6/15/2011 KW

Lentiviral production and infection protocol for second generation lentivirus such as pLKO.1 or pHIV-ZSgreen

A. producing lentiviral particles

Day 1.) Plate 2x10^6 HEK293T cells into a PLL-coated 60-mm dish. Use 4 mL of antibiotics-free medium.

Day2.) Transfetion.

pLKO.1 or pHIV-ZSgreen 3 μg.
psPAX2 2.25 μg.
pMD2.G 0.75 μg.----------in 100-μL Opti-MEM (Gibco-Invitrogen #11058-021)

10 μL Lipofectamin 2000 + 90 μL Opti-MEM.

Incubate for 5 min, then mix with plasmid solution. Incubate for another 15 min and add to the dish from Day1. Incubate overnight.

Note: Can be done by calcium phosphate but I found the viral titer is higher when Lipofectamin 2000 is used. Please use calcium phosphate for large scale production followed by concentration of the viral solution (see below).

Day3.) Aspirate supernatant and add fresh DMEM with 10%FBS (with antibiotics). Incubate for 24 h.

Day4.) Collect supernatant and add fresh medium. Incubate for 24 h. Store the supernatant at 4ºC.

Note: the conditioned medium should become "yellow-ish" in the end.

Day5.) Collect supernatant and combine with the supernatant from Day 4. Filter with .45-μm pore filter. Use immediately or store at -80ºC in aliquots.

Day6.) Titrate viral transduction unit (TU) if the viral construct contains fluorescent markers such as GFP or RFP. Refer to the Werb lab protocol for details. http://anatomy.ucsf.edu/werbwebsite/protocols.htm

B. infecting lentivirus

Day 1.) Plate 1-2x10^5 cells into a 12-well plate. Infection.

Note: cell number varies depending on cell types and viral titer.

Day2.) Infection.

Mix the original viral solution (supernatant from day 5 above) with culture medium (total 1 mL). Add 8 μg/mL polybrene. Replace the medium with lentiviral solution. Incubate overnight. – check with Bingnan to see if we should add a slow centrifuge step to increase infection efficiency – he tried this.

Day3.) Aspirate supernatant and replate the cells into 12-well plates as multiple (3-4) replicates. Incubate overnight.

Day4.) Add 1-2-μg/mL puromycin to select the infected cells if needed. Or check infection efficiency by FACS for GFP/RFP-expressing constructs.

C. Large scale production of lentivirus

Refer to the Werb lab protocol for details.
http://anatomy.ucsf.edu/werbwebsite/protocols.htm

For centrifugation, we use SW28 rotor with ultra-clear centrifuge tube (1x3.5 inch, #344058 Beckman) for 35-mL conditioned medium/tube. Centrifuge speed at 20000 rpm for 2 h 45 min. (Nomura lab ultracentrifuge)