**Time: 2024.12.21-2024.12.28**

1. **Experiment:** Single-Cell Sorting and Planting
2. **Time:** 2024.12.21-2024.12.28
3. **Member:** Fan Yang, Qiwen Jiang, Xinxin Zhang, Kaiqing Zhang, Meng Sun
4. **Principle:**

Fluorescence-activated cell sorting (FACS) uses target cells' specific fluorescent signals to isolate individual cells into 96-well plates, ensuring clonal growth from a single cell. Second, in vitro culture mimics physiological conditions to sustain cell viability and proliferation, while inverted microscopy tracks clonal expansion. For sequencing, gentle centrifugation preserves cell integrity during collection; cell lysis releases nucleic acids, enabling high-quality sequencing of clonally pure single-cell samples.

5. **Material:** NK cell media (Gibco, supplemented with 5% FBS and 500 IU/mL IL-2), IL-2 (Gibco), 5% hAB serum (Gibco), Trypan Blue
6. **Method:**

(1) Single-Cell Sorting and Planting:

① FACS was performed under the following conditions: Nozzle size: 100 μm. Sort mode: Single-cell deposition (1 drop/well). Gating strategy: Virus-infected cells were gated based on fluorescence intensity.

② Single cells were sorted directly into 96-well plates pre-filled with 150 μL conditioned medium (complete medium + 10% FBS + 1% penicillin-streptomycin).

③ Negative control wells (medium only, no cells) were included in each plate.

(2) Culture and Monitoring:

① All 20 plates were transferred to cell incubator (37 ℃, 5% $CO_2$) and cultured for 7 days.

② Half-medium change was conducted on Day 3:75 μL of spent medium was aspirated and replaced with 75 μL fresh pre-warmed medium.

③ Clonal growth was monitored daily using an inverted microscope.

(3) Cell Collection and sequencing:

① The 96-well plate is gently tapped to resuspend cells and avoid aggregation.

② The suspension from each well is transferred into pre-labelled centrifuge tubes.

③ The cells were centrifugated at 300 × g for 5 minutes at 4 ℃ and the supernatant is carefully aspirated without disturbing the pellet.

④ Optionally, the cell pellet is then resuspended in ice-cold PBS, followed by repeated centrifugation to remove residual culture medium.

⑤ Finally, the processed cells are directly subjected to lysis and sequencing services were outsourced to Shanghai Biomarker Technologies Co., Ltd.

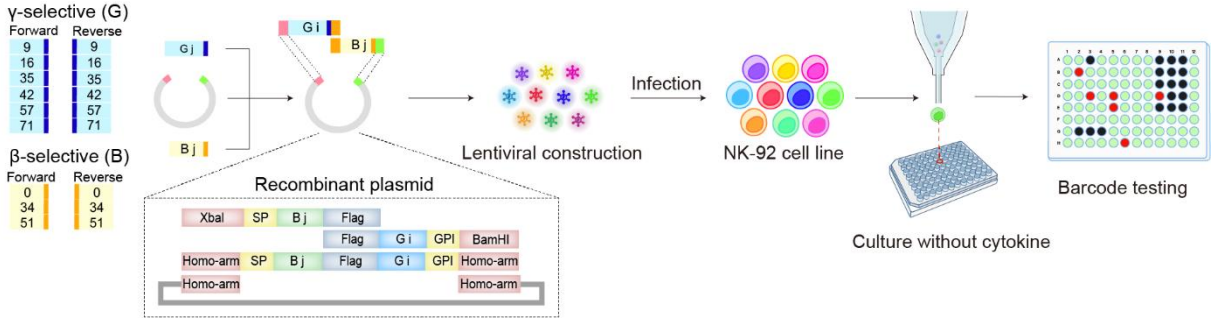


**Fig 1.** Single-Cell Sorting and Planting

Screening for gene combinations that significantly promote NK cell proliferation by infecting NK-92 cells with lentivirus. IL-2β and IL-2γ gene fragments were designed and recombined into various

combinations via homologous recombination to construct a plasmid library. The plasmid library was packaged into lentivirus and used to infect NK-92 cells. After culturing for 7 days under cytokine-free conditions, barcode testing was performed to identify significantly proliferating NK cells, and the best-matching gene combination was selected.