**Cortical Neuron Culture**

**Reagents and animals:**

**Mice or rat**

E17-18

**Neuronal Media (Make Fresh)**

Total Volume = 12ml/plate \* # of 96 well plates (Thermo Scientific 167425)

* + Neurobasal (Thermo 21103049 )
  + Pen/Strep 1:100
  + B27 Plus (Thermo A3582801) 1:50
  + Glutamax (Thermo 35-050-061) 1:100

**Trypsin Solution**

3ml of .05% Trypsin and 1:50 of DNase 10mg/ml stock (Worthington Biochemical LS002139) per pool of cortices

**Plates**

1. Coat plates for minimum 4 hours at 37ºC with Poly-D-Lysine (PDL; Thermo ICN10269490) 1mg/ml diluted 1:10 in sterile water.
2. Wash 3x with water for 3 minutes each (can be stored at -20 ºC)

**Procedure:**

**Dissociated Culture**

1. Dissect cortices into a tube containing **Hibernate E** (Thermo A1247601) on ice
2. Remove **Hibernate E** and add 3ml of **pre-warmed** **Trypsin solution** +60ul DNase (10mg/ml)
3. Triturate 3-5x to break up cortices into pieces and incubate for **25 minutes** **at 37ºC**
4. Centrifuge at **500 x g for 5 min** andremove as much **Trypsin solution** as possible
5. Resuspend to 3ml with **Neuronal Media** and Triturate 40x with P1000
6. Filter cell suspension through 100uM filter
   * Wash filter with an additional 1ml **Neuronal Media**
7. Take 10ul of resuspended neurons and add 190ul Trypan blue
   * Load 10ul on a hemocytometer and count the number of neurons
8. Resuspend the desired number of cells in your plating volume (30,000 per one 96 well, 120,000 per 24 well)
9. Immediately after plating, do not move the plate for 10 minutes
   * Leave it in the hood during this time