Retroviral and Lentiviral Infection of Target Cells

Protocol

1. Thaw the recombinant retrovirus or lentivirus supernatant in a 37°C waterbath and remove it from the bath immediately when thawed.
2. Prepare polybrene stock to a concentration of 0.8mg/mL.
3. In the early morning, infect the target cells in a 6-well plate with 2mL/well supernatant in the presence of 8μg/mL polybrene (add 20μL of the stock polybrene to 2mL of the viral supernatant, 1:100 dilution). Place the remainder of the viral supernatant in the fridge for the second infection in the afternoon.
4. 6-8 hours later, remove the viral supernatant (from the first infection) from the wells and re-infect the cells with 2mL of fresh supernatant (with polybrene).
5. For lentiviruses, one infection (incubated overnight) works well with most target cells. Dilute lentivirus with fresh complete medium (1:1) if cytotoxicity is a problem.
6. The next day, remove the viral supernatant and add the appropriate complete growth medium to the cells and incubate at 37°C.
7. 72 hours after incubation, subculture the cells into 2 x 100mm dishes and add the appropriate selection drug for stable cell-line generation. For most cell lines the selection concentration is between 0.2μg/mL (often around 0.3μg/mL).
   Note: For the EGFP retrovirus, the selection marker is Puromycin.
8. 10-15 days after selection, pick clones for expansion and screen for positive ones.

Notes

- After thawing, we recommend that the supernatant not be frozen again for future use since the viral titer will decrease significantly.
- Infection of MDA-MB-468 cells would be a good control for EGFP viruses.