Lentiviral titering by limiting dilution

I. Materials

6-well cell culture treated plates

15 mL conical vials

Polybrene (Hexadimethrine bromide; Sigma #H9268) or Protamine sulfate (Sigma #P4020)

Puromycin Dihydrochloride (Sigma #P8833)

Crystal Violet Solution (Sigma #HT90132)

Dulbecco's Phosphate Buffered Saline (PBS)

Human and mouse cell line and appropriate growth media.(For example, A549 cell and F-12K culture medium containing 10% fetal calf serum and 1 X Pen/Strep).

II. Instructions

A. Optimization of lentiviral infection

1. Lentiviral infections should be optimized for each cell-line. For example, the cell seeding density, the puromycin concentration, cytotoxicity of polybrene and time course should be test before cell-based assay.
2. Growth rate of cell is very greatly. Adjust the number of cell plated to accommodate a confluency of 50% upon transduction.
3. To make sure the cell is always in the fastest growth phase, never let the cell grow more than 80% confluence.
4. Depending on the experimental setting, different types of cells can be used in order to determine the infectious titer.

B. Procedure

1. (DAY 1) Plate 2 x 10^5 A549 cells per well in a 6-well plate and incubates at 37 °C, 5 % CO2 for 18-20 hours.
2. (DAY 2) Make a stock solution of F-12K culture medium with 8 ug/ml polybrene.
3. Thaw lentivirus stock at room temperature and prepare 2 mL 10-fold serial dilutions ranging from 10^{-2} to 10^{-6} in 15 ml conical vials. Mix gently by inverting the tubes 10 times.
4. Add 1 ml F-12K culture medium containing polybrene to one well as a mock control. Then add 1ml of each of diluted virus to the remaining wells of the plate. Incubate at incubator at 37 °C, 5 % CO2 for 18-20 hours.

5. (DAY 3) Remove the medium containing virus from well and replace with 2 mL of F-12K culture medium (without polybrene).
6. (DAY 4) Remove the medium and replace with 2 mL of F-12K culture medium containing puromycin (2 μg/mL) to select stably transuded cells.
7. (DAY 5-14)Replace medium with fresh medium containing puromycin every 3–4 days.
8. (Day 14)Remove the medium and gently wash each well with 3ml PBS once. Add 1 ml crystal violet solution and incubate 10 minutes at room temperature.
9. Remove crystal violet solution, then gently wash well with 3 ml PBS twice. Count the blue-stained colonies.
10. Titer of the lentiviral stock was determined by: Number of clonies × Folds of dilution (transducting units/ml; TU/mL).

Example: The colony counts were shown at table,

Dilution	Mock	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
number of colony	No colonies	UD*	UD	UD	38	4

*UD: undeterminable

Thus, the titer of the lentiviral stock is 3.9 x 10^6 TU/ml (*i.e* . average of 3.8 x 10^5 and 4 x 10^6)