Description

Firefly Luciferase-mCherry Lentivirus (Puromycin) are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viruses contain firefly luciferase and mCherry (Luc2-P2A-mCherry) driven by an EF1a promoter, and a puromycin selection marker (Figure 1). Luciferase and mCherry are co-expressed under the EF1A promoter in cells, allowing for flexibility in the detection of transduced cells.

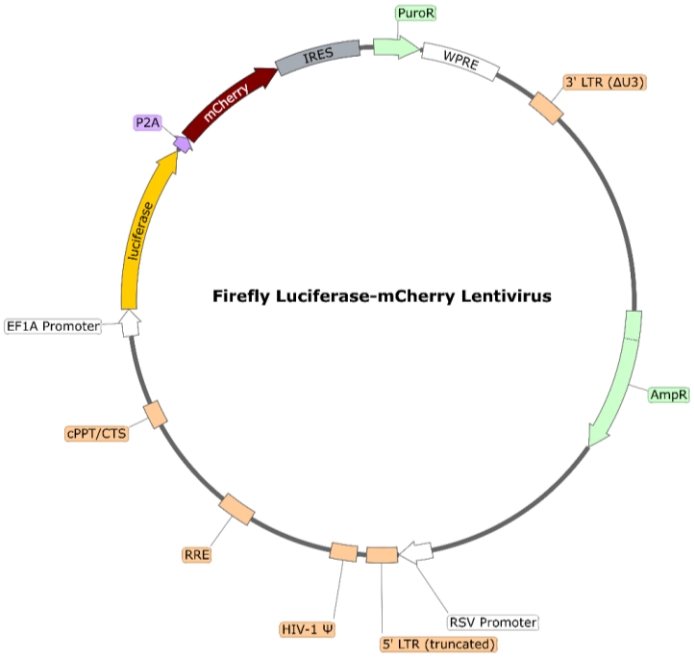

Figure 1. Schematic of the lenti-vector used to generate the Firefly Luciferase-mCherry Lentivirus (Puromycin). This is a SIN vector.

Background

Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. mCherry is a monomeric red fluorescent protein derived from DsRed found in the sea anemones *Discosoma*. It belongs to the mFruit family of monomeric red fluorescent proteins, which are improved versions of mRFP1 (monomeric red fluorescent protein 1) in terms of brightness and photostability. The use of fluorescent proteins allows for direct visualization of transfected or transduced cells under a fluorescent microscope or analysis by flow cytometry. The use of lentiviruse to introduce both luciferase and mCherry is a convenient strategy that allows expression of the markers in almost all mammalian cells and to easily determine transduction efficiency and access cellular responses.

Application(s)

- Positive control in experiments involving transduction.
- Optimize transduction assays and track reporter protein expression over time.
- Generate cell pools or stable cell lines expressing firefly luciferase and mCherry following puromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells in 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 for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

Biosafety

The lentiviruses are produced with a 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.

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 and reagents used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well clear-bottom tissue culture-treated assay plates	
96-well white, clear-bottom tissue culture-treated assay plates for luminescence measurements	
Flow cytometer or fluorescence microscope	
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 Firefly Luciferase-mCherry lentivirus. 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 target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target gene with the appropriate antibiotic prior to carrying out the reporter assays.

Day 1:

1. Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well in 100 µl of Thaw Medium 1 into a clear-bottom 96-well microplate.

2. Incubate the cells at 37°C with 5% CO_2 overnight.

Day 2:

1. Add 1 µl of Firefly Luciferase-mCherry lentiviruses into each well.

2. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer 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 overnight.

   Note: Alternatively, cell plating and transduction can be performed at the same time.

Day 3:

1. Remove the medium containing the lentiviruses from the wells.

2. Add 100 µl of fresh Thaw Medium 1 to each well.

   Note: If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

Day 4-5:

1. 48-72 hours post-transduction, the transduced cells are ready for analysis by the methods of interest. To determine luciferase activity, add ONEStep™ Luciferase reagent to the cells. The expression of mCherry in the target cells can be examined under a fluorescence microscope or by flow cytometry.

Notes

To generate a Firefly Luciferase-mCherry 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, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, following by clonal selection.

Figures and Validation Data

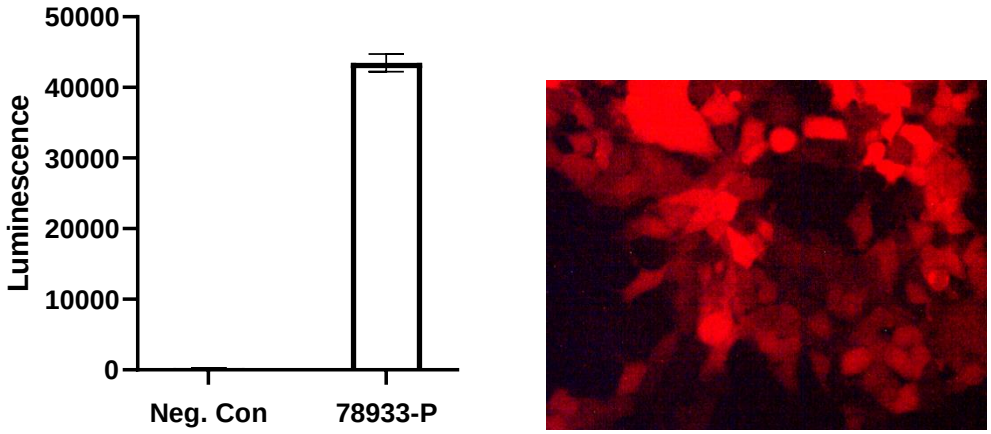

Figure 2. Luciferase activity and mCherry expression in HEK293 cells transduced with Firefly Luciferase-mCherry Lentivirus (Puromycin).
Left: 10,000 HEK293 cells were transduced with 0.5 µl of Firefly Luciferase-mCherry Lentivirus (Puromycin). 48 hours post-transduction, luciferase activity was measured with ONE-Step™ Luciferase. Right: 10,000 HEK293 cells were transduced with 0.5 µl of Firefly Luciferase-mCherry Lentivirus (Puromycin). 66 hours post-transduction, the expression of mCherry was assessed with a fluorescence microscope.

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

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

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 µl x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 µl x 2
Firefly Luciferase (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Renilla Luciferase Lentivirus (G418 or Puromycin)	79565	500 µl x 2
Expression Negative Control Lentivirus (G418 or Hygromycin or Puromycin)	79902	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (EF1A Promoter/Geneticin, Hygromycin or Puromycin)	78740	500 µl x 2

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