15-9-20

Seed 2 T25 flasks of cells to be **70-90%** confluent at transfection.

For each flask:

- 9 ug of pOG44 (433 ng/ul, so it will be **21 ul** of pOG44 in each transfection)
- 1 ug of RRP6-3XFLAG plasmid (1058 ng/ul, so it will be ~ **1 ul**)
- P3000™ Reagent (2 µL/µg DNA)
- 3.75 or 7.5 µL of Lipofectamine 3000

1. Seed cells to be 70–90% confluent at transfection
2. Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes)– Mix well

| Number of flask | 1 | 2 |
|---|---|---|
| Lipofectamine 3000 | 3.75 | 7.5 ul |
| Opti-MEM™ Medium | 125 µL | 125 µL |

3. Prepare master mix of DNA by diluting DNA in OptiMEM™ Medium, then add P3000™ Reagent– Mix well

250 ul of Opti-MEM™ Medium + 42 ul of pOG44 + 2 ul of RRP6-3XFLAGplasmid + 40 ul of P3000TM Reagent (2 µL/µg DNA)

4. Add Diluted DNA to each tube of Diluted LipofectamineTM 3000 Reagent (1:1 ratio = 125 ul:125 ul)
5. Incubate for 10–15 minutes at room temperature.
6. Add DNA-lipid complex to cells
7. **Approx. 24 h later** split the cells and place them in T175 flask without antibiotic.
8. **Approx. 3 h later** aspirate media. Add media containing 100 ul/ml Hygromycin B and 10 ug/ul Blasticidin and 1% pen/strep.
9. Change media every 4-5 days
10. After 2 weeks colonies that are visible to the naked eye should have appeared.
11. Split cells and grow continuously in DMEM/10%FBS/1%pen/strep containing selection antibiotic.
12. Make a frozen stocks of obtained cell line