Transduction Protocols:

1. Adhesive cells:

Day 0: Seed the cells in complete medium at an appropriate density and incubate overnight. (NB: at the time of transduction, cells should be 10% ~50% confluent.)
For example, seed Hela cells at 0.5 x 10^5/ml x 0.5ml in a well of a 24-well plate;

Day 1: Remove the culture medium from the cells. Add fresh complete medium (NB: use as little media as possible at transduction). Thaw the pre-made lentiviral stock at room temperature. Add the appropriate amount of virus stock to obtain the desired MOI. Return cells to a 37°C/CO_2 incubator.
For example, add 50 µl of lentiviral stock to the cells in 24-well plate above (to achieve a MOI of 5).

Day 3: At ~72hr after post transduction, check the transduction rate with a suitable filter under a fluorescent microscope, or calculate the exact transduction rate by Fluorescence Activated Cell Sorting (FACS) or any flow cytometry system (such as a Guava machine). **Note:** see filter setting below for each fluorescent protein.
Note: For some cell types such as primary cells, it may take longer for maximal expression. In some cases this may not be until 1 week post-transduction.

Day 3: (optional): Transduced cells can be sorted via FACS, or selected by a specific antibiotic (see each product vector map for its antibiotic marker). A pilot experiment should be conducted to determine the kill curve for your specific cell line, for example, Bsd concentrations can range from 0.5ug ~10ug/ml.

2. Suspension cells:

- Grow your cells in complete suspension culture medium, shaking in a flask in a CO_2 incubator.
- Measure the cell density. When cells have grown to ~3 x 10^6 cell/ml, measure the cell viability (should > 90%) and then dilute to 1 x 10^6 cell/ml in complete medium.
- Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particles into the diluted cells at ratio of: **100ul virus per 1ml cells** (Note: depend upon the cell types; you may need to use more or less virus). Grow cells in a flask, shaking in a CO_2 incubator.
- At 48 hours after transduction, add an equal amount of fresh medium containing antibiotics, dependent upon the particle type. Grow cells shaking in a CO_2 incubator.
- At 72 hours after transduction (some cell lines may need longer), check for fluorescence under a microscope or calculate the transduction efficiency using a cell sorting machine (like FACS or a Guava machine).

  (Note: GFP filter wavelength: Ex450-490 ~Em525; RFP filter: ~Ex545/~Em620).