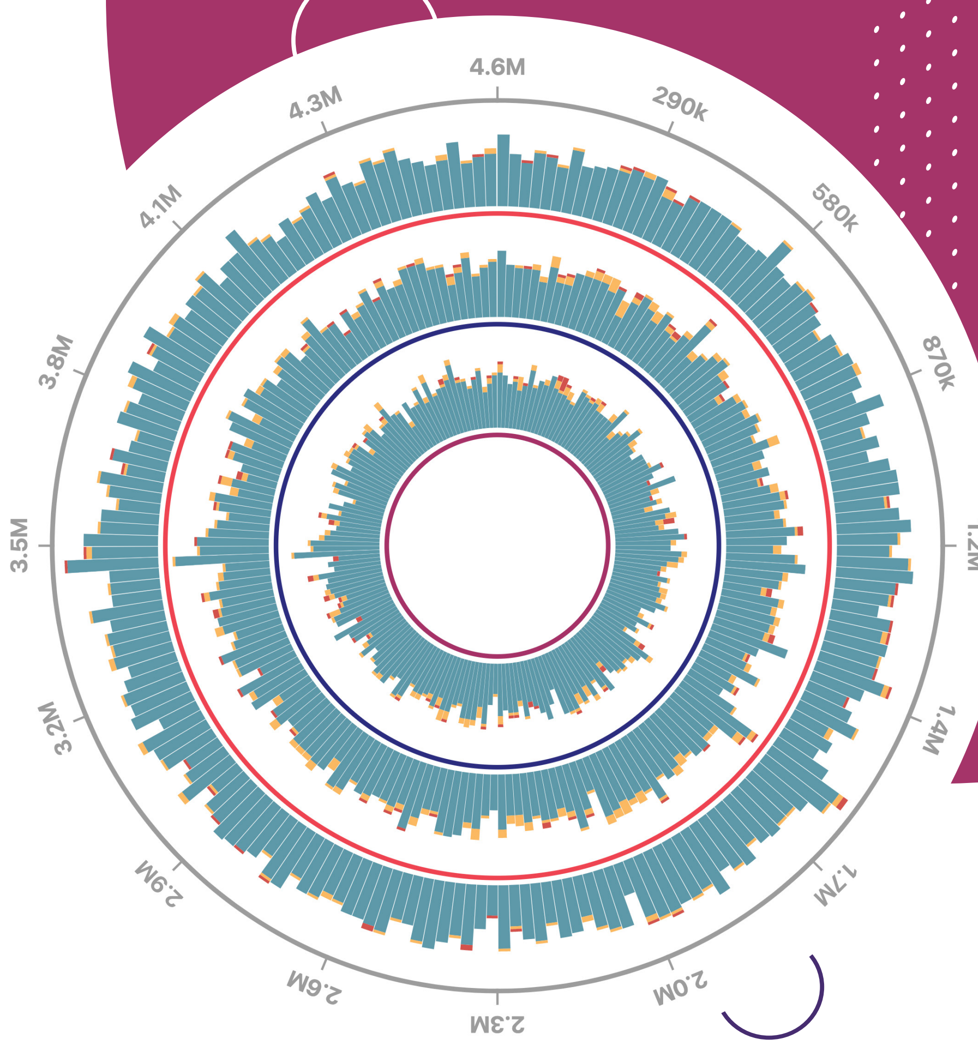
The New Era of CRISPR-based Genome Engineering



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Genome Editing at an Unprecedented Scale

While gene editing has always attracted interest for treating genetic diseases, scientists now use the technique to engineer genomes for many applications, including antibiotic discovery and enhanced metabolite production. Genome engineering has come a long way over the years, with very rapid and recent advances resulting from the advent of CRISPR gene editing technologies. Using these innovations, synthetic biologists are redesigning systems found in nature by making large functional changes in existing organisms to quickly develop new biological tools, products, and insights.

