Lentiviral particle transduction protocol

The LipExoGen Lentiviral Expression System™ uses a third-generation, replication-incompetent, VSV-G pseutdotyped HIV-1-based lentiviral particle to enable effective transduction and expression of your gene of interest in dividing or non-dividing mammalian cells. The lentiviral particles are purified by PEG precipitation and sucrose gradient centrifugation and are suspended in serum-free RPMI 1640 medium.

For maximum transduction efficiency, the following protocol is recommended.

Adherent cells Adherent target cells (3-5x10^5 cells/well) should be plated and incubated overnight at 37°C, 5% CO_2, in a 24- or 12-well cell culture plate. On the following day, replace the medium with fresh culture medium and add polybrene at a final concentration of 6-9 µg/ml and mix by swirling. Then, add the appropriate amount of freshly thawed lentiviral particles and mix again by swirling. After the lentiviral particles have been added to the target cells, incubate the cells at 37°C, 5% CO_2 for a minimum of 48-72 hours. If the cells exhibit toxicity to polybrene and/or lentivirus, change to fresh medium 16 hours after infection. Regardless, the cells should be passaged 48-72 hours after infection and cultured overnight before proceeding with drug selection or analysis of gene expression.

Floating cells Floating cells in the exponential phase of growth can be pooled and transduced directly without plating overnight. On the day of transduction, the cells should be pooled together and resuspended in a 24-well plate (3-5x10^5 cells/well) with fresh culture medium. Add polybrene for a final concentration of 6-9 µg/ml and mix by resuspending the cells. Then, add the appropriate amount of freshly thawed lentiviral particles and mix again by resuspending. Centrifuge the plate for 2 hours (about 2500 rpm, 30°C) and then incubate the cells at 37°C, 5% CO_2 for a minimum of 48-72 hours. If the cells exhibit toxicity to polybrene and/or lentivirus, pellet the cells in a centrifuge tube, remove the medium, and resuspend the cells with fresh culture medium before returning to culture. Regardless, the cells should be passaged 48-72 hours after infection and cultured overnight before proceeding with drug selection or analysis of gene expression.

Determining the optimal amount of lentivirus All of our lentiviral particles are provided in a stock solution of about 1x10^8 lentiviral particles/mL. This is not the same as transduction efficiency, which we express in transduction units (TU) and determine in-house using HEK293FT cells. It is important to realize there will be variability in actual transduction efficiency between individual lentiviral particles offered by us (including different versions of the same lentivirus product), as well as differences in transduction efficiency due to the unique conditions which are applicable to you. When planning your experiments, you need to consider the many factors that can influence TU. These include the number of tandem repeats and inverted repeats (i.e. for TF reporter lentiviruses), the size of the insert between LTRs (including how many promoters, the size of the reporters, size of the gene, etc.), the nature and condition of your cells (e.g. primary cells vs cell lines), attention to proper storage requirements, as well as other factors. Larger inserts between LTRs will generally be more difficult to transduce than smaller inserts, especially for primary cells. For our TF reporter lentiviruses, you should plan on using about 20-50 uL per test for the ones with RFP or GFP only (single reporters, no luciferase), and about 50-100 uL per test for the ones with luciferase (as well as dual-reporters such as GFP+RFP, or anything else which would increase the insert size significantly). The number of cells you use may also affect your results and should be planned according to your own situation. In HEK293FT cells, we can achieve medium to high transduction using only 20 uL for most of our lentiviruses when applied to 3-5x10^5 cells in a 24-well plate. If you are using our luciferase TF reporters, we suggest a higher cell number, between 1-2x10^6 cells, and about 50-100 uL of virus to start. The same thing applies to primary or other difficult to transduce cells. Finally, if you are planning on transducing your cells with multiple viruses (for example, internal control plus reporter lentivirus), we strongly recommend performing this in two separate transductions and waiting until the

cells have completely recovered after the first drug selection before continuing on to the second transduction. All of the tips described above are mostly applicable to the TF reporter lentiviral particles, because of the presence of repeats in these constructs. For ORF cDNA and shRNA lentiviruses, as well as our dual-reporter imaging lentiviruses, you should be able to use about 20 uL as a good starting amount and 3-5x10^5 cells, including the constructs containing luciferase. General considerations should be applied based on the size of insert for ORF cDNA fragments. Bigger inserts will be more challenging to transduce compared to smaller ones. Lentiviral particles are supplied in a volume of between 380-500 uL per vial to reach the TU stated for each product. After thawing the lentivirus for the first time, before making aliquots, we recommend that you spin down the vial on a small benchtop mini-centrifuge for 10-20 seconds and make aliquots of the supernatant. Note, Jurkat and other T cells might be more sensitive to puromycin than other cells. You need to be careful with the dose you use to avoid killing all the cells.

Storage of the lentiviral particles Upon receipt, the frozen vial should be promptly stored at -80^{o}C and thawed just prior to first use. Any leftover lentivirus should be aliquoted and immediately returned to -80^{o}C. Multiple freeze-thaw cycles should be avoided as this may reduce TU.

The RFP reporter has its excitation/emission peaks at 587/610 nm