Retroviral and Lentiviral Infection of Target Cells

Protocol

NOTE: To ensure antibiotic selection is working properly, it may be necessary to complete a killing curve first to find the optimal antibiotic concentration for selection.

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. Plate the target cells in a 6-well plate 24h before infection with the density around 30-40% confluence.
4. 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.
5. 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).
6. 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.
7. The next day, remove the viral supernatant and add the appropriate complete growth medium to the cells and incubate at 37°C with 5% CO_2.
8. 72 hours after incubation, subculture the cells into 2 x 100mm dishes and add the appropriate selection drug for stable cell-line generation.

   Note: If the selection marker is Puromycin, for most of cell lines, the selection concentration is between 0.2-1.0 μg/mL (often around 0.3 μg/mL). If the selection marker is geneticin (G418), the selection concentration for mammalian cell is between 200-1500 μg/ml.
9. Remove and replace antibiotic containing medium every 3-4 days using the antibiotic concentration in step 8 for selection.
10. 10-15 days after selection, pick clones for expansion and screen for positive ones. Once selected and isolated, maintain the cells with the following suggested antibotic concentration: 200 μg/ml of G418, or 0.2 μg/mL of Puromycin.

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 GFP viruses.