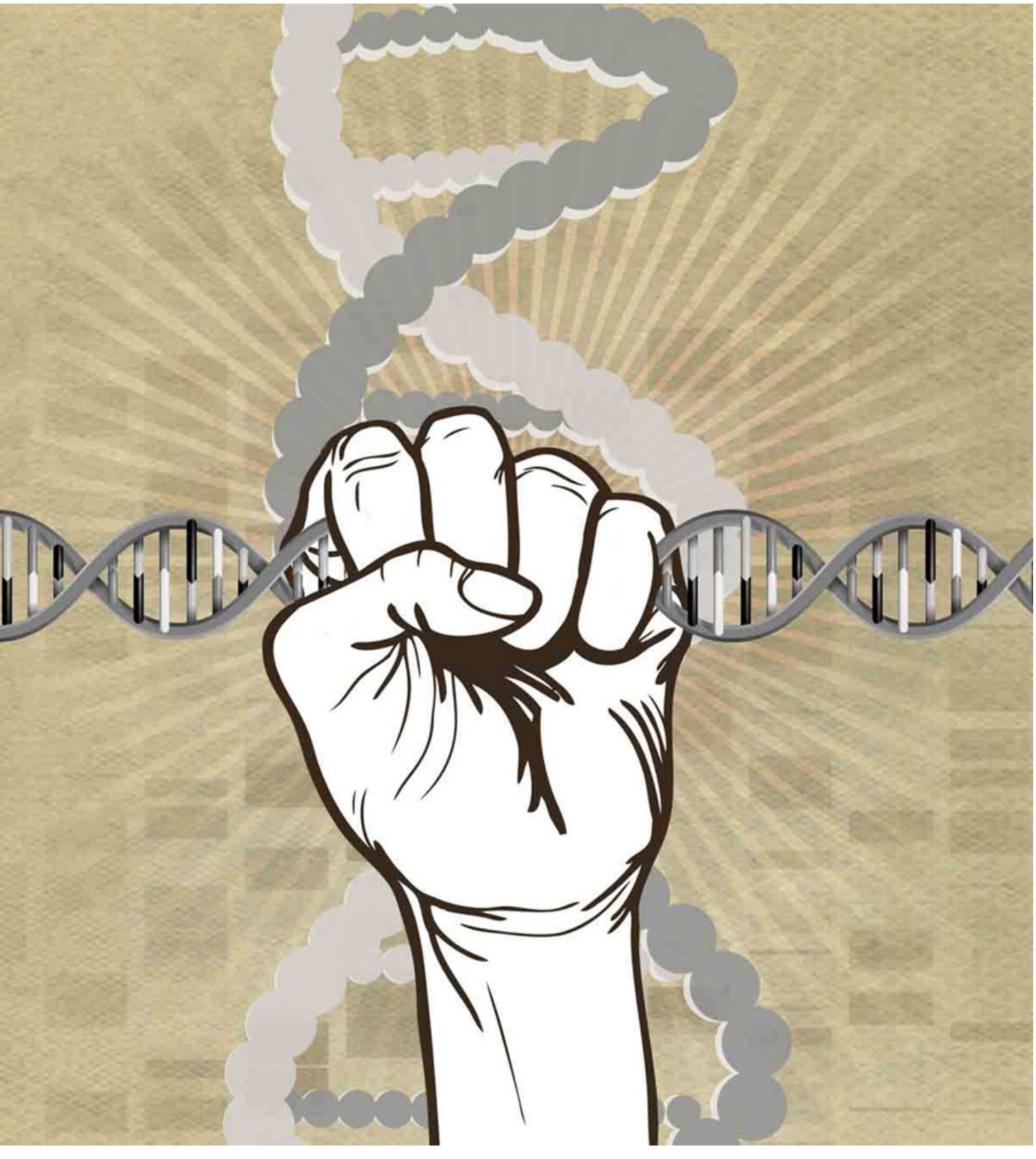
FROM THE JUNE 2016 ISSUE

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 | Monday, May 02, 2016

RELATED TAGS: GENETIC ENGINEERING, GENETICS



Alison Mackey/Discover/Juli Hansen/Shutterstock

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.

