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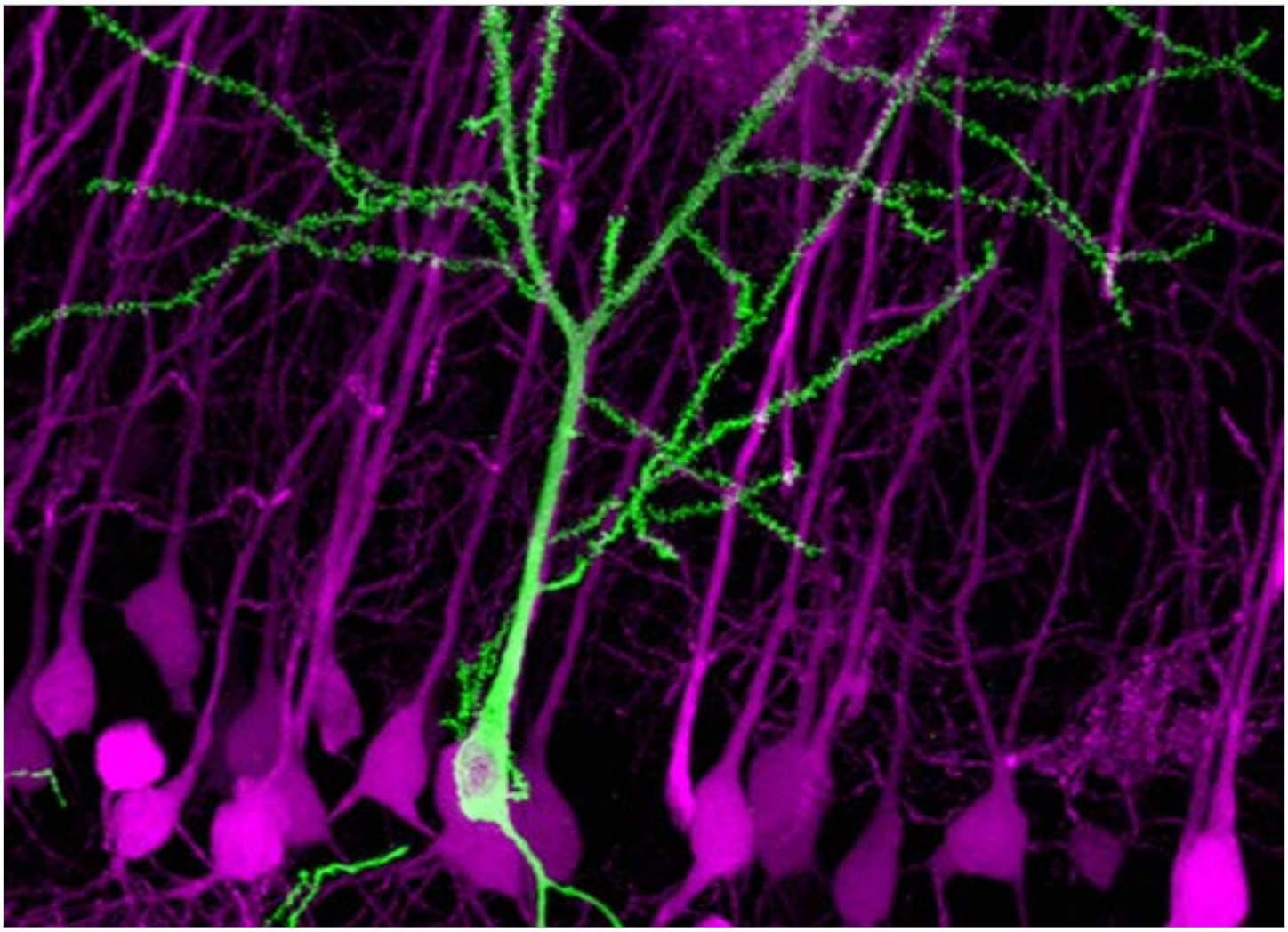
Genetic Engineering & Biotechnology News

