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Inscripta Looks to Combine CRISPR, Single-Cell Genomics, Synthetic Biology in New Platform

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NEW YORK – Kevin Ness, the CEO of gene editing company Inscripta, has long had a vision of doing for genome writing what Illumina did for genome reading — to make genome engineering as innovative, as scalable, and as widespread as genome sequencing is today.

To do that, Inscripta has been developing an integrated suite of software, reagents, nucleases, and an automated benchtop instrument that are designed to work together in a way that seems to combine the principles behind CRISPR gene editing, single-cell genomics, and synthetic biology into one technological platform.

That would make sense, considering the expertise the company's officers bring to the table. Ness himself cofounded QuantaLife (which was later acquired by Bio-Rad) and 10x Genomics. And the chairman of the board is John Stuelpnagel, cofounder and first CEO of Illumina.

The firm started slowly — in December 2017, it released a new CRISPR enzyme it had developed free of charge to the research community. The enzyme, called MAD7, is part of the so-called Madagascar family of novel RNA-guided nucleases that Inscripta is developing. MAD7 was initially characterized in *Saccharomyces cerevisiae* and *Escherichia coli*. The firm made it available for research purposes with no upfront licensing fees, and said it would charge a nominal fee if the enzyme was used in the manufacturing of a product.

The response was huge, according to Ness. In March 2018, he told GenomeWeb that the idea with the MAD7 giveaway had been to put gene editing and engineering tools in the hands of researchers who too often couldn't get access to them.

"What we're trying to do is liberate the scientific community as a whole — not just academics, but also the commercial sector — and really spur innovations. And so instead of hoarding this technology, we decided to put it out there for full open access," he said at the time.

It was part of a carefully crafted business strategy. "If we can spark and create more genome editors now, later we can launch our full tool set and solution, and there'll be a market and more customers," Ness added.

In fact, that strategy is starting to pay off as researchers are beginning to publish studies on their own discoveries using MAD7. Most recently, a team from the Mayo Clinic and Iowa State University posted a paper on the BioRxv preprint server on their work using MAD7 in zebrafish and human cells.

In July 2018, Inscripta announced that it had successfully characterized MAD7 in mammalian cells, and subsequently released a new enzyme in the Madagascar family called MAD2. Importantly, Inscripta used the expertise of its customer base to improve its own product and give the customers what they wanted — working with the research

community to use, test, and improve MAD7, and send specific feedback to the company, rather than company executives trying to figure out what their customers wanted and then selling it to them.

Further, the mammalian characterization of MAD7 and the release of MAD2 were steps in getting Inscripta to its ultimate goal: creating a full suite of gene editing tools, including instruments, software, and reagents that all work together "to do the next generation of gene editing experiments that can't even be done today," Ness said at the time.

Now, Inscripta is taking the next step in what will eventually be the full release of its benchtop Digital Genome Engineering platform — the company's Executive Director of Data Science Richard Fox has released results from a 200,000-edit library that was used to make a large variety of edit types to an *E. coli* biosynthesis pathway.

Though Inscripta's focus remains on gene editing, this new glimpse at the overall technology strategy shows the company widening its focus to make it more about genome engineering at a large scale.

"You have many spots of the genome where, at each spot, you have the full flavor of genetic perturbation or change, and doing that with many experiments all at once where all of those experiments can be tracked — and that's ultimately the integrated platform that we want to put forward," Ness said in an interview this week. "This platform gives you a holistic toolset to hit the genome at many spots so that you can ultimately engineer biology for a broad set of applications."

Overall, Inscripta's idea for a genome-editing platform is technology that writes genomic modifications into any location within the genome in a rationally controlled and precise manner, with no random side effects.

"We've designed our platform to be compatible with that and to [produce] the full flavor of edit types," Ness said. "You can't just do knockouts to take your wild-type to the phenotype you care about. You have to have insertions, deletions, swaps. And it's probably going to be combinations of knockouts and up-and-down regulation, insertions, deletions, swaps. We've designed the platform to be there."

