**E17 to P1 Cortical Cultures (Ghosh Lab Protocol)**

**1. Prepare solutions**: Enzyme (E), heavy inhibitor (HI), light inhibitor (LI), culture media. Place at 37 C.

**2. Prepare for dissection**: Spray dissection area with 70% EtOH. Sterilize dissection instruments by immersion in 70% EtOH for 10 minutes. Air dry dissection instruments by propping on petri dish covers. Measure out HBSS in two 50 ml tubes. One tube should have approx 40 mls; the other about 15 mls. Place on ice.

**3. Dissection**:

(i) Embryonic: Euthanize pregnant rat with $CO_2$ (3-5 mins). Remove uterus and place in 15 ml HBSS in 100 mm petridish. Move to dissection scope.
Newborn: Euthanize by decapitation. Place in 15 ml HBSS and move to dissection scope.

(ii) Use fine scissors or forceps to remove bone (cranium) overlying brain.

(iii) Transfer brains to new HBSS in 35 or 60 mm plates.

(iv) Remove pia, starting at the bottom of the brain.

(v) Cut brain along midline. Pinch off thalamus at the ventral medial surface.

(vi) With the medial surface of the brain facing you, remove the hippocampus by pinching with a pair of forceps.

(vii) Pinch off the basal ganglia and infratemporal cortex (roughly the ventral 1/3 of the brain). You should now be left with a bowl which is the neocortex.

(viii) Transfer the cortex to new HBSS and cut it into small pieces (roughly 1/2 mm)

(ix) Use a cut/flamed pasteur pipet to transfer pieces of cortex to 1/2 of the enzyme soln (E) in a 15 ml tube.

**4. Dissociation**:

(i) Leave tissue in 'E' at 37 C for 20 mins. Add remaining 1/2 of enzyme soln., and leave cells at 3l C for an additional 20 mins. Rock the tube occasionally. At the end of this period move 'E' with tissue, HI, LI, media to TC hood.
**[during the enzymatic step clean up dissection area, and wash dissection instruments very well with water]**

(ii) Remove 'E', leaving tissue at the bottom of the tube.

(iii) Rinse cells 1X with LI.

(iv) Remove LI, add HI, leave tissue in HI for 2 mins at 37 C.

(v) Remove HI, rinse tissue 1X with 5 mls media.

(vi) Remove media, add 5 mls new media.

(vii) Triturate tissue gently about 10-20 times with 5 ml pipet. This shouls result in virtually complete dissociation. Allow any debris to settle, and transfer media with cells to new tube.

(viii) Take a 30 µl aliquot of cells and count using hemacytometer.

**5. Plating**:

(i) Dilute cells in media as appropriate and plate as follows:
24 well plates: 2-3X$10^5$ per well
60 mm plates: 3X$10^6$ per well

(ii) Rock plate back and forth, and sideways gently to ensure even cell distribution. Place in 5% $CO_2$ incubator.

(iii) 2-4 hrs later replace media with fresh warm media (500µl per well for 24 well plates, 3 ml per 60 mm plate).

*At all times be gentle to your cells!*