MIXED GLIAL CULTURES (primary, cortical, mouse)

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1. Dissect the cerebral cortices+hippocampus of 6-8 neonate mice (P1-P2) and place in cold PBS.

2. Remove the meninges.

3. Mince the tissue (e.g. with small scissors) and place in a 50ml tube.

4. Centrifuge 3min at 1000rpm (=200g).

5. Discard the supernatant and re-suspend in 12 mL warm Trypsin-EDTA (GIBCO #25200-072). Mix by inversion

6. Incubate 10min at 37°C in a water bath with agitation (100 rpm)
(we used to do this incubation for 25min but the last batches of trypsin seem to be more potent and we do this incubation now for 10 min only).

7. Stop trypsinization with 12ml DFF10 + 0.5ml DNase I
DFF10 is DMEM:F12 1:1 (GIBCO #31330-038; with Gln and HEPES) + 10%FBS + antibiotics (Pen/Strp). DNase I (Sigma D-5025), 150.000 units/10 ml PBS; store at -20°C

8. Mix by inversion, 10-12 times, until the mucus formed by sticky DNA disappears. Then, pipette up and down repeatedly until clumps of tissue are not visible anymore. This may take 40-60 up and down pipettings. This step is important to get a single cell suspension.

9. Centrifuge 7 min 1,000 rpm (=200 x g).

10. Remove carefully supernatant and discard.

11. Add 20 mL of medium (DFF10, see above) and homogenize pellet with pipette.

12. Filter cell suspension with a 105 μm mesh (Spectrum (1-800-6343300) #146436))

13. Count cells (typically we obtain 4-5 x 10^6 cells/pup)

14. Bring cells to a final density of 300,000 cells/ml with DFF10

15. Seed at 150μl, 300μl, 500μl, 2.5ml per well of a 96-, 48-, 24- or 6-well plate, respectively.

Typically we obtain 4-5 x 10^6 cells per pup.
In 96- or 48-well plates, the wells in the periphery are not seeded with cells but with water (+antibiotics/fungicide). Medium is changed fully at DIV 5 and then once a week. Cells are confluent at DIV 8-12.