Title	Differentiating Neural Stem Cells into Neurons
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Adapted from -	Gibco Protocol
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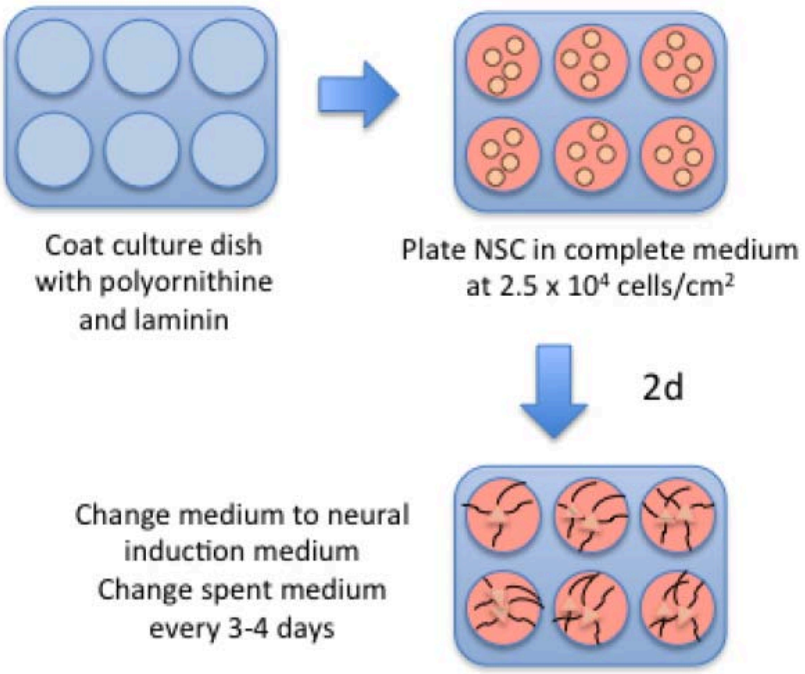
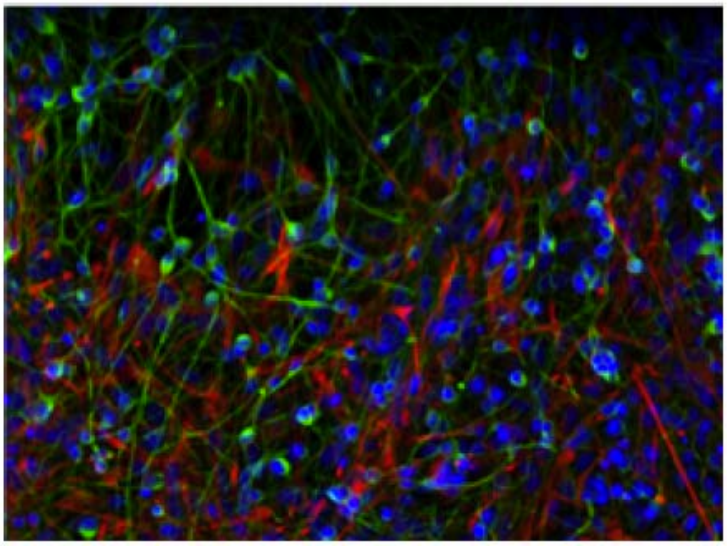
❖ Introduction:





Human iPSC-derived neuronal culture: Neurons/Tuj1 (Green); Glia/GFAP (Red) Nuclei/DAPI (Blue) - Rakesh Karmacharya, MD, PhD (Broad/McLean) from Harvard Stem Cell Institute

❖ Protocol:

Neural stem cells (NSCs) will proliferate as progenitors a few times even after the complete growth medium is replaced with the appropriate differentiation medium. If the cells reach 90% confluency, it might be necessary to split the cells at a 1:2 ratio. However, do not split the cells once they reach day 9-10 of differentiation when they can get damaged during the passaging process.

1. Plate neural stem cells on a polyornithine and laminin- coated culture dish in complete StemPro NSC SFM at 2.5 × 10^4 or 5 × 10^4 cells/cm2.
2. After 2 days, change the medium to neural differentiation medium. Change the spent medium every 3 to 4 days.
3. If expedited differentiation is desired, add 0.5 mM of dibutyryl cAMP (Sigma, Cat. no. D0627) to the differentiation medium daily starting at day 7 of differentiation for 3 days.

IMPORTANT! Do not expose cells to air at any time after they have differentiated into neurons.

❖ Materials:

polyornithine and laminin-coated culture dish		
complete StemPro NSC SFM medium		
neural differentiation medium		
dibutyryl cAMP		
StemPro NSC SFM Complete Media		
Component	Final concentration	Amount
KnockOutTM D-MEM/F-12	1X	97 mL
GlutaMAXTM-I Supplement	2 mM	1 mL
bFGF (prep as 100 µg/mL stock)	20 ng/mL	20 µL
EGF (prep as 100 µg/mL stock)	20 ng/mL	20 µL
StemPro® Neural Supplement	2%	2 mL
Neural Differentiation Medium		
Component	Final concentration	Amount
Neurobasal® Medium	1X	97 mL
B-27® Serum-Free Supplement	2%	2 mL
GlutaMAXTM-I Supplement	2 mM	1 mL

❖ Troubleshooting:

❖ References: