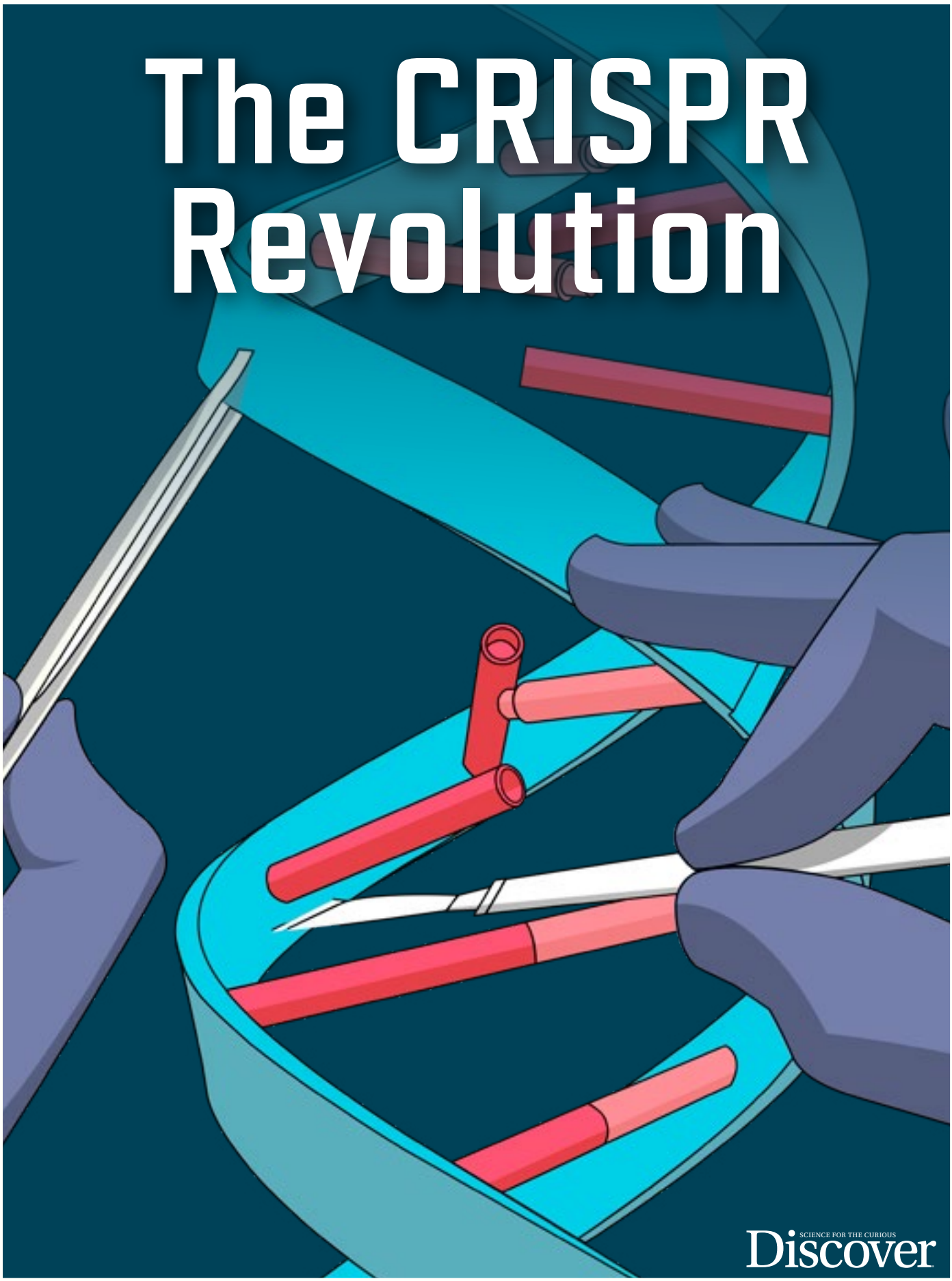
The CRISPR Revolution



PERCEPTION7/SHUTTERSTOCK

SCIENCE FOR THE CURIOUS
Discover

JAMES STEINBERG

BIG IDEA

The CRISPR Antidote

Scientists hacked the machinery of cellular warfare to splice genes. Now they've found a way to guard against it, too.

AN ARMS RACE is playing out inside your body. It's part of an invisible war that's raged for billions of years. When viruses hunt and infect bacteria, the bacterial survivors store pieces of their vanquished foes — DNA snippets — within their genomes so that next time, they can detect and defend against the attack. In response, viruses evolve their own counterattack.

The bacteria's natural defense system is called CRISPR-Cas9. And in 2012, biochemist Jennifer Doudna, together with French microbiologist Emmanuelle Charpentier, upended genetics with an ingenious idea. What if scientists could exploit CRISPR as a gene-editing tool? Since then, Doudna and others have hacked these cellular weapons in an effort to treat diseases and create stronger crops. Now scientists are attempting another task: avoiding unintended mutations resulting from their gene edits.

To grasp the tool's precision, imagine the letters of a genome — G, A, T, C — typed into a stack of books dozens of stories high. A guide RNA shepherds Cas9 — which acts like a pair of DNA scissors — to the right spot, where it zooms in on just 20 letters and lets scientists change a few.

"CRISPR-Cas9 lets you find the right spot," says Joseph Bondy-Denomy, a microbiologist at the University of California, San Francisco. "That's a big deal."

Indeed, a global gene editing revolution is underway. Lawyers battle over patent rights. CRISPR startups are selling stocks on the NASDAQ. And in a milestone this year, Oregon Health and Science University researchers used CRISPR to successfully correct heart disease-causing genes in human embryos. It was the first U.S. CRISPR experiment on humans.

But despite its track record, sometimes CRISPR brings unintended consequences — gene edits in undesired locations. Scientists call these "off-target effects." Cas9's scissors don't always stop once the targeted cuts are made. Sometimes the scissors will roam for another day or two, cutting other sites that resemble the target but aren't quite a perfect match.

"If left to their own devices, over time, [CRISPR proteins] might have the ability to cause trouble," says Doudna, who is also a University of California, Berkeley, professor.

In May, a group of ophthalmologists and others sounded the alarm bells in a letter published in *Nature Methods*. The team used CRISPR to fix a blindness-causing gene in mice. But when they re-examined the mice, they found hundreds of unintended genetic mutations. Headlines about off-targets ensued, and CRISPR stocks tanked.

Doudna challenges the group's methods and thinks that, in general, the off-target fear is overblown. Scientists knew about these mutations, and the technology is more than accurate enough for academic research purposes. The problems begin only as scientists move CRISPR into complex clinical trials.

Bondy-Denomy, the UCSF microbiologist, appears to have found a "natural" way to combat these off-target effects. His research focuses on the arms race between bacteria and viruses, and last year, Bondy-Denomy started testing out a hunch. If bacteria defend against viruses using CRISPR, he reasoned, then viruses likely have a response to counteract it. He was right. Viruses do produce "anti-CRISPR" proteins that grab Cas9 and impair its gene-editing ability. He published his results in *Cell* in January 2017. "This is basically an off switch," he says.

By summer, Doudna, Bondy-Denomy and their collaborators had used this viral counterpunch to reduce off-target effects. In *Science Advances*, the team detailed how they used CRISPR to make edits and then deployed anti-CRISPR to stop the Cas9 scissors from running amok.

The technique could help CRISPR move from the lab toward more therapeutic applications where absolute precision is required, Doudna says. Other teams are exploring different ways to avoid off-target effects, too. For example, the team that edited human embryos earlier this year saw no off-target effects, thanks to prep work aimed at keeping CRISPR on a shorter leash.

The technique could help CRISPR move from the lab toward more therapeutic applications.

