BPS Bioscience

CRISPR/Cas9 Human Kinase Knockout Lentivirus Library (Array Format)

#78487

User Manual

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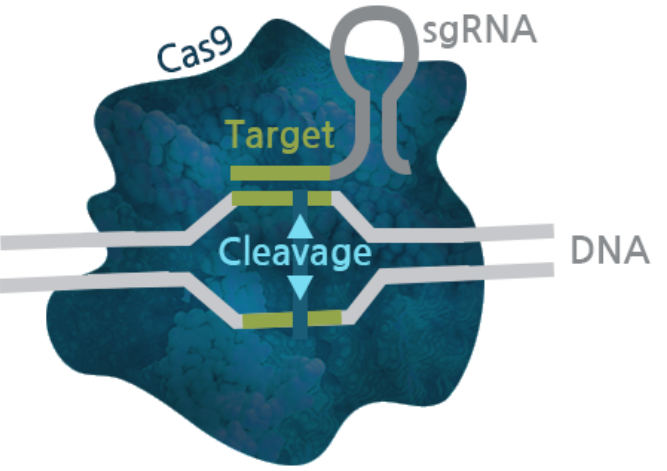

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I. Introduction

CRISPR/Cas9 genome editing is adapted from the bacteria's innate antiviral immune response (Figure 1). Bacteria capture and store DNA fragments from invading viruses within a region of their own genome. These CRISPR sequences (Clustered Regularly Interspaced Short Palindromic Repeats) protect the bacteria from future infections. When the bacteria are infected by a virus with a DNA sequence complementary to these CRISPR sequences, nuclease Cas9 (CRISPR associated protein 9) is recruited to specifically cleave the invading DNA, resulting in its degradation.

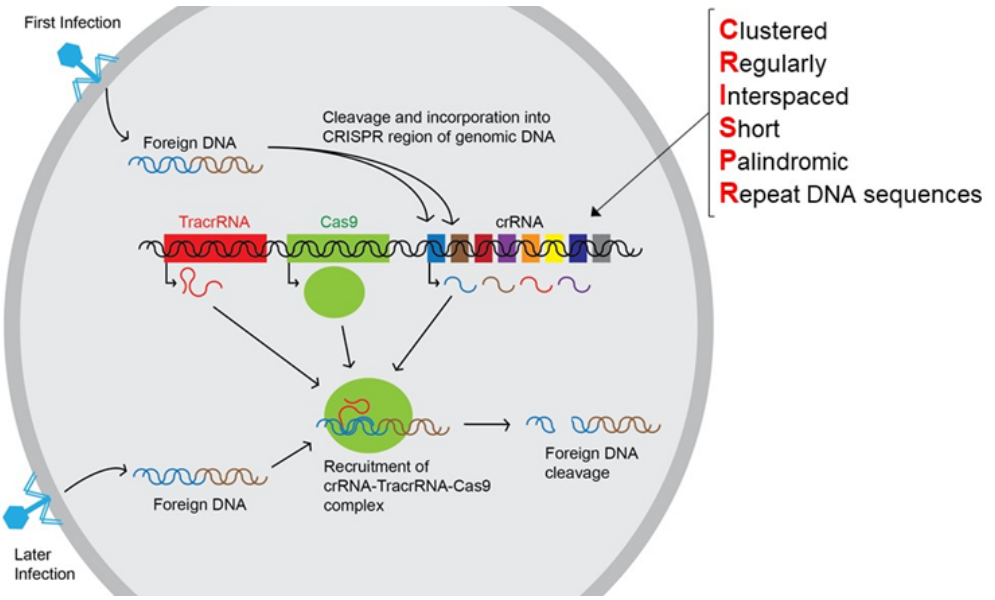


Figure 1: Overview of CRISPR/Cas9 in Bacteria

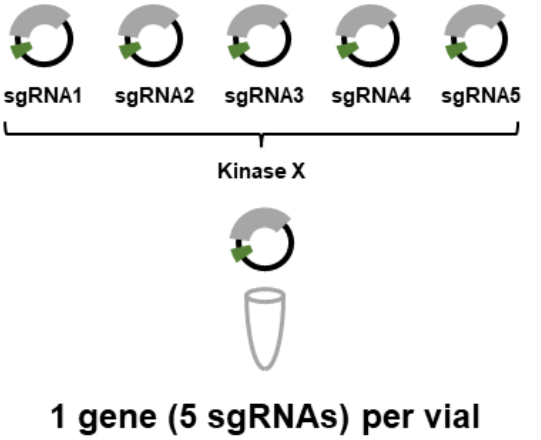
Mammalian CRISPR/Cas9 gene editing systems exploit the ability of Cas9 to use CRISPR sequences as a guide to recognize and cleave complementary strands of DNA. By expressing the Cas9 nuclease in mammalian cells and introducing a synthetic single guide RNA (sgRNA), Cas9 can be recruited to the DNA coding region of a specific gene of interest to introduce a double-stranded break. In mammalian cells, this double-stranded break is repaired through Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR). Non-Homologous End Joining often introduces the deletion or insertion of several base pairs at the cut site, resulting in a frame shift and in the functional inactivation of the targeted gene.

