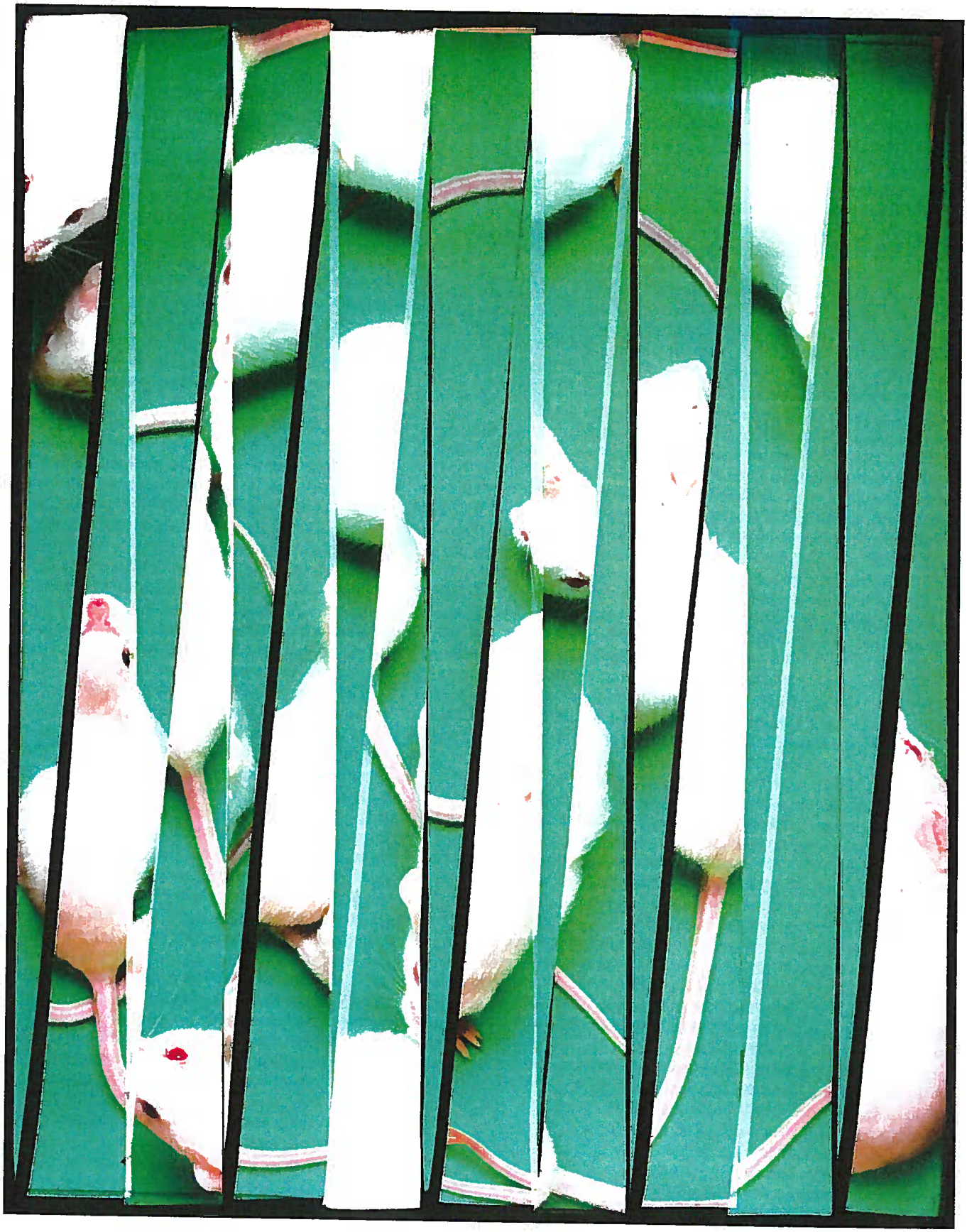
LIFE, THE REMIX

A new technique that lets scientists edit DNA with ease is transforming science—and raising difficult questions

By Alice Park

PHOTOGRAPHS BY HANNAH WHITAKER FOR TIME



Kathy Niakan's laboratory at London's Francis Crick Institute is the size of a walk-in closet, but between its walls she's working on one of the most expansive frontiers ever contemplated by science.

