New Scissors for Cutting Chromosomes

Investigators have devised a new technique for cutting large chunks of DNA exactly where they want

RESTRICTION ENZYMES HAVE BEEN the workhorses of genetic engineering for nearly 20 years now. These enzymes, which cut DNA at very specific sites, have made possible the astounding progress in gene cloning and sequencing. But indispensable as they are, these scissors have their limitations. Even the best among them, the "rare cutters," chop big chunks of DNA into too many pieces for easy handling. The problem is becoming particularly acute now that researchers are gearing up to map and sequence the very large genomes of humans and other complex organisms.

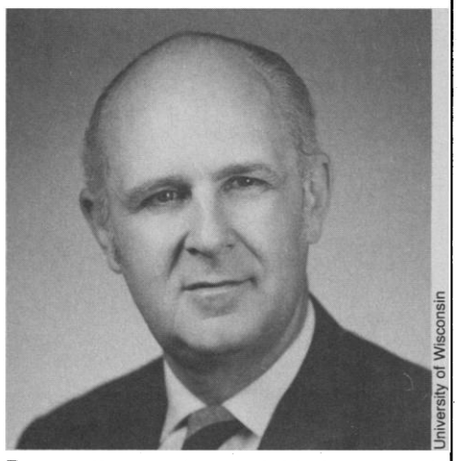
But help is on the way. Michael Koob and Waclaw Szybalski of the University of Wisconsin have come up with a simple solution to this long-standing problem in genetic engineering. They have created a new type of scissors that will cut a huge piece of DNA exactly where they want and no place else. Their technique promises to become a nifty new tool for dissecting large genomes.

The two researchers scored their first successes with the technique a couple of years ago, but then they used only small plasmid DNAs (*Science*, 16 August 1988, p. 1084). What people are excited about now is that they have shown that the method actually works in the big genomes it was designed for. Szybalski described their new results in yeast at the recent genome mapping and sequencing meeting at Cold Spring Harbor Laboratory. "Elegant," says geneticist Maynard Olson of Washington University, one of the organizers of the meeting. Olson adds that "it is rare for a new method to appear with such gorgeous data."

The problem with restriction enzymes is that even the rare cutters recognize a specific group of just eight bases and then cleave the DNA at that site. And the bigger the genome, the more often that combination of eight bases will appear. That means that the relatively small *Escherichia coli* genome will be cut about 72 times, the genome of the yeast *Saccharomyces cerevisiae* about 230 times, and the human genome about 50,000 times. "The thing falls into 50,000 pieces and you can't manage it" says Szybalski.

What's really needed, Koob and Szybalski reasoned a few years ago, is a restriction enzyme that recognizes a much longer stretch—say 15 or 20 bases. Statistically, that particular combination would show up hardly ever, even in a huge genome. A 20-base recognition site, for instance, will occur once every trillion bases, or once in every 1000 human genomes, says Szybalski.

And since nature doesn't make one, they jury-rigged an exceedingly rare cutter, and its recognition site, themselves. At the heart of their approach is a way to modify the DNA to essentially "erase" all but one of the cutting sites for the restriction enzyme they use. They call it the Achilles' heel cleavage, borrowing from the Greek myth about the great warrior of the Trojan War. According to the myth, Achilles' mother, Thetis, dipped him into the River Styx, which made him invulnerable to arrows—except at the one place on his heel where she held him. In Szybalski and Koob's version, the DNA, too, is rendered invulnerable to restriction enzymes, except at just one site. But here the "hand" is, if you will, a DNA-binding protein, and the River Styx is an enzyme that inactivates all the restriction sites except the one beneath that hand.



Rare cutter. *Waclaw Szybalski has just used the new scissors to cut yeast DNA.*

Their first task was to select the DNA-binding protein itself—one that could recognize and protect 15 or 20 bases and give the researchers the high specificity that they wanted. They settled on the *lac* repressor, a bacterial protein that binds to a 20-base sequence, known as the *lac* operator, but some other DNA-binding proteins would work just as well, says Szybalski.

The yeast genome does not normally contain binding sites for the *lac* repressor. So using a bit of genetic engineering, Koob and Szybalski introduced the operator into a predetermined spot on chromosome 5, next to a known gene. They used a synthetic *lac* operator designed to contain recognition sites for two commonly used restriction enzymes, Hha I and Hae II.

When the researchers then added the *lac* repressor to the yeast cell, the protein quickly found the operator on chromosome 5 and bound there, covering up the two restriction sites, much as Thetis' hand covered Achilles' heel. Next, Koob and Szybalski added an enzyme, methyltransferase, that inactivates all the restriction sites—except for the two hidden under the *lac* repressor. Finally, they removed the *lac* repressor and other proteins, leaving the yeast genome with just one recognition site each for the two enzymes.

And just as predicted, when Szybalski and Koob added the restriction enzyme Hae II to a mixture of all 16 yeast chromosomes, it cut once and only once on chromosome 5. What's more, says Szybalski, they can use this technique to cut anywhere they want, depending on where they insert the *lac* operator, which is a trivial task in yeast but could be trickier in mammalian cells.

Other investigators are experimenting with similar approaches to cutting DNA at unique sites. Scott Strobel and Peter Dervan at the California Institute of Techology, for instance, just reported progress with a chemical cleavage method, also in yeast (*Science*, 6 July, p. 73), but nothing has yet approached the efficiency or specificity of the Achilles' heel technique.

Szybalski predicts the technique will be extremely versatile in mapping and sequencing large genomes, like the human genome. For one, it provides a way to break them into manageable chunks that can then be sequenced with the ease of *E. coli*, says Szybalski. And by using other "blocking proteins," instead of the *lac* repressor, it should be possible to cut chromosomes at the beginning of genes, which would be handy not only for finding genes but also for sequencing them.

All of this remains to be demonstrated in mammalian cells, notes Charles Cantor, head of the genome center at Lawrence Berkeley Laboratory, who nonetheless calls the new method "impressive." But Szybalski harbors no doubts about the technique: "This is the first and only way to cut a big genome in one specific place, with very high efficiency." And he is already gearing up to use the new tool as part of a University of Wisconsin effort to sequence the *Drosophila* genome, which contains a hefty 150 megabases. ■ LESLIE ROBERTS