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UC Davis Scientists Pioneer CRISPR Protocol with Cas9 Nickase

At the University of California, Davis, mouse mutation expert Angus Lee has adopted CRISPR technology for genome editing, and recently published promising results from a new targeting method. He relies on all-in-one kits from System Biosciences to power his experiments, save valuable time, and help him build better models.

Angus Lee spends a lot of time thinking about how to build a better mouse. As supervisor of the Murine Molecular Constructs Laboratory, part of the Mouse Biology Program at the University of California, Davis, Lee is responsible for generating mouse models for any number of genetic mutations requested by his extensive customer base.

The rise of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) as a genome editing method in the last two years has dramatically changed how Lee approaches his work, as well as what his customers can do. One of the biggest differences between CRISPR and previous protocols for generating a mouse mutant is turnaround time: this new tool allows Lee to deliver modified mice to customers in three or four months, compared to a year or more with traditional embryonic stem cell methods.

Particularly for projects where customers must create a mutant, test it, and then tweak that mutation and go through the process all over again, the time savings afforded by CRISPR has revolutionized what people can imagine and test. “Using CRISPR is really attractive to us to get the desired mouse mutants to the customer faster and cheaper,” Lee says.

But CRISPR is still a new approach, and scientists around the world are honing best practices for using it. Lee, who needs robust and well-validated workflows to deliver high-quality mouse mutants to the investigators who rely on him, recently completed a project designed to evaluate a CRISPR protocol involving a mutated version of the Cas9 enzyme to eliminate off-target effects seen with the more commonly used wild-type nuclease. He turned to System Biosciences (SBI), a company that offered the first commercial products for CRISPR experiments, and selected an all-in-one kit that streamlined his experiments.

Lee’s project was a success, and was recently published in a journal of the Federation of European Biochemical Societies [1]. Today, Lee and his team use SBI’s pre-made Cas9 SmartNickase™ mRNA and gRNA synthesis kit as a routine part of their CRISPR protocol.

Nuclease vs. Nickase

At the Mouse Biology Program, established in 1997 and now serving investigators around the world, Lee works with scientists who need mouse models for truly cutting-edge research — so he is always on the lookout for innovative and effective approaches to designing mouse mutants.

For Lee and his colleagues, CRISPR was a welcome change from a tedious homologous recombination method that involved designing a DNA construct, introducing that construct into embryonic stem cells, creating a chimera, breeding the chimera, and ultimately checking to see if germline transmission of the targeted mutation had occurred. Even when everything went smoothly, the process took a full year. When there were problems, it could take much longer. CRISPR offered the opportunity to lop off more than half of the time, labor, and cost that went into mouse mutants — and helped investigators accelerate their research.

The first CRISPR paper was published in June of 2012; since then, this genome editing tool has seen rapid adoption. "This technology has been established very quickly and it has been demonstrated to apply to many species," says Lee, who notes that scientists continue to tune a number of parameters for the most effective results.

For Lee, two parameters in particular were worth a closer look. For one, the first papers detailing the use of CRISPR in mice used hybrid strains, rather than the inbred strains more commonly used in labs. He thought CRISPR results from an inbred strain would be more relevant to the community. The second parameter was the enzyme. While the wild-type Cas9 nuclease is used more often, researchers had demonstrated that the enzyme caused a host of unpredictable, off-target effects. Cas9 nickase, a newer, mutant version of the enzyme for CRISPR projects, appeared less prone to those errors. "Most people at the time were using nuclease, but we wondered whether nickase would work for the generation of a conditional allele," Lee says. "There were several reports of off-target effects with the Cas9 nuclease, which have caused concern because you don't want other mutations occurring."

For his project, he chose one gene, the isoprenoid synthase containing domain (Ispd). Lee's goal was to insert loxP sites flanking critical exons — a process known as floxing — to create a conditional allele of the gene. The project wasn't just tinkering: floxing to make a conditional allele had not been previously demonstrated in any reproducible or effective manner using Cas9 nickase.

Then Lee chose his reagents: the time-saving Cas9 SmartNickase and gRNA synthesis all-in-one kits from SBI. He had plenty of options; back in 2012, SBI's R&D team immediately recognized the scientific opportunity CRISPR presented and conducted an all-hands-on-deck effort to make reagents available less than a year after the first paper came out. Today, SBI's offerings include a custom service team that handles the vector and RNA design work for clients.

For this project, Lee and his collaborator Kent Lloyd — director of the Mouse Biology Program at UC Davis — microinjected one-cell mouse zygotes with Cas9 nickase mRNA, two single guide RNAs, and a DNA template encoding the two loxP sites targeting the second exon in Ispd, as they reported in the paper. The zygotes were then surgically implanted into female mice for gestation. One of the mice born (representing about 8% of live pups) expressed the conditional allele; when that mouse reproduced, the new allele was transmitted to her pups. "To our knowledge, this is the first study to demonstrate the successful generation of a conditional knockout (floxed) allele in mice using paired [Cas9 nickase] with a single DNA template expressing a loxP flanked critical exon for targeting by homologous recombination,"

they note in the paper. "This approach demonstrates the relative ease and reliability of generating conditional alleles using Cas9n and a single DNA template."

Lee was pleased with the results, noting that the use of two guide RNA sequences was important for limiting off-target effects. He also likes how quickly results can be checked: the first generation of pups can be analyzed for the desired mutations (as well as for off-target effects) as soon as they're born. CRISPR also enables the introduction of multiple mutations at once, streamlining a process that might take several generations of mice with other methods.

Based on results from the initial project, Lee is pursuing ways to increase the effectiveness of CRISPR experiments using nickase, which is known to be slightly less powerful than nuclease. "We know that we can enhance the efficiency in the future with better design strategy," Lee says. "I'm happy about demonstrating that conditional allele creation can be done, and now there's just some work to improve efficiency."

All-in-One Kit

As he conducts that work, Lee will continue to use CRISPR reagents from SBI. "You can do everything all in one kit," he says. "It allows us to assemble the guide RNA sequence easily, and it's bundled with the *in vitro* transcription reactions so once you have your guide RNA in a plasmid, then you can use the same kit with different reagents to transcribe your guide RNA sequence into mRNA and inject it into the mouse embryo."

Lee has run about 10 more projects using SBI's Cas9 SmartNickase mRNA, Cas9 SmartNuclease mRNA, and gRNA synthesis kits, and has been impressed with the reliable results. He has tried other kits, both homemade and commercial, but SBI reagents are a lab favorite. "This all-in-one gRNA synthesis kit is what we use the most," he says.

An advantage of buying from SBI is the technical support service available to customers seeking advice for their experiments. "I love the tech support because you can call them or email and they respond quickly," Lee says.

He notes that the SBI kits offer a great way to get started with genome editing experiments. "I would recommend it to anyone who's interested in CRISPR," Lee says. "It's easy to learn and already includes most of the things that you need."

1. Lee, Angus Yiu-fai, and Kevin C. Kent Lloyd. "Conditional Targeting of *Ispd* Using Paired Cas9 Nickase and a Single DNA Template in Mice." *FEBS Open Bio* 4 (2014): 637–642. *PMC*. Web. 3 Dec. 2014.