Sometime soon, Niakan will place a human embryo on the platform of her microscope. With one hand, she will steady the embryo—an egg that has been fertilized by a sperm but hasn't yet begun the cell division that eventually leads to a person. With the other, she will maneuver a tiny pipette up against the embryo and inject a specially prepared liquid. If all goes as expected, the liquid will alter the DNA at the core of the cell—literally rewriting the embryo's genetic code. At that point, Niakan will have effectively edited this potential human being. She isn't interested in creating designer humans; instead, she's trying to learn how healthy humans are made, by identifying which DNA sequences are crucial to helping a human embryo develop normally.

This research would be significant enough all on its own. Niakan, a 38-year-old Ph.D. from UCLA, is trying to override nature's selections, instead generating an outcome that she has designed. But what's truly remarkable is that her work represents just one front of a broad revolution in genetics sparked by the technique called CRISPR-Cas9. Just four years old, this discovery is transforming research into how to treat disease, what we eat and how we'll generate electricity, fuel our cars and even save endangered species. Experts believe that CRISPR can be used to reprogram the cells not just in humans but also in plants, insects—practically any piece of DNA on the planet. On June 2, a scientist at MIT and Harvard's Broad Institute announced the development of a related CRISPR technique that can edit RNA, which is responsible for regulation and expression of genes. If DNA is the genetic alphabet, RNA spells actual words. In plain terms, that means the already vast possibilities for CRISPR got even bigger.

So while Niakan moves forward with her work, scientists around the world are exploring other ways to deploy this powerful new tool. At the University of California, Riverside, a team is reprogramming a yeast strain to convert sugars into the components of biofuels. A plant pathologist at Pennsylvania State University has created a mushroom that doesn't brown. At Temple University in Philadelphia, scientists have used CRISPR to successfully excise HIV from human cells in a lab—and in living animals infected with the virus. Scientists envision creating cows that make more milk, tomatoes that don't taste like water and—that stuff of science fiction—the ability to bring back extinct species. In July, the National Institutes of Health (NIH) will issue recommendations on the first bid to test a CRISPR-based medical treatment, on people with myeloma, by taking out their blood cells and revving up their cancer-fighting genes with CRISPR and then returning the newly edited disease-free cells.

Talk to any biologist, geneticist or botanist right now and you will hear a level of excitement that comes only from the emergence of something truly groundbreaking. If the evolution from giant mainframes to personal computers forever changed technology, CRISPR promises to do something similar for genetics—democratizing the power to improve on nature for scientists at nearly all levels of expertise in practically every field. There have been other techniques for altering DNA, but those were expensive and complicated. CRISPR is neither. "It's a game changer," says David Baltimore, a Nobel laureate for his discoveries in viral cancer genetics.

The potential is enormous, but to many, the risks are equally great. Even well-intentioned scientists don't understand all the possible downstream effects of unleashing altered organisms into the wild—including the human gene pool. The simplicity that makes CRISPR so powerful raises the possibility that terrorists or rogue states could deploy it as a weapon—a fear that led Director of National Intelligence James Clapper to include gene-editing methods like CRISPR on a list of mass-destruction threats earlier this year. But no matter the dangers, rewards or questions, this technology is being used now. Will scientists know what to do with it?

IT'S FITTING that the first experiment using CRISPR to edit human embryos will take place at the institute named after Francis Crick. Together with James Watson in 1953, Crick unveiled how DNA is structured. Their discovery launched the modern genetic revolution, because revealing the way DNA is put together allowed researchers to start taking it apart. That, in turn, led them to understand how genetic aberrations contribute to disease.

