SynthImmunol_NMU Notebook No.3

Time: 2024.05.06-2024.05.09

1. **Experiment:** Lentivirus Package
2. **Time:** 2024.05.06-2024.05.09
3. **Member:** Xinyu Zhu, Xudong Tang, Song Zhang
4. **Material:** Lenti-293 T cells, 5% CO_2 incubator, 5% FBS, CPT Transfection Kit (Viraltherapy)
5. **Method:**

(1) Preparation of Cells Before Packaging

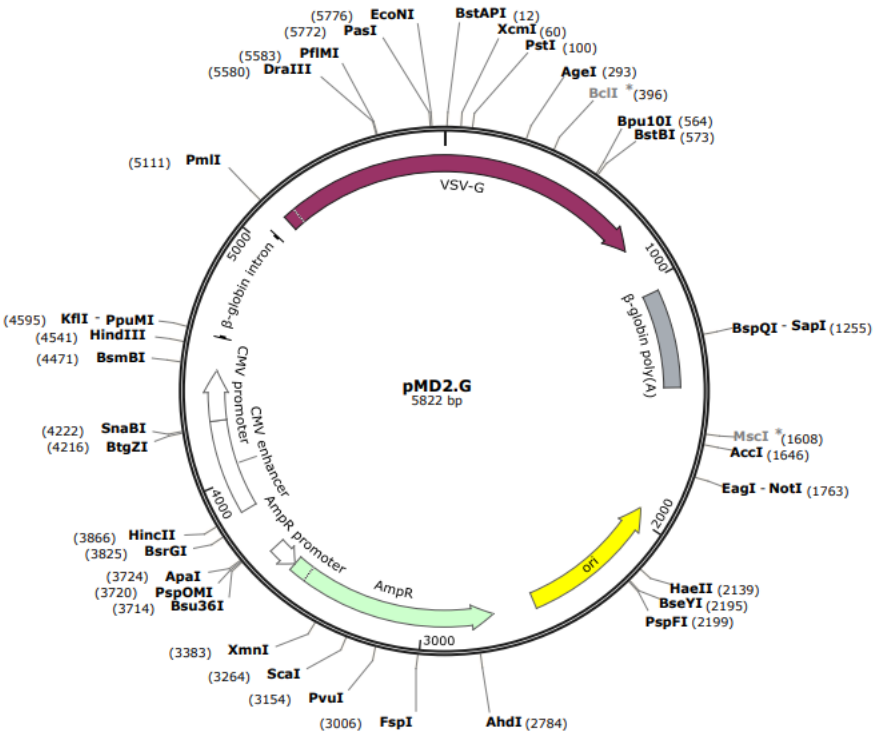
One day before transfection, Lenti-293 T cells was passaged and evenly plated on 100 mm culture dishes (at 8-10×10^6 cells per dish). Cells were cultured at 37°C in a 5% CO_2 incubator. The medium was replaced with 10.5 mL of pre-warmed, resistance-free medium (5% FBS) 2-4 hrs before transfection.

(2) Lentivirus Packaging

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

The plasmid backbone (pMD2G and pCMVR8.74) was as follows.



②Gently mix the contents of Tube B using a pipette and slowly add it dropwise to Tube A. Change to a new pipette tip and mix using the bubble method, ensuring that bubbles are introduced slowly and the mixing is completed within 2 minutes. Let the mixture sit at room temperature for 30 minutes.

③Slowly add the mixture dropwise to the transfection mixture in the cell culture plate. Gently mix and incubate at 37°C in a CO_2 incubator for 16 hrs. After 16 hrs, discard the medium and replace it with 15

ml of fresh complete medium for continued incubation. After 36 hrs post-medium change, collect the first round of virus supernatant and store it temporarily at 4°C. Then, add 15 ml of fresh medium. After 72 hrs of transfection, collect the supernatant again and combine it with the first collection.

④Thoroughly mix the collected virus supernatants, centrifuge at 3000 rpm and 4°C for 10 minutes, discard the pellet, and filter the supernatant through a 0.45 μM filter to collect the filtrate.

⑤Transfer the filtered virus supernatant into pre-sterilized ultracentrifuge tubes. Weigh and balance using an electronic balance, keeping the volume below 40 ml. Place the tubes symmetrically in the ultracentrifuge and centrifuge at 20,000 rpm (82,700 g) and 4°C for 2.5 hrs.

⑥Carefully remove the ultracentrifuge tubes from the rotor, discard the supernatant, and invert the tubes on sterile paper towels to allow any remaining supernatant to drain. Remove any residual droplets, and a visible pellet should be present at the bottom of the tubes.

⑦Based on the amount of virus pellet, add 150 μL of DMEM medium to each tube to dissolve the virus pellet, cover the tubes, and dissolve at 4°C for 60 minutes, gently shaking every 15 minutes. Centrifuge at 500 g for 1 minute at 4°C to concentrate the solution at the bottom of the tube.

⑧Gently resuspend the pellet using a 200 μL pipette, avoiding foam formation. Pool the liquid from all tubes into a single EP tube. Aliquot the concentrated virus suspension into 50 μL portions, store in labeled tubes, and quickly freeze using crushed dry ice before storing at -80°C.