

Description

CBL-B is an E3 ubiquitin-protein ligase which has been identified as a negative regulator of T-cell activation. Using CRISPR/Cas9 to inactivate CBL-B has been shown to be sufficient to inhibit T-cell expansion.

The CBL-B CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a CRISPR/Cas9 gene driven by an EF1A promoter, along with 5 sgRNA (single guide RNAs) targeting human CBL-B driven by a U6 promoter (Figures 1 and 2).

The integrating lentivirus integrates randomly into the cellular genome to express both Cas9 and the sgRNA. Puromycin selection forces high expression levels of both Cas9 and the sgRNA, and can be used with the integrating lentivirus to quickly and easily achieve high knockdown efficiencies in a cell pool. Efficiencies may vary, depending on the cell type and the gene of interest.

Application

1. Transient knock-down of CBL-B in target cells
2. Generation of a stable CBL-B knock-out cell pool following puromycin selection and limited dilution

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^6$ 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. Titters can drop significantly with each freeze-thaw cycle.

Biosafety

None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Figures and Validation Data

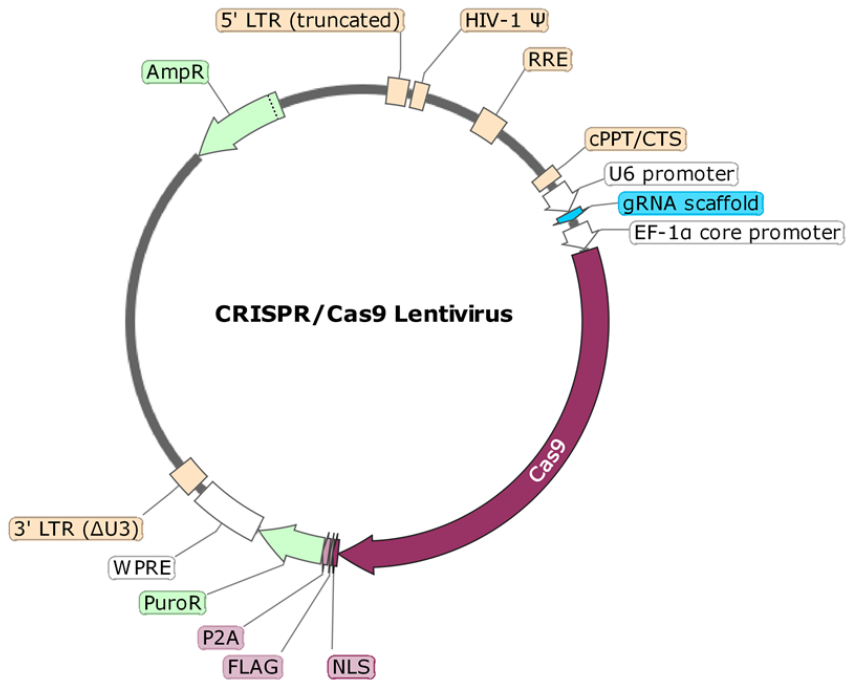


Figure 1. Schematic of the Lenti-vector used to generate the CBL-B CRISPR/Cas9 Lentivirus

Gene Target:	sgRNA Sequence
CBL-B	TGTGGGATGTCTCGACTCCTAG
CBL-B	CTTCATCTCTTGATCAAAG
CBL-B	TTCCGCAAATAGAGCCCA
CBL-B	TGAATTAGATCCAGGCGAGG
CBL-B	TGCACAGAACTATCGTACCA

Figure 2. List of sgRNA Sequences in the CBL-B CRISPR/Cas9 Lentivirus

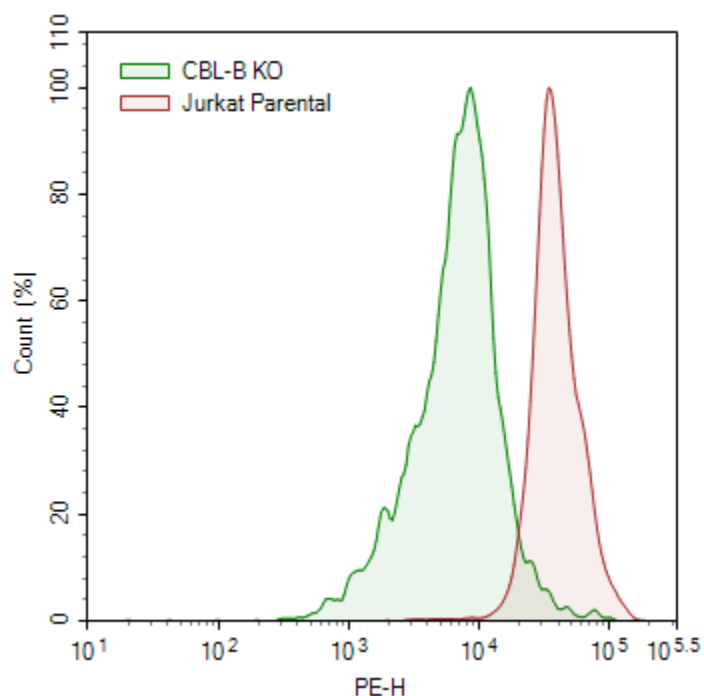


Figure 3. Knock-down of CBL-B in Jurkat cells.

Parental Jurkat cells were transduced via spinoculation with CBL-B CRISPR/Cas9 lentivirus. 24 hours after transduction, cells were selected for 72 hours with puromycin, stained with anti-human CBL-B antibody (Proteintech, #12781-1-AP) and PE-conjugated anti-Rabbit secondary antibody (BioLegend, #406421), then analyzed by flow cytometry. Parental Jurkat cells are shown in red, and the transduced cells are shown in green.

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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CBL-B TR-FRET Assay Kit	79575	384 rxns.
CBL TR-FRET Assay Kit	79786	384 rxns.
CBL-B, His-Avi-Tag	80414	100 µg
CBL-B, GST-Tag (Human)	80415	100 µg
CBL-c, FLAG-Tag	100332	100 µg
CBL-B, His-Avi-Tag, Biotin-labeled (Human)	80412-1	25 µg
CBL-B (Y363F), His-tag, Biotin-labeled (Human)	80413-1	25 µg
CBL-B (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78344	500 µl x 2