Mapping the human genome, which was completed between 2001 and 2003, gave researchers the next important tool: the master plan that can be studied for clues about the functions of all our genes. Enzymes that could splice aberrant DNA came next, in the 1970s, but their unpredictability required deep

expertise and a lot of luck to target the genome at just the right places. Newer methods, with inscrutable names like "zinc fingers" and "TALENs," have recently dominated genetic-editing experiments, but these still lacked the accuracy to make most doctors comfortable enough to use them to treat genetic diseases in humans.

It turns out that the master key for unlocking DNA editing was waiting to be discovered inside a cup of yogurt. In 2007 a group of dairy scientists were trying to understand why a variety of bacteria that gives yogurt its tang was constantly getting infected by viruses that altered the taste of the product. When they sequenced the genome of the *Streptococcus thermophilus* bacteria, they kept hitting odd repeated fragments of DNA. "We thought they were annoying," says Rodolphe Barrangou, one of the researchers, who is now an associate professor of food science at North Carolina State University.

Eventually Barrangou and others realized that the repeated fragments weren't random or something to be ignored; they were the bacteria's way of keeping a genetic record of viruses that had infected them—a crude but very effective immune system. In between the repeated sections of DNA were snippets of the virus' genes; when the same virus attempted to reinfect the bacteria, it would gravitate toward its matching section on the bacterial genome and bind to it. That summoned a powerful enzyme that effectively snipped the virus out, leaving the bacteria free from infection.

This was a critical insight. Scientists had previously named the repeated segments "clustered regularly interspaced short palindromic repeats"—hence, CRISPR. But the real breakthrough was figuring out how to put CRISPR to use in something other than a strain of bacteria in a breakfast food.

During the summer of 2012, two groups teamed up to figure it out: one led by Jennifer Doudna, from the University of California, Berkeley, who is an expert on RNA and first became intrigued by biology while growing up in Hawaii; and another headed by Emmanuelle Charpentier, then at Umea University in Sweden and now at the Max Planck Institute. By sharing their research with each other, they discovered that a particular enzyme in the cells—named Cas9—could function as a powerful pair of molecular scissors. CRISPR, they discovered, could be programmed to target a specific section of DNA by loading it with its matching RNA sequence. Once paired, the Cas9 enzyme would cut out the matched section. "I had this gut feeling that this could be something really, really exciting and interesting," says Doudna, 52. "I remember looking at the data and realizing we could create an engineered version that was more simplified."

KATHY NIAKAN

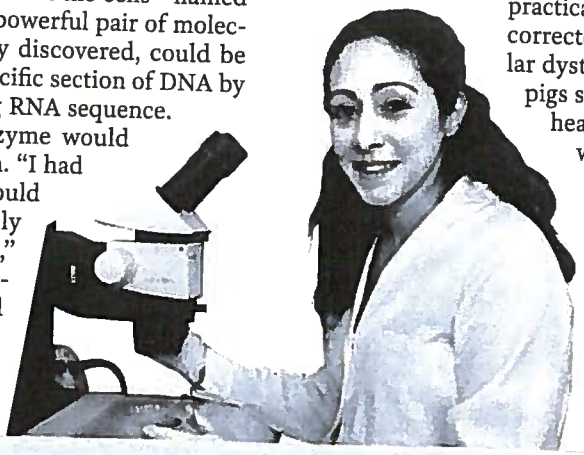
She will be the first person to use CRISPR in a sanctioned study on human embryos.

ON WHY SHE IS USING CRISPR

"We have very little understanding of what it takes for a healthy human embryo to develop successfully. CRISPR can get at the genes responsible for that and maybe lead to healthier pregnancies and fewer miscarriages."

ON USING CRISPR RESPONSIBLY

"Being a scientist also means being a human being. Studies with human embryos are a sensitive topic, so we owe it to the public to be transparent and let them know why we're doing this so nobody is caught off guard. Scientists need to discuss their research really, really openly."



