This step dissociates intestinal organoids to a single cell suspension for single cell RNA-sequencing and/or organoid passaging.

1. Aspirate old media.
2. ~~Wash 2 mL cold PBS once in~~ 6-well.
3. Remove the organoid PBS and replace with 1.5 mL of Cell Recovery Solution.
4. Using a p1000 pipette, scrape the gel off the bottom of the plate and pipette up with the cell recovery solution. Transfer to a 1.5 mL Eppendorf tube.
5. Incubate on a rotating nutator for 20 minutes in 4C cold room.
6. Centrifuge the sample at 400 *g* for 5 min. If Matrigel has not fully dissolved by inspecting on a glass slide under microscope, maintain on ice for further five minutes and repeat until this is the case.
7. Remove supernatant and re-suspend in 2.5 mL of pre-warmed TrypLE; incubate for 10 min in a water bath at 37°C. The sample should be assessed under a microscope to assess whether we achieve single-cell. If not, incubate for another 5 minutes.
8. Quench 2.5 mL of cold complete DMEM and centrifuge at 400 *g* for 5 min, remove supernatant and resuspend in 2.5 mL of ~~DMEM (~~4°C).
9. ~~Repeat this step for a total of three times.~~
10. Aspirate and resuspend in 100 uL of 0.04% BSA PBS. Countess.

Flow

For 0.125 mL virus = 65,900 live cells at a 20% viability
For 0.500 mL virus = 57,500 live cells at a 25% viability

pipette 10 X
quench - 10 ml DMEM
900 g / 5 min
pellet.
Spin down
resuspend - pipette 10 X
500 µl
flow tube

ERM : 1:5000
DAPI