Description

The Kinase CRISPR/Cas9 lentivirus is designed to target a specific kinase of interest for knockout. The replication-incompetent, HIV-based, VSV-G pseudotyped lentiviral particles are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. The SIN 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. Each vial of lentivirus consists of a mixture of lentiviral particles targeting 5 different sgRNAs per gene.

The lentivirus integrates randomly into the cellular genome to express both Cas9 and the sgRNAs. Because it contains Cas9, the lentivirus 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 sgRNAs. 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.

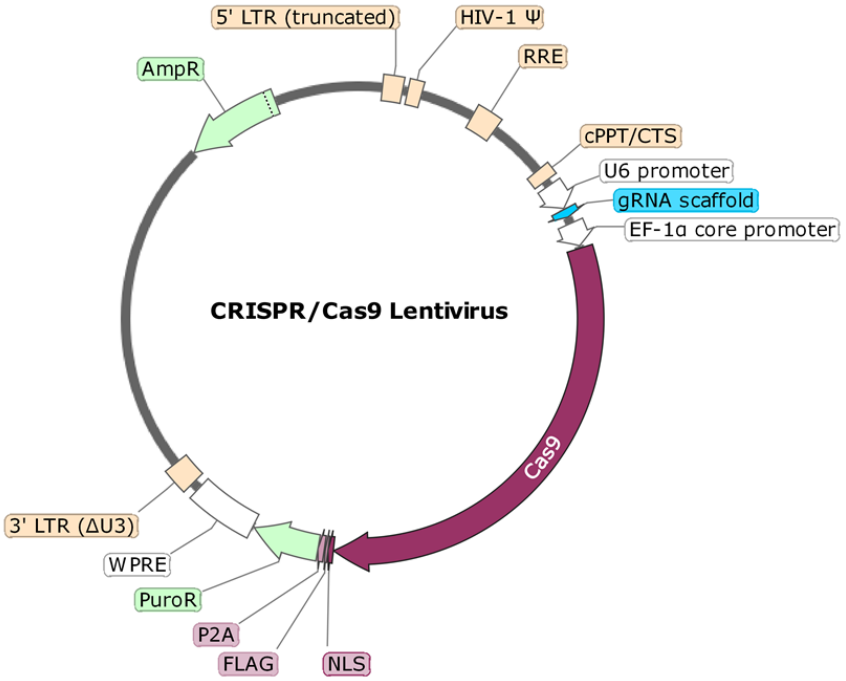

Figure 1: Schematic of the lentivector used to generate each kinase CRISPR/Cas9 Lentivirus.

Applications

1. Transient knockout of the kinase of interest in a cell pool
2. Generation of a stable knockout cell line following puromycin selection and limiting dilution

Formulation

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

Titer

Two vials (200 µl x 2) of lentivirus at a titer ≥1 x 10^7 TU/ml. The titer varies with each lot and is provided with each shipment.

Storage

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

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.

Protocols

The protocols described here are provided as an indication of the methods used to generate validation data. The transduction conditions (e.g., MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements.

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% CO2 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% CO2 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
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.

1. Resuspend the target cells in 2 ml of their normal growth medium at a density of 10^6 cells/ml.
2. Add the appropriate amount of virus to reach the desired MOI.
3. Optional: add polybrene to the cells to increase transduction efficiency (we suggest 8 µg/ml).
4. Gently invert the cells to mix and incubate at room temperature for 10-20 minutes.
5. Spinoculation: centrifuge the cells at 800 x *g* for 30 minutes at 32°C.
6. Remove the virus-containing supernatant, resuspend the cells in 2 ml of fresh growth medium, and transfer to a new well or flask.
7. Incubate the cells for 24-72 hours at 37°C in 5% CO_2 before beginning selection with puromycin.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq. For all further questions, please email support@bpsbioscience.com.

Notes
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.

Related Products

Products	*Catalog #*	*Size*
CRISPR Kinase Knockout Lentivirus Library (Array)	78487	1 Array
Custom panels	Custom order	various

Custom Panels
Build your own Kinase Knockout Lentivirus Panel directly on our website. Our customer service will provide you a quote for any build. Visit webpage.