Multiplex Automated Genome Engineering (MAGE)

Multiplex Automated Genome Engineering, or MAGE, is a genome editing technique that enables scientists to quickly edit an organism's DNA to produce multiple changes across the genome. In 2009, two genetic researchers at the Wyss Institute at Harvard Medical School in Boston, Massachusetts, Harris Wang and George Church, developed the technology during a time when researchers could only edit one site in an organism's genome at a time. Wang and Church called MAGE a form of accelerated evolution because it creates different cells with many variations of the same original genome over multiple generations. MAGE made genome editing much faster, cheaper, and easier for genetic researchers to create organisms with novel functions that they can use for a variety of purposes, such as making chemicals and medicine, developing biofuels, or further studying and understanding the genes that can cause harmful mutations in humans.

MAGE is a technique that researchers can use to edit DNA. DNA is made up of two long strands bound together by bonds formed between compounds called nucleotides, which function like letters in an instruction manual. There are four kinds of nucleotides in DNA, including adenine, guanine, cytosine, and thymine, which are often shortened to A, G, C, and T respectively. Because of their physical structures, adenine and thymine can bond together, but not with guanine and cytosine, and vice versa. Every set of three nucleotides, called a codon, corresponds to a specific amino acid, which are the building blocks of proteins. Amino acids can be combined in many different ways to produce a wide variety of proteins that perform specific functions within a cell. The order of nucleotides in DNA tell the cell what kinds of proteins to produce and when. A string of nucleotide sequences that encode for a product is called a gene.

Before biologists can use techniques like MAGE to edit a genome, they first have to know the order of nucleotides in that genome. To do that, they can use genome sequencing, a process of reading a strand of DNA to understand the order of nucleotides in it. Understanding the order of nucleotides in DNA can help researchers understand how and why organisms function the way they do, like reading an instruction manual. Genome editing, on the other hand, enables genetic engineers who use techniques like MAGE to change the function of a cell by manipulating its genetic code, like rewriting the instruction manual. Engineers can remove nucleotides, insert extra ones, or substitute nucleotides, which changes the order of the DNA. That means the DNA would code for a different amino acid, which then changes the function of the resulting protein.

While even just one edit can affect the cell, making several of those changes at many sites within a genome can significantly change the way a cell functions. By changing the genetic code, engineers can essentially program cells to stop making the products that they normally would, to produce a greater or lesser number of products, or even to create different products altogether. Engineers can then let an edited cell reproduce and divide, spreading the change to other cells. With enough genetically modified cells, genetic engineers can potentially change the functions of whole organisms. Scientists could only do such research at a slow rate prior to Wang and Church's production of MAGE.

Wang, who was working towards earning his PhD in biophysics, was a graduate student in Church's lab at Harvard Medical School when the pair co-developed MAGE technology. At the time, Church's lab focused on developing transformative technologies to read and write genetic codes. As a result of working with Church to create MAGE, Wang was recruited to be an assistant professor of Systems Biology at Columbia University in New York City, New York, in 2013. According to Virginia Cornish, his mentor at Columbia University, Wang stood out from other researchers because of his role in developing MAGE. As of 2020, Wang continues to work as an assistant professor at Columbia

University and runs a synthetic biology lab that focuses on genetically engineering bacteria. Church continues to run his lab and work as a professor of genetics at Harvard Medical School.

Wang and Church developed MAGE at a time when genome sequencing technologies were becoming increasingly accessible following the completion of the Human Genome Project, which is a worldwide endeavor to sequence the entirety of the human genome that cost 2.7 billion US dollars. By the time Wang and Church created MAGE in 2009, sequencing technologies had dropped to only costing about 200,000 US dollars. As of 2020, sequencing costs under 1000 dollars and scientists expect that number to continue decreasing. The cost of sequencing lowered as new technologies emerged that were quicker, less expensive, and easier than previous technologies.