However, this gene-editing antidote could have another important use. Security experts, including former Director of National Intelligence James Clapper, worry that CRISPR makes things easier for would-be bioterrorists. Bondy-Denomy says if someone launched a CRISPR attack on humans or our crops, anti-CRISPR could work as an antidote. DARPA, the U.S. military research agency, liked the idea enough to give Doudna and Bondy-Denomy a grant to continue making Cas9 safer.

While Bondy-Denomy doubts CRISPR will ever be deployed in a human battle, he can at least be confident in knowing anti-CRISPR has already proven itself in the cellular arms race. — ERIC BETZ

ALISON MACKEY/DISCOVER; DNA: JULI HANSEN/SHUTTERSTOCK

THE REVOLUTION WILL BE EDITED

IN THE SAN FRANCISCO BAY AREA, FROM GLOBAL CORPORATIONS TO KIDS, EVERYONE IS EMBRACING THE BREAKTHROUGH GENE-EDITING TECHNOLOGY CRISPR.

BY **JEFF WHEELWRIGHT**
PHOTOS BY **ERNIE MASTROIANNI/DISCOVER**

Smacking a bell on his desk, George Cachianes summons the class to order. Twenty-six teenage biotechnologists cluster at three tables. Cachianes teaches Principles of Biotechnology — he calls it "Welcome to Graduate School" — to a select group of juniors and seniors at Abraham Lincoln High School in San Francisco.

His Room 22 is not just a classroom, but a functioning laboratory. Its equipment, acquired through grants and donations, can handle tasks such as sequencing DNA and analyzing proteins. But today's lesson is about a newer technology, a means for altering the genes of any organism — and, potentially, its offspring. It's called Crispr-Cas9.

An elegant tool with an inelegant name, Crispr-Cas9 has electrified the biotech world. Molecular biologists, biomedical researchers, and movers and shakers throughout the life sciences have adopted it. Compared with earlier methods to tweak the genomes of bacteria, plants, laboratory mice and human cells, the Crispr-Cas9 gene-editing method is fast, precise and cheap, an order of magnitude better than the others. What's more, it's simple enough for high school kids to use.

Barely 4 years old, Crispr-Cas9 was pioneered by Jennifer Doudna, a scientist across the bay at the Berkeley campus of the University of California. Doudna and her collaborator Emmanuelle Charpentier, from the Max Planck Institute for Infection Biology in Berlin, were studying how bacteria recognize and chop up invading viruses to eliminate them as a threat. The two realized that the bacterial defense system could be harnessed to scientists' own ends. They designed what Doudna calls a "programmable DNA-cleaving enzyme."

The system has two major parts. Crispr, the programmable part, is the mechanism by which bacteria identify and target foreign genes introduced by viruses. In bacteria, Crispr produces a type of RNA dubbed guide-RNA. The guide-RNA — think of it as the navigator — delivers the enzyme Cas9 to just the right place in the foreign genome, whereupon Cas9 performs a disruptive cut. Doudna's team reprogrammed the natural operation by synthesizing their own version of the guide-RNA. Together, the synthetic guide-RNA and Cas9 form a complex capable of editing any gene.

Long before the Crispr-Cas9 breakthrough, however, the Bay Area was a biotech boomtown. Reaching from swanky San Francisco east to erudite Berkeley and industrious Oakland, and south to the fiefs of Apple, Facebook and Google in Silicon Valley, the Bay Area contains the largest biotechnology complex in the U.S., and new ventures roll out almost daily. That sector of the economy generates almost $100 billion and more than 100,000 jobs. Cachianes boasts that he has former students working in one or another of 1,600 firms. The region's only rival is Cambridge, Mass., where Harvard and MIT are the innovators in biotech and the life sciences.

The feverish uptake of Crispr-Cas9 has reshuffled careers and empowered new users at all echelons of science in the Bay Area: visionaries at Berkeley, East Bay entrepreneurs, Silicon Valley corporate types, the DIY community and, yes, teenagers.

George Cachianes (right) shares a laugh with his biotechnology students at San Francisco's Abraham Lincoln High School.

THE STUDENT INTERN

Lincoln High School senior Vanessa Arreola, bound for UC Berkeley in the fall, illustrates the workings of the Crispr-Cas9 gene editor for her classmates.

Soon after Crispr-Cas9 came out, Cachianes put a demonstration into his curriculum: His Lincoln High students transform *E. coli* bacteria, which are relatively easy to work with. Cachianes obtained bacteria genetically engineered to express a red fluorescent protein, RFP for short. The mission of the students' Crispr-Cas9 project is to shut off the RFP gene and return the bacteria to their normal color.

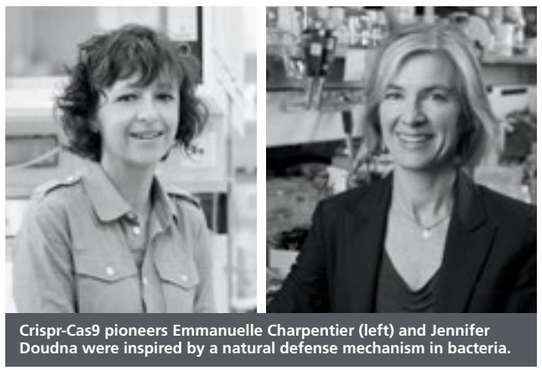
On the day I visit, the class listens to fellow student Vanessa Arreola share what she learned about the technique during a summer internship at the Gladstone Institutes, a private research foundation in San Francisco. As a lab tech, she didn't manufacture the Crispr-Cas9 complex herself, but she did use it.

Dark-haired, petite, with turquoise nail polish, Arreola rattles off facts, cramming a summer's learning into 20 minutes. "It's easy to understand what Crispr-Cas9 does," she begins. "It's hard to understand *how* it does it."

She sketches the system in its natural state, as scientists have observed it in bacteria and related organisms called Archaea. Crispr stands for Clustered Regularly Interspaced Short Palindromic Repeats. These are the bits of foreign DNA that the bacteria have taken up during their immunological response, which can be triggered again and again. Arreola shows how the Crispr DNA produces the guide-RNA for the Cas9 enzyme. "Working with RNA is hard," she says, at which Cachianes jumps in: "You know what I say — RNA is so unstable that if you give it a dirty look, it falls apart."

A hand goes up. "Is this like gene splicing?" Yes, Arreola replies. It looks like her peers are getting it.

Next, she turns to the thrust of her summer project: editing stem cells from the heart to learn more about a congenital heart disorder. Researcher Casey Gifford, Arreola's mentor at Gladstone, asked me not to name the disorder or the gene that her lab is tinkering with — she doesn't want to alert any possible competitors. "With Crispr-Cas9, it's so easy," Gifford says. "I have a head start. But others could quickly do it."

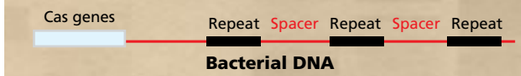


Crispr-Cas9 pioneers Emmanuelle Charpentier (left) and Jennifer Doudna were inspired by a natural defense mechanism in bacteria.