Loss-of-function or CRISPR/Cas9 knockout screens are powerful tools to identify new drug targets and signaling partners. The CRISPR/Cas9 system is characterized by a high knockout efficiency, often allowing clearer interpretation of results compared to screens using shRNA (short hairpin RNA) or siRNA (short interfering RNA) in which residual levels of expressed proteins may complicate analysis.

The CRISPR/Cas9 library is provided in a ready-to-use lentiviral format that can easily be applied to almost any mammalian cell type, including primary and non-dividing cells. This CRISPR/Cas9 library targets all known kinases and pseudo-kinases, with 5 sgRNAs per gene. Additionally, because these lentiviruses contain Cas9, they can be used in any target cell regardless of whether the cells already express Cas9.

II. Description

The CRISPR/Cas9 Kinase Knockout Lentivirus Library (Array Format) targets 619 human kinases and pseudo-kinases. A table containing the complete list of kinases and pseudo-kinases can be downloaded as an excel file from our website. The Array consists of a series of vials, with each vial containing a mixture of integrating CRISPR/Cas9 lentiviral particles targeting 5 sgRNAs for a specific gene (1 vial per gene, 5 sgRNAs per vial). The Array also includes a total of 150 control sgRNAs that do not target any gene (combined into 30 vials containing 5 control sgRNAs per vial). Thus, the Array contains a total of 649 vials and 3,245 sgRNAs.



The VSV-G pseudotyped lentiviral particles are replication incompetent and are ready to infect almost all types of mammalian cells, including primary and non-dividing cells.

The SIN (self-inactivation) lentiviral backbone contains the Cas9 gene (*Streptococcus pyogenes* CRISPR-associated protein 9) driven by an EF1a promoter, an sgRNA driven by a U6 promoter, and a puromycin selection marker (Figure 2).

The lentiviruses integrate randomly into the cellular genome. Since the lentiviruses contain Cas9, they can be used in any target cell regardless of whether the cells already express Cas9. Puromycin selection ensures high expression of both Cas9 and the sgRNA. Knockout efficiencies will depend on the cell type and the gene of interest. Stable CRISPR/Cas9 knockout cell lines can also be generated following limiting dilution.

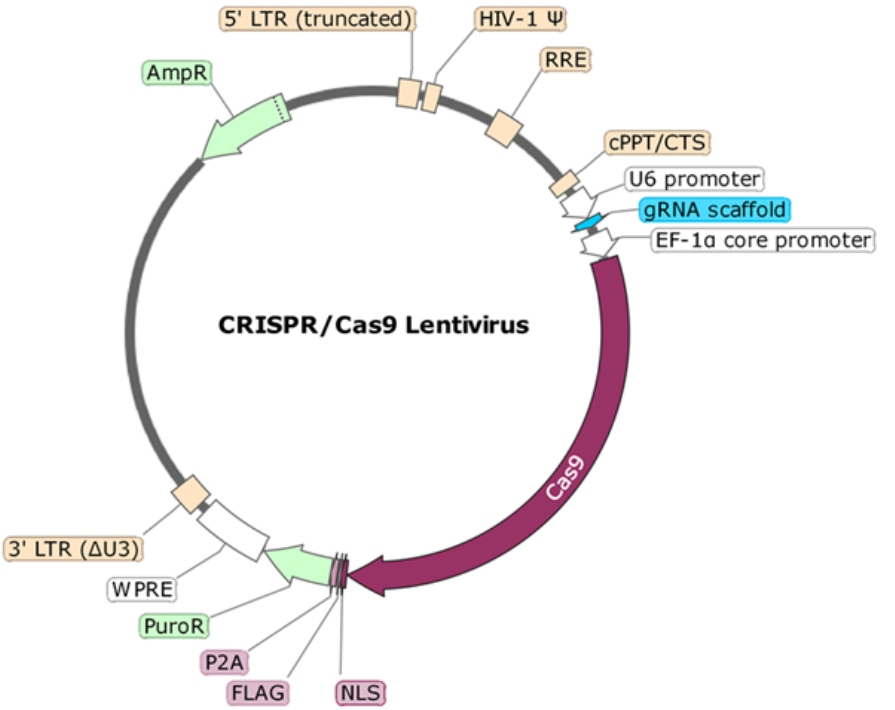


Figure 2: Plasmid Map for Mammalian Expression of Cas9 and sgRNAs.