The Best of Genome Editing 2016



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Virus Hacks Host Genome, Steals CRISPR to Protect Itself

http://www.genengnews.com/gen-news-highlights/virus-hacks-host-genome-steals-crispr-to-protect-itself/81252833/?kwrd=genome

Viruses have often been described as the ultimate parasite, shedding all of their nonessential parts and leaving behind an extremely efficient genetic transfer apparatus. Phage viruses have evolved to infect various bacteria proficiently and hijack their replication machinery to make more viruses. Yet, this often doesn't preclude a different virus from concomitantly infecting the same bacterium and competing with or overtaking the original phage invader.

However now, researchers from the University of British Columbia (UBC) have recently described their findings surrounding a phage virus that infects major freshwater bacteria, which appears to use stolen bits of immune system DNA to highjack its host's immune response. Specifically, the investigators found that cyanophage N1 carries a CRISPR DNA sequence, which is generally used by bacteria to fight off viral infection.

"Here we present the first genomic characterization of viruses infecting Nostoc, a genus of ecologically important cyanobacteria that are widespread in freshwater," the authors wrote. "Cyanophage N-1 encodes a CRISPR array that is transcribed during infection and is similar to the DR5 family of CRISPRs commonly found in cyanobacteria. The presence of a host-related CRISPR array in a cyanophage suggests that the phage can transfer the CRISPR among related cyanobacteria and thereby provide resistance to infection with competing phages."

"This is the first evidence we've seen that a virus can donate an immunity system via CRISPR," explained senior study author and University of British Columbia virologist Curtis Suttle, Ph.D. "This is like a hacker compromising a computer system,

Light micrograph of cyanobacteria from the Anabaena genus. [DOE]

and then immediately patching it to ensure other hackers can't break in."

CRISPR's— clustered regularly interspaced short palindromic repeats— are libraries of DNA typically used by bacteria to identify, and then destroy, infecting viruses. In the past several years, researchers have adapted the bacterial defense mechanism as a gene editing and manipulation technique with great therapeutic potential.

The UBC team stated that the N1 virus likely uses the theft to help cyanobacteria—from the ecologically important Nostoc and Anabaena genera—to fight off infection by other viruses, while continuing to be a suitable host for itself.

"Bacteria and their viruses have a shared evolutionary history stretching for billions of years," Dr. Suttle

noted. "So at some point along the way N1 stole a defensive CRISPR array from Nostoc or a close relative."

The findings from this study were published recently in mBio in an article entitled "Viruses Infecting a Freshwater Filamentous Cyanobacterium (Nostoc sp.) Encode a Functional CRISPR Array and a Proteobacterial DNA Polymerase B."

Both groups of cyanobacteria are abundant members of habitats ranging from the bottom of ice-covered polar lakes and hypertrophic coastal lagoons to rice paddy soils and rock-pool communities. Their ancestors were responsible for producing the oxygen on Earth, and close relatives of Nostoc are still responsible for providing a huge portion of the air we breathe.

"Filamentous cyanobacteria belonging to the genus Nostoc are widespread and ecologically important in freshwater, yet little is known about the genomic content of their viruses," the authors remarked. "This first genomic analysis of cyanophages infecting filamentous freshwater cyanobacteria reveals that their gene content is unlike that of other cyanophages." GEN

A New CRISPR System for RNA

http://www.genengnews.com/gen-news-highlights/a-new-crispr-system-for-rna/81252789/?kwrd=genome

While the CRISPR/Cas9 genome editing system has been "burning up the charts", so to speak, with its extraordinary versatility and potential for treating a host of diseases, until now its editing capabilities have been limited to DNA. Whereas DNA editing makes permanent changes to the genome of a cell, a CRISPR-based RNA-targeting approach would allow investigators to make temporary changes that can be tuned up or down, and with greater specificity and functionality than existing methods for RNA interference.

