**Human organoid version (From PB, Sep 11, 2023)**

1. **Do all this in lenti-room!!!**
2. AK and AKS human organoids in falcon, seed on 24-well NUNC plate
3. Choose 1 NTC and 2 shRNA
4. Add 500ml virus + 0.8 ul Polybrene + 1 ul Rocki (prevent cell death) (1 ml in total)
5. Centrifuge 600g/1hr in 32 degree, tape on plate
6. Put to 37 degree/CO2 for 4 hrs
7. Collect in falcons, 6 tubes
8. Spin down 900 g/5 min
9. Remove supernatant
10. Plate the organoids + Matrigel
11. 100 or 150 ml / 6 well (depend on number of organoid)
12. ERN media (not WRN)
13. Culture for 2 days
14. Optional: split organoids, to save Matrigel, use ERN:Matrigel = 1:1
15. Puro selection until all control (normal organoids in a new well) die. The stock conc of puro is 10 mg/ml (in brown glass bottle) and our target conc is 4 ug/ml. So, add 0.4 ul per 1ml (0.8 ml per 2ml, per well), mix with 1ml pipet. ERN is previous, so we don’t dilute puro with ERN medium before using.
16. Split organoids to remove dead cells, 200 ml Matrigel per well
17. Puro selection again when you see organoids rebuild from fragments
18. Puro selection and split should last for 1 week at least (Don’t do further WB/qRT-PCR before complete puro selection, it just waste your time.)

**Lentiviral Infection of Organoids**

Cultured organoids were transferred in suspension (in 50% L-WRN conditioned media) into a 15 ml tube, centrifuged at 200 x g for 5 min, resuspended in 200 μl trypsin-EDTA, and incubated at 37°C for 5 min. Washing media (1 ml) was added and the organoids were dissociated by vigorous pipetting.

Organoids were centrifuged again at 200 x g for 5 min and then the cells were resuspended in 250 μl solution containing lentivirus, 8 μg/ml polybrene and 10 μM Y27632 (R&D). Each suspension with a single lentivirus was then transferred to a single well of a 48 well plate, which was sealed with Parafilm (Bemis Co.) and centrifuged at 600 x g at 32°C for 1 h.

Plates were then incubated at 37°C for 6 h to allow transduction.

Cells were resuspended in conditioned organoid media (1 ml per well) and transferred to a 1.5 ml tube for centrifugation at 200 x g for 5 min.

Cells were resuspended in 20 μl Matrigel and cultured via our organoid culture method as listed above, except that the medium was supplemented with the appropriate antibiotics (e.g., puromycin) for 7 days to deplete non-transduced organoid cells.