III. Advantages

- Ready to use
- Comprehensive panel: 619 kinases and pseudokinases (5 sgRNAs each) and 150 control sgRNAs (3,245 sgRNAs in total)
- Versatile format
  - Array: order the entire kinome packaged as one gene per vial (649 total vials)
  - Individual Kinase: order a lentivirus targeting a single, specific kinase of interest
  - Custom Panel: design your own kinase panel (visit BPS Bioscience custom services on our website to order)
- All-in-One: each vial contains lentiviral particles to transduce the Cas9 gene, 5 sgRNAs targeting the kinase of interest, and a puromycin selection marker. There is no requirement for the target cell to already express Cas9.
- Each sgRNA lentiviral particle is individually constructed, sequence-verified, individually cultured, and titered to ensure high quality and representation across the entire library.
- VSV-G pseudoviruses transduce most mammalian cells, including primary and non-dividing cells.
- Safe: requires only Biosafety Level 2 (BSL-2).

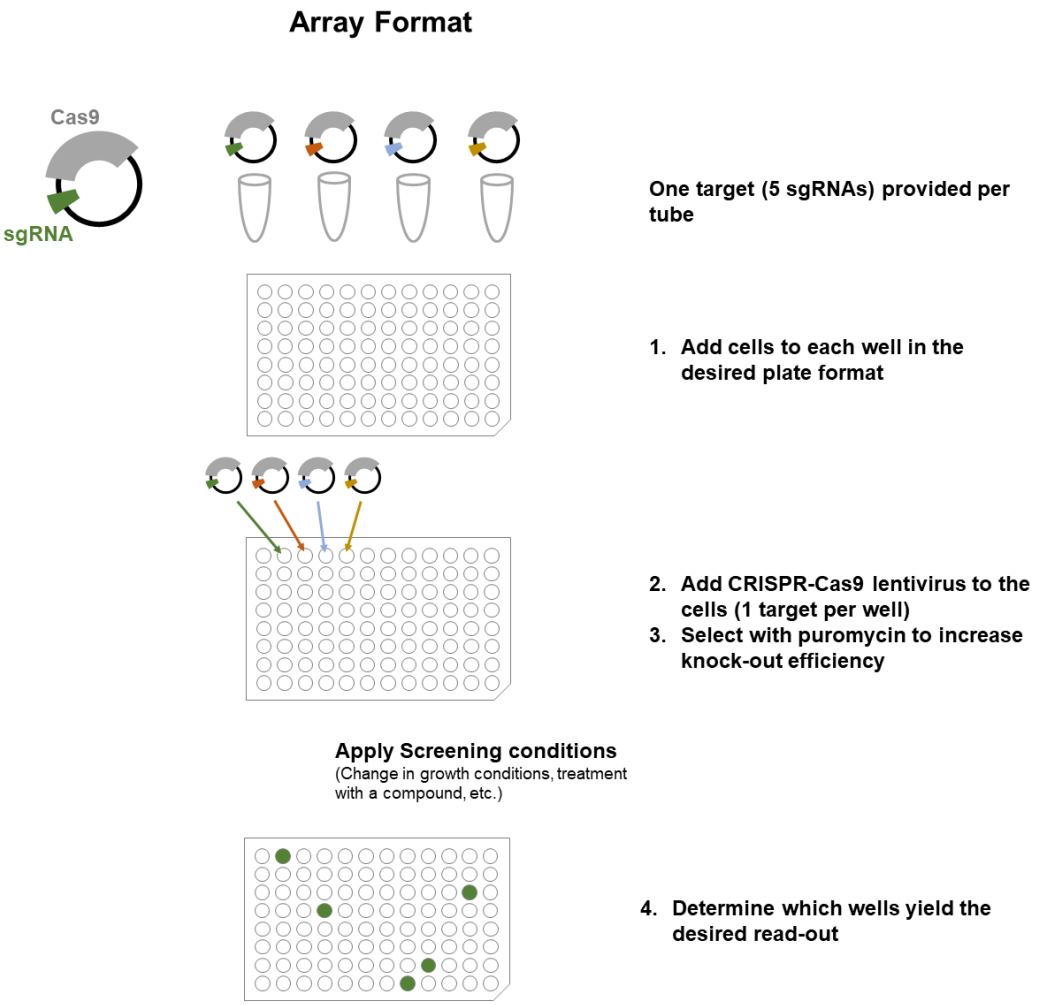


Figure 3: CRISPR/Cas9 Screening in the Array Format

IV. Formulation, Titer, Shipping and Storage

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Each of the 649 vials comes at a titer ≥1 x 10^7 TU/ml. The titer varies slightly with each lot; the exact titer is provided with each shipment. Each lentivirus was individually titered to ensure representation across the entire library. Cell titers were determined by measuring the lentiviral structural protein p24 (using Lenti-X GoStix Plus by Takara).