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

Ernie Mastroianni/Discover

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."

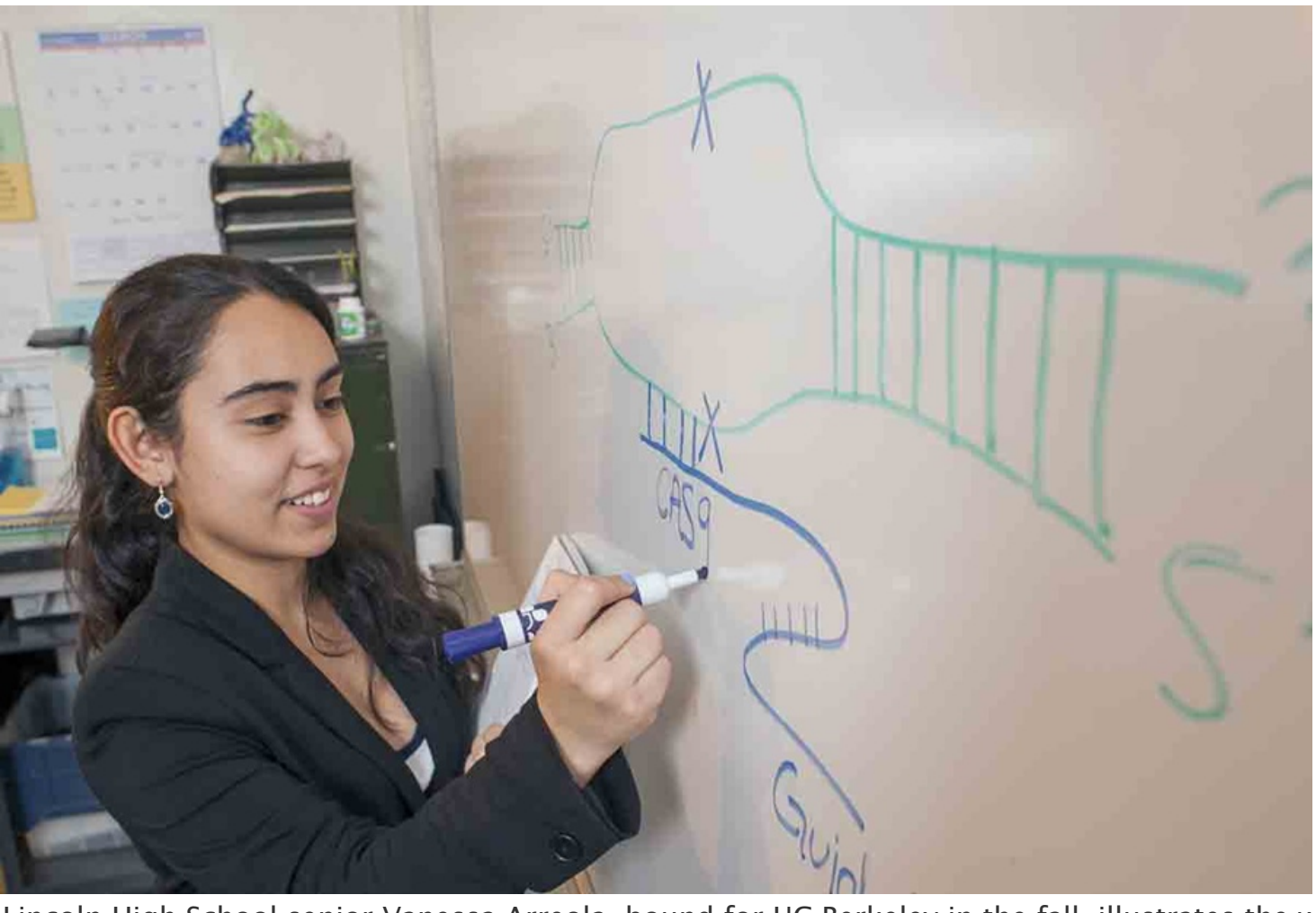
The system has two major parts. Crispr, the programmable part, is the mechanism by which bacteria identify and target foreign genes introduced by viruses. In bacteria, Crispr produces a type of RNA dubbed guide-RNA. The guide-RNA — think of it as the navigator — delivers the enzyme Cas9 to just the right 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.