FRANCIS CRICK INSTITUTE

The two teams moved quickly to publish their findings, and in August 2012, the CRISPR-Cas9 technology was revealed to the world in a scientific journal. Scientists working in fields as varied as cancer, food science and the energy sector immediately knew their worlds were forever changed. "It was like setting a match to tinder," says Thomas Barnes, chief scientific officer at Intellia, a biotechnology company cofounded by Doudna to try to use CRISPR to treat disease. There was such a backlog of knowledge about genes and such an unmet need for ways to manipulate them that the technique was immediately heralded as monumental. "The moment CRISPR was introduced, everyone immediately knew what to do," says Barnes. "All the things they thought should be done could now actually be done."

The fervor intensified six months later, when the Broad Institute's Feng Zhang, an associate professor of brain and cognitive sciences and biological engineering at MIT, took CRISPR to the next level. "I thought, Let's try to see if we can use this technology inside the human cell," says Zhang, 34. "I thought, If that can work, this can be transformative."

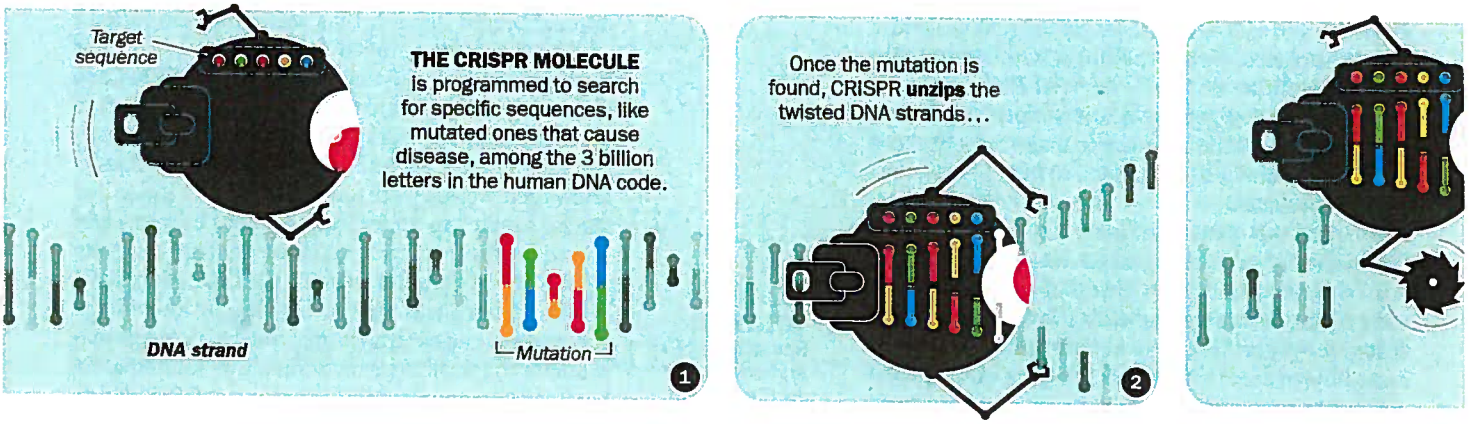
It was. Zhang's work demonstrated that CRISPR could be used to precisely and efficiently edit the DNA of human cells. And with that, the revolution was under way.

TODAY YOU CAN GO ONLINE to any number of biological-supply companies and order your own CRISPR kit for as little as $130. The technique is being used in hundreds of labs across the U.S. and around the globe. At New York City's Memorial Sloan Kettering Cancer Center, cancer biologist Scott Lowe is developing therapies that turn on and off genes in tumor cells to make them easier for the immune system to destroy. Before CRISPR, figuring out what effect a particular gene had on cancer required breeding mice that lacked the gene to see how their cancers progressed or didn't—a months-long endeavor. "Now CRISPR makes it very easy in an afternoon to knock out a gene and study what effect it has on the tumor," Lowe says.

