Lentivirus Protocols

Before you get started

Do your research

The following protocols are intended to be general guidelines and are not optimized for your specific cell line or animal model. We recommend that you do a literature search to find a protocol that closely aligns with your experimental conditions for optimal results.

Aliquot the virus

Freeze-thaw decreases lentivirus titer dramatically. Store the viral stock immediately upon arrival. Aliquot to desired vials right before use.

Follow biosafety procedures

Lentiviruses are classified as BSL-2 organisms, so be sure to follow the recommended NIH guidelines. This includes the required training and use of a Class II biological safety cabinet.

Transduction of Adherent Cells (*in vitro*)

Day 1

Plate cells so that they will be 40-70% confluent the following day. The amount you seed will depend on the doubling time of your cells. As a general guideline, you could seed at a density of 50,000 cells per well for a 24-well plate or at a density such that they would become confluent in 48 hours. Add 500 µL of complete medium to each well and incubate at 37°C with 5% CO_2 overnight. You may use a different sized plate, but be sure to adjust the number of cells and the amount of medium accordingly.

Day 2

Remove medium by aspiration and replace with fresh complete medium with a final concentration of 5-8 µg/mL polybrene*. Gently mix and add the appropriate amount of lentiviral stock to obtain desired MOI (final volume should be ~500 µL/well). For a pilot study we recommend testing a variety of MOIs to find the optimal concentration. We recommend using MOIs of 0 (uninfected control), 5, 10, or up to 30 for hard-to-transduce cell lines. Incubate cells at 37°C with 5% CO_2 overnight.

*Polybrene increases transduction efficiency, but is toxic to some cell lines (e.g., primary neurons). If you observe toxicity, you can either leave out polybrene entirely or substitute with protamine sulfate.

Day 3

Remove medium and replace with fresh complete medium. If you observe toxicity, you may decrease the infection time to between 4-20 hours. If you are selecting cells based on puromycin resistance, add puromycin no earlier than 24 hours post-infection. The optimal amount of puromycin to add should be determined prior to this experiment (see “Calculate a kill curve” section on page 3).

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Day 5+

If using antibiotic selection, replace medium with fresh puromycin-containing medium every 3 days until resistant colonies can be identified.

If you are using a fluorescent reporter, you can begin looking for expression ~72 hours post-transduction. To establish a stable cell line, you can use FACS to sort for the population of cells expressing GFP, RFP, or your fluorescent reporter of choice.

Transduction of Suspension Cells (*in vitro*)

Day 1

If using a 24-well plate, resuspend suspension cells in fresh, complete medium containing 5-8 µg/mL polybrene* and aliquot 500 µL into each well. We recommend seeding at 5-10x10^5 cells/well but it will depend on the cell type. Add the desired amount of lentivirus to each well. For a pilot experiment, we recommend using MOIs of 0 (uninfected control), 5, 10, or up to 30 for hard-to-transduce cell lines. Close the lid, mix gently, and wrap the edges of the plate with parafilm. Centrifuge the plate(s) at 1,200 x g for 2 hours at 25°C. Remove parafilm and incubate for 3 hours. Add 500 µL of fresh medium (no polybrene) to each well (total volume of ~1 mL/well). Incubate overnight at 37°C with 5% CO_2.

*Polybrene increases transduction efficiency, but is toxic to some cell lines (e.g., primary neurons). If you observe toxicity, you can either leave out polybrene entirely or substitute with protamine sulfate.

Day 2

16-24 hours post-transduction, spin down suspension cells and resuspend in complete medium (no polybrene) to a density of 1-5x10^5 cells/mL. Incubate for an additional 1-2 days.

Day 3-4

Split the cells 1:4, 1:8, or desired amount to avoid confluence. Incubate for an additional 2-3 days in complete medium. If you are using antibiotics to generate a stable cell line, this is the step to add it to the medium. The optimal amount of antibiotic (e.g., puromycin) should be determined prior to this experiment (see “Calculate a kill curve” section on page 3).

Day 5+

If using antibiotic selection:

Replace medium with fresh antibiotic-containing medium every 2-3 days and split cells as needed until resistant colonies can be identified.

If using fluorescence microscopy/FACS:

Begin looking for expression ~72 hours post-transduction. Be sure to split cells as needed to avoid confluence. To establish a stable cell line, you can use FACS to sort for the population of cells expressing GFP, RFP, or your fluorescent reporter of choice.

Injection (*in vivo*)

To determine the optimal amount of lentivirus to use for mice or other small animal injections, we recommend first testing three or more doses. Assuming you are using average-sized adult mice, doses of 10^6, 10^7, and 10^8 IFU can be used as starting points to find the optimal dose. Please note that these are general guidelines for mice and may not apply to larger animal models, such as pigs or non-human primates.

Additional Information

Calculate a kill curve (optional)

If using antibiotic selection, first determine the antibiotic's kill curve (minimal amount needed to kill untransduced cells). Add a range of concentrations of the antibiotic to cells that are 40-70% confluent. Following antibiotic addition, examine viability daily and replace with fresh antibiotic-containing medium every 3 days. You should be able to determine the optimal dose within 4-14 days. For puromycin, we recommend using 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/mL.

Physical vs Functional Titer

Viral titer can be expressed in a variety of formats, such as viral particles per mL (VP/mL), plague-forming units per mL (PFU/mL), transduction units per mL (TU/mL), or infectious units per mL (IFU/mL). VP/mL is a measurement of how much virus is present (physical titer), whereas TU/mL, PFU/mL, and IFU/mL are equivalent measurements of how much virus actually infects the target cell (functional titer). Functional titer is considered the more accurate measurement and is generally 10-100 fold less than the physical titer. Vigene's lentiviral titers are expressed as IFU/mL. You can use the formulas below to calculate the amount of virus needed for your experiment.

Calculating the Volume of Virus Needed

Lentivirus IFU needed = Desired MOI x Number of cells

$$\text{µL of viral stock to use} = \frac{\text{Lentivirus IFU needed}}{\text{Viral titer (IFU/mL)}} \times 1{,}000\ (\text{µL/mL})$$

E.g., If your desired MOI is 20 and you want to infect 50,000 cells, you will need 10^6 IFU. If the titer is $1x10^9$ IFU/mL, add 1 µL of the lentivirus stock to the medium.

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