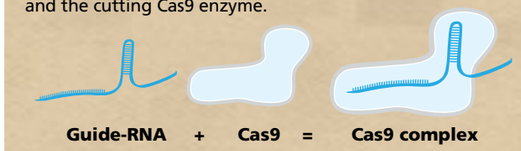
CHARPENTIER: HELMHOLTZ/HALLBAUER&FIORETTI; DOUDNA: FOUNDATION FOR THE NATIONAL INSTITUTES OF HEALTH; CRISPR ILLUSTRATION: ALISON MACKEY/DISCOVER

CRISPR-CAS9 HOW IT WORKS

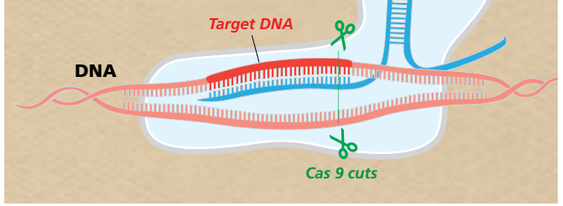
1 Bacterial DNA has unusual repeating sequences that are separated by spacers — short, non-coding segments sometimes inappropriately called "junk DNA." These repeating sequences have been dubbed CRISPR (or Crispr), for Clustered Regularly Interspaced Short Palindromic Repeats. Near each Crispr sequence are genes for a variety of Cas (Crispr-associated) enzymes, including Cas9.



2 When faced with an external threat such as an invading virus, Cas enzymes produce a kind of "most wanted" poster: They snip off bits of the invading viral DNA and stuff them into the spacers, where they can be used as RNA guides to recognize future invaders. Researchers use this natural defense mechanism in bacteria as the basis for the Crispr-Cas9 gene-editing system, creating synthetic guides to search out whichever specific string of DNA bases the researchers choose. You can think of the system in two parts: the guiding Crispr and the cutting Cas9 enzyme.



3 When the guide-RNA locates its target DNA, it latches on, and then Cas9 cleaves through both strands of the DNA double helix. The cut DNA is then either left as-is, silencing it, or repaired by using the gene editor to slip in a new, functioning segment.



THE VISIONARY

After the Doudna-Charpentier paper in 2012 announcing Crispr-Cas9, Doudna and her associates at UC Berkeley began getting requests from investigators elsewhere for help in using the technique. The requests turned into "a flood," says Jacob Corn, a biochemist who used to work at Genentech, a major biotech company in South San Francisco.

In 2014, Doudna asked Corn to manage the flood but also to be proactive: to refine the new technology and to promote it through a new institute at the university. Corn leads the flagship lab of the Innovative Genomics Initiative (IGI), a consortium of 10 Bay Area researchers. They share ideas and laboratory materials, hold meetings and lead courses for researchers from outside the area.

The excitement over Crispr-Cas9 reprises the hopes raised for gene therapy a generation ago. Gene therapy was going to take the fruits of the Human Genome Project, the recorded sequence of human DNA, and use the information to correct scores of conditions. It hasn't happened, in part because the methods for delivering copies of healthy genes to unhealthy cells were too crude, and in part because the most common human diseases do not have clear-cut genetic targets. That doesn't faze Alex Marson, an IGI investigator at the University of California, San Francisco. "After decades of making maps and getting the details of the genome," he says, "now it's time to go in, to change sequences, and see how function is altered."

The new method has been a boon to Marson's career. When Crispr-Cas9 came along, "I dove into it."

Corn, who experiments with Crispr-Cas9 in his own lab, is just as enthusiastic. "It's a special time. There's a lot of *Sturm und Drang* around Crispr-Cas9," he says. "What can it do? What can it not do? People are realizing that it'll work all over the place. The explosion is coming from a bottleneck of backed-up experiments.

"It's hard to keep up. Right now there are up to three Crispr-Cas9 papers published [in journals] every day — that's 21 a week. And that's probably an undercount; some researchers haven't published because they got scooped by others.

"Yes, people had been doing genome editing since the '90s. There were tools to do this, but not everyone had them," Corn adds, referring to other proteins that can cut specific genes, but cost more and require more time.

Crispr-Cas9 has leveled the playing field, Corn says. Investigators with big labs and deep pockets could afford earlier gene editing tools, but Crispr-Cas9 presents almost no barrier to entry. As Corn elaborated in a blog post: "This turns an exclusive practice, where people with questions had no way to get answers, into an inclusive one, where many questions lead to many answers. I often call this the 'democratization' of gene editing."

Scientific director Jacob Corn leads the flagship lab of the Innovative Genomics Institute at UC Berkeley. The consortium is a place for researchers to share ideas and resources.

TOP PHOTO: LI KA SHING FOUNDATION

The Innovative Genomics Institute is housed in the gleaming Li Ka Shing Center at UC Berkeley.

THE ENGINEER

The IGI laboratory at Berkeley consists of row upon row of white-coated young people bent over their work, some with their backs to one another, others conferring head to head. Corn takes me to the bench of a postdoctoral researcher named Mark DeWitt. Hired in 2014, DeWitt heads the IGI team tasked with curing sickle cell disease in mice — his is one of a number of research teams pursuing the idea.

DeWitt's experiments are intended to be a proof-of-principle that Crispr-Cas9 can help the hundreds of thousands of people, most of them in Africa and India, who carry two flawed copies of the HBB gene. This gene makes hemoglobin, the stuff of red blood cells. In the disease state, the blood cells are sickle-shaped instead of round, leading to stabs of pain and eventual organ damage. The plan is to extract blood-forming stem cells from a patient's bone marrow and correct as many copies of the mutated gene as possible. Theoretically, when the stem cells are returned to the patient, they will generate enough of the normal hemoglobin to counter the symptoms, if not eliminate the disease.

DeWitt explains that Crispr-Cas9 is used both to insert the adverse mutation into mice, and then to reverse the defect by installing the normal version of HBB, known as wild-type. In both cases, the cut by Cas9 triggers an automatic repair process at the site of the gene. In repairing the biochemical break, the DNA incorporates the variant of HBB that the researcher has provided: the mutated form to cause the disease and then the normal form to cure it.

Assembling a Crispr-Cas9 package is not all that difficult, DeWitt says. Engineered separately, the purified Cas9 enzyme and the guide-RNA solutions are swirled in a tube and come together in a protein complex. He calls the third ingredient the template, or donor DNA: "It's the DNA that encodes the edit you want to make, in this case wild-type HBB."

DeWitt adds the Crispr-Cas9 complex and DNA replacement parts to a solution containing mutated stem cells. Using

IGI lab postdoctoral researcher Mark DeWitt demonstrates how Crispr-Cas9 works with a 3-D model of the gene-editing system.

electroporation — in Corn's words, "we run an electric current and make holes in the cells" — he'll inject the complex and replacement parts through the cells' protective membrane.

DeWitt places a plastic tray about the size of a microwave dinner, with 16 thimble-sized wells filled with the culture, under the hood of the electroporation device. Invisibly, in less time than it takes to zap a frozen meal, a portion of the cells are transformed, one string of DNA switched for another.

The first team to achieve a genetic rewrite of sickle cell and of thalassemia, a related blood disorder, will hit the jackpot. Recently, Corn, DeWitt and others of the IGI team published a paper on their promising results in mice. Before moving on to human trials, they will need to study all instances of "off-target" effects: Years before Crispr, the viruses employed to deliver DNA in gene therapy trials occasionally damaged the whole system, causing cancer. The problem was that researchers couldn't direct the packet to the proper place on the chromosomes.

Caribou Biosciences CEO and co-founder Rachel Haurwitz and Chief Science Officer Andy May set up shop to change the world in a former bakery located in a busy commercial area of Berkeley.

THE PROSPECTORS

Doudna founded Berkeley-based Caribou Biosciences early in 2012, just as her paper with Charpentier was published. The company is her commercial stake in Crispr-Cas9, and, backed by UC Berkeley's lawyers, she's launched a patent action against MIT researcher Feng Zhang, who contends that he invented the technique about the same time and has the right to commercialize it. Caribou aims to bring Crispr-Cas9 to all domains of the life sciences, from agricultural and industrial biotech to medicine and molecular biology. The company's coolly boastful motto: "Engineering any genome, at any site, in any way."

