Large Scale Lentivirus Infection

1. Suspend 60 million cells per library in 30~35 mL virus-containing media and add 8ug/mL (final conc.) polybrene. (example, 240 million cells with 110 ml of virus-containing media and then add polybrene stock to get final conc. for whole genome with 10sgRNAs.).

2. Aliquot to 6-well plates (~5-6 mL per well) and spin 1000x g for 2hr at 33C.

3. Harvest cells and spin down.

3. Resuspend cells carefully with 120 mL fresh medium/ 60 million cells (in T175 flask).

4. Grow cells and check infection by mCherry or BFP % using flow cytometry. You may need to split/dilute again before adding drug (keep the density around 500000/ml)

5. If 30-50% infected (takes 2days in general), Start puromycin selection (0.75 ug/mL or minimum required for 80-90% killing) for 2~3 days.

6. Spin cells and resuspend in puro-free medium; recover for 2 days.

7. Measure mCherry/BFP again by flow cytometry.

8. Freeze down T0 sample and combine libraries to get equal # of infected cells, and begin experiment.