The platform is also high-throughput and as cost-effective as possible, enabling researchers to do multiple rounds of editing. Finally, he added, the platform integrates unique, readable barcodes into each edit, so that researchers can track and monitor their edits and designs in each genome, and feed the most successful ones into the next round of editing. That tracking system is also machine-learning compatible.

"We've put all of that into an easy-to-use single push-button system that the basic researcher could use, so that everyone across the global community gets the same data," Ness said. "You don't have to be an expert to use this."

In delving into the details, he described how the system is designed to work at the single-cell level. Although it's compatible with 96-well plates, the platform is essentially meant to turn each well in that plate into its own plate, and each cell in that well into its own well. In other words, rather than running 96 experiments in each plate, if each well contains 100,000 cells, then Inscripta believes that its system can be used to run 100,000 separate gene editing experiments in each well.

This is achieved through its integrated, optimized set of reagents, guide RNAs, nucleases like MAD7, and tracking barcodes that have been designed to work together in large editing libraries. Further, Inscripta has engineered homology arms that are covalently linked to the guide RNAs and the nucleases, ensuring an optimized version of homology-directed repair (HDR) once an edit is made in the genome of the target cell.

In eukaryotic cells, the repair of double-strand breaks occurs primarily either through HDR — in which a donor template of DNA or sequence homology is used to repair the break and thereby create a specific and new genotype in the cell — or through non-homologous end-joining (NHEJ). NHEJ is a homology-independent pathway ion which the two ends of broken DNA join together, and typically occurs much more frequently than HDR, even when a donor template is present. Inscripta claims to have optimized its genome editing system to circumvent the unreliable nature of HDR.

The Inscripta genome editing CRISPR complex is built to include a nuclease such as MAD7 to do the cutting, repair machinery, and an Inscripta library that is a molecule made up of a guide RNA, a homology arm, and the company's tracking barcodes covalently linked together. Tens of thousands of these libraries can be put into one well in each plate.

In order to optimize the repair mechanism, the company's researchers worked to ensure that they had the correct concentration of reagent at the right location of the cell at the right time, and to maintain those factors in a kinetically constant state, Ness said.

"It is a challenge and it is one of the main hurdles that we've overcome," he added. "Our platform is software, instrument, and reagents, so we get to control things in a manner that most other CRISPR approaches don't because they usually don't have all three of those handles."

While such a system could theoretically be designed to work with naturally occurring nucleases such as Cas9 or Cas12, the kinetics and time constants have been optimized for Inscripta's Madagascar nucleases and would have to be realigned for other types of enzymes.

"We're introducing single-cell writing technology," Ness said.

For Fox, the power of this technology was made apparent when he used it in a specific protein engineering experiment that he said would not have been possible without the Inscripta platform.

Directed evolution is a method used in protein engineering that mimics natural selection. It consists of subjecting a gene to iterative rounds of mutagenesis, selection, and amplification. But it can be tedious and hard to do, and it's impossible to find all beneficial variants in a given protein strain, given that most proteins have about 10^{390}variants. That problem is compounded enormously when applied to entire gene pathways or whole genomes.

When DNA shuffling — a technique that allows for accelerated and directed protein evolution *in vitro* and combines independently isolated mutations from various genes into a single progeny — and machine learning are applied, the process of finding beneficial mutations in proteins becomes somewhat easier. The real trick is being able to apply those same principles of efficient protein engineering to pathways and entire genomes.

"For proteins, you can do some reasonably good editing at scale, but it's just one locus in the genome. A full editing capability would allow you to intervene at the protein level, but also all kinds of other elements within the genome, whether they're ribosome-binding sites, or promoters, or terminators," Fox said. "You want to be able to make both small edits and large edits for fully unlocking the variety of edit types that are going to drive biology in ways that are important to you, [in a] number of disparate targets in the genome."

Right now, he added, the available gene editing and engineering tools will allow for a low number of edit types at a low number of genomic locations through knock-ins and knock-outs; a high number of edit types at a low number of genomic locations through protein engineering, directed site mutagenesis, and expression engineering; and for a low number

of edit types at a high number of genomic locations through genome-wide base editing and genome-wide knock-out screens. What's missing is the ability to do a high number of edit types at a high number of genomic locations, and Inscripta is aiming to fill that gap.