Caribou's one-story office and lab are in a slightly scruffy neighborhood near the freeway on the Oakland side of Berkeley. I meet there with Chief Scientific Officer Andy May. At 43, May is one of the older employees at the company, which numbers two dozen and counting.

"Why does the sign in your waiting room say 'The Bakery'?" I ask.

"Because this used to be a Twinkie factory," he says.

According to May, Crispr-Cas9 is the biggest thing to come along in molecular biology since the development of PCR, or polymerase chain reaction, in the 1980s. Thanks to PCR, scientists could amplify DNA in great volumes and thereby "read" the genetic sequences of all sorts of organisms in all their variations. "We've had the ability to read genetic information, but we haven't had the ability to write it back into cells," May says. "Now we can write it back and edit it. We've closed the cycle."

Rachel Haurwitz, Caribou's 30-year-old CEO and co-founder, who earned her Ph.D. in molecular and cell biology under Doudna, joins us in the conference room. Caribou has secured funding from corporate giants DuPont and Novartis for Crispr-Cas9 projects, though Haurwitz won't tell me what they are specifically. "We're a platform technology company," she says. "We make Cas9 proteins, and we have the tools, the bioinformatics, to analyze large numbers of experiments. We're driving to understand the details, to gain understanding and explain why it's safe."

Haurwitz has honed her sales pitch. "To really benefit mankind," she says, "you have to commercialize. There is investor excitement, but they understand that [the payout] could be a decade away. We've been building the plane as we've been flying it. If you win, it's a tremendous win."

From Caribou I crawl south through the traffic to Santa Clara, at the base of the San Francisco Peninsula. Here is the headquarters of Agilent Technologies, a worldwide supplier for the biotech industry and other scientific research with $4 billion in annual revenue. The two employees I meet with — Stephen Laderman, director of Agilent Research Laboratories, and Laurakay Bruhn, who oversees product development for Crispr-Cas9 — are a generation older than May and Haurwitz. Their conference room is twice as large and their chairs twice as plush. Without doubting the importance of what they call "the gene-editing explosion," they aren't yet sure of the value of Crispr-Cas9 to their company.

"It's a future play," Laderman says cagily. "We're not just making the tool, [but also] making measurements that determine what the tool did." By measurement, he means the validation of experiments, the part that investigators sometimes neglect. It's one thing to buy a Crispr-Cas9 package, a cool new tool for your project, and another thing to ensure that it has worked. For example, Agilent might help a drug company determine any off-target effects of a new gene-therapy product.

Caribou's May and Haurwitz, who offer the same service, imply they'd do it better. They refer to Agilent as "a hardware company, selling picks and shovels."

"We do sometimes talk about ourselves as selling picks and shovels," Laderman concedes. Adds Bruhn: "We make RNA really well. [Customers] don't want to make their own picks. They want to find the gold."

THE WIZARDS

UC Berkeley developmental biologist Nipam Patel uses Crispr-Cas9 to edit the genomes of butterflies, crustaceans and other animals to learn how an organism forms.

The experimental focus and putative benefits of Crispr-Cas9 have for the most part centered on human beings and our biomedical concerns, with some nervous speculation about permanently enhancing our genes. Tests last year by Chinese scientists on human embryos prompted an international meeting to discourage this kind of research.

The fact is that any creature's DNA can be altered permanently — it's happening right now in the UC Berkeley lab of Nipam Patel. A developmental biologist, Patel edits the genomes of "non-traditional model species," including butterflies and crustaceans. By turning genes off in embryos, Patel and his team have gained insights into developmental pathways, such as how a butterfly grows distinctive wing patterns.

Crispr-Cas9 has accelerated the pace of exploration in his lab by a factor of 10. "The savings are enormous," Patel says. "It costs $75 to knock out a gene. That's crazy, compared to what we used to pay." Patel used to work with a technique called RNA-interference, or RNAi, which he says was 10 times as costly. "And only some worked well," he says. "It took us two years with RNAi to transform three genes versus just months to transform seven genes with Crispr. The Cas9 enzyme is efficient and robust. The animal is changed right from the get-go."

The genes that interest Patel determine body plans, controlling how and when a nascent organism forms its limbs, mouth parts, antennae, etc. A subset of such genes, known as Hox genes, is found across the animal kingdom, in humans as well as fruit flies. Patel zeroes in on the Hox genes of a tiny crustacean, *Parhyale hawaiensis*, commonly known as a beach hopper. When Crispr-Cas9 knocks out a Hox gene in an embryonic beach hopper, strange things ensue, like a clawed foot forming where a swimming foot ought to be, or an antenna growing out of a mouth. The opposite tack, knocking-in, inserts foreign DNA into a Hox gene, resulting in, for instance, a claw that glows green under fluorescent light.

When I visited, two biologists, Erin Jarvis and Arnaud Martin, were harvesting fertilized eggs from female beach hoppers swimming around in a petri dish. Next, the researchers injected Crispr-Cas9 packets into the eggs before the cells divided, to transform as many cells as possible in the developing crustaceans. The operations took place under a microscope with barely visible needles and probes; the investigators must have supremely steady hands. "No caffeine on injection days," quips Jarvis.

Asked whether the power they wielded through Crispr-Cas9 gave them pause, Jarvis offers the standard justification: "We're working to understand genetic processes. It adds to the basic science, so we can understand more about ourselves."

Graduate student Erin Jarvis, a member of Patel's lab, prepares to inject a tiny dose of Crispr-Cas9 into embryos of *Parhyale hawaiensis*, a crustacean commonly known as a beach hopper.

Martin says, "It's *the* tool to modify nature. But when do we stop engineering nature? It's kind of like Frankenstein."

The discussion turned to gene drives, so far just a concept. A mutation could be implanted in a critical mass of mosquitoes or rodents or some other pest, and the mutation would spread through the population for good or for ill. Would biologists do the right thing? And the thing they thought was right — would it work as planned?

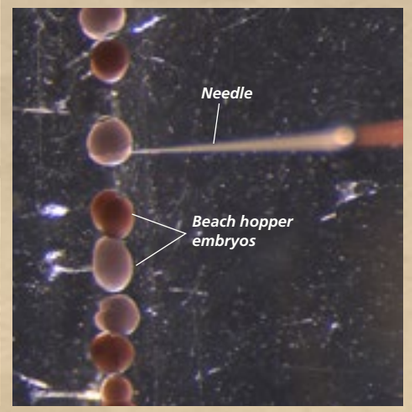
"I hope I'm a good wizard," says Martin. "I'm afraid of the magic, though."

IT SLICES! IT SPLICES!

CRISPR-CAS9 GENE EDITING CAUGHT IN THE ACT

MAKING MUTANT BEACH HOPPERS

Beach hopper embryos (top left), half a millimeter across, are lined up and prepared for an injection by Erin Jarvis, a graduate student at the University of California, Berkeley. Without intervention, the embryos would develop into normal crustaceans (middle left). But the hair-thin needle (near left) delivers a minuscule dose of Cas9 protein and guide-RNA — just 50 trillionths of a liter. In a matter of hours, it will create mutations that permanently alter the beach hopper's DNA. For example, in one experiment researchers gave mutant hoppers forward-walking legs (below right) instead of jumping legs, as seen in a wild beach hopper (below left, colorized for identification). Both are scanning electron microscope images.



PRETTY IN PINK YEAST

The common brewer's yeast *Saccharomyces cerevisiae* is creamy white in its natural state (left). It takes on a decidedly pink cast (right) after self-described biohacker Josiah Zayner manipulates it with Crispr-Cas9. The gene editor cuts the yeast's *ADE2* gene and inserts a few extra nucleotides using donor DNA. "The pink color is a well-known consequence of this mutation," he says.

