Lentivirus transduction protocols (for reference only)

Many factors can affect transduction efficiency. Not all viral particles floating in culture medium can eventually transduce (or infect) the cells. Some additives such as polybrene can enhance the transduction efficiency. But cell type is the main factor to determine the transduction efficiency. An actively dividing cell line gives much higher transduction rate than non-dividing cell types. If you transduced non-dividing cells, a higher Multiplicity of Infection (MOI) has to be used for your optimal expression. Please refer to our recommended transduction protocols below as general reference.

MOI is the average copy number of lentiviral particles per genome of target cell in the infected cell population. Since not all particles can be infected cells, the MOI does not directly correspond to percentage of infected cells. MOI is calculated by counting the number of the cells and the number of the viral particles to be transduced. The number of viral particles per cell is defined as multiplicity of infection (MOI). A higher MOI generates more integration and as a result, higher level of expression. To obtain optimal expression for your specific application, a range of MOIs (e.g. from 1 to 20) should be tested. For example, to achieve single copy integration, theoretically the MOI has to be used at less than one (such as e.g. MOI=0.3). Practically, at MOI =0.3, dependent upon the cell types, only 5% ~ 20% cells will be transduced and the majority of transduced cells should only have one copy of insert. For most cases, you may want to simply add 50ul of our premade LVP into one well of a 24-well plate without worrying too much about MOI.

1. Adhesive cells:

Day 1: Remove the culture medium. Add fresh, warm, complete medium (0.5 ml). Thaw the pre-made lentiviral stock at room temperature. Add the appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C / CO_2 incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. If you cannot use all virus at one time, you still can re-freeze the virus at -80°C for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At ~72 hr after transduction, check the transduction rate under a fluorescent microscope with a suitable filter, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any other flow cytometry system (such as Guava machine).

Day 3 + (optional): Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literature about how to generate stable cell lines.)

Day 3+ (optional): Functional assay for your target in transduced cells.

Note 1: **A quick application protocol is:** add 50 µl virus into one well of a 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after adding the virus (no need to change medium during the course), visualize the positive rate under a fluorescent microscope. For stable cell line generation, transfer the cells into selective antibiotic containing medium, or sort the cells through their fluorescent signal and then select the cells by antibiotics.

Note 2: For some cell types such as primary cells it may take a longer time for maximal target expression; in some cases, maximal expression may not be detected until 1 week post-transduction.

2. Suspension cells:

- Grow your cell in your complete suspension culture medium, including shaking if required in a CO_2 incubator
- Measure cell density. When cells reach about 3 x 10^6 cells/ml, measure cell viability which should be > 90%, then dilute cells to 1 x 10^6 cell/ml in complete medium
- Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at a ratio of: **50µl or 100µl virus per 0.5 ml cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in a flask in the CO_2 incubator with shaking if necessary.
- At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics (Note: each type of particles contains an antibiotic marker and the amount of antibiotic to be used depends on the cell types). Grow in CO_2 incubator.
- At 72 hours after transduction, check fluorescence under the microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
- You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;
CFP filter: ~Ex436 ~Em480;
YFP filter: ~Ex500 ~Em535; (has overlapping spectrum with GFP)