Researchers were able to better understand how genomes worked as the access to sequencing technology increased, which enabled advanced genome editing technologies to emerge. Prior to MAGE, scientists commonly edited genomes using targetable nucleases, which are proteins that scientists can construct to recognize and cut specific sequences of DNA. Although that technology was precise and effective, it did not let engineers edit a genome in multiple places. Instead, they would have to introduce a mutation to one spot in the cell, then allow the cell to reproduce so they have multiple copies of the genome in case they make any mistakes before they could edit the genome at another site. When Wang and Church introduced MAGE in 2009, it enabled genetic engineers to introduce mutations at multiple target sites at the same time, therefore decreasing the time it takes to make multiple edits.

Genetic engineers using the MAGE technique introduce mutations through short strands of single stranded DNA, or ssDNA. When using MAGE, genetic engineers introduce more than one ssDNA at a time. They do that by constructing multiple ssDNAs that target different areas of a cell's genome and simultaneously introduce them to a colony of cells that have the same original genome. Then, other cells in the colony take up ssDNAs carrying different mutations at different parts of their genome, which ultimately leads to a wide range of variations of the original genome once the cells begin to replicate.

After the cells divide, researchers put the colony of cells through more division cycles, adding more ssDNA with a variety of mutations each time. Mutations are not inherently beneficial or harmful to cells, rather they can create diversity in a population and drive evolution. Because MAGE is so rapid, there is little time for any sort of biological correction mechanisms to correct the mutations. Therefore, those mutations accumulate and combine within different cells, changing their functions in unique ways. Instead of producing a colony of cells all manipulated in the same way as previous genome editing practices had accomplished, MAGE supplied a diverse colony containing many different strains of cells, which scientists could use to investigate new technologies. To make the process even easier, Wang and Church built machines programmed to automatically carry out the MAGE technique unassisted. The machines are about a meter and a half long and contain growth chambers to maintain healthy cell cultures as well as different compartments where the machine can repeatedly deliver DNA into the population of cells. According to Wang and Church, the MAGE device makes the process faster and more reliable.

Wang and Church proved the efficiency and reliability of the MAGE technique and machines when they published the 2009 paper "Programming Cells by Multiplex Genome Engineering and Accelerated Evolution," in the journal Nature with a group of collaborators. In the paper, they detail how MAGE operates and describe an experiment in which the researchers used MAGE to edit the genome of the Escherichia coli bacteria to overproduce lycopene, an antioxidant with potential anticancer properties. To do that, the authors introduced mutations at twenty-four different sites within the E. coli genome, where they expected the mutations would have the greatest effect. There, they repeated the MAGE process for thirty-five cycles. The process resulted in 15 billion new strains of bacteria, each strain with a uniquely modified genome. According to the authors, some of the bacteria even produced five times more lycopene than they expected.

Following the success of Wang and Church's 2009 experiment, the pair began to employ the MAGE technique in bigger projects. Around that time, Church was managing and advising several different biotechnology groups, with whom he eventually used the technology to engineer new biological products. In 2010, Church's California-based biotechnology company, LS9, won the Presidential

Green Chemistry Award for engineering bacteria to convert sugar into a diesel-like fuel. In 2011, another one of Church's biotechnology firms, Joule Unlimited, won the Wall Street Journal Technology Innovation Award for engineering bacteria that could convert sunlight, carbon dioxide, and water into a kind of renewable fuel. Both of those alternative fuels contain alkanes, which are molecules made of hydrogen and carbon that are compatible with automobile engines and can therefore provide a sustainable replacement for gasoline. Additionally, scientists could use a similar process in order to create medicine or to engineer organisms that are resistant to pathogens, which they could then use to create vaccines or treatments for diseases in humans.