The CRISPR Revolution

While CRISPR sequences were first identified in 1987,[1] it wasn't until 2012 - when programmability was first demonstrated - that scientists began to co-opt this microbial defense system to rapidly edit nucleic acids. Researchers generally use a simple CRISPR system consisting of a Cas nuclease, typically Cas9, and a guide RNA (gRNA) for precise targeting. Once the gRNA-enzyme complex cuts genomic DNA, the cellular DNA repair machinery attempts to repair the double-stranded break resulting in an insertion, deletion, or compositional change in the existing sequence. CRISPR gene editing technology has been a game-changer in the genetic engineering field as researchers now make gene edits in record time. The field is increasingly advancing due to CRISPR gene editing tool updates, such as optimized Cas proteins with enhanced efficiency and decreased off-target effects.

Large-Scale Limitations

CRISPR-based gene editing has great potential, but current technologies are often inadequate for high-throughput applications such as genome-scale engineering because they are limited in their efficiency, scalability, and diversity of edit types. While a vast improvement on traditional mutagenesis techniques, CRISPR can still be laborious on the large scale, as researchers typically perform gene editing experiments in 96-well plates, with each well serving as a partition for a single set of editing reagents. Scientists individually load combinations of cells, gRNAs, and donor DNAs in separate wells. Even with the benefit of liquid handling automation, the effort required to engineer genomes using this approach creates a significant bottleneck in high throughput workflows.

While traditional CRISPR-based methods considerably speed up gene editing, they are insufficient for generating all of the intended diversity in a genome at the same time or within the same experiment. Generating diversity would ideally encompass a variety of edit types such as insertions, deletions, and substitutions across a genome. However, CRISPR methods are predominantly used only for gene knock-outs. Genome wide CRISPR methods are often used to make imprecise gene knockout libraries while more precise editing has largely been restricted to short tracts of DNA at a single locus.

A Solution for Genome Engineering and Discovery

The Onyx™ platform by Inscripta drives a CRISPR-based technology consisting of an instrument, reagents, and software that enables scientists to rapidly generate a variety of edits at genome scale in *Escherichia coli* and *Saccharomyces cerevisiae*.[2] This platform is unique as it fully integrates the end-to-end genome engineering workflow from electronic edit design, through DNA synthesis and cloning, to the delivery and analysis of engineered strains.

When working to solve a problem, synthetic biologists engage in a Design-Generate-Test-Learn (DGTL) cycle to engineer and improve upon biological solutions. The Onyx™ platform takes researchers through every step of the DGTL cycle faster than ever before.

In the first step, researchers use cloud-based InscriptaEngineer™ software to **design** their experiment by choosing their host strain, target sites to be edited (single genes, pathways, or even whole genomes), and the edit types (insertions, deletions, or substitutions) to be generated. Inscripta then manufactures the custom editing DNA libraries and sends them to the researchers, along with microfluidic components and reagents as a complete custom editing kit.

Next, researchers rapidly **generate** an edited cell library by loading the edit library, host cells, and kit components into the Onyx™ benchtop instrument and running the CRISPR-based reaction. Using cells as partitions and placing the CRISPR components on plasmids greatly improves the editing workflow as each cell is transformed with its own plasmid, which contains a unique target.[2] After 2-3 days of on-instrument run time, depending on the host organism, a library consisting of millions of precisely edited single cells with phenotypes of potential interest is ready to be analyzed.

Researchers move on to **test** the cell library for desired phenotypes, from growth experiments to desired product titers and beyond. The edits responsible for promising phenotypes are identified through sequencing of the edit-specific barcodes and analysis by InscriptaResolver™ software. Even screening just a portion of the cell library can identify constructs with significant improvements or novel biological functions.

Finally, using InscriptaResolver software, researchers analyze their data, compare results from multiple experiments and understand the impacts of different genetic edits to **learn** and extract value from their data sets. Iterative turns through the DGTL cycle enable the researcher to further improve cellular capabilities and rapidly evolve strains of interest by producing large functional changes in biological systems.

