Manual

Lenti-Fire™

Lentiviral transduction of Target cells

with Firefly luciferase Luc2

Make your own Firefly luciferase cell lines – it's easy – use a Lenti!

Order Fast at www.invivoimagingsolutions.com

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Introduction

Lenti-Fire™ are ready made lentiviral particles allowing fast production of stable firefly luciferase luc2 expressing cell lines from both dividing and non-dividing mammalian cells with high efficiency. Just expose your target cells to Lenti-Fire™ and get expression of luc2 in 72 hours.

Product Description

Lenti Fire™ combines the most sensitive in vivo optical imaging reporter namely the firefly luciferase Luc2 construct from the pGL4 plasmid with the best gene delivery system, a 3rd generation replication incompetent lentiviral vector.

Luc2 is the best and brightest firefly luciferase construct for deep tissue in vivo bioluminescence imaging. This enhanced luciferase is codon optimized for mammalian cell cytoplasmic expression. Luc2 emission spectrum peaks at 600nm in mammalian cells. The substrate is regular D-luciferin.

An Ubiquitin C promoter drives high constitutive expression of the transgene circumventing the issue of CMV promoter silencing, observed in certain cell lines.

Lentiviral vectors are safe, viral based gene delivery systems that generate long term, sustained expression of transgenes. Lentiviral vectors are able to stably integrate into quiescent, non-dividing as well as dividing cells without silencing and without immune response. Transgene efficiency of close to 100% is reported and lentivirus infects a broad array of species and the most resilient cells.

How does it work?

Upon viral transduction, your target host cells are exposed to viral RNA and preformed viral enzymes such as RT and integrase etc. The viral RNA is reverse-transcribed into DNA, and a subsequent preintegration complex is actively imported into the nucleus, and stably integrated into the host genome. One or two days after, the lentiviral genome is integrated into the host genome and luciferase is expressed.

General Considerations for viral transduction

The following information should be considered before one attempts target cell transduction:

- The transduction efficiency of target cells varies significantly under different experimental conditions, including virus concentration, exposure time to virus, and growth area of cells. To determine the viral concentration required to provide the desired multiplicity of infection (MOI) for your target cells, perform several transductions with different concentrations of viral particles. Results from these test transductions should be used to determine an optimal concentration that yields the highest percentage of infected cells based on luciferase expression.
- Recombinant gene expression can be measured directly 48 – 72 hours after transduction ("transient transduction"), but selecting stably transduced cells will require additional time after transduction. The decision to use "transiently transduced" cells or "selected stable" cells will depend on the nature of your target cells, biological assay and transduction efficiency.

Terminology

TU/ml (transduction units/ml) or viral titer - the relative concentration of transduction-competent pseudoviral particles;

MOI (multiplicity of infection)—the ratio of transduction pseudoviral particles (TU) to the number of cells being infected. For example, if 1×10^6 cells are to be infected at an MOI of 1, then 1×10^6 TU should bevadded to the cells;

Transduction Efficiency—the average copy number of expression constructs per genome of target cell in the transduced population.

Biosafety

Our Lenti-Fire lentiviral particles are derived from a 3rd-generation, self-inactivating recombinant lentiviral vector system with enhanced biosafety and minimal relation to the wild-type, human HIV-1 virus. The lentiviral particles produced with this system are replication-incompetent and designed with a number of safety features to enhance its biosafety.

Despite the consideration of the safety features discussed, it is highly recommended that any manipulation with lentiviral vectors, including viral production and transduction, be performed under Biosafety Level 2 (BL-2). All published BL-2 guidelines with proper waste decontamination should be strictly followed. In addition, exercise extra caution when creating lentivirus carrying potentially harmful or toxic genes (e.g. activated oncogenes).

For more information about the BL-2 guidelines and lentivirus handling, refer to "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, published by the Center for Disease Control (CDC). This document may be downloaded at the following address:
http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

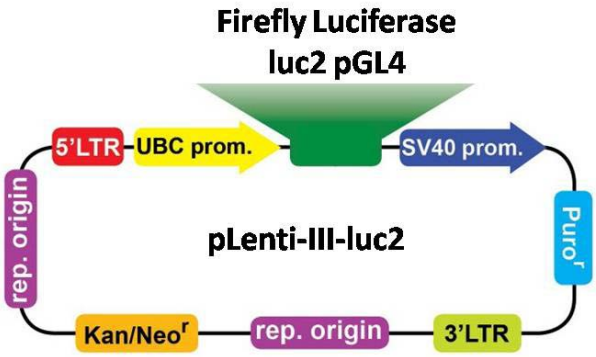
It is also important to consult with the health and safety officers at your institution for guidelines regarding the use of lentiviruses, and to always follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times
- Always work with pseudoviral particles in a Class II culture facility.
- All procedures are performed carefully to minimize splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before.
- disposal by an approved decontamination method such as autoclaving.

