Improve Your Gene-Editing Success With CRISPR-SNIPER Technology
REPROCELL is the global life science leader providing a unique combination of fresh human tissue and stem cell expertise, products, services and technologies to empower your drug discovery. With scientists and labs on three continents, REPROCELL has translational solutions to help support you at every stage of your drug discovery journey. Whether you are looking for fresh human tissue samples, assays or gene-editing, our global team has the resources and expertise to support you.
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Introduction

The discovery of both CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and associated proteins, the Cas nucleases, has revolutionized the field of gene-editing. Characterization of the role of the CRISPR-Cas system in pathogen detection, the immune response, and DNA repair mechanisms, has enabled this naturally occurring biological system to be harnessed and developed as a valuable research tool.

CRISPR-Cas technology has unlocked new and improved opportunities in biological and genetic research, food production, and increasingly, in the development of gene therapy and cell-based therapeutics.

However, as with all new developments, the full potential of the technology can only be reached if it is scientifically and commercially transferable, cost-effective, and user-friendly. Use of conventional CRISPR-Cas technology is limited for some gene-editing applications including simultaneous multiple gene knock-out, knock-in of longer length DNA fragments and single nucleotide modification.

Here, we highlight CRISPR-SNIPER, a timesaving CRISPR-Cas technology with built-in optimization capacity and improved gene-editing efficiency that overcomes the limitations of conventional CRISPR-Cas and broadens application of the gene-editing technology.

Delivered by REPROCELL, CRISPR-SNIPER takes gene-editing to new and improved levels of success.

“We may be nearing the beginning of the end of genetic diseases.”

— Jennifer Doudna
The CRISPR-Cas System

Alternative gene-editing technologies, such as TALENs (transcription activator-like effector nucleases) and zinc finger nucleases (ZFNs), paved the way for precise genome modification techniques. However, they have several limitations in use.

TALEN-based systems differ from the CRISPR-Cas system in that they are protein-based DNA editing techniques whereas the CRISPR-Cas system is a small RNA-mediated sequence-specific cleavage technique. The ability of the CRISPR-Cas system to target a gene of interest using a single guide RNA (sgRNA) means that the system is highly programmable and nucleotide specific — one of the key elements to its success as a research tool.

Generating sgRNA is a relatively straight-forward laboratory process enabling the adaptation of CRISPR-Cas methods to many different applications. The technology was initially developed as a method to knock out target genes in functional studies, however, recent technical advances have extended this remit to selectively activate or repress target genes, purify specific regions of DNA, image DNA in live cells, and precisely edit DNA and RNA sequences.

The type II CRISPR-Cas9 system, the most commonly used system, has more target sites than ZFNs and TALENs, and numerous Cas9 variants which enhance its ability to perform systematic analyses of gene function and allow specific genome targeting in many different organisms, including mammalian cells.

The principle of the CRISPR system is simple: a non-coding RNA molecule guides the Cas9 nuclease to a gene sequence inducing a site-specific cleavage (Figure 1).

DNA damage is then repaired by cellular DNA repair pathways — non-homologous end joining repair (NHEJ) or homology-directed repair (HDR). The NHEJ pathway is efficient but error-prone, introducing insertions and deletions (INDELs) that may affect gene function. INDELs can then be characterized to identify and select the mutations of interest.

By contrast, the HDR pathway is less efficient but with high-fidelity making it useful to generate specific nucleotide changes ranging from a single nucleotide change to large insertions. The NHEJ pathway is naturally the preferred option for most cell types, and additional components are needed to drive the repair mechanism towards the more specific HDR pathway.
Figure 1. The CRISPR-Cas9 Complex. A short synthetic RNA containing a sequence required to bind Cas9 nuclease and a user defined sequence specific for the target guides the RNA/nuclease complex to the gene of interest. The target sequence must be unique within the genome and located near a protospacer adjacent motif (PAM) – a binding signal for Cas proteins. Binding of the sgRNA to Cas9 activates the nuclease activity and allows DNA cleavage to occur.
The basic CRISPR-Cas experimental workflow is a 3-stage process

1. **Design:** Choose the best and/or specific components for a particular cell type e.g. sgRNA and Cas nuclease

2. **Delivery:** Choose the best format (plasmid DNA, RNA, ribo-nucleoprotein) and delivery method (electroporation, lipofection, virus) to allow the editing process to take place within a cell

3. **Analysis:** Characterize the edits using PCR and Sanger or **Next Generation Sequencing** methods to determine gene editing efficiency and select mutations of interest

The complete workflow can take several months, and each stage has its own set of challenges. Cas9 nuclease activity, target site selection and sgRNA design, delivery methods, off-target effects, and the incidence of HDR are all factors potentially affecting the efficiency and specificity of the system. Developments in informatics and machine learning help abrogate factors such as target selection and sgRNA design, and molecular modification of the Cas nucleases can increase activity.

