Time: 2024.04.08-2024.04.22

1. **Experiment:** Experiment technique learning
2. **Time:** 2024.04.08-2024.04.22
3. **Member:** Song Zhang, Yaqi Gao, Xinyu Zhu, Xiaoyuan Chen, Yinran Luo, Hanyue Liu, Xudong Tang
4. **Summary:**

(1) Construction of the plasmids of CAR

① Time: 2024.04.08-2024.04.11

② Member: Xinyu Zhu, Yinran Luo

③ Summary:

Principle:

Plasmid construction is a commonly used experimental technique in the field of genetic engineering to insert a target gene into an appropriate plasmid vector for gene cloning, expression, and delivery. The principle of the assay is based on DNA recombinant technology, which precisely attaches the target gene to the plasmid vector by utilizing restriction enzyme cleavage and ligation of the end by ligase.

Method:

- The reactions were maintained at 37°C for 1 hr, and inactivated at 65°C for 5 mins.
- The products were stored at 4°C.
- The products were analyzed using 1.5% agarose gel electrophoresis at 120V for 45 mins, and target bands were subsequently recovered.
- The linear vectors and the target segments were recycled and then connected.
- The whole system was incubated at 16°C for 30 mins, inactivated at 65°C for 10 mins, and then stored at 4°C.
- The connection products were transformed into Stbl3 by T4 ligase (Rapid DNA Ligation Kit, Genstar).
- Following clone verification by sequencing, bacterial cultures (500 mL) were grown in 2L flasks, and plasmids were extracted using the NucleoBond Xtra Midi Maxi EF plasmid purification kit (Macherey-Nagel 740420.10).

(2) Lentivirus packaging

① Time: 2024.04.12-2024.04.16

② Member: Xiaoyuan Chen, Hanyue Liu, Xudong Tang

③ Summary:

Principle:

The core principle of lentivirus packaging experiments is to co-transfect the lentivirus transfer plasmid inserted into the target gene into packaging cells with other plasmids (packaging plasmids and envelope plasmids) that help the virus assembly and maturation. With the help of packaging plasmid, the plasmid was transferred to produce viral RNA and necessary proteins. The envelope plasmid provides the envelope protein required for the surface of the virus particle. This process eventually leads to the formation of lentiviral particles carrying the target gene.

Method:

- 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 hrs before transfection.
- 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 homogenous mixtures in tube B were added dropwise into A using a pipette gun and placed for 30 mins at room temperature.

(3) NK92 expansion

① Time: 2024.04.17-2024.04.21

② Member: Song Zhang, Yaqi Gao

③ Summary:

Principle:

The growth of NK92 is characterized by suspension growth. Cytokines are the key to maintain NK cell survival and support NK cell proliferation in vitro, and the expansion of NK cells by using cytokines such as IL-2 alone is relatively modest.

Method:

- NK92 cell suspension from the stock is transferred to a tissue culture flask containing RPMI-1640 medium (Gibco).
- An appropriate seeding density of 2-5×10^6 cells per 10-15 mL of medium should be ensured.
- 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.
- The medium was then replaced by fresh RPMI-1640 (Gibco).
- Cells are passaged when reach 70-80% confluency, whose suspension was 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.
- Cells were counted and checked for morphology to ensure healthy growth.