Lentiviral Library Array

Cat number	Genes	Titer
78487	1 gene per vial, 619 genes	> 1 x 10^7 TU/ml per gene

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store at -80°C. Avoid repeated freeze-thaw cycles as titers drop significantly with each freeze-thaw cycle.

V. Biosafety

The lentiviruses are produced with the SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

VI. Applications

Many applications require a simple phenotypic readout and are therefore amenable to high throughput screening. Complex readouts depend on the technical capabilities of each laboratory.

1. Viability Screening

Perform a knockout screen in 96-well plates and measure the resulting cell viability or cell proliferation to determine which gene knockout causes the cells to die or to stop growing.

This type of screen identifies genes involved in cell survival or genes required for cell proliferation.

2. Synthetic Lethality Screening

Perform a knockout screen in 96-well plates, add compounds of interest to the knockout cells, and measure the resulting cell viability to identify genes which, when depleted, cause the target cells to become sensitive to the compounds being tested.

3. Drug Resistance Screening

Perform a knockout screen in 96-well plates and add a drug of interest. Then, measure the cell survival or cell proliferation to determine which genes, when knocked out, enable the cells to become resistant to the drug. Identification of these genes may provide insight into pathways of drug resistance.

4. Transcriptional Activation (cell reporter system)

Perform a knockout screen in 96-well plates utilizing cells expressing a luciferase or fluorescent reporter under the control of a specific promoter sequence. Identify genes which, when knocked out, induce either the activation or inactivation of the signaling pathway being probed by the reporter.

5. Cell Signaling (protein expression or phosphorylation)

Perform a knockout screen in 96-well plates to determine which kinase is involved in either the regulation of a specific phosphorylation event or that controls the expression levels of a protein of interest. Readouts may include flow cytometry, Western blots, etc.

VII. Protocols

Preparation

The CRISPR/Cas9 lentiviruses contain everything needed to generate knock-out cells (the Cas9 nuclease, sgRNA, and the tracrRNA). Therefore, they can be used in any target cell, regardless of whether those cells are already expressing Cas9.

If puromycin selection or enrichment is going to be used at any time during the experiment, perform a killing curve on your target cells in advance to determine the minimum concentration of puromycin required to kill non-transduced cells. For more information, read our protocol on how to perform a kill curve:

bpsbioscience.com/cell-line-faq.

Determining the MOI

Multiplicity Of Infection (MOI) is the ratio of the number of infective virions per number of target cells. For example, if 100,000 infective particles are added to 1 million cells, the MOI is 0.1.

The optimal MOI of any experiment depends not only on the cell type, but also on the experimental design and readout. In the array format, the greatest integration and knockout efficiencies are desired. Therefore, an MOI of up to 5-10 may be appropriate.

Transduction

For adherent cells, the virus can be added directly to the cells. Optional: Adding polybrene increases the transduction efficiency, but polybrene can also be toxic to cells if added for too long or at a high concentration. If the cells are known to be stress-sensitive, perform a 24-hour dose response with polybrene to optimize the concentration of polybrene to be used.

1. Plate the cells in normal growth medium 24 hours prior to transduction. Cells should be plated at approximately 50% confluency.
2. Incubate the cells at 37°C in 5% CO_2 overnight.
3. Add the appropriate volume of virus directly to the cells for the desired MOI.
4. Optional: add polybrene to the cells to increase transduction efficiency (we suggest 8 µg/ml).
5. Incubate the cells at 37°C in 5% CO_2 for 24-48 hours.
6. Replace the lentivirus-containing culture medium with fresh culture medium. Add the appropriate concentration of puromycin to select for transduced cells.
7. If needed, split the cells according to your laboratory's standard protocol, and maintain the cells in selection medium.
8. Colonies should form within 7-14 days after transduction, depending on the cell type.

