CRISPR-SNIPER Gene Editing Service

Advanced Gene Editing for Challenging Cases

stemgent GenAhead Bio

A JOINT VENTURE

CRISPR, but better

At REPROCELL, we have collaborated with GenAhead Bio to provide CRISPR-SNIPER Gene Editing Services. This novel approach to genome modification makes it possible to achieve otherwise challenging mutations.

This makes CRISPR-SNIPER the most efficient gene-editing technique on the market. It greatly increases the likelihood of project success — saving you time, effort and money.

The Benefits of SNIPER†

- ✔ **Increase** screening specificity
- ✔ **Track** iPSC differentiation
- ✔ **Solve** challenging cases
- ✔ **Achieve** multiplex gene knock-out and knock-in
- ✔ **Save** time and money

† **S**pecification of **N**ewly **I**ntegrated **P**osition and **E**xclusion of **R**andom-integration.

	CRISPR System	CRISPR-SNIPER System
Target cells	Mainly cell lines	Cell lines and iPSCs
SNP knock-in %	< 1%	10-30%
Max. insertion size	~ 2 kbp	5-7 kbp
Conditions tested	~ 1	6-12
Biallelic modifications	✗	✔
KI reproducibility	Low	High

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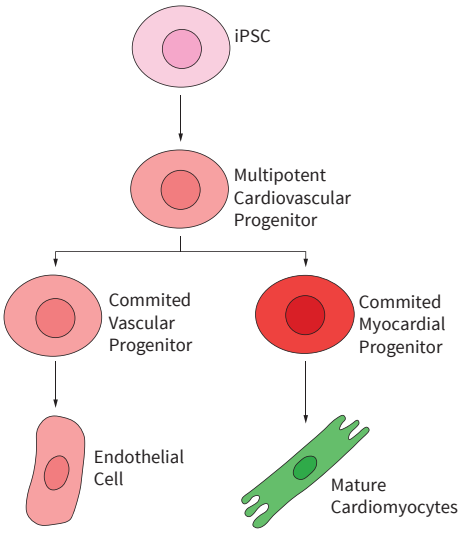
Five examples of CRISPR-SNIPER in action

1. Insertion of large gene fragments

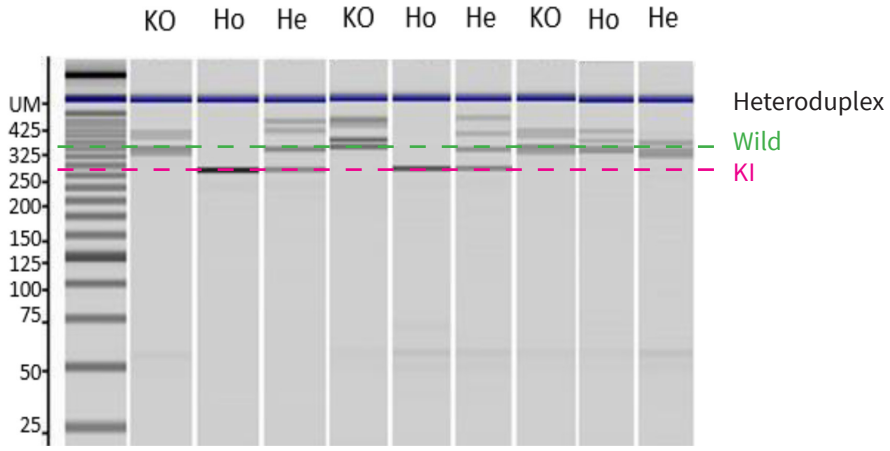
Normally, the rate of insertion decreases dramatically when your gene of interest (GOI) exceeds 2000 bp. As SNIPER allows optimization of gene editing conditions, it can be used in synergy with CRISPR to knock-in genes up to 7000 bp in size. This is particularly useful for tracking gene expression, as it makes the insertion of functional gene segments less challenging.

