Behind the wall	1056
Going the TALEN route	1057
Editing deeper	1058
Buy or build	1058
Box 1: Building a core facility with a commercial tool	1056
Box 2: TALENs in a FLASH	1057

Genome-editing tools storm ahead

Vivien Marx

The menu of maturing, diversifying methods calls for careful selections in experimental design.

With a rapidly growing fan base, gene-editing tools enable experiments from basic biomedical research to crop science. Researchers use these tools to edit genomes at sites of their choosing. “The tools are now really good, and the applications are expanding,” says Andrew Scharenberg, chief scientific officer of Cellectis therapeutics, which focuses on biomedical applications of this technology.

Gene-editing tools are meeting the real world in an ongoing clinical trial sponsored by Sangamo BioSciences, whose results serve as a bellwether for the emerging industry. “It shows you the power of the tool and how far-reaching it is,” says Nathan Wood, vice president of synthetic biology at Life Technologies. With its drug candidate, Sangamo harnesses its intellectual property portfolio relating to a type of gene editor called a zinc-finger nuclease (ZFN), which can cut DNA at a predetermined point, leading to the ferrying in of a new gene or regulatory element or disrupting an existing gene.

In the trial, ZFNs are used to engineer a specific gene deletion into each patient’s CD4⁺ T cells, a type of white blood cell. Where the gene edit is successful, the door through which human immunodeficiency virus (HIV) enters cells and assaults the immune system—the CCR5 co-receptor—is disrupted. According to results that Sangamo made public in September, CD4 counts have increased in study participants in a statistically significant manner.

One trial participant, Matthew Sharp, learned that he was HIV positive over 20 years ago. He battles HIV as a patient and an AIDS activist, and his lowered CD4⁺ counts have made him vulnerable to infections. He is hopeful that science might help to change

his current drug regimen. “One day, after more research with this zinc-finger technology, my dream is to be able to stop my anti-retroviral medications,” Sharp said in one account about how he is faring¹.

The gene-editing community is watching this trial “very carefully,” as it represents a first clinical test for the technology, says Dominic Esposito, who works with gene-editing tools on a daily basis and who has launched a gene-editing core facility (**Box 1**). He runs the protein expression lab at Frederick National Laboratory for Cancer Research, which is affiliated with the US National Cancer Institute. He says that scientists need to choose mindfully from among the diversified set of gene editors, matching them to their experiment.

A wide choice of tools

The techniques to engineer and tailor targeted nucleases originated in academic labs and continue to develop there as well as in industry^{2,3}. Johns Hopkins University researcher Srinivasan Chandrasegaran figured out how to hitch the nuclease domain of a restriction enzyme, FokI, to zinc-finger proteins, thereby creating ZFNs.

These enzymes generate targeted double-strand DNA breaks that are fixed by the cell’s own repair pathways, through either nonhomologous end joining or homologous recombination. As discovered

Cellectis says it can make up to 17,000 nucleases a year.

by Maria Jasin and colleagues, a double-strand break in a particular gene greatly stimulates the efficiency with which it can be targeted by homologous recombination⁴. Both repair processes are now harnessed by scientists for gene editing, either to ferry in new genes or to knock existing genes out.

Some gene-editing tools have been around nearly two decades; others are younger or even brand new. In addition to ZFNs, the gene-editing toolbox includes meganucleases, also called homing endonucleases, and transcription activator–like effector nucleases (TALENs), which also induce double-stranded breaks³.

Transcription activator–like (TAL) effectors are virulence factors from *Xanthomonas* bacteria, and they give these plant pathogens their bite: the bacteria are bad news for cotton and rice. Building in part on decades of experience in ZFN development, scientists have rapidly discovered how to harness the DNA-binding domains of these proteins for gene editing in cultured cells and in several organisms.

