SEQUENCING genomes has become easy. Understanding them remains incredibly hard. While the trickle of sequence information has turned into a raging torrent, our knowledge isn’t keeping up. We still have very little understanding of what, if anything, all our DNA does.

This is not a problem that can be solved by computers. Ultimately, there is only one way to be sure what a particular bit of DNA does—you have to alter it in real, living cells to see what happens. But genetic engineering is very difficult and expensive.

At least, it used to be. Last month, two groups announced that they had performed a mind-boggling feat. They targeted and disabled nearly every one of our genes in cells growing in a dish. They didn’t knock out all the genes in each cell at once, of course, but one gene at a time. That is, they individually modified a staggering 20,000 genes. "It’s truly remarkable," says Eric Lander, director of the Broad Institute of MIT and Harvard, who led one of the studies. "This is transformative."

To put it into perspective, in 2007 an international project was launched to target and "knock out" each of the 20,000 genes a mouse possesses. It took the collective effort of numerous labs around the world more than five years to complete, and it cost $100 million. Now two small teams have each done something similar in a fraction of the time and cost. The secret, as simple and powerful a way of editing genomes.

The term breakthrough is overused, but this undoubtedly is one. "It’s a game-changer," says Feng Zhang, also at the Broad Institute, who led the other study.

The technique, unveiled just a year ago, is generating tremendous excitement as its potential becomes clear. It is already starting to accelerate the pace of research—Lander and Zhang used it to find out which genes help cancer cells resist a drug, for instance. In years to come, it is likely to be used in gene therapy, and to create a new generation of genetically engineered organisms with extensive but precise changes to their genomes. And if we ever do decide to genetically modify people, this is the tool to do it with.

While genetic engineers have done some amazing things, their first tools were very crude. They bombarded cells with extra DNA—sometimes literally—in the hope that it might occasionally get added to a cell’s genome. But there was no way to control where in the genome it went, and if added DNA ends up in the wrong place it can cause havoc. Also, this approach does not allow for any tinkering with existing genes, which is the key to finding out what they do and their variants do.

So in the past couple of decades the focus has switched to genome editing. To visualise how it works, imagine the genome as a collection of cookbooks written on long scrolls of paper and cared for by blind librarians. The librarians try to repair any damage but because they can’t read they are easily tricked.

If you cut a scroll in two, the librarians will join the pieces together again but in the process they often wreck the recipe. In other words, you can disable, or "knock out," a gene by cutting it.

What’s more, if you add an extra piece of paper and then cut a scroll in two, the librarians will often assume the piece was cut from the scroll and add it in where the cut was made. In this way, segments of DNA can be added exactly where you want.

So the secret of genome editing is to cut DNA at just the right spot, and let the cell’s DNA repair mechanisms do the rest for you. In practice, this means finding a molecule that, if added to a cell, will bind to a specific DNA sequence and cut the DNA at that point. There are natural proteins that do exactly this, but the chances of finding an existing protein that happens to target the one site in the entire genome that you are interested in are vanishingly small. Indeed, artificial proteins that bind to a specific DNA sequence have to be designed, made and tested for each site you want to make. That can and is being done in many research labs around the world. Indeed, this kind of gene editing could soon be used in gene therapies to treat everything from sickle cell anaemia to HIV. Yet although there are now various tricks for speeding up the process of creating a designable DNA-binding protein, it is still far from easy. It takes still take months or years of work to do yourself, or cost tens of thousands of dollars to have it done for you.

To complicate matters further, much of the underlying technology has been patented.

Now, though, there is an alternative that is much faster, cheaper and—so far—freely available to all. The story of how it came
Superfast editing

The key to genome editing is the ability to target and cut specific DNA sequences. The CRISPR technique offers this advantage.

OLD METHOD
For each change, a protein that binds to the target DNA has to be designed and tested. That could take months or years.

CRISPR METHOD
An RNA guide, which is cheap and easy to make, guides the Cas9 cutting enzyme to the target DNA.

Organisms with three or more changes can be generated in a single step. Saving years of work.

about begins in the late 1980s. While studying the genome of the E. coli bacterium, a group in Japan noticed a peculiar series of repeating sequences, separated by what appeared to be random bits of DNA that were later called “spacers.” These characteristic sets of repeats and spacers are now known as clustered regularly interspaced short palindromic repeats, or CRISPR (pronounced “cris-prp”). CRISPRs have turned out to be extremely common. They are present in half of all bacteria and a quarter of all eukaryotes. This means they must do something incredibly useful. But what? Their purpose remained elusive until 2005, when three groups reported that the apparently random pieces of spacer DNA actually match parts of the DNA of viruses that attack single cells. To biologists such as Eugene Koonin of the National Institutes of Health in Bethesda, Maryland, this immediately suggested a purpose: perhaps these spacers are the equivalent of a series of “wanted” posters, allowing cells to recognize and destroy viral DNA. In other words, an immune system.

In 2009, further studies had confirmed this suspicion and also revealed how the system works. The process begins with an RNA copy being made of a spacer and its flanking repeats. This RNA then joins up with a CRISPR-associated, or Cas, protein. The RNA provides a “blueprint” for the enzyme, and the Cas protein uses the RNA to bind to any matching viral DNA in the cell, and the Cas protein will slice it up.