For example, you may want to insertfluorescent reporter genes to enable rapidoptimization of differentiation protocols or to permit tracking of specific cell populations during differentiation. Alternatively, you may want to knock-in antibacterial resistance genes to assist cell selection.



2. Insertion of Biallelic Mutations

Achieving the correct disease phenotype may require the insertion of heterogenous or homogenous mutations. However, with conventional gene editing it is challenging to achieve biallelic gene modifications. Using SNIPER, gene editing conditions can be optimized sto allow the creation of heterozygous or homozygous mutants for disease modelling.



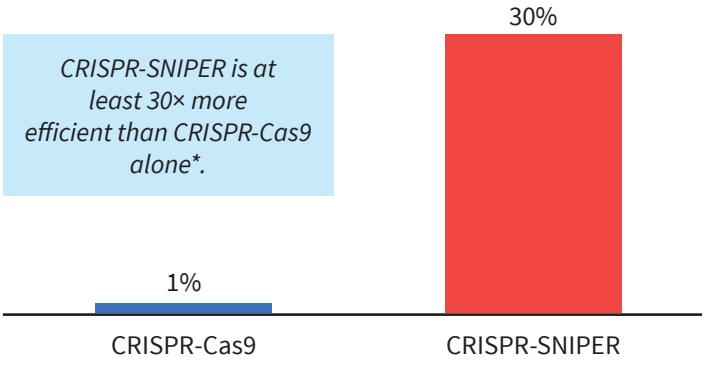
SNIPER enables editing of both DNA strands to create a homozygous (Ho) and heterozygous (He) genotype in multiple iPSC clones.

3. Increased Screening Efficiency

Thanks to SNIPER's enhanced screening efficacy, the system can detect at least 30X more positive clones than conventional screening techniques*.

In this way, SNIPER enhances CRISPR-Cas9 gene editing by making it easier to detect cells that contain your desired mutation. This means that CRISPR-Cas9 projects that were once unfeasible can be achieved.

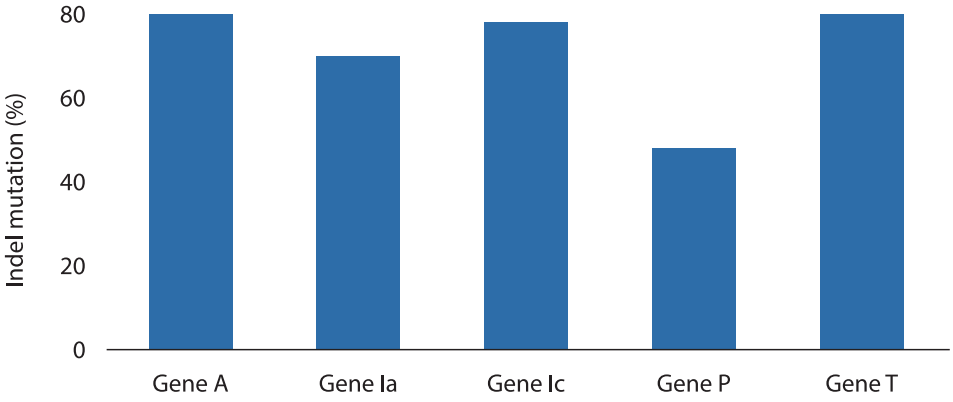
CRISPR-SNIPER is at least 30× more efficient than CRISPR-Cas9 alone.*



*For SNP modification, SNIPER screening detects positive clones at a 30× higher frequency (30%) compared with conventional screening (1%).

3. Knock-out of multiple genes without increasing cell passage number

Editing multiple genes normally involves sequential gene editing experiments, each increasing cell passage number. With the CRISPR- SNIPER system you can edit up to five genes at once, thereby avoidig teh effects of extended passaging, such as slow growth, formation of genetic abnormalities, and difficulties in differentiation. A further advantage of this property is the ability to assess the effect of your modification on the interaction of numerous pathway components at once.

