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Lentiviral Infection of Target Cells

Important notes: The transduction efficiency of mammalian cells varies significantly under different experimental conditions. This includes virus concentration, exposure time to the virus and growth area of the well or plate used for the infection.

To determine the viral concentration required to provide the desired multiplicity of infection (MOI) for your target cells, it is advisable to perform several test transductions with reporter viral particles (e.g. GFP Control LV006 or ß-gal Control LV007 lentiviruses) at a range of different volumes such as 1 µl, 5 µl, 10 µl, and 100 µl. Results from these preliminary tests can be used to determine an optimal concentration that will yield the highest percentage of successfully infected cells.

The Spinoculation Protocol is recommended for transduction of suspension cells (T cells, B cells, PBMC etc.) or hard-to-transduce cell types. The Spinoculation Protocol includes an additional spin step that concentrates the virus at the surface to increase transduction efficiency.

Downstream assays should be carried out 48-72 hours following transduction if no antibiotic selection is used. The decision to directly assay without selection will depend on the transduction efficiency and proliferation rate of your target cells and also the biological assay(s) you will be performing.

For cells with a high infection efficiency (e.g. HEK 293, HT1080, HeLa, MDA-MB-468 cells etc) most biological assays can be performed without a need for selection. For cells that are more resistant to infection, it is desirable to select only the clones that stably express the lentivector construct for downstream experimental assays.

Standard Protocol

The following protocol has been provided as a general guideline only, to be used as a starting point for determining optimal conditions for target cell transduction:

1. Plate the 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 if required) and incubate the cells at 37°C with 5% CO_2 overnight.

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.

2. Prepare a mixture of complete media with polybrene at a concentration of 8 µg/ml. Remove the growth media from the wells and replace with 0.5 ml of the polybrene-media-mix per well (adjust volume as necessary if using a different size plate). If the transduction efficiency of the target cells is low, add in ViralEntry™ Transduction Enhancer G515 at 1:100 (or your own optimized dilution ratio).

3. Once an effective MOI has been determined for the target cells through preliminary test infections, use the appropriate volume of virus to infect your cells. You should include a transduction well with a positive GFP control virus and an appropriate blank control viral construct. Leave one well of uninfected cells as an additional standard control. Following the infection, incubate the cells at 37°C with 5% CO_2 overnight.

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4. Remove the culture medium and replace with 1 ml of complete medium. Incubate the cells at 37°C with 5% CO_2 overnight.

5. The following day, split the cells 1:3 or 1:5 (depending on the growth rate of your target cells) and continue incubating for 48 hours in complete media.

6. The infected cells can then be selected for stable expression using appropriate antibiotic selection at a minimum concentration, as determined by a killing curve. Downstream expression can then be assayed by a number of techniques, including Western blot or RT-PCR.

Spinoculation Protocol

The following protocol has been provided as a general guideline only, to be used as a starting point for determining optimal conditions for target cell transduction. Following volumes are for 6-well plate:

1. Resuspend the target cells in fresh pre-warmed complete culture medium at concentration of 10^5 -10^7 cells/ml in a final volume of 8 ml. Aliquot 2 ml into each of 4 x15 ml sterile conical tubes.

2. Once effective MOI has been determined for target cells through preliminary test infections, use the appropriate volume of virus to infect cells with polybrene at concentration of 8 µg/ml. If transduction efficiency of target cells is low, add in ViralEntry™ Transduction Enhancer G515 at 1:100 (or at own optimized dilution ratio). You should include a transduction well with a positive GFP control virus and an appropriate blank control viral construct. Leave one conical tube as uninfected cells as an additional standard control.

3. Gently mix and incubate cells for 20 minutes at room temperature in the tissue culture hood.

4. Centrifuge cells for 30 minutes at 800 x g at 32°C.

5. Remove virus containing medium and resuspend cell pellet with 2 ml of fresh complete culture media. In a 6-well plate, transfer each suspended cell pellet into its own well. Incubate cells for 18-72 hours.

6. Transfer cells into separate sterile 15 ml conical tubes, and centrifuge for 5 minutes at 200 x g. Aspirate media and replace with 2 ml complete media (with appropriate selection antibiotic-optional). Transfer cells into separate tissue culture plates, and incubate overnight.

7. The following day, split cells 1:3 or 1:5 (depending on the growth rate of your target cells) and continue incubating for 48 hours in complete media.

8. The infected cells can then be selected for stable expression using appropriate antibiotic selection at a minimum concentration, as determined by a killing curve. Downstream expression can then be assayed by a number of techniques, including Western blot or RT-PCR.

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