Already, CRISPR is producing clear results in practically every corner of biology. Researchers have corrected the genetic defect in Duchenne muscular dystrophy in mice and deactivated 62 genes in pigs so that organs grown in the animals, such as heart valves and liver tissue, won't be rejected when scientists are ready to transplant them into people. In China, researchers report that they have accomplished in dogs, rabbits, goats and monkeys what human bodybuilders yearn for: a way to quickly build muscles to hulklike proportions. Also in China, plant scientists are editing out genes that make wheat susceptible

HOW CRISPR EDITS DNA

Every cell in the body carries a copy of genetic code—a blueprint for who we are. CRISPR allows scientists to edit that code with more control than ever before



to mildew, potentially leading to hardier crops.

Malaria researchers are exploring a number of ways that CRISPR can be used to manipulate mosquitos to make them less likely to transmit the malady. (Since only females bite and spread the parasite, for example, they're editing in sterilizing changes so the females can't reproduce. Eventually, the hope goes, malaria will cease to be transmitted.)

Some even see the technology as an answer to the growing problem of plastic waste. In Japan, scientists found a bacterium that can chew up the main element in landfill staples like plastic shopping bags—but very slowly. They're investigating ways to use CRISPR to rev up the plastic-degrading gene and turn such microbes into garbage-eating machines.

Even endangered species might be getting the CRISPR treatment. George Church, a professor of genetics at Harvard Medical School, is exploring the possibility of saving the Asian elephant by giving it an entirely new habitat in the relatively human-conflict-free tundra of Siberia. What he hopes will keep the species alive are genes from the extinct woolly mammoth. "It dawned on me that this could be possibly the most exciting part of a new conservation strategy where the goal is not so much to bring back extinct species but to enliven the ecosystem and help endangered species," says Church.

In other words, if you can imagine something that involves genetics, there is probably a scientist somewhere thinking about how to use CRISPR for it. "Right now the only limiting factor in CRISPR is our imagination," says N.C. State's Barrangou. "The question now is, Where can you not use it?"

AN EQUALLY IMPORTANT QUESTION might be, Where should you not use it? CRISPR research keeps accelerating and not just because of the excitement of scientific discovery. The biotech industry is poised for huge profits as everything from CRISPR disease treatments to CRISPR pigs and even mushrooms comes to market.

EMMANUELLE CHARPENTIER & JENNIFER DOUDNA
The two collaborated to develop CRISPR-Cas9, the most accurate and reliable way of editing DNA.

DOUDNA ON CRISPR'S POTENTIAL
"The thing that makes it both wonderful and a bit awesome in a scary way is that it is so easy to employ. But we can't put the genie back in the bottle. It's here. We have to go with it."