View the video now

Now, researchers from the Broad Institute of MIT and Harvard—led by Feng Zhang, Ph.D., who aided in the initial developments CRISPR/Cas9—Massachusetts Institute of Technology, the National Institutes of Health, Rutgers University-New Brunswick, and the Skolkovo Institute of Science and Technology have published data that characterizes a new CRISPR system that targets RNA, rather than DNA—opening up a powerful avenue in cellular manipulation.

View the video now

The research team was able to identify and functionally characterize C2c2, an RNA-guided enzyme capable of targeting and degrading RNA. Their findings revealed that C2c2—the first naturally occurring CRISPR system that targets only RNA to have been identified and initially discovered by this collaborative group in October 2015—helps protect bacteria against viral infection. The scientists were able to demonstrate that C2c2 can be programmed to cleave particular RNA sequences in bacterial cells, making it a valuable addition to the molecular biology toolbox.

"C2c2 opens the door to an entirely new frontier of powerful CRISPR tools," explained co-senior study author Feng Zhang, Ph.D., a Core Institute Member

of the Broad Institute. "There are an immense number of possibilities for C2c2, and we are excited to develop it into a platform for life science research and medicine."

The RNA-focused action of C2c2 complements the CRISPR/Cas9 system, which targets DNA, the genomic blueprint for cellular identity and function. The capacity to target only RNA, which helps carry out the genomic instructions, offers the ability to manipulate RNA precisely in a high-throughput manner—and manipulate gene function more broadly.

The findings from this study were published recently in Science in an article entitled "C2c2 Is a Single-Component Programmable RNA-Guided RNA-Targeting CRISPR Effector."

"The study of C2c2 uncovers a fundamentally novel biological mechanism that bacteria seem to use in their defense against viruses," noted co-senior author Eugene Koonin, Ph.D., senior investigator and leader of the Evolutionary Genomics Group at the NIH. "Applications of this strategy could be quite striking."

Currently, the most common technique for performing gene knockdown is small interfering RNA (siRNA). According to the research team, C2c2 RNA-editing methods suggest greater specificity and hold the potential for a wider range of applications. For example, adding modules to specific RNA sequences to alter their function—i.e., how they are translated into proteins—would make them valuable tools for large-scale screens and constructing synthetic regulatory networks. Moreover, researchers could harness C2c2 to tag RNAs fluorescently as a means to study their trafficking and subcellular localization.

View the video now

In the current study, the team was able to target precisely and remove specific RNA sequences using C2c2—lowering the expression level of the corresponding protein. This suggests C2c2 could represent an alternate approach to siRNA, complementing the specificity and simplicity of CRISPR-based DNA editing and offering researchers an adjustable gene "knockdown" capability using RNA.

C2c2 has some key advantages going for it that should easily enable it to be developed into a major molecular tool: C2c2 is a two-component system, requiring only a single guide RNA to function, and it is genetically encodable—meaning the necessary components can be synthesized as DNA for delivery into tissue and cells.

"C2c2's greatest impact may be made on our understanding the role of RNA in disease and cellular function," remarked co-first author Omar Abudayyeh, a graduate student in Dr. Zhang's laboratory. GEN

CRISPR Crossing New Barriers

http://www.genengnews.com/insight-and-intelligence/crispr-crossing-new-barriers/77900666/?kwrd=genome