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.

Ernie Mastroianni/Discover

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."

How Crispr-Cas9 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

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.

Ernie Mastroianni/Discover

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."

THE ENGINEER

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

Ernie Mastroianni/Discover

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 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.

THE PROSPECTORS

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.

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.

Ernie Mastroianni/Discover

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.

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.

Ernie Mastroianni/Discover

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.”

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.”

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?”

Ernie Mastroianni/Discover

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.”

Zayner's DIY Crispr-Cas9 kit.

Ernie Mastroianni/Discover

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 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.

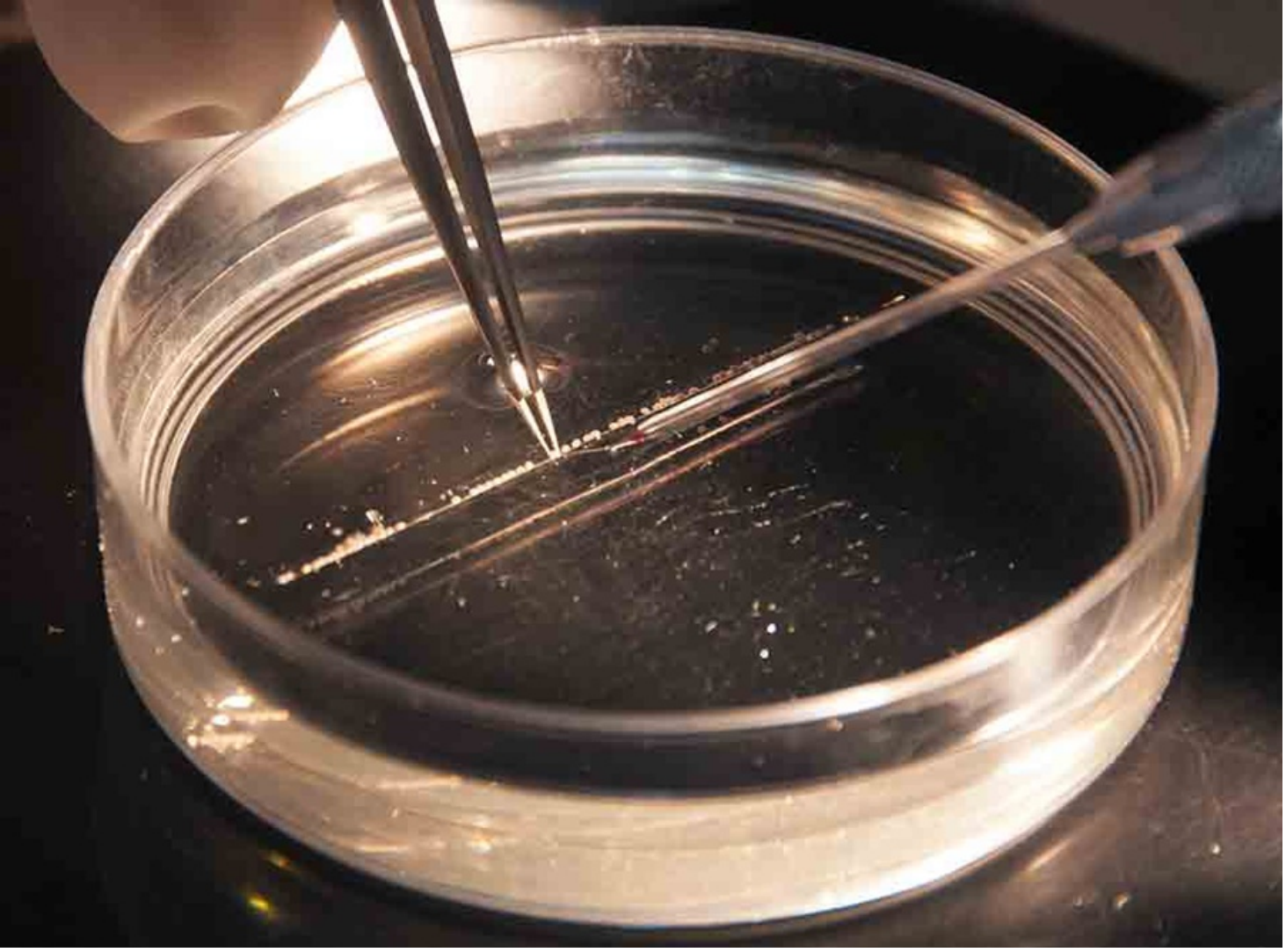
"Researchers have millions and so companies mark up the price," he grumbles as we move into the living room. "Why should I have to pay to access publicly funded research? I think science needs democratization. The public doesn't have the necessary DIY protocols for Crispr-Cas9. What is the best Cas9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

