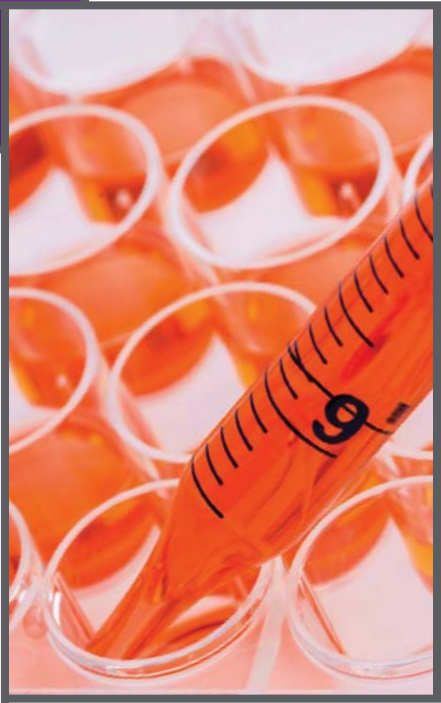
Guide-it™ CRISPR/Cas9 Gesicle Production System



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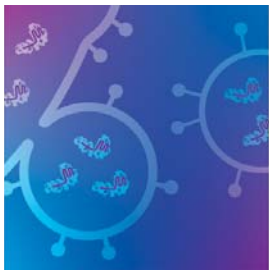
TaKaRa Clontech

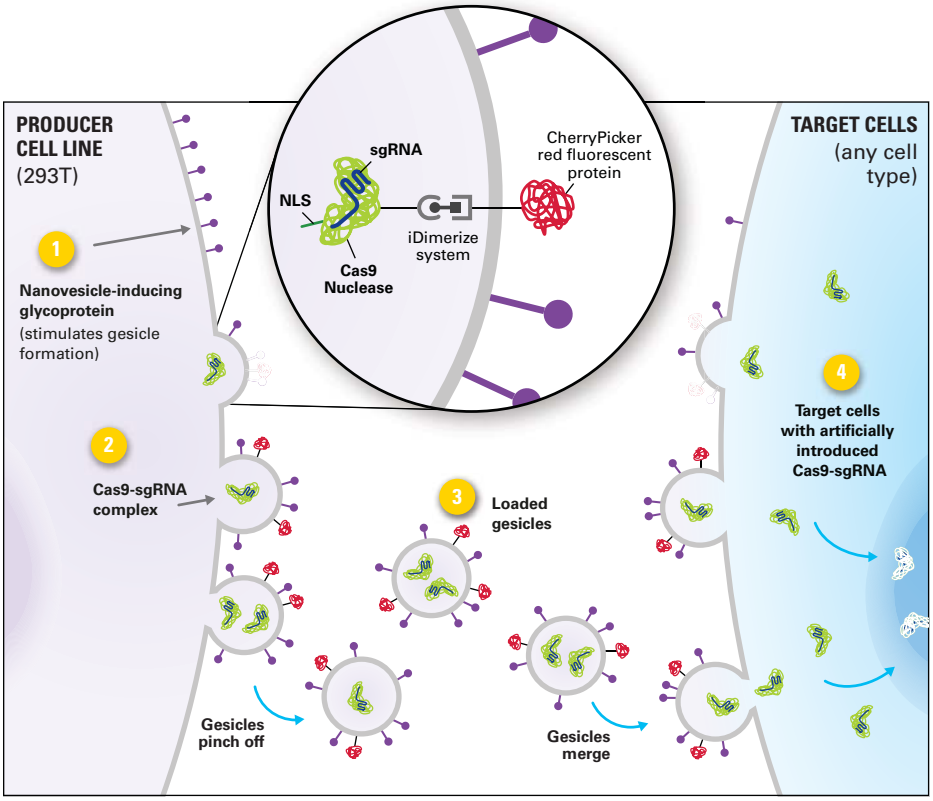
Guide-it CRISPR/Cas9 Gesicles

Delivery of Cas9-sgRNA to a Broad Range of Cell Types



What are Gesicles?