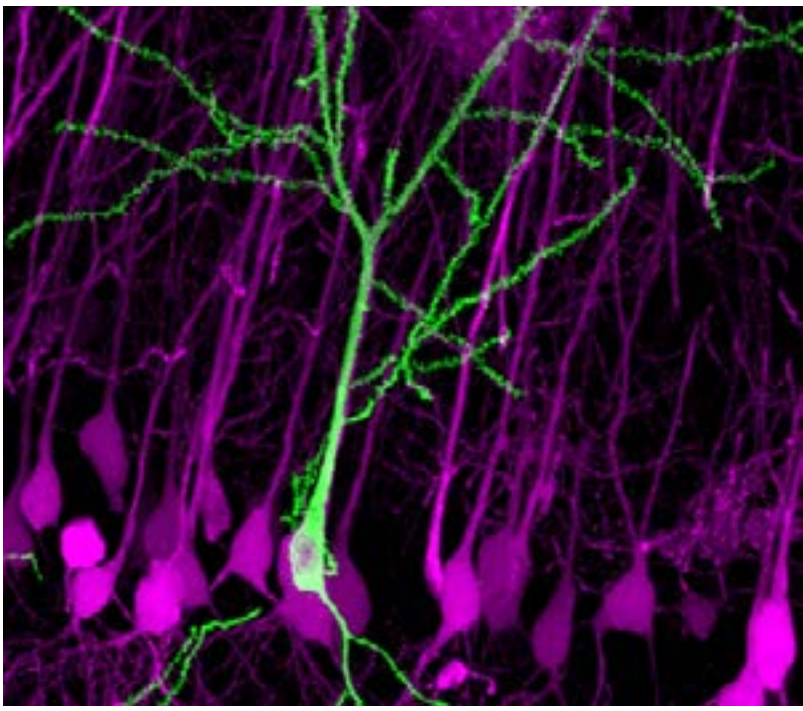
Researchers Are Developing Ways to Edit Some of the Most Difficult-to-Edit DNA-Neuronal DNA

Emma Yasinski

Ryohei Yasuda, Ph.D., scientific director, and his team at the Max Planck Florida Institute of Neuroscience (MPFI) are working to understand the way individual cells in our brains change as we learn and form memories. One of their main goals is to understand how different proteins behave and impact the structure and function of an individual cell, but, much like the field of genetics was once limited by the inability to visualize the structure of DNA, their research has been limited by their ability to locate and visualize the many different types of proteins within a single cell. Current imaging methods do not provide contrast and specificity high enough to see distinct proteins. Plus, the best methods are time-consuming and expensive; it can take a year or more to develop engineered models.

Over the past few years, the development of CRISPR technology has helped scientists overcome countless genetic engineering challenges, and allowed them to edit genes with unmatched precision and speed, massively increasing clarity and cutting the cost of research requiring genetic engineering. The technique has been used in myriad ways to increase understanding and treatment of diseases and disorders, but some cells are more difficult to edit than others. Brain cells have proven especially difficult to manipulate using CRISPR.

Recently, MPFI researchers Takayasu Mikuni, Ph.D., M.D., and Jun Nishiyama, Ph.D., M.D., and Dr. Yasuda were able to harness the power of the CRISPR/Cas9 system in order to create a quick, scalable, and high-resolution technique to edit neuronal DNA, which they called "SLENDR," (single-cell labeling of endogenous proteins by CRISPR/Cas9-mediated homology-directed repair.) Using the technique, the researchers labeled several distinct proteins with fluorescence, and were able to observe protein localization in the brain that was previously invisible. That's just the start of what researchers may be able to accomplish using this reliable, new technique for inserting genes into neurons.



Confocal microscopic image of the hippocampus showing immunoreactivities for mEGFP (magenta) and the HA tag (green) fused to ß-Actin.

CRISPR/Cas9 and Neurons

CRISPR is a tool built into bacterial DNA that the organisms use to fight infections. When a virus invades and attempts to insert its infectious DNA into that of a bacterial cell, a special section of the bacterial DNA, called CRISPR, cuts the viral DNA and renders it unable to wreak havoc on the bacteria. The organism then inserts a copy of the viral DNA into its own DNA to work as a type of adaptive immune system, to better recognize and defeat the invader in the future. As scientists have begun to understand how this system works, they have manipulated it to target and damage specific, functional genes in a variety of organisms, and in some cases, insert a new gene in its place.

