CRISPR Challenges: Why This Transformative Technology Still Needs Improvement

The promise of CRISPR editing will not be realized without meaningful innovation in scalability, efficiency, and access

Performed responsibly, genome engineering has the potential to address challenges in how we feed, fuel, and heal humanity. Unfortunately, current editing techniques suffer from limitations in scalability, efficiency, and access that must be overcome in order for the research community to achieve this potential.

The goal of establishing biological engineering as a predictable science has been thwarted repeatedly by the inherent complexity of biological systems combined with our lack of understanding of their governing rules. In this situation, more rudimentary types of engineering — such as perturbing a system to study how it responds, or tweaking systems at the gene or protein level to see how changes affect phenotype — must suffice.

Forward biological engineering relies on iterative cycles of design, create, test, and learn (DCTL™). The key element for success with this approach is to create ample diversity to generate a range of variations in the phenotype of interest. Libraries of potential phenotype-altering edits must be large enough to increase the likelihood of hitting upon the right modification for the desired trait. Large-scale diversity generation should make it possible to test thousands, perhaps hundreds of thousands, of individual ideas rapidly and efficiently. Ideally, the most promising changes would then be recombined in new ways and tested many more times to identify optimized combinations and fine-tune the results as needed. For the best outcomes, this work must be equally feasible at the gene, pathway, and genome level.

The introduction of CRISPR gene editing methods has significantly advanced what is possible in biological engineering pipelines, allowing for precise intervention across the entire genome. CRISPR's relative ease of use and effectiveness, compared to previous editing techniques, have been transformative for the gene editing community.

Still, real improvement is required before genome-scale, CRISPR-based workflows can be as robust and reliable as they should be. In order for CRISPR to truly revolutionize genome engineering — and to serve as the much-needed cornerstone in biological engineering efforts to address major challenges in healthcare, agri-culture, and energy — it must become more scalable, efficient, and accessible.

CRISPR Successes

Today's CRISPR methods have been remarkably effective for certain uses. Single base editors, for instance, make it possible to introduce simple changes in many places across the genome. Knocking out small sections of DNA is also a tried-and-true method for generating useful diversity. For studying the results of a small number of edits or comparing the phenotypic outcome of one modification to another, CRISPR is an excellent choice.

Perhaps the most important success story from CRISPR so far, though, is the broader change it has delivered to molecular biology labs around the world. Since biology first became a scientific discipline, much of it has been observational in nature, particularly at the genome level. CRISPR has enabled an important shift in mindset, enabling genome biology to become a full-fledged interventional science. Scientists can now perturb a system by introducing precise changes throughout the genome and then analyze the effects of those changes. This is a major step toward mastering the rules of biology, which will fundamentally change what is possible through biological engineering.

But much progress is still needed to truly make the most of CRISPR technology.

Challenge: Scalability

The most urgent need in current CRISPR pipelines is a significant improvement in the ability to generate diversity in a scalable manner, which includes introducing both large numbers of edits as well as a broad variety of edit types. Scientists today must choose a small fraction of possible edits to test for their biological trait of interest; there is no feasible means of evaluating tens of thousands of genomic changes except for overly simplistic knockout screens or single-base editors.

Consider a common need in molecular biology laboratories: determining which previously identified mutations (of varying sizes) might be causal for a certain disease or trait. Often, scientists engaged in this process begin with a list of thousands of mutations that should all be recapitulated in a clean background for the most rigorous results. Since this is not possible with current CRISPR workflows, researchers instead are forced to gamble on testing just a few dozen, or at most a few hundred, and hope that the causal mutation happens to be among them.

Large-scale protein mutagenesis projects face the same dilemma. Scientific success hinges on being able to target as many proteins as possible across a metabolic pathway, for which researchers must have the capacity to make multi-base pair coding changes for amino acid mutations at all relevant points in the genome. Typically, though, experimental realities limit them to modifying just a few loci.

The diversity challenge affects not just how many edits can be made, but also the variety of edits that can be made. Recent projects have demonstrated that thousands of single-base edits can be made in one experiment. While this is impressive, it does not fully reflect the diversity of edits that should be tested in a robust editing pipeline. For a much deeper understanding of biology — and to rapidly advance genome engineering efforts — scientists must have the ability to introduce larger inserts or replace entire elements such as promoters, terminators, and transcription factor binding sites, to name just a few.

Finally, we must enable combinatorial editing to unlock the full potential of edit diversity, something that is not possible even at low throughput with today's methods. Combining mutations allows for testing of the effects of multiple edits at once. For example, researchers could test a terminator change paired with protein coding edits, bringing together the power of regulating expression and altering proteins in a single experiment. This best represents the process of natural sexual evolution and is most likely to deliver major improvements in genome function or enhancing a specific trait. It is also needed for characterizing important sequence-to-function relationships.