Spinoculation

1. For cells grown in suspension, spinoculation can increase the transduction efficacy. Protocols should be optimized for specific types of suspension cells, but here is a protocol that can be used as a starting reference. Volumes are given for a 6-well plate format, although cell densities and volumes can be adjusted to fit other formats as well.
2. Resuspend the target cells in 2 ml of their normal growth medium at a density of 10^6 cells/ml.
3. Add the appropriate amount of virus to reach the desired MOI.
4. Optional: add polybrene to the cells to increase transduction efficiency (we suggest 8 µg/ml).
5. Gently invert the cells to mix and incubate at room temperature for 10-20 minutes.
6. Spinoculation: centrifuge the cells at 800 x *g* for 30 minutes at 32°C.
7. Remove the virus-containing supernatant, resuspend the cells in 2 ml of fresh growth medium, and transfer to a new well or flask. Incubate the cells for 24-72 hours at 37°C in 5% CO_2 before beginning selection with puromycin.

Validation data

Flow cytometry data can be found on our website:

https://bpsbioscience.com/crispr-cas9-kinase-knockout-lentivirus-library-array-format-78487

VIII. References

1. Shalem, O, *et al*. (2014). Genome-Scale CRISPR/Cas9 Knockout Screening in Human Cells. *Science* **343** (6166), 84-87.

2. Sanjana, NE, *et al*. (2014). Improved vectors and genome-wide libraries for CRISPR screening. *Nature Methods* **11** (8), 783-784.

IX. License Disclosure

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

X. Related Products

Individual CRISPR/Cas9 Kinase Knockout lentiviruses may be ordered individually from our website (BPS Bioscience #78488).

https://bpsbioscience.com/kinase-human-crispr-cas9-lentivirus-integrating-78488

Small panels of CRISPR/Cas9 Kinase Knockout Lentiviruses may be ordered via Custom Products and Services.

Visit https://bpsbioscience.com/crispr-kinasepanel for more information on kinase panels.

Custom Products and Services

Our team of highly experienced scientists will generate custom CRISPR/Cas9 knock-out, knock-in, activation cell lines, as well as reporter lentiviruses and cell lines in more than 70 different cell types, targeting whichever gene(s) you are interested in. The development process is comprised of separate milestones where data is provided after each milestone completion. Each project is customized for the desired deliverables by working directly with you. Contact us to learn more about what we offer.

sales-team@bpsbioscience.com

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Cas9-expressing Cell Lines

Cat number	Product	Size
78072	Cas9 A549 Cell Pool	2 vials
78134	Cas9 A549 Cell Line (High/Low expression)	2 vials
78089	Cas9 Daudi Cell Pool	2 vials
78157	Cas9 Daudi Cell Line	2 vials
78073	Cas9 HCT116 Cell Pool	2 vials
78135	Cas9 HCT116 Cell Line (High/Low expression)	2 vials
78166	Cas9 HEK293 Cell Line	2 vials
78161	Cas9 HeLa Cell Pool	2 vials
78070	Cas9 Jurkat Cell Pool	2 vials
78136	Cas9 Jurkat Cell Line (High/Low expression)	2 vials
78179	Cas9 MCF7 Cell Pool	2 vials
78069	Cas9 MDA-MB-231 Cell Pool	2 vials
78150	Cas9 MDA-MB-231 Cell Line (High/Low expression)	2 vials
78087	Cas9 Neuro2A Cell Pool	2 vials
78071	Cas9 Raji Cell Pool	2 vials
78156	Cas9 Raji Cell Line	2 vials

Cas9 Protein

Cat number	Product	Size
100206	Cas9 (His-tag, *S. pyogenes*)	50 – 500 µg

CRISPRa (Activation) Cell Lines

Cat number	Product	Size
78080	CRISPRa (SAM) Jurkat Cell Line	2 vials
78192	CRISPRa (SAM) HEK293 Cell Line	2 vials
78193	CRISPRa (SAM) HeLa Cell Line	2 vials
78194	CRISPRa (SAM) HepG2 Cell Line	2 vials

Cas9 Lentivirus

Cat number	Product	Size
78066	Cas9 Lentivirus (Puromycin Selection)	2 vials (500 µl)
78067	Cas9 Lentivirus (Hygromycin Selection)	2 vials (500 µl)
78432	Cas9 Lentivirus (Neomycin Selection)	2 vials (500 µl)
78119	CD5 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78198	CD5 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78207	FCGR2A CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78199	FCGR2A CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78056	CD47 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78063	CD47 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78435	CIITA CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78434	CIITA CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78054	CTLA4 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78061	CTLA4 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78053	Lag3 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78060	Lag3 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78052	PD-1 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78059	PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78057	PD-L1 CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78064	PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78058	TIGIT CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78065	TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)
78055	TCR CRISPR/Cas9 Lentivirus (Integrating)	2 vials (500 µl)
78062	TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	2 vials (500 µl)

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