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Editorial overview: The next-generation of genome editing: The future is now

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As we approach the first quarter mark of the 21st century, we have witnessed the dawn of a new era in genome engineering, the science of rewriting the genetic code of living systems. Nowhere else is this best illustrated than by the recent approval of Casgevy (exagamglogene autotemcel), a cell-based gene therapy for sickle cell disease that utilizes CRISPR-Cas9 to enhance the production of the fetal form of hemoglobin, a remarkable achievement made in just under a decade since the initial groundbreaking adaptation of the technology for genome editing in eukaryotic cells. This exceptionally short timeframe for clinical implementation emphasizes not only the immense power of CRISPR-Cas-based approaches for solving previously intractable biomedical problems but also the enormous potential of genome editing at large for impacting science and engineering in the decades ahead.

Yet despite the astonishing advances that have fueled this first wave of success, there remains a critical need for more robust, flexible, precise, and safer genome-engineering tools. This need arises, in part, from the fact that the earliest generations of targeted genome-editors relied on DNA double-strand breaks (DSBs) to create their edits, a process that not only can lead to a high-percentage of unproductive modifications at the target DNA sequence but one that also poses serious risk for triggering chromothripsis and other chromosomal aberrations. Nonetheless, new and improved technologies have emerged that are now capable of modifying DNA with greater precision and fewer collateral effects, with base editors [1], prime editors [2], and recombinase/integrase-based systems [3] representing three such examples.

The articles in this Special Issue highlight these and other advances in this rapidly growing field, a field whose evolution has been catalyzed in part by an improved knowledge of the mechanisms governing DNA repair, the development of sophisticated methods for characterizing editing outcomes, and the creation of new approaches for building better genome-editors.

For instance, while gene-editing tools are fundamental to the process of modifying a target DNA sequence, it is the cellular DNA repair mechanisms that, in most cases, are responsible for facilitating the change itself. For this reason, it's becoming increasingly essential for genome engineers to possess a detailed understanding of the DNA repair mechanisms triggered by the various classes of editors. With this in mind, Gvozdenovic et al. review current knowledge on the DNA repair pathways most relevant

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to emerging next-generation genome-modifying technologies and propose strategies for modulating these mechanisms to control specific outcomes [4].

Additionally, while next-generation genome editors offer improved specificity and, in some cases, nearly undetectable levels of editing at off-target sites, a major limitation in the field remains a lack of easy-to-implement approaches for reliably assessing these undesirable outcomes. In this Issue, Rao et al. describe the current landscape of methods for inventorying off-target effects for various next-generation editors, including base editors and prime editors [5], while Park et al. summarize advances in characterizing the large-scale genomic modifications that can often go undetected following an editing event [6].

Unsurprisingly, as the most commonly utilized next-generation gene-editing platforms are typically ribonucleoproteins, protein engineering has emerged as an exceptionally powerful approach for not only enhancing but also expanding the functional capabilities of these systems. These innovations are reviewed in this Issue by Winter et al. [7] Similarly, as computational tools are playing an increasingly central role in the development of new genome-modifying technologies, Fong et al. also discuss in this Issue the applications and limitations of machine learning-based models for genome engineering [8].

In contrast to these efforts, a distinct yet often equally successful approach for overcoming the limitations of existing gene-editing tools is the discovery of new systems from diverse microorganisms, many of which can function by unique and occasionally safer mechanisms, often with improved or expanded functional capabilities. Raftopoulou et al. provide a timely overview of the tools currently used to not only discover but also characterize and validate such unique systems [9].

When highlighting genome editing, it's impossible not to discuss applications, which are vast. As many biomedical applications of next-generation editors have been extensively covered in recent reviews elsewhere, we instead focus on two emerging applications: the development of diagnostic tools capable of rapidly detecting pathogens and genotypes [10] and the use of genome-wide screens to uncover genes and pathways involved in various physiological and pathological processes [11].

Finally, regardless of the application, an important challenge facing the effective implementation of any genome-modifying technology is its delivery. While genome editors can be delivered to cells as DNA, RNA or ribonucleoprotein complexes, each approach has its own distinct advantages and drawbacks. In this Issue, Kabadi et al. discuss advances in the implementation of viral-based systems for delivering genome editors, an approach that, while often superior in terms of efficiency to other delivery modalities, remains controversial in terms of safety [12]. Chen et al. in turn highlight recent advances, challenges, and emerging opportunities for non-viral-based methods for delivering editors [13], an area of considerable promise but one that still is hampered in many situations by inefficiency.

We are excited about the breadth of topics covered in this Special Issue and are eager to witness the next quarter century of growth for this field, a quarter century that we expect will continue to see genome engineering reshape the biomedical sciences.

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