Time: 2024.10.26-2024.12.01

1. **Experiment:** Lentivirus Packaging and Infection of CAR on NK-92 cell line
2. **Time:** 2024.10.26-2024.12.01
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4. **Principle:**

   Lentiviral packaging is a technique to generate replication-incompetent recombinant lentiviral particles for efficient delivery of exogenous genes into target cells. Its core principle relies on the split of viral functional components into separate plasmids, which are co-expressed in packaging cells to assemble infectious but non-replicating viral particles.
5. **Materials:** Lenti-293 T cells, 5% CO_2 incubator, 5% FBS, CPT Transfection Kit (Viraltherapy), Centrifuge 5810 R (Eppendorf, 5810R), Eclipse TS100 (Nikon, TS100), 96-well plates, 5% CO_2 incubator, complete medium (RPMI-1640 with 10% FBS), polybrene.
6. **Method:**

   (1) One day before transfection, Lenti-293 T cells was passaged and evenly plated on 100 mm culture dishes (at 8-10×10^6 cells per dish). Cells were cultured at 37 °C in a 5% CO_2 incubator. The medium was replaced with 10.5 mL of pre-warmed, resistance-free medium (5% FBS) 2-4 hours before transfection.

   (2) A mixture of twenty-three plasmids in equimolar amounts was prepared, and transfection was performed using the calcium phosphate transfection method with the CPT Transfection Kit (Viraltherapy). The transfection mixture composition was as follows:

Table.1 Transfection mixture composition

Transfection tube	Reagent	For 1×15cm Dish	For 10×15cm Dish
A	Buffer A	500 μL	45 mL
	lentiviral plasmids	21 μg	210 μg
	pMD2G	7 μg	70 μg
B	pCMVR8.74	14 μL	140 μL
	sterile water	Up to 450 μL	Up to 4.5 mL
	Buffer B	50 μL	500μL

   (3) The homogenous mixtures in tube B were added dropwise into A using a pipette gun and placed for 30 mins at room temperature.

   (4) Cell Inoculation:

   ① Q Collect NK-92 cells from a culture flask.

   ② Centrifuge at 300 × g for 5 mins at room temperature.

   ③ Carefully remove the supernatant and resuspend the cell pellet in an appropriate volume of complete medium (e.g., RPMI-1640 with 10% FBS).

   ④ Calculate the required volume of cell suspension to achieve a final concentration of 1×107 cells/mL.

   ⑤ Add the calculated volume of cells to an appropriate volume of complete medium to achieve a final concentration of 1×107 cells/mL.

   ⑥ Dispense 100 μL of the 1×107 cells/mL suspension into each well of a 96-well plate. Incubate the plates at 37 °C in a 5% CO2 incubator for 24 hours to achieve approximately 50% confluence using a light microscope to observe the cells. At 50% confluence, the cells should cover approximately half of the well surface. You'll see some areas with dense cell layers and others with less or no cells.

   (5) Infection: To each well, 4 μL of a 25 × polybrene infection-enhancing solution was added. Considering

the multiplicity of infection (MOI) and the virus titer, the specific volume of the virus, calculated as (MOI × cell number) / virus titer, was added to the wells. The plates were then incubated at 37 °C for 12-16 hours.