INJECTION IMAGE BY ERIN JARVIS/PATEL LAB; BEACH HOPPER MUTATION IMAGES BY ARNAUD MARTIN/PATEL LAB

THE BIOHACKER

Self-described "independent scientist" Josiah Zayner says gene-editing technology should be available to all: "Why should I pay to access publicly funded research?"

If it's that easy, why only write about Crispr-Cas9? I wanted to try my hand, you know, *transform* something.

I turn to Josiah Zayner, a 35-year-old rebel with a Ph.D. For Zayner, the term *biohacker* embraces DIY as a political cause.

In the kitchen of his suburban apartment, not far from the San Francisco airport, traffic noise seeps in from the street. We put on disposable gloves — "Though we don't really need them," he says — and he hands me a test tube. "Have you ever pipetted before?"

A pipette is like a large medicine dropper that can dispense precise amounts. My first job is to squirt a solution of calcium chloride into the tube.

"To do a transformation, you have to trick the bacteria," he says. "You add some calcium chloride to neutralize the charge of the DNA, allowing it to permeate the cell membrane."

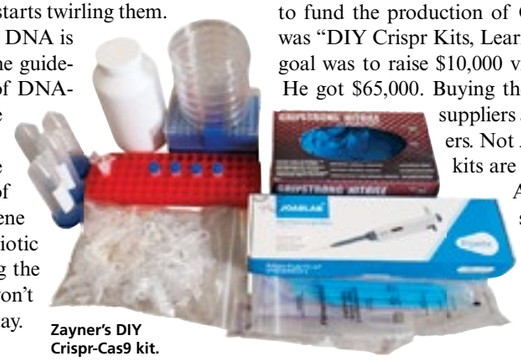
I press the plunger with my thumb. Next?

"Now we need to get some bacteria in there. They've been sitting around in my fridge. Let's scrape some off."

The biohacker movement is about non-scientists, quasi-scientists and a substantial number of moonlighting professional scientists who are taking molecular biology into their own hands with big and little "why not?" projects. Think yeast cultures expressing different colors under fluorescent lights, or cheese made not from cows but from microorganisms implanted with genes for milk proteins. Those are just two of the projects emanating from hangouts hosted by Berkeley BioLabs, or Oakland's Counter Culture Labs, or BioCurious in Sunnyvale. From the Counter Culture Labs website: "Our goal is to demystify and democratize this technology, putting tools into the hands of those who want to learn."

So Zayner, charging little for his time, would empower the likes of me, at least for tonight. Until recently his days were spent at NASA; his assignment was to develop bacteria that could degrade plastic on long space missions. He didn't like the job very much. He considered joining a leading biotech company, but that would have meant changing his appearance: two-tone hair and studs lining both ears. For the time being, he's making a go as an "independent scientist."

He picks up two test tubes and starts twirling them. The bacteria are in. The template DNA is in. The Cas9 enzyme is in, and the guide-RNA. A plasmid, a simple kind of DNA-delivery vehicle, will move a gene for antibiotic resistance into the bacterial cells, jump-starting the Crispr-Cas9 system. The object of the experiment is to mutate a gene in the bacteria, giving it antibiotic resistance, then prove it by dosing the cultures with antibiotics. We won't know the outcome for about a day. (Spoiler alert: success.)



Zayner's DIY Crispr-Cas9 kit.

Zayner's take on democratization has a harder edge than the IGI vision. Sure, it's good that Crispr-Cas9 is being commoditized, as the hard parts of fabrication are taken over by industry. Addgene, a company in Cambridge, Mass., reports that "since 2012 it has distributed some 50,000 Crispr-Cas9 plasmids to 15,000 scientists around the world." Patel and Corn may be delighted by the falling costs, but Zayner thinks that paying $65 for a Crispr-Cas9 plasmid from Addgene is too much.

"Researchers have millions and so companies mark up the price," he grumbles as we move into the living room. "Why should I have to pay to access publicly funded research? I think science needs democratization. The public doesn't have the necessary DIY protocols for Crispr-Cas9. What is the best Cas9 sequence to use? Where do you get the chemicals?

"You have this potentially awesome therapy. What if, at hacker spaces, you had 1,000 people working on Cas9? People would come in and contribute stuff. You'd have to educate them, but then you would unleash them."

In November, Zayner mounted an Indiegogo campaign to fund the production of Crispr-Cas9 kits. The online pitch was "DIY Crispr Kits, Learn Modern Science By Doing." The goal was to raise $10,000 via crowdsourcing within a month. He got $65,000. Buying the components, he negotiated with suppliers abroad and individual manufacturers. Not Addgene or Agilent? No way. "My kits are ridiculously cheaper," Zayner says. At press time, he was about to start shipping the kits. D

Contributing editor ***Jeff Wheelwright*** *is author of* The Wandering Gene and the Indian Princess: Race, Religion, and DNA.

Can CRISPR Feed the World?

Biologists have a new tool to save oranges and other crops — if the public can stomach it.

BY **ERIC BETZ**

WILLIAM ZUBACK/DISCOVER; ORANGE: NATALY STUDIO/SHUTTERSTOCK

LARRY BLACK kneels in the sandy soil beside a bushy orange tree flush with ripening fruit, his brow glistening in the hot Florida sun. He pinches a sprig of young leaves and pulls it in at eye level. "You see him?" Black says. "He's tiny." A grayish speck flutters off. It's the Asian citrus psyllid — smaller than a grain of rice, but big enough to possibly destroy Florida's citrus industry.

The tree's yellow-blotched leaves betray a symptom citrus growers have come to expect. It's sick. And so is nearly every mature citrus tree in the state.

Black's family has raised oranges here since the 1850s. For five generations, they've faced hurricanes, frost and pests. But over the past decade or so, they've seen this tiny bug become their worst calamity, decimating the state's iconic orange trees by ferrying a disease called citrus greening, or Huanglongbing (HLB) — the yellow dragon disease.

"Pre-HLB, a grower planted a grove of trees and expected them to live for a generation," says Black, who runs Peace River Packing Co. in Fort Meade. "And that's just not a reality anymore."

Standing beside Black is Fred Gmitter, a citrus breeder and geneticist at the University of Florida. His skin is sun-weathered and freckled, forged by decades of walking groves just like this one.

Gmitter picks two leaves and holds them out. He explains that the bacteria behind citrus greening, *Candidatus* Liberibacter asiaticus, invade and clog a plant's phloem — the internal plumbing system for circulating sugar. Sugar gets stuck in the leaves, messing with photosynthesis. The roots starve. Surviving trees often bear sour and misshapen fruit.

"It's like an atom bomb going off in the tree," Gmitter says.

At the industry's height in 1997, Florida's nearly 1 million acres of citrus could've covered Rhode Island. Growers harvested a whopping 244 million boxes. This year's predicted haul: 46 million boxes, the worst since World War II, thanks to a one-two punch from greening and Hurricane Irma. The U.S. Department of Agriculture has spent nearly half a billion dollars fighting the disease, and yet these days, that jug of OJ in your fridge likely is mixed with Brazilian oranges.

But Florida growers see reasons for hope. Scattered among the sick and dead trees, Gmitter has found some strong survivors. These trees still get infected but show fewer symptoms and grow healthy fruit. Inside their genes, scientists are hunting for a cure.

"For the industry, immunity is the thing you're looking for. That's the long game," says Tim Eyrich, the head researcher at Southern Gardens Citrus, one of the state's largest growers. "And immunity is probably going to come through some type of genetics."

Walk the juice section at your local grocer, and you'll find bottle after bottle stamped "non-GMO." This means the DNA of the ingredients inside haven't been edited by science to include foreign genetic material. But despite the labels, many growers believe they won't survive in the long term without a solution that includes genetically modified organisms (GMOs).

