**Single Cell suspension from organoids**

**Reagents and Instruments needed**

1. Triple plus media (cold)

2. Dispase Media (Triple plus media containing 1mg/ml Dispase): **this should be freshly prepared**

3. Fire-polished pipettes

4. 15mL conical tubes

5. TriplE express

6. Thermomixer R (in the hood)

**Get ready in TC hood:**

Bunsen burner and striker-be sure to turn gas off when done in hood and at main switch.

glass Pasteurs: Fire-polish pipets to ~1/2 original diameter

1ml, P220 and P20 Pipettemen and tips

In an ice bucket:

+++ media

**Begin:**

-Put15ml conical on ice

-Aspirate off media

-Add 500µl of 1mg/mL Dispase media in each well you are going to harvest. Incubate cells at 37 C for 20 min.

-Harvest organoids adding 500µl of cold triple plus to the existing 500µL in each well. Pipet up and down several times before transferring to the 15 ml conical on ice. Add ice cold triple plus up to 10 mL.

**IMPORTANT**: Do not harvest more than 10 wells (from 24-WP) in the same 15mL conical tube. In that case you can use 50mL conical tube instead and filled up with cold media.

-Spin 750 rpm 4°C for 8 minutes. You will see collapsed MG within the conical tube up to 2mL.

-CAREFULLY aspirate off down to just above 2mls.

-Use fire polished Pasteur to pipet up and down 10 times: see clumps breaking up,

-Add triple plus media (up to 10mL) and respin at 750rpm 4C 5min

-Remove as much media as possible and add 1mL of TriplE; pipet up and down several times. Incubate the conical tubes at 37C for 5min in the thermomixer (500rpm mixing).

-After 5 minutes you will see again floating matrigel (it is NOT DNA). Add 1mL of Dispase media and pipet up and down several times. Put the tube back to the thermomixer and incubate for additional 25 min at 37C 500rpm mixing.

-Put the tube on ice and dilute with cold triple plus (up to 10mL). Spin at 1100 rpm 3 minutes (cells are treated as 2D cells).

-Aspirate off media and resuspend in Complete media (or culturing media) before counting and assessing viability using the Countness. Based on our experience if you harvest less than 6 wells you should resuspend the pellet in 500µL; more than 6 wells you can resuspend in 1mL of media.