Fixation of organoids: Sethi Lab

1. When organoids are confluent in 6-well plate (minimum 2 wells for each prep), wash them with PBS.
2. Add 10% formalin 2 ml/well directly, keep at room temperature on rocker 3-4 hrs.
3. Pipette and collect cells into 1.5ml tubes. Most of the Matrigel should be dissolved after formalin fixation.
4. Spin down (2000 rpm, 3 minutes), aspirate the supernatant carefully and add remaining formalin and organoids. Spin down (2000 rpm, 3 minutes), aspirate the supernatant carefully.
5. Add 1ml PBS to wash the organoids, spin down and carefully aspirate the supernatant as much as possible with pipette.
6. Microwave 2% agar (loosen the cap).
7. Add 50-80ul agar solution for one well, resuspend cell pellets and then put tubes immediately on ice.
8. Using a thin spatula or needle, scoop the solidified agar with organoids onto a cassette. Put the cassette into 70% ethanol.

Notes:

1. Prepare 2% agar: 1g noble agar powder and 50mL of deionized water
2. Most of the Matrigel should be dissolved after fixation. If not, resuspend with formalin once again and incubate the tubes in room temperature.
3. When aspirate the supernatant, be careful. Always leave 100ul leftover and aspirate the pipette.