Efficient delivery of active Cas9 protein and target-specific sgRNA to a broad range of cell types

Guide-it CRISPR/Cas9 Gesicle Production System

- Highly efficient gene modification via gesicles—demonstrated with a fluorescent protein model system
- Knockout via gesicles is comparable to plasmid-based delivery in easier-to-transfect cell types
- Gesicle treatment easily surpasses plasmid-based delivery for knockout studies in harder-to-transfect cell types
- Gesicles can be used for efficient knockout of an endogenous gene in Jurkat cells grown in suspension

Introduction

CRISPR/Cas9 techniques have simplified many of the challenges associated with gene editing. However, efficient gene modification remains reliant on successful delivery of both the Cas9 endonuclease and single-guide RNA (sgRNA) to the cells of interest. Plasmid-based delivery methods can be sufficient for delivery in some cell lines, but efficiency is often low for primary and suspension cells. In contrast, viral-based delivery methods are capable of transducing a wider range of cells, but can pose technical issues regarding viral production and safety. Moreover, both plasmid and viral delivery result in persistent overexpression of the Cas9 endonuclease, which can result in off-target editing of similar genomic sequences.

The Guide-it CRISPR/Cas9 Gesicle Production System is a complete and simple method for creating cell-derived nanovesicles that deliver active Cas9 protein complexed with a gene-specific sgRNA of your own design. Due to the non-persistence of Cas9, gesicles leave no additional footprint, reducing potential off-target effects. Gesicles are nontoxic and include surface proteins that mediate binding and fusion with the cellular membranes of target cells. These critical features enable efficient Cas9 delivery and gene editing in a broad range of cell types.

Results

Model system for analysis of genome modification with gesicles

Gesicle functionality was first evaluated using a model system where a fluorescent protein, ZsGreen1, was targeted for knockout (Figure 1). Briefly, cell lines were created that contained an integrated *ZsGreen1* expression cassette. Using an sgRNA targeted against *ZsGreen1*, successful Cas9-mediated cleavage can be measured by loss of ZsGreen1 expression when analyzed by flow cytometry.

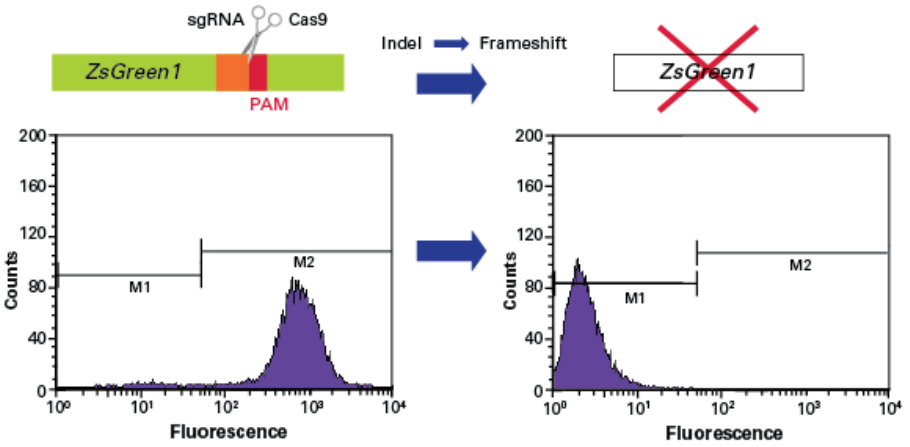

Figure 1. Model system for knockout of a fluorescent protein via gesicle treatment.

Knockout via gesicles is efficient and comparable to plasmid-based delivery in easier-to-transfect cell types

Using the model system, gesicle performance was evaluated in multiple cell types. Various *ZsGreen1* cell lines were either cotransfected with expression plasmids encoding Cas9 and the sgRNA against *ZsGreen1* or were treated with gesicles containing a Cas9-sgRNA ribonucleoprotein (RNP) complex, wherein the target of the complexed sgRNA was *ZsGreen1*. Cells were analyzed by flow cytometry six days later. For cell types traditionally considered to be easier to transfect, *ZsGreen1* knockout efficiency was similar between gesicle- and plasmid-treated cells (Figure 2). Thus, these data indicated that gesicle-based delivery of Cas9-sgRNA RNP complexes is comparable to plasmid-based delivery for cells amenable to transfection.