Lenti-Fire™ Features:

- An enhancer deletion in the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral vector following transduction and integration into genomic DNA of the target cells.
- Utilization of an RSV promoter upstream of 5'ΔLTR allows efficient Tat-independent production of viral RNA.
- The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (gag/pol/rev), and their expression is derived from different plasmids, which all lack packaging signals. These plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus.
- None of the gag, pol, or rev genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.
- Most commercial retroviral vectors are limited in gene delivery applications because of their restricted tropisms and generally low titers. For recombinant lentiviral vectors, these limitations are resolved by pseudotyping the vector with the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) envelope. This significantly increases viral particle stability, broadens target cell range and facilitates high transduction efficiency.

Lenti-Fire™ Map:



Transduction Procedure:

The following protocol provides general guidelines as a starting point to determine optimal conditions for your target cells and experiments.

Day 1

1. Plate target cells in a 24-well plate 24 hours prior to viral infection at a density of 0.5×10^5 cells per well. Add 0.5 ml of complete optimal medium (with serum and antibiotics) and incubate cells at 37°C with 5% CO2 overnight. Desired confluency is 70-90%. Do not use freshly thawed cells. Allow at least one passage after thaw prior to transduction.
   Note: *It is possible to use other plate formats for transduction. In this case, the amount of cells should be adjusted depending on the growth area of the well/plate.*

Day 2

2. Thaw the lentivirus in a 37°C water bath. Remove immediately form waterbath when thawed. Place on ice.
3. Prepare a mixture of complete medium with Polybrene(*) if desired at a final concentration of 5µg/ml. Remove media from plate wells and replace with 0.5 ml of this prewarmed Polybrene/media mixture per well (for 24-well plate).
4. Infect target cells by adding several different amounts of viral stock (example: 1µl, 5µl, 10µl, and 100µl of virus). Mix the virus with the medium gently by rotation or inversion. Do not vortex. The optimal MOI depends on the permissiveness of the cell line and can vary from 1-100. This can only be determined by trial and error. Incubate cells at 37°C with 5% CO2 overnight.

Day 3

5. Remove the culture medium and replace with 1ml of complete prewarmed medium. Incubate the cells at 37°C with 5% CO2 overnight.

Day 4

6. The following day, split it 1:3 to 1:5, depending on the growth rate of your target cells, and continue incubating for 48 hours in complete medium.

Day 6

7. The infected target cells can be either analyzed for transient expression or selected for stable expression using appropriate selection markers (puromycin or G418). Luciferase Luc2 expression can be screened for by imaging of the cells in well plates (black polystyrene, clear bottom) in e.g. an in vivo imaging system. Add 150µg/ml D-luciferin to the culture medium. This will allow to quantify photon emission form the cells. Use puromycin section to generate stable cell lines. Isolate resistant clones and image for luciferase expression. Use serial dilution and imaging to isolate single clones with highest luciferase expression. Grow monoclonal cell lines for a month, imaging routinely to verify stability of luciferase expression.

*(Polybrene®-Hexadimethrine Bromide; Sigma Cat. No. H9268). Transduction of lentivirus into mammalian cells may be enhanced if cells are transduced in the presence of Polybrene®. This is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined (usually in the range of 2 – 10 µg/ml). Excessive exposure to Polybrene (>12 hr) can be toxic to some cells (e.g. primary neurons). It is recommended to determine cellular toxicity to polybrene beforehand.

Polybrene® Stock Solution:

1. Prepare a 5 mg/ml stock solution in deionized, sterile water.
2. Filter-sterilize and dispense 1 ml aliquots into sterile microcentrifuge tubes.
3. Store at -20°C for long-term storage. Stock solutions may be stored at -20°C for up to 1 year. Do not freeze/thaw the stock solution more than 3 times as this may result in loss of activity. The working stock may be stored at +4°C for up to 2 weeks.

Troubleshooting Guide

Problem	Possible Cause	Solution
No transgene expression.	promoter silencing.	This occurs with CMV promoters when Lentiviral vector may integrate into a chromosomal region that silences the CMV promoter. Therefore the Ubiquitin C promoter was chosen. Screen multiple antibiotic-resistant clones and select the one with the highest expression levels.
	viral stock stored incorrectly.	Aliquot and store at –80°C. Avoid freeze/thaw.
	Low transduction efficiency: Polybrene not used.	Use imaging to check efficiency of transduction of target cells.
	Target cells not transducible with lentiviral vectors.	Transduce target cells in the presence of Polybrene.
	MOI too low.	Use higher MOI.
	Antibiotic concentration too high.	Determine antibiotic sensitivity of target cells by performing a killing curve. Use minimum antibiotic concentration required.
	Cells harvested too early for assay.	Perform expression assay 72 hours post-transduction to allow the accumulation of expressed protein.
Cytotoxic effects of target cells.	Large volume of viral supernatant used for transduction.	Use less viral supernatant; dilute viral supernatant further with fresh medium(1:3 to 1:5) for transduction.
	Polybrene concentration too high.	Use less or omit Polybrene during transduction.
	Antibiotic concentration too high.	Use minimum antibiotics for effective selection.
	Gene of interest toxic to cells.	Try a different cell line or inducible promoter.

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