# E14 Rat Cortical Culture

1. Anesthetize pregnant Long-Evans Rat (E14).
2. Remove uterus and place in ice-cold HBSS.
3. Remove embryos from uterus.
4. With a pair of forceps remove the skin overlying cranium, and the cranium (very soft at this age).
5. Scoop out the brain and place in fresh cold HBSS.
6. Try to remove as much of the pia as possible. (This can be difficult - it is better to leave some of the pia on at this stage rather than damaging the underlying tissue.)
7. Dissect out the neocortex. (Laterally we go down as far as the ganglionic eminence, and medially to the hippocampal anlage. Make sure not to take the ganglionic eminence (unless you want striatal precursors) or any of the diencephalon). The piece of cortex should appear as a thin sheet of cells in the shape of a shallow bowl.
8. Transfer cortex (from about 8 embryos) to 3 mls of L15-SFM media (37 C).
9. Gently triturate cells (4X) with a fire-polished glass pasteur pipet. Let the large chunks settle. Transfer supernatant to a new test tube (A). (We usually examine an aliquot of cells from tube 'A' after steps 9, 10, and 11 to make sure that clusters are being retained (5-20 cell clusters) and that the cells are not being singly dissociated. If most cells are singly dissociated, be gentler with the trituration in the next round and reduce the number of times you triturate the tissue)
10. Add 3 mls L15-SFM to chunks from step '9' and repeat trituration as before. Transfer supernatant to test tube A.
11. Add 3 mls L15-SFM to chunks left from step '10' and repeat as before. (This can be sequentially repeated several times if necessary, but typically we do 3 sequential triturations.)

12. Tube 'A' should now have about 9 mls of media with cells. Gently resuspend the cells and take a small sample for cell counts on a hemacytometer. You should see a mixture of singly dissociated cells and small clusters of cells (5-20 cells per cluster). Calculate number of clusters per volume and plate 50-100 clusters per well in a 24-well costar plate (coated with poly-lysine and laminin) in 400-500 µl media per well. Add bFGF to a final concentration of 10ng/ml. Supplement bFGF every 3rd day.

When doing the procedure for the first time it is useful to fix a plate 6 hrs after plating and immunostain the culture with anti-Nestin antibodies to determine the percentage of cells that are undifferentiated. Over 90% of the cells should be Nestin-positive.

**HBSS (for dissection)**
10X HBSS (Gibco #310-4180) 50 ml
1 M Hepes (pH 7.4) 1.25 ml (=2.5mM)
1 M Glucose 15 ml (=6.5 mg/ml)
100 mM $CaCl_2$ 10 ml (=2mM)
100 mM $MgSO_4$ 5 ml (=1mM)
1 M $NaHCO_3$ 2 ml (=4mM)
Add sterile $dH_2O$ to a total vol. of 500 ml. Keep at 4 C and use within 1 month.

**L15-SFM (50 mls)**
L15 (w/o bicarb/glutamine; ICN 12-510-54) 46 ml
1M $NaHCO_3$ 1.25 ml
1M Glucose 1.5 ml
200 mM Glutamine 250 µl
Pen Strep 500 µl
N2 supplement (Gibco) 500 µl