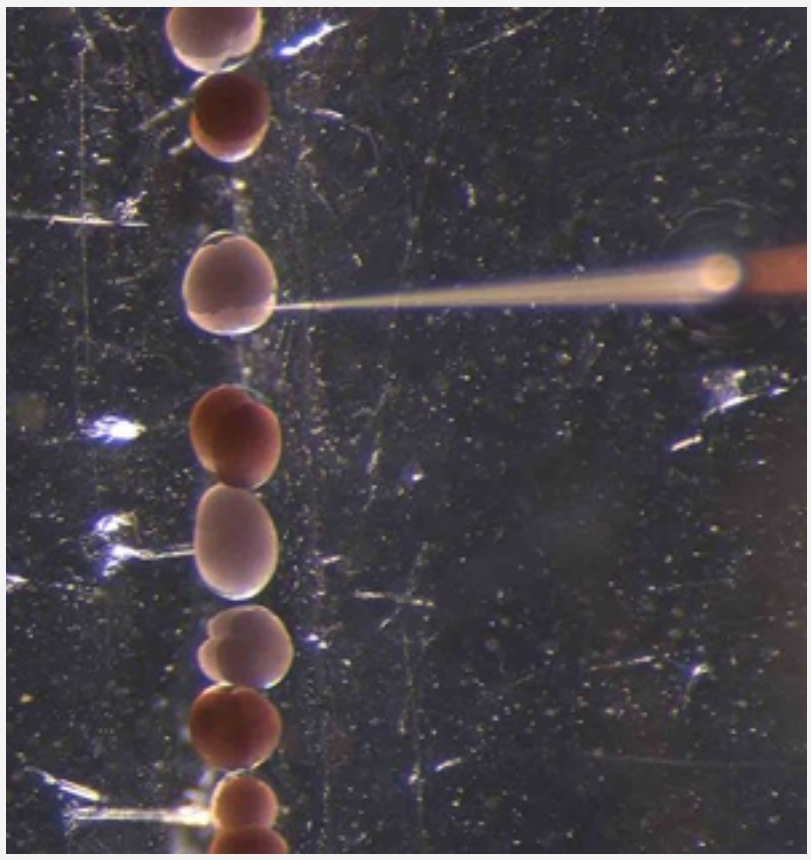
In November, Zayner mounted an Indiegogo campaign to fund the production of Crispr-Cas9 kits. The online pitch was "DIY Crispr Kits, Learn Modern Science By Doing." The goal was to raise $10,000 via crowdsourcing within a month. He got $65,000. Buying the components, he negotiated with suppliers abroad 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.

It Slices! It Splices!