Gesicles are cell-derived nanovesicles containing active Cas9 protein complexed with a single guide RNA (sgRNA) specific to a target gene



System Overview

1. Gesicle formation is induced by glycoproteins on the surface of 293T producer cells that have been cotransfected with our gesicle packaging mix and a target-specific guide RNA plasmid.

2. Utilizing the iDimerize™ system, a small ligand is added to load the Cas9-sgRNA ribonucleoprotein complex into the gesicle through interaction with the membrane-bound CherryPicker™ red fluorescent protein (RFP) on the gesicle surface.

3. Loaded and RFP-labeled gesicles pinch off from the producer cells and are collected from the supernatant, yielding a concentrated stock of Cas9-sgRNA gesicles.

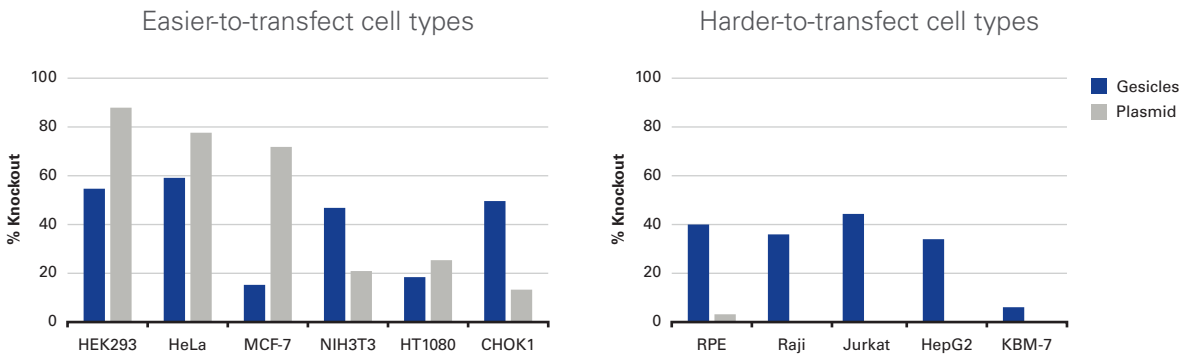
4. Harvested gesicles can be applied to a broad range of target cell types, to which they fuse, transiently labeling the cells red and releasing the Cas9-sgRNA complex into the cell. The presence of a nuclear localization signal (NLS) on the Cas9 protein and the absence of the dimerizer ligand in your cell culture medium ensures that the complex is transported to the nucleus after dissociating from the RFP.

Why use Gesicles?

- Efficient delivery of active Cas9 protein and target-specific sgRNA to a broad range of cell types
- Cas9 protein delivery eliminates genomic integration and reduces off-target effects
- Tight control over dose and timing of delivery and editing

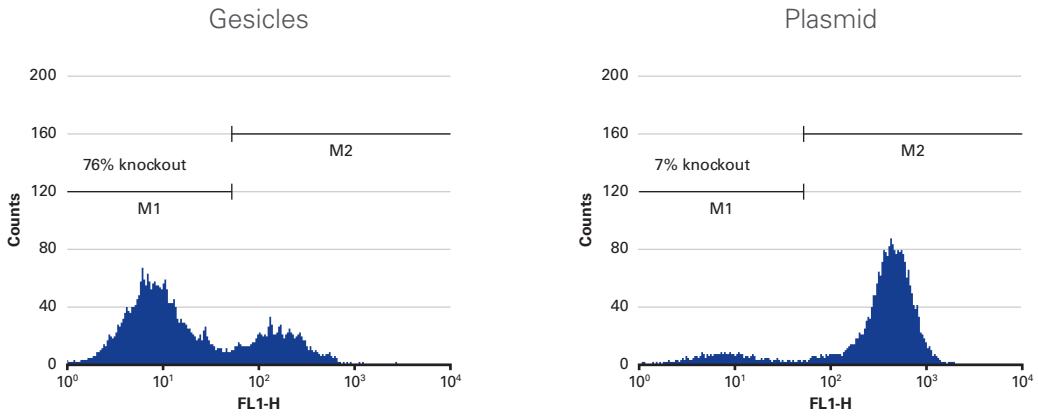
Efficient Delivery to a Broad Range of Cell Types