Charpentier, left, and Doudna

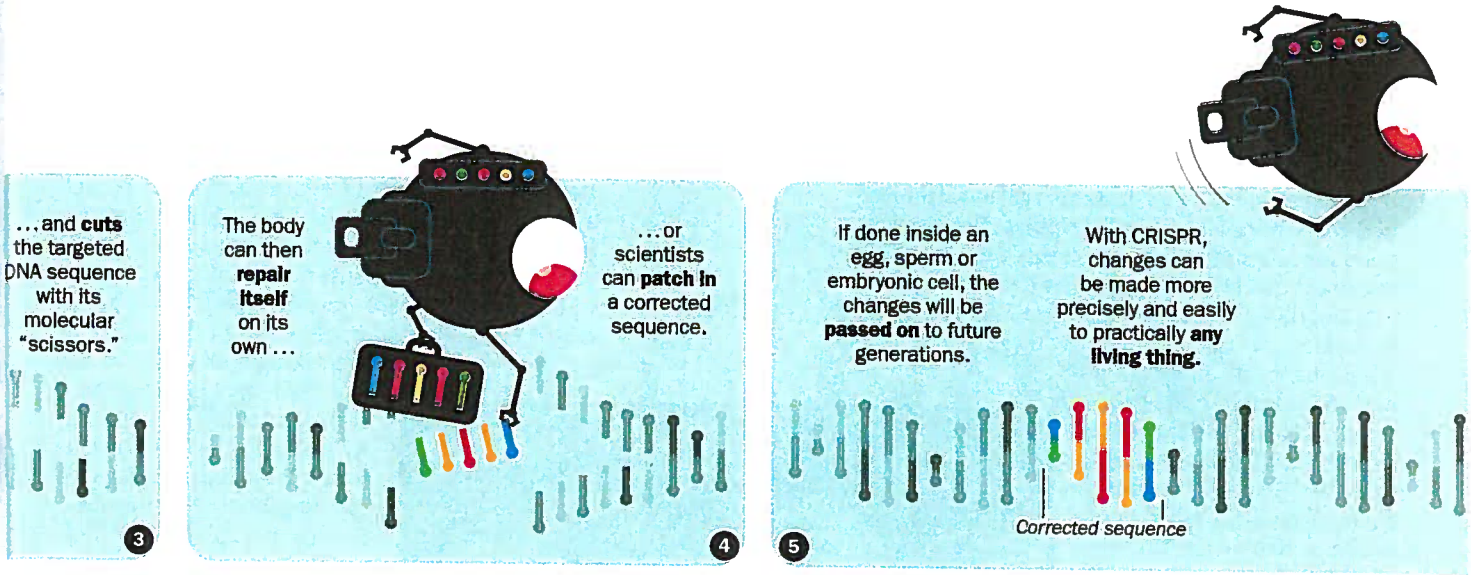
The Broad Institute's Zhang and Berkeley's Doudna are both co-founders of biotech companies as well. They are also embroiled in a high-stakes patent battle over whose institution may license the rights to use CRISPR for all these promising applications—and that battle is ongoing.

The speed with which CRISPR has infiltrated so many areas of science is sobering to those most familiar with what the technology can do. "I worry a lot," says Harvard's Church. "And I have every reason to encourage citizens at large to worry as well."

So, apparently, does the national-security establishment. CRISPR means that most microbes driving infectious diseases are just a few DNA edits away from becoming superstrains that could wipe out unprepared populations. That's the thinking that prompted Director of National Intelligence Clapper's classification of CRISPR as a weapon of mass destruction. With the tools easily bought online, it would be theoretically possible to engineer a killer mosquito that transmits a deadly disease, or a DNA-damaging virus, that could infect human cells and decimate the population.

Doudna, chastened by the lightning pace, began to speak out about her concerns. "The science is moving so fast, and I'd spend the day talking to colleagues who were excited about it, answering emails about new ways of using it and reviewing papers about CRISPR and then come home to have dinner with neighbors who were not scientists, and I realized they have no idea what's going on," she says. "Here we have a technology that enables us to alter human evolution. I realized I might need to get involved in more public discussion about this technology."

In January 2015, she encouraged other leaders in the field to gather in Napa Valley for a summit, the first of its kind, to discuss its place in science. The idea was to discuss CRISPR's potential impact on society and debate the ethical issues involved to get ahead of some of the anything-goes appli-



GRAPHIC BY LON TWEETEN FOR TIME. CHARPENTIER, DOUDNA: AP. ZHANG: BLN GIBBS—THE ELI AND EDYTHE BROAD FOUNDATION

For more on these ideas, visit **time.com/CRISPR**

cations. They focused on what they felt was the most controversial use—editing human reproductive cells like eggs, sperm and those in embryos, which would be able to pass on their changes to future generations.

By the end of the lively debate, the 13 scientists, ethicists and lawyers at the summit agreed that using CRISPR to modify human reproductive cells, so called germ-line changes, that would result in pregnancy or treatments in people, should not be attempted by scientists for the time being.

Their position was based on the reality that precise as CRISPR is, the technique still isn't perfect. Even more uncertain, the group said, were the long-term consequences of altering genomes. Snipping out a disease-causing gene might treat the ailment, but evolution makes it clear that any change in genes or characteristics in a living thing may affect its ability to survive and reproduce in other ways down the line. It's well established, for example, that the mutation responsible for sickle-cell anemia also tends to protect people from developing malaria. What other risk-benefit balances would this kind of genetic editing disturb?