Crispr-Cas9 Caught in the Act



Ernie Mastroianni/Discover

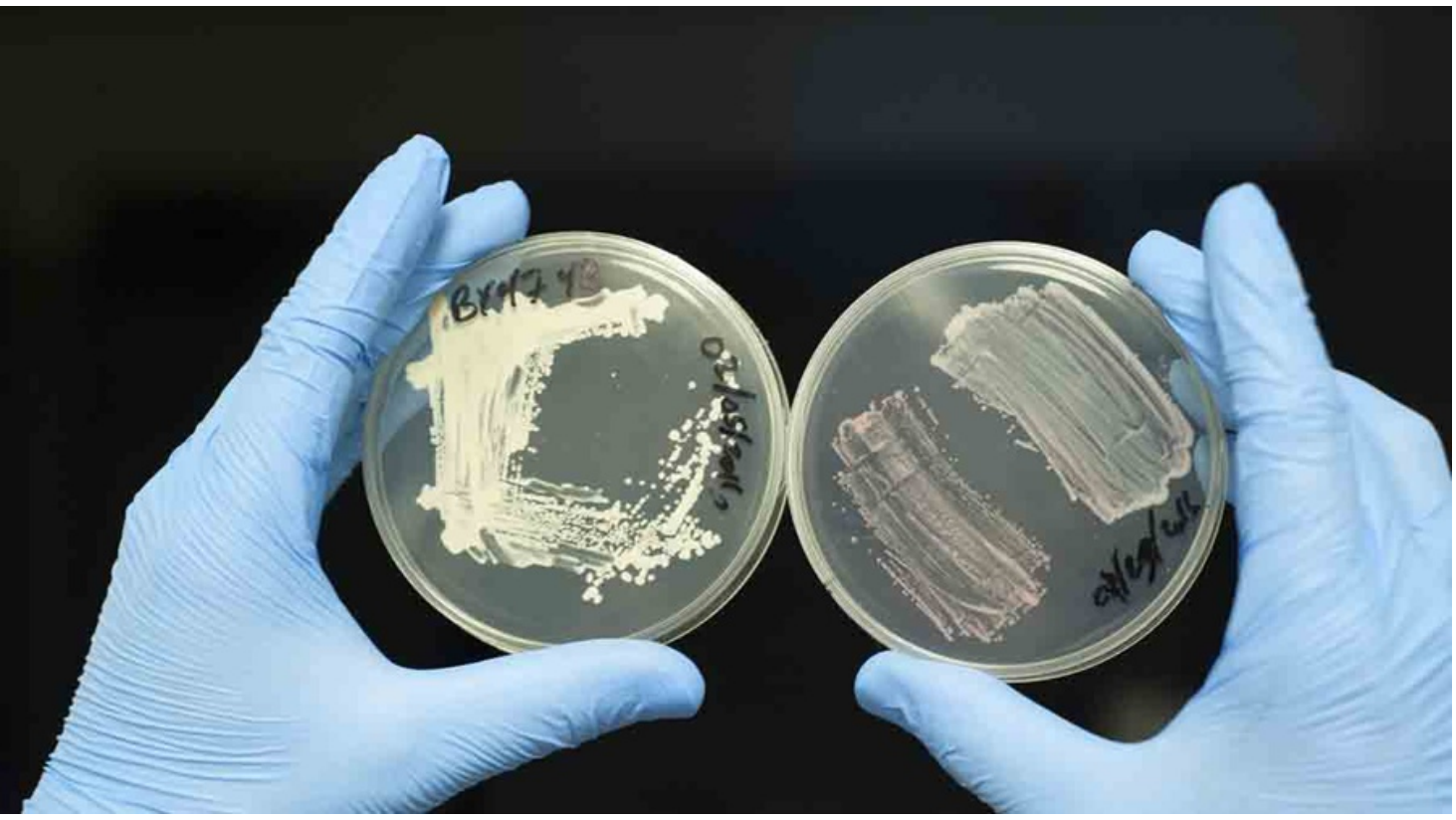


Erin Jarvis/Patel Lab

Making Mutant Beach Hoppers: Beach hopper embryos (top), 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. But the hair-thin needle (right) 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**.**

Arnaud Martin/Patel Lab

Pretty in Pink Yeast: The common brewer's yeast Saccharomyces cerevisiae is creamy white in its natural state (below left). It takes on a decidedly pink cast (below 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.



Ernie Mastroianni/Discover