Knockout of ZsGreen1 in Multiple Cell Types



Gesicles containing Cas9-sgRNA protein complexes outperform plasmid transfection in harder-to-transfect cell types. Cell lines were created that contained an integrated *ZsGreen1* fluorescent protein expression cassette. In this system, successful Cas9-mediated cleavage can be measured by loss of ZsGreen1 expression. These cell lines were treated with gesicles loaded with Cas9-sgRNA protein complexes (with the sgRNA generated against *ZsGreen1*), and then analyzed by flow cytometry. Cas9-sgRNA protein complex delivery and ZsGreen1 knockout via gesicles was efficient and comparable to plasmid-based delivery in easier-to-transfect cell types (left graph) and surpassed the results achieved via plasmid-based delivery in harder-to-transfect cell types (right graph).

Knockout of an Endogenous Gene in Jurkat Cells



Efficient knockout of an endogenous protein (CD81) using gesicles containing Cas9-sgRNA complexes. The cell-surface protein receptor CD81 was knocked out in Jurkat cells using either plasmid cotransfection of Cas9 DNA and sgRNA, or gesicles preloaded with a Cas9-sgRNA ribonucleoprotein complex. The knockout efficiency was measured six days later via antibody labeling of the membrane receptor followed by flow cytometry analysis. Results for delivery via gesicles were significantly greater than results achieved with plasmid transfection.

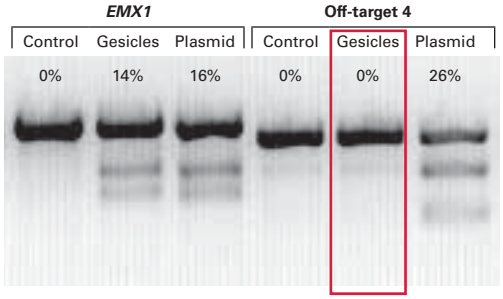
Active Protein Delivery Reduces Off-Target Effects

Persistent overexpression of the Cas9 endonuclease (as observed with plasmid or viral delivery) can result in off-target editing of similar genomic sequences. Gesicles minimize the probability of off-target effects through precise control over dose and delivery of the protein.

Target sequence *EMX1*:
GAGTCCGAGCAGAAGAAGAAGGG

Potential off-target sequence:
GAGTCTAAGCAGAAGAAGAAGAG

PAM site



Cleavage indicates Cas9-mediated editing

***EMX1* gene**

CCTGAGTCCGAGCAGAAGAA GAAGGGCTCCCAT
GGACTCAGGCTCGTCTTCTT CTTCCCGAGGGTA

EMX1 gene

EMX1 sgRNA1

Off-target 4

CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
GTATCATCTGTTCTCAGATTCGTCTTCTT CTTCTCTCGGTG

OT4 *EMX1*

off target 4

Plasmid transfection

CCTGAGTCCGAGCAGAAGAA GAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGA- GAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAG-- ----GGCTCCCAT
CCTGAG---------- -AAGGGCTCCCAT
CCTGAGTCCGAGCAGAAG-- GAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCAT

CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
---------- --------CCAC
CATAGTAGACAAGAGTCTAAGCAGAAGA- -----GAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAA--A GAAGAGAGCCAC
CATAGTAGACAAGAGTCTA---------- GAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAAAGAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAAAGAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAAAGAAGAGAGCCAC

