

## THE RUNNERS-UP &gt;&gt;

## Build Your Own Genome

A technical tour de force grabbed headlines around the world for synthetic biology this year. In what was hailed as a defining moment for biology and for biotechnology, researchers at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, and San Diego, California, built a synthetic genome and inserted it into a bacterium in place of the organism's original DNA. The new genome caused the bacterium to produce a new set of proteins.

The synthetic genome was an almost identical copy of a natural genome, but ultimately, researchers envision synthetic genomes custom-designed to produce biofuels, pharmaceuticals, or other useful chemicals. Also this year, researchers at Harvard University improved their high-throughput method of modifying existing genomes for such purposes, and other synthetic biologists showed that RNA-based “switches” can get cells to behave differently in response to certain signals.

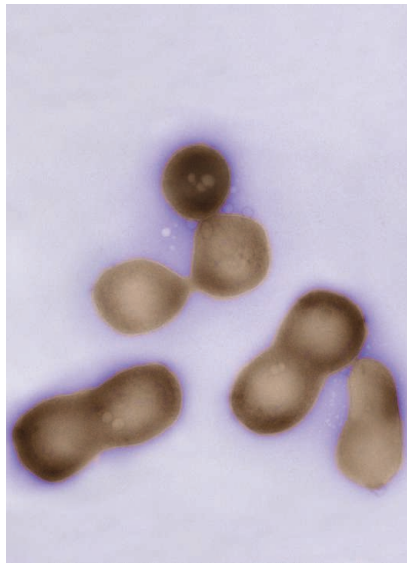
J. Craig Venter and his team built its \$40 million genome from smaller pieces of store-bought DNA. First they stitched the synthetic DNA together in stages in yeast; then they transplanted it into a bacterium, where it replaced the native genome.

Although not truly “artificial life,” as some media declared, this success prompted a congressional hearing and a review by a presidential commission on the ethics of synthetic biology.

It's far from the only synthetic biology game in town, however.

In 2009, Harvard's George Church introduced a technique called multiplex genome engineering, which adds multiple strands of DNA to bacteria every couple of hours, rapidly generating genetically engineered organisms with extensively revamped genomes. This year, his team came up with a cheaper way to produce the DNA strands used to modify the genome, in hopes of making this approach cost-effective for industrial use.

Teams led by Caltech's Niles Pierce, Stanford University's Christina Smolke, and Boston University's James Collins have come up with ways to change a cell's behavior by modifying its regulatory pathways. In some cases, they add specially designed RNA molecules that can sense molecules in the cell associated with, say, cancer or inflammation. Once that happens, they cause the cell to produce a protein that may sensitize the cell to drugs or cause it to undergo programmed cell death. Another team made a riboswitch that caused bacteria to seek out and destroy the herbicide atrazine. Such devices are much closer than synthetic and modified genomes to having practical applications.



**Life recreated.** Scanning electron microscope image of bacteria with synthetic genomes.

In the DNA. Some living humans may have Neanderthal ancestors.



## Reading the Neanderthal Genome

Thirteen years ago, when researchers sequenced just a few snippets of mitochondrial DNA from a Neanderthal, the breakthrough made headlines worldwide. This year, researchers published a draft of the Neanderthal nuclear genome—and their first analysis of what these 3 billion bases of DNA reveal about the evolution of these extinct humans and us.

Using new methods to sequence degraded fragments of ancient DNA (see “Insights of the Decade,” p. 1616), researchers spliced together a composite sequence from three female Neanderthals who lived in Croatia 38,000 to 44,000 years ago, to reconstruct about two-thirds of the entire Neanderthal genome. For the first time, scientists could compare in detail the genomes of Neanderthals and of modern humans.

Reading this sequence, the researchers concluded that modern Europeans and Asians—but not Africans—have inherited between 1% and 4% of their genes from Neanderthals. Apparently, Neanderthals interbred with modern humans after they left Africa at least 80,000 years ago but before they spread into Europe and Asia. If correct, this stunning discovery challenges a model that says that as modern humans swept out of Africa, they completely replaced archaic humans such as Neanderthals without interbreeding.

The Neanderthal genome also gives researchers a powerful new tool to fish for genes that have evolved recently in humans, since they split from Neanderthals. The catalog includes 78 differences in genes that encode proteins that are important for wound healing, the beating of sperm flagella, and gene transcription. Several encode proteins expressed in the skin, sweat glands, and inner sheaths of hair roots, as well as skin pigmentation—all differences that reflect adaptations to new climates and environments as modern humans spread around the globe.

The researchers have also identified 15 regions of interest that differ between humans and Neanderthals, including genes that are important in cognitive and skeletal development. When mutated in humans, some of these genes contribute to diseases such as schizophrenia, Down syndrome, and autism, or to skeletal abnormalities such as misshapen clavicles and a bell-shaped rib cage.

