**Single cell dissociation and sorting of gastric organoids grown in 6 well plates.**

1. Aspirate media and wash Matrigel dome with 2ml of ice-cold PBS without disturbing the Matrigel dome.

2. Transfer Matrigel containing organoids to 15ml falcon containing ice cold Cell Recovery Solution. I use a cell scraper to detach Matrigel from the plate and aspirate it up with 1ml of cell recovery solution. I use 8-10ml of cell recovery solution for 6-7 drops of Matrigel (~1 well of a 6 well plate). Make sure cell recovery is ice cold.

3. Rotate in cold room for 20-25 minutes

4. Spin down ~ 1300rpm x 3min. At this point you should see a pellet of whole organoids and no remaining Matrigel. Depending on the number of droplets of Matrigel sometimes you still have residual Matrigel – I generally aspirate that Matrigel to get close to the organoid pellet.

5. For RNA/Protein extraction I stop here and use Trizol or RIPA buffer respectively.

6. For ATAC (and ChIP) I now resuspend the pellet in 1-2ml 0.05% trypsin and pipet around 10-15 times.

7. Transfer the 15ml falcon to 37-degree water bath for 1min. Remove from water bath, clean with ethanol. After the first incubation you see a string of tissue in the trypsin. I pipet another 10-15 times.

8. Take 10ul on a slide and assess dissociation under microscope. If single cells then move to step 9. If still clumps go back to step 7.

9. Inactivate trypsin with FBS containing media. Spin down at 1500rpm x 5 mins.

10. Resuspend pellet in 1ml of FACS buffer (DMEM without phenol red + 1% FBS + ROCK inhibitor). Add 1ul of DAPI solution.

11. Filter through sorting tubes and proceed. Keep on ice.