THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Lentivirus Packaging in 96-Well Plate

Plasmids: pCMV-DR8.2 (1μg/μl), pCMV-VSVG (1μg/μl), transfer plasmid (40ng/μl)
Transfection reagent: Lipofectamine 2000
Virus harvesting medium:
DMEM + 10% FBS + 1g/ml BSA + 1% P/S
500 ml Hyclone DMEM high glucose
50 ml FBS (Hyclone SH30071.03)
64 ml BSA (10g/100ml in DMEM, Hyclone BSA, SH3057402)
6ml P/S (antibiotics)

1. Continually pass 293T cells in 1:2 for three times before seeding the cells into 96-well plate;
Day 0
2. Dilute the cells to a final concentration of $5*10^5$ cells/ml in antibiotics-free culture medium;
3. Seeding 100μl 293T cells into each well of 96-well plate (i.e., $5*10^4$ cells/well);
4. Incubate the cells for overnight (about 16~17 hours) at tissue culture incubator;
Day 1
5. Dilute the transfer plasmid to a final concentration of 25 ng/μl;
6. Transfer 4 μl of each transfer plasmid to the corresponding well in a new 96-well plate;
7. Prepare packing plasmid DNA (pCMV-DR8.2 + pCMV-VSVG) mixture as shown below
Add 11ug (pCMV-DR8.2), 1.1ug (pCMV-VSVG), 2.31 ml OPTI MEM to a 5ml-tube ® mix well® add 21μl to each well of the above new 96-well plate;
8. Dilute lipofectamine 2000 with OPTI MEM
Add 55 μl lipofectamine 2000 to 2.75 ml OPTI medium ® mix well ® incubate at room temperature for 5 min;
9. Add 25 μl to each well of the new 96-well plate containing plasmids;
10. Incubate at room temperature for 20 min;
11. Transfer 50 μl of DNA-lipofectamine mixture to the cells in each well of 96-well plate;
12. Centrifuge the cells at 2000 rpm (about 890xg) for 30 min at room temperature;
13. Incubate the cells at incubator for overnight;
Day 2
14. Carefully remove the culture medium (~120 μl) from each well after 18 hours culture;
15. Add 200 μl BSA-containing DMEM medium to each well;
16. Incubate the cells at tissue culture incubator for 48 hours;
Day 4
17. Spin down the cells at 2000rpm for 10 min;
18. Carefully transfer 190 μl of culture medium from each well into the corresponding new well of a new 96-well plate;
19. Pool 5 μl from each well together for later virus titer;
20. Seal the plate with sterile sealing film and label the plate;
21. Store the plate at -80 C before cell infection;