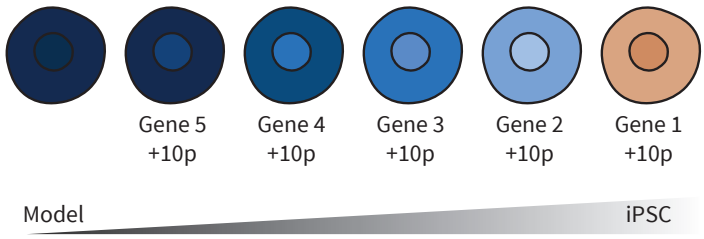


Multiplex gene KO can be achieved using a range of gRNA's with different cleavage capabilities. By optimizing the editing efficacy for each gRNA, a KO model with multiple mutations can be created after just one round of gene editing.

4. Making challenging modifications possible

Thanks to the increased accuracy of SNIPER screening, you can now fulfill gene editing projects that may be impossible using CRISPR-Cas9 alone. This is because SNIPER combines a checkerboard of culture conditions with digital PCR to pre-screen for clones most likely to possess your desired modification. By increasing screening sensitivity, CRISPR-SNIPER makes a wider range of genome modification projects possible -including SNPs, large gene insertions and function gene insertions.

With SNIPER you can edit up to five genes simultaneously.

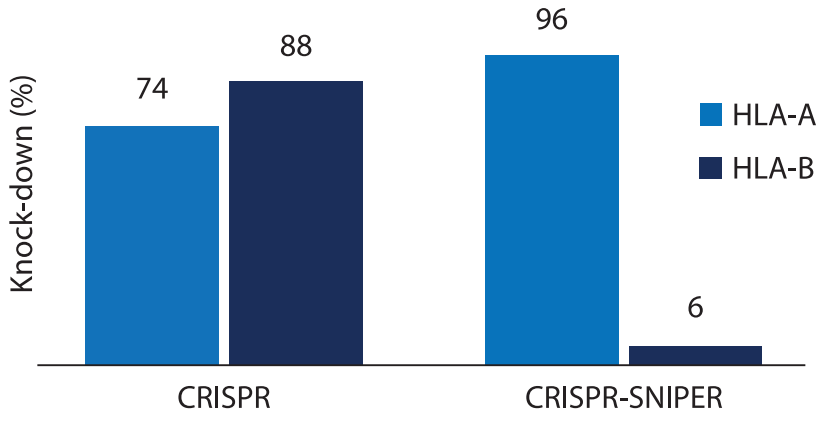


5. Achieving more accurate gene editing

It can be difficult to obtain your desired mutation if another sequence shares high homology to your GOI. Byoptimizing the culture conditions, guide RNA, and adding nickase to each gene editing experiment our scientists can increase the specificity of CRIPSR gene editing even further - ensuring that we only provide the cells you want.

The graph below illustrates that our optimized gRNA results in selective knock-down of HLA-A, whilst theHLA-B gene remains undisturbed. This is a large improvement over the results attained with non-specificgRNA used in conventional gene editing, which results in knock-down of both alleles rather than one.

Compared with conventional gene editing techniques, SNIPER can isolate similair mutants at a high success rate.



Your Custom Gene Editing Project

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Stage 1 Bulk screening for optimal conditions

Stage 2 Scheduled cloning

Stage 3 Cell expansion and QC

Action Points

- Optimize gRNAs
- Set Optimal Conditions
- Perform Transfection
- Complete First Screening

Check Points

- Confirm transfection condition by bulk screening with PCR

Action Points

- Select Positive Clones
- Pick-up Positive Clones
- Perform Second screening

Check Points

- Verification of Gene Editing Success via PCR

Action Points

- Expansion of Positive Clones
- Quality Checks
- Cryopreservation of Cells
- Delivery of Cryovials

Check Points

- Verification of DNA sequence
- Screening Undifferentiated Marker Expression
- Mycoplasma Testing

Make REPROCELL your one-stop partner for gene editing

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