However, some have expressed concern with genome engineering in general because they suggest that it is an unnatural manipulation of nature. From an agricultural perspective, people have criticized genetically modified organisms, or GMOs, in foods as potentially less healthy, unsafe, or bad for the environment. However, scientists overwhelmingly agree that GMOs are not unsafe to consume and are often quite beneficial for the environment. People may also fear that researchers may use genome engineering technologies to manipulate human genomes in unethical ways. Despite concerns about its potentially unethical applications, Church has maintained that all new technologies have risks, and that those risks should not be a reason to avoid moving forward.

While controversy pervades every application of genome engineering, scientists continue to refine technologies like MAGE to test new hypotheses. Church contributed his experience with MAGE to facilitate the controversial de-extinction projects led by Revive & Restore, a nonprofit organization using biotechnology to attempt to bring extinct organisms, such as passenger pigeons and woolly mammoths, back to life. Although many people are excited for the possibilities of de-extinction, some critics worry that by making extinction seem reversible, de-extinction may ultimately undermine the conservation movement by decreasing public concern for other ongoing extinctions. As of 2020, Church's lab continues to work on developing techniques like MAGE to be able to edit eukaryotic cells and perhaps eventually human cells.

The larger scientific community has generally accepted MAGE as an expansion of the genome engineering toolkit, and have begun using it to manipulate genomes in ways that could have a wide variety of applications. Those include the possibility to mass produce industrial proteins for medical purposes, or to create biofuels that can mitigate the effects of global warming. MAGE has given genetic engineers the ability to make edits at a genome-wide scale with a more efficient scope. As genetic engineering continues to advance with technologies like MAGE, engineers can harness it in news ways that change the way humans can create medicine, produce agriculture, or live sustainably.

Sources

1. Bao, Zehua, Ryan E. Cobb, and Huimin Zhao. "Accelerated Genome Engineering Through Multiplexing." Wiley Interdisciplinary Reviews: Systems Biology and Medicine 8 (2016): 5–21. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4715650/ (Accessed July 26, 2020).
2. Bonde, Mads T., Sriram Kosuri, Hans J. Genee, Kira Sarup-Lytzen, George M. Church, Morten O. A. Sommer, and Harris H. Wang. "Direct Mutagenesis of Thousands of Genomic Targets Using Microarray-Derived Nucleotides." ACS Synthetic Biology 4 (2015): 17–22. https://pubs.acs.org/doi/10.1021/sb5001565 (Accessed July 26, 2020).
3. Clayton, Susan. "Preserving the Things We Value." Center for Humans & Nature, 2015. https://www.humansandnature.org/conservation-extinction-susan-clayton (Accessed July 26, 2020).
4. Columbia University Department of Pathology and Cell Biology. "Harris H. Wang, PhD." Columbia University. https://www.pathology.columbia.edu/profile/harris-h-wang-phd (Accessed July 26, 2020).
5. Gallagher, Ryan R., Zhe Li, Aaron O. Lewis, and Farren J. Isaacs. "Rapid Editing and Evolution of Bacterial Genomes Using Libraries of Synthetic DNA." Nature Protocols 9 (2014): 2301–16. https://isaacslab.yale.edu/sites/default/files/gallagher_isaacs_nprot.2014.082_0.pdf (Accessed July 26, 2020).
6. "Harvard Molecular Technologies." Harvard Molecular Technologies. http://arep.med.harvard.edu/ (Accessed July 26, 2020).