Once the section of DNA is damaged, the technique relies on the cell to naturally repair its own DNA. There are two methods that the cell might use to accomplish this. One is homology-directed repair (HDR), the other is non-homologous end joining (NHEJ). HDR rebuilds or replaces the damaged locus of the genome, whereas NHEJ reattaches the damaged ends. When the reattachment occurs following the degradation of the ends, it often leads to the deletion of function of the gene ("knock-out" the gene). If a cell uses HDR to repair itself, scientists can include a desired gene in the CRISPR system that will be inserted into the DNA to replace the damaged gene.

Despite the impressive power of CRISPR system, its use in brain cells has been limited because by the time the brain has developed, its cells are no longer dividing. Most mature brain cells will repair themselves using NHEJ. The researcher can't give the cell a gene to insert if it's not going to insert one to begin with. While scientists can use CRISPR relatively easily to damage and knock out certain genes through NHEJ in the brain, the lack of cell division has made it very difficult for them to knock in desired sequences to genes, through HDR, with reliable precision. That's where the SLENDR technique comes in.

SLENDR

SLENDR combines the power of the CRISPR/Cas9 system with the specificity and timing of in utero electroporation. Electroporation is a well-known technique used for introducing new material into cells and creating genetic knock-outs and knock-ins. Using in utero electroporation allows researchers to insert the CRISPR/CAS9 system into prenatal models, where brain cells are still developing and dividing. Thus, the broken DNA is still being repaired via HDR, giving researchers the opportunity to precisely modify a gene. This is a big deal. "I believe that SLENDR will be a standard tool for molecular and cellular neurobiology," said Dr. Yasuda. "SLENDR provides a valuable means to determine subcellular localization of proteins, and will help researchers to determine the function of the proteins."

In the recent study, the researchers at MPFI inserted a gene that made proteins of interest fluoresce under the microscope. They were even able to reliably label two different proteins with distinct colors at the same time in the same cell. The researchers were able to use the technique to visualize the proteins both in vivo and in vitro. And they were able to do it in a matter of days rather than years.

With existing knowledge of how brains develop, researchers can adjust the timing and position of the electroporation in utero to accurately target cells that will go on to populate particular cortical layers of the brain, even if they haven't differentiated and moved to that layer yet.

The recent study used the technique primarily to tag certain proteins within brain cells and observe their behavior. But, with continued optimization, the method has the potential to elucidate immeasurable brain activities in both normal and diseased brains, and lead to a deeper understanding of brain function. "The most important part is that precise genome editing is possible in the brain. That's what's important," said Dr. Nishiyama, post-doctoral researcher who worked on the study. "That's the biggest thing." Neuroscientists would be remiss to ignore its worth and not explore its potential. GEN

CRISPR Moves from Butchery to Surgery

http://www.genengnews.com/gen-articles/crispr-moves-from-butchery-to-surgery/5759/?kwrd=genome

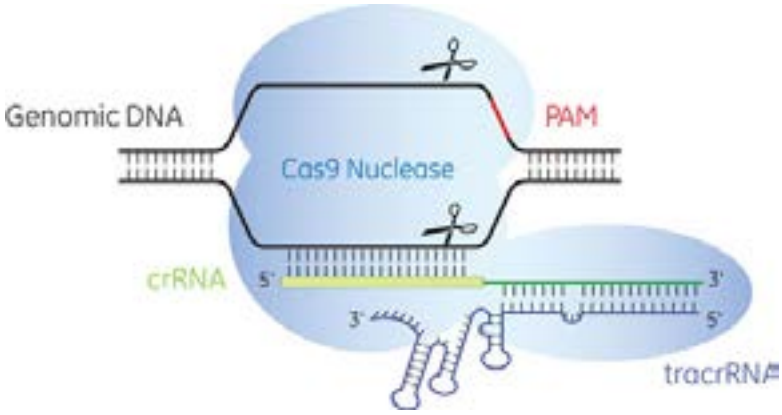
More Genomes Are Going Under the CRISPR Knife, So Surgical Standards Are Rising

Meghaan M. Ferreira, Ph.D.

