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Primary cortical neuron isolation and culture

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Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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Abstract

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1 **Dissection and Digestion** - Micro-dissect cortices from P1 rat pups of both sexes (Sprague Dawley, Charles River Laboratories, SD-001). - Digest cortices in papain (~7.5 units/ml) at 33°C for 45 minutes.

2 **Cell Preparation** - Triturate the digested tissue in low and high ovomucoid solutions. - Resuspend cells in panning buffer (DPBS (GIBCO 14287) supplemented with BSA and insulin). - Pass cells through a 20 µm mesh filter (Elko Filtering 03-20/14).

3 **Negative Panning** - Incubate filtered cells on negative panning dishes coated with: - Bandeiraea Simplicifolia Lectin 1 (x2) - Goat anti-mouse IgG+IgM (H+L) (Jackson ImmunoResearch 115-005-044) - Goat anti-rat IgG+IgM (H+L) (Jackson ImmunoResearch 112-005-044) antibodies.

4 **Positive Panning** - Incubate cells on positive panning dishes coated with mouse anti-L1 (ASCS4, Developmental Studies Hybridoma Bank, Univ. Iowa) to bind cortical neurons. - Collect adherent neurons by forceful pipetting with a P1000 pipette.

5 **Neuron Isolation and Plating** - Pellet isolated neurons (11 minutes at 200 g). - Resuspend neurons in serum-free neuron growth media (NGM): - Neurobasal, B27 supplement, 2 mM L-Glutamine, 100 U/ml Pen/Strep, 1 mM sodium pyruvate, 4.2 µg/ml Forskolin, 50 ng/mL BDNF, and 10 ng/mL CNTF. - Plate 70,000 neurons onto 12 mm glass coverslips coated with 10 µg/ml poly-D-lysine (PDL, Sigma P6407) and 2 µg/ml laminin. - Incubate at 37°C in 10% CO2.

6 **Media Changes and Maintenance** - On day in-vitro (DIV) 2: - Replace half of the media with NGM Plus (Neurobasal Plus, B27 Plus, 100 U/mL Pen/Strep, 1 mM sodium pyruvate, 4.2 µg/ml Forskolin, 50 ng/ml BDNF, and 10 ng/ml CNTF). - Add AraC (10 µM) to stop the growth of proliferating contaminating cells. - On DIV 3: - Replace all the media with NGM Plus. - On DIV 6 and DIV 9: - Replace half of the media with NGM Plus.

7 **Lentivirus Infection Protocol** - On DIV 2: - Add 100 µl of supernatant containing lentivirus plus polybrene (1 µg/ml) to the AraC NGM mixture. - On DIV 3: - Completely wash out the lentivirus and replace with NGM Plus containing 100 ng/ml BDNF.