Challenge: Efficiency

As CRISPR editing becomes more widely adopted and the scale of experiments increases, it will also be important to improve efficiency in these workflows — from edit rate to trackability.

Efficiency in edit rate, or the fraction of cells in a population containing your target edit, is not a problem when editing is performed at low throughput. However, as researchers seek to scale their experiments, editing rates can drop precipitously. For pooled editing, the fraction of cells with a desired edit can be very low. This poor conversion translates to limited success in experimental outcomes. Inefficiency at higher throughput is currently a major problem for scientists interested in using CRISPR, and unfortunately it is one that is not well understood throughout the gene editing community.

When it comes to trackability, there are two distinct needs. First, there's the experiment-level tracking that must happen for robust scientific results. Each desired edit should be monitored to confirm that the CRISPR technique made the intended changes. With simple edits this is currently feasible, but as more complex edits and particularly as combinatorial editing become possible, today's tracking methods will not suffice. For example, combinatorial modifications may not permit the use of certain barcoding methods to monitor CRISPR edits. Multiplexed edits and iterative cycles of edits will only yield reliable results if scientists can reliably track all changes introduced throughout a genome.

In addition, tracking must occur at a higher level to ensure biosecurity and responsible use of CRISPR pipelines. Just as the gene synthesis community has coalesced around security guidelines about specific sequences that have been deemed unsafe to recreate, the gene editing community will likely also develop guidelines designed to reduce the risk of bioterror and other potential misuses of this technology. If that does not happen, regulatory agencies may step in and establish security frameworks to govern the use of CRISPR. Anyone involved in the routine application of CRISPR workflows — from tool or reagent vendors to scientists performing the work — should proceed with the expectation that biosecurity rules are coming, and do everything possible to ease their adoption.

Challenge: Access

Access to CRISPR technology is a multi-faceted problem for scientists today. While this gene editing method is simpler to use than previous techniques, such as zinc finger nucleases or TALENs, it is far from plug-and-play. Industrial-scale labs have little trouble committing the time and resources to set up and troubleshoot a CRISPR workflow, but many smaller labs find the challenges of implementing CRISPR to be remarkably onerous. The design processes involved for nucleases, cassettes, and guide RNAs are notoriously difficult and laborious.

The cost of running CRISPR experiments — particularly as researchers scale up from a handful of edits to something more ambitious — can also be prohibitive. This is true both for academic labs, where grant funding is often limited, and for biotech or bio-industrial labs, which face steep royalties for using the most well-tested CRISPR nucleases. Cas9 and other nucleases are patented and often command royalties of double-digit percentages, terms that are far too rich for many startup companies or even better-funded organizations. Due to ongoing legal disputes about which patents will hold up in court, there is a continual sense of uncertainty that keeps some corporations

from even attempting to adopt CRISPR technology.

A New Approach

Novel CRISPR-powered technology was designed to address the current limitations of gene editing. This system — consisting of a benchtop instrument, consumables, and software — is undergoing validation on projects in multiple academic and bio-industrial laboratories.

The technology, developed by Inscripta, Inc., dramatically increases the scale of Digital Genome Engineering, making it possible to perform CRISPR-based forward engineering experiments through high-throughput diversity generation and multiplexed combinatorial editing. The approach expands the number and variety of edits that can be made. It also allows for the development of machine learning models to formulate and test new hypotheses and generate new cycles of library designs.

Recent projects have made clear the potential of such a system. In one early experiment, scientists made more than 16,000 precisely selected changes across 19 genes in *E. coli* to assess the effects on lysine production. This increased the variant space covered by 1,000 times what was done in previous experiments, and did so in just 10% of the time. A subsequent experiment increased throughput even more, spanning 200,000 edits.

In another validation project, scientists conducted an experiment to recreate an earlier project to enrich for a certain trait in *E. coli*. The original project relied on random mutagenesis and identified almost 650 SNPs. The new experiment included that entire set of SNPs, and added 52,000 more. Importantly, many of the new edits consisted of multiple base pair changes, an edit type inaccessible through random mutagenesis or single-base pair editing technologies. Results confirmed the original findings but also identified other relevant edits, including the most successful — a two-base change that could not have been found with the original adaptive evolution technique.

The new benchtop system is easy to use, allowing even small labs to implement CRISPR workflows. It also incorporates biosecurity safeguards to promote responsible use of genome engineering.

Ultimately, this approach will give scientists a more comprehensive view of the phenotypes they study by allowing them to test far more genomic changes and to rapidly select the most effective ones. This should have far-reaching benefits for agricultural and bio-industrial science, healthcare, and alternative energy.

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