Scientists recently convened at the CRISPR Precision Gene Editing Congress, held in Boston, to discuss the new technology. As with any new technique, scientists have discovered that CRISPR comes with its own set of challenges, and the Congress focused its discussion around improving specificity, efficiency, and delivery.

In the naturally occurring system, CRISPR-Cas9 works like a self-vaccination in the bacterial immune system by targeting and cleaving viral DNA sequences stored from previous encounters with invading phages. The endogenous system uses two RNA elements, CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA), which come together and guide the Cas9 nuclease to the target DNA.

Early publications that demonstrated CRISPR gene editing in mammalian cells combined the crRNA and tracrRNA sequences to form one long transcript called asingle-guide RNA (sgRNA). However, an alternative approach is being explored by scientists at the Dharmacon subsidiary of GE Healthcare. These scientists have a system that mimics the endogenous system through a synthetic two-component approach that preserves individual crRNA and tracrRNA. The tracrRNA is universal to any gene target or species; the crRNA contains the information needed to target the gene of interest.



The Dharmacon subsidary of GE Healthcare provides the Edit-R Lentiviral Gene Engineering platform. It is based on the natural S. pyrogenes system, but unlike that system, which uses a single guide RNA (sgRNA), the platform uses two component RNAs, a gene-specific CRISPR RNA (crRNA) and a universal trans-activating crRNA (tracrRNA). Once hybridized to the universal tracrRNA (blue), the crRNA (green) directs the Cas9 nuclease to a specific genomic region to induce a double- strand break.

MilliporeSigma's CRISPR Epigenetic Activator is based on fusion of a nuclease-deficient Cas9 (dCas9) to the catalytic histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300. This technology allows researchers to target specific DNA regions or gene sequences. Researchers can localize epigenetic changes to their target of interest and see the effects of those changes in gene expression.

Predesigned Guide RNAs

In contrast to sgRNAs, which are generated through either in vitro transcription of a DNA template or a plasmid-based expression system, synthetic crRNA and tracrRNA eliminate the need for additional cloning and purification steps. The efficacy of guide RNA (gRNA), whether delivered as a sgRNA or individual crRNA and tracrRNA, depends not only on DNA binding, but also on the generation of an indel that will deliver the coup de grâce to gene function.

"Almost all of the gRNAs were able to create a break in genomic DNA," said Louise Baskin, senior product manager at Dharmacon. "But there was a very wide range in efficiency and in creating functional protein knock-outs."

To remove the guesswork from gRNA design, Dharmacon developed an algorithm to predict gene knockout efficiency using wet-lab data. They also incorporated specificity as a component of their algorithm, using a much more comprehensive alignment tool to predict potential off-target effects caused by mismatches and bulges often missed by other alignment tools. Customers can enter their target gene to access predesigned gRNAs as either two-component RNAs or lentiviral sgRNA vectors for multiple applications.

"We put time and effort into our algorithm to ensure that our guide RNAs are not only functional but also highly specific," asserts Baskin. "As a result, customers don't have to do any design work."

Donor DNA Formats

Knockout experiments are a powerful tool for analyzing gene function. However, for researchers who want to introduce DNA into the genome, guide design, donor DNA selection, and Cas9 activity are paramount to successful DNA integration.

MilliporeSigma offers two formats for donor DNA: double-stranded DNA (dsDNA) plasmids and single-stranded DNA (ssDNA) oligonucleotides.

The most appropriate format depends on cell type and length of the donor DNA. "There are some cell types that have immune responses to dsDNA," said Gregory Davis, Ph.D., R&D manager, MilliporeSigma. The ssDNA format can save researchers time and money, but it has a limited carrying capacity of approximately 120 base pairs.