7. Keim, Brandon. “Genome Engineering Goes High Speed.” Wired, 2009. https://www.wired.com/2009/07/cellfactories/ (Accessed July 26, 2020).
8. Lajoie, Marc J., Alexis J. Rovner, Daniel B. Goodman, Hans-Rudolf Aerni, Adrian D. Haimovich, Gleb Kuznetsov, Jaron A. Mercer, Harris H. Wang, Peter A. Carr, Joshua A. Mosberg, Nadin Rohland, Peter G. Schultz, Joseph M. Jacobson, Jesse Rinehart, George M. Church, and Farren J. Isaacs. “Genomically Recoded Organisms Expand Biological Functions.” Science 342 (2013): 357–60. http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC4924538&blobtype=pdf (Accessed July 26, 2020).
9. “MAGE Summary.” Harvard University. http://2011.igem.org/Team:Harvard/Technology/MAGE (Accessed July 26, 2020).
10. Mannion, Antoinette M., and Stephen Morse. “Biotechnology in Agriculture: Agronomic and Environmental Considerations and Reflections Based on 15 Years of GM Crops.” Progress in Physical Geography: Earth and Environment 36 (2012): 747–63. http://epubs.surrey.ac.uk/745768/ (Accessed July 26, 2020).
11. Marchant, Jo. “Evolution Machine: Genetic Engineering on Fast Forward.” NewScientist, 2011. https://www.newscientist.com/article/mg21028181-700-evolution-machine-genetic-engineering-on-fast-forward/ (Accessed July 26, 2020).
12. Marx, Vivien. “Harris Wang.” Nature Methods 15 (2018): 301. https://www.nature.com/articles/nmeth.4676.pdf?origin=ppub (Accessed July 26, 2020).
13. Nair, Prashant. “Profile of George M. Church.” Proceedings of the National Academy of Sciences 109 (2012): 11893–5. https://www.pnas.org/content/pnas/109/30/11893.full.pdf (Accessed July 26, 2020).
14. National Human Genome Research Institute. “DNA Sequencing Costs: Data.” National Institutes of Health. https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data (Accessed July 26, 2020).
15. Pimm, Stuart. “Opinion: The Case Against Species Revival.” National Geographic News, 2013. https://www.nationalgeographic.com/news/2013/3/130312--deextinction-conservation-animals-science-extinction-biodiversity-habitat-environment/ (Accessed July 26, 2020).
16. Singh, Vijai, and Darren Braddick. “Recent Advances and Versatility of MAGE Towards Industrial Applications.” Systems and Synthetic Biology 9 (2015): 1–9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4688408/ (Accessed July 26, 2020).
17. “The 1000 Dollar Genome.” Illumina. https://www.illumina.com/content/dam/illumina-marketing/documents/company/featured-articles/the-1000-dollar-genome.pdf (Accessed July 26, 2020).
18. “The GMO Debate.” Cornell Alliance for Science, 2018. https://allianceforscience.cornell.edu/blog/2018/08/the-gmo-debate/ (Accessed July 26, 2020).
19. The Wyss Institute. “MAGE: Multiplex Automated Genomic Engineering.” The Wyss Institute. https://wyss.harvard.edu/technology/multiplex-automated-genomic-engineering-mage/ (Accessed July 26, 2020).
20. Thompson, David B., Soufiane Aboulhouda, Eriona Hysolli, Cory J. Smith, Stan Wang, Oscar Castanon, and George M. Church. “The Future of Multiplexed Eukaryotic Genome Engineering.” ACS Chemical Biology 13 (2018): 313–25. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5880278/ (Accessed July 26, 2020).
21. Wang, Harris H., Farren J. Isaacs, Peter A. Carr, Zachary Z. Sun, George Xu, Craig R. Forest, and George M. Church. “Programming Cells by Multiplex Genome Engineering and Accelerated Evolution.” Nature 460 (2009): 894–8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4590770/ (Accessed July 26, 2020).
22. Wang, Harris H., Hwangbeom Kim, Le Cong, Jaehwan Jeong, Duhee Bang and George M. Church. “Genome-Scale Promoter Engineering by Coselection MAGE.” Nature Methods 9 (2012): 591–3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3428217/ (Accessed July 26, 2020).
23. Yong, Ed. “Hacking the Genome with a MAGE and a CAGE.” Discover, 2011. https://www.discovermagazine.com/planet-earth/hacking-the-genome-with-a-mage-and-a-cage (Accessed July 26, 2020).