As researchers close in on the few genes that separate us from Neanderthals, they are also trying to decipher how differences in genetic code alter proteins produced in the lab. This year, scientists inserted 11 pairs of single peptides into eukaryote cells to test for differences in gene expression. With luck, they may pinpoint some of the genes that equipped us to survive while Neanderthals went extinct.



## Next-Generation Genomics

Genomics researchers savored the fruits of massively parallel sequencing in 2010. Cheaper, faster “next generation” machines have taken hold over the past 5 years; this year they yielded important results from several large projects.

One ambitious effort, the 1000 Genomes Project, seeks to find all single-base differences—or single-nucleotide polymorphisms (SNPs)—present in at least 1% of humans. It completed three pilot studies this year, which together identified 15 million SNPs—including 8.5 million novel ones. The information will help scientists track down mutations that cause diseases.

Researchers also finished cataloging all the functional elements in the genomes of the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*; the results are expected to be published by year’s end. In human DNA, the complete genome sequences of two Africans from hunter-gatherer tribes, the oldest known lineages of modern humans, confirmed the extensive genetic diversity within those groups. Researchers also produced a draft of the Neandertal genome (see p. 1605) and deciphered the genome from 4000-year-old hair preserved in Greenland’s permafrost.

The cornucopia of results also included surveys of all the transcribed DNA—the so-called transcriptome—and of protein-DNA interactions, as well as assessments of gene expression and the identification of rare disease genes.

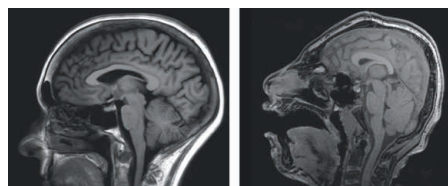
## Souped-Up Cellular Reprogramming

Changing a cell’s fate by adding extra copies of a few genes has become routine in labs around the world. The technique, known as cellular reprogramming, allows scientists to turn back a cell’s developmental clock, making adult cells behave like embryonic stem cells (see “Insights of the Decade,” p. 1612). The resulting induced pluripotent stem cells (iPSCs) are helping scientists to study a variety of diseases and may someday help to treat patients by supplying them with genetically matched replacement cells.

This year, scientists found a way to make reprogramming even easier using syn-

thetic RNA molecules. The synthetic RNAs are designed to elude the cell’s antiviral defenses, which usually attack foreign RNA. The technique is twice as fast and 100 times as efficient as standard techniques. And because the RNA quickly breaks down, the reprogrammed cells are genetically identical to the source cells, making them potentially safer for use in therapies.

Early evidence suggests that the RNA approach reprograms the cell more thoroughly than other methods do, yielding a closer match to embryonic stem cells. The method can also prompt cells to become nonembryonic cell types. By inserting synthetic RNA into a cell that codes for a key gene in muscle tissue, for example, the researchers could turn both fibroblasts and iPSCs into muscle cells.



## Homing In on Errant Genes

Scientists who study rare genetic disorders hit on a powerful strategy for finding the culprit DNA this year. Using cheap sequencing techniques and a shortcut—sequencing just the 1% of the genome that tells cells how to build proteins—they cracked several diseases that had eluded researchers until now.

The old way to track down the cause of Mendelian disorders, or diseases caused by a mutation in a single gene, was to study DNA inheritance patterns in families. That approach doesn’t work when few relatives with the disease can be found or when a

mutation isn’t inherited but instead crops up spontaneously.

In late 2009, geneticists began sequencing just the exons, or protein-coding DNA, of patients with Mendelian disorders. (A few teams sequenced the patients’ entire genome.) This “exome” sequencing yielded a long list of mutations that the scientists then winnowed, for example, by ignoring those that don’t change protein structure or that many people carry. The end result: the faulty DNA underlying at least a dozen mystery diseases—including genes that lead to severe brain malformations, very low cholesterol levels, and facial deformities that look like a made-up Japanese Kabuki performer.

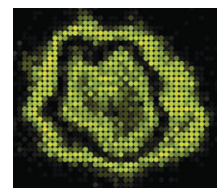
Finding the gene behind a rare disease can lead to better diagnosis and treatments and to new insights into human biology. Scientists hope to use exome sequencing to tick off the causes of more than half of some 7000 known or suspected Mendelian diseases that still don’t have a genetic explanation.

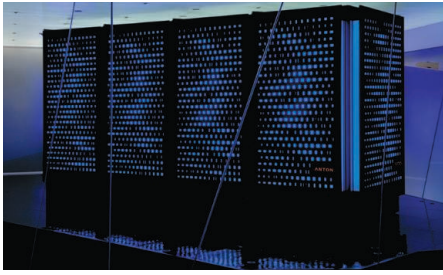
## Quantum Simulators Pass First Key Test

Like a student who sneaks a calculator into a test, physicists have found a quick way to solve tough mathematical problems. This year, they showed that quantum simulators—typically, simulated crystals in which spots of laser light play the role of the crystal’s ions and atoms trapped in the spots of light play the role of electrons—can quickly solve problems in condensed-matter physics.

