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\textsuperscript{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 500\(\mu\)l DMEM in a 1.5ml microfuge tube at RT.
4. Remove DMEM and add 500\(\mu\)l HBSS. Incubate in CO\textsubscript{2} incubator at 37°C for 5 min.
5. Weakly dissociate the tissue using yellow tip (P200 tips) and add 50\(\mu\)l Trypsin-EDTA/tube and incubate at 37°C for 5 min.
6. Add 50\(\mu\)l DNasel (Roche, stock 5 mg/ml) per tube and incubate at 37°C for 5min.
7. Dissociate tissue with the yellow tip extensively. Add 500\(\mu\)l 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 20\(\mu\)l 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 \(\mu\)l of bGFP (2 \(\mu\)g/ml, 100x stock) and 1.2 \(\mu\)l of EGF (100 \(\mu\)g/ml, 5000x stock). Transfer the cell suspension into the plate and gently mix. Grow them in CO\textsubscript{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 (> 100\(\mu\)m) 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.

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

Growth Medium (50 ml)
- F12/DMEM (1:1)
- N2 Supplement 1x
- 5mM Hepes
- 1x P/S
- Gentamycin

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