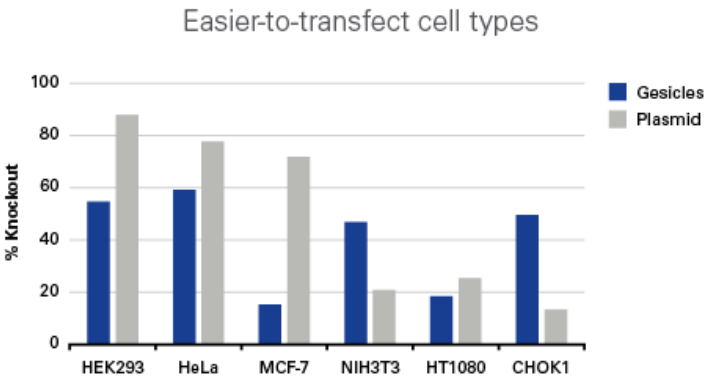

Figure 2. Knockout efficiency of fluorescent reporter in easier-to-transfect cell types.

Knockout via gesicles is efficient and surpasses plasmid-based delivery in harder-to-transfect cell types

Concurrent with the experiment described above, gesicle- and plasmid-mediated delivery was also evaluated in cell types considered harder to transfect. For these cell types, delivery via plasmid did not result in efficient knockout of *ZsGreen1*. In contrast, delivery via gesicles resulted in efficient editing (Figure 3). Taken together, these results indicate that the editing efficiency of gesicles surpasses plasmids, making gesicles an effective tool for hard-to-transfect cells.

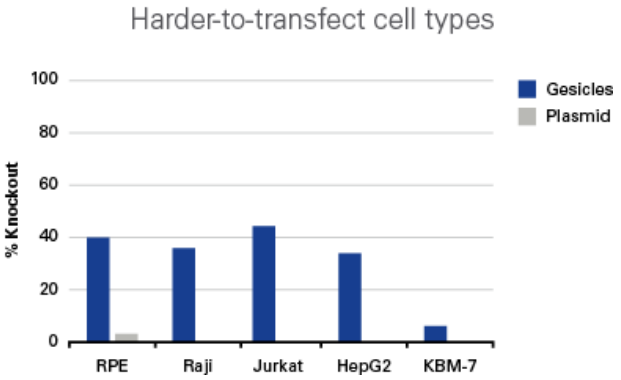

Figure 3. Knockout efficiency of a fluorescent reporter in harder-to-transfect cell types.

Gesicles can knock out an endogenous gene in Jurkat cells grown in suspension

Finally, gesicles were evaluated for the knockout of a physiologically relevant, endogenous gene in Jurkat cells grown in suspension. The knockout targeted *CD81*, which codes for a cell surface protein expressed in many mammalian cells and has been implicated in Hepatitis C, HIV, and influenza pathogenesis. Jurkat cells were either cotransfected with expression plasmids for Cas9 and *CD81*-specific sgRNA or treated with gesicles preloaded with a Cas9-sgRNA RNP complex targeting *CD81*. Knockout efficiency was evaluated via antibody labeling for CD81 followed by flow cytometry analysis (Figure 4). Plasmid-based delivery resulted in very low efficiency; only 7% of cells lost CD81 expression. Conversely, gesicles had high efficiency, with 76% of Jurkat cells lacking detectable levels of CD81. Consequently, gesicles outperform plasmid-based techniques with the ability to efficiently target endogenous genes in difficult-to-transfect cells.

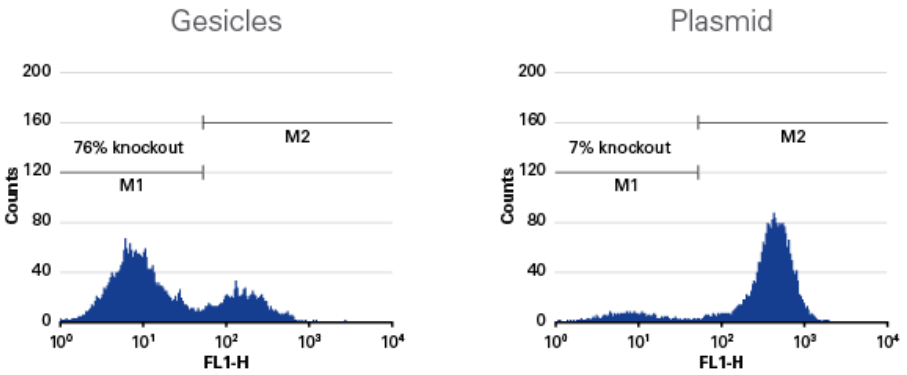

Figure 4. Efficient knockout of an endogenous protein (CD81) in Jurkat cells using gesicles.