Cas9 Gesicles

CCTGAGTCCGAGCAGAAGAA GAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAG-- ----GGCTCCCAT
CCTGAGTCCGAGCAGAAGAA -AAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGAA ----------CAT
CCTGAGTCCGAGCAGAAGAA G---GGCTCCCAT
CCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCAT
CCTGACTCCGAGCAGAAGAAAGAAGGGCTCCCAT
CCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCAT

CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
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CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC
CATAGTAGACAAGAGTCTAAGCAGAAGAA GAAGAGAGCCAC

No off-target editing when using gesicles

The use of gesicles decreases off-target effects. HEK 293T cells were either simultaneously cotransfected with plasmids encoding Cas9 DNA and a sgRNA against *EMX1,* or treated with gesicles loaded with Cas9-sgRNA ribonucleoprotein complexes. After 72 hr, the *EMX1* gene and a potential off-target locus (off-target 4) were analyzed using the Guide-it Mutation Detection Kit (Cat. # 631443). With the gesicles, no off-target effect could be detected (top panel). Sequencing data for the different clones were aligned with the underlined wild-type sequence, revealing a range of deletions and insertions (indels; highlighted in red). Gesicles correctly edited the *EMX1* gene with no off-target effects, whereas plasmid cotransfection resulted in indels in both the target site, *EMX1,* as well as off-target site 4 (bottom panel).

Complete Kit for Producing Custom CRISPR/Cas9 Gesicles

The Guide-it CRISPR/Cas9 Gesicle Production System contains everything you need to easily produce gesicles that will efficiently target your gene of interest

Lyophilized packaging mixes contain:

- Xfect Transfection Reagent

And coding sequences for:

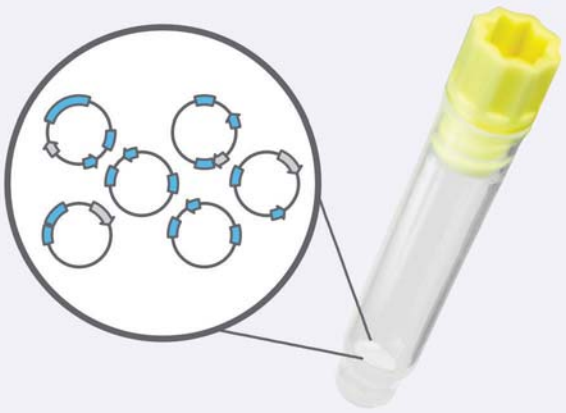
- Nanovesicle-inducing glycoprotein
- Cas9 endonuclease
- CherryPicker RFP

Also included:

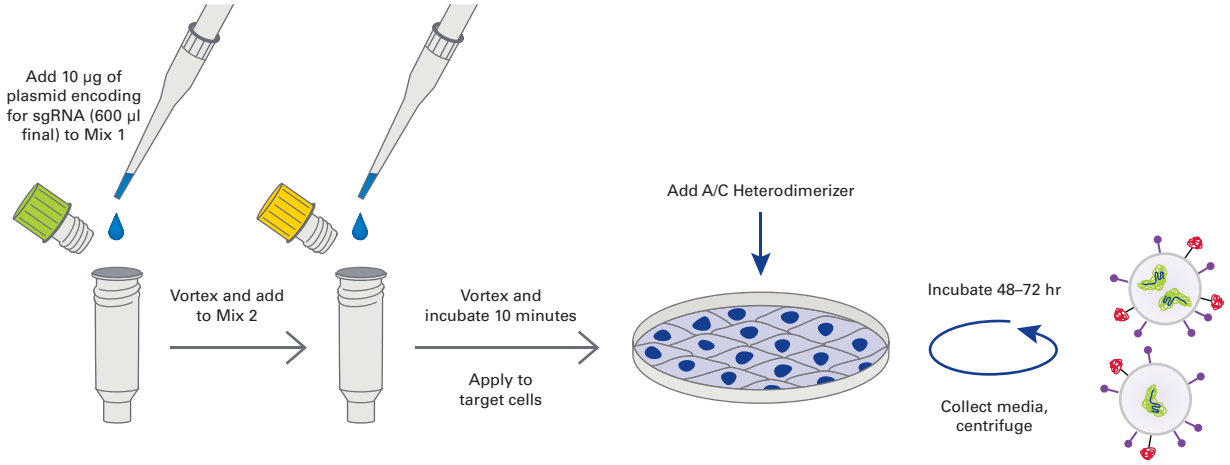
- Pre-linearized vector for cloning your target sgRNA

