Papain Dissociation of Cortical Neurons
McConnell Lab Protocol
Updated 29 May 2014
For use with the Worthington kit (http://www.worthington-biochem.com/PDS/cat.html)

Day Before:

1) Make ovomucoid-inhibitor solution ("High Inhibitory" or "HI"):
   -Add 32ml EBSS (vial 1) to albumin-ovomucoid mixture (vial 4)
   -Mix and equilibrate in incubator
   -Store at 4 deg.
   -Good for at least a few days
2) UV-treat hood overnight (alternatively, treat for one hour morning of FACS)
3) Make CSMN buffer (recipe below) (good for 2 days stored at 4 deg)
4) Treat plates with poly-D lysine
   -Coat 3 coverslips for each of 2x24-well plates with PDL (stock is 100X, add 100ul to 10ml water)
   -Let sit for 1 hour
   -Remove PDL and wash 2x with sterile H2O
   -Add CSMN buffer to wells and store in incubator

Morning of:

1) Thaw Kynurenic acid (KYNA)
2) Place EBSS (provided in the kit) in the incubator
3) Remove embryos from pregnant dam and place in cold HBSS on ice. Optional: Check for fluorescence under microscope.
4) Dissect embryos in cold HBSS. Store neocortical tissue in 15-ml Falcon tube on ice.
5) Place HI in the incubator with the lid slightly open for no longer than 35 minutes. (Note: do this just after dissection is complete.)

Dissociation:

1) Add 5-10ml EBSS to papain vial (vial 2). Place at 37 deg for 10 min. (Do this just before finishing the last dissection so papain has time to go into solution.)
2) Add 500ul EBSS to DNase vial (vial 3). Mix gently.
3) Make dissociation solution (Recipe for 1 dissociation):
   -Take 5ml papain in EBSS and add to new Falcon tube
   -Add 250ul DNase in EBSS
   -Add 5ul KYNA
4) Add all of the dissociation solution to tissue
5) Leave in incubator for 25-30 minutes, disturbing the tube every 5-10 minutes.
6) Gently triturate cells until they appear mostly dissociated, then centrifuge cells for 10 mins at #4 setting on McConnell centrifuge (~500g).

7) While centrifuging, make the low ovomucoid inhibitor solution ("Low Inhibitory" or "LI") (Recipe for 1 sample):
   -2.7ml EBSS
   -300ul HI
   -150ul DNase
   -3ul KYNA
8) Aspirate supernatant from cells.
9) Add all of the LI solution to the cells and carefully re-suspend (~3ml LI per sample)
10) In a new tube, add 5ml HI, and layer all of the cells in LI on top, for 8ml total
11) Centrifuge on #4 for ~7 mins
12) While centrifuging, add 20ul KYNA to 20ml CSMN buffer (or 40ul KYNA to 40ml CSMN).
13) Remove supernatant from cells.
14) Re-suspend cell pellet in ~500ul CSMN buffer and transfer to filter tube. (Note: if >6 embryos were used, consider re-suspending in >500ul CSMN
15) Spin filter cells using filter tube for 1 minute on #4. (Note: Each filter tube only holds 500ul, so may need to split sample into two tubes.)
16) Re-suspend filtered cells (already in 500ul+ CSMN buffer).
17) Spot 1ul onto each pre-FACS coverslip, wait 3 mins for cells to settle, then add 1-1.5 ml CSMN buffer, incubate at 37 deg
18) Sort in FACS facility.
    Note: Cells seem to like being collected in a bacterial dish taped to the lid of 6-well plate containing 2-3 ml CSMN buffer (If more solution needs to be added to the cells being sorted, add CSMN **not** saline). If doing RNA or DNA analysis, collect cells in a microcentrifuge tube containing 500ul CSMN buffer or RNALater. Store cells on ice.
19) If culturing cells, collect everything from the dish into a falcon tube (~8ml total).

To Process After FACS:

1) Spin cells for 10 mins at #4 setting.
2) Carefully remove supernatant.
3) For cells in RNALater, resuspend in fresh RNALater and store at 4 deg.
4) For cells that were in CSMN, spot 1ul onto post-FACS coverslips, let sit 3 min, add 1-1.5ml CSMN buffer.
5) Incubate coverslips at 37 deg for 90 mins.
6) Remove CSMN buffer from plated cells and fix in 4% PFA for 5 mins.
7) Remove PFA and store in PBS at 4 deg.

CSMN buffer recipe (20 ml):

Ingredient	Amount	Final Concentration
DMEM	9.5 ml	50%
Neurobasal	9.5 ml	50%
Pyruvate	200 ul	1mM
L-Glutamine	200 ul	2mM
Insulin	200 ul	5ug/ml
Pen/Strep	200 ul	100U/100ug/ml
Sato	200 ul	1X
2M Glucose	350 ul	35mM
4% BSA	17 ul	0.34%