Conclusions

The Guide-it CRISPR/Cas9 Gesicle Production System provides a method for the delivery of active Cas9-sgRNA RNP complexes to target cells for CRISPR/Cas9 gene editing. This technology enables efficient modification of target loci in a broad range of cell types. Gesicle-based delivery is comparable to plasmid-based delivery methods in easier-to-transfect cell types, while also surpassing these methods in harder-to-transfect cells. Finally, gesicles leave no additional footprint and allow tight control over the dose of Cas9, leading to a decreased chance of off-target effects.

Methods

Production of gesicles containing Cas9 protein and sgRNA

The workflow and mechanism of gesicle production is covered in more detail on our CRISPR/Cas9 gesicle technology overview page. Target sgRNA against either *ZsGreen1* or *CD81* was cloned into the pre-linearized pGuideIt sgRNA1 vector included in the Guide-it CRISPR/Cas9 Gesicle Production System. This cloned plasmid was added to the provided Guide-it CRISPR/Cas9 Gesicle Packaging Mix. The mix contains lyophilized Xfect Transfection Reagent premixed with an optimized formulation of plasmids encoding Cas9 and all the other elements needed for gesicle production. The packaging mix was added to the Gesicle Producer 293T Cell Line in the presence of the provided A/C Heterodimerizer ligand. Gesicles were collected from the media 48–72 hours later, concentrated via centrifugation, and stored at -70°C until use on target cells.

Knockout of *ZsGreen1* in a broad range of cell types

HEK 293T, HeLa, MCF-7, NIH3T3, HT1080, CHOK1, RPE, Raji, Jurkat, HepG2, and KBM-7 cells were seeded in 24-well plates at a density of 5.0 x10^5. After 24 hours, cells were either cotransfected with 500 ng each of expression plasmids encoding Cas9 and a sgRNA targeting *ZsGreen1* using Xfect Transfection Reagent or treated with 30 µl of Cas9 gesicles (produced as described above). Six days later, cells were analyzed by flow cytometry for expression of *ZsGreen1*.

Knockout of *CD81* in Jurkat cells

Jurkat cells were seeded in 24-well plates at a density of 5.0 x10^5. After 24 hours, Jurkat cells were either cotransfected with 500 ng each of expression plasmids encoding Cas9 and an sgRNA targeting *CD81* using Xfect Transfection Reagent or treated with 30 µl of Cas9 gesicles (produced as described above). Six days later, cells were labeled with an antibody specific for CD81 and analyzed by flow cytometry.

Related Products

Cat. #	Product	Size	License	Quantity	Details
632617	Gesicle Producer 293T Cell Line	1 mL		*	

The Gesicle Producer 293T Cell Line is a subclone of the transformed human embryonic kidney cell line, HEK 293, which is highly transfectable and supports high levels of protein expression. The cell line also constitutively expresses the simian virus 40 (SV40) large T antigen. Gesicles are produced in these cells via co-overexpression of packaging mix components, which include a nanovesicle-inducing glycoprotein and a protein that is displayed on the cell surface and mediates binding and fusion with the cellular membrane of target cells. Simultaneous overexpression of another protein cargo can result in incorporation of that protein within the gesicles. When combined with a gesicle production system, these cells are capable of producing high gesicle yields.

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632616	Guide-it™ CRISPR/Cas9 Gesicle Packaging Set	10 Rxns		*
632613	Guide-it™ CRISPR/Cas9 Gesicle Production System	1 System		*
632612	pGuide-it-sgRNA1 Vector System	1 System		*
631317	Xfect™ Transfection Reagent	100 Rxns		*
631318	Xfect™ Transfection Reagent	300 Rxns		*

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