Production of Pseudovirus in 293T Cells

Stamatatos Lab
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Day 1

1. Plate 6ml of 293T cells ($0.5x10^6$/ml) in complete DMEM (500ml DMEM + 50ml heat-inactivated FBS + 10ml L-Glutamine Pen-Strep) into 10cm tissue culture treated dish (note: all steps should be performed with sterile technique in tissue culture hood).

Day 2

2. Combine 36ul of GeneJuice with 264ul of Optimem, mix by vortexing, and incubate 5 minutes at room temperature.
3. Add 12ug of DNA (pNL4-3 env-/rev- + env plasmid), mix by gentle pipetting, and incubate for 15 minutes at room temperature. (GeneJuice:DNA ratio is 3:1; Backbone:Env DNA ratio should be optimized, generally 5:1 is sufficient).
4. Add mixture dropwise to plate, in concentric manner, rock to mix (do not swirl as it will result in the mixture concentrating in center of plate), and incubate 4-6 hours at 37°C.
5. Aspirate media from plate, replace with 9ml pre-warmed complete DMEM, and incubate for 3 days at 37°C.

Day 5

6. Remove supernatant to 15ml conical tube and spin at 1200 rpm for 5 minutes at 4°C.
7. Aliquot supernatant into cryovials and store at -80°C.