BOX 1 BUILDING A CORE FACILITY WITH A COMMERCIAL TOOL

Although some academic groups build core facilities on their own, in other cases companies shape gene-editing core facilities. With support from Cancer Research UK, Ian Prior at the University of Liverpool set up one such facility in January of this year, in which he trains scientists to use a proprietary gene-editing system by Horizon Discovery. "It's a research facility, not a service facility," he says. Pharmacologists, stem cell biologists and cancer researchers edit the genomes of cells they need.

The benefit for Horizon is customer exposure as well as access to cell lines developed in the labs that the company can potentially commercialize. Horizon Discovery's platform uses adeno-associated viruses (AAVs) to stimulate homologous recombination and introduces a drug-resistance cassette in the process. Researchers use drug resistance to sort successfully edited cells.

In his own research, Prior had been looking for a way to avoid overexpressing proteins. Gene-editing tools let scientists introduce mutations, and genes can be expressed in the cell's "original context." TALENs or ZFNs may be right for the job, but building these tools takes both time and money, and Prior was concerned about validation testing of commercially available gene editors. He chose to partner with Horizon because of the drug resistance-based method the company's technology uses to validate results and off-target effects.

At the Frederick National Laboratory for Cancer Research, Dominic Esposito has created a service core facility, also with the Horizon system. Many of his customers seek to make genomic changes mimicking disease mutations and examine the effect of a particular mutation in cell lines. Esposito and his colleagues make and deliver the virus, and then the researchers do the downstream experiment and screen for correct clones.

Esposito considered zinc-finger technology for his facility but found it too costly for the US National Institutes of Health researchers he serves. In the past he has worked with TALENs, but he now focuses mainly on the AAV technology. "I have been a big fan of TALENs from the original papers," he says, but they still seemed too much of an investment for him. "You have to make the TALEN construct and the targeting construct that you are going to use for your knock-ins." He acknowledges that using the AAV system requires experience making high-titer viral preparations. "One of our strong suits here is virology," he says. The advantage of AAV is easy delivery into cells, but there is a downside: each virus-modified cell line takes 3–6 months to make.

Horizon Discovery

Horizon sells applications of gene-editing tools rather than the reagents themselves, says Rhodes.

Many scientists criticize the AAV system because of the long production time frame. "That's been the knock on the technology," says Eric Rhodes, Horizon's chief technology officer, commenting on the criticism. The time factor is a challenge the company is currently addressing, he says.

Collaboration with labs is helping to grow the company's business. Partnerships can lead to commercialization agreements for cell lines. Horizon's customers are mostly pharmaceutical and biotechnology companies, and there are plans to expand its range of cell lines for applications beyond oncology. The gene-editing tools are not the company's main commodity, but rather, he says, "the cell lines that already have the modifications in them."

A new tool on the distant horizon is an RNA-based system derived from a bacterial line of defense against viral attack. A team from the University of California, Berkeley, the University of Vienna and Umeå University in Sweden suggests that a dual-RNA structure directing a site-specific nuclease could be the basis of a gene-editing tool that is "efficient, versatile and programmable"⁵. Commenting on this work, Rodolphe Barrangou from DuPont Nutrition and Health points out that a nucleic acid-based member of the gene-editing tool family, unlike ZFNs and TALENs, would not require redesign for every DNA target⁶.

Behind the wall

Much ZFN know-how sits behind an intellectual property wall. Sangamo has set up an exclusive arrangement to share reagents and methods with Sigma-Aldrich. The market potential and broad applicability of ZFNs caught Sigma's eye, explains Greg Davis, who develops gene-editing technology at

Sigma Life Science

The potential of ZFNs caught Sigma's eye, says Davis.

for ZFNs. "Academia is a very big market for us," grabbing the "lion's share" of the sales volumes, he says. Scientists are working on human, mouse and rat cells and are expanding to new model organisms. Sigma's assays are also finding their place in the drug-discovery market. Choosing established ZFN technology over newer gene-editing tools means "you're reducing your amount of risk," says Davis.

Gene-editing companies seek to differentiate their tools in several ways. One

Sigma Life Science.