"Within 10 years, there might not be any orange juice left," says Brian Staskawicz, a plant disease expert at the University of California, Berkeley. "So you ask the people, do you want a GMO orange tree, or you want no orange juice? Take your pick."

The choice might not be quite so black and white. A new gene editing technology called CRISPR lets scientists create genetic mutations in a more natural way that's also faster and cheaper than previous techniques.

"It's different from a classical GMO in that we're not adding a genome from another organism," Gmitter says. Instead, by knocking out a few existing genes, researchers are trying to engineer a tree resistant to greening.

Citrus greening is transmitted by the tiny Asian citrus psyllid. The disease was first detected in Florida in 2005 by state agriculture workers.

FROM LEFT: MICHAEL ROGERS/UNIVERSITY OF FLORIDA/IFAS/CITRUS RESEARCH AND EDUCATION CENTER; ERNIE MASTROIANNI/DISCOVER (2)

Larry Black, a Florida orange grower, has planted a grove of Sugar Belles, a citrus variety more tolerant of a disease that's killing citrus trees around the world.

This technicality — that it's not transgenic, like putting a fish gene in a tomato — is leading U.S. government regulators to take a hands-off approach. No final decision has been announced, but so far regulators say the CRISPR'd crops are non-GMO. The agriculture industry could have the benefits of genetic modification without the stigma.

It's not just oranges being infected. Pests and diseases destroy up to 30 percent of global crops. From bananas and tomatoes to wheat, rice and potatoes, a surprising number of common foods are in peril. And such blights disproportionately affect the developing world because fertilizers, pesticides and genetic engineering are either unavailable or prohibitively expensive.

Modern food is grown in monocultures — where crops are genetically very similar — to make harvesting easier. But that means when a disease strikes, all the plants get sick. In addition, globalization has meant that diseases can spread faster and farther, and the warmer temperatures from climate change can attract pests to new regions. Meanwhile, farmers must grow 70 percent more calories by 2050 to feed some 10 billion people.

Pests and diseases destroy up to 30 percent of global crops. From bananas and tomatoes to wheat, rice and potatoes, a surprising number of common foods are in peril.

CRISPR could help solve these problems.

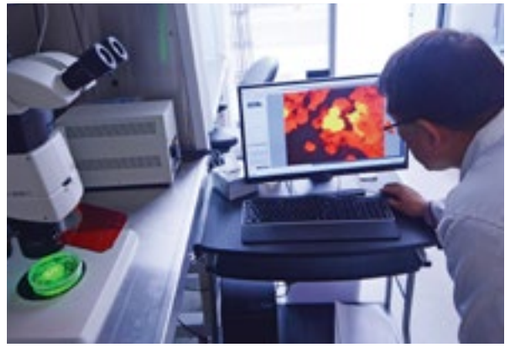
DEMOCRATIZING GMOS

Gmitter still remembers the day in 2005 when state agriculture workers found citrus greening in a backyard near Miami International Airport. Even before greening arrived, Florida growers were worried about it. A century earlier, the disease had devastated groves in China.

As it spread across Florida — as well as Brazil, China and dozens of other countries — Gmitter and a small team of international citrus scientists persuaded industry groups to give them about $6 million to sequence the orange tree's genome. "If we have the blueprint for the citrus tree — if we have the catalog of genes — this becomes a toolbox," Gmitter recalls thinking. Inside that toolbox, he hoped to find a solution.

By the time they released the first citrus genome sequence in 2011, the cost of sequencing technology was already plummeting. The next year brought the birth of a radically new way to genetically engineer life: CRISPR.

Each time a virus attacks bacteria, those bacteria save a snippet of the invader's DNA in their genome. They use this snippet as a kind of mugshot to spot and remove the virus when it attempts another invasion. Molecular biologist Jennifer Doudna of UC Berkeley, working with French biologist Emmanuelle Charpentier, discovered



Nearly all of Florida's orange trees show signs of citrus greening (left). UC Berkeley researcher Myeong-Je Cho (above) is learning to use CRISPR on a variety of plants. Petri dishes (right) in a fridge-sized incubator at UC Berkeley contain green shoots that have been gene edited.

A Florida orange shows signs of citrus greening.

Citrus Greening

OTHER NAMES: Huanglongbing (HLB), yellow dragon disease.

CAUSED BY: *Candidatus* Liberibacter asiaticus bacteria, spread by the Asian citrus psyllid insect.

AFFECTED: Citrus plants, including oranges, grapefruits, lemons, limes and tangerines.

SYMPTOMS: Yellow shoots; dark aborted seeds; mottled or patchy discoloration to leaves; mature fruit that is small, hard, misshapen, partially green and falls from its stem prematurely; bitter taste.

CURE: None. Infected trees usually die within a few years.

that this natural defense system could also be employed as a kind of DNA scissors. This tool, called CRISPR-Cas9, can target and cut with incredible efficiency.

So far, the hype has centered on combating human disease, like when an American-led team of scientists corrected heart disease-causing genes in a human embryo last year. But CRISPR has already pushed well beyond biomedical breakthroughs. Researchers at Penn State University, for example, edited the genes of a common white button mushroom so that the fungi resisted browning. On the livestock side, biologists at the University of Missouri used CRISPR to breed a litter of pigs that are unharmed by a disease that costs the industry $600 million each year.

Hundreds of millions of dollars are now going toward applications in agriculture. From DuPont Pioneer to Monsanto, major seed corporations are trying to cash in. But plant disease experts say the real revolution will come when gene editing is used on crops overlooked by large agriculture companies.

Doudna sees CRISPR as the democratization of gene modification, where we could even have gene-edited plants growing in our backyard gardens. "It is such an accessible technology," she says. Last year, the Innovative Genomics Institute (IGI) at UC Berkeley — a lab co-founded by Doudna — launched a $125 million initiative to unleash CRISPR on agriculture and other areas outside of medicine. "There may be even more applications for CRISPR in agriculture than there are in human biology," says Staskawicz, whom Doudna tapped to lead IGI's crop efforts.

A PLANT FROM ONE CELL

Breeders spent more than a century creating genetic crosses to increase disease resistance, Staskawicz says, yet scientists only recently figured out how it worked: Plants and animals both rely on a major class of disease resistance genes.

Many bacterial diseases infect plants using what scientists call a type-III secretion system. That's a rather boring name for a robust, destructive little molecular machine. This machine's main objective is to inject proteins that disarm the plant's immune system. But the battle isn't totally one-sided. Once the disease resistance genes kick in, they trigger a cascade of effects to fight off the infection.

You can get those disease resistance traits through crossbreeding, but doing so also pulls in genes that could

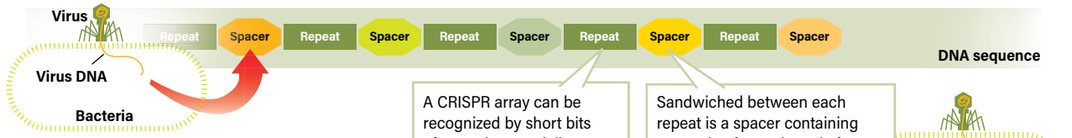
CLOCKWISE FROM BOTTOM LEFT: ERNIE MASTRODIANNI/DISCOVER (2); ERIC BETZIG/DISCOVER (2)

TOP: WILLIAM ZUBACK/DISCOVER. BOTTOM: ALISON MACKEY/DISCOVER

the basics

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. It's part of a natural bacterial defense system that scientists are now using to cut DNA more precisely than any previous method of genetic engineering. Here's a simplified look at how it works.

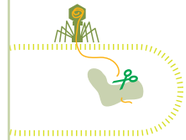
IN NATURE



Certain bacteria have an adaptive immune system. Each time a virus attacks one of these types of bacteria, a snippet of the invader's genetic code is stored away in the bacteria's DNA for future reference in a sequence called a CRISPR array.