References

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Design – Generate – Test – Learn – Repeat

New CRISPR-based editing technology is revolutionizing synthetic biology. Scientists can now rapidly engineer cell libraries with multiple edit types throughout the genome. Multiple iterations of the Design-Generate-Test-Learn cycle have the potential to deliver huge phenotypic leaps in extremely short periods of time.

Design

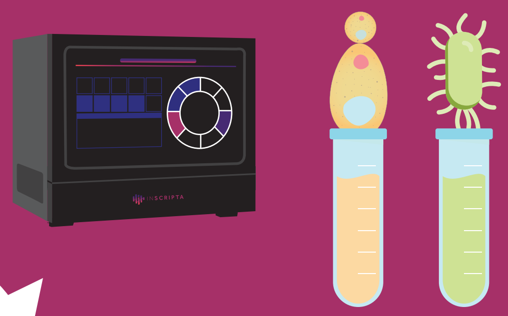
Utilize InscriptaDesigner™ cloud-based software to design a genome engineering edit library. Multiple edits can be made in single genes, pathways, or genome-wide.

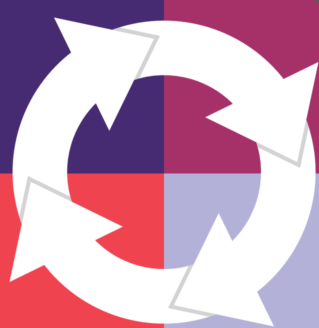
- Select a host strain and targets for editing
- Design the edit library
- Order reagents

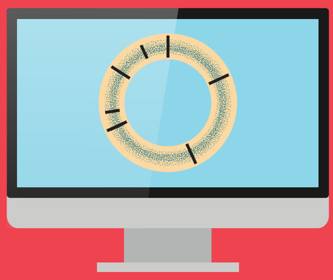
Generate

Run the automated Onyx™ instrument to perform CRISPR-based genome editing on the benchtop.

- Load reagents and cells to be edited
- Start the run
- Collect the edited cells in 2-3 days



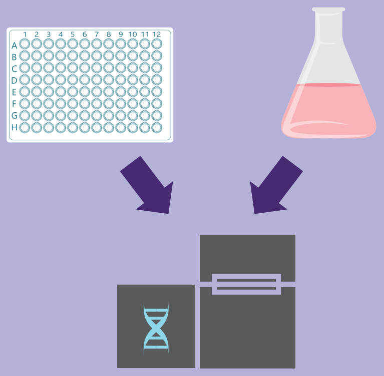




Learn

Use InscriptaResolver™ cloud-based software to identify edits of interest and guide the next experiment.

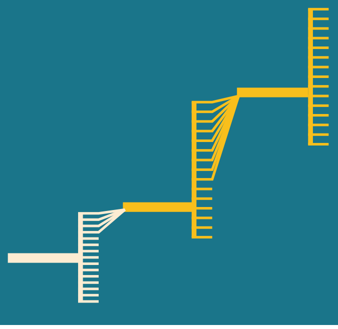
- Connect results from multiple experiments
- Devise combinatorial optimization strategies
- Unpack the phenotypic contributions of different genetic edits



Test

Employ Onyx™ assays and InscriptaResolver™ software to assess the genome engineering metrics and identify edits of interest.