Sales volumes last year for ZFN reagents, which include a custom ZFN service, pre-designed knockdown ZFNs and targeted integration kits, "have more than doubled," says Keith Hansen, Sigma's product manager

aspect is to what degree functional validation is performed and the way the companies help scientists assess potential off-target effects, which result when the nuclease

Horizon Discovery

Some pipelines use adeno-associated viruses to create gene-edited cell lines.

cuts at sites in the genome besides the one of interest. Sigma tests each ZFN it ships, Hansen says. The company also applies bioinformatics tools to assure that the ZFN target site is “unique within that genome,” says Davis. This approach is useful, for example, when synthesizing a nuclease to use near a specific single-nucleotide polymorphism.

In the past, ZFN quality has come at a price. Long gone are the days when the Sigma ZFN price tag was an eye-popping \$250,000. Altered production schemes and strategies have led to price drops by orders of magnitude. The road is paved for gene-editing tools to be commodities. “We do want it to be a commodity, we do want it to be ubiquitous so that everyone turns to this without even thinking about it,” says Paul Brooks, who manages market segments at Sigma. Extending beyond the nuclease reagents themselves, Sigma and other companies are also moving toward gene-edited products such as engineered animals or cell lines.

The diversity of tools has created a wealth of commercial and open-source offerings, which compete for researchers’ attention and wallets as the marketplace evolves. For example, as Life Technologies’

Wood explains, his company and Cellectis have both submitted patent applications for their TALENs, which have not yet been issued. Although some scientists express concern over a looming intellectual property battle, others believe this situation will not crimp research.

Going the TALEN route

Life Technologies licensed TALEN technology from plant biologists at Martin Luther University and the Two Blades Foundation, which supports research in genetic methods to confer disease resistance to crops.

Life Technologies shares the commercial TALEN market with Cellectis, which has slightly over 200 employees. Cellectis licensed TALEN gene-editing technology from the University of Minnesota and Iowa State University.

“We have a broader portfolio than Cellectis has,” Wood says. He explains that his company has a range of TAL effector-based tools with different functionalities: for example, they may be nucleases, gene activators or repressors. The company also offers a multisite cloning system. “A customer can decide what payload they want to deliver

TALENs are fusions of transcription activator–like effectors of plant pathogens to the FokI nuclease.

could make them themselves or than the competition,” he says. TAL-effector delivery from Life Technologies takes an average of 8 days.

Life Technologies plans to develop these tools to allow scientists to apply TAL effectors in more diverse experiments. Products have seen “significant adoption” in the stem cell, plant and cell engineering community, and uptake is “rapid” in the transgenic animal community, Wood says. Future applications include crops, biofuels and biotherapeutics.

Designing and producing gene-editing tools requires much know-how and experience, explains Wood, some of which comes from the synthetic biology company GeneArt, which Life Technologies acquired in 2010.

Neal Stewart at the University of Tennessee, a Life Technologies customer, wants to make synthetic promoters to express plant genes and use TAL effectors as an expression tuner. “Of course, we are looking also at TALENs, too, for genome editing, but our main gig has been gene expression,” he says of his project, a collaboration with Life Technologies.

Sigma Life Science

The market is growing for gene-editing nucleases.

to the genome and put it into the multicloning site,” he says. “We deliver these products to researchers faster than they

BOX 2 TALENS IN A FLASH

Modifying genes in a high-throughput fashion, even pathway-wide or genome-wide, is a possibility raised by gene-editing tools. Many construction routes exist for TALENs, but gel isolation of fragments or the need to characterize intermediate constructs gets in the way of high-throughput assembly. Keith Joung and his team have developed a method for large-scale TALEN assembly called fast ligation-based automatable solid-phase high throughput, or FLASH⁷.