What’s more, the cut-up pieces of viral DNA are sometimes spliced into the CRISPR region to form new spacers. In this way the wanted spacers can be kept up to date as viruses change their appearance. Learning how to fight off specific viruses is something that bacteria do better than we do. As the experts say, it’s a natural immune system.

“Extremely powerful”
Initial work proved disappointing, because the DNA-cutting Cas protein turned out to be an intricate complex made of several different proteins, which would be tricky to work with. Then, in 2009, Charpentier’s team made a key discovery. They were studying the CRISPR-Cas system in bacteria that often infect people, called Streptococcus pyogenes. They discovered it was quite different to other systems. “Instead of a complex of proteins it used only one,” says Charpentier.

This was more complicated in another way—it required not one but two pieces of RNA to guide the DNA-cutting enzyme, now called Cas9, to the target site. However, in 2012 Charpentier and DouAdvina together showed that those two RNAs can be combined into a single artificial RNA molecule that guides the Cas9 protein just as effectively. What they had created was a potentially very simple yet extremely powerful genome-editing tool. Because an RNA molecule does the targeting, there is no need to design new proteins to do this. All researchers need to do is make an RNA that contains a 20-base-pair sequence complementary to the target DNA, as well as the usual sequences needed for the guide RNA to hook up with the Cas9 protein. “It’s a much simpler way to do site-specific targeting,” says DouAdvina.

Crucially, making custom pieces of RNA is far quicker and cheaper than designing proteins. Automated machines can churn out short DNA or RNA sequences in hours, at a cost of just a few dollars. But Charpentier and DouAdvina’s study involved targeting bits of DNA floating loose in a tube. Would the bacterial system work inside the complex cells of animals and plants? DouAdvina’s team set out to test this but they were beaten to it by Zhang’s team and that of George Church at Harvard University, both of which reported in the first week of 2013 that the CRISPR-Cas9 system worked beautifully in human cells (Science, vol 339, p 823). This was a vital demonstration says DouAdvina, whose own study appeared just three weeks later.

After that, everybody wanted to try CRISPR-Cas9. “It’s exploded, and it’s continuing to move very quickly,” says Charles Gerbasch at Duke University in Durham, North Carolina. “That’s testament to how easy it is to use, and how robust the tool is.”

Already, the CRISPR-Cas9 system has been shown to work in a wide variety of organisms, including mice, zebra fish and fruit flies. It also works in plants, including rice and wheat, the most widely grown crops. No one has yet done a direct study comparing the CRISPR system with other genome-editing methods, so it is not yet clear which is more accurate in terms of hitting only the target DNA, and more efficient in terms of the percentage of cells successfully modified. For sheer speed and ease of use, CRISPR wins hands down.

The key to the gene editing method was found in a S. pyogenes. And those are not the only advantages: because the system evolved to target multiple viruses, it can be adapted to modify more than one gene in a cell at a time. In bacteria, the CRISPR system is already multiplex, so when I first learned about it, multiplex genome engineering was the first thing that came to mind,” says Zhang.

He has shown that it is feasible to edit at least five genes at once (Cell, vol 153, p 930). “Privately people have told me they’ve modified many more genes simultaneously,” says Zhang. With other techniques, making multiple changes is much more difficult. Church has developed an “evolution machine” potentially capable of making thousands of changes to cells (Nat Biotechnol, 27 June 2012, p 34), but it is complicated and works only with bacteria for now. Why modify so many genes at once? “Look at the mutations associated with disease,” Zhang says. “There’s never a single mutation responsible for diabetes, or heart problems – it’s always a combination.” To study their effects, you have to create lots of animals possessing these particular combinations.

That used to mean engineering several strains of mice each with one modification; then spending years crossing them breedings to create a strain with all of the modifications (see diagram, far left). Now it can be done in one step, by using the CRISPR system to make multiple changes to a mouse embryo while it is still at the single-cell stage. The ability to take two or more can now be done in six weeks, says Zhang. “That’s a big difference.” For those who have spent years trying to just one or two specific changes to plants or animals, this is revolutionary.

Make your own drugs
And as if this were not enough, this is not all that the CRISPR system can do. The activity of genes is controlled by proteins known as transcription factors. These proteins recognise and bind to specific DNA sequences near a gene and, by boosting or blocking the activity of the enzymes that transcribe the DNA into messenger RNA, either increase that gene’s activity or switch it off. Design your own transcription factors, and you can control gene activity—an ability that has all kinds of uses in research and medicine. But most attempts so far have involved designing new proteins, which as we have seen is not easy.

Gerbasch and a few others realised that the Cas9 protein can be modified so that instead of cutting DNA it acts as a transcription factor. In July, his team proved this works in human cells (Nature Methods, vol 10, p 972).

That opens up all kinds of possibilities. For instance, the approach could be used to treat autoimmune diseases such as arthritis by getting cells with a person’s body to make more of a specific and inflammatory protein. “Rather than providing the protein as a drug, we could induce cells to make themselves,” says Gerbasch.

“The field has exploded and it’s continuing to move very quickly. That’s testament to how easy the method is. Did you know that there are over 200,000 scientists working on CRISPR today?”

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U-Bit

To make quantum theory real, we must create the most powerful entity in the universe

LIFE RECODED

Rewriting genes just got a lot easier

AGONY AND ECSTASY
Epilepsy holds the key to finding inner peace

FROZEN IN TIME
The place where the ice age lives on

PSYCHO BIOTICS
Gut bugs that mess with your mind