However, even with these optimizations and improvements, the process can still take many months to optimize and complete, and the gene-editing efficiency plus the number of positively edited clones may still be inadequate. A key problem with conventional CRISPR-Cas is that experimental failure is only recognized after many weeks or even months of effort have been expended. Reducing timelines and improving editing efficiency are necessary if the technology to progress and develop.
“Successful genome editing requires significant expertise in many areas. Your company has a deep understanding of them; our team are very impressed.”

— CRISPR-SNIPER Customer
Advancing CRISPR-Cas technology with SNIPER Screen

CRISPR-SNIPER technology recently introduced by REPROCELL in collaboration with GenAhead Bio, takes the CRISPR-Cas system to the next level — streamlining timelines, reducing overall costs, and increasing the efficiency and potential application of the system. The technology addresses key areas of the 3-stage process — the analytical stage and the optimization steps required for the design and delivery stages.

The analytical stage of traditional CRISPR-Cas technology is generally the most onerous, labor-intensive, and costly stage, and often ends in failure. Following delivery and transfection of the system components to allow gene-editing to occur, a limiting dilution of the cells is performed, and individual clones are then picked for analysis. Depending on the efficiency of the system, this process may be repeated several times, and on average, hundreds of clones selected and checked (Figure 2).

**SNIPER (Specification of Newly Integrated Position and Exclusion of Random-integration)** combines a checkerboard of culture conditions with highly sensitive digital PCR to empirically pre-screen and identify clones most likely to be positive for the desired gene-edit. As a result, fewer clones are selected for checking, more positive clones are identified, and the screening process is significantly reduced from around 8 weeks to 2 weeks (Table 1, Figure 2).

<table>
<thead>
<tr>
<th>Screening</th>
<th>Average no. of clones selected</th>
<th>Positive clones (%)</th>
<th>Average duration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNIPER</td>
<td>50</td>
<td>20 - 30</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Conventional</td>
<td>&gt;200</td>
<td>0.1 - 1</td>
<td>8</td>
</tr>
</tbody>
</table>

*Table 1. Comparison of conventional and SNIPER screening workflows for a typical knock-in gene editing study.*

"Using a tool like CRISPR to make any mutation we want, big or small, speeds up our ability to decode the genome, to figure out the function of every gene. That’s a major goal of Biology”
— Geraldine Seydoux
In addition to use as a screening and analytical tool, the addition of SNIPER to CRISPR-Cas systems can also be utilized to facilitate optimization steps required for the design and delivery stages of the technology. Component design, multiple component delivery and transfection strategies can be assessed under different culture conditions within the checkerboard to optimize and select the best combination for a cell type and transfection conditions, negating the need for multiple optimization steps in the pre-analysis stages. Crucially, through the application of CRISPR-SNIPER, success or failure is identified at an early stage in the workflow, ensuring that time and costs are kept to a minimum for unsuccessful gene-editing attempts.

CRISPR-SNIPER technology is extremely versatile, maximizing gene-editing efficiencies in numerous different cell lines, including induced pluripotent stem cell (iPSC) lines. Due to the improved detection capability of SNIPER screening, fragile cell types more likely to be negatively affected by gene-editing procedures do better using CRISPR-SNIPER protocols.

As well as the technical advantages offered by the CRISPR-Cas9 system, the addition of the SNIPER screen accelerates the identification of successful editing for all knock-in, knock-out, and single nucleotide base-pair change applications.
Multiplex Gene Knock-Out

Potent and precise knock-out (KO) of genes has traditionally been an important research tool for determining the function of a gene. CRISPR-SNIPER significantly streamlines the ‘loss of function’ process with elevated levels of specificity and efficiency. The addition of a SNIPER screen enables a multiplex approach to gene KO to be undertaken. Conventional CRISPR-Cas9 methods allow for the step-wise KO of multiple genes. However, several culture passages in between each gene KO procedure are required, resulting in an extended experimental process that may adversely affect the viability and function of cells. This is a common issue for iPSC lines where extended passage could lead to decreased growth rates, formation of genetic abnormalities, and inhibition of differentiation capacity.

By using SNIPER technology, several genes can be targeted at once, thereby reducing the number of passages and the time required to obtain a viable multiple gene KO cell product (Figure 3).

Figure 3. SNIPER knock-out gene editing. Panel A: Edits were generated using a sgRNA specific for the Human Leukocyte Antigen (HLA) class A gene. Selective and complete knock-down of the HLA-A gene was achieved by day 7 of culture. Panel B: 5 sgRNA constructs specific for each gene were included.
Long-length DNA Knock-In

Specific knock-in (KI) of genes or ‘gain of function’ has many potential applications, from the addition of a reporter gene for live imaging of cells and identification or purification purposes to gene repair or replacement required for many potential gene therapy options.