A CRISPR array can be recognized by short bits of repeating, partially palindromic DNA sequences. (A palindrome is a string of letters that reads the same backward and forward, e.g. CAATAAC.)

Sandwiched between each repeat is a spacer containing a sample of genetic code from previous viral invaders. The next time the same virus attacks, the spacer will be used as a search-and-destroy template for the bacteria's CRISPR system.



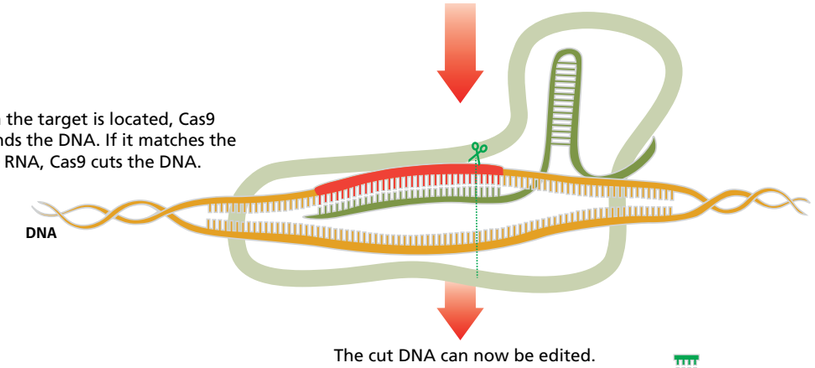
IN THE LAB

Scientists realized they could repurpose this system and program it to seek out any string of genetic code.

The CRISPR-Cas9 system has two main components:

Together, they seek out the target DNA sequence, encoded in the guide RNA.

When the target is located, Cas9 unwinds the DNA. If it matches the guide RNA, Cas9 cuts the DNA.



The cut DNA can now be edited.

SOURCES: Innovative Genomics Institute, Harvard Medical School

Plant Genetics Through Time

Humans have altered their food for thousands of years.

> 10,000 B.C.

FIRST FARMERS: Humans begin domesticating plants for food.

EARLY 1900s

PLANT BREEDING BECOMES SCIENCE: Sir Rowland Biffen crosses breeds to create disease-resistant wheat. Scientists can now select for traits instead of relying just on chance.

1994

FIRST COMMERCIAL GMO: Calgene (today owned by Monsanto) launches slow-ripening Flavr Savr tomatoes. It's a hit with consumers, but soon is canceled in part due to high costs.

1999

OPPOSITION GROWS: A Cornell University study implies GMO corn pollen endangers monarch butterflies. Experiments by the USDA rebut the finding, but the perception sticks.

2003

FRESH REGS: The European Union passes strict rules on GMOs. Many EU countries later ban farming them.

2012

NEW EDITS: Scientists show they can edit genes with CRISPR, and it's used on plants the following year. The method outshines existing tech.

2016

NO DIFFERENCE: A two-year study by the National Academy of Sciences finds no significant difference between GMOs and non-GMOs in risk to health or environment.

diminish the crop. CRISPR's precision lets scientists select specific genes from a plant's relative — wild or domestic — and insert only the desired traits. Scientists can also simply knock out a gene that leaves a plant susceptible to disease.

One of the biggest challenges has been delivering the CRISPR components into seeds. And in a newly minted lab at the IGI, plant scientist Myeong-Je Cho is trying to figure out how to use CRISPR on plant seeds, including those from the cacao tree, which is hobbled by a disease that threatens livelihoods in the developing world. Before joining IGI in 2016, Cho worked at the ag corporation DuPont Pioneer. IGI hired him because of his approach to using CRISPR on seeds.

Past techniques inserted CRISPR into cells using *Agrobacterium*, a bacteria that can also carry the location for the scissors to cut. But that method is still transgenic. Cho's approach puts CRISPR directly into cells. Staskawicz says it's a significant advance.

Almost all American-grown corn and soybeans come from genetically modified seed.

To demonstrate, Cho grabs a scalpel and dissects a tiny flower. Donning a white lab coat, he cozies up to his weapon of choice: a gene gun. There's no pistol grip or trigger; it's just a tiny box that holds a petri dish full of plant embryos. Instead of bullets, the gun shoots hundreds of thousands of gold particles coated in CRISPR components. He fires and — pop! — they splatter like a shotgun blast. The particles penetrate the plant cells inches below, delivering CRISPR.

"If you look at it under a microscope, there are many, many holes," Cho says.

The technique relies on a remarkable capability of plant cells called totipotency. In humans, only stem cells have the ability to become any body part. But for plants, each and every cell can form everything.

"A single cell has the potential to become a whole plant," Cho says.

If Cho can make CRISPR work on cacao and other plants, the new crops will keep the same properties as their parents — the refined product of thousands of years of breeding — but exclude the genes that make the crops susceptible to disease. After he's done with the gene gun, Cho shows off sparkling white refrigerator-sized incubators full of petri dishes. Inside each perfectly stacked container is a clump of what looks like pre-chewed food. Many sport little green shoots that'll grow up to be genetically modified broccoli, rice, wheat, cacao, pepper and tomato. Each is part of IGI's efforts to fix one crop problem or another.

"The technology is robust, and it's simple," Staskawicz says. "A lot of people can do it, and you don't need fancy equipment."

FRANKENFOOD FREAKOUT

Most of us don't think about it, but we eat GMO foods every day. Almost all American-grown corn and soybeans come from genetically modified seed. The two crops are used as sweeteners and fillers in an amazing array of processed foods.

Wheel your cart around a supermarket, and you'll push past aisles of GMO foods, such as breads, cereals and crackers, as well as yogurt, milk and meat. Even cheese is made from genetically engineered rennet — the enzyme that curdles milk — instead of traditional rennet from animal stomachs.

But not long after engineered corn and soybeans hit the market in the mid-'90s, the term *GMO* got tangled together with concerns about pesticides and patented seeds. And there's good reason for that. The first wave of genetically engineered foods was all about farmers' needs (like crops that withstand pesticides and net higher yields) and corporate profits (from selling those pesticides).

The public disdain created a bizarre supermarket reality — a GMO-free zone in the produce section. The agriculture industry is convinced we'll accept genetic engineering in processed foods yet recoil at GMO whole foods.

There is one exception: the papaya. Some 30 years ago, Hawaii's papaya industry — like the citrus industry today — was decimated by an unstoppable disease. Cornell University scientist

NATALIE ERHOVA/SHUTTERSTOCK

Nian Wang, a microbiologist at the University of Florida's Citrus Research and Education Center (CREC), examines containers of edited citrus stored in the incubating room. He and his team have identified 13 genes that may be linked to citrus greening.

Dennis Gonsalves came up with a GMO papaya that survived the virus, and he gave away the seeds for free. The plant saved the industry.

Interestingly, surveys show Americans aren't sure what they think about GMOs. A 2016 Pew poll revealed that the vast majority has heard just "a little" or "nothing" about the subject. About half of Americans believe they eat some or no genetically modified food. But among the 16 percent who say they care deeply about GMOs, the perception is largely negative. That's despite the scientific consensus — including a large-scale report from the National Academy of Sciences in 2016 — that GMOs are safe and nutritionally identical to conventional crops.

Marketing hasn't helped the stigma. "Non-GMO" labels adorn all manner of products, regardless of whether a transgenic version exists. Even the orange juice industry — whose farmers are helping fund a GMO solution — labels containers.

"[Food producers] see it as a revenue driver," says Tim Eyrich, vice president of research at Southern Gardens Citrus. "That's why we see non-GMO Himalayan salt, whereas the last time I looked, sodium chloride doesn't have DNA. That's a marketing thing. And that's an education thing. But people buy it."