In addition to selecting an appropriate donor DNA format, controlling where, how, and when the Cas9 enzyme cuts can affect gene-editing efficiency. Scientists are playing tug-of-war, trying to pull cells toward the preferred homology-directed repair (HDR) and away from the less favored nonhomologous end joining (NHEJ) repair mechanism.

One method to achieve this modifies the Cas9 enzyme to generate a nickase that cuts only one DNA strand instead of creating a double-strand break. Accordingly, MilliporeSigma has created a Cas9 paired-nickase system that promotes HDR, while also limiting off-target effects and increasing the number of sequences available for site-dependent gene modifications, such as disease-associated single nucleotide polymorphisms (SNPs).

"The best thing you can do is to cut as close to the SNP as possible," advised Dr. Davis. "As you move the double-stranded break away from the site of mutation you get an exponential drop in the frequency of recombination."

Ribonucleoprotein Complexes

Another strategy to improve gene-editing efficiency, developed by Thermo Fisher, involves combining purified Cas9 protein with gRNA to generate a stable ribonucleoprotein (RNP) complex. In contrast to plasmid- or mRNA-based formats, which require transcription and/or translation, the Cas9 RNP complex cuts DNA immediately after entering the cell. Rapid clearance of the complex from the cell helps to minimize off-target effects, and, unlike a viral vector, the transient complex does not introduce foreign DNA sequences into the genome.

The QX200 Droplet Digital PCR System from Bio-Rad Laboratories can provide researchers with an absolute measure of target DNA molecules for EvaGreen or probe-based digital PCR applications. The system, which can provide rapid, low-cost, ultra-sensitive quantification of both NHEJ- and HDR-editing events, consists of two instruments, the QX200 Droplet Generator and the QX200 Droplet Reader, and their associated consumables.

To deliver their Cas9 RNP complex to cells, Thermo Fisher has developed a lipofectamine transfection reagent called CRISPRMAX. "We went back to the drawing board with our delivery, screened a bunch of components, and got a brand-new, fully optimized lipid nanoparticle formulation," explained Jon Chesnut, Ph.D., the company's senior director of synthetic biology R&D. "The formulation is specifically designed for delivering the RNP to cells more efficiently."

Besides the reagent and the formulation, Thermo Fisher has also developed a range of gene-editing tools. For example, it has introduced the Neon® transfection system for delivering DNA, RNA, or protein into cells via electroporation. Dr. Chesnut emphasized the company's focus on simplifying complex workflows by optimizing protocols and pairing everything with the appropriate up- and downstream reagents.

From Mammalian Cells to Microbes

One of the first sources of CRISPR technology was the Feng Zhang laboratory at the Broad Institute, which counted among its first licensees a company called GenScript. This company offers a gene-editing service called GenCRISPR™ to establish mammalian cell lines with CRISPR-derived gene knockouts.

"There are a lot of challenges with mammalian cells, and each cell line has its own set of issues," said Laura Geuss, a marketing specialist at GenScript. "We try to offer a variety of packages that can help customers who have difficult-to-work-with cells." These packages include both viral-based and transient transfection techniques.

However, the most distinctive service offered by GenScript is its microbial genome-editing service for bacteria (Escherichia coli) and yeast (Saccharomyces cerevisiae). The company's strategy for gene editing in bacteria can enable seamless knockins, knockouts, or gene replacements by combining CRISPR with lambda red recombineering. Traditionally one of the most effective methods for gene editing in microbes, recombineering allows editing without restriction enzymes through in vivo homologous recombination mediated by a phage-based recombination system such as lambda red.

On its own, lambda red technology cannot target multiple genes, but when paired with CRISPR, it allows the editing of multiple genes with greater efficiency than is possible with CRISPR alone, as the lambda red proteins help repair double-strand breaks in *E. coli.* The ability to knockout different gene combinations makes Genscript's microbial editing service particularly well suited for the optimization of metabolic pathways.

