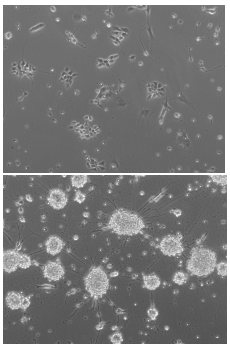
Neurosphere culture

(adapted from Dr. Changmee Kim)

1. Dissect E12.5~E13.5 embryos in cold PBS in a Petri dish on ice.
2. Dissect out the dorsal thalamus with fine forceps with the guide of EGFP from the *Gbx2^{Cre}* allele. Alternatively, the thalamus can be dissected out from brain slices based on the morphological landmarks.
3. Store the dissected tissue from each embryo in 500µl DMEM in a 1.5ml microfuge tube at RT.
4. Remove DMEM and add 500µl HBSS. Incubate in CO_2 incubator at 37°C for 5 min.
5. Weakly dissociate the tissue using yellow tip (P200 tips) and add 50µl Trypsin-EDTA/tube and incubate at 37°C for 5 min.
6. Add 50µl DNaseI (Roche, stock 5 mg/ml) per tube and incubate at 37°C for 5min.
7. Dissociate tissue with the yellow tip extensively. Add 500µl FBS to neutralize Trypsin activity. Triturate with the pipettor for 10 sec.
8. Centrifuge at 800 rpm for 5 min at RT.
9. Remove the media carefully using a pipette. NEVER USE ASPIRATOR!!!
10. Add 1ml Growth Media and resuspend with a blue tip (P1000 tip). Take out 20µl cell suspension to determine number of cells with a hemacytometer. (0.8~0.9 10^6 cells from the DT (one side) and maybe 100x more cells from the cortex of E13.5 embryos)
11. Add 5ml Growth Media to a 6cm plate. Add 60 μl of bGFP (2 μg/ml, 100x stock) and 1.2 μl of EGF (100 μg/ml, 5000x stock). Transfer the cell suspension into the plate and gently mix. Grow them in CO_2 incubator for one week. Change only a half of the media (7.5ml) every other day and freshly add bFGF and EGF.
12. After about one week, primary neurospheres (> 100µm) can be used for experiments or passaged. Transfer the cells into a conical tube and settle down by spinning at 500 rpm and repeat from step 4.



Neurospheres from DT (top) and Ctx, in 72 hr.

Reagents:

F12/DMEM (Invitrogen)
100x N2 Supplement (Invitrogen)
100x Pen/Strep (Invitrogen)
1mg/ml gentamycin (Sigma)
1M HEPES solution (Sigma, H0887)
HBSS without Ca2+, Mg2+
0.25% Trypsin-EDTA

<u>Growth Medium</u> (50 ml)

F12/DMEM (1:1)	
N2 Supplement 1x	0.5 ml
5mM Hepes	0.25 ml
1x P/S	0.5 ml
Gentamycin	100 μl

bFGF (cat # 133-FB), EGF (cat # 2028-EG): R&D Systems 1-800-343-7475
Freezing Medium, 2x (2ml DMSO + 2ml FCS + 6ml culture medium)
trypsinization with 0.05% Trypsin-EDTA