**Lipofectamine 293 T Transfection**

* **293T culture medium**: DMEM, 10% fetal calf serum (FCS), penicillin and streptomycin

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 to the ‘+’ tube and mix
4. **Add ~2-3ug of transfection DNA, 1uL Hit60, and 1uL VSVG to “+” tube**
5. Transfer the contents of the ‘L’ tube into the ‘+’ tube and mix
6. Let sit for 5 minutes at room temperature
7. Vacuum media from a small flask of 293T cells
8. Add contents of the Eppendorf tube onto 293T cells
9. Incubate for 3 hours, **then add fresh DMEM with serum on top of what’s there**
10. DAY 2
11. 24 hours post-infection check that 293T cells were successfully transfected and are GFP+ using the microscope
12. 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 293T cells (See optional step 6)
2. Aspirate media from the target cells (B16 and MC38) 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 **5 uL polybrene** (final concentration of 10 ug/mL) to the target cells
5. **After 6 hours, add fresh media to top of old media**
6. Leave both the target cells and the 293T 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