Pooled and Arrayed Library Strategies

Scientists are using CRISPR technology for a pplications such as metabolic engineering and drug development. Yet another application area benefitting from CRISPR technology is cancer research. Here, the use of pooled CRISPR libraries is becoming commonplace. Pooled CRISPR libraries can help detect mutations that affect drug resistance, and they can aid in patient stratification and clinical trial design.

Pooled screening uses proliferation or viability as a phenotype to assess how genetic alterations, resulting from the application of a pooled CRISPR library, affect cell growth and death in the presence of a therapeutic compound. The enrichment or depletion of different gRNA populations is quantified using deep sequencing to identify the genomic edits that result in changes to cell viability.

MilliporeSigma provides pooled CRISPR libraries ranging from the whole human genome to smaller custom pools for these gene-function experiments. For pharmaceutical and biotech companies, Horizon Discovery offers a pooled screening service, ResponderSCREEN, which provides a whole-genome pooled screen to identify genes that confer sensitivity or resistance to a compound. This service is comprehensive, taking clients from experimental design all the way through to suggestions for follow-up studies.

Horizon Discovery maintains a Research Biotech business unit that is focused on target discovery and enabling translational medicine in oncology. "Our internal backbone gives us the ability to provide expert advice demonstrated by results," said Jon Moore, Ph.D., the company's CSO.

In contrast to a pooled screen, where thousands of gRNA are combined in one tube, an arrayed screen applies one gRNA per well, removing the need for deep sequencing and broadening the options for different endpoint assays. To establish and distribute a whole-genome arrayed lentiviral CRISPR library, MilliporeSigma partnered with the Wellcome Trust Sanger Institute. "This is the first and only arrayed CRISPR library in the world," declared Shawn Shafer,

Ph.D., functional genomics market segment manager, MilliporeSigma. "We were really proud to partner with Sanger on this."

Pooled and arrayed screens are powerful tools for studying gene function. The appropriate platform for an experiment, however, will be determined by the desired endpoint assay.

Detection and Quantification of Edits

Finally, one last challenge for CRISPR lies in the detection and quantification of changes made to the genome post-editing. Conventional methods for detecting these alterations include gel methods and next-generation sequencing. While gel methods lack sensitivity and scalability, next-generation sequencing is costly and requires intensive bioinformatics.

To address this gap, Bio-Rad Laboratories developed a set of assay strategies to enable sensitive and precise edit detection with its Droplet Digital PCR (ddPCR) technology. The platform is designed to enable absolute quantification of nucleic acids with high sensitivity, high precision, and short turnaround time through massive droplet partitioning of samples.

Using a validated assay, a typical ddPCR experiment takes about five to six hours to complete. The ddPCR platform enables detection of rare mutations, and publications have reported detection of precise edits at a frequency of <0.05%, and of NHEJ-derived indels at a frequency as low as 0.1%. In addition to quantifying precise edits, indels, and computationally predicted off-target mutations, ddPCR can also be used to characterize the consequences of edits at the RNA level.

According to a recently published Science paper, the laboratory of Charles A. Gersbach, Ph.D., at Duke University used ddPCR in a study of muscle function in a mouse model of Duchenne muscular dystrophy. Specifically, ddPCR was used to assess the efficiency of CRISPR-Cas9 in removing the mutated exon 23 from the dystrophin gene. (Exon 23 deletion by CRISPR-Cas9 resulted in expression of the modified dystrophin gene and significant enhancement of muscle force.)

Quantitative ddPCR showed that exon 23 was deleted in ~2% of all alleles from the whole-muscle lysate. Further ddPCR studies found that 59% of mRNA transcripts reflected the deletion.

"There's an overarching idea that the genome-editing field is moving extremely quickly, and for good reason," asserted Jennifer Berman, Ph.D., staff scientist, Bio-Rad Laboratories. "There's a lot of exciting work to be done, but detection and quantification of edits can be a bottleneck for researchers."

The gene-editing field is moving quickly, and new innovations are finding their way into the laboratory as researchers lay the foundation for precise, well-controlled gene editing with CRISPR. GEN