THE AUTHOR FILE

Greg Davis

Gene editing becomes faster and more facile.

Double-stranded DNA kept slowing Greg Davis down. The scientist at Sigma-Aldrich was exploring zinc-finger nucleases (ZFNs), a newly licensed technology for gene editing. These enzymes can be designed to cut chromosomes at a given sequence, allowing synthetic DNA to be incorporated into the break by a cell's own repair machinery. Unlike other genome-editing techniques, it worked across a range of species, including human cells.

James Visser, Sigma-Aldrich

Fuqiang Chen and Greg Davis

But the synthetic DNA came in the form of plasmids, circles of thousands of base pairs. The time from designing a plasmid to actually having it delivered is a frustrating two weeks, or longer for difficult designs. In this issue of *Nature Methods*, Davis and colleagues describe a faster approach, using single-stranded oligonucleotides to efficiently alter human cells.

Using plasmids to alter DNA, says Davis, is like rewriting an entire page in a novel to change one word. "We could edit a single base pair, but we had to make a large molecule to do the change." What happens with an oligo, he explains, is like erasing and replacing a single sentence on the metaphorical page. Oligos are not only cheaper, but they can be available the next day.

Davis was not the first to try to replace plasmids with smaller DNA constructs. Much work has been done to incorporate single-stranded DNA into cells, some of it using ZFNs. But Davis and colleagues had a resource that other researchers did not: a large set of highly active ZFNs. That meant his team could easily introduce a cut in the chromosome that was close to the site of a desired mutation.

One of the most important pieces of equipment, says Davis, was actually not in the laboratory but over Davis's desk. "[First author] Fuqiang [Chen] and I spent a lot of time talking on the whiteboard, planning experiments. We'd draw a schematic of the chromosome, where the ZFN cuts, where is the mutation that we want to make, and what the oligo has to look like to deliver that."

With the oligo in hand, the idea was simple enough to test. The researchers first targeted a well-known site called *AAVS1*. They designed an oligo to insert an easily detected 6-base sequence and introduced that DNA, along with an appropriate ZFN pair, into cells. The gene editing occurred with greater than 20% efficiency, an amazingly high rate. "I just didn't believe it," Davis recalls. "It was like cold fusion." Moreover, the technique worked in all seven different cell types tested. "We had to make sure it worked for everybody," explains Davis.

They chose the *AAVS1* locus mainly for its convenience. To know whether the technique could be really useful, the researchers had to show that it could work with a sequence chosen to answer a biological question. They did this by editing the sequence encoding the kinase RSK2 at its natural location in the genome, changing a crucial amino acid to render it resistant to a small-molecule inhibitor. "That closed the loop on the editing and the phenotype," says Davis.

But an oligo-ZFN combination can create more than point mutations: it can also delete large regions, as close in as 100 base pairs from the ZFN cut site and as far out as 100,000 base pairs. In one cancer cell line, the sequence targeted for deletion was triploid, and using the ZFN-oligo combination the researchers made 1-kilobase deletions at all three locations with an efficiency of about 3%. In addition, small genetic elements, such as the Cre recombinase binding site, *loxP*, can also be inserted at the site of deletions.

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Performing large deletions in mammalian cells with ZFNs had already been reported, but doing so required two pairs of ZFNs designed for each end of the deletion. Now, a single ZFN pair can cause a wide range of genomic deletions specified by designed oligos. Ironically, that could mean that researchers will actually be able to perform more experiments with fewer ZFN purchases. "We've created a two-for-one situation. We joked about that a little bit," says Davis. But the situation can also be interpreted a bit differently, he adds. Even after a recent 50% price drop by Sigma Life Science, some researchers may only be able to afford one custom-made ZFN pair rather than two. Now they will be able to perform experiments they might not have been otherwise able to perform. That, in turn, should fuel additional development, says Davis. "In academia, people rely on grant money. We rely on product sales."

Monya Baker

Chen, F. *et al.* High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases. *Nat. Methods* **8**, 753–755 (2011).