Cellomics Technology
innovative tools for genetic engineering

Transducing Target Cells with Lentivirus

The following protocol is a general method for transducing adherent cells in six-well plate. Use it as a starting point for determining the optimal transduction conditions for your target cells.

1. Plate target cells in 2ml complete growth medium, 12–18 hr before transduction.

   Note: Use heat-inactivated FBS for transduction.

2. Prepare transduction medium: Add polybrene to 2 ml of complete growth medium to desired concentration. The optimum final concentration of polybrene may be determined empirically but generally falls within a range of 2–12 μg/ml.

   Note: Polybrene is a polycation that reduces charge repulsion between the virus and the cellular membrane. Excessive exposure to polybrene (>24 hr) can be toxic to cells.

3. Thaw aliquots of your lentiviral stocks. Mix gently, but do not vortex. Note that each freeze-thaw cycle will decrease titer by 2–4-fold. Add proper volume of the lentiviral stocks into prepared virus transduction medium to obtain the desired MOI, the total volume of virus represents no more than 1/3 the final volume of prepared virus transduction medium.

4. Remove the plate(s) of target cells from cell culture incubator. Aspirate culture medium. Add prepared transduction medium with virus to the cells. (Optional) Centrifuge the cultures at 1,200 x g for 60–90 min at 32° C or room temperature (Centrifugation can significantly increase infection efficiency). Incubate the plate(s) at 37°C for 8–24 hr in a CO_2 incubator. If you are concerned exposure to the polybrene, limit the transduction to 6–8 hr.

5. Remove and discard the virus-containing transduction medium and replace it with fresh growth medium. Continue to incubate the cells for 24–48 hr to allow the expressed protein to accumulate in the target cells. Harvest the cells for analysis or proceed with selection using the appropriate antibiotic.