Titration of Lenti-Vectors

- Medium: DMEM with Glutamine and high Glucose (4.5g/l)
  + 10% FBS
  + 1mM Sodium Pyruvate
  + 20mM HEPES (or 25mM)
  + Penicillin/Streptomycin

- 8mg/ml Polybrene in PBS (= 1000x stock solution. Available e.g. from Sigma)

Day 1:

- Seed 50,000 293T cells per well in 500µL medium in 24-well plate (or NIH-3T3 cells for Eco pseudotypes).
- Wait for the cells to attach (2 to 5 hours).
- Add Polybrene to final concentration of 8µg/ml (or change medium, 500µl per well with 8µg/ml Polybrene)
- Add viral particles containing supernatant to the cells, e.g. 1ul per well in triplicate. Doing this the first time try the following amounts: 0.1µl; 1µl; 10µl and 100µl per well (see information below).
- Centrifuge the plate for 1 hour, 1000g, 24°C.

Day 2:

- Change medium, use 1ml per well (without Polybrene).

Day 4:

- Analyze the cells in a flow cytometer and calculate the titer.

Calculation of the titer

The titer should be calculated from wells showing between 5% and 20% positive cells ideally. Higher transduction rates lead to multiple integrations per cell and thus underestimation of the titer [Fehse et al. 2004, Pois(s)on - it's a question of dose..., Gene Ther. 11(11)]. Therefore different amounts of supernatant have to be used for the titration, 0.1µl for high titer constructs (e.g. containing eGFP only) and 1µl for standard constructs. 10µl and up may be necessary for low titer constructs co-expressing problematic cDNAs.

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T = N*P/V       T: titer
                N: number of plated cells
                V: volume of added supernatant
                P: proportion of transduced cells
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Example:
1µl of supernatant yielded 12% of GFP-positive cells. That means that at the time point of transduction 12% of the 50000 cells got transduced by a vector particle.

$T = 50\,000 * 0.12 / 0.001ml = 6 \times 10^6 /ml$

It is difficult to compare the titer between different laboratories. For example some labs do not perform the centrifugation step (called spinoculation or spin-inoculation) or do not use Polybrene or use Protamine sulfate instead et cetera. Also very important is the cell type that is used for a titration, on different cell lines the titer may differ more than 10-fold. There is no “titer of a vector preparation”, there is only a “titer of a vector preparation titrated under conditions XY on cell line Z”.