Physicists usually invent theoretical mod-

els to explain experiments. They might approximate a magnetic crystal as a three-dimensional array of points with electrons on the points interacting through their magnetic fields. Theorists can jot down a mathematical function called a Hamiltonian encoding such an idealization. But “solving” a Hamiltonian to reveal how a system behaves—for example, under what conditions the electrons align to magnetize the crystal—can be daunting.





## Molecular Dynamics Simulations

Sometimes brute force is the way to go, particularly when using computers to simulate the gyrations proteins make as they fold. Such simulations are a combinatorial nightmare. Each two neighboring amino acids in a protein chain can bind to one another at two different angles, each of which can have three conformations. So a simple protein with 100 amino acids can fold in  $3^{198}$  different ways. Getting at the atomic detail is even scarier. Proteins sort through all these possibilities in milliseconds or less. Computers take far longer.

Protein-folding experts have long turned to supercomputers for help. But even these behemoths struggle to track the motions long enough to simulate the complete folding process. Two years ago, researchers in the United States unveiled a new supercomputer hardwired with 512 computer chips tailor-made to speed the calculations of the way neighboring atoms in a protein and the surrounding water interact. That enabled them to gain another burst in speed. As a result, the group reported this year that they've been able to track the motion of atoms in a small protein 100 times longer than previous efforts could do—long enough to see the protein wind its way through 15 cycles of folding and unfolding. Next up, the group is already turning to novel machines with 1024 and 2048 chips to improve simulations of larger proteins.

However, physicists can tailor a quantum simulator to a particular Hamiltonian and let the experiment solve the theoretical problem. Five groups reproduced the results for four previously solved Hamiltonians. Three even mapped “phase diagrams” akin to the one that shows the temperatures and pressures at which water becomes a gas, liquid, or solid.

Physicists hope quantum simulators will crack Hamiltonians that have not been solved—such as one for high-temperature superconductors. But first they had to show that the things could reproduce known results. Check.



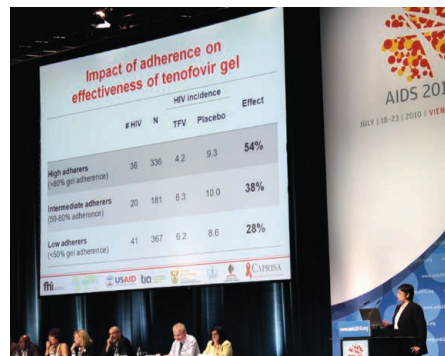
## Rats Redux

Today, most lab cages house mice, but the tenant of choice used to be rats. The reason: Rats are more like us. The human heart, for example, beats about 70 times a minute; a rat's heart, 300 times; a mouse's, 700. Electrical signal patterns in rat and human hearts are also similar. Rats, being more intelligent than mice, might also be better models of human neural diseases such as Alzheimer's and Parkinson's. And rats are bigger and easier to handle for lab work.

Then, in 1989, researchers learned to delete specific genes to make “knockout mice.” The technique they used, called homologous recombination of embryonic stem cells, didn't work in rats. So mice became the preferred

experimental animal in various studies, from developmental biology to drug development.

That too may pass. In 2009, researchers adapted to rats a method, previously used in fruit flies and zebrafish, that uses enzymes called zinc finger nucleases to knock out genes. In August, another group announced a tweak that produced “knockout rats” by the same genetic trick used for knockout mice. Also this year, several groups reported advances in using transposons, DNA sequences that jump from one location to another within a genome, to generate rats with genetic mutations—animals useful for developmental biology and disease research. As a result of such techniques, knockout and genetically modified rats may soon displace their smaller cousins in lab cages around the world.



## HIV Prophylaxis

From the start of the AIDS epidemic through 2009, only five of 37 large-scale studies that attempted to prevent HIV yielded convincing, positive results. Then, this past July and November, two trials of different, novel HIV-prevention strategies unequivocally reported success. AIDS researchers all but danced with joy.

The first result stole the show at the jam-packed XVIII International AIDS Conference held in Vienna, Austria. A vaginal gel

that contains the anti-HIV drug tenofovir reduced HIV infections in high-risk women by 39% over a 30-month period. Nearly 900 South African women participated in the study, half receiving the microbicide and the others an inert gel. Among “high adherers,” women who used the microbicide exactly as instructed, its efficacy reached 54%.

Last month, the first-ever study of oral pre-exposure prophylaxis made headlines with results even more encouraging. The subjects, 2499 men and transgender women who have sex with men, were recruited from six countries. Half were asked to take Truvada, a combination of tenofovir and emtricitabine, each day. After an average of 1.2 years, the treated group had 43.8% fewer infections than the group that took a placebo. Again, better adherence equaled better efficacy: In a small substudy, efficacy increased to 92% in participants who had measurable levels of Truvada in their blood.

Neither approach is a magic bullet, AIDS researchers say. But in combination with other measures, they could usher in a new era of HIV prevention.