**Lipofectamine Plat-E Transfection**

* **Plat-E culture medium**: DMEM, 10% fetal calf serum (FCS), 1 μg/mL puromycin, 10 μg/mL blasticidin, penicillin and streptomycin
* **Plat-E transfection medium**: DMEM, 10% FCS, penicillin and streptomycin
* Start with cells that are roughly 30-40% confluent on Day 1

DAY 1

1. Pipette 700 uL DMEM without serum into two Eppendorf tubes
   1. Label one ‘L’ and one ‘+’
2. Add 20 uL Lipofectamine to the ‘L’ tube and mix
3. Add 20 uL Plus Reagent and ~2-3 ug transfection DNA to the ‘+’ tube and mix
4. Transfer the contents of the ‘L’ tube into the ‘+’ tube and mix
5. Let sit for 5 minutes at room temperature
6. Vacuum media from a small flask of Plat-E cells
7. Add contents of the Eppendorf tube onto Plat-E cells
8. Incubate for 2-3 hours, then add fresh DMEM with serum on top of what’s there

DAY 2

1. 24 hours post-infection check that Plat-E cells were successfully transfected and are GFP+ using the microscope
2. Aspirate and replace media

DAY 3

1. 48 hours post-infection collect supernatant from cells and pass through a 0.45 um filter into a 15 mL tube. Replace DMEM on Plat-E cells (See optional step 6)
2. Aspirate media from the target cells (DO11, RMA, etc.) and replace with 2 mL of fresh media
3. Take 2 mL of the filtered virus and add to the target cells (1:1 ratio with media)
4. Add 1 uL polybrene (final concentration of 2.5 ug/mL) to the target cells
5. After 4-6 hours, add fresh media to top of old media
6. Leave both the target cells and the Plat-E cells in the incubator until 72 hours from initial infection

DAY 4

1. OPTIONAL: 72 hours post-infection repeat steps 1-6 on Day 3, either for reinfection of the original target cells and/or infection of new target cells

DAYS 5-7

1. Check GFP expression of transfected cells
2. Sort GFP+ cells on the FACSAria