Explant Culture in Matrigel™

1. Sacrifice the timed-pregnant female (E12.5-E13.5), and then swab its abdomen with 70% alcohol and open it to expose the embryos. Dissect embryos in cold PBS.
   **Used sterilized instruments for dissection*.

2. For thalamus (Th) or medial ganglionic eminence (MGE) explants:
   a. Excise the brains from the skull of embryos, and remove all visible pia in ice cold Krebs buffer.

   b. Brains are embedded in 4% low melting point agarose, and cut into 300 μm coronal sections with a vibratome in cold Krebs buffer. (see protocol for Culture of Brain Slices).

   c. The Th or MGE is dissected out with guidance of *Gbx2-GFP* fluorescence, and cut into pieces of approximately 300 μm in diameter with thin tungsten needles.

3. Transfer the explants into a drop of approximately 35 µl matrigel* in a 6cm Petri dish.
   **Matrigel is ready-to-use. It should always be placed on ice, otherwise it would polymerize at room temperature. Aliquot (1ml each) and store matrigel at -80˚C.*

4. For axon outgrow assay, add aggregates of transfected 293T cells to the matrigel and position the aggregate and explant, 100-300μm apart, using tungsten needles (Place the petri dish on ice all the time, otherwise matrigel will polymerize).

5. Transfer petri dish to room temperature to allow matrigel to solidify. Cover gels with serum free culture medium, and incubate at 37˚C in a 5% CO_2 environment.

6. Analyze the axon outgrowth after 24-72 hours. Mount cultures on slides and image on a Zeiss Axio Imager microscope. Excluded cultures in which explants lacked neurite outgrowth or are positioned either <75 µm or >200 µm away from the cell aggregate.

7. The attractive or repulsive effects of guidance cues are quantified by blind scoring using a virtual quadrant strategy. Explants form thee or four independent experiments are scored.

8. Images are subdivided into proximal and distal regions, and axon outgrowth originating from proximal and distal sides is determined by measuring the area,

or the total fluorescence associated with the area using ImageJ software (NIH). (This measurement takes into account both the number and length of the axons, parameters that cannot be accurately measured individually in these three-dimensional cultures.) To avoid variability due to shape and orientation of explants, exclude fluorescence associated with the body of the explant from the analysis. The ratios of the proximal and distal areas is compared using Student's *t* test after logarithmic transformation and verification of homogeneity of variance (Bartlett test).

Serum–free medium (use same day as prepared)

Neurobasal medium	95 ml (Gibco)
B-27 supplement	2 ml (Gibco)
50% glucose	1 ml
100X glutamine	1 ml
Pen/strep 100X	1 ml
Gentamicin	200 μl

PBS (1X, PH 7.2)	Invitrogen	20012-027
Matrigel™ basement membrane matrix	BD Biosciences	354234
Neuroblast medium	Invitrogen	21103-049
N-2 supplement	Invitrogen	17502-048
B-27 supplement	Invitrogen	17504-044
Penicillin-streptomycin	Invitrogen	15140-122

References:

Bonnin A, Torii M, Wang L, Rakic P, Levitt P. 2007. Serotonin modulates the response of embryonic thalamocortical axons to netrin-1. Nat Neurosci 10(5):588-597.

Brose K, Bland KS, Wang KH, Arnott D, Henzel W, Goodman CS, Tessier-Lavigne M, Kidd T. 1999. Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 96(6):795-806.