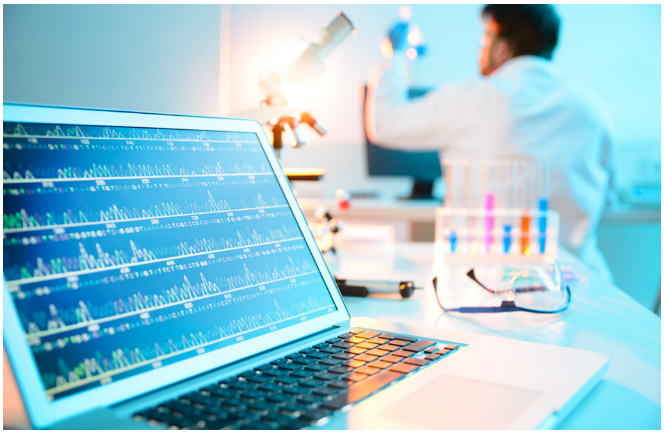
- Analyze cell library composition
- Screen the edited cell library for desirable phenotypes
- Sequence barcodes or the microbial genome to identify edit



Repeat

Perform multiple iterations of genome editing and screening to rapidly make large phenotypic leaps.

- Quickly combine multiple edits providing desired phenotypes
- Engineer an optimized strain in record time



Evolution on the Benchtop

Synthetic biologists create new products with scientific or commercial value; however, forward biological engineering is a challenge because of the sheer scale of editing and analyzing entire genomes. While the Design-Generate-Test-Learn cycle helps scientists organize the steps involved in genome engineering, deciding which genetic changes will generate the largest phenotypic benefits is a daunting task. To be successful, scientists require mechanisms for large-scale diversity generation and evaluation. Whether researchers are trying to optimize a known phenotype or engage in hypothesis-free discovery, they can quickly obtain large phenotypic leaps by creating a variety of gene edits throughout a genome and recombining or further editing organisms.

Uncovering Potential with Diversity Generation

Many secrets of the genome remain hidden, even in well-studied organisms, such as *Escherichia coli* and *Saccharomyces cerevisiae*; therefore, there is great potential for discovery in forward biological engineering experiments. To optimize biological systems and uncover novel biology, scientists must first make a large number of mutations to generate testable diversity throughout a genome.

When designing genome editing experiments, scientists must choose their edit locations and decide how they will screen those engineered variants for positive hits. One common strategy is to use prior knowledge to make a range of edits in specific genomic locations and screen all of those variants. This strategy typically maximizes the hit rate, but often produces a low number of total hits. Alternatively, maximizing the total number of hits can be a powerful diversification strategy. By creating genome-wide edits coupled with an efficient screening method, the success rate may be low, but the number of positive hits will often be higher than the higher hit rate strategy due to the sheer number of variants tested.

Depending on an organization's screening resources, testing thousands of strain variants may be functionally out of reach; however, researchers can also discover desirable phenotypes in large and diverse genetic populations by cultivating and analyzing pools of edited strains.

Rapid Evolution Through Combinatorial Optimization

By combining positive hits, researchers can rapidly generate organisms that have made large phenotypic leaps. Scientists can perform iterative cycles of combinatorial optimization, where they repeatedly combine edits from strain variants with desirable phenotypes and rapidly create increasingly fit offspring.[1] This strategy essentially mimics evolution by natural selection, but does so rapidly and on the benchtop, as rounds of recombination, like sexual reproduction, mates the DNA of organisms with desired traits.[2] In addition, using the Onyx™ platform, scientists can further infuse the system with additional genetic diversity by performing genome-wide editing all over again on an interesting variant and continue screening for strains with increased fitness.

References

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Learn More About Forward Engineering in Biological Systems

Genome-Wide Precision Editing Enables Endless Applications

Synthetic biologists apply genome engineering to numerous fields, including chemicals, fuels, agriculture, and healthcare, to produce new products and renewable resources. The use of CRISPR editing methods has accelerated the discovery of useful strain variants and has enabled the rapid improvement of existing processes.

Improved Growth Inhibitor Tolerance

Microbial fermentation produces a variety of valuable commercial chemicals ranging from pharmaceuticals and food products to fuels and materials. The sometimes harsh media conditions of these fermentations and the metabolic imbalances of engineered strains pose a major challenge to the economics of many commercial fermentations. Scientists can now apply CRISPR-based methods to rapidly develop bacterial strains with tolerance to growth inhibitors or restored metabolic flux imbalances to improve the productivity of a wide variety of fermentation processes.

