stop linking amino acids together, much as a period ends a sentence. In this study, the researchers engineered *E. coli* bacteria by replacing each stop codon bearing the pattern of DNA bases thymine-adenine-guanine, or TAG, with a different (but nonharmful) stop codon reading TAA.

Replacing each instance of that single codon, which appears 314 times in the *E. coli* genome, involved a two-step process. "We're speeding up evolution," Church explains. The first technique, called multiplex automated genome engineering (MAGE), can make rapid, specific changes in cells: researchers introduce pieces of synthetic DNA into bacteria and then select resulting strains that possess the desired properties (in this case, with some of the TAG codons replaced by TAA). They then use a method called conjugative assembly genome engineering (CAGE) that draws on bacteria's natural ability to swap genetic material. By selecting strains with the most TAA codons, the team could eventually create a strain in which every instance of the TAG codon has been replaced.

The goal is to create a bacterium that is resistant to infection by viruses.

The larger goal, Church says, is to create a bacterium that is resistant to infection by viruses. Viruses can't make their own proteins, so they hijack host genomes and force them to make viral proteins; Church's team wants to scramble the genetic code in a way that leaves its functions intact but makes it unrecognizable to viral invaders. The TAG and TAA codons are "synonymous"—they both have the same function in the genome—so by replacing each TAG stop codon with a TAA equivalent, Church's team can then delete the machinery in the cell responsible for reading TAG. The cell will function as if everything is normal, but a virus expects to see the code in its original form. "If you make the code different enough," Church explains, "that organism becomes resistant to all viruses."

Viral resistance is only one of the ways these editing techniques can be used to hack genomes. "Our goal is to change every base pair," Church reports. His approach differs from that of a team at the J. Craig Venter Institute that synthesized a genome from scratch and transplanted it into a cell last year. Rather than copy something from life, Church says, his team's goal is to radically change an existing genome's properties. He estimates that creating the entire *E. coli* genome would cost several million dollars, whereas his editing technique is much cheaper and more flexible, and at each stage of the process provides researchers with feedback about how their changes have affected the cell. With a directed evolution approach, he says, "If you select for what you want, the end product is exactly what you want."

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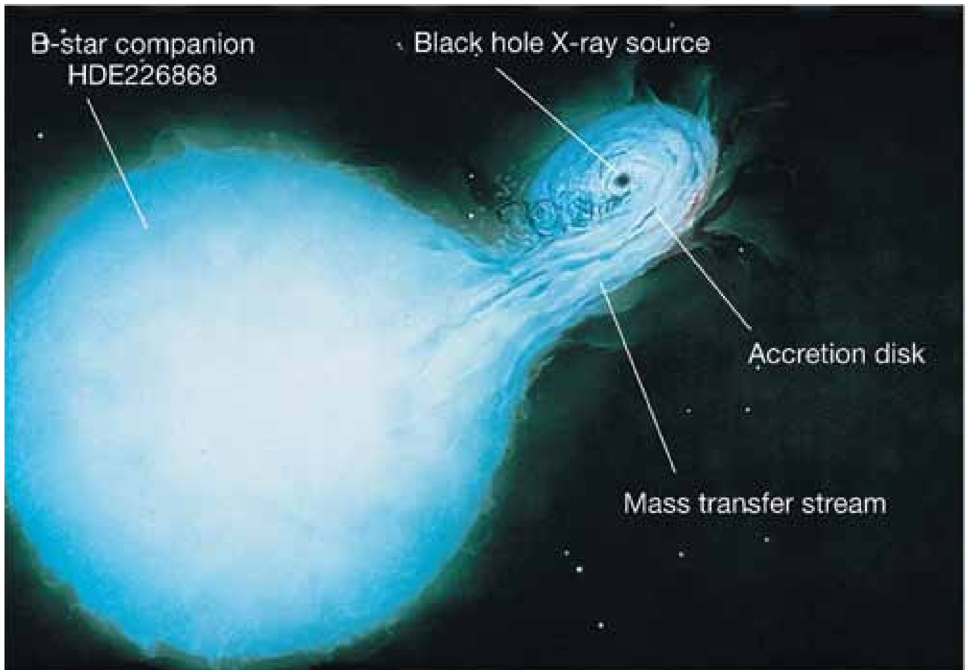
*For more information on Church's work, see "DNA as Data," January-February 2004, page 44.*




## A Bet and a Black Hole

**The idea that objects exist** whose gravity is so powerful that light cannot escape them has been around for centuries. But it was not until instruments aboard a rocket detected x-rays from an unseen source in the constellation Cygnus in 1964 that researchers considered the possibility that they had in fact discovered a black hole, an object from which nothing, including light, can escape. Seven years later, astronomers discovered a star in Cygnus orbiting something that could not be seen. "The dark object's gravity seemed to be tearing gas from its bright companion," says author and astronomer Ken Croswell, Ph.D. '90, "and as the gas took the final plunge [see illustration], it became so hot it emitted x-rays." But not everyone believed a black hole was the cause; in 1974, Stephen Hawking even bet another physicist that it wasn't. Now the controversy (which Hawking conceded long ago, based on indirect measurements) has been definitively put to rest by Mark Reid and colleagues at the Harvard-Smithsonian Center for Astrophysics, who were able to calculate an accurate distance to Cygnus X-1, making possible an inference of its mass. Furthermore, they calculated that the gas closest to the dark object orbits it almost 670 times per second—a phenomenal rate that is half the speed of light—clear evidence of an object whose gravitational pull is so strong that it could only be a black hole.

~JONATHAN SHAW



*Illustration by Lola Judith Chaisson*