Organoid Processing Protocol

Optimized for airway organoids cultured as in Hynds et al. Methods in Molecular Biology 2016:
https://link.springer.com/protocol/10.1007%2F7651_2016_5

Materials/Equipment:

15 ml falcon tubes
V-bottomed 96-well FACS plate
1.5 ml Eppendorf tubes
4% PFA
70% EtOH
PBS
HistoGel (1 ml aliquots in 1.5 ml Eppendorf tubes)
Tray of ice
Plate-compatible centrifuge
Heat block

Procedure:

1. Pipette 6 ml ice-cold 4% PFA into 15 ml falcon tubes (1 per condition).

2. Transfer organoids to 15 ml falcon tubes on ice using a 200 ul pipette with the ends of the tips cut (combining 6-12 wells of a 96-well plate for histology processing).

3. Fix organoids for 30 mins on ice. During this time, pre-cool a labelled V-bottomed 96-well plate on ice, set centrifuge to 4°C and warm an aliquot of HistoGel to 65°C on a hot block.

4. Centrifuge 15 ml falcon tubes at 400 x g for 5 mins to pellet organoids (pellet should be visible).

5. Remove PFA being careful not to disturb pellet.

6. Resuspend pellet in 200 ul ice-cold PBS and transfer to a pre-cooled, pre-labelled 96-well FACS plate using a 200 ul pipette with the ends of the tips cut off (1 well per condition).

7. Centrifuge plate at 400 x g for 5 mins.

8. Carefully remove supernatant from wells using a 1 ml pipette.

9. Resuspend the pellet of organoids in 120 ul HistoGel using a 200 ul pipette with the ends of the tips cut off.

10. Allow HistoGel to set on ice for 10 mins.

11. Remove HistoGel from wells by gently pushing a 200 ul pipette tip down the wall of the well.

12. Store in labelled 1.5 ml Eppendorf tubes containing 500 ul 70% EtOH until processing for histology using routine dehydration to paraffin methods.