In one example, researchers performed massively parallel genome engineering using Inscripta's Onyx™ technology to enhance *Escherichia coli* growth in the presence of inhibitory compounds common in biomass hydrolysates.[1,2] Using the Onyx technology, scientists designed six edit libraries—one knockout and five constitutive synthetic promoter insertions with increasing expression strengths (promoter ladder)—targeting all 4,336 annotated protein coding *E. coli* genes. Once the edited cell libraries were generated, the researchers pooled the resulting cells and inoculated them into flasks containing media with one of the inhibitory compounds. After a growth period, they sequenced DNA isolated from each flask across the identifying barcode region to determine which edits conferred resistance to each compound.

For the inhibitor furfural, scientists found that alterations in at least a quarter of the *E. coli* genes led to either an increase or a decrease in growth. While some of these genes were previously identified for roles in furfural tolerance,[3,4,5] many were novel.[1] Additionally, compared to control growth conditions, 246 genes that conferred growth inhibition in furfural when knocked out also resulted in a growth benefit when over-expressed, suggesting that the edits successfully saturated the genome.[1]

Increasing Lysine Output

Commercially produced lysine by microbial fermentation is an important addition to animal feed; therefore, scientists are engineering the lysine metabolic pathway in bacterial strains to increase production. Researchers used the Onyx™ technology to produce knockout and promoter ladder edit libraries to rapidly optimize the *E. coli* lysine pathway in a single experiment.

Researchers made 200,000 edits in coding and non-coding regions of the *E. coli* genome, and then screened those edited cell libraries for lysine over-producers that grew in the presence of a toxic lysine analog, S-(2-aminoethyl)-L-cysteine (AEC). After barcode sequencing of the treated and control populations, investigators identified edits in numerous genes that imparted the desired phenotype. In addition to finding hits within the known lysine biosynthesis pathway, researchers found edits in 17 uncharacterized genes with no known function that increased lysine production.[6]

Additionally, individually beneficial edits were then serially combined onto a top single-edit strain containing a high strength promoter in front of known lysine pathway gene *dapA*. After two combinatorial cycles, the original *dapA* promoter variant strain carried three total edits and produced 14,000-fold greater lysine than wild type *E. coli*.[6]

Antibiotics and Beyond

Synthetic biologists are also using modern genome engineering strategies to identify new bacterial targets for antibiotic development, a much-needed area of study considering the ongoing antibiotic-resistance crisis. Antibiotic mechanisms of action are complex, from inhibition of metabolic activity to damaging cell components. A high-throughput approach using pooled edit libraries enables scientists to find bacterial genes essential for conferring antibiotic resistance as possible new antibiotic targets.

Scientists hope to discover previously unknown resistance mechanisms using CRISPR-based techniques.

Even well-studied genomes, such as that of *E. coli,* contain many mysteries. By employing massively parallel genome engineering, scientists can uncover new functions and make large phenotypic leaps that lead to novel discoveries. High-throughput CRISPR-based techniques combined with iterative DGTL cycles speed up genome evolution to an unprecedented rate.

References

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Learn More About Onyx™ Applications

Any edit. Any location.

The Onyx™ Digital Genome Engineering Platform looks like nothing in your lab—because it IS like nothing in your lab. Now you can design, engineer, evaluate and track results, right on your benchtop.

Unprecedented scale

Build the largest massively parallel, multiplexed libraries.

Make diverse edits including insertions, deletions, substitution and more.

Rapidly connect multiple experiments, leveraging machine learning processes.

Superior performance

High edit rates ensure your designs are realized.

Barcode every cell, track every edit.

Rational design approach affords you precise control.

Greater access

The Onyx workflow seamlessly integrates software, consumables and instrument into a fully automated solution that requires minimal gene-editing expertise.

Inscripta's favorable licensing terms ensure that scientists own their discoveries and subsequent inventions.

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