While Niakan plans to use CRISPR on human embryos, she will not allow them to develop beyond seven days—or about when they've divided enough times to have 200 to 300 cells. (U.K. law prohibits letting human embryos in research to progress past 14 days.) Doudna says the summit's attendees, including her, support such use of CRISPR. But with research and commercialization evolving so quickly, it isn't hard to imagine some next steps—including some with decidedly eugenic overtones. If IVF clinics gain the ability to edit out severe genetic diseases, will some move on to creating babies tailored to parents' preferences for height or intelligence or athletic ability?

Robin Lovell-Badge, an embryologist and geneticist at Crick, notes that there are currently no U.S. laws governing the type of research that is done on human embryos with private money. For now, the NIH forbids public funds to be used on research that uses CRISPR on human embryos. But most commercial IVF clinics already have the microscopes needed to use CRISPR on embryos since they routinely use it to inject sperm into eggs for fertilization. "That really scares me, because you can imagine someone with a big ego, whether it's a patient or a clinician, wanting to be the first to use CRISPR to treat something, or rogue IVF clinics offering services guaranteeing offspring will have this or that trait," Lovell-Badge says. "That really scares me and my colleagues."

The line between what is considered abnormal and normal in an embryo or even a fetus could become fraught. "If we start to say certain people with genetic conditions should not exist, then what message does that send to people who already have that same disorder?" says Calum MacKellar, director of research for the Scottish Council on Human Bioethics. "As a society we will get to the point of saying that certain people are no longer equal. And that's a terrible situation to be in."

ARE THERE CONSTRUCTIVE WAYS of developing appropriate limits and guidelines? The leaders in the field, including Doudna, have triggered an ongoing discussion in the scientific community. For now, the National Academy of Sciences has called for researchers to voluntarily refrain from using CRISPR on human embryos that are meant to come to term, calling such studies "irresponsible" at this point. Such guidelines, while not binding in any legal or regulatory way, can still provide a crucial framework for shaping the way powerful technologies like CRISPR are used. That's especially true in the U.S., where studies not funded by the government are not bound by any federal laws overseeing human-embryo research.

That's likely just a stopgap until some sort of national legislation is passed to govern how the research proceeds and how it is applied—similar to the U.K. law that prohibits Niakan's embryos from being transplanted or brought to term.

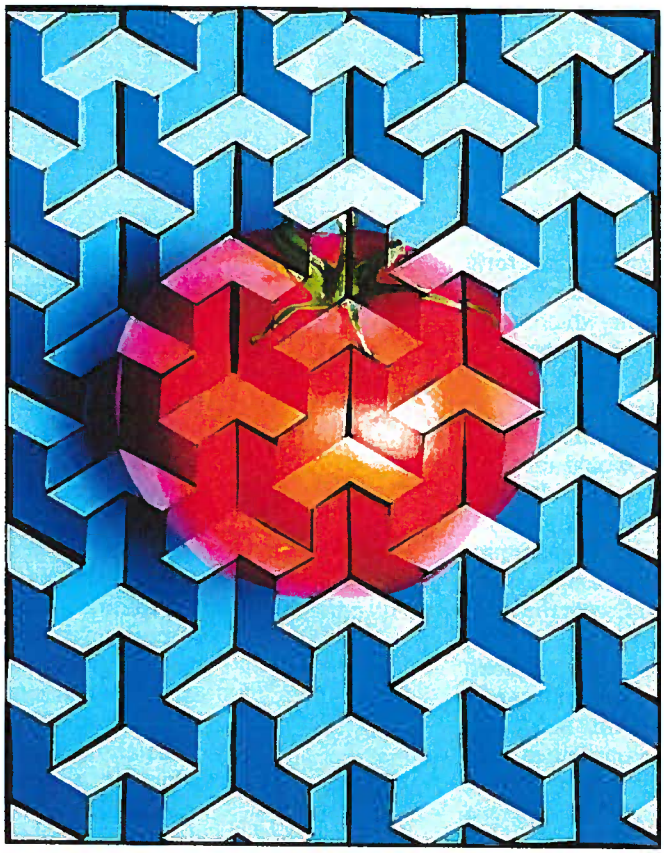
FENG ZHANG

He was the first person to demonstrate the use of CRISPR on human DNA. He now holds the patent for the technology, which is being contested.

