Dissecting Medium

1. Autoclave 2.38 g HEPES in 450 ml water in a 500 ml bottle
2. Add

Volume	Solution	Stock
14.25 ml	NaCl	29.2 g in 150 ml
12.5 ml	KCl	0.93 g in 125 ml
1.8 ml	KH_2PO_4	0.68 g in 50 ml
1.0 ml	$MgSO_4 \cdot 7H_20$	1.23 g in 50 ml
5 ml	$Na_2EDTA \cdot 2H_2O$	0.019 g in 50 ml
2 ml	$NaHCO_3$	4.20 g in 50 ml
5 ml	100X Pen/Strep	Gibco # 15070-014
50 ml	heat-inactivated FCS	
8.5 ml	$CaCl_2$	1.47 g in 100 ml (or 1.68 g $CaCl_2 \cdot 2H_20$)

Mix well and store for 2 weeks or less at 4˚C.

Dispase

0.015 g dispase (Gibco # 17105-041) in 10 ml PBS

Culture Medium

1:1 DMEM/F12 (Gibco #'s 11995-024 and 11765-013)
1X Pen/Strep
1X Insulin-Transferrin-Selenium (Gibco # 51500-023)
20 ng/ml bFGF (Gibco # 13256-029)

Matrigel

40234B from Collaborative Research (Becton Dickinson)

Procedure

1. Dissect embryos from decidua in Dissection Buffer. Works best with 8.75 day embryos: embryos that have completed turning and have 10 or more somites. These can be collected in the afternoon of day 8 for CD1 mice. Other strains are likely to be slower, and might best be dissected the morning of the following day.
2. Cut a trunk segment using the somite number as a guide (e.g. somites 4 to 6 inclusive). Works best for pieces about 3 somites in length.
3. Cut away tissue ventral to the somites.
4. Put the piece in 0.15% dispase in PBS for 10 to 20 minutes at RT--when the other tissues have begun to peel away from the neural tube, then proceed to next step.
5. Pipette up and down a few time to remove somites and extra tissue from neural tube.
6.Transfer neural tube to dissecting media to stop the protease.
7. Transfer neural tube to a plate covered with Matrigel (35 mm plate covered with 1 ml Matrigel, gelled 20 minutes at 37 ˚C in 5% CO_2 incubator) in a minimal volume, draw up excess medium with pipet or Kimwipe
8. Cover the tissue with a 10 µl drop of Matrigel.
9. Put in 5% CO_2 incubator at 37˚C for 15 minutes.
10. Cover plate with 1 ml of culture medium.