The large-scale experiment the company chose to perform to demonstrate the capability of its platform involved engineering *E. coli* to efficiently produce the amino acid lysine. It's considered a challenge to engineer bacteria that will efficiently produce large amounts of lysine, but the amino acid is a billion-dollar commodity and is added to everything from animal feed to certain medications.

As such, the lysine biosynthesis pathway in *E. coli* has been well studied for decades. "What's typical in any kind of metabolic engineering project is to go after the protein or proteins that are of interest, and we certainly did that," Fox said. "But we also wanted to emphasize and explore the power of going genome-wide."

So, Fox's team at Inscripta plumbed the literature for studies detailing *E. coli* variants that are known to affect the efficiency of lysine biosynthesis, and then took an agnostic, genome-wide approach, adding thousands more variants, including knock-outs and a ladder of promoter insertions for every gene in *E. coli.* The resulting libraries created 200,000 edits in the *E. coli* genome.

The resulting engineered *E. coli* cells were several dozenfold to several hundredfold improved over the wild-type starting strain, Fox said. While some of those improvements came from edits to dapA, which is a well-known target in the lysine pathway, others came from other proteins — including 17 new targets the team identified outside the lysine pathway — as well as a variety of edit types, rather than just knock-ins or knock-outs.

"We have amino acids, we have frameshifts, we have promoters, we have stops, we find them all and we find them not only within the pathway, but we also find them genome-wide," Fox said. "This is really exciting because all this diversity that has heretofore been locked up from us is now available for forward engineering."

The time it took to do all this was about two months, Fox said, including designing, building, and performing quality control measures on the libraries. Eventually, such a process could take less than a month, he added, noting that such a screen would technically be impossible to do with currently available technology.

One early-access user of the technology estimated that building a library of only 5,000 edits that would add promoter swaps to every essential gene in the *E. coli* lysine pathway would take approximately three man-years of a postdoc's time, Inscripta Chief Commercial Officer Jason Gammack noted.

"This goes to the dilemmas of the imagination. When I asked [the early-access customer] how he would think about designing this experiment, he simply said he wouldn't because he couldn't actually execute the experiment," he added. "So, this shows the limitations of the current technology."

For Ness, the possibilities for this kind of technology are almost endless. Enabling scientists to create genomes with the types of mutations they want in an almost unrestricted manner could allow Inscripta to play in dozens of markets in the US economy over the next 25 years, some of them worth billions of dollars. Some of these markets — such as pharmaceuticals, waste management, cosmetics, or alcohol production — may seem obvious. But if Inscripta's genome engineering technology works as the company claims it does, it has the potential to branch out into less obvious markets such as materials production, airlines, and cars.

Gammack noted that early research is being done on high-tech polymers that are applied to the outsides of airplanes to reduce drag. These polymers are made from monomers that are then polymerized to resemble shark skin. In another example, he added, yeast could be engineered to make bricks, which could be used to build homes in developing countries or resource-poor areas.

"Only the limits of the imagination are in play," he said. "All of these areas are currently being touched, but only really scratched because of the current limitations of the technology."

However, Gammack added, in addition to opening the door to multiple billion-dollar markets, the platform is also a "hypothesis-validation machine," designed to make basic science research easier and more efficient. "[Researchers] want to do that genotype-phenotype association. They want to quickly iterate on their phenotypes. Our platform technology will allow that to happen as well."

The final part of the Inscripta platform — the benchtop instrument — is slated to be released in the fourth quarter of this year, though some early-access customers are using it now, Ness and Gammack said. In fact, early access users include pioneers in the field of CRISPR research, such as the Broad Institute and the University of California, Berkeley.

The company's barcodes are readable on standard sequencers, however, and the rest of the software-nuclease-reagent library packages are made to work on lab equipment that's currently available.

"It's a really powerful platform, and we're getting it out to the world," Ness said, adding that the power of the technology will "start to displace the existing technologies that will kind of become antiquated."