

But he also believes that a meeting to address potential problems makes sense. “I think,” he says, “it would be entirely appropriate to convene a meeting like Asilomar to discuss the current state and future of biological technology.” This June, as leaders in the field meet to share their latest ideas about what can now be created, perhaps they will also devote some thought to what shouldn't.

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W. Wayt Gibbs is senior writer.

MORE TO EXPLORE

An Expanded Eukaryotic Genetic Code. Jason W. Chin et al. in *Science*, Vol. 301, pages 964–967; August 15, 2003.

Genetic Circuit Building Blocks for Cellular Computation, Communications, and Signal Processing. Ron Weiss et al. in *Natural Computing*, Vol. 2, No. 1, pages 47–84; 2003.

The M.I.T. Synthetic Biology Working Group: syntheticbiology.org

The M.I.T. Registry of Standard Biological Parts: parts.mit.edu