Protocol: SNaP neurosphere formation

As described in this preprint

Day 0

1. Prepare 45mL NES complete with 50uM (1:200) ROCK Inhibitor (Y27)
   NOTE: this is 5X as much ROCK Inhibitor as is used for normal cell passaging
2. Add 1mL accutase, incubate at 37C for 5 more minutes
3. Add 5mL NES base and wash well to detach and singularize cells
4. Transfer to 15mL conical tube and take samples for counting
5. Centrifuge at 1,200 RPM for 5 minutes
6. Aspirate and resuspend cells in NES complete +Y27 to a dilution of 60,000 cells/mL
7. Mix thoroughly and transfer 150uL to each well of a low-attachment 96-well U-bottom plate, and place in incubator

Day 2

8. Prepare 17mL NES complete+Y27 (still at high concentration; 50uM)
9. Aspirate 75uL from each well, and replace with 150uL of fresh NES complete+Y27
   a. When doing all feeds from this point onward, 'blast' bottom of well to kick up debris, allow neurosphere to settle to bottom, then aspirate spent medium containing debris and detached cells

Day 3

10. Using blast technique, remove 125uL spent medium and replace with 150uL NES complete (no Y27)

Day 4+

11. Once again using blast technique, replace medium with NES complete. Remove 150uL and add 150uL fresh NES complete.
12. Continue replacing 150uL NES complete every other day

Recipes

NES Base
240mL DMEM/F12
2.5mL Pen/Strep
2.5mL MEM-NEAA
5ml Glutamax

Complete
50 ml NES base
1ml B27 w/o Vitamin A
500ul N2 supplement
5ul bFGF
5ul EGF

[Sterile filter]

Feeding schedule

Day 2
Day 3
Day 4
Day 6
Day 8
Day 10
Day 12
Day 14
Day 16
Day 18
Day 20
Day 22
Day 24
Day 26
Day 28
Day 30

Protocol: Cerebral organoid formation

As described in this manuscript and this JoVE video protocol

Day 0

1. Prepare 45mL E8 media with 50uM (1:200) ROCK Inhibitor (Y27)
   NOTE: this is 5X as much ROCK Inhibitor as is used for normal cell passaging
2. Add 1mL accutase, incubate at 37C for 5 more minutes
3. Add 5mL E8 media and wash well to detach and singularize cells
4. Transfer to 15mL conical tube and take samples for counting
5. Centrifuge at 1,200 RPM for 5 minutes
6. Aspirate and resuspend cells in E8 media +Y27 to a dilution of 60,000 cells/mL
7. Mix thoroughly and transfer 150uL to each well of a low-attachment 96-well U-bottom plate, and place in incubator

Day 2

8. Prepare 17mL E8 media + Y27 (still at high concentration; 50uM)
9. Aspirate 75uL from each well, and replace with 150uL of fresh E8 media + Y27
   a. When doing all feeds from this point onward, 'blast' bottom of well to kick up debris, allow organoid to settle to bottom, then aspirate spent medium containing debris and detached cells

Day 3

10. Using blast technique, remove 125uL spent medium and replace with 150uL E8 media (no Y27)

Day 4+

11. Once again using blast technique, replace medium with Neural Induction (NI) media. Remove 150uL and add 150uL fresh NI.
12. Continue replacing 150uL NI media every other day; clear neuroepithelial structures should start to form within in a week.

Day 16+

13. After roughly two weeks, organoids will outgrow the 96-well plates. Cut a P1000 tip so that the organoids can fit through the opening, and transfer organoids to ultra-low attachment 24-well plates or a spinner flask.
14. Continue feeding with NI every other day

Recipes

Neural Induction media (NI)
240mL DMEM/F12 with Glutamax
2.5mL N2 supplement
2.5mL MEM-NEAA
250 µg Heparin

[Sterile filter]

Feeding schedule

Day 2
Day 3
Day 4
Day 6
Day 8
Day 10
Day 12
Day 14
Day 16
Day 18
Day 20
Day 22
Day 24
Day 26
Day 28
Day 30