

## **Lenti spinoculation for organoid**

From Paul Doan

- 0544T AK organoid (APC, KRAS mutant from CRC patient)
- from 4 full well in 6-w
- triple Express, pipette mix, add in Falcon, incubate 37\*c 4 mins
- quench with DMEM, centrifuge 900g, 5 mins
- remove media, matrigel
- add new ERN human media + Polybrene (final 8 ug/mL after virus vol), 0.5 mL/conditions (6 Paul + 2 DAPK3 = 8 total)
- add suspension to Nunclon plate (24-w)
- add virus 500 uL
- > Condition
- Paul Perturb-seq library 10x conc LV : 0.5 mL, 0.25 mL, 0.125mL, 0.0625, neg (no LV, +puro), No LV no puro
- top up DMEM to 500 uL
- PCC09-DAPK3-g1, g2 (2) : 500 uL
- > spinoculation
- centrifuge 37\*c, 600g, 1 hr in Lentiroom
- incubate 3 hr in incubator
- aspirate media to Falcon tubes, add DMEM 3 mL
- centrifuge 900g 5 mins, remove media
- add matrigel+ERN media 150 uL
- plate in 6-w nun-clon
- add human organoid ERN media

Plan : start puro after D1-2