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Sculpting Genomes with a Hammer and Chisel

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Abstract

With a flood of new tools for genome engineering, we can benefit from choosing the tool that best fits the job.

One can only imagine the excitement of the Iron Age stone carvers who first obtained iron tools over 3,000 years ago. The hammer revolutionized rock quarries, while the chisel allowed carvers to sculpt features with unprecedented precision. The masterpieces we still admire today are a testament to the success of these unnamed carvers, but we can only imagine the hundreds of shattered statuary limbs and cracked aquiline noses that resulted before the right tools were found for each job.

Genome engineering has recently made stunning advances with the emergence of powerful site-specific nucleases. Like the early stone carvers, we are just beginning to learn how to properly use these new tools. Unlike the early carvers who could see each chip of stone, we are largely engineering in the dark. We sequence the intended mutation site to claim success, but whole-genome sequencing is still too expensive to routinely check the true fidelity of each mutational event.

Two recent papers that probe the fidelity of the clustered, regularly interspaced short-palindromic-repeat (CRISPR/Cas) system highlight the surprising extent of possible off-target damage (1, 2). Remarkably, some CRISPRs actually mutated predicted off-target sites at a *higher* frequency than the intended sites (1). In contrast, studies using zinc-finger nucleases (ZFNs), and transcription-activatorlike effector nucleases (TALENs) have demonstrated higher fidelity, though the off-target potential of these platforms certainly still exist (3-5). Although definitive studies to elucidate ideal genome engineering methods await affordable wholegenome sequencing, it is worth considering the potential metrics for choosing the right tools. The three factors that are most important are accessibility, robustness, and fidelity.

Accessibility to genome engineering platforms differs markedly due to technical and legal barriers. Just a few years ago, site-specific genome engineering tools were out of reach for most researchers. Now, we can choose among three major platforms: ZFNs (6), TALENs (7, 8), and CRISPRs (9-11). Each system has different architecture and history that affect accessibility. ZFNs are the pioneers of the site-specific nuclease toolbox, but are the most difficult to build. Although ZFNs can work remarkably well (5, 12), making a potent ZFN requires significant investment in protein engineering to refine the activity and specificity. In contrast, TALENs have a modular design that allows a molecular biology lab to build a series of TALENs targeting a unique DNA sequence in just a few days (3, 13, 14). The

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CRISPRs are the easiest to build, since sequence specificity is encoded in a short guide RNAs (gRNA) that can be produced hundreds at a time (9-11).

The legal barriers to accessibility may be as daunting as the technical ones (15). The field is awash with patents and companies maneuvering for a lead position. Unlike other molecular biology reagents (such as restriction enzymes or antibodies), purchasing site-specific nucleases commonly requires material transfer agreements that contain "reach through rights" making claims to the cells or animals that have been modified by the nucleases. For instance, plants, animals and induced pluripotent stem (iPS) cells made with some purchased TALENs or ZFNs cannot be put to commercial use without permission of the nuclease provider(15). Fortunately for researchers, there are increasing sources of site-specific nucleases that allow users to retain "freedom to operate" with resulting engineered materials.

Robustness of a nuclease to make an intended mutation would seem to the most important criterion for a successful engineering platform, but this can be deceiving if one does not consider the potential for high off-target activity that may also be occurring. Certainly, the excitement at being able to make sitespecific mutations is understandable, since it has been so difficult in the past. But the recent studies showing high offtarget activity of the CRISPR system reveal the risk of focusing on targetsite robustness alone (1, 2).

Fidelity in genome engineering is most critical for making human iPS cell disease models or for gene correction for human therapy, as a single basepair change can make the difference between health and a lethal disease. Even for the more specific platforms (ZFNs and TALENs), the human genome is vast and off-target effects may confound disease models or therapy. Unlike many biochemical reactions that have millions of substrate molecules, a site-specific nuclease should ideally act at only two genomic sites per cell—the maternal and paternal gene alleles. Although nuclease excess can drive up recombination rates, they can also act off-target. Indeed, studies show that expressing higher amounts of nuclease can result in greater off-target effects (1, 5). If fidelity is desired, it is safer to choose a cell that was exposed to the least (not the most) nuclease molecules. While choosing engineering conditions with lower mutagenic rates might seem counterintuitive at first, these are likely to be the conditions that provide the highest fidelity clones. Other improvements in fidelity may be achieved with extended DNA recognition sites, temporal control of nuclease activity or heterodimeric nuclease pairs.

Of course fidelity is not always all-important. Organisms such as flies, worms or fish can be bred rapidly to remove off-target mutations in subsequent generations. The flexibility and high activity of CRISPRs should make them very useful in these model organisms. In addition, the ability to rapidly home to diverse locations in the genome suggests that CRISPRs will provide valuable tools for mammalian biology. Nuclease-dead versions of CRISPRs have already proven to be effective for inhibiting/activating gene expression (16-19) and may reshape epigenetic features in ways that we have previously not imagined. Perhaps CRISPR nucleases can be used as sledgehammers for large-scale mutagenic screens in model organisms, and nuclease-dead CRISPRs will provide the epigenetic finish when sculpting genomes.

The Iron Age stonemasons could see their mistakes in broad daylight to evaluate each new tool. By comparison, genome engineers can rarely take a full portrait of their work, even when it is finished. Nevertheless, we are at the beginning of a new age. We have marvelous new tools for shaping genomes that we are just beginning to learn to use. As whole-genome sequencing continues to improve, the lights are slowly illuminating our workshop to reveal unwanted changes induced by new engineering technologies. Perhaps in the future we will devise more accurate engineering systems to better control the spatial and temporal

choreography of recombination for truly precise genome editing. In the meantime, we can enjoy wielding our new tools, largely in the dark, and hoping for a masterpiece.

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