Description

The BCMA CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based, VSV-G pseudo-typed lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses contain a CRISPR/Cas9 gene, driven by an EF1A promoter, and with 5 sgRNA (single guide RNA) targeting human BCMA (B- cell maturation antigen) driven by a U6 promoter (see Table 1 for sgRNA sequences). Simultaneous expression of Cas9 and BCMA sgRNA allows the generation of BCMA-knockout cells with one single transduction step. The lentiviruses also contain a puromycin selection marker (Figure 1).

The non-integrating lentivirus is made with a mutated integrase, resulting in only transient expression of Cas9 and sgRNA. Although using the non-integrating lentivirus results in lower knockdown efficiency, Cas9 is not permanently expressed, which lowers the risk of off-targeting, and there are no random integrations into the cell's genome. Despite transient expression of Cas9 and sgRNA, knockout cell lines can still be generated using cell sorting or limiting dilution due to the permanent changes in the genomic DNA from the Cas9 nuclease activity and NHEJ repair.

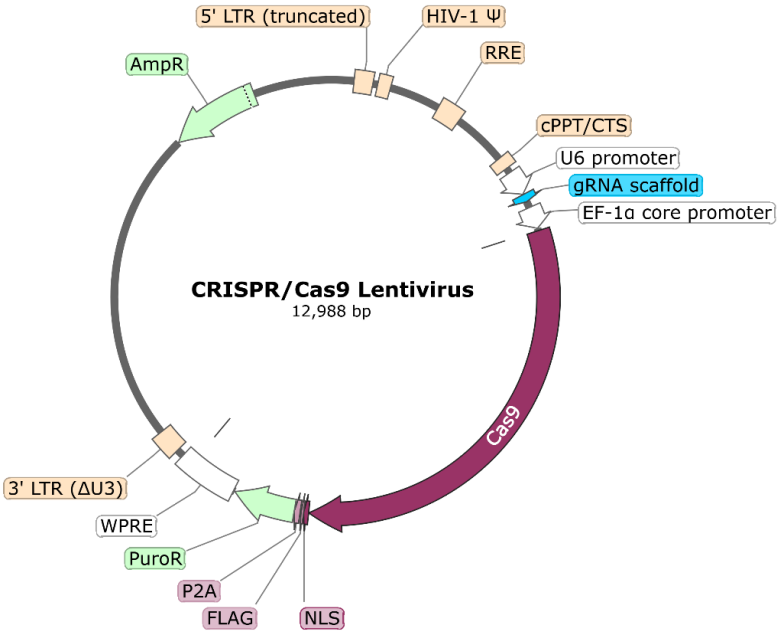

Figure 1. Schematic of the lenti-vector used to generate the BCMA CRISPR/Cas9 Lentivirus.

Table 1: List of sgRNA Sequences in the BCMA CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
TNFRSF17 (BCMA)	TNFRSF17-1	CCTCTAACATGTCAGCGTTA
TNFRSF17 (BCMA)	TNFRSF17-2	TGTCAACTTCGATGTTCTTC
TNFRSF17 (BCMA)	TNFRSF17-3	CGAGTACACGGTGGAAGAAT
TNFRSF17 (BCMA)	TNFRSF17-4	TTCACTGAATTGGTCACACC
TNFRSF17 (BCMA)	TNFRSF17-5	GTGTTTTTAAACTCGTCCTT

Note: Puromycin selection should not be used for more than 48 hours post-transduction, which may lower knockout efficiency.

Background

B-cell maturation antigen (BCMA), also known as CD269 or tumor necrosis factor receptor superfamily member 17 (TNFRSF17), is a cell surface receptor of the TNF receptor superfamily that recognizes B-cell activating factor (BAFF) and is involved in B cell proliferation and maturation. BCMA is preferentially expressed in mature B lymphocytes and the soluble form of BCMA can be found at higher levels in the serum of Multiple Myeloma (MM) patients. BCMA is a highly attractive target antigen for immunotherapy. BCMA, similarly to CD19, is restricted in expression to mature B cells allowing the progenitor's population to be spared during treatment and to replenish the patient's B cell population. BMCA targeting therapies include bispecific antibodies, antibody-drug conjugates, and chimeric antigen receptor (CAR) T cells. To date, the FDA has approved two BCMA CAR-T therapies for the treatment of MM, that resulted in promising outcomes for patients. Further studies will allow a better understanding of the role of BCMA in cancer and fine tune cancer therapy tools.

Application

- Knock-out of BCMA in cells.
- Generation of BMCA knock-out cell pools.
- Generation of a stable BCMA knock-out cell line following transient puromycin selection (48 hours maximum) and single-cell selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥1 x 10^7 TU/ml. The titer will vary with each lot; the exact value 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 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

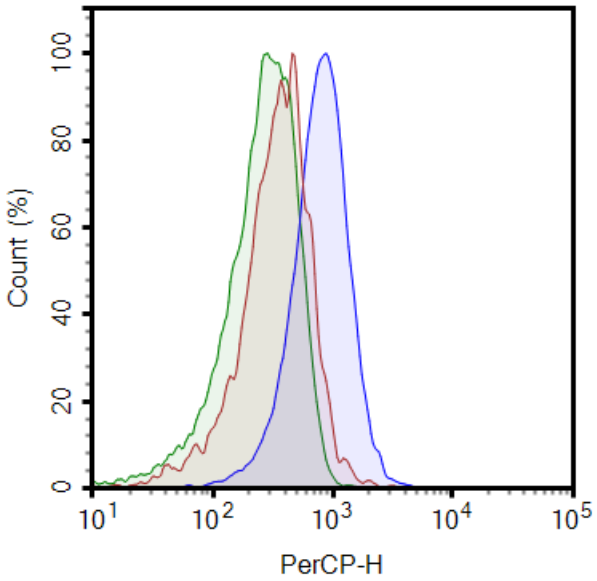

Figure 2. Flow cytometry analysis of BCMA expression in RPMI-8226 cells transduced with BCMA CRISPR/Cas9 lentivirus.
RPMI-8226 cells were transduced by spinoculation with 5,000,000 TU/well of BCMA CRISPR/Cas9 lentivirus. 24 hours after transduction, cells were selected with puromycin for 24 hours. Cells were stained with PerCP/Cyanine-labeled anti-human BCMA antibody (BioLegend #357509) and analyzed by flow cytometry. Stained parental cells are shown in blue, unstained parental cells are shown in green, and cells transduced with BCMA CRISPR/Cas9 lentivirus are shown in red. Prolonged puromycin selection will result in a higher % of cells not expressing BCMA.

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.

Related Products

Products	*Catalog #*	*Size*
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065	500 µl x 2
LAG3 CRISPR/Cas9 Lentivirus (Non-Integrating)	78060	500 µl x 2