In this process, a DNA fragment that encodes a TAL-effector repeat is labeled and ligated to another DNA fragment that encodes four specific TAL-effector repeats. The next step is attachment to a streptavidin-coated magnetic bead. Then, more DNA fragments that encode preassembled TAL-effector repeats are sequentially ligated, thus leading to an array of desired length and DNA binding specificity. In the last step, the array is cleaved from the bead.

“FLASH assembly on beads means all the manipulations can be done by a liquid-handling robot,” says Joung. DNA fragments

encoding up to 96 different TAL-effector repeat arrays can be made in 1 day, which can then be cloned and their sequence verified in expression vectors in 1 week. “The method is very robust in our hands, and we’ve recently made it even more robust by switching from streptavidin-coated magnetic beads to streptavidin-coated wells in a 96-well plate,” he says.

For researchers lacking robotic helpers, the same method works with a multichannel pipette, which can make up to 24 TALENs at once in the same time frame, he says. Even when the robot runs only periodically, FLASH can make over 1,500 TALENs. A few research groups are in the process of collecting FLASH reagents to set up core facilities to supply their institutions with TALENs.

With this process in place, Joung says, the bottleneck can shift away from working out production methods. Scientists can now explore ways to use large numbers of TALENs: notably, techniques for introducing TALENs into cells or organisms in a high-throughput fashion and for assessing their effects in an equally speedy way.

FLASH assembly allows quick and automated production of TAL-effector repeat arrays.

In the past, he has considered other gene-editing tools as well. “I looked quite hard at ZFNs for engineering male sterility traits in plants, but decided to go with site-specific recombinases for that one application,” says Stewart.

Collectis also has a broad customer base for its TALENs, from academia to pharmaceutical and biotech firms, and collaborations with industrial companies such as Bayer Crop Science and Total. “The array of applications is extremely large,” says André Choulika, Collectis chairman and CEO.

Revenues last year reached nearly €20 million (\$26 million), and last fall the firm raised €50 million (\$65 million). TALEN sales have accelerated over the last 12 months, he says.

The company offers an array of functional assessments for TALENs. For the design process, the company uses in-house algorithms to analyze the target sequence for genome redundancy and potential off-target sites, says Philippe Duchateau, who directs Collectis’s nuclease group. After synthesis, a TALEN is sequenced and tested in a yeast assay and, depending on the project, may also be tested in mammalian cells for its capacity to induce mutagenesis.

According to Choulika, his firm has the world’s largest nuclease-producing capacity: it can design and produce up to around 17,000 nucleases a year. The company has increased its R & D efforts in therapeutics, which is where he believes “the real leap” in the field will occur.

Editing deeper

Besides having a role at Collectis therapeutics, Scharenberg has a lab at the University of Washington and sees patients as a pediatrician at Seattle Children’s Hospital. Editing T cells, as in the Sangamo trial, is a “proximal application” for gene-editing tools. Eventually it could be possible to reach into blood stem cells to edit genes involved in inherited immune deficiencies.

As familiarity with gene-editing tools spreads, experiments will grow in complexity. Prices will continue to drop as companies offer more prebuilt TALENs, further enabling larger-scale projects, says J. Keith Joung, a researcher at Massachusetts

General Hospital. Scientists will be looking at the performance of several TALENs in parallel, an endeavor reflected in the emergence of core facilities (‘cores’) at academic institutions, such as the Universities of Utah and Wisconsin. Some companies expand their customer base by helping create such cores (**Box 1**).

Companies might be hard-pressed to compete if academics create high-quality TALEN libraries. But the tools must be built and tested at large scale, which may give companies an edge. Firms can put people, storage and methods in place for the repeated manufacturing processes, says Scharenberg. “That’s where I think companies work really well.”

Some companies are exploring niches that predate ZFNs, notably homing endonucleases. “The ease of engineering is what has driven TALENs to the top of the heap, there’s no question about it,” says Jordan

Jarjour, cofounder and director of research and development at Pergen, a company spun out of the Northwest Genome Engineering Consortium.