Conventional CRISPR techniques are limited by the length of DNA that can be inserted and subsequently detected. The addition of SNIPER to CRISPR-Cas workflows allows for the accurate detection of both short and longer length DNA inserts incorporated using CRISPR-Cas9 and the HDR pathway (Figure 4).

Detection of longer length inserts using conventional CRISPR methods is often difficult due to the extended homology regions or arms of the insert. SNIPER screen utilizes a high-fidelity probe and digital PCR system to accurately detect the inserted DNA and enable detection of longer length inserts up to 2kb. The addition of the screen increases the potential use of CRISPR-Cas9 technology to applications where larger DNA insertions are required to obtain the edit of interest.

Figure 4: Comparison of a long and short sequence specific oligo donor in a typical knock-in gene editing study. Longer length homology arms inhibit reliable detection using conventional CRISPR techniques.

<table>
<thead>
<tr>
<th>DNA insert</th>
<th>Homology Arm (bp)</th>
<th>Knock-in Efficiency (%)</th>
<th>Analysis Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short (100 bp)</td>
<td>~50 bp</td>
<td>0%-35%</td>
<td>qPCR</td>
</tr>
<tr>
<td>Long (2 kbp)</td>
<td>~1 kbp</td>
<td>22%-74%</td>
<td>SNIPER</td>
</tr>
</tbody>
</table>
Single Nucleotide Base change applications

The largest class of known human pathogenic mutations, by far, is the single nucleotide polymorphism (SNP). Installing, changing, or reversing pathogenic SNPs efficiently and cleanly within a vast genome can be difficult using conventional CRISPR techniques. For single nucleotide base change generation, the inclusion of an additional selection marker, such as PuroR or GFP, is not an attractive option.

Consequently, screening of large numbers of colonies is required to identify positive clones, resulting in a significant investment in time and resources. Addition of SNIPER to single base change modification screening results in a 100-fold or greater increase in the fraction of positive clones, greatly improving this resource-intensive step. This increase in efficiency is crucial to reliably differentiate between colonies heterozygous or homozygous for a SNP (Figure 5).

**Figure 5:** CRISPR-SNIPER Detection of SNP conversion in iPSC line. Knock in mutants were generated by inserting DNA containing the SNP of interest. SNIPER screen detected the number of colonies positive for either the homozygous (33%) or heterozygous (26%) version of the SNP. Wild type (KO) SNP colonies were also quantified (33%).
“You have great CRISPR technology. I’d like to ask for another project, but am worried you are busy with other companies’ requests!”

— CRISPR-SNIPER Customer
REPROCELL CRISPR-SNIPER Gene-editing Services

Unlike comparable gene-editing companies and providers, REPROCELL offers a modular and flexible CRISPR-SNIPER approach that benefits many different and complex gene-editing needs including the experimentally challenging constructions such as marker insertions and single nucleotide base changes (Figure 7). Importantly, the modules are applicable to a variety of cell types, most notably iPSCs and both adherent and suspension cancer cells.

**Figure 7:** With CRISPR-SNIPER, Two types of service menus are offered. (1) Design and Feasibility study and (2) Full package service. The process of Design and Feasibility study is developed using our expertise in optimal gRNA designs and gene-editing to achieve success rate as high as 95% — even for SNP conversion.
Module 1:
Design and Feasibility Package

This module enables quick and efficient optimization of individual components, delivery and culture conditions for a specific target and cell type.
REPROCELL requests only the sequence and locus of your target gene with no additional information or end-user protocols required. Our in-house scientists will custom design and manufacture the sgRNA and donor DNA fragments for your experiment and will test up to 6 transfection conditions to identify the best component design and delivery condition with the highest transfection efficiency.

The end point is a bulk unselected clone stock ready for individual clone selection, expansion and characterisation.

Module 2:
Full-Service Package

Module 2 offers a more comprehensive and extensive approach, providing a full gene-editing work-flow, clonal selection, expansion and/or characterization services. The end point for module 2 is the delivery of individually selected and expanded clones with the target gene, along with editing efficiency and cell characterisation data.

CRISPR/Cas9 gives us the ability to delete every single gene in our genome...this could help us identify a new wave of drug targets.”

— Sylvie Guichard
References


Useful Resources

[CRISPR-SNIPER Gene Editing Service](#)

[iPSC Genome Editing by CRISPR-SNIPER](#)

[Blog post:] What is CRISPR-SNIPER Gene Editing?

[Blog post:] 5 ways SNIPER can enhance your CRISPR gene editing

[Webinar:] CRISPR/Cas9 using SNIPER Technology — Managing Challenging Cases
REPROCELL. Better translating discoveries into therapies.