Available separately:

- Gesicle Producer 293T Cell Line



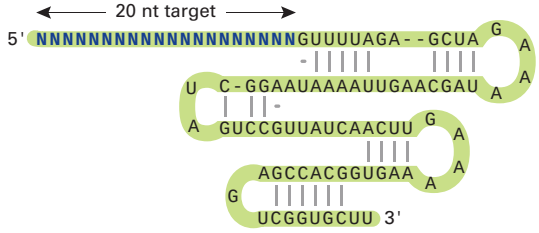
Gesicle Production

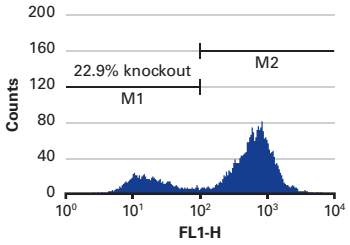


1. Your target-specific sgRNA is cloned into a pre-linearized expression plasmid, pGuide-it-sgRNA1
2. The cloned expression plasmid with your sgRNA is mixed with dH_2O and the Guide-it CRISPR/Cas9 Gesicle Packaging Mixes 1 and 2
3. After 10 minutes of incubation, the transfection-ready mix is applied to 293T-based gesicle packaging cells in the presence of the heterodimerizer ligand
4. Gesicles containing active Cas9 protein complexed with your sgRNA are collected 48–72 hours later
5. Gesicles are concentrated via centrifugation and can be used immediately, or stored at –70˚C for more than 1 year

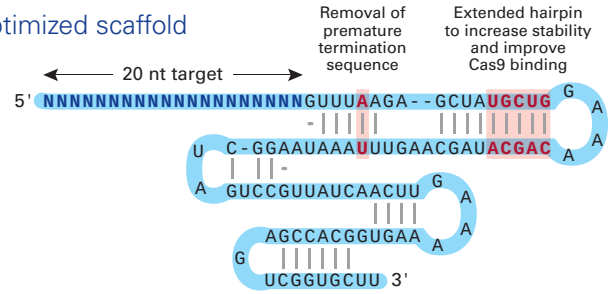
Gesicles Utilize an Improved sgRNA Scaffold that Ensures High Editing Efficiency

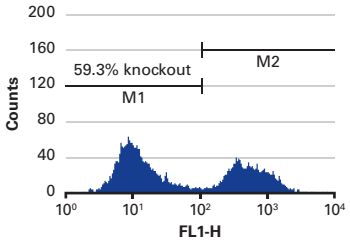
Traditional scaffold





Optimized scaffold





Editing efficiency is increased by an improved sgRNA scaffold design. HT1080 cells containing an integrated fluorescent protein expression cassette were treated with gesicles produced with either the traditional, or an optimized, sgRNA scaffold targeting *AcGFP1*. The knockout efficiency was measured six days later by flow cytometry analysis. The optimized sgRNA scaffold increased knockout efficiency by 36.4%. Thus, only the pGuide-it-sgRNA1 vector containing the optimized scaffold is recommended for gesicle production.

Visit www.clontech.com/sgRNA-design for more sgRNA design tips

PRODUCTS

Cat. #	Product	Package Size
632613	Guide-it CRISPR/Cas9 Gesicle Production System (includes Cat. # 632612 and 632616)	10 rxns
632612	pGuide-it-sgRNA1 Vector System	10 rxns
632616	Guide-it CRISPR/Cas9 Gesicle Packaging Set	10 rxns
632617	Gesicle Producer 293T Cell Line	1 ml

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