Description

The Cas9 Lentiviruses are replication incompetent, HIV-based VSV-G pseudo-typed lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a Cas9 gene driven by an EF1A promoter, along with a Puromycin selection marker.

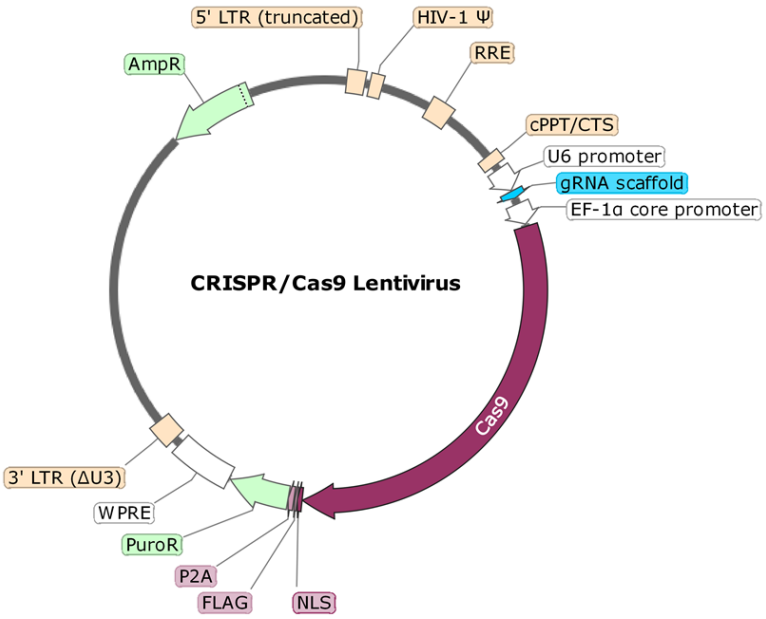

Figure 1: Schematic of the lenti-vector used to generate the Cas9 Lentivirus.

Background

Cas9 (Streptococcus pyogenes CRISPR associated protein 9) is an endonuclease enzyme that, when recruited to a specific DNA sequence by a sgRNA (single guide RNA), introduces a double stranded break into the DNA. This double stranded break is repaired in mammalian cells either through Non-Homologous End Joining or Homologous Recombination. Non-Homologous End Joining often results in the deletion or insertion of several base pairs at the cut site, which, when resulting in a frameshift, causes the functional inactivation of the targeted gene. Cas9 Lentivirus can be used to generate Cas9 expressing cells in almost any mammalian cell line. Cells stably expressing Cas9 can then be transduced or electroporated with sgRNA targeting a gene of interest to quickly generate knock-out cell pools or cell lines. The use of Cas9 technology has revolutionized the cell biology field, and its power can be harnessed for therapeutical applications.

Application

- Expression of Cas9 in target cells
- Generation of a stable Cas9 over-expressing cell line following puromycin selection

Formulation

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

Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥1 x 10^6 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

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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 pseudotyped 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.

Validation Data

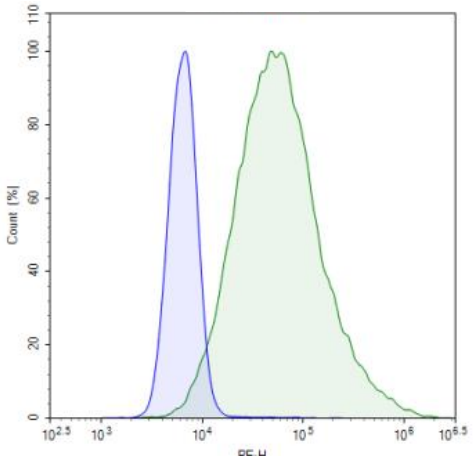

Figure 2: Cas9 expression in A549 cells transduced with Cas9 Lentivirus (Puromycin Selection) (BPS Bioscience #78066).
A549 cells were transduced with 5 x 10^6 TU/well for 72 hours and analyzed by flow cytometry. Cells were stained with PE anti-FLAG antibody (BioLegend #637309). Non-transduced, parental A549 cells are shown in blue, and the transduced cells are shown in green.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. 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.

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Related Products

Products	*Catalog #*	*Size*
Cas9, His-tag (S. pyogenes)	100206	50 µg
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
Cas9 Expressing Jurkat cells	78070	2 vials
Cas9 Expressing MDA-MB-231 cells	78069	2 vials
Cas9 Expressing A549 cells	78072	2 vials
Cas9 Expressing HCT116 cells	78073	2 vials
Cas9 Expressing Raji cells	78071	2 vials

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