That confounds plant scientists, who see gene editing's potential to fix all manner of ills, from crop diseases to pesticide overuse.

"Here's the real problem: We need food," says Staskawicz, pointing to the world's growing population. "And you've got to do it in some sort of environmentally sustainable fashion. You've got to really reduce farmer inputs — things like pesticides and fertilizers. These things all contribute to global warming."

A CAUTIONARY TALE

Molecular biologist Diana Horvath understands how hard it is to bring GMO produce to market. She gave up her venture capital job to cofound the non-profit 2Blades. Her goal was to move plant disease breakthroughs from the lab to the field.

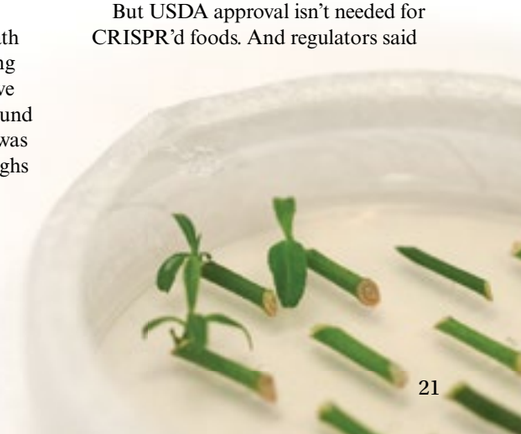
In 2004, she found a poster child for a "good" GMO. Tomato farmers had been battling a disease called bacterial leaf spot, which shrivels plants. Growers try to control it with copper-laden sprays, even though the bacteria is now resistant.

But peppers, a close relative to tomatoes, contain a gene that gives them immunity to the disease. Staskawicz's lab found a way to insert that gene into tomato plants, making them immune. During field trials, Florida farmers grew more food without using the traditional chemicals. And yet GMO tomatoes didn't pan out. Receiving U.S. Department of Agriculture approval is expensive, and growers wouldn't gamble on a crop the public might reject.

But USDA approval isn't needed for CRISPR'd foods. And regulators said

Citrus shoots grow in a petri dish at CREC's Core Transformation Lab, where scientists use gene editing in the fight against citrus greening.

ERNIE MASTROIANNI/DISCOVER (2)

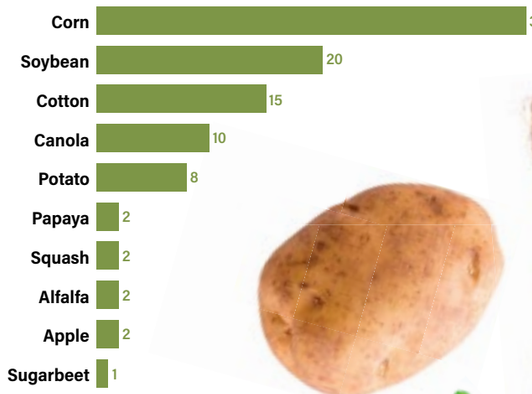


University of Florida citrus breeder and geneticist Fred Gmitter (left) has created several new citrus varieties now stored in an enclosed facility that isolates them from insects and other outside contaminants. Gmitter peels a Bingo (above), a breed that's more tolerant of greening.

Genetically Grown in the USA

Public opinion polls show Americans aren't sure how much genetically modified food they're eating. So far, most transgenic GMOs — those edited to have genes from multiple species — go into livestock feed and processed foods containing corn or soy. Only a smattering of other GMO crops have been approved by the U.S. Department of Agriculture.

USDA approved GMO crops*



*Not all approved varieties shown are currently grown. List excludes a handful of other approvals.

SOURCE: U.S. Department of Agriculture

SOYBEAN: NATTAPOLSTUDIO/SHUTTERSTOCK. POTATO: DANZA/SHUTTERSTOCK. CORN: ALEX STAROSELTSEV/SHUTTERSTOCK

Penn State's non-browning mushrooms aren't GMOs because there's no "introduced genetic material."

Of course, consumer sentiment could halt CRISPR'd crops anyway. And growers quickly backed off 2Blades' tomatoes even though U.S. law doesn't require obvious GMO labels.

Citrus could provide the test case. Some Florida growers have sold their citrus fields to developers, while others have simply abandoned their orchards. But those who are still in the game — such as Black, the Florida grower — know all about the latest tech, including CRISPR. "Most growers look forward to a genetic solution," Black says.

CRISPR HOPE

Just outside microbiologist Nian Wang's cramped office at the University of Florida's century-old Citrus Research and Education Center in Lake Alfred, a cadre of young scientists work diligently along lab benches. Parts of the building date back to the 1930s. Yet in these crowded and somewhat dated quarters, Wang and his team have pushed the limits of scientific knowledge.

ERNIE MASTROIANNI/DISCOVER (2)

All citrus plants are genetically very similar, but that doesn't mean the genome is simple. Wang's team has found it challenging to employ CRISPR's DNA scissors. "Citrus is not the model system," says Vladimir Orbovic, who helps Wang and other citrus scientists conduct their experiments in the center's Core Transformation Lab. "It's a very complicated crop."

Ironically, scientists haven't been able to grow the bacteria effecting greening in a lab, making it harder to study. Another hurdle is that greening is a relatively new disease. Gmitter and his colleagues have studied the evolutionary history of citrus. Their results show that, while the plant was first domesticated in Asia thousands of years ago, greening showed up only in recent centuries. The disease is so new that even wild trees aren't immune.

But Orbovic says that if they can get CRISPR to work in a complicated system like citrus, their methods could prove extremely useful for editing other crops, too. And over the past year, they've had a breakthrough. Wang's team has identified 13 potential genes that cause citrus to be susceptible to greening. His team is now trying to knock out those genes with CRISPR.

"We don't really know which one is the right one," Wang says. "So we do all of them, and hopefully we get one of them right."

As each plant is edited, the fruits of their labor are stashed next door in a makeshift incubating room. The room is a mess. Over-the-counter grow lights nurture a mélange of petri dishes and vials stacked on cardboard atop discount-store shelving units. A citrus sapling inside one of these vials — sealed with plastic film and a rubber band — could be the salvation of an industry. But it will take awhile to find out.

Citrus trees take years to reach maturity. After editing an orange tree's cells, Wang's team will have to wait as long as two years to expose the plant to citrus greening. Only then will researchers know the tree is immune. Even then, they will have to wait another couple of years for the immune plant to produce fruit to ensure the oranges still taste good.

But Wang's work has given the industry some hope. A short walk from his office building is a greenhouse repository called "the ark." This is where the saplings go after outgrowing their vials. Inside, Wang shows off a healthy young citrus tree. His team used CRISPR to make it resistant to citrus canker, a disease that's simpler to tackle than greening.

FARMING REALITIES

About an hour south of the Lake Alfred labs, Black parks his pickup in front of a grove of freshly planted trees. They're Sugar Belles and Bingos, new varieties bred to compete with California Cuties.

He can't afford to wait for a CRISPR solution; he's got to plant today, and these varieties are more tolerant of greening. He can still turn a profit farming citrus if he can keep the trees alive for 15 years.

He recently almost lost them. After Hurricane Irma, Black returned to discover 90 mph winds had blown over 4,000 young trees. His company had to restake each one. But Black shrugs it off, recalling generations of calamities that have reshaped the industry.

"This is just agriculture," Black says. "It happens. Today's problem always seems worse than all those that have come before." D

__Eric Betz__ is a Discover *associate editor. His last feature was December 2017's cover story on NASA's mission to a far-off world.*

To see more images and a video of how the Florida citrus crisis is impacting growers, visit **DiscoverMagazine.com/Florida**