Time: 2024.06.25-2024.06.26

1. **Experiment:** Flow cytometry sorting
2. **Time:** 2024.06.25-2024.06.26
3. **Member:** Yaqi Gao, Xinyu Zhu, Xiaoyuan Chen, Yinran Luo
4. **Material:** RPMI-1640 medium (10-15 mL per culture flask), FLAG-APC antibody (BioLegend, 637308) at 10 μL per 2200 μL of PBS (1:200 dilution) for initial labeling and 2 μL per 1 mL of PBS (1:500 dilution) for secondary labeling, Phosphate-buffered saline (PBS).
5. **Method:**
   (1) NK92 cell suspension from the stock is transferred to a tissue culture flask containing RPMI-1640 medium. An appropriate seeding density of 2-5×10^6 cells per 10-15 mL of medium should be ensured.
   (2) The culture flask is placed in a CO_2 incubator set at 37°C with 5% CO_2 for 24 hrs. Cell growth should be monitored to ensure that cells are in the logarithmic phase.
   (3) Replace the medium by removing the old medium and adding fresh RPMI-1640 medium.
   (4) When cells reach 70-80% confluency, cells are passaged. The cell suspension is collected, and centrifuged at 300 × g for 5 mins. The supernatant is discarded, and the cells are resuspended in a fresh medium before seeding into new culture flasks.
   (5) Cells should be regularly counted and their morphology checked to ensure healthy growth.
   (6) NK cells suspension was expanded to over 10^7 and centrifuged at 1500 rpm for 5 mins. 30 mL of PBS was used to wash the cells.
   (7) The cells were resuspended by 2200 μL of PBS and 10 μL of FLAG-APC antibody (BioLegend, 637308) was added to label the cells. The cells were then incubated in the dark for 15 mins.
   (8) The cells were washed again by 30 mL of PBS. The supernatant was discarded, and the cells were resuspended again by 500 μL of PBS.
   (9) The positive cells were sorted based on the CAR expression, from top 10% to 20%.
   (10) The sorted cells were cultured to proliferate to 2×10^7 and the CAR-positive cell proportion is detected by FACS.
   (11) 1 mL of cell suspension was taken. After the three-mins placement on ice, the cells were centrifuged at 3000 rpm for 5 mins and collected.
   (12) The supernatant was discarded completely and the cells were resuspended in 1 mL of PBS.
   (13) 2 μL of FLAG-APC antibody (BioLegend, 637308) was added to label the cells.
   (14) The cells were then incubated in the dark at room temperature for 15 mins. 1 mL of PBS was added to terminate the reaction and the cells could be collected after centrifuging at 3000 rpm for 5 mins.
   (15) The cells were resuspended by 1 mL of PBS and the supernatant was discarded. The cells were resuspended in 50 μL of ice-cold PBS, and flow cytometry analysis could be carried on.
6. **Result:**

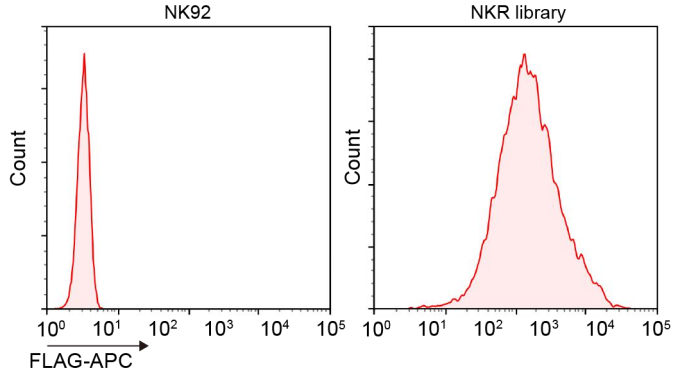

Fig.1 Repertoire construction