ON CRISPR'S POWER

"This is a tool that allows us to manipulate the DNA inside of a cell. That's something we couldn't do very well before—but it's something we wanted to do for a very long time."

The reason for the permissive legal environment in the U.S. has a lot to do with politics. Nearly every previous attempt to regulate embryo experiments was swept quickly into a polarizing debate over abortion and failed to address legitimate scientific questions about the potential value of the research. "We are probably behind the eight ball on addressing the questions that gene editing raises from an ethical standpoint," says Dr. Ezekiel Emanuel, chair of medical ethics and health policy at the University of Pennsylvania School of Medicine.



Abroad, absent strict rules, more eye-raising studies have proceeded. In 2015, Chinese scientists reported the first use of CRISPR on human embryos, albeit ones that were not genetically normal (unlike the healthy ones that Niakan intends to study), and the blowback from scientists around the world was swift. But the study, as alarming as it was to many ethicists and scientists, shows how eager people are to push the limits of what CRISPR can do.

Baltimore, the Nobel laureate who was involved in the discussions over recombinant DNA techniques in the 1970s, looks to that history for hope that we can navigate the promise and pitfalls safely. Back then, Congress passed a law creating a government panel to review all proposed studies involving the technology until scientific experts were comfortable that individual researchers would use it responsibly. Private companies also signed on to have their work sanctioned by the committee in order to maintain their legitimacy and avoid the appearance of going rogue with a potentially dangerous technology. Decades later, stem-cell science forged ahead despite a federal ban, because private funders stepped in to advance the research rigorously and responsibly. Professional groups of stem-cell scientists also provided guidelines to help shape the direction and quality of studies and nurture the struggling new field.

But for now, the only agreement among experts is that using CRISPR to treat humans—including editing the genomes of eggs, sperm or embryos that are allowed to develop into human beings—is premature.

> 'We are probably behind the eight ball on addressing the questions that gene editing raises from an ethical standpoint.'
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> **DR. EZEKIEL EMANUEL,** University of Pennsylvania

IN HER CRICK INSTITUTE LAB, Niakan is well aware of the precedent that her work will set for how CRISPR will be used in human embryos for years to come. She points to two cabinets devoted to the paperwork for tracking every embryo that she receives and its journey through the other embryo research she does in her lab. "We are inspected on a constant basis," says Niakan, referring to the U.K. regulators. "They want to make sure the embryos are being used for this specific research project and that they are traceable from the time they enter our facility to the time they are used in the project. They also check that the people who donate them are given the proper informed consent about the research."

So she is proceeding—carefully—with that first sanctioned edit of a normal human embryo, hoping to learn about the earliest steps in human development. By selectively snipping out genes that previous research suggests might be important in helping early embryos thrive, she can come up with a list of genes that all healthy embryos need. When she splices out each gene in question, the DNA will attempt to repair itself. She knows the repairs will likely fail since the disruption is so dramatic. The gene will no longer be able to make whatever contribution it has to the embryo's development, setting off a chain reaction that will prevent the embryo from developing further. But from that failure new knowledge will be acquired—knowledge that would be "technically virtually impossible" to get without CRISPR. What she learns could help prevent miscarriages and help more couples struggling with infertility to start families.

That's what keeps Niakan focused on completing her groundbreaking CRISPR experiments—and intent on including the public in the conversation. "I think it's important to be transparent and to be open about why we are picking certain genes and why we are doing this study. Most scientists don't do that. But in this case, I want them and the public to appreciate the logic of what I'm doing," Niakan says. "I hope everybody working on CRISPR now and in the future will be that transparent." ◻