Lentivirus Infection

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Materials and Reagents

1. Cells
2. Polybrene (hexadimethrine bromide) (Sigma-Aldrich, catalog number: H9268)
3. Puromycin
4. Human or mouse cell line and appropriate growth media reagents required for cell-based assay
5. Polybrene appropriated antibiotics for selection purpose

Equipment

1. Tissue culture Incubator
2. 6 cm tissue culture plates (Thermo Fisher Scientific)

Procedure

Note: Lentiviral infections should be optimized for each cell line and cell-based assay. For example, the following parameters should be tested before starting large-scale infections to determine the optimal conditions for a given experiment:

1. *Cell seeding density.*
2. *Amount of lentivirus.*
3. *Puromycin concentration.*
4. *Timecourse.*

A. Seed cells at appropriate density in 6 ml in 6 cm plates.

1. Adherent cells: seed 1 day prior to infection.
2. Suspension cells: seed day of infection in media containing polybrene.

B. Add virus to cells:

1. (Adherent cells): Remove growth media and add fresh media containing polybrene. Alternatively, remove a portion of the growth media and supplement with media containing polybrene. Adjust volumes and polybrene concentration to achieve the correct final polybrene concentration (8 µg/ml).

C. Viral infection:
   1. Incubate cells overnight.
   2. Change media 24 h post-infection. Remove media and replace with 6 ml fresh growth media. If antibiotics selection is desired, use fresh growth media containing antibiotics. *Note: Puromycin concentration should be optimized for each cell line; typical concentrations range from 2-5 μg/ml.*

D. Incubate cells, replacing growth media (with antibiotics, if desired) as needed every few days. Incubation periods are highly dependent on the post-infection assay.
   Note: All lentiviral procedures should be carried out in accordance with biosafety requirements of the host institution.