Jarjour finds TALENs well suited to preclinical proof-of-concept work. For a potential therapeutic, however, his bet is on homing endonucleases because of their ease of delivery and specificity. Other gene editors carry risks of cleaving off-target sites. “I don’t think I would personally want a TALEN hanging around in a stem cell or any cell that has multipotent capabilities,” he says. He and his colleagues have developed a high-throughput flow cytometry-based protein engineering platform for meganucleases that he puts to work for customers and partners, one of which is Collectis.

Buy or build

The academic community has created resources to help scientists build their own gene-editing tools. The resource portals <http://taleffectors.com/> from the lab of Feng Zhang at MIT and <http://www.TALEngineering.org/>, maintained by the Joung lab, cater to the build-your-own-TALEN group. The Zinc Finger Consortium <http://www.zincfingers.org/>, founded by Joung and Daniel Voytas of the University of Minnesota, offers protocols, reagents, software and know-how for building ZFNs. It has been less active lately because of the high level of interest in TALENs, Joung says. “However, we still believe that zinc-finger nucleases have certain advantages relative to TALENs, and we are continuing

to develop the technology in my lab,” he says, adding that tool choice should suit the research project.

The decision to buy or build will depend on what will be quicker and more reliable in a given situation. “Are you going to spend more time

and effort—essentially cost—doing it yourself?” is the question scientists should ask themselves, says Eric Rhodes, chief technology officer at Horizon Discovery, which sells cell lines that have been modified using gene-editing tools. “If you go to a company, I think they’ll build it quicker,” says Scharenberg. “You may have some val-

“The array of applications is extremely large,” says Choulika.

For large-scale TALEN assembly, FLASH might do the trick, says Joung.

“If you go to a company, I think they’ll build it quicker,” says Scharenberg.

idation data, which is added value for the price.”

The buy-or-build decision can depend on project scale. If less than half a dozen TALENs are needed, ordering them might be more efficient, says Joung. When dozens, hundreds or thousands of TALENs are needed, that decision shifts. “I think it makes the most sense to make them yourself, and then the only question is what method you use,” he says. For researchers wanting thousands of nucleases, his response is FLASH, a method geared toward high-throughput construction of TALENs (**Box 2**).

“Off-target effects are certainly still an open question for TALENs,” and they are an issue with ZFNs too, says Joung. These off-target events are rare, meaning that it is not cost-effective to use DNA sequencing

as a detection tool. Outside of therapeutic applications, off-target effects may be less problematic. For example, researchers may use gene editors to confirm findings from RNAi experiments, and “they will interpret results keeping in mind that there may be off-target effects,” he says.

To address the potential confounding effect of off-targets, scientists may design TALENs for multiple sites in their target gene of interest. Joung says that if experiments with multiple TALENs deliver the same result, “it strengthens the interpretation that the effect they are seeing is due to the specific effect of TALENs on their target gene of interest.”

The scientific community is working on ways to comprehensively identify off-target sites and better decipher how these effects

come about. “Once we understand them, then we can contemplate how to reduce them,” Joung says. “But we need to figure out what they are first.”

1. Sharp, M. *HIVandHepatitis.com* <<http://www.hivandhepatitis.com/hiv-treatment/hiv-cure/3275-gambling-for-a-cure-for-hiv>> (2011).
2. Porteus, M.H. & Carroll, D. *Nat. Biotechnol.* **23**, 967–973 (2005).
3. Bogdanove, A.J. & Voytas, D.F. *Science* **333**, 1843–1846 (2011).
4. Jasin, M. & Berg, P. *Genes Dev.* **2**, 1353–1363 (1988).
5. Jinek, M. *et al. Science* **337**, 816–821 (2012).
6. Barrangou, R. *Nat. Biotechnol.* **30**, 836–838 (2012).
7. Reyon, D. *et al. Nat. Biotechnol.* **30**, 460–465 (2012).

Vivien Marx is technology editor for *Nature* and *Nature Methods*. (v.marx@us.nature.com).