Description

The CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus (Notch Signaling Pathway) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses contain a firefly luciferase reporter driven by multiple copies of the CSL responsive element (CBF1/RBPJκ/Suppressor of Hairless/Lag-1) located upstream of the minimal TATA promoter. The lentiviruses also contain a puromycin selection marker (Figure 1). After transduction, Notch signaling can be monitored by measuring luciferase activity.

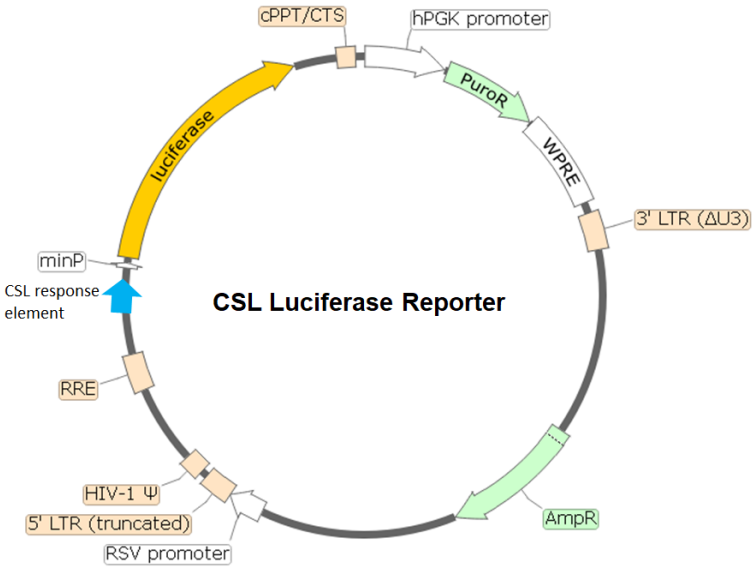

Figure 1. Schematic of the lenti-vector used to generate the CSL (CBF1/RBP-Jk) Luciferase *Reporter Lentivirus.*

Background

The Notch signaling pathway controls cell fate decisions in vertebrate and invertebrate tissues, and it is involved in embryonic development, tissue homeostasis, and regulation of immune and angiogenic systems. Notch signaling is triggered through the binding of a transmembrane ligand, present in opposing cells, to one of the four existing Notch transmembrane receptors (Notch1/ Notch2/Notch3/Notch4). This results in proteolytic cleavage of the Notch receptor, releasing the constitutively active intracellular domain of Notch (NICD). NICD translocate to the nucleus and associates with the transcription factor CSL (CBF1/RBPJκ/Suppressor of Hairless/Lag-1) and coactivator Mastermind to turn on the transcription of Notch-responsive genes. Dysfunction of Notch signaling has severe consequences, from developmental disorders to cancer (such as T cell acute lymphoblastic leukemia, T-ALL, and urothelial bladder cancer). The use of Notch inhibitors, mainly gamma-secretase inhibitors, has shown promise in cancer therapy and in regenerating tissues. Further studies will deepen our understanding of Notch signaling and will benefit future therapeutic approaches.

Application

- Screen for activators or inhibitors of the Notch signaling pathway in cells using luciferase activity as readout.
- Generate CSL-responsive Luciferase Reporter cell pools or stable cell lines by puromycin selection.
- Generate a Notch1dE/CSL Luciferase Reporter cell system when used in combination with Notch1dE Lentivirus (BPS Bioscience #78747).

Formulation

The lentivirus particles were produced in 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 >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 SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and 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.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
HEK293 Cells	ATCC #CRL-1573
Thaw Medium 1	BPS Bioscience #60187
Notch1dE Lentivirus	BPS Bioscience #78747
96-well tissue culture, clear-bottom, white plate	Corning #3610
One-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using the CSL (CBF1/RBP-Jk) Luciferase Reporter and Notch1dE Lentivirus (BPS Bioscience #78747) and it is a general guideline only. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy it may be necessary to select the cells expressing the reporter gene with puromycin, creating a cell pool or stable cell line, prior to carrying out the reporter assays.

Day 1:

1. Seed HEK293 cells at a density of 5,000-10,000 cells per well in 90 µl of Thaw Medium 1 into white, clear bottom 96-well microplate.

2. To each well, add 2 µl of CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus and 5 µl of Notch1dE Lentivirus.

   Optional: Add polybrene to each well to a final concentration of 5 µg/ml.

3. Gently swirl the plate to mix.

4. Incubate the plate at 37°C with 5% CO_2 for 48-66 hours.

Day 3:

1. Add 100 µl/well of ONE-Step™ Luciferase assay reagent.

2. Incubate the plate at Room Temperature (RT) for ~15 to 30 minutes.

3. Measure luminescence using a luminometer.

Validation Data

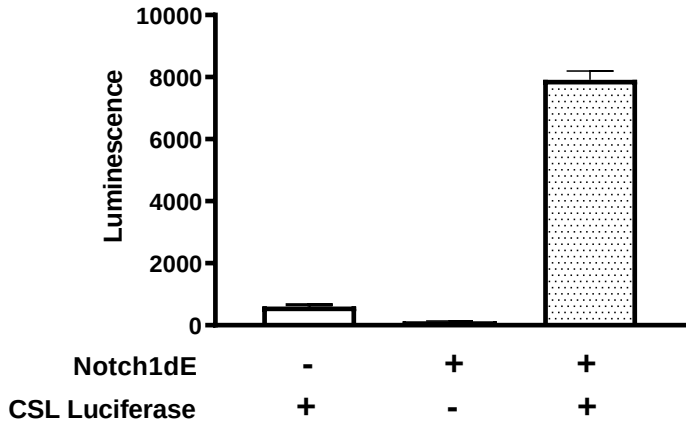

Figure 2. CSL driven luciferase reporter activity in HEK293 cells transduced with CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus and/or Notch1dE Lentivirus.
Approximately 10,000 HEK293 cells/well were transduced with 40,000 TU/well of CSL (CBF1/RBP-Jk) Luciferase Reporter Lentivirus and/or 40,000 TU/well of Notch1dE Lentiviruses. After 48 hours of transduction the luciferase activity was measured with ONE-Step™ Luciferase. Luciferase activity was detected only when both viruses were used. Results are shown as the raw luminescence reading.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Notes

1. To generate a CSL Luciferase Reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, FAQs (bpsbioscience.com)) for antibiotic selection of transduced cells, followed by clonal selection.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase Lentivirus (BPS Bioscience #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. This negative control is important to establish the specificity of any treatments and to determine the background reporter activity.
    b. Renilla Luciferase Lentivirus (G418 or Puromycin) (BPS Bioscience #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the control of a CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.
    c. Firefly Luciferase Lentivirus (BPS Bioscience #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a CMV promoter. It serves as a positive control for transduction optimization studies.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

References

1. Lu F.M., *et al.*, 1996 *Proc. Natl. Acad. Sci. USA* 93(11): 5663-5667.
2. Kanungo J., *et al.*, 2008 *J. Neurochem.* 106: 2236-48.
3. Cao L. *et al.*, 2023 *Blood Adv.* 10.1182/bloodadvances.2023010380

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
Notch1dE Lentivirus	78747	500 µl x 2
CSL Reporter – HEK293 Cell Line	79754	2 vials
Notch Signaling Pathway Notch1/CSL Reporter – HEK293 Recombinant Cell Line	60652	2 vials
Notch1 Pathway Reporter Kit (Human)	79503	500 reactions
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Renilla Luciferase Lentivirus (G418 or Puromycin)	79565	500 µl x 2
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2