Retroviral infection in pancreatic organoids

Chang-il Hwang

Materials

1. Confluent organoids (one well in 48 well plate)
2. Concentrated retrovirus in Organoid media (+ Rho kinase inhibitor, Y27632). Virus needs to be concentrated by RetroX concentrator (Clontech) and resuspended with Organoid media (+Rho kinase inhibitor)
3. Fire polished glass pipette
4. TripLE express (Invitrogen)
5. 5% FBS + DMEM + P/S
6. Triple +++ Advanced F12/DMEM (ice-cold)
7. Matrigel
8. 1:4 diluted Polybrene in PBS
9. Complete Organoid media (+ Rho kinase inhibitor, Y27632)

Procedures

1. Add 500 ul ice-cold Triple +++ into the well, and pipette up and down to break organoids, and transfer into 10ml ice-cold Triple +++ medium
2. Centrifuge at 750rpm for 5min at 4’c
3. Remove 8~8.5ml of media and leave 1.5~2ml media
4. Pipette up and down with a fire polished glass pipette 10 times
5. Add Triple +++ media up to 10ml
6. Centrifuge at 750rpm for 5min at 4’c
7. Carefully remove media
8. Add 1ml TripLE express and incubate at 37’c for 5min
9. Add 9ml 5% FBS DMEM P/S to quench TripLE express
10. Centrifuge at 750rpm for 5min at 4’c
11. Resuspend cell pellet with 250ul retrovirus with 1ul 250X Polybrene (Dilute 1000X stock in PBS)
12. Transfer 250ul cells with retrovirus into a single well of 48 well plate (low attachment plate)
13. Centrifuge at 600g (~1700 rpm) for 1 hour at room temperature
14. Incubate at 37’c CO2 incubator for 6 hours
15. Resuspend cells with 1ml Triple +++ media and transfer to 15ml tube, and add 9ml ice-cold Triple +++ media
16. Centrifuge at 750rpm for 5min at 4’c
17. Remove the supernatant
18. Resuspend with 100~ 200ul Matrigel and plate into 48 well plate (25ul / well)
19. Incubate at 37’c incubator for 5min
20. Add 250ul of Complete Organoid media (including RhoK inhibitor)
21. Select for antibiotics 2 days after infection