**Basic organoid skills**

**Mouse organoids splitting**

1. Pre-warm NUNC 6-well plate + Prepare fresh Matrigel on ice
2. Remove old medium
3. Trypsinizing organoids in room temperature (1ml Tryple)
4. Mash the Matrigel and organoids, keep it in incubator for 5min
5. Prepare 4-5ml CM, add 2ml CM to mix/stop Trypsinizing, collect in 10ml Falcon
6. Spin down 900rcf/5min, then remove CM and top Matrigel
7. Use 200ml pipet to remove all the old Matrigel
8. Add 200ml new Matrigel (or ERN or WRN) to each well (on ice to avoid solidification)
9. Mix in Falcon, avoid bubbles
10. Distribute mixed Matrigel to 4-6 drops, using small pipet
11. Keep in incubator for 20-30 min, plate should be upside down
12. Add 2ml fresh new medium per well (50ml WRN + 100 ul Rocki)
13. Split again in 4-5 days

**Mouse organoids freezing**

1. Add 1 ml freezing medium, mash the Matrigel
2. Transfer the organoids to 2ml tube
3. Put it to -80 degree freezer

**Collect protein from organoids**

**Collect RNA from organoids**