Advancing Biotherapeutic Manufacturing Through Precision Gene Editing Platforms

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1 Abstract

Gene editing technologies, particularly the CRISPR-Cas9 system, have emerged as powerful tools in the bioproduction industry, enabling precise genetic modifications in host cells such as Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK293) cells, among others. Despite widespread interest, challenges persist in achieving reliable and reproducible outcomes due to the lack of standardized workflows.

At Revvity, we have developed and optimized robust gene editing pipelines for the efficient isolation, identification, and characterization of knockout (KO) and knock-in (KI) clones across multiple cell types. Our platform includes a high-precision CRISPR-based alternative capable of generating KO edits independent of gene copy number and introducing KI edits of DNA fragments up to 13 kb, with or without homology arms. This enables the integration of complex genetic constructs with high efficiency.

For more advanced applications, we have established a multiplex gene editing approach using CRISPR-Cas9, targeting up to four loci simultaneously. Given the elevated risk of chromosomal rearrangements in multiplex editing, we employ Targeted Locus Amplification (TLA) to confirm on-target specificity and genomic integrity at the edit site.

These innovations demonstrate a comprehensive, scalable workflow for gene editing projects in bioproduction, improving both reliability and precision across a wide range of applications.

2 Method

Revvity has access to a variety of gene editing technologies for the enhancement of expression platforms that can be used in research and manufacturing of biotherapeutics, such as:

Research cell line

CRISPR-Cas9:

- **Simple** reagent design; easy to implement
- **Fast** and **precise** editing performance
- Extremely **efficient** compared to other gene editing platforms

Commercial cell line

Recombinant adeno-associated virus (rAAV):

- Design of edits is **straightforward**
- **Does not integrate** in the host genome
- **Reliable** method for gene editing
- **Commercial rights granted** to edited cells
- **Less efficient** compared to CRISPR-based platforms

CRISPR-based alternative platform:

- Successfully **validated** in CHO cells
- **Less off-target edits** compared to CRISPR-Cas9
- **High editing efficiency** and comparable timeline to CRISPR-Cas9
- **Commercial rights granted** to edited cells

3 Case study 1: High efficiency multiplexed gene editing for bioproduction cells

I- Introduction

To improve the phenotype of bioproduction cell lines, it may be necessary to KO multiple genes. Our current workflow for the generation of a single gene KO, using CRISPR, enables us to develop edited cells in less than 6 months (**Fig. 1**).

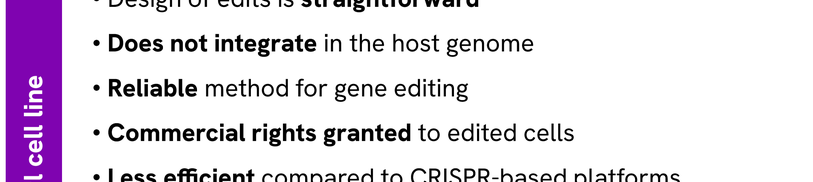


Fig. 1: Gene editing workflow using CRISPR platform.

Therefore, we wanted to take advantage of our expertise and tools to target multiple loci, without impacting this timeline. As proof of concept, CRISPR-Cas9 was used to simultaneously KO four genes within the CHO genome. The four genes selected encode for impurities found in the supernatant of the CHOSOURCE™ GS KO cell line.

II- Results

The first step of our process was to determine the target genes copy number in the CHOSOURCE GS KO cell line using droplet digital (dd)PCR. The analysis showed that each gene was found to have two copies in the CHOSOURCE GS KO cell line.

Next, the transfection protocol was optimized to allow the simultaneous targeting of the four genes and the editing efficiency on each locus was assessed at the pool stage, using an in-house algorithm (**Fig. 2**).

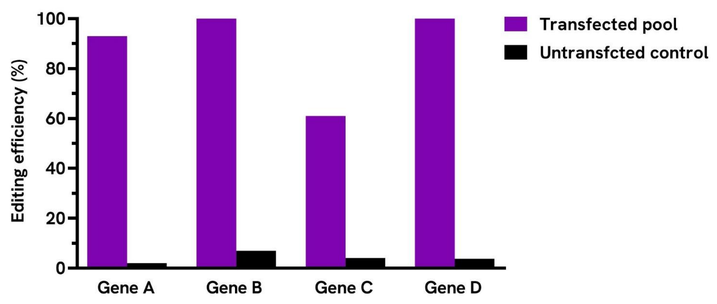


Fig. 2: Editing efficiency (%) obtained in four target loci in the transfected pool (purple). For each gene, a non-transfected control sample (black) was used for background analysis.

The transfected pool was subjected to limiting cell dilution to isolate and identify KO clones. A total of 298 clones were screened using an amplicon-based next-generation sequencing (NGS) approach, to identify KO clones carrying out-of-frame indels on both alleles of the four target genes. Seventeen putative KO clones were identified, and their genotype was further confirmed by analyzing Sanger sequencing traces using an in-house TIDE-like algorithm. The data obtained for one KO clone is shown in **Table 1**.

Target gene	Copy number	Amplicon-based NGS sequencing		Sanger sequencing	
		Detected indels (bp)	%	Detected indels (bp)	%
Gene A	2	+1	97.7	+1	91.9
Gene B	2	NA	NA	-2	47.5
				-5	44.2
Gene C	2	-2	58.7	-2	48.8
		+1	40.4	+1	45.9
Gene D	2	+2	98.6	+2	94.0

Table 1: Data from amplicon-based NGS and Sanger sequencing in one KO clone.

With the double-strand breaks created following simultaneous gene targeting, there is a risk of unwanted chromosomal rearrangement. Thus, TLA was performed to further validate the edited clones (**Table 2**).

Targeted loci	Breakpoint reads	Indels (bp)	Breakpoints in locus (%)	WT reads (%)	Amplicon-NGS detection	Sanger sequencing detection
Gene A	2	-457	25.0	×	×	×
		+1	75.0	×	✓	✓
Gene B	2	-5	48.0	×	N/A	✓
		-2	52.0	×	N/A	✓
Gene C	2	-2	48.0	×	✓	✓
		+1	52.0	×	✓	✓
Gene D	2	+2	60.0	×	✓	✓
		-300	40.0	×	×	×

×; No detection, ✓; Detection

Table 2: Events detected by TLA in each of the targeted loci, in one KO clone.

Data acquired from TLA confirmed the genetic validation obtained by amplicon-based NGS and, Sanger sequencing analysis using an internal TIDE-like algorithm. In addition, TLA-NGS analysis identified a large deletion which could not be detected by previous methods. Most importantly, TLA confirmed absence of complex chromosomal rearrangements between the simultaneously targeted loci, which further validates the robustness of the multiplexed gene editing approach used.

Finally, mass spectrometry analysis was performed for functional validation in the parental line and two edited clones. The target genes were shown to be inactivated in the KO clones (**Table 3**).

Target gene	Protein in parental cell line (%)	Protein in KO cell line (%)	
		Clone 1	Clone 2
Gene A	0.21	0	0
Gene B	0.46	0	0
Gene C	0.30	0	0
Gene D	0.2	0	0

Table 3: Mass spectrometry results indicating the percentage of protein detected in parental and edited cells for the four genes targeted.

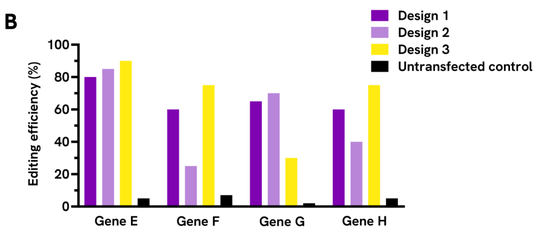
4 Case study 2: CRISPR-based alternative technology for CHO cell genome editing

I- Evaluation of CRISPR-based alternative technology for KO

Genes with copy number ranging from one to three (**Fig. 3A**) were selected to assess performance of the CRISPR-based alternative technology. High editing efficiency was achieved with at least one design tested for each of the genes selected (**Fig. 3B**). Performance comparison between CRISPR-Cas9 and CRISPR-based alternative technology showed both technologies perform similarly, when studying four individual genes (all with copy number of 2) (**Fig. 3C**).

A

Target gene	Copy number
Gene E	1
Gene F	2
Gene G	2
Gene H	3



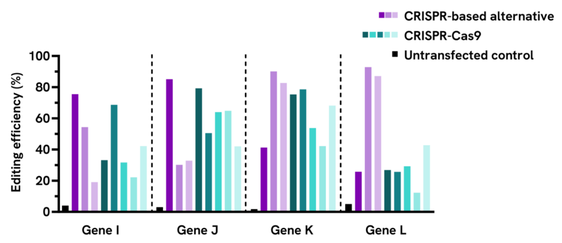


Fig. 3: Evaluation of a CRISPR-based alternative technology for the generation of KO CHO cells. A: Selection of genes with different copy number. B: Editing efficiency (%) obtained for each target gene, using different designs, in the transfected pools. C: Editing efficiency (%) obtained for each target gene, using various designs, in transfected pools using either CRISPR-based alternative or CRISPR-Cas9 reagents.

II- Evaluation of CRISPR-based alternative technology for KI

CRISPR-based alternative technology was tested with CHOSOURCE GS KO cell line to KI DNA fragments of varying sizes (from 1.5 kb to 13 kb) using either Homology Directed Repair (HDR) (**Fig. 4A**) or Non-Homologous End Joining (NHEJ) (**Fig. 4C**). For each method, several clones were successfully isolated and shown to have on-target integration (**Fig. 4B** and **4D**), indicating CRISPR-based alternative technology as a tool for successfully generating KI clones.

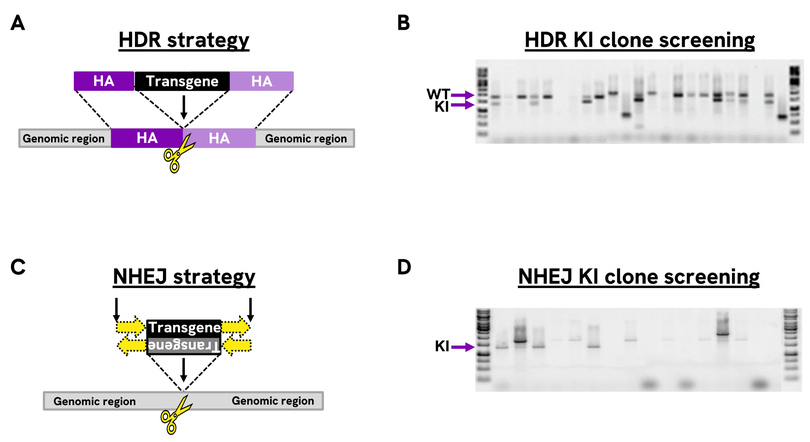


Fig. 4: CRISPR-based alternative technology for KI of DNA of various sizes. A: HDR strategy used for KI of 1.5 kb insert. B: Clone screening PCR showing presence of KI band. C: NHEJ strategy used for large DNA cassette (13 kb) KI, using CRISPR-based alternative technology. D: Clone screening PCR showing presence of KI band.

5 Conclusion

- **Gene editing enables the development of bioproduction cell lines with enhanced phenotypes.**
- **Leveraging Revvity's gene editing expertise and tools, we established a robust pipeline for multiplexed editing, successfully targeting four genes simultaneously with CRISPR-Cas9. Genetic validation was performed using multiple approaches, including confirmation of gene knockouts at the protein level.**
- **In parallel, a CRISPR-based alternative technology demonstrated comparable performance to CRISPR-Cas9 for generating both KOs and KIs. This platform delivers high editing efficiency with timelines equivalent to CRISPR-Cas9, offering a versatile option for cell line engineering.**

The CHOSOURCE™ Platform is available for research, clinical, diagnostic, and commercialization applications, including services, under specific licenses from Revvity.