Retroviral 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 retroviruses) at a range of different volumes such as 100 µl, 200 µl, and 600 µl in a 6 well plate. Results from these preliminary tests can be used to determine an optimal concentration that will yield the highest percentage of successfully infected cells.

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 high infection efficiency such as HEK 293, HEK 293T, HT1080, HeLa, 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 retrovector construct for downstream experimental assays.

Standard Protocol
The following protocol has been carried out using HEK 293T cells as target cells and it is provided as a general guideline only. It can be used as a starting point for determining optimal conditions for target cell transduction:

DAY 1:
1. Plate the target cells in a 6-well plate, 24 hours prior to viral infection at a confluency of 5-10%. Add 2 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.

DAY 2:
2. When the cells are 20-40% confluent, remove growth medium. Overlay the cells with 100 µl of retrovirus per well, or the pre-determined volume based on preliminary test infections. Bring final volume to 1ml with serum free media. Add 8 µg/ml of polybrene(G062) to the media/virus mixture. Following the infection, incubate the cells at 37°C with 5% CO2 for 4-6 hours.

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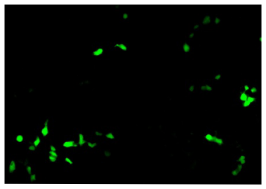
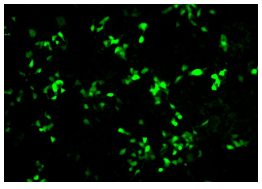
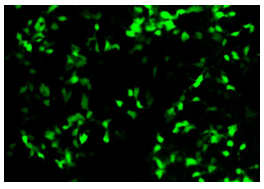
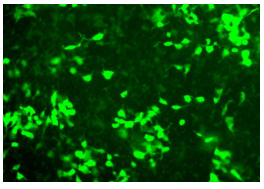
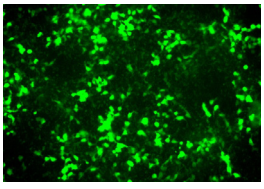
3. After 4-6 hours add 1ml of complete medium to the infected wells. Incubate cells overnight at 37°C with 5% CO_2.

Note: 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.

DAY 3:
4. Remove the infection mixture and replace it with 2 ml of complete medium. Incubate the cells at 37°C with 5% CO_2 overnight.

Note: The infected cells can then be evaluated for gene expression, fluorescence signal (if applicable), drug selection etc.

As an example here, we have infected HEK 293T cells in a 6 well plate with a range of different volumes (300-500 µl) of Retro-CMV-GFP Retrovirus (RVP003) and observed for GFP signal 48-72h post-transduction.

300 µl	350 µl	400 µl	450 µl	500 µl
				

* For other culture dishes determine appropriate volumes based on 20-40% cell confluency.
* For titers higher than 10^6, change volumes accordingly.