

Time: 2024.06.19-2024.06.20

- Experiment:** Lentivirus Packaging
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- Member:** Song Zhang, Xinyu Zhu, Hanyue Liu
- Material:** Lenti-293 T cells, 5% FBS, CPT Transfection Kit (Viraltherapy)
- Method:**

- One day before transfection, Lenti-293 T cells was passaged and evenly plated on 100 mm culture dishes (at $8-10 \times 10^6$ 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 transfection mixture composition was as follows:

Transfection tube	Reagent	For $1 \times 15\text{cm}$ Dish	For $10 \times 15\text{cm}$ Dish
A	Buffer A	500 μL	45mL
	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 4.5mL
	Buffer B	50 μL	500 μL

- The homogenous mixtures in tube B were added dropwise into A using a pipette gun and placed for 30 mins at room temperature.
